UCRL-19708
c. 2

STIMULATION OF GENETIC RECOMBINATION AMONG LAMBDA BACTERIOPHAGES BY ULTRAVIOLET RADIATION: THE MECHANISM AND THE RELEVANCE OF DNA REPAIR PROCESSES

Raymond Milton Baker
(Ph. D. Thesis)

February 1970

AEC Contract No. W-7405-eng-48

TWO-WEEK LOAN COPY

This is a Library Circulating Copy which may be borrowed for two weeks. For a personal retention copy, call Tech. Info. Division, Ext. 5545

UCRL-19708 c.2

LAWRENCE RADIATION LABORATORY
UNIVERSITY of CALIFORNIA BERKELEY

4b

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

TABLE OF CONTENTS

	Page
ABSTRACT	4
Section	
I. INTRODUCTION	7
A. Rationale and Summary	7
B. Background Review	16
1. Bacteriophage λ	16
2. DNA Repair	20
3. Mechanism of Recombination; Relation to DNA Repair	29
4. Stimulation of Genetic Recombination	40
II. MATERIALS AND METHODS	45
A. Bacteriophages	45
B. Bacterial Strains	47
C. Media	50
D. Preparation of Phage Stocks	51
E. Ultraviolet Irradiations	53
F. Phage Titrations and Complementation Tests	54
G. Phage Crosses	56
H. Determination of Recombination Frequencies	59
I. Indicator System for Assay of HCM-labeled Parental Phages	61
III. RECOMBINATION AMONG UV-IRRADIATED LAMBDA BACTERIOPHAGES IN UV-SENSITIVE HOST BACTERIA	64
A. UV Enhancement of Recombination Frequencies	64
1. HCR+ and HCR- Host Strains; Host Cell Reactivation of Recombination-Promoting UV Lesions	64

Page

2. Recombination-Deficient Host Bacteria 78

The Possibility of Replication-Bypass of UV Photoproducts in λ Phage; Post-Replication Repair 87

3. Other UV-Sensitive Host Bacteria 92

B. Multiplicity Reactivation of UV-Irradiated λ Phages . . 96

1. HCR+ and HCR- Host Bacteria; The Relation between UV-Induced Recombination and Multiplicity Reactivation 96

2. Other UV-Sensitive Host Bacteria 104

IV. RECOMBINATION OF UV-IRRADIATED PARENTAL LAMBDA PHAGE GENOMES 109

A. Recombination of Unirradiated HCM-labeled Parental Phages 109

1. Host-Controlled Modification 109

2. Recombination by Breakage and Reunion of Parental DNA Strands 113

3. Properties of the HCM Label on Parental Recombination 115

B. Enhancement of Recombination among HCM-labeled Parental λ Phages; UV-Induced Recombination by Breakage and Reunion 118

C. Recovery and Viability of UV-Irradiated HCM-labeled Parental λ Phages 132

1. Relative Viability of HCM-labeled Phages Recovered from HCR+ and HCR- Host Bacteria 135

2. UV-Induced Decrease in the Recovery of Parental HCM-labeled Phages 144

D. The Kinetics of UV Enhancement of λ Phage Recombination; a Diffuse Recombinagenic Effect 161

Page

V. INDIRECT STIMULATION OF LAMBDA PHAGE RECOMBINATION BY UV-IRRADIATION OF HCR+ and HCR- HOST BACTERIA 178

A. The Context 178

B. UV-Sensitivities of the Host Capacities of HCR+ and HCR- Bacteria 182

C. Enhancement of Recombination Frequencies for Unirradiated Infecting λ Phages 188

D. Enhancement of Recombination Frequencies for UV-Irradiated Infecting λ Phages 196

VI. SOME GENERAL CONCLUSIONS 204

A. The Mechanism of UV-Induced Recombination 204

B. Relevance of DNA Repair to UV-Induced Recombination . . . 206

C. The Mode of UV Stimulation of λ Phage Recombination . . . 208

ACKNOWLEDGMENTS 215

APPENDICES

I. The Origin of an Anomalous Secondary UV Enhancement of Recombination Frequency among λsus Phages in HCR-Host Bacteria 216

II. Control Crosses of Unirradiated HCM-labeled λ Phages. . . 220

1. HCM-labeled single recombinants 223

2. HCM-labeled double recombinants 230

III. An Hypothesis Concerning the Nature of UV-Inactivation and UV-Reactivation of Bacteriophage λ--a Lethal Defect Which May Occur in Semi-Conserved UV-Irradiated Genomes . 236

REFERENCES 246

Stimulation of Genetic Recombination among Lambda Bacteriophages by Ultraviolet Radiation: the Mechanism and the Relevance of DNA Repair Processes

ABSTRACT

Raymond Milton Baker

The stimulation of genetic recombination among λ bacteriophages by UV radiation has been studied in UV-sensitive and wild type E. coli K12 host bacteria. The UV enhancement of recombination frequencies was assayed for all progeny phages and for progeny that contained parental phage DNA strands labeled by means of host-controlled modification (HCM).

The direct stimulation of λ recombination by UV-irradiation of the infecting phages is even greater in excision-defective bacteria, which are deficient in host cell reactivation (HCR) of the phages, than in wild type host cells. The UV lesions that promote recombination are repaired by HCR with the same efficiency as are potentially lethal UV lesions.

The dose-response for UV enhancement of λ recombination is the same in HCR+ recA and recB host bacteria which are deficient in their own recombination as in wild type REC+ host strains. However, the UV survival of λ phages is somewhat lower in mutant recA cells than in wild type host bacteria.

There is substantial multiplicity reactivation of UV lesions in phage λ. Under conditions of comparable recombination among the phages, the dose reductions due to multiplicity reactivation of UV

lesions are similar in HCR+ and HCR- host bacteria, but a much greater survival increase is evident in the HCR- strains.

The UV-induced recombination of λ proceeds by breakage and reunion of parental DNA strands. Light UV-irradiation of the infecting phages causes comparable increases in the frequencies of recombinants among all progeny phages and among progeny that contain HCM-labeled--λ·K12(P1)--parental DNA strands.

The HCM-labeled progeny recovered from multiple infections with UV-irradiated phages of excision-defective HCR- (but not HCR+) bacteria contain nearly as many host cell reactivable UV lesions as the infecting phages. The HCM-labeled progeny probably contain mainly conserved parental DNA. It is likely that UV-induced HCM-labeled recombinants are formed by breakage and reunion of infecting phage chromosomes prior to their replication.

With increasing UV dose to the infecting phages there is a decreased recovery of progeny that retain a parental HCM label. The possible significance of this effect is discussed.

Over intervals of the λ chromosome thought to differ in the ratio of physical to genetic length, the UV enhancement of recombination frequency varies as the genetic length of the interval. In excision-defective HCR- host bacteria, the enhancement of recombination frequency is proportional to a power less than one of the number of functional recombinagenic lesions in the phage DNA.

The indirect enhancement of recombination among unirradiated λ phages caused by heavy UV-irradiation of the host bacteria was also examined. This effect is less marked than the direct enhancement

caused by irradiation of the phage DNA itself. The indirect enhancement is apparently due to increased recombination by breakage and reunion of all phage genomes throughout the infection. It is observed for lower UV doses to excision-defective HCR- host cells than to HCR+ bacteria. This study also furnished information concerning UV-inactivation of the host capacity for λ phage.

The experimental findings are considered with regard to possible mechanisms by which UV lesions might stimulate recombination. The preferred hypothesis is that UV-induced structural defects in DNA lead to an increased efficiency of pairing among chromosomes precedent to recombination by breakage and reunion. No evidence was found to imply that the UV-induced recombination is directly associated with any mode of DNA repair.

I. INTRODUCTION

I-A. Rationale and Summary

Genetic recombination is the phenomenon in which chromosomes containing homologous genetic material "mate" to yield new, intact chromosomes with associations of genes different from that of either parent. Recombination is observed among the complex chromosomes of higher, eucaryotic, cells after both meiosis and mitosis; it is the exclusive mechanism of genetic reassortment among the single DNA molecules that constitute the simple chromosomes of procaryotic cells and viruses.

Natural frequencies of genetic recombination in micro-organisms can be enhanced by exposure of their genetic substance to ultraviolet light (UV) and numerous other agents that produce specific lesions in DNA (115, 117, 243a). The molecular mechanism by which these agents stimulate recombination is unknown. There is now conclusive evidence that most natural recombination among bacterial viruses occurs by breakage and reunion of the mating chromosomes (158, 222); on the other hand, it was long felt that several peculiar genetic characteristics of UV-induced recombination were best explained by theories postulating "copy-choice" of the mating molecules (117, 144, 87).

A variety of structural defects in DNA, including lesions induced by UV, can be repaired in bacteria by enzymic systems that act to preserve the integrity and viability of the primary genetic material (186, 89, 102). Such processes include the excision of photoproducts, with replacement of the defective regions in the DNA strands before duplication of the chromosomes (195, 176, 104), and replication-bypass of photoproducts followed by post-replication repair of any consequent defects

in the daughter chromosomes (192, 26, 109). It has been proposed that some of the enzymes which participate in DNA repair may also be involved in genetic recombination (104, 38, 109). Moreover, the concomitant induction of recombination and repair events in UV-irradiated genomes suggests the possibility that these phenomena may be not only responses to a common stimulus but also manifestations of the same series of events at the molecular level.

The experiments described in this report were undertaken with a view to establishing the mechanisms by which UV-induced recombination of the dependent bacterial virus λ is stimulated and executed, with particular reference to the possible relevance of DNA repair processes mediated by the host bacterial cells. The principal tactics directed to this end were the utilization of host bacteria known to be defective in DNA repair and of parental λ bacteriophages whose DNA strands were biologically labeled by means of host-controlled modification.

Ultraviolet-induced damage to λ bacteriophages can be corrected in wild type E. coli host bacteria by a process known as host cell reactivation (HCR), which is closely related, perhaps identical, to the excision-repair process that operates upon the bacterial DNA (67, 187, 183). In order to ascertain whether the UV-induced recombination among λ phages is associated with excision-repair processes or whether, on the contrary, the recombination-promoting UV lesions are susceptible to repair by host cell reactivation, genetic crosses of UV-irradiated phages were performed in wild type (HCR+) and mutant, excision-defective (HCR-) host strains and the results were compared (section III-A.1). It was found that the UV enhancement of phage recombination is even greater in the HCR- than in the HCR+ host strains. The recombination-promoting lesions

are repaired by host cell reactivation with the same efficiency as are potentially lethal UV lesions, indicating that the stimulation of recombination is due to unexcised but host cell reactivable UV photoproducts in the phage DNA.

The UV enhancement of λ phage recombination was also examined in REC- host bacteria (section III-A.2), which are defective in functions necessary to recombination and repair of their own DNA although they retain capacity for host cell reactivation (38, 37); it has been proposed that certain REC- bacteria may be defective in post-replication repair that proceeds by a mechanism of UV-induced recombination (109). The dose-response for UV enhancement of λ phage recombination was found to be essentially the same for rec+, recA, and recB host bacteria. On the other hand, the recA gene does make a small but significant contribution to the repair of potentially lethal UV lesions in the λ phage DNA. It is possible that the recA allele affects a secondary, perhaps post-replication, repair mode in the UV-irradiated phages without significantly influencing the stimulation of phage recombination by unexcised UV photoproducts.

The multiplicity reactivation of UV-inactivated phages in multiply-infected host bacteria is believed to be effected by recombination among the irradiated phage genomes (150, 183). In order to clarify the relation of this phenomenon to the UV enhancement of phage recombination, the multiplicity reactivation effect was also assayed for the crosses of UV-irradiated λ phages in wild type and repair-deficient host cells (section III-B). The principal UV stimulation of phage recombination was apparent at substantially lower doses than was multiplicity reactivation. The survival increase due to multiplicity reactivation is much

more evident in excision-defective HCR- than in HCR+ host strains, but in all hosts multiplicity reactivation caused comparable reductions in the effective UV dose under conditions of comparable phage recombination. These results suggest that the multiplicity reactivation is due in large part to UV-induced recombination and demonstrate that host cell reactivation and multiplicity reactivation are not sequential processes.

In order to investigate whether the UV-induced recombination occurs by a mechanism involving breakage and reunion of the DNA strands of the parental chromosomes, crosses were performed in which the DNA of the infecting λ phages was labeled by means of host-controlled modification (HCM) (6, 111), and the recombination frequencies among the HCM-labeled progeny phages were assayed. Characteristics of the HCM-labeled recombination of unirradiated parental chromosomes were defined in extensive control crosses (Appendix II). The results from crosses of UV-irradiated HCM-labeled phages imply that for relatively low UV doses the UV-induced phage recombination in both HCR+ and HCR- host strains is due almost exclusively to breakage and reunion of the parental DNA strands of conserved and semi-conserved infecting chromosomes (section IV-B). Moreover, it appears that multiple UV-induced recombinations often occur in the course of a single mating of two phage genomes. For higher UV doses, in an excision-defective HCR- host, recombination between unrepaired parental DNA and the newly synthesized genomes contributes significantly to the enhancement of overall recombination.

The HCM label also permits assay of host cell reactivable UV lesions retained in parental DNA which is recovered from infections of the host bacteria with UV-irradiated λ phages (section IV-C.1). The HCM-labeled progeny phages from an HCR+ host do not retain any host

cell reactivable UV lesions. However, the HCM-labeled progeny from crosses in an excision-defective HCR- host strain exhibit almost as many potentially lethal UV lesions as do the infecting phages, a slight discrepancy being attributable to residual HCR. This finding supports the view that UV photoproducts are removed from the irradiated phage DNA during host cell reactivation, and further suggests that the HCM-labeled progeny from crosses of UV-irradiated phages contain principally conserved parental DNA.

The relative recovery of HCM-labeled progeny phages decreases markedly with increasing UV dose to the labeled infecting phages (section IV-C.2). While the causes of this effect could not be conclusively identified, it is possible that a primary component of the decrease is due to nuclease degradation of semi-conserved parental phage DNA. In light of previously reported characteristics of the phenomenon known as UV-reactivation (183, 168), this interpretation would suggest that much of the normally observed UV-inactivation of λ phages is caused by an interference of excision and degradative processes with a post-replication repair process, each acting upon defects in semi-conserved parental DNA which result from replication-bypass of UV photoproducts (Appendix III).

With a view to elucidating the nature of the stimulus which UV lesions provide to recombination (section IV-D), the enhancement of recombination was assayed over intervals of the λ chromosome that differ in the ratios of their physical to their genetic lengths (120). The UV stimulation of recombination was proportional to the genetic, rather than the physical, lengths of the intervals between marked loci. In addition, there are indications that the stimulation of phage recombination in an HCR- strain is proportional to a power less than one of the

number of unexcised recombination-promoting lesions in the parental phage DNA. Taken together, these observations suggest that the UV lesions may exert a diffuse recombinagenic effect, which is propagated with diminishing intensity along the irradiated DNA. This interpretation is consistent with kinetic equations which can be applied to describe the data.

The experiments outlined above concern the "direct" enhancement of recombination among lightly UV-irradiated λ phages in unirradiated host bacteria. A small "indirect" enhancement of recombination frequencies occurs for crosses of unirradiated λ phages in heavily UV-irradiated host bacteria (130). The characteristics of this indirect enhancement effect were investigated, utilizing wild type and excision-defective host bacteria and HCM-labeled parental phages. The principal objective was to determine to what extent this phenomenon is comparable to the direct stimulation of recombination by irradiation of the phage DNA itself.

For crosses of unirradiated phages, with increasing UV dose to the host bacteria the frequency of exchanges by breakage and reunion among both parental and newly synthesized phage genomes increases up to about fivefold the level observed in unirradiated host cells (section V-C). The indirect enhancement of phage recombination occurs at lower doses in the HCR- than in HCR+ host bacteria, implying that the indirect stimulation is also due to unexcised but host cell reactivable UV lesions, in this instance located in the bacterial DNA. Both direct and indirect enhancements of phage recombination occur in crosses of lightly UV-irradiated phages infecting irradiated HCR+ bacteria (section V-D). The competitive inhibition of residual excision caused by UV-irradiation of the HCR- host bacteria further increases the enhancement of parental

phage recombination induced directly by UV photoproducts in the phage DNA.

Results obtained in these experiments also indicate that the capacity of the excision-defective HCR- host cells to support λ phage growth is less sensitive to lethal UV damage in the bacterial genome than is the capacity of HCR+ hosts (section V-B). Furthermore, for both HCR+ and HCR- host bacteria, as the capacity for λ phages is inactivated in heavily UV-irradiated cells there is a failure of multiplicity reactivation, as well as host cell reactivation, of UV-irradiated infecting phages. With increasing UV dose to the host bacteria the initial replication of an increasing fraction of the infecting phage genomes is apparently blocked because of the inactivation of a requisite host factor.

The principal conclusions of this study with specific regard to the direct enhancement of genetic recombination among UV-irradiated λ bacteriophages are: A) The UV stimulation of recombination is due to unexcised UV photoproducts in the phage DNA that are repairable by host cell reactivation with the same efficiency as are potentially lethal UV lesions. B) The UV-induced recombination of λ phages proceeds by a mechanism of breakage and reunion of parental DNA strands and at low to moderate doses occurs primarily among conserved and semi-conserved infecting phage chromosomes, early in the infection. C) The stimulation of recombination is proportional to the genetic rather than the physical length of the intervals between loci in the chromosome; the UV-induced stimulus to recombination is most likely diffused along the chromosome.

These results are quite compatible with a model that explains the UV stimulation of recombination as due to an enhanced efficiency of the

pairing between homologous DNA molecules which is prerequisite to their recombination (190, 41). The mating of chromosomes would be facilitated by the presence in the DNA of UV photoproducts, including intra-strand pyrimidine dimers (228, 191), which increase the susceptibility of the irradiated molecules to local denaturation (153). No evidence was found in these experiments to indicate that the UV-stimulated recombination event itself differs in any substantial respect from the breakage and reunion of chromosomes that is characteristic of recombination among unirradiated λ phage genomes (157), or to imply that the UV-induced recombination is directly associated with any mode of DNA repair.

The results for indirect enhancement of phage recombination by irradiation of the host bacteria offer an interesting counterpoint to those for the direct stimulation of recombination by irradiation of the phage DNA itself. The findings are consistent with an interpretation that the irradiated bacterial DNA can serve as a catalyst to mediate an enhanced efficiency of pairing among all phage genomes during the infection. The indirect stimulation may proceed by essentially the same mechanism envisioned for the direct stimulation of mating observed for UV-irradiated infecting phages.

One would anticipate that the explanation advocated for the stimulation of genetic recombination by ultraviolet radiation might be generalized to account for the effects upon recombination of some other agents which produce specific repairable lesions in DNA. In order to inquire into this possibility, genetic crosses of λ phages exposed to nitrogen mustard (HN2) have been performed in wild type HCR+ and excision-defective HCR- host bacteria. The results suggest that phage recombination is stimulated by HN2-induced intra-strand guanine-guanine crosslinks which

are excised in HCR+ but not in the HCR- host bacteria, whereas the principal lethal HN2-induced lesion is an inter-strand crosslink (139a, 139b). These experiments will be reported in detail elsewhere (10, 13b--see also remarks in section VI-C). This finding supports the notion that the salient property of one class of recombinagenic lesions is the introduction of a local distortion into the secondary structure of the DNA, which facilitates local denaturation of the molecule and pairing of homologous chromosomes.

A brief review of principal background material to the experiments discussed in this report is presented in the following section (I-B).

Portions of the work described in Chapter III have been reported previously (12, 11, 89, 13a).

I-B. BACKGROUND REVIEW

I-B. 1 Bacteriophage λ

Escherichia coli bacteria are the natural hosts of λ bacteriophage. λ is a temperate virus--capable of existing not only as a free infectious particle or vegetative agent within the host cell but also as a "prophage," its genetic substance integrated into the host chromosome. In the latter case the potential for vegetative phage development is conferred as a genetic trait to the progeny of the host, which is referred to as "lysogenic"; "induction" of the prophage to an active vegetative state can be effected by exposure of the lysogenic cell to various perturbing agents such as UV or HN2. The physiology and genetics of λ lysogeny and vegetative growth have been extensively investigated (reviews: 116, 18, 199, 54, 49).

As a free particle, λ consists of a tail 1400 A in length attached to a head of 540 A diameter which contains a single linear double-stranded DNA molecule of molecular weight ca. 30 to 33×10^6 daltons (ca. 5×10^4 nucleotide pairs) (126, 35). One would estimate that this complement should encode sufficient information for about 30 to 40 distinct polypeptides; about 25 λ genes have now been distinguished and the functions of many of these have been identified (54, 49). Consistent with its relatively limited genetic complement and its potential for lysogenization of the host, λ behaves as a dependent virus (206, 87): λ does not destroy the host genome in the course of vegetative development, as do "autonomous" T-even coliphages, and throughout the phage infection the capacity of the host to support the growth of λ is more sensitive to destructive radiation and chemical treatments than its capacity for

the larger, autonomous coliphages (206, 208). The dependence of λ implies that many functions required for the development of the phage in addition to the metabolic machinery necessary to protein synthesis may be provided by genes residing in the host genome.

After adsorption of a free λ phage to the host and injection of its DNA genome into the cell, the infection may follow either a lytic or a temperate course, apparently depending upon the outcome of a competition between processes of duplication and repression of the infecting genomes (117). In the latter case, vegetative development of the phage is curtailed by the action of a repressor protein for which the phage itself carries the gene (113, 177). Physical integration of the phage genome into that of the host in lysogenization--and excision of the phage genome in induction--is accomplished by a site-specific recombination event between the circular bacterial chromosome and a circular form of the λ DNA (33, 199). In the case that the phage infection follows a lytic course, synthesis of the phage DNA proceeds directly after infection and within about 25 minutes the first infectious progeny phages appear within the cell (229). Lysis of the host usually occurs about 50 minutes after infection (this interval is referred to as the "latent period"), with the liberation of around 100 progeny phage particles per cell.

Lambda DNA passes through an intriguing sequence of molecular configurations in the course of vegetative development. Short complementary stretches of single-stranded DNA extend beyond each end of the linear double-stranded molecule found in free phage particles, and soon after entering the host cell the phage chromosome forms a circle which is stabilized by hydrogen bonding between these cohesive end segments

(92, 20). One of the single-strand gaps present in this structure is then closed by the formation of a covalent bond to complete one of the phosphodiester backbones; later, after the introduction of a quaternary coil conformation into the circular DNA molecule, the remaining single-strand gap is closed (20, 169, 133). The actual vegetative replication of the phage DNA appears to take place while it is in a circular form with a single-strand break (171). There is also evidence for concatenates of vegetative λ DNA considerably longer than a single chromosome, which may represent structures generated in the course of replication or recombination, or intermediates in the conversion of vegetative to mature phage DNA (234, 250). The significance of these various chromosomal configurations is not yet understood.

The genetic map of λ bacteriophage (Fig. 4 below) is a single linear linkage group with a genetic length of about 15 recombination units or probably around 0.7 Morgans (where 1 recombination unit corresponds to 1% recombination among the progeny from a genetic cross, and a Morgan is the interval within which there is 1 recombination in the ancestry of the average progeny (114, 122, 2)). Genetic recombination is more rare among λ phages than among the autonomous T-even coliphages. This limited level of interchange is primarily attributable to the occurrence of few mating events during vegetative development within the host cell--around 0.5 to 1. matings in the ancestry of the average λ genome compared to 5 to 10 for a T-even genome (244, 122)--and might be in part related to the frequency of coiled forms of intracellular λ DNA.

In general, the kinetics of recombination among λ phages follow the predictions of the Visconti-Delbruck theory, which assumes that recombination events occur during pairwise matings that are random with respect to time and partner (227, 244, 122). A progressive increase in the frequency of recombinant phages is observed from the time the first infectious particles appear intracellularly until lysis of the cell (244). There is no positive interference of recombinations between λ phages; rather, there is a low generalized negative interference--a slight but significant tendency for λ chromosomes to undergo more than one, statistically independent, recombination in a single pairing of the parental genomes (122). A high, localized negative interference is observed over short lengths (ca. 1500 nucleotides) of the chromosome (2). As for T-even phages, the reciprocal products of λ recombination are not recovered with comparable frequencies from individual host cells (122, 115).

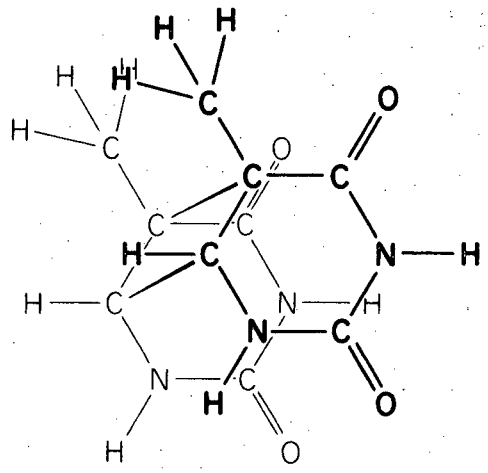
I-B.2 DNA Repair

Structural defects induced in DNA by a variety* of lethal and mutagenic agents can be repaired in bacterial cells. The best characterized repairable lesions are intra-strand cyclobutane-type pyrimidine dimers (Fig. 1a) induced by UV (228, 191) and di(guanin-7-yl) cross-links (Fig. 1b) formed by the bifunctional alkylating agent nitrogen mustard (138, 149). The first repair process to be characterized biochemically was photoreactivation, a highly specific in situ cleavage of UV-induced pyrimidine dimers in an enzyme-substrate complex activated by visible light (190, 183). Of concern in this report are the more general repair phenomena that occur in the absence of exposure to visible light and are therefore termed "dark repair," including processes of excision-repair and post-replication repair of lesions induced by UV radiation (reviews: 183, 192, 209, 89, 193, 102).

The concept of excision-repair is founded upon three principal types of evidence. First, many microbial mutants have been isolated that are exceptionally sensitive to radiation and chemical mutagens; the sensitivity of these mutants is attributable to a deficiency in their capacity to effect enzymatic repair of damage in their DNA (182, 183, 209). Second, in many microbial strains UV-induced pyrimidine dimers in the DNA are excised during a period immediately after irradiation and appear in the soluble fraction of the cells (195, 23b, 193).

---

* The category of known repairable defects includes lesions induced by Mitomycin C (24a), X-rays (156, 24b), decay of incorporated P^{32} (170), methyl methane sulfonate (209, 23a), nitrous acid (104), nitrosoguanidine (242), acridine dye-sensitized photoeffects (86, 68), thymine starvation (77, 40), and mild thermal treatment (27), in addition to UV-induced pyrimidine dimers and di-guanyl crosslinks produced by nitrogen and sulfur mustards.

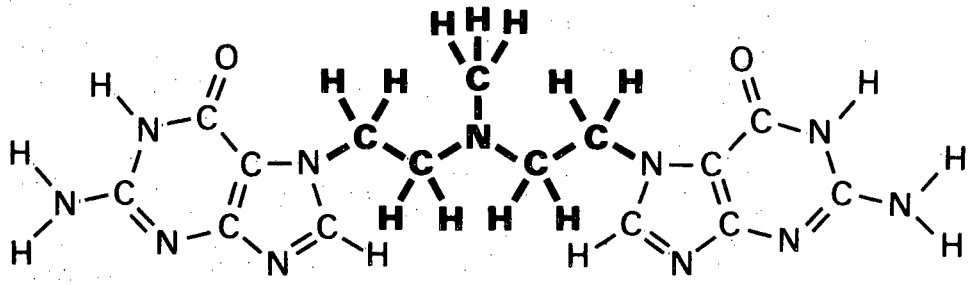


Thymine dimer

DBL 696-4749

Figure 1. a (top): The most common type of pyrimidine dimer formed in DNA by ultraviolet light. Adjacent thymine moieties in the same strand are linked by a cyclobutane ring.

b (bottom): Guanine-guanine cross-linking by nitrogen mustard. The 7-nitrogen atoms of two guanine moieties in opposite (or the same) strands of DNA are alkylated by the two "arms" of the bifunctional HN2 molecule.



GUANINE HN2 GUANINE

DBL 679-1823

Some radiation-sensitive mutants fail to remove these photoproducts from their DNA. Third, regions of localized non-conservative DNA synthesis are observed in the chromosomes of UV-irradiated micro-organisms (176, 77). This synthesis is referred to as "repair replication" and is believed to represent the replacement of damaged segments of the DNA strands.

On the basis of such evidence, several models of excision-repair have been proposed (Fig. 2) (253). According to the "cut and patch" model, the repair is envisioned as proceeding in four discrete steps: 1) excision of a lesion from the DNA by means of single-strand cuts that bracket the defect; 2) local degradation of the interrupted strand; 3) resynthesis of the degraded strand utilizing the complementary strand as template; and 4) rejoining of the phosphodiester backbone of the repaired strand by formation of a covalent bond across the remaining single-strand break. In an alternative "patch and cut" scheme, the first step is conceived as a simple incision, introducing a single-strand cut adjacent to the lesion, and steps 2 and 3 (above) are envisioned as concomitant, the original damaged strand being peeled away from its complement in the face of an advancing point of repair-replication.

Although these models of the excision-repair process no doubt represent oversimplifications, enzymatic activities consistent with each of the postulated repair steps have been demonstrated in *in vitro* systems. Extracts of *M. lysodeikticus* and *E. coli* exhibit an endonuclease activity that introduces single-strand breaks preferentially into UV-irradiated DNA (210, 182, 215, 75). The number of breaks approximates the number of dimers present in the DNA (193), and the endonuclease activity is absent in certain UV-sensitive mutants of *M. lysodeikticus* (75). The extracts

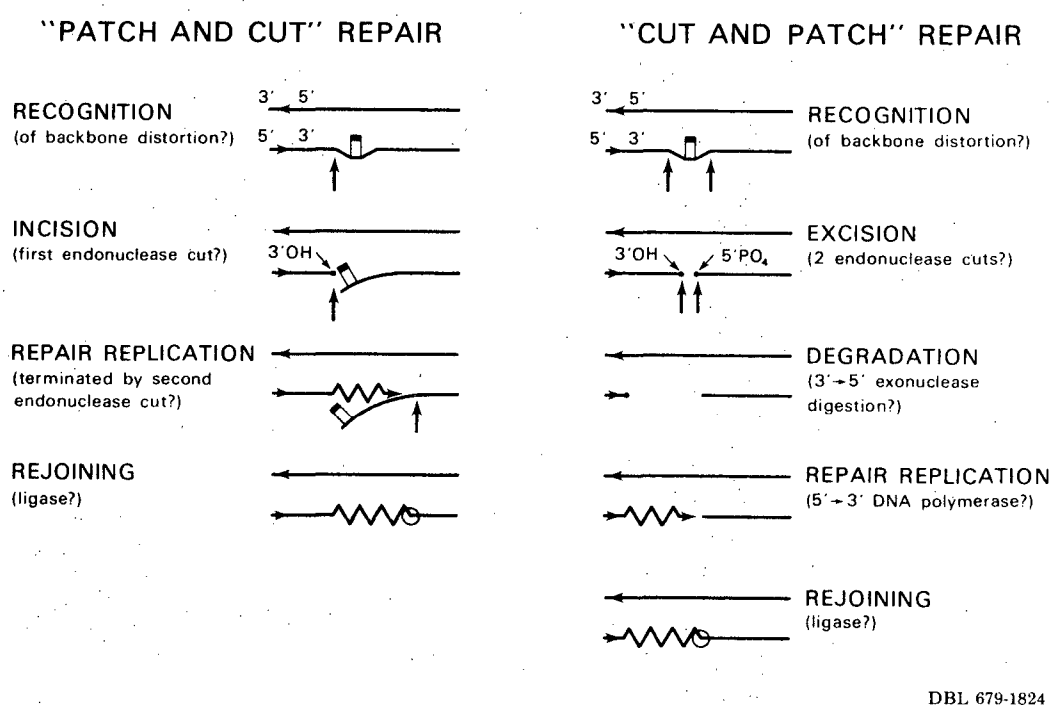


Figure 2. Two explicit hypotheses regarding the enzymic steps in excision-repair. The principal difference between the models lies in their conception of excision and repair-replication as concurrent or sequential steps. (The "cut and patch" scheme may be modified to indicate a single endonuclease cut followed by exonuclease degradation.)

also contain an exonuclease activity that releases thymine dimers preferentially from the damaged DNA (36, 215, 75). These endonuclease and exonuclease activities have recently been purified extensively and their specificity confirmed (124). The E. coli DNA polymerase of Kornberg has a specificity similar to that required for repair-replication type synthesis with concomitant exonuclease digestion of the defective segment (178, 135). Sealase/ligase enzymes that close single-strand gaps in DNA have been isolated from E. coli (179, 70, 141, 186).

It is presumed that the excision-repair process is a general one, since the sequence of repair steps outlined above could account for the correction of a variety of defects, and striking correlations have been observed in the sensitivities of microbial mutants to divers inactivating agents (104, 209, 89). The fact that some mutants are relatively sensitive to one type of agent and resistant to another may be taken to indicate either a) that the repair sequence has been blocked at a point that allows correction of one type of defect but not the other, b) that different "recognition" enzymes are initiating repair of the different lesions, or c) that different modes of repair are operating to correct the lesions.

A second mode of "dark repair" involves bypass of a UV-induced photoproduct in the course of DNA replication followed by post-replication repair of the defective region in the daughter chromosome. Contemplated in theory for some time (22, 242), this mechanism of repair has recently been demonstrated in UV-irradiated E. coli that are deficient in excision-repair (184, 26, 109). These mutants are capable of surviving a UV dose that is calculated to induce on the order of 50

potentially lethal pyrimidine dimers per chromosome. Post-irradiation DNA replication proceeds at a slightly reduced rate in these cells, and analysis of the average single strand molecular weights of the newly synthesized DNA suggests that the daughter strands contain gaps caused by the bypass of UV photoproducts; these gaps disappear upon subsequent incubation of the cells (184). Immediately after replication in the course of bacterial conjugation, pyrimidine dimers remaining in the parental DNA strand transferred from the donor to the recipient bacterium are susceptible to repair by photoreactivation but not by excision (109). Since excision, but not photoreactivation, requires a double-stranded substrate (182, 249), the gaps in the newly synthesized strand are most likely located opposite the dimers in the template strand. Post-replication healing of these gaps might not occur in a doubly mutant excision-defective recombination-deficient strain (see section I-B.3 below), for which a single pyrimidine dimer in the chromosome may prove lethal (108). In an excision-defective recombination-proficient strain the bypassed pyrimidine dimers remain in viable progeny DNA and continue to induce mutations for several generations (26).

Howard-Flanders and collaborators suggest that the post-replication repair of double-strand defects resulting from bypass of photoproducts proceeds by a mechanism of "sister-strand exchanges," which by redistributing the lesions among the strands of the daughter chromosomes could serve to restore information in defective regions (184, 109). Other possibilities are that the gaps are filled in by a relatively slow--possibly random--polymerization of nucleotides opposite the damaged template (242), or that the gaps are closed by a type of "copy-choice repair replication" utilizing an intact strand from another daughter

chromosome as template (28). Infidelity of this repair mechanism may be responsible for the UV induction of mutations (242, 28, 243b).

The post-replication repair of gaps caused by replication-bypass of UV photoproducts may be accomplished by the same pathway that effects a pre-replication repair of lesions due to such agents as X-rays (102, 243a). This would account for the X-ray sensitivity of those bacterial mutants thought to be defective in the post-replication repair of UV damage. Failure of the mechanism involved in post-replication repair could conceivably render single-strand gaps from whatever source more susceptible to destructive nuclease attack. It is not yet clear to what extent the enzymic processes necessary to excision-repair and post-replication repair (and/or repair of X-ray damage) might overlap (cf., 104, 125, 23a).

A simplified categorization of the genotypes and phenotypes of those radiation-sensitive mutants of E. coli K12 discussed in the present report is presented in Table I.

DNA repair phenomena are also observed in bacteriophage infection. Here one must again distinguish between autonomous and dependent types of phages. The UV-sensitivities of the T-even group of autonomous bacteriophages are not affected by the repair proficiency of the host cell (56), but at least two genes in the T4 phage itself--v and x--are known to affect its UV survival (79, 81). The v gene controls excision of thymine dimers from irradiated phage DNA (193); endonuclease and exonuclease activities consistent with excision-repair have been found in extracts of T4-infected cells (215). It is reported that T4 mutants defective in the genes for DNA polymerase and polynucleotide ligase are

Table I - A simple categorization of the radiation-sensitive strains of *E. coli* K12 bacteria discussed in this report. (Abbreviations: R = resistant; S = sensitive; + = present; - = absent; i or ± = intermediate.)

CLASS	LOCUS	Radiation Sensitivity		Recombination	HCR of λ	UV-R of λ	Reference
		UV	X-rays				
Wild type	--	R	R	+	+	+	
HCR	uvrA	S	R	+	-	±	(106,154, 209)
	uvrB	S	R	+	-	±	
	uvrC	S	R	+	-	±	
	uvrD	S	i	+	-	+	(168)
"EXR"	lex	S	S	±	+		(104,102)
REC	recA	S	S	-	+	-	(37,241, 57,168)
	recB	S	S	-	+		
	recC	S	S	-	+		

also more sensitive to UV (17). In general, the intrinsic UV-sensitivities of the other autonomous coliphages are similar to that of the T4v mutant--and significantly greater than that of dependent coliphages such as T1 and λ (206, 183).

UV damage to the dependent λ phage can be repaired in wild type host bacteria by a process known as host cell reactivation (HCR), which is thought to be similar--probably identical--to the excision-repair of UV-damaged bacterial DNA (67, 129, 187, 183). The host cell reactivation of λ appears to depend completely upon the excision enzymes of the bacterial cell to remove UV photoproducts from the phage DNA, for all host strains that are defective in excision (the uvrA, uvrB, and uvrC mutants of Table I) exhibit an HCR- phenotype (106). Recently isolated bacterial strains that are mutant at a uvrD locus are HCR- even though--in contrast to uvrA/B/C mutants--they are able to introduce single-strand breaks and extensively degrade irradiated phage DNA (168, 197). All other repair-deficient bacterial mutants exhibit an HCR+ phenotype. It is conceivable that some of the functions required to complete excision-repair of λ phage can be provided by the phage itself. Alternatively, the four uvr loci may define the entire repair system that operates in excision-repair and HCR, a different repair mode being affected in other radiation-sensitive bacterial mutants (125, 209).

I-B.3 Mechanism of Recombination; Relation to DNA Repair

Historically, two general hypotheses have been advanced to account for the mechanism of genetic recombination:

a) Recombination by breakage and reunion (164, 42, 157, 87). According to this model, homologous chromosomes come together and pair along certain regions, the specificity of the alignment presumably being dictated by the nucleotide sequences within the DNA of homologous genes. The paired strands are then broken at homologous sites, and the broken ends rejoined crosswise so that recombinant chromosomes are produced.

b) Recombination by copy choice (16, 90, 144, 87). This model envisions recombination as occurring during the replication of paired DNA molecules. Thus, it is suggested that a replica being synthesized on one chromosomal template might somehow be switched to a template on the homologous chromosome, so that the newly constructed DNA molecule contains genetic information derived from each of the paired homologues. Although originally conceived in terms of a conservative duplication of the genomes, a tenable copy-choice model must now incorporate the concept of semi-conservative DNA replication (cf., 87). A hybrid version of the breakage and reunion and copy-choice models, known as "break and copy," envisions the recombination as involving restoration of a chromosomal fragment through replication that is extended onto a template provided by a paired homologous chromosome (160).

An impressive amount of genetic evidence has been arrayed to support one or the other of these hypotheses or their variants (158, 87, 212). Evaluation of this evidence is rendered all the more difficult from the present standpoint because of the potential intervention of DNA repair processes to generate pure recombinant chromosomes by the

repair of internally heterozygous molecules (vide infra). However, two fundamental distinctions may be drawn between these categories of recombination models. First, a copy choice or break and copy scheme requires that recombination be associated with DNA replication, whereas recombination by breakage and reunion could occur entirely in the absence of normal DNA replication. Second, only a recombinant formed by strict breakage and reunion would be composed mainly of DNA derived from both strands of each parental molecule. It is on the basis of these distinctions that the breakage and reunion model of recombination has prevailed in recent years.

Meselson and collaborators, working with prelabeled λ phages, have established that some recombinant phage chromosomes are composed almost entirely of parental DNA and that there is a correspondence between the amount of DNA and the extent of genetic information that a parental phage contributes to a recombinant (160, 131, 157) (see also discussion in section IV-A.2). More recently, Tomizawa and Anraku have used labeled parental T4 phages to study the sequence of events in the formation of recombinant T4 chromosomes (224, 3, 4, 222). They have demonstrated: i) that the first step in recombination is the formation of a "joint molecule" consisting of overlapping components of the parental DNA molecules united end to end by means of hydrogen bonds; ii) that these joint molecules are then transformed into intact recombinant molecules in which the components derived from different parental structures are covalently bonded; and iii) that, while prior protein synthesis is required for the formation of joint molecules and very limited DNA synthesis is necessary for the transformation of joint to recombinant molecules, the entire process can occur in the absence of detectable DNA replication. These experiments provide conclusive evidence that natural recombination

among bacteriophages can proceed by a mechanism quite closely related to simple breakage and reunion.

DNA repair processes may be relevant to the phenomena of genetic recombination in three ways. First, recombination according to a mechanism of breakage and reunion would seem to require certain steps that are topologically indistinguishable from those envisioned for excision-repair. Second, DNA repair might be invoked to account for certain unusual genetic phenomena, such as gene conversion and conceivably even high, localized negative interference, which are related to recombination but are not easily explained on the basis of a simple breakage and reunion model. Third, it is possible that the process of post-replication repair proceeds by a mechanism involving a form of recombination, or at least enzymic steps which are also necessary to recombination.

Howard-Flanders and Boyce have proposed that the terminal steps of recombination by breakage and reunion include steps also involved in excision-repair (103, 104). Thus the primary steps of recombination could be viewed as: 1) pairing of homologous DNA molecules, followed by the introduction of single-strand breaks and the association of complementary single strands from the two parents in a manner such that a "joint molecule" of the type depicted in Fig. 3 - "H" is generated; 2) degradation of unpaired single strands to eliminate the regions of overlap between the recombined components (Fig. 3 - "I"); 3) repair-replication to fill in any single-strand gaps in the recombinant molecule; and 4) the rejoining of the phosphodiester backbones of the molecule so that the recombinant components are united by covalent bonds

Figure 3. A hypothetical scheme for genetic recombination, illustrating various conceivable roles of repair processes in the breakage and reunion of DNA molecules. The sequence A through H is an adaptation from that proposed by Whitehouse in his "polaron hybrid" DNA model of crossing over (236, 237). H is a form suggested by Tomizawa and Anraku for the T4 "joint molecule" (224). The steps in the transition from H through J are those proposed by Howard-Flanders and Boyce (104). J is the structure suggested by Meselson for the λ recombinant molecule (157). Note that the intact recombinants contain regions of structural heterozygosity (HR_1 and HR_2), in which the complementary strands are of different parental origin. The scheme depicted here also results in a region of gene conversion (GCR), or non-reciprocal recombination, in which one of the parental genes is represented on three strands and the other on only one strand of the two recombinant molecules. Subsequent repair of mismatched bases due to genetic heterozygosity in these regions could lead to the elimination of one of the parental alleles.

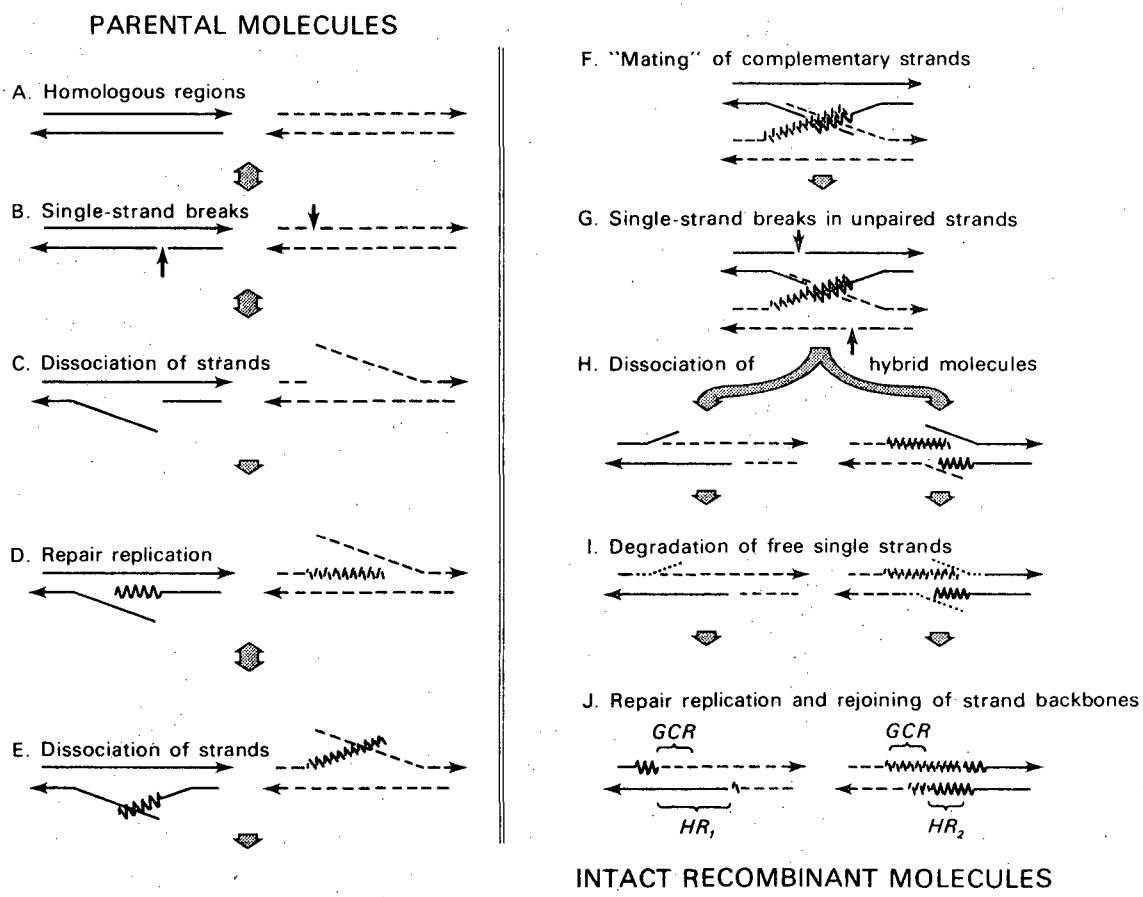

PARENTAL MOLECULES
A. Homologous regions
B. Single-strand breaks
C. Dissociation of strands
D. Repair replication
E. Dissociation of strands
F. "Mating" of complementary strands
G. Single-strand breaks in unpaired strands
H. Dissociation of hybrid molecules
I. Degradation of free single strands
J. Repair replication and rejoining of strand backbones
GCR
GCR
HR1
HR2
INTACT RECOMBINANT MOLECULES
DBL 670-1837

Figure 3

(Fig. 3 - "J"). This repair sequence would be consistent with the observed progression from a hydrogen-bonded joint molecule to an intact covalently bonded recombinant molecule in T4 phage (222). Moreover, there are indications that recombination in phage λ may be accomplished by the removal and resynthesis of small portions of the molecule, such as would result from repair in the region of overlap connecting the recombined components (157).

The possibility that the excision enzymes themselves might be essential to recombination was eliminated by the finding that bacterial mutants that are defective in excision are able to support normal recombination (104). The principal evidence in support of common pathways in DNA repair and genetic recombination has been the isolation of "REC-" mutants of bacteria, which are both deficient in recombination and extremely sensitive to UV and X-rays (38). Several varieties of REC- mutants have been found in E. coli (Table I); they exhibit somewhat different phenotypes corresponding to mutations at three (recA, recB, and recC) or more loci on the bacterial chromosome (37, 107, 226, 241, 57).

It was at first thought that the REC- mutants might be defective in an enzymic process common to excision-repair and recombination, but subsequent studies have tended to contradict this interpretation. The REC- strains apparently are capable of substantial excision-repair; a recA mutant, for example, successfully repairs approximately 95% of UV-induced pyrimidine dimers even though relative to the wild type it is nearly 200 times as sensitive to UV and its efficiency in recombinant formation is only about 10^{-4} (39, 104). This type of mutant retains the ability to excise (39), repair replicate (175), and heal single-strand breaks in λ DNA in vitro (69) and in vivo (24b); moreover, recA bacteria are capable

of liquid holding recovery (66)--an enhancement of survival following UV-irradiation which is thought to derive from prolonged excision-repair during an interval of suspended growth in non-nutrient medium (83).

The defect in the REC- strains may involve control of single-strand breakdown (39, 102). Mutants at the recA locus exhibit a high degree of DNA turnover even when they have not been irradiated, and recB mutants show less than normal DNA turnover (104, 57). These observations raise the question of whether the concomitant occurrence of radiation-sensitive and recombination-deficient phenotypes might not be a pleiotropic effect due to the disruption of essentially independent enzymic processes.

There are several lines of evidence that the recA mutation may inactivate a mode of DNA repair other than excision-repair. First, doubly mutant uvrArecA bacteria are about 40 times more sensitive to UV than the singly mutant uvrA cells, a difference too great to be attributed to a simple neutralization of residual excision activity (108). Strains carrying multiple uvr- mutations to excision-deficiency are no more than two to three times more sensitive to UV than single uvr- mutants (106, 168). Second, the extensive DNA degradation observed in recA mutant strains following UV-irradiation is not dependent upon the excision capacity of the bacterial cells (108). Rather, the sites most sensitive to degradation may be adjacent to the replicating point in the irradiated DNA (101). Third, UV mutagenesis--which is thought to be associated with replication-bypass of UV photoproducts (242, 28)--is suppressed in recA mutant bacteria (163, 243b).

Howard-Flanders and collaborators have suggested that the UV-sensitivity of recA and recB bacteria is due to a deficiency in post-replication repair of defects caused by replication-bypass of UV

photoproducts, and further contended that the post-replication repair normally proceeds by a recombinational mechanism involving sister-strand exchanges among the daughter chromosomes to reconstruct a viable genome (184, 109, 108). While there is convincing evidence to support the former interpretation, the latter view is open to question on the grounds that a common failure of recombination and the post-replication repair in REC- bacteria need not indicate that these phenomena proceed by closely analogous mechanisms. It is not clear that the usual enhanced recombination of semi-conserved irradiated DNA (vide infra) is a response to defects caused by replication-bypass as opposed to the UV photoproducts themselves.

The possible relations between recombination and repair are also under scrutiny in bacteriophages. The T4x mutant is 70% more sensitive to UV than the wild type T4 (79), and recombination frequencies are reduced about threefold in crosses of T4 where both parents carry the x mutation (81). However, the nature of the altered function is unknown. T4 phages mutant in genes 43 (DNA polymerase) and 30 (ligase) are significantly more sensitive to UV (17), but the relevance of these genes to recombination is not yet clear (222, 179, 17).

Distinct mutants of bacteriophage λ have been isolated which are defective in general vegetative recombination--λred (53, 201, 200)--and in site-specific recombination of the type implicated in integration and excision of the prophage--λint (73, 55, 199). To date, the red mutants have been classified into three complementation groups, two of which relate to the gene for λ exonuclease and the third to the gene encoding β, of unknown function (200).

Essentially normal levels of vegetative recombination are observed among λred+ phages in a recombination-deficient bacterial host (226, 30). Vegetative recombination among λred phages is reduced approximately tenfold in a REC+ or recB bacterial host and falls to 1/30 to 1/1000 the normal level in a recA host (53, 201). There are thus at least three distinct recombination systems operating in the course of a λ phage infection (viz., Rec, Red, and Int), and the bacterial recombination system is able to provide for a limited amount of recombination among the dependent phages.

The λred mutants are slightly more UV-sensitive than the λred+ when assayed on uvrA or recA host bacteria and about 30% more sensitive than the λred+ on a doubly mutant uvrArecA host (53). It is clear that the lambda Red system has some influence upon repair of the phage DNA in repair-deficient host cells, although this effect is dwarfed by the approximately sixfold difference in UV-sensitivity of λ phage on uvr+ (HCR+) and uvr- (HCR-) host bacteria.

A phenomenon relevant to both recombination and repair is the <u>internal heterozygote</u>, in which a region of the chromosome carries information for different alleles on the complementary DNA strands (91, 143, 130, 189). The generation of heterozygotes is probably a natural consequence of recombination by a breakage and reunion mechanism (Fig. 3), and in fact most phage chromosomes containing internally heterozygous regions are found to be recombinant for outside markers (143, 130). It has been demonstrated that in some circumstances internal heterozygotes of phage λ can be repaired by excision and correction of mismatched bases (233, 94, 47--see also 205). Variation in the efficiency of this type of repair

might account in part for the widely differing frequencies of heterozygotes found among various kinds of phage.

The phenomenon of gene conversion (146, 162b, 100, 61) could also be accounted for by the action of DNA repair processes on recombinants (see 205 for a contrary hypothesis). Most extensively studied in fungi, gene conversion is classically exemplified by aberrant segregation at a heteroallelic locus in meiosis so that a 3:1 ratio of alleles is observed among the products rather than the usual 2:2 Mendelian ratio. Such a meiotic "conversion" is often accompanied by reciprocal recombination for outside markers.

Holliday has suggested that gene conversion may be attributable to the repair of internal heterozygotes that arise through the pairing of heteroallelic parental strands (e.g., Fig. 3-"J") in breakage and reunion recombinants (96, 100). Moreover, an apparent non-reciprocal recombination of closely linked markers that is not correlated with recombination for outside markers could be generated by repair of mismatched bases in transiently paired parental strands (e.g., Fig. 3-"F"). If the parental molecules were to reanneal rather than consummate the recombination, the result would be introduction of an internal heterozygosity into each of the reconstituted parental molecules. Further repair of the newly formed heterozygotes could with 50% probability convert one parental allele to the other, the net effect being an information transfer unaccompanied by an exchange of physical substance (96, 89, 61). Mutations to radiation-sensitivity in Ustilago maydis have marked effects on mitotic recombination and possibly gene conversion (99).

Whitehouse has advanced a rather elaborate model of recombination and gene conversion that implicates DNA repair processes also in the formation of the initial "joint" recombinant molecule--as in Fig. 3 -

"A" through "H" (236, 237, 238). He has also pointed out that, in a recombinant molecule from a multi-factor cross of closely linked markers, random repair of internal heterozygotes could result in an apparent clustering of recombination events, i.e., a high, localized negative interference (see 100). However, this hypothesis may be inconsistent with the finding that in yeast conversion events thought to result from heterozygote repair involve the incorporation of information from a single parent over a relatively extended portion of the chromosome, 500 to 1000 nucleotides (61).

I-B.4 Stimulation of Genetic Recombination

The frequency of genetic recombinants formed in a cross of homologous chromosomes which differ at genetically marked loci is a measure of the genetic linkage of the loci, or their tendency to segregate together in the products of a randomly occurring genetic exchange. Any treatment that serves to increase the number of effective recombination events or to disrupt the physical continuity of chromosomes should decrease genetic linkages. Many lethal and mutagenic agents which introduce lesions into DNA have been found to be recombinagenic as well (117, 243a). The best characterized of these effects is the UV enhancement of recombination.

In 1955, Jacob and Wollman (115) reported that light UV-irradiation of λ phages before, or immediately after, infection of the host bacteria causes a marked increase in phage recombination frequencies. Their description of the principal features of this effect may be summarized as follows: a) The UV enhancement of recombination frequency is linear with dose until attainment of either genetic equilibrium (50% recombinant progeny) or, for UV doses which inactivate about 70% or more of the infecting phages, a plateau of maximal enhancement at a level about five times the normal recombination frequency. b) The slope of the enhancement response and the level of maximal enhancement vary with the genetic linkage between the markers crossed. c) The UV enhancement of recombination is photoreactivable. d) The induced recombination events occur during the early part of the latent period. e) The UV-stimulated recombination includes a large number of seemingly non-reciprocal double crossovers. f) For crosses in which only one of the parental phage genotypes is irradiated the enhancement is about half as great as when

both phage types are irradiated, and the irradiated parent appears to serve as a donor of genetic fragments to the unirradiated phage type.
g) The UV-induced increase in recombination frequencies is accompanied by an increase of around 100-fold in the frequency of internal phage heterozygotes (189, 231), from around 10^{-4}% to about 1% (115, 130).

Further studies have demonstrated that UV-irradiation also enhances recombination among T-even phages (59, 62), increases P22 and λdg phage transduction frequencies (67, 154), decreases the linkage between genetic loci in bacterial conjugation (117, 240), induces haploidization and recombination between the exogenote and endogenote in partially diploid bacteria (41), and stimulates mitotic recombination and gene conversion in fungi (119, 181, 98). In each instance the characteristic features of the UV-induced recombination are consistent with those described by Jacob and Wollman for UV-induced λ phage recombination.

The mechanism of the UV enhancement of recombination is unknown. Originally, it was proposed that the characteristics of UV-induced recombination might be most easily explained in terms of a copy-choice model, with UV lesions in the parental DNA strands envisioned as provoking switches of a replica back and forth between mating templates (115, 206). Considerable doubt is cast upon this interpretation by the recent accumulation of evidence that microbial recombination proceeds mainly by breakage and reunion type mechanisms, and that various anomalies associated with recombination might be accounted for by DNA repair (section I-B.3 above). Thus while it could be argued that at present there is no unequivocal experimental evidence excluding all possibility that <u>UV-induced</u> recombination might occur by a semi-conservative copy-choice

mechanism, such an hypothesis now seems neither necessary nor warranted by any collateral evidence.

On the other hand, it is by no means certain that the UV-induced recombination is accomplished by breakage and reunion of chromosomes analogous to that observed for vegetative recombination among unirradiated λ phages. In addition to (a) simple breakage and reunion, other possible mechanisms which might be considered for the UV-induced recombination are: (b) a variety of non-conservative copy-choice DNA synthesis during repair of single-strand interruptions (28, 243a); (c) exchanges of single-strand segments among the mating chromosomes (109, 240), perhaps through a mechanism related to that which effects single-strand recombination of donor and recipient DNA in bacterial transformation (63, 21, 172a); (d) informational exchanges through repair of internal heterozygotes in transiently paired strands from different chromosomes (96, 98).

For heuristic purposes it is helpful to distinguish between the mechanism by which the UV-induced recombination may be executed and the recombinagenic stimulus which UV-induced lesions furnish to initiate genetic exchanges. Thus one may consider an independent categorization of the ways in which UV lesions might lead to recombination: 1) By introducing slight structural deformities into the irradiated DNA, UV lesions promote the development of regions of local denaturation (153). Such an effect might facilitate the pairing of strands from homologous chromosomes (190, 41), which otherwise could be a rate-limiting step of recombination (221). 2) UV lesions might facilitate recombination by presenting impediments to DNA replication, and thereby prolonging the time available for pairing of homologous chromosomes in close proximity and/or a particularly favorable conformation (98, 97) or otherwise inducing a metabolic state conducive to recombination (96, 100).

3) Excision-repair of UV photoproducts, with concomitant introduction of single-strand interruptions and unpaired single strands, might facilitate the mating and recombination of DNA strands from different chromosomes (100). 4) The introduction of single-strand gaps in DNA as a consequence of replication-bypass of UV photoproducts could stimulate pairing or even set in motion a specialized recombinational system to effect post-replication repair (109, 100). Post-replication repair might occur by a mechanism of copy-choice repair replication (28) or single-strand exchanges (184) (cf., above), which could lead to recombination among chromosomes of different genotype as well as among the replication products of the same chromosome. Howard-Flanders and collaborators have suggested that UV-induced bacterial recombination is primed by double-strand defects caused by replication-bypass; they have shown that post-replication repair of DNA transferred from an irradiated donor in bacterial conjugation does not occur prior to UV-induced recombination with the recipient genome (109, 240).

An indirect effect of UV to increase λ recombination frequencies, apparently distinct from that discussed above, is observed when unirradiated phages are crossed in very heavily UV-irradiated host bacteria (129, 130). Such a procedure can lead to a two- to five-fold increase in recombination frequencies and a more than 100-fold increase in the frequency of internal phage heterozygotes. This effect has been provisionally attributed to prolonged mating among the parental phages as a result of inhibited replication and maturation of the phage DNA (130).

A number of other agents in addition to UV have been found to stimulate recombination or decrease genetic linkages in microbial genetic systems. Among these agents are X-rays (78, 152), decay of

incorporated P^{32} (117), thymine starvation (65) and 5-fluorodeoxyuridine (60, 65), nitrous acid (252, 251), nitrosoguanidine (251), bifunctional (but not monofunctional) alkylating agents (165, 248), and mitomycin C (95). Most of these agents have also been found to induce the development of prophage in lysogenic bacteria. While all of them affect DNA synthesis to some extent, a more striking and consistent correlation among them is their common induction of repairable lesions in the DNA. One would hope that an elucidation of the mechanism of UV stimulation of recombination might provide some indication as to a general mode of action of these recombinagens.

II. MATERIALS AND METHODS

II-A. Bacteriophages

λc67, λc68, and λc71 are clear-plaque mutants, isolated by A. D. Kaiser, that are defective in the cIII, cII, and cI cistrons respectively* (123). λsusB1, λsusG9, λsusO29, λsusP3, λsusQ73, and λsusR5 are suppressor-sensitive mutants* isolated by A. Campbell (32). The approximate location of these markers on the genetic map is shown in Figure 4. Samples of the λc mutants and strains of E. coli C600 lysogenic for the various λsus mutants were obtained from Katherine Brooks.

Multiple λ mutants carrying the c71 and one or more sus markers were prepared by crossing UV-irradiated single mutants in E. coli AB1886 (uvrA-). It will be seen from the results presented below in Chapter III that this procedure yields a high frequency of phage recombinants. The desired genotypes were selected from the progeny of these crosses by means of complementation tests of individual picked plaques.

P1kc phage (142) utilized in the preparation of P1 lysogens was obtained from Neil Willets. Lysates of P1 were prepared by the confluent lysis technique (1).

---

* The cI, cII, and cIII cistrons affect repression, and the phages mutant in these functions show greatly decreased frequencies of lysogenization relative to the wild type (123). λsusB1, λsusG9, and λsusR5 are mutant for late phage functions. The B cistron product is necessary for the conversion of freshly synthesized phage DNA to an infectious form, and the G cistron function is required for the production of functional phage tails (232). The R cistron codes for the phage lysozyme (34). λsusQ73 is defective in a cistron that supplies a general inducer of late phage functions, while λsusO29 and λsusP3 are mutant in functions essential to vegetative DNA replication (29, 48, 121).

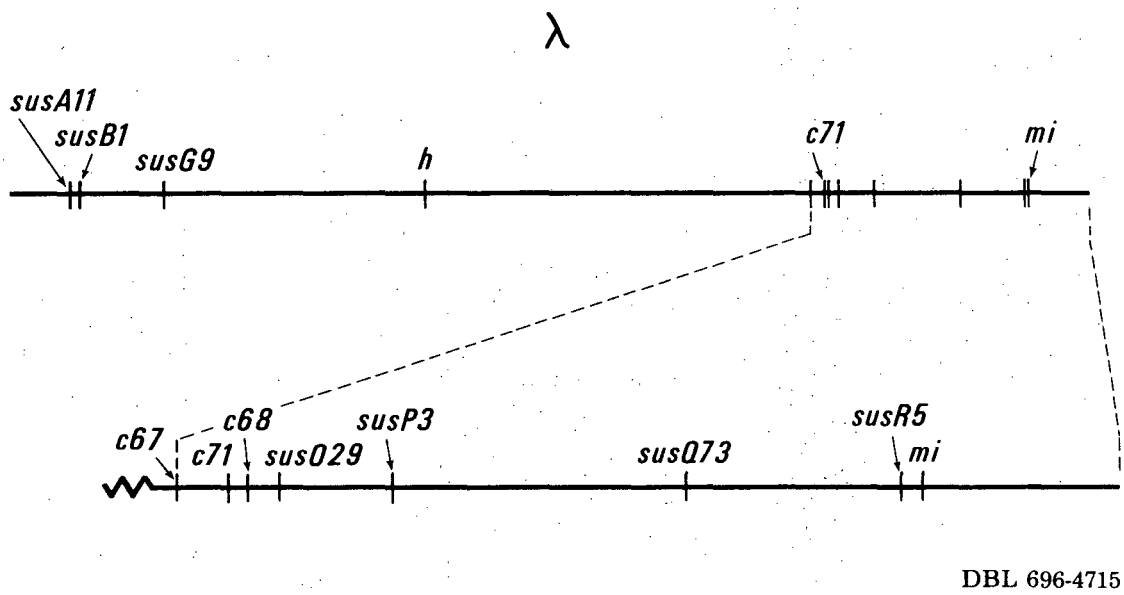


Figure 4. The genetic map of vegetative λ bacteriophage, showing the location of markers discussed in the text. The c and c26 loci referred to in Chapter IV are situated near c71. Relative genetic distances are based on data from several sources (2, 120, 123, 31, and this report) and are expressed in crossover units normalized according to the mapping function of Amati and Meselson (2). The relative positions of these markers on the genetic map correspond roughly to their physical location on the bacteriophage chromosome, except for a distortion introduced by the high ratio of physical to genetic length in the susP3-susQ73 interval (94, 120).

II-B. Bacterial Strains

The characteristics and sources of the *E. coli* K12 bacterial strains employed as hosts in these studies are given in Table II. All bacterial strains were re-isolated shortly before their use in major experiments.

Derivatives of strains C600, W3350, AB1157, and AB1886 lysogenic for phage P1kc were prepared as follows. A high titer suspension of P1kc, prepared by several cycles of growth by confluent lysis on the strain to be lysogenized, was spotted on a lawn of soft agar seeded with that strain. After overnight incubation, cells were scraped from the resulting zone of confluent lysis, washed, and plated out for isolation of individual colonies. Colonies were screened for lysogenicity by selecting those that produced lysis when replica plated onto a lawn of AB1157 indicator and examining their properties of host controlled restriction and modification of λ phage. By these criteria, approximately 1% of the clones derived from the original zone of confluent lysis were classified as P1 lysogens.

The concentration of bacterial cells in a liquid suspension was routinely estimated by measuring the relative absorbance at 520 mμ (for tryptone broth cultures) or 450 mμ (for suspensions in simple media), using a Bausch & Lomb Spectronic 20 or a Zeiss spectrophotometer.

Table II - Bacterial Strains Employed

Strain	Characteristics[a]	Source	Reference
C600	F-,Thr-,Thi-,Lac-,$T1^r$,$T5^r$,Pm+.	D.Freifelder	(5)
W3350	F-,gal-1,gal-2,Pm-.	"	(32)
AB1157	F-Arg-,His-,leu-8,pro-2, thr-4,ara-14,gal-2,lac-1, mtl-1,xyl-5,Thi-,$T6^r$,Sm^r, Pm+[b].	P.Howard-Flanders	(105)
AB1884	Same as AB1157 except uvrC-34, Pm±[b].	"	(106)
AB1885	Same as AB1157 except uvrB-5, Pm±[b].	"	(106)
AB1886	Same as AB1157 except uvrA-6.	"	(105,106)
AB2470	Same as AB1157 except recB-21, Pm±[b].	"	(104,57)
AB2494[c]	F-,His-,leu-8 &/or thr-4, met-B,pro-2,gal-2,xyl-5, Sm^r, lex-1, Pm±[b].	A.J.Clark	(104,102, 110a)
JC1557	F-,leu-2,his-1,arg-6,met-1, lac-1,4, $T1^r$,Sm^r, Pm+.	"	(39)
JC1569	Same as JC1557 except recA-1.	"	(39,241)
C600(P1kc)		from C600 by lysogenization.	
W3350(P1kc)		from W3350 by lysogenization.	
AB1157(P1kc)		from AB1157 by lysogenization.	
AB1886(P1kc)		from AB1886 by lysogenization.	

Footnotes to Table II - Bacterial Strains Employed

(a) The bacterial strains utilized in these studies are described in terms of mating type, auxotrophic and fermentative markers, resistance to T coliphages and streptomycin, and radiation sensitivity. Symbols beginning with an upper case letter refer to phenotypic properties; symbols beginning with a lower case letter refer to genotypic characters (43). The following abbreviations are used: F- = non-donor in conjugation, thr = threonine, arg = arginine, his = histidine, leu = leucine, pro = proline, met = methionine, thi = thiamine (B_1), lac = lactose, gal = galactose, ara = arabinose, mtl = manitol, xyl = xylose, $T1^r$, $T5^r$, $T6^r$ = resistant to T1, T5, T6 coliphage respectively, Sm^r = resistant to streptomycin, Pm = permissive for λsus mutants, uvr- = UV-sensitive, rec- = recombination-deficient, lex- = x-ray sensitive.

(b) The strains of AB1157 and AB1886, obtained from Howard-Flanders in 1964, are permissive for all λsus mutants tested. Strains of AB1157 (designated AB1157N), AB1884, AB1185, and AB2470 obtained in 1966 were found to permit the growth of some λsus mutants but not of others, e.g., λsusG9. It is thus likely that the strains obtained in 1964 and 1966 differ with respect to alleles at one or more of the suppressor loci. In all other respects the characteristics of the strains were as reported in the literature.

(c) The AB2494 strain was found to be quite slow-growing, with a doubling time of around an hour in tryptone broth (all cells in the culture were viable). A spontaneously occurring mutant of this strain with a somewhat faster growth rate was isolated for use as a host for phage crosses and as an indicator for plating phage. The UV-sensitivity and host cell reactivation properties of this substrain are identical to those of the original AB2494, and control crosses indicated that the UV enhancement of phage recombination is comparable in the two strains.

Note that aside from the lex-1 allele the AB2494 strain is not isogenic with the lex+ AB1157 strain utilized for control experiments. AB2494 is an arg+uvrA+ recombinant from a cross of AB2474 (uvrA6, lex-1) with Hfr J2, where AB2474 is a nitrosoguanidine-induced mutant of AB1886(uvrA6) (110a).

II-C. Media

The standard medium used for growth of bacterial cultures and incubation of phage crosses was tryptone broth: 1% tryptone + 0.5% NaCl in distilled water, with 10 μg/cc thiamine hydrochloride added after autoclaving. This medium was supplemented with 1% Difco agar for phage assay plates and with 0.65% agar for top agar. Dilutions were carried out in M9 or in a solution consisting of 0.01 M KH_2PO_4, 0.01 M K_2HPO_4, 0.001 M $MgSO_4$, 0.0001 M $CaCl_2$ in distilled water. Purified phage stocks were kept at 4°C. in a storage medium composed of 0.5 M NaCl, 0.001 M $MgCl_2$, 0.01% gelatin, 0.01 M Tris pH 7.8 in distilled water. The samples of anti-λ serum used in these studies were kindly provided by Mark Achtman.

II-D. Preparation of Phage Stocks

UV inductions of λ lysogens were performed according to the following procedure. The lysogenic bacteria were grown to a concentration of about 2×10^8 cells/cc in tryptone broth, collected by centrifugation or millipore filtration, resuspended in 3/4 volume of 0.01 M $MgSO_4$, and starved for an hour at 37°C. with vigorous aeration. The suspension was then exposed, in a thin layer and with agitation, to about 400 ergs/mm^2 of UV radiation. One-quarter volume of 4× concentrated tryptone broth was added and the culture incubated with vigorous aeration until lysis was complete, as judged by a minimum in the absorbance at 520 mμ. The lysed culture was sterilized by addition of 0.5% - 1.0% $CHCl_3$ and clarified by centrifugation or passage through celite and a millipore filter.

Stock of λ phages carrying a clear plaque mutation were prepared by vegetative growth on E. coli C600 or C600(Plkc) host bacteria. The host strain was usually grown to a concentration of 1.5×10^8 cells/cc in tryptone broth and then seeded with phage to a concentration of 6×10^7 phage/cc. After allowing the phage to adsorb for 15 minutes, the culture volume was doubled by addition of broth. The culture was then incubated with vigorous aeration to lysis, usually about 3 hours, sterilized, and clarified as above.

These procedures could be scaled to final volumes ranging from 20 cc to 6000 cc. The yield was usually $1 - 3 \times 10^{10}$ phage/cc in the clarified lysate. A New Brunswick Microferm Fermenter was utilized for incubation of UV induction cultures and vegetative growths carried out in large volumes. In order to insure maximum genetic homogeneity of phage preparations, lysogens or clear mutants were subjected to several serial single colony or single plaque isolations to obtain the inocula for growth of large stocks.

Large stocks of the λ phages to be used in the experiments described below were purified and preserved by a slight modification of a procedure obtained via David Freifelder: After sterilization and clarification of the lysate, the phage were precipitated by addition of 30% wt/vol $(NH_4)_2SO_4$. The precipitate which settled out at 4°C. was collected by centrifugation at 10,000 × g for 60 minutes and resuspended in 60cc-90cc of phage storage medium. This phage suspension was then subjected to two cycles of alternate centrifugations at 10,000 × g for 10 minutes to remove debris and at 52,000 × g for 120 minutes to pellet the phage. The final phage pellet was allowed to resuspend in several cc of phage storage medium and banded overnight in CsCl ($\rho \sim 1.5$ gm/cc) at 100,000 × g. The band was collected, dialyzed against three changes of storage medium, and diluted to a 5cc-10cc volume for storage. The total number of viable phage present in such a purified stock was approximately 50% that present in the original clarified lysate.

Highly purified λ phage stocks are unstable at concentrations below about 5×10^{11} phage/cc in simple media, but they can be stabilized by addition of a low concentration of protein to the storage medium. This stabilization can also be effected by gelatin, which has the advantage for these studies of an insignificant UV absorption at 254 mμ. Hence all phage stocks were stored, diluted, irradiated, etc., in the phage storage medium described in section III-C, which contains 0.01% gelatin; no decay of viable titer was observed at any phage concentration over a period of several years.

II-E. Ultraviolet Irradiations

Most ultraviolet irradiations were performed with an 8-watt GE germicidal lamp, emitting primarily 2537 A radiation (118), at a dose rate of approximately 10 ergs/mm^2/sec. Aliquots of phage, suspended in the storage medium at a concentration of 4×10^{10}/cc ($O.D._{254m\mu} \sim 0.2$) or less, were irradiated in a layer 1-2 mm deep (2-3 cc) in a 10 cm glass petri dish. (Phage prepared in this way could be stored indefinitely in screw cap tubes with no change in viable titer or recombination properties, so that the same phage suspensions could be utilized in a series of experiments in different bacterial host strains over a period of weeks or even months.) The UV irradiations for bacterial survival curves were performed in a similar manner, with the cells suspended in dilution medium at a concentration of 5×10^6/cc.

Irradiations of bacteria to be used as hosts in phage crosses (Chapter V) were performed with two 15-watt GE germicidal lamps at a dose rate of approximately 40 ergs/mm^2/sec. (The UV dose scales in the data concerning irradiation of host cells have been adjusted to correspond to the 10 ergs/mm^2/sec dose rate.) Ten cc aliquots of starved cells, suspended in 0.02 M $MgSO_4$ at a concentration of 4×10^8/cc, were irradiated in 10 cm petri dishes with vigorous mechanical agitation.

The UV sources were calibrated by potassium ferrioxalate actinometry (118). All experiments susceptible to photoreactivation were performed under dim or yellow light.

II-F. Phage Titrations and Complementation Tests

All phage stocks and samples were routinely titrated on AB1157 or AB1886 indicator bacteria in order to determine total phage titers. λsus mutants and λsus cross lysates were titrated on W3350 indicator to monitor the levels of λsus+ revertants and determine the titers of λ++ recombinants respectively (see section II.H). Titers were usually based on counts of at least two, usually three, plates at dilutions yielding 50-400 plaques/plate (a linear range). In rare cases a greater number of plates were counted in order to score 50-100 plaques of phage present at low concentration in the sample.

Cultures of indicator bacteria were prepared by 4-5 hours aeration of unaerated overnight cultures in tryptone broth. For most UV survival assays the phage were mixed on the plates with top agar seeded with the indicator strain. For all other assays the phage were routinely preadsorbed to the stationary phase indicator bacteria--approximately 2×10^9 cells/cc in broth--for 15-60 minutes at room temperature before plating. (This preadsorption facilitated plate-counting and increased the efficiency of plating (EOP) of the phages on AB1157 and AB1886 by approximately 20%; preadsorption did not affect the EOP on W3350 indicator nor did it significantly affect the results of UV survival assays except for very low doses.) The plates were scored after at least 14 hours' incubation at 37°C.

Tests for intercistronic complementation among λsus mutants were conducted according to a modification of a procedure obtained from K. Brooks. The two phages to be tested were spotted together--for each phage ca. 0.02 cc of a suspension at 10^8 phage/cc--on a lawn of top agar

seeded with stationary phase W3350 nonpermissive indicator, allowed to dry, and incubated. Confluent lysis, in contrast to a small number of plaques due to λsus+ revertants or λ++ recombinants, indicated complementation. This procedure was adapted to test for complementation between phages initially present as prophages in separate lysogens by spotting ca. 10^6 log phase cells of each lysogen on the lawn and exposing the plate to an inducing dose of UV before incubation. Similarly, complementation between a free phage and a phage initially present as a prophage could be tested by introducing the free phage after induction and several hours' incubation of the lysogen.

II-G. Phage Crosses

Equal numbers of the phage mutants to be crossed were mixed to a final concentration of 4×10^{10} phage/cc in phage storage medium and aliquots of this suspension were UV-irradiated as described above. The host bacteria were grown to a concentration of 4×10^{8} cells/cc in tryptone broth with vigorous aeration, removed from the growth medium by millipore filtration, resuspended in 0.01 M or 0.02 M $MgSO_4$, and starved for about 60 minutes at 37°C. (122, 64). The concentration of starved cells was then adjusted to approximately 4.4×10^{8} cells/cc* ($O.D._{450m\mu}$ ~ 1.0). For each cross an 0.9 cc aliquot of the cell suspension was infected with 0.1 cc of a phage preparation, to give a multiplicity of about 5 of each parental phage type per cell. The phages were allowed to adsorb for 20 minutes at 37°C. Unadsorbed phage were then neutralized by addition of anti-λ serum to a final k ~ 0.5-1.5 (1), as required, and incubation for a further 20 minutes. (This treatment reduced the background level of unadsorbed infecting phage particles 10^{3}- to 10^{4}-fold to a concentration 0.02% - 0.5% that of the infected cells.) The infected cells were diluted 100- to 1000-fold into tryptone broth and a 10 cc aliquot was incubated for 90-120 minutes in a 125 ml Erlenmeyer flask with vigorous agitation. The cross was terminated by incubation of the lysate with 0.5% - 1.0% $CHCl_3$, after which samples of the lysate were diluted into phage storage medium for later titration.

---

* The viable titers per O.D. unit of JC1569(recA-1) and AB2470(recB-21) suspensions were 0.60 and 0.25 respectively those of the JC1557 and AB1157 controls, because a large fraction of the cells in such Rec- cultures are not viable. The concentration of these Rec- strains was adjusted to the same O.D. (rather than viable titer) as their controls in order to preserve a constant multiplicity of phage particles/adsorbing cells.

At the appropriate times during this procedure, samples were taken in order to assay the concentration of viable cells infected, the percentage of phages unadsorbed to host cells, the titer of phage un-neutralized by anti-serum action, and the concentration of infected complexes in the cross. Unadsorbed and un-neutralized free phage were assayed as the titer of plaque-forming units (PFU) remaining after incubation of the sample with 0.5% $CHCl_3$ in phage storage medium. A sample of known titer was assayed as a control on $CHCl_3$ inactivation of the free phage, which was occasionally significant.

By performing the various steps of this procedure consecutively for different crosses (staggering the various adsorptions, dilutions, etc.) it was possible to perform from 12 to 36 crosses in tandem utilizing the same preparations of host bacteria and virtually identical experimental conditions. A single experiment then yielded complete data curves for variations of phage recombination frequency, etc., and survival of multiply-infected cells as PFU (i.e., active infected complexes) as a function of dose to infecting phages or to cells. This tactic eliminated scatter that would normally be expected from day to day variations in the conditions of the crosses. The curves presented in the text for recombination and multiplicity reactivation of λsusB1 + λsusG9 phages in AB1157 and AB1886 hosts (Figs. 7, 9, 16, and 17) represent the results of several series of crosses performed on different days but randomized with respect to the UV doses to infecting phages. All other curves represent results derived from a single cross series.

Phage adsorption in these experiments was usually 75% - 90% except for a somewhat reduced adsorption to the JC1557 and JC1569 strains. For example, adsorption of unirradiated λc67 + λc68 phages to the various host strains was assayed as: AB1157 - 87%, AB1886 - 96%, AB1885 - 90%,

AB1884 - 93%, AB2470 - 88%, AB2494 - 75%, JC1557 - 42%, JC1569 - 48%. Control experiments indicated that UV-irradiation of the phages or of the host cells did not significantly affect phage adsorption. The burst size was approximately 30-50 for crosses of λsusB1 and λsusG9 and approximately 70-140 for the other crosses considered.

II-H. Determination of Recombination Frequencies

λsus mutants grow on the permissive indicator strains AB1157 and AB1886, but not on the non-permissive indicator W3350. λsus+ revertants (present at frequencies from 10^{-7} to 10^{-4} in a typical λsus stock) and λ++ recombinants from crosses of λsus mutants grown on both types of indicator. The recombination frequency among the progeny from a cross of λsus mutants was measured by dividing the lysate titer on W3350 by the lysate titer on AB1157 or AB1886 to obtain the ratio (titer of λ++ progeny): (titer of total progeny phages). In general the resolution of this assay is limited to 0.01% - 0.1% by intercistronic complementation among a mixture of λsus mutants on the non-permissive indicator.

Wild type λ++ recombinants from crosses of λc67 and λc68 form very turbid plaques, while the plaques of the parental and λc67c68 recombinant phages are relatively clear. Lysates from these crosses were plated on AB1157 and the recombination frequency (titer of λ++ progeny):(titer of total progeny phages) was measured by dividing the number of turbid plaques by the total number of plaques. A sufficient number of plaques was counted to insure the scoring of at least 100 recombinants from crosses of irradiated phages and 25-50 recombinants from the zero point crosses of unirradiated phages.

Unless specified otherwise in the caption, the brackets or indicated errors on the recombination frequencies reported in the data describe the range of frequencies observed from single titrations of duplicate phage crosses. (The duplicate crosses were always performed on the same day under identical conditions; however, the duplicate cross

lysates were routinely titrated on different days using different preparations of indicator bacteria.) The results were not significantly affected by increasing the sample through additional crosses or titrations. Note that the recombination frequencies reported in the data refer to only one of the two recombinant phage genotypes.

II-I. Indicator System for Assay of HCM-labeled Parental Phages

In many of the phage crosses performed in this study the parental phage DNA strands were labeled by means of host-controlled modification (HCM). This label allows the superposition of a selection for DNA strands derived primarily from parental phages upon the assays for total and recombinant progeny phages of a cross. The basis of the HCM label is discussed in detail in Section IV-A. The K12-K12(P1) indicator system utilized for assay of the various phage types from these crosses is defined in Table III, in terms of the efficiencies of plating (EOP's) of the phages on the various indicator strains.

Since the K12(P1) lysogens segregate non-lysogenic cells at a significant frequency upon serial passage, K12(P1) indicator cultures were inoculated from recently re-isolated single colonies. The restriction character of these cultures was routinely monitored in order to insure EOP's for $\lambda \cdot K$ of 1 to 4×10^{-5} in the titrations of HCM-labeled cross progeny.

Legend to Table III: EOP's of Phage Types Recovered from a Cross of λsus β•P and λsusγ•P in Type K-HCM Host Bacteria

λ phages synthesized in nonlysogenic strains of E. coli K12 (λ•K) plate with 100% efficiency on those K12 strains but are restricted to an EOP of about 2×10^{-5} on K12 strains lysogenic for bacteriophage P1. By contrast, phage grown in a K12(P1) strain (λ•P) plate at 100% efficiency on both K12(P1) and K12 hosts. When the progeny of a cross of λsus•P phages in a nonlysogenic K12 host are plated on K12(P1) indicators, the permissive (column 2) and restrictive (column 3) characters of the indicators allow a selection for total or recombinant progeny carrying the parental-HCM specificity. (The +/- symbols in the table refer to the ability of the phage to plate on a host with the permissive or restrictive characters, respectively, of the indicator; the numbers represent the resultant efficiencies of plating.)

Table III

EOP OF PHAGE TYPES RECOVERED FROM λ sus β · P x λ sus γ · P IN TYPE K-HCM HOST BACTERIA

Indicator bacteria			λ · P Parental-HCM phage types		λ · K Progeny-HCM phage types	
			Infecting parental genotypes λ sus β · P λ sus γ · P and		Parental genotypes λ sus β · K λ sus γ · K and	
Strain	Permissive character: EOP of λ sus / EOP of λ^+	Restrictive character: EOP of λ · K / EOP of λ · P	Recombinant genotype I λ sus β sus γ · P	Recombinant genotype II λ^{++} · P	Recombinant genotype I λ sus β sus γ · K	Recombinant genotype II λ^{++} · K
AB 1157 [HCR^+]	Pm^+	R_K	+ +	+ +	+ +	+ +
AB 1886 [HCR^-]	1.0	1.0	1.0	1.0	1.0	1.0
W 3350 [HCR^+]	Pm^- ~10^{-5}	R_K 1.0	− + ~10^{-5} *	+ + 1.0	− + ~10^{-5} *	+ + 1.0
AB 1157 (P1) [HCR^+]	Pm^+	R_P	+ +	+ +	+ −	+ −
AB 1886 (P1) [HCR^-]	1.0	~10^{-5}	1.0	1.0	~10^{-5}	~10^{-5}
W 3350 (P1) [HCR^+]	Pm^- ~10^{-5}	R_P ~10^{-5}	− + ~10^{-5} *	+ + 1.0	− − ~10^{-10} *	+ − ~10^{-5}

Titer on: *AB 1157* → Total phage
W 3350 → Total λ^{++} recombinant phage
AB 1157 (P1) → Total parental-HCM phage
W 3350 (P1) → Parental-HCM λ^{++} recombinant phage

DBL 696-4716

III. RECOMBINATION AMONG UV-IRRADIATED LAMBDA BACTERIOPHAGES IN UV-SENSITIVE HOST BACTERIA

III-A. UV Enhancement of Recombination Frequencies

In a consideration of the possible relevance of host cell DNA repair processes to the UV enhancement of λ phage recombination, two questions of primary interest are: 1) Can the UV lesions that promote λ recombination be repaired by host cell reactivation? and 2) Are host cell repair enzymes involved in the UV-stimulated recombination event? The most direct approach to these questions is to examine the UV stimulation of λ phage recombination in host bacteria that are known to be defective in DNA repair.

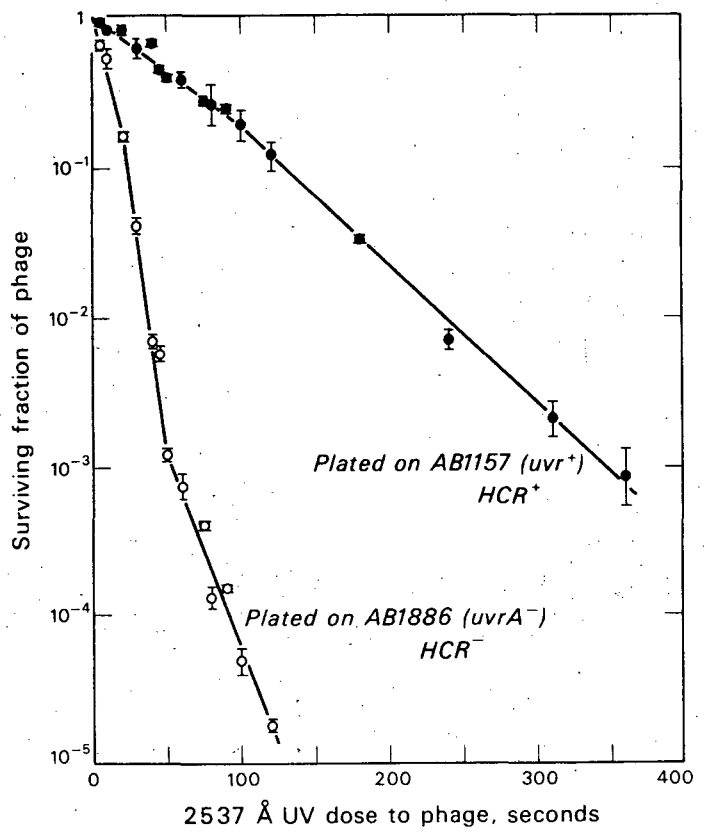
III-A.1 HCR+ and HCR- Host Strains; Host Cell Reactivation of Recombination-Promoting UV Lesions

The UV survival curves for a λ phage mixture plated on two principal bacterial strains utilized in these studies are shown in Fig. 5. AB1157(uvr+) is a wild type, HCR+ host strain. The HCR- AB1886(uvrA) strain is mutant at a genetic locus controlling excision of structural defects from DNA and is unable to remove UV-induced pyrimidine dimers (105, 23b). The difference in phage survival on the two bacterial strains is due to the fact that potentially lethal UV photoproducts in the phage DNA are repaired by host cell reactivation in the uvr+ host but not in the uvrA host strain.

It is thought that the phenomenon of host cell reactivation is analogous, perhaps identical, to excision-repair of bacterial DNA (183). All bacterial mutants that are known to be defective in excision-repair

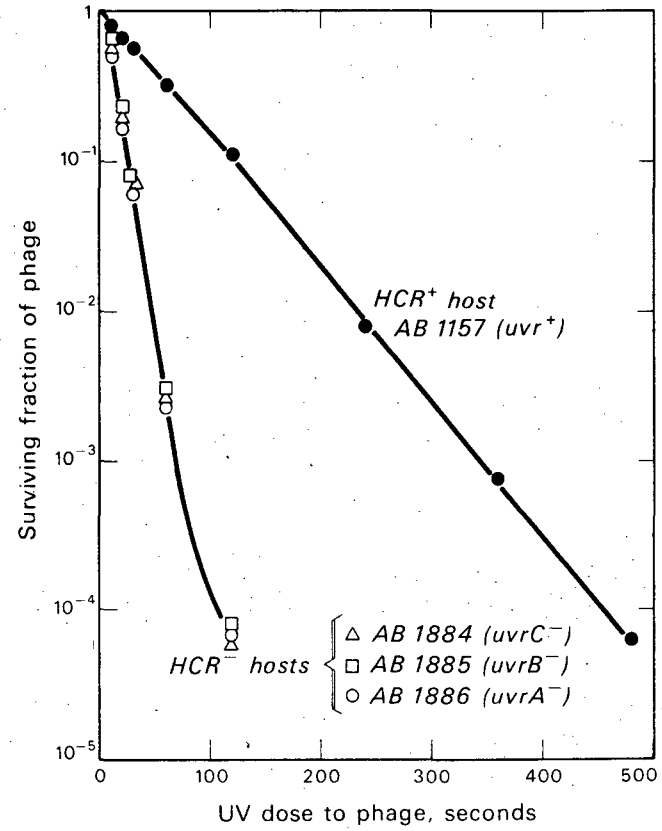
Figure 5. UV survival curves for a mixture of λsusB1 and λsusG9 plated on AB1157(uvr+) and AB1886(uvrA) host bacteria. For each indicator strain, the survival curve of each phage type plated separately is the same as that for the mixture of the phage types. In this figure, the brackets on the data points indicate the standard deviations of the survivals assayed for the same phage sample in three separate platings on different days.

Figure 6. UV survival curves for a mixture of λc67 and λc68 plated on the HCR+ AB1157(uvr+) and on HCR- AB1884(uvrC34), AB1885(uvrB5), and AB1886(uvrA6) host strains. The UV survivals of λc67 and of λc68 assayed separately on the AB1157 and on the AB1886 indicators are the same as those of the mixture of phage types.



DBL 675-1621

Figure 5



DBL 696-4717

Figure 6

also exhibit a diminished capacity for host cell reactivation (Table I). Mutations responsible for inability to perform the initial excision step of excision-repair map at one of three widely separated loci on the bacterial chromosome--uvrA, uvrB, and uvrC (106, 225, 168). The HCR- phenotype of uvrB and uvrC mutants is evident in Fig. 6, which shows the UV survival curves of another λ phage mixture on the host strains AB1157(uvr+), AB1886(uvrA), AB1885(uvrB), and AB1884(uvrC).

The variations of phage recombination frequency with UV dose that are observed upon crossing UV-irradiated λsusB1 and λsusG9 phages in the uvr+ and uvrA hosts are shown in Fig. 7. At zero dose, the phage recombination frequencies in these two hosts are essentially the same. In the uvr+ host the recombination frequency rises linearly with dose to a plateau at a level about ten times the initial frequency. These kinetics are similar to those originally described by Jacob and Wollman (115). The result is quite different when crosses of irradiated phages are performed in the uvrA host. The initial rate of increase in recombination frequency is about five times <u>greater</u> in this repair-deficient host. Furthermore, in the uvrA host the proportion of λ++ recombinant progeny continues to rise with UV dose, at a reduced but constant rate, beyond the level at which it reaches a plateau in the uvr+ host.

In order to determine whether this same pattern of UV-enhanced recombination is exhibited in HCR- hosts mutant at other loci, crosses of irradiated λc67 and λc68 were performed in AB1157(uvr+), AB1886(uvrA), AB1885(uvrB), and AB1884(uvrC) host strains. The results, shown in Fig. 8, confirm the findings discussed above and indicate that the kinetics of recombination enhancement are essentially the same in all three excision-defective HCR- host strains.

Figure 7. Dose-response curves for the UV enhancement of recombination frequency in crosses of λsusB1 with λsusG9 in AB1157 (uvr+) and AB1886(uvrA) host bacteria. The crosses were carried out by multiple infection of the host cells, according to the procedure described in section II-G, with the same irradiated phage mixtures whose viability in single infection is shown in Fig. 5. Unless specified otherwise, the brackets on indicated recombination frequencies will always refer to the range of values determined from single titrations of two replicate crosses.

Figure 8. Dose-response curves for the UV enhancement of recombination frequency in the cross λc67 × λc68 in AB1157(uvr+), AB1884(uvrC), AB1885(uvrB), and AB1886(uvrA) host bacteria--for the same phage preparations that are described in Fig. 6.

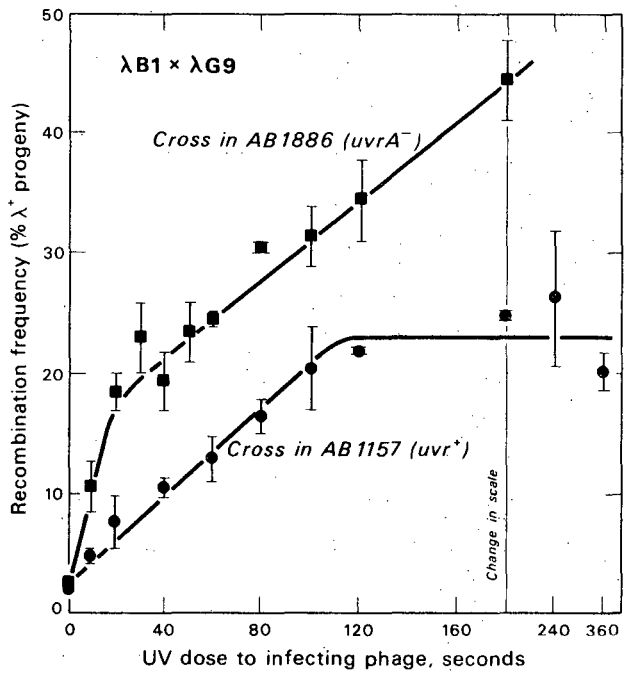


Figure 7

DBL 672-1522

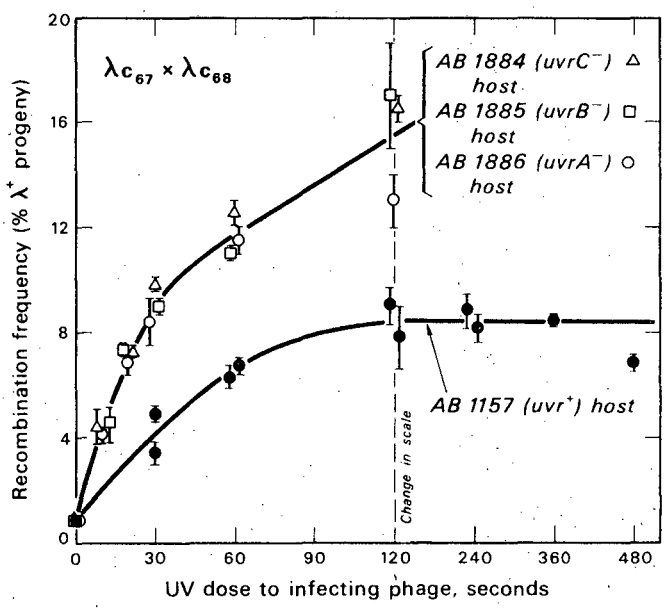


Figure 8

DBL 696-4718

UV-irradiation is more effective in stimulating recombination among λ phages in the HCR- hosts. In Figs. 7 and 8 the dose-response curves for crosses in the uvr- host strains lie everywhere above the corresponding curves for the uvr+ host. One infers that some of the UV lesions promoting recombination are repaired by excision in the uvr+ but not in the uvrA, uvrB, and uvrC strains.

It is important to ascertain whether the recombination-promoting lesions are repaired by host cell reactivation with the same efficiency as are potentially lethal UV lesions. The curves presented in Figs. 9 and 10 indicate that they are, at least in the low dose range. In order to construct these figures, the UV dose scale has been eliminated between the dose-response curves for UV inactivation of the infecting phages (Figs. 5 and 6) and for UV stimulation of recombination frequency (Figs. 7 and 8, respectively). Thus, for each cross and UV dose, the phage recombination frequency observed in a particular host strain is plotted against the survival of the parental phages in single infection of that host. The distance from the origin along the survival axis is directly proportional to the average number of lethal "hits" per phage particle.* It is thought that lethal hits are unrepaired structural defects in the DNA (88, 192, 102). The purpose of Figs. 9 and 10 is to present the recombination frequencies in the various hosts as a function of the number of unrepaired UV lesions in the phage DNA rather than as a function of the initial number of lesions as in Figs. 7 and 8. This change of variables effectively eliminates any differences in phage

---

* Assuming a Poisson distribution of lethal hits among the population of UV-irradiated phages, the surviving fraction $N/N_o = Exp(-H)$, where H is the average number of hits per phage.

Figure 9. The UV enhancement of recombination frequency from the crosses of λsusB1 with λsusG9 in AB1157(uvr+) and AB1886(uvrA) host bacteria, plotted from the data in Figs. 5 and 7 as a function of the fraction of infecting phages which survives in single infection of the host.

Figure 10. The UV enhancement of recombination frequency from the cross λc67 × λc68 in AB1157(uvr+), AB1884(uvrC), AB1885(uvrB), and AB1886(uvrA) hosts, plotted from the data in Figs. 6 and 8 as a function of the fraction of infecting phages viable in single infection of the host bacteria.

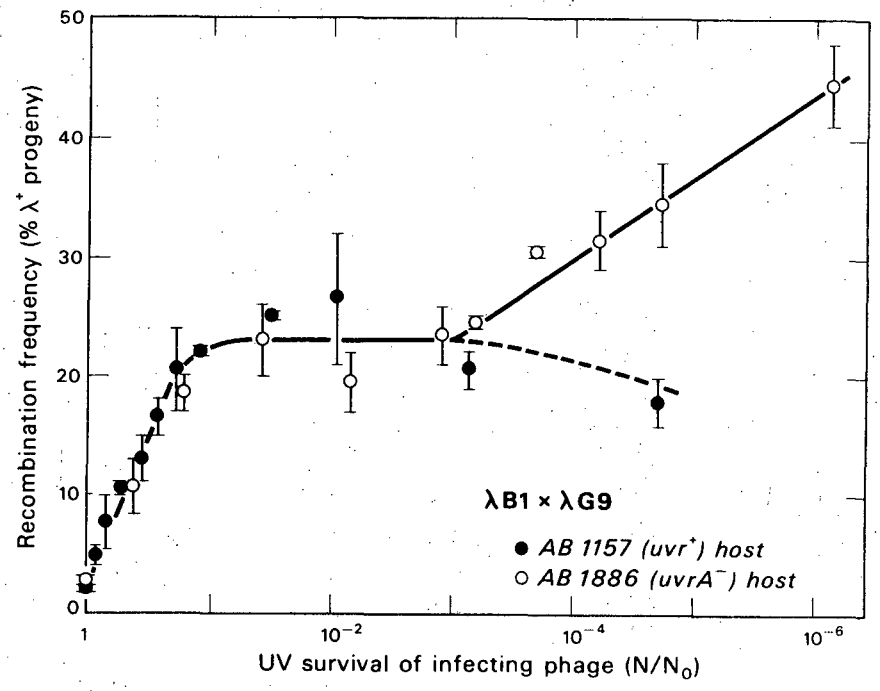


Figure 9

DBL 675-1620

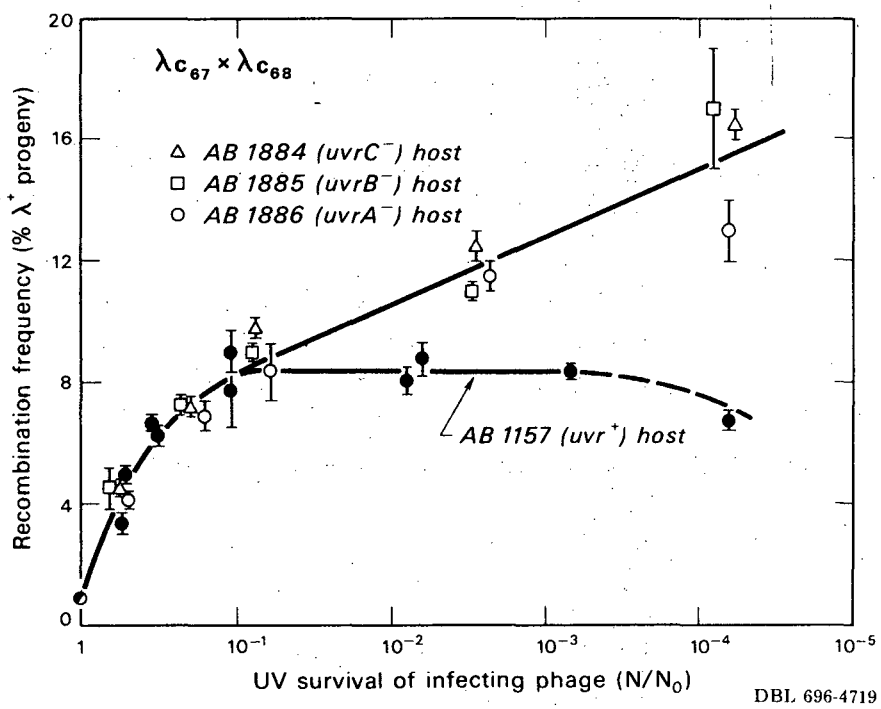


Figure 10

DBL 696-4719

recombination frequencies that correlate with the differing capacities of the host strains to reactivate potentially lethal UV lesions in singly infecting phage chromosomes.

It is clear from Figs. 9 and 10 that for the phage survival range in which UV-stimulated recombination rises to a plateau in the HCR+ host, and in which the initial primary increases in recombination occur in the three HCR- hosts, the recombination frequencies are the same function of the number of phage-lethal lesions in the HCR+ and HCR- host strains. The differences in phages survivals are accompanied by corresponding differences in phage recombination frequencies. These results prove that in this survival range the recombination-promoting and the potentially lethal UV lesions have the same sensitivity to excision. A corollary of this conclusion is that excision is not involved in either the stimulation or the execution of the UV-induced recombination. This finding is consistent with the possibility that the recombination-promoting and potentially lethal lesions are in fact identical. Since both types of lesion are photoreactivable, they are presumed to be--or to derive from--UV-induced pyrimidine dimers in the phage DNA (247, 193).

For phage survivals below 10^{-1}, the phage recombination frequencies in the HCR+ host remain at plateau levels of maximal enhancement, with perhaps a slight decline at quite low phage survivals (Figs. 9 and 10). In the HCR- host strains, however, for the lower phage survivals there are further, secondary, enhancements of phage recombination frequencies at reduced efficiencies. In order to clearly delineate the several possible sources of such secondary enhancements in the HCR- hosts, it is necessary to distinguish between the results obtained in

the crosses of λsusB1 with λsusG9 (Fig. 9) and those from the crosses of λc67 with λc68 (Fig. 10).

For the crosses of λsusB1 with λsusG9, UV-irradiation of the infecting phages causes an increase in the recombination frequencies from about 2.4% for unirradiated phages to a plateau level near 25% λ++ progeny. Additional stimulation of genetic exchanges among the vegetative phage chromosomes notwithstanding, further increases in the observed recombination frequencies, such as that found in the HCR- host for phage survivals below 10^{-3} (Fig. 9), should be precluded by the generation of equal numbers of the two parental and two recombinant genotypes in the pool of recombining genomes.* By contrast, the question of genetic equilibrium is not pertinent to the levels of UV-induced recombination in the crosses of λc67 with λc68. In this case the natural recombination frequency of about 0.85% is enhanced to a plateau level of 8.5% in the HCR+ host; in the HCR- hosts the recombination frequencies continue to increase for phage survivals below 10^{-1}, but do not exceed 20% for the highest dose assayed (Fig. 10).

The secondary enhancements of phage recombination frequencies in HCR- host strains thus pose two distinct problems for consideration: 1) Why do the recombination frequencies for crosses of λsusB1 with λsusG9 in the HCR- host appear to increase <u>past</u> the level of genetic equilibrium for phage survivals below <u>10^{-3}</u>? and 2) Why do the recombination frequencies for the more closely linked c67 and c68 loci <u>continue</u>

---

* It is a moot question whether the plateau for the crosses of λsusB1 with λsusG9 in the HCR+ host is due to attainment of genetic equilibrium or to saturation of the enhancement just short of equilibrium. The observation that the recombination frequency between the more closely linked c67 and c68 markers is enhanced with analogous dose-response kinetics suggests that the latter interpretation is correct.

to increase in the HCR- strains for phage survivals below 10^{-1} whereas a plateau level is attained in the HCR+ host?

It is likely that the secondary increase in the susB1-susG9 recombination frequency in the HCR- host is an artifact, attributable to the progressive UV-inactivation of complementation among the quasi-defective λsus mutants and the selective growth advantage of the λ++ recombinant when there is but one functional parental phage genome per infected complex. Evidence to support this interpretation is presented in Appendix I. This explanation accounts nicely for the facts that in Fig. 9 the percentage of λ++ recombinant progeny appears to reach a plateau before increasing a second time at very low phage survivals and that it then rises past the conventionally defined level of genetic equilibrium.

There is no reason to expect that the λ++ recombinant genotype should have any selective advantage in crosses of heavily irradiated λc67 and λ68. The secondary enhancement of recombination frequency between these two loci in the HCR- hosts (Fig. 10) is probably a true indication of an increase in the number of intracellular mating events. A likely explanation is that this secondary enhancement is due to recombination late in the phage infection between parental and newly synthesized phage chromosomes, stimulated by unexcised recombination-promoting UV lesions in parental phage DNA strands. This type of exchange would occur much more rarely in an HCR+ host due to continuing excision-repair of the UV photoproducts (see section IV-D.1 below). Hence one observes a plateau in the HCR+ host, presumably due to maximum UV stimulation of recombination early in the phage infection, whereas the UV enhancement of overall recombination continues at a reduced

rate with increasing dose in the HCR- strain.

It is also conceivable that a secondary enhancement of recombination frequencies at low phage survivals could be an artifact of multiplicity reactivation. This possibility will be considered in section III-B.1 below.

In both the HCR+ and HCR- host bacteria, the primary UV stimulation of λ phage recombination occurs in the dose range for which there is at least 10% survival of the infecting phages (Figs. 9 and 10)--i.e., less than an average of about 2.3 lethal hits per phage. From information in the literature it is possible to estimate the range of initial UV photoproducts per phage in which this primary enhancement occurs. The UV dose rate in these experiments was approximately 10 ergs/mm^2/sec at 2537 Å. Setlow and Carrier have determined that 1 erg/mm^2 of 2650 Å UV radiation induces approximately 1.0×10^{-6} pyrimidine dimers per nucleotide in *E. coli* DNA in solution (196). Action spectra for inactivation of *E. coli* (118) and for inactivation and cross-reactivation of T-even phage (166) imply that irradiation at 2537 Å induces at least 75% as many pyrimidine dimers/erg/mm^2 as at 2650 Å. The DNA of λ phage contains approximately 1×10^5 nucleotides (35), with overall base composition and nearest neighbor frequencies virtually identical to those of *E. coli* (213). Assuming that the conformation of the phage chromosome within the protein coat does not significantly affect the yield of photoproducts, one would then estimate that under the experimental conditions here:

$$1 \text{ sec UV} = (10 \text{ ergs/mm}^2)(0.75 \times 10^{-6} \text{ dimers/nucleotide/erg/mm}^2)$$
$$\times (1 \times 10^5 \text{ nucleotides}/\lambda \text{ phage chromosome})$$
$$\cong 0.8 \text{ pyrimidine dimers}/\lambda \text{ phage chromosome.}$$

From the dose-response curves shown in Figs. 7 and 8 one can then conclude that the UV enhancement of recombination in the HCR+ host occurs for doses up to approximately 90 initial pyrimidine dimers per phage and the primary enhancement in the HCR- hosts for doses up to approximately 18 initial pyrimidine dimers per phage.

The results of this calculation may be restated from another viewpoint: even in the excision-defective uvr- host bacteria, the ratio of initial pyrimidine dimers to lethal hits per infecting phage is around 8 over the first decade of UV inactivation. (The value of this ratio does not decrease by more than 1/3 on the exponential portion of the survival curve, at higher UV doses to the phages.*) Since one or two unrepaired UV-induced defects might suffice for an inactivating hit (88, 183, 102), one is led to anticipate that there may be substantial "residual HCR" activity in the uvr- mutant host bacteria (80, 84) or that some type of replication bypass of UV-induced photoproducts--perhaps analogous to that observed in irradiated E. coli chromosomes (26, 184)--can be effected in irradiated λ phage chromosomes. Of course, these alternatives are not mutually exclusive.

---

* The UV survival curves of λ on both wild type and uvr- indicator strains have a slight shoulder (see ref. 129); the exponential segments of the inactivation curves extrapolate back to intercept the ordinate at values ranging from 1.2 to 2.0 in different experiments. On the exponential portions of the survival curves (e.g., for surviving fractions of 10^{-1} to 10^{-3}), the calculated ratios of initial pyrimidine dimers to phage lethal hits are approximately 35 for the AB1157(uvr+) host (compared to about 40 over the first decade of inactivation) and about 6 in the uvr- strains.

The comparable values of the ratios of initial pyrimidine dimers to phage lethal hits for various T4 phage mutants (pp. 13, 19) are - T4v+x+: 13; T4v-x+: 6; T4v-x-: 3 (190, 183, 81, 246, 188), where the experimentally determined values for thymine-containing dimers have been arbitrarily adjusted upwards 25% to include all pyrimidine dimer photoproducts (196).

If the recombination-promoting UV lesions in the λ phage DNA are repairable by HCR, as concluded above, and substantial residual HCR activity is present in the excision-defective uvr- host bacteria, the recombination frequency of lightly UV-irradiated λ phages in uvr- host cells should be further enhanced under conditions in which the residual excision activity has been inhibited. The results of experiments discussed in Chapter V, in which uvrA host bacteria were themselves UV-irradiated in order to inhibit residual excision, fulfill this prediction. The data imply that unirradiated uvrA host cells retain sufficient residual HCR activity to correct, in multiple infections, about 70% - 80% of the potentially lethal and recombination-promoting lesions induced by a 5-second UV dose to the parental phages (cf., also, section IV-D.1, Fig. 28). This amounts to repair of around 3 pyrimidine dimers per phage chromosome or perhaps as many as 30 dimers per infected complex. While furnishing further evidence for the conclusion that the recombination-promoting UV lesions in λ phage DNA are repairable by HCR, this result indicates that residual HCR could account in large part for the fairly high ratio of initial pyrimidine dimers to lethal hits for the UV-inactivation of λ phages infecting even HCR- host bacteria.

III-A.2 Recombination-Deficient Host Bacteria

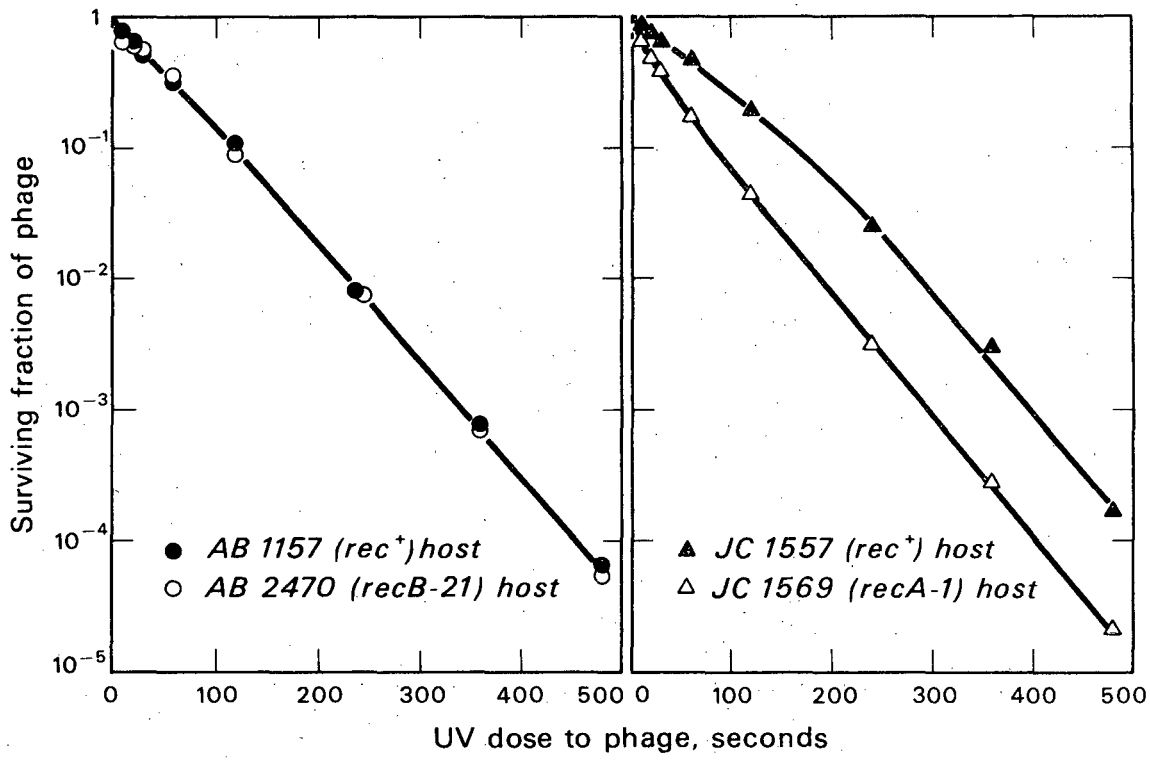
The REC- mutant strains of *E. coli* bacteria are quite sensitive to UV and deficient in their own recombination (38, 37), yet they are able to excise UV-induced pyrimidine dimers and host cell reactivate UV-irradiated λ phage (39, 226). The recombination of unirradiated wild type λ phages is about the same in REC+ and REC- hosts (226, 30),

since the Red recombination system encoded by the phage genome itself is normally the principal mediator of vegetative phage recombination (53, 201). However, the host cell Rec recombination system is capable of supporting a significant amount of recombination among λred (recombination-deficient) mutant phages (53, 201).

The survival and recombination of UV-irradiated λred+ phages in recA-1 (39, 241) and recB-21 (104, 57) host bacteria have been examined in order to determine if the REC- mutations that impair host cell recombination and DNA repair also affect UV-induced phage recombination. The results indicate that the UV enhancement of phage recombination is essentially the same in the REC+ and REC- host bacteria. On the other hand, the UV survival of the λ phages is somewhat lower in the recA than in the rec+ or recB host cells. It is possible that the recA mutation affects a secondary mode of repair in the UV-irradiated phages without significantly influencing the stimulation of phage recombination by unexcised UV-induced photoproducts.

The UV survivals of λ phages on the wild type AB1157(rec+,uvr+) and the recB mutant AB2470(recB-21,uvr+) host strains are shown in Fig. 11; it is clear that the UV survivals of λ plated on these two indicators are identical. The recB mutant is known to have a reduced capacity to degrade its DNA following UV- or X-irradiation (104, 57). By contrast, recA-1 mutant recombination-deficient bacteria exhibit extensive degradation of their DNA following irradiation and an abnormally high rate of DNA turnover even in unirradiated cells (39, 104). The curves in Fig. 12 describe the UV-inactivation of λ phages plated on the JC1569(recA-1, uvr+) and the wild type JC1557(rec+,uvr+) host strains. The phage

Figure 11 Figure 12



DBL 696-4720

Figure 11 (left). UV survival curves for a mixture of λc67 and λc68 phages in single infection of AB1157(rec+) and AB2470(recB-21) hosts.

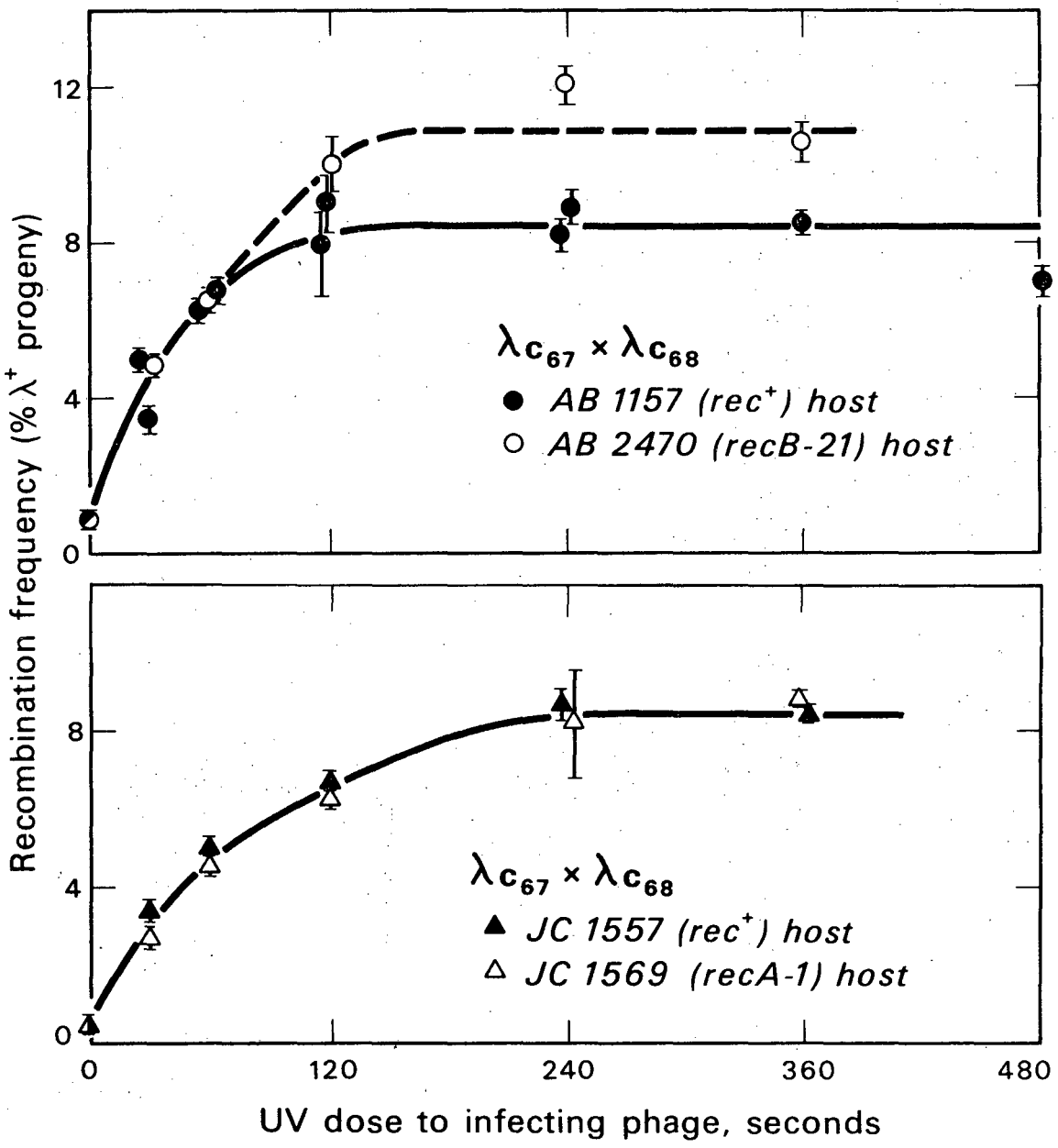
Figure 12 (right). UV survival curves for a mixture of λc67 and λc68 phages in single infection of JC1557(rec+) and JC1569(recA-1) host strains. On each indicator the two phage types have the same UV-sensitivity. The identical irradiated phage preparations were used for the experiments described in Figs. 6, 8, 10, 11 through 15, and 18 through 20.

survivals on the recA and recA+ indicators diverge substantially for low UV doses; the recA strain can be phenotypically classified as HCR+, however, because the survival of UV-irradiated phages is much greater on this host than on a uvr- strain (compare Figs. 8 and 12) and because the slopes of the phage survival curves on the recA and recA+ hosts are the same for moderate to high UV doses.

The UV enhancements of phage recombination frequencies for crosses of λc67 with λc68 in the recB, recA, and wild type host bacteria are shown in Figs. 13 and 14. The dose-response kinetics for the enhancement of phage recombination are essentially the same in the two REC- strains as in their respective REC+ controls. The plateau of enhancement for the recB host appears to be slightly higher than that for the recB+ strain; in several different experiments it was observed that the phage recombination frequencies in the recA host are often slightly, but not significantly, lower than those observed for the same UV dose in the recA+ host strain.

These data indicate that the host recombination functions that are defective in the recA and recB mutant bacteria are not required for the UV stimulation of λred+ phage recombination. This result is consistent with the possibility that the UV-stimulated recombination as well as vegetative recombination among unirradiated λ phages is mediated principally by the phages' Red recombination system, which is known to effect genetic exchanges by a mechanism of

Figure 13 (top). Dose-response curves for the UV enhancement of recombination frequency in the cross λc67 × λc68 in AB1157(rec+) and AB2470(recB-21) bacteria.



DBL 696-4721

Figure 14 (bottom). Dose-response curves for the UV enhancement of recombination frequency in the cross λc67 × λc68 in JC1557(rec+) and JC1569(recA-1) host bacteria.

breakage and reunion of the phage chromosomes (160, 157, 53, 201).* (The results of experiments concerning the UV enhancement of recombination among mutant λred phages, which are being conducted in other laboratories (198), are not yet available.)

The dose-response curves describing the UV enhancement of λ phage recombination are essentially the same for the recA and recA+ hosts (Fig. 14), whereas there is a substantial difference between the UV survival curves of the infecting phages on these indicators (Fig. 12). These data suggest that there is little or no correlation between the effects of the recA mutation in the host bacteria upon the viability and upon the enhanced recombination of UV-irradiated λ phages, contrary to the result expected if the function which is mutant in the recA bacteria is involved to comparable extents in the UV-stimulated phage recombination and in repair of the irradiated phage DNA.

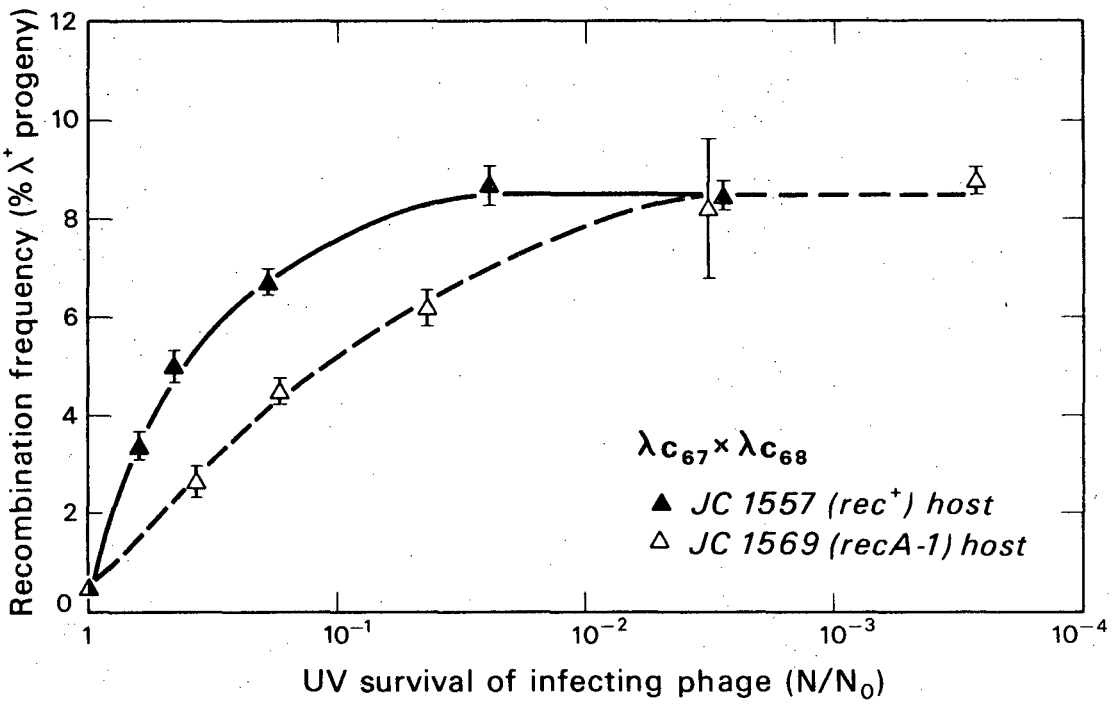
The recombination frequencies observed in the recA and recA+ hosts are plotted in Fig. 15 against the survival of the infecting

---

* Experiments similar to those described here in Figs. 13 and 14 for λ have recently been reported for coliphage S13, a small dependent phage containing single-stranded DNA of molecular weight ca. 2×10^6 daltons that encodes only about 7 polypeptides (202). This phage is dependent in large part upon the host cell recombination apparatus; the principal mechanism of recombination among S13 phages is blocked in a recA, though not in a recB, mutant host strain (218, 219). Extracellular UV-irradiation of S13 phages leads to a 20-30 fold increase in recombination frequencies from crosses in a rec+ or recB host, but there is no enhancement of the residual recombination in a recA mutant host strain (219). That the recA function is necessary to the UV enhancement of S13 recombination frequencies is consistent with the idea that no significant influence of the recA mutation upon the UV enhancement of λ recombination was observed here because of the presence of functional red genes in the phages. It is also noteworthy that the principal mechanisms of natural and UV-stimulated recombination in S13 may share at least one common step and could be the same (219).

phages on their respective hosts. As a function of lethal hits to the infecting phages, the slope of the initial recombination enhancement in the recA+ strain is about three times that of the recombination enhancement in the recA host. For sufficiently low phage survivals, however, the phage recombination frequency is sustained at the same plateau level in both host strains. The curves in Fig. 15 imply that, in contrast to the case for the excision-defective uvr- host strains (Figs. 9 and 10), in the recA host some of the unrepaired--or perhaps incompletely repaired--potentially lethal UV lesions in the phage DNA do not contribute to an enhanced frequency of recombinants among the progeny phages recovered from the crosses.

The data described in Figs. 14 and 15 must be qualified in one particular. In contrast to the case for multiply infected recA+ host bacteria (including the recB mutant), fewer recA host cells yielded progeny phages from crosses of even lightly UV-irradiated infecting phages than from identical crosses of unirradiated phages (see section III-B below, re Fig. 20). For example, there were only about 55% as many active recA infected complexes for a cross of phages irradiated for 60 seconds as for a cross of unirradiated phages, while for comparable crosses in recA+ host strains there were the same number of active infected complexes for these cases. The extent of UV-enhanced recombination among the phages in the inactive recA complexes is not reflected in the data described in Fig. 14. Since the subpopulation of infected bacteria which gave rise to the UV-induced recombinants from the recA crosses could have been significantly enriched for those complexes able to support successful repair (and possibly also UV-induced recombination) of the irradiated infecting phages, it is conceivable that the UV-induced



DBL 696-4722

Figure 15. The UV enhancement of recombination frequency from the cross λc67 × λc68 in JC1557(rec+) and JC1569 (recA-1) bacteria, plotted from the data in Figs. 12 and 14 as a function of the fraction of infecting phages which survives in single infection of the host bacteria.

recombination among λ phages in the recA host strain may be somewhat less efficient relative to that in the recA+ host than is implied by the curves in Fig. 14.

On the other hand, the result indicated in Fig. 15 is <u>not qualitatively</u> affected by taking this reservation into account. If the curves in Fig. 15 are corrected for a hypothetical bias due to inactive recA infected complexes--by altering the phage survival scale to show the relative survivals for single (Fig. 12) versus multiple (Fig. 20) infections of the host or even to indicate the relative survivals of the infected complexes themselves (Fig. 20)--the initial slope of the curve for crosses in the recA+ host is nevertheless twice as great as that of the curve for crosses in the recA host strain.

Most generally, one might infer from Fig. 15 that in the recA mutant bacteria the excision-repair of UV lesions in the phage DNA is impaired at a step subsequent to that which effectively neutralizes the recombinagenic stimulus of a photoproduct or that a second mode of DNA repair is affected which can repair potentially lethal but not recombination-promoting UV lesions (these possibilities are not mutually exclusive). Since the recA and recA+ bacteria are known to have similar capacities to excise pyrimidine dimers (39), either interpretation supports the idea that the simple removal of photoproducts from the UV-irradiated DNA suffices to curtail their effect upon recombination whether or not repair of the damaged segments of the genomes is successfully completed. These results do raise the question of whether the recA and recA+ bacteria differ only in their ability to support a step of HCR other than excision or whether the recA mutation might affect a post-replication repair mode in λ phage.

III-A.2 (cont.) The possibility of replication-bypass of UV photoproducts in λ phage; post-replication repair

Although the increase in UV-sensitivity of λ phages on recA mutant strains* is slight compared to that observed on uvr- (HCR-) host strains, it is of particular interest here because it probably reflects the same aberration in DNA repair processes acting upon the irradiated phages that is responsible for the UV-sensitivity of the recA bacteria themselves. The efficiency of excision-repair (HCR) may be reduced somewhat in recA mutant bacteria--perhaps as a result of impaired control of single strand breakdown following the excision of photoproducts from irradiated DNA.** This possibility is underscored by the fact that there is little or no residual HCR (84) activity for low UV doses to doubly mutant uvrA6,recA-13 bacteria (108)--in contrast to the case for singly mutant uvrA6 cells (sections IV-C.1, V-B, D below). However, there is some evidence that the UV-sensitivity of the recA strains is due principally to inactivation of another mode of DNA repair in irradiated bacterial genomes (108, 66).

It has been proposed that the recA-dependent repair mode consists in the post-replication healing of gaps present in newly synthesized

---

* UV survival curves of λ that are quite similar to those compared in Fig. 12 with respect to the recA-1 mutation of the host have recently been reported for recA-13 (53), 152rec- (68), and rec-42 (168) mutant host strains also.

** It is not clear to what extent the extensive degradation of DNA observed in recA mutant strains might be non-specific--deriving, for example, from an increased susceptibility of single-strand breaks in the DNA, from whatever source, to nuclease attack. In any case, the extreme nature of the defects in these REC- bacteria is attested by the fact that about 40% and 75%, respectively, of the cells in log phase cultures of the recA-1 and recB-21 strains were inviable as colony-forming units and unable to host a successful infection of even unirradiated λ phages.

DNA strands as a result of replication-bypass of UV photoproducts in irradiated template strands (184, 109). An extended version of this hypothesis would attribute the post-replication repair to exchanges among the sister strands of the daughter chromosomes in recombination events which are induced by the double-strand defects resulting from bypass (109, 240). These ideas are outlined above in the introductory review--sections I-B.2, 3, 4. While the nature of the involvement of "UV-induced" recombination in post-replication repair is open to question, there can scarcely be any doubt that replication-bypass of UV photoproducts does occur in E. coli bacteria (26, 109); and, while bypass is observed in singly mutant excision-defective cells, this mode of coping with UV damage is apparently ineffective in doubly mutant uvrA,recA and probably uvrB,recB bacteria (109, 108).

There is presumptive evidence to support the view that an analogous, although probably less vital, bypass/post-replication repair mode exists for UV-irradiated λ phage, and that the post-replication repair mechanism is dependent on the function provided by the recA gene of the host bacteria or a red gene of the phage itself. Data reported by Echols and Gingery (Fig. 3 in ref. 53) shows that mutations to either red14 in the phage or recA-13 in the host bacteria combine with a uvrA mutation in the host to exert additive effects upon the UV-sensitivity of λ phage. Furthermore, this data also indicates that the red and the bacterial recA mutations interact synergistically to increase the UV-sensitivity of the phage on the HCR- host by about 30%. These observations suggest that the phage's red and the host's recA genes provide functions that partially overlap not only in support of vegetative phage recombination, but also in participation in a secondary DNA repair mode distinct from

excision-repair. A mutation in either one of these genes alone causes an increase in only the initial slope of the phage survival curve, as exemplified by the data in Fig. 12 for λred+ phage on the recA-1 and recA+ host bacteria; this effect could be attributed to the preferential survival as plaque-forming units of those infected complexes in which the non-mutant gene is able to sustain optimal function of the red-recA dependent repair mechanism.

The fact that even in HCR- host bacteria around 6 to 8 pyrimidine dimers per infecting phage chromosome are required to induce a phage-lethal hit (section III-A.1) is consistent with the notion that some form of replication-bypass of UV photoproducts can occur in phage λ. Such bypass might well necessitate a post-replication repair of defects in the daughter chromosomes analogous to that which is thought to occur in the bacteria. Thus it is reasonable to tentatively identify the red-recA dependent repair mode in λ as post-replication healing of gaps present in semi-conserved parental DNA as a result of replication-bypass of UV photoproducts. For either HCR+ or HCR- hosts, the increase in phage survival that results from presence of the red+ and recA+ alleles (op. cit., 53) could correspond to bypass, with successful post-replication repair, of around 30% of the potentially lethal photoproducts initially induced in the infecting phage chromosomes, in addition to the repair effected by conventional (or residual) HCR.

The plausibility of this hypothesis is supported by the recent finding that UV-induced mutation of either the bacterial or an infecting λ phage genome is suppressed in recA mutant E. coli K12 strains (163, 243b), in apparent accord with theories that UV-induced mutation is usually associated with post-replication repair of gaps caused by bypass of the

mutagenic photoproducts (243a, 28). Furthermore, there is evidence that lesions induced in either E. coli bacteria or infecting λ phage by dye-sensitized photodynamic action can also be repaired by a recA dependent mechanism that operates independently of HCR (86, 68).

On the basis of this indirect evidence, it will be adopted as a working hypothesis here that a bypass/post-replication repair mode, analogous to that in the E. coli host bacteria, normally functions to alleviate UV damage in λ phage. While a more definitive formulation of this concept surely must await further studies, the presumption of a bypass/post-replication repair mode for λ phage is significant for a general interpretation of several experimental results discussed in the present report (e.g., sections IV-D.1, 2 and Appendix III). Of course, this assumption is also obligatory in order to entertain the suggestion that UV-stimulated recombination might be an inter-molecular manifestation of post-replication repair, consisting in single-strand exchanges primed by double-strand defects which result from replication-bypass of UV photoproducts (184, 109, 240).

In the context of these ideas, the results presented in Figs. 12, 14, and 15 above may be reappraised with regard to the possibility that the UV-induced phage recombination is associated with post-replication repair of semi-conserved parental phage DNA.* Because the exact

---

* The point at issue here is not whether UV-induced recombination can occur between genomes which contain irradiated DNA in semi-conserved form, but whether UV-induced recombination occurs principally among semi-conserved parental genomes as a consequence of the priming action of double-strand defects caused by replication-bypass. Given that replication-bypass of UV photoproducts is possible (26, 184) and that unexcised UV photoproducts promote recombination (section III-A.1 above, 104), one would expect to observe recombination of semi-conserved irradiated

natures of the defective functions in REC- bacterial strains are not understood at present, it is difficult to assess whether the concomitant inactivation of bacterial recombination and post-replication repair in recA mutant cells might signify an intimate relation between the overall mechanisms of these phenomena. If analogous processes occur in the dependent λ phage, this difficulty might be circumvented by focusing consideration upon the "sectors" of UV-induced recombination and post-replication repair in the phage which are sustained by the recA gene of the host as opposed to a red gene of the phage itself. The data in Figs. 12, 14, and 15 provide the relevant information concerning survival and recombination of UV-irradiated λred+ phages in recA and recA+ host bacteria.

As noted in previous discussion, the curves for UV enhancement of λ phage recombination as a function of phage-lethal hits in the recA and recA+ host strains (Fig. 15) imply that in the recA mutant either a) the excision-repair of UV lesions in the phage DNA is impaired at a step subsequent to the removal of the recombinagenic photoproducts, or b) another mode of DNA repair is affected, with the consequence that for a given UV does there is an increase in the number of effectively

---

genomes induced by unexcised, bypassed photoproducts regardless of the nature of the recombinagenic stimulus. Such recombination may account for many of the exchanges induced by high UV doses to λ phages infecting excision-defective HCR- host strains (Fig. 10 and elsewhere, below). It is now obvious that the enhanced recombination induced in bacterial matings by irradiation of the donor cells (117) is caused by unexcised UV photoproducts in the male DNA which have been bypassed during replication associated with chromosome transfer in conjugation (109, 230). The UV enhanced recombination of single-stranded ØX174 and S13 phages (219) probably occurs between double-stranded molecules (185) following bypass of UV photoproducts (151) during synthesis of the initial double-stranded replicative form from an extracellularly irradiated phage.

lethal lesions with little or no change in the number of recombinagenic lesions, or c) both. If the main effect of the recA mutation upon the UV survival of infecting λ phages is to impair the post-replication healing of gaps caused by replication-bypass, these results would suggest that the UV-induced phage recombination is substantially less dependent upon the function provided by the host recA gene than is the presumed post-replication repair mode. The conclusion would be that in λ phage the phenomena of UV-induced genetic recombination and post-replication repair are probably not intimately related. Moreover, if the mechanisms of natural and UV-induced λ phage recombination are essentially the same, this interpretation would be consistent with independent data (53) indicating that the viability of UV-irradiated λ phages is somewhat more dependent upon the recA allele of the host bacteria than upon a red allele of the phage itself while the red gene mediates around 90% of the recombination among unirradiated phages.

III-A.3 Other UV-Sensitive Host Strains

The UV stimulation of λ recombination has also been examined briefly in a host strain mutant at the lex locus. The AB2494(lex-1) mutant strain is HCR+ but UV- and X-ray-sensitive; it exhibits excessive DNA degradation after UV-irradiation and somewhat less efficient recombination than the wild type, and could be defective in a single-strand breakdown associated with repair (104, 102). The UV-sensitivity of λ phages seems to be slightly increased in the lex-1 mutant host strain, but even for high UV doses to the phages the surviving fraction on AB2494(lex-1) is about one-half that found on the lex+ AB1157 host (Table IV).

The lex locus of E. coli K12 may be analogous to the exr locus of E. coli B. The lex-1 and exr (B_{s-1}/B_{s-2}) mutations map in the same region of the bacterial chromosomes, and the K12 and B mutants share those radiation-response phenotypes that have been examined in both strains, but the identity of the loci has not been established (155, 102, 243b). It is known that both the E. coli K12 lex (as well as recA) mutants and the E. coli B exr mutants are stable against UV-induction of mutations (242, 243b), possibly as a consequence of a defect in post-replication repair processes (243a, 28), and there is some indication that the multiplicity reactivation of T1 phages may be suppressed in an exr- host (see section III-B.2 below).

In order to determine whether the lex locus significantly affects natural or UV-induced λ phage recombination, several crosses were performed in the AB2494(lex-1) host strain. The results are shown in Table IV, in comparison with data for the lex+ AB1157 host from the same experiments. For a given UV dose, the phage recombination frequencies were enhanced by approximately the same factor in the mutant lex host and in the wild type strain, although the absolute recombination frequencies from crosses in the lex strain were usually somewhat lower. No significance can be attached to the slight variations in λ phage survival and recombination in the AB2494(lex-1) and lex+ AB1157 host strains, since--the lex locus aside--these strains are not strictly isogenic (see Table II and accompanying footnote, section II-B). The AB2494(lex-1) bacteria usually yielded smaller bursts of progeny phages from the crosses.

Another radiation-sensitive host strain that might yield significant results concerning the UV stimulation of recombination is the uvrD

CROSS	UV dose to phages	Surviving Fraction of Phage (in single infection)		Recombination Frequency (% λ++ Progeny)			
		AB2494(lex-1) Indicator	lex+ AB1157 Indicator	AB2494(lex-1) host		lex+ AB1157 host	
λsusB1 x λsusR5	0	- -	- -	2.8 ± 0.5	(4)	4.1 ± 0.2	(3)
λc67 x λc68	0	1.0	1.0	0.4	(1)	0.84 ± 0.08	(4)
	60	0.28	0.38	2.8	(1)	6.5 ± 0.4	(4)
	120	0.053	0.093	4.9	(1)	8.4 ± 1.4	(4)
	240	0.0059	0.014	6.1	(1)	8.4 ± 0.7	(4)
	360	0.00033	0.00064	- -		- -	
λsus029 x λsusR5	0	- -	- -	0.79 ± 0.14	(4)	1.7 ± 0.2	(3)
λsusQ73 x λsusR5	0	- -	- -	0.42 ± 0.03	(2)	0.47 ± 0.03	(2)
	180	- -	- -	4.2 ± 0.7	(2)	3.1 ± 0.2	(2)

Table IV - UV survival of λ phage and UV enhancement of λ phage recombination frequencies in AB2494(lex-1) host bacteria in comparison to results for the lex+ AB1157 host. The data for the crosses of different phage types were procured in separate experiments. Recombination values are based upon the numbers of crosses indicated in parentheses; the indicated errors are standard deviations.

mutant (Table I) of Ogawa et al. (168). The uvrD strains are probably defective in the regulation of single-strand degradation or repair synthesis associated with host cell reactivation; they are HCR- yet are able to excise photoreactivable UV lesions, degrade irradiated DNA, and heal single-strand gaps in λ phage (168, 197). Normal recombination frequencies are observed among unirradiated λ phages in a uvrD host strain (168), which indicates that not all of the functions required to complete host cell reactivation are essential to the formation of viable recombinant molecules. One might expect the dose-response kinetics for UV stimulation of λ recombination in a uvrD mutant host to be the same as those for a wild type host, since the recombination-promoting lesions would be excised from the irradiated DNA with the same efficiency in both instances. This experiment has not yet been performed, as the uvrD mutants became available too recently for inclusion in the work reported here.

III-B. Multiplicity Reactivation of UV-Irradiated Lambda Phages

UV-damaged bacteriophages that are not viable in single infection can sometimes interact in a multiply infected cell so as to produce viable progeny (150, 129). This phenomenon, known as multiplicity reactivation, is believed to arise from the generation of a viable chromosome by recombination and/or functional complementation among the damaged phages (59, 183). Because the multiple infection of a cell with UV-irradiated λ phages may lead to both UV enhancement of recombination and multiplicity reactivation, it is important to examine the relation between these two phenomena.

III-B.1 HCR+ and HCR- Host Bacteria

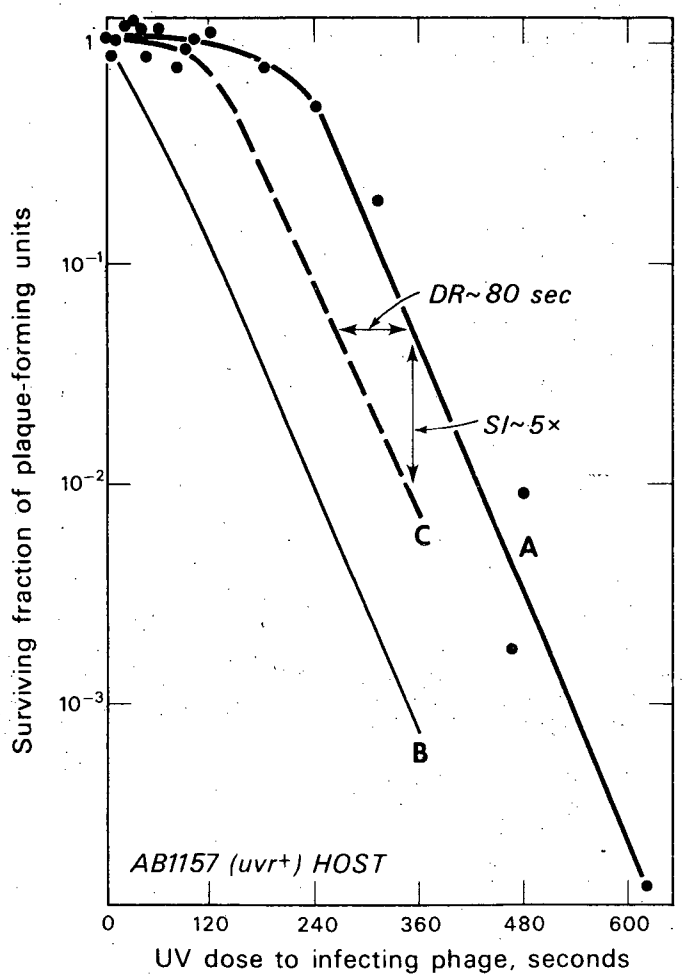
The curves in Figs. 16 and 17 show the UV survivals of a phage mixture in single and multiple infection of the AB1157(uvr+) and AB1886 (uvrA) host strains. The dashed lines represent the survivals calculated for multiply infected complexes in which no multiplicity reactivation occurs; they are equivalent to "multi-target" counterparts of the survival curves for phage in single infection.* The experimental points in these figures represent the relative survivals as plaque-forming units of the

---

* These theoretical multi-target curves were calculated according to the equation $N/N_o = 1 - Exp(-MS)$, where M = the average multiplicity of infection per cell (measured by dividing the actual number of phage adsorbed by the number of viable cells infected), and S = the fraction of infecting phages that are viable in single infection of the host. Assuming that these viable phages are distributed among the cells according to a Poisson distribution, the quantity $1 - Exp(-MS)$ expresses the fraction of multiply infected cells that contain one or more phage viable in single infection. For $MS \ll 1$ this expression reduces to $N/N_o \cong MS$.

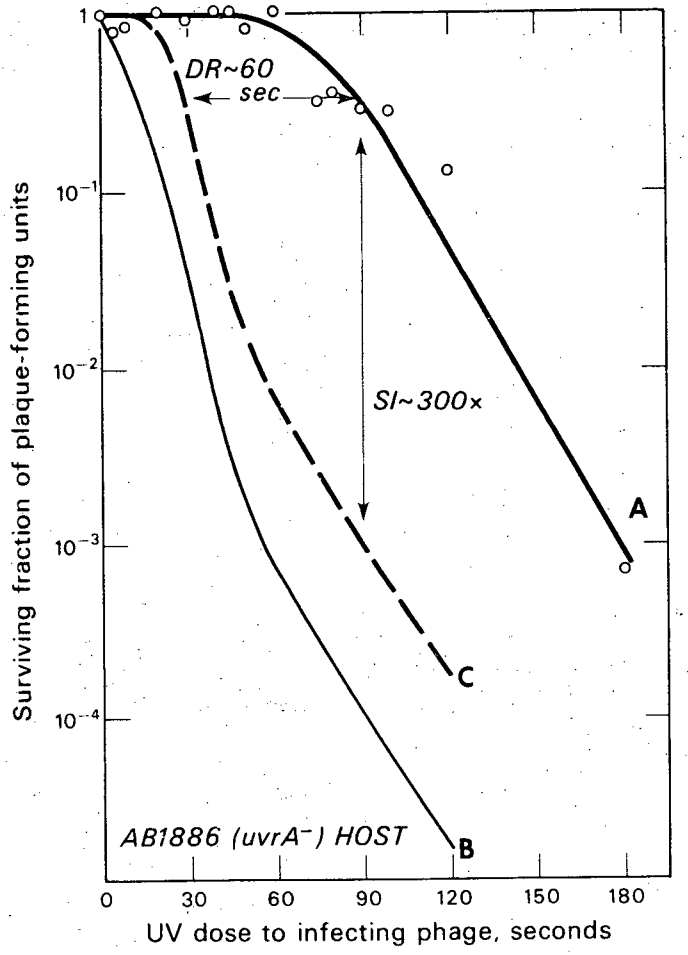
Figure 16. Multiplicity reactivation of UV-irradiated λ phages in the AB1157(uvr+) host. Curve A: the UV survival of a mixture of λsusB1 and λsusG9 in multiple infection of AB1157 cells. The host bacteria were infected with about 10 phages each in crosses of λsusB1 with λsusG9. The data points indicate the relative fraction of infected complexes which formed plaques on a permissive indicator strain as a function of the UV dose to the infecting phages. Curve B: the UV survival of the same infecting phage mixture in single infection of AB1157 (in this case from Fig. 5). Curve C: the survival curve calculated for the multiply infected complexes on the assumptions that each cell is infected with 10 phages and that only those cells will lyse which receive at least one phage active in single infection. The extent of multiplicity reactivation is characterized by the parameters dose reduction (DR) and survival increase (SI).

Figure 17. Multiplicity reactivation of UV-irradiated λ phages in the AB1886(uvrA) host. Curves A, B, and C as in Fig. 16 except with reference to the AB1886 host. Note that Figs. 16 and 17 (and Figs. 18 and 19 below) are not drawn to the same scale.



DBL 677-1708

Figure 16



DBL 677-1709

Figure 17

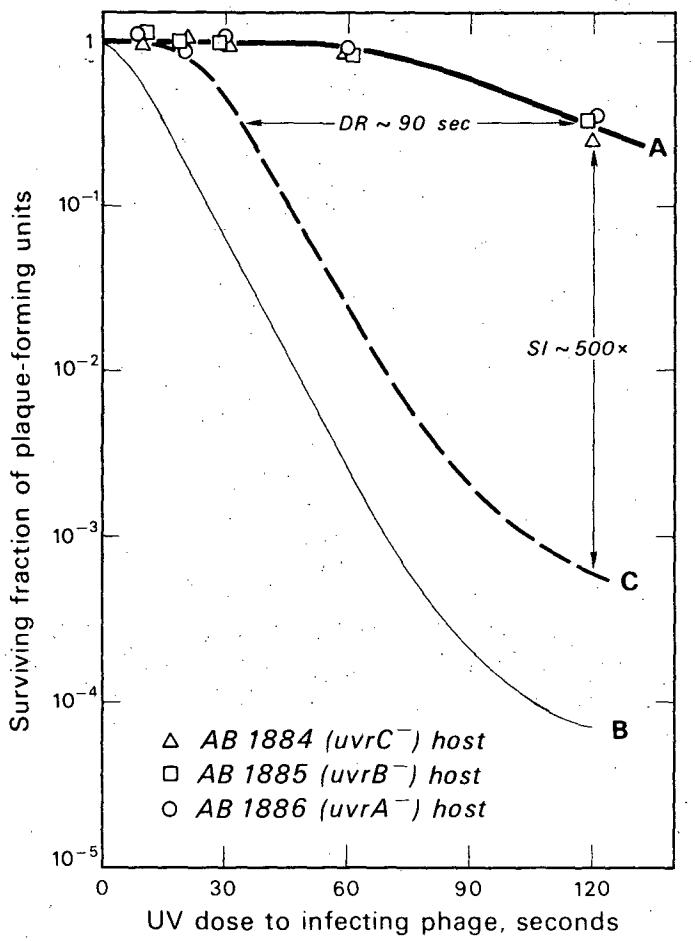
multiply infected host cells in the crosses of UV-irradiated λsusB1 and λsusG9, discussed above in section III-A.1. It is clear from Figs. 16 and 17 that for both the uvr+ and uvrA host strains the experimentally observed survival of multiply infected complexes is greater than would be predicted from the calculated curve. This enhanced survival results from multiplicity reactivation.

An apparent UV stimulation of phage recombination could conceivably arise as a consequence of multiplicity reactivation. Thus, when cells are infected with UV-irradiated phages in a genetic cross, an increased recombination frequency among the progeny phages might arise from a preferential lysis of those infected complexes in which an unusually large amount of recombination leads to multiplicity reactivation (78, 183). It is obvious from Figs. 16 and 17 that such an explanation cannot account for the primary UV enhancement of λ recombination frequencies. In the phage survival range where this initial increase in recombination occurs (Figs. 9 and 10), all of the multiply infected cells of both uvr+ and uvrA host strains continue to produce viable progeny phages. This conclusion is further supported by the data shown in Figs. 18 and 19, describing the relative survival of the multiply infected complexes in crosses of UV-irradiated λc67 and λc68 phages in the uvrA, uvrB, uvrC, and uvr+ host strains.

At higher UV doses that do cause inactivation of the multiply infected complexes the behavior of the phage recombination frequencies is different in the uvr+ and the uvr- host strains. The recombination frequencies in the uvr+ host have reached the plateau level and do not

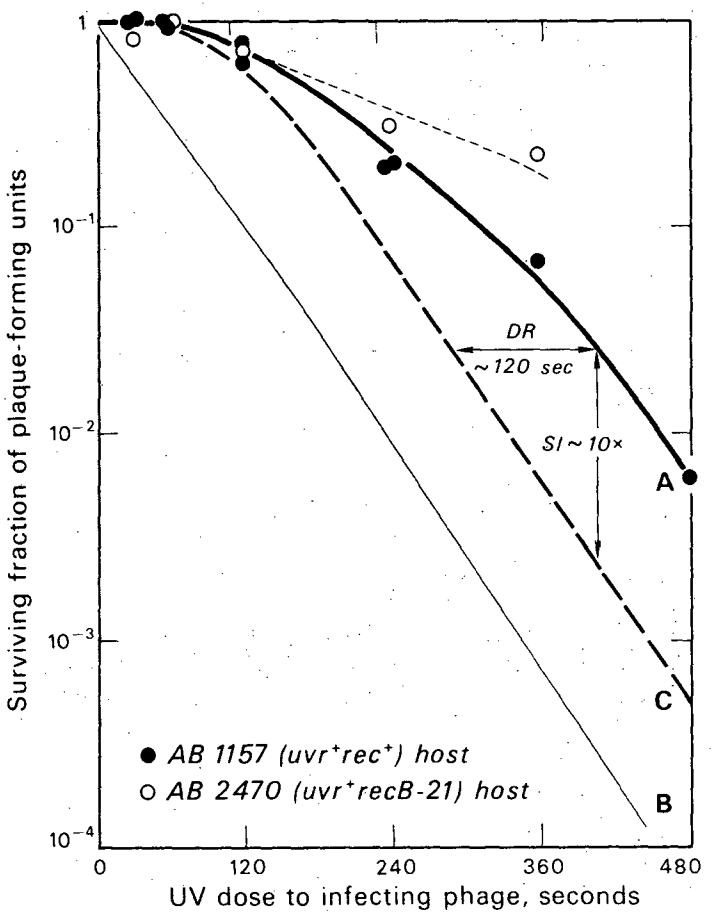
Figure 18. Multiplicity reactivation of UV-irradiated λ phages in the AB1884(uvrC), AB1885(uvrB), and AB1886(uvrA) host strains--with data points from the survival of infected complexes in the crosses of λc67 with λc68 at a multiplicity of about 9 phages adsorbed per cell. Otherwise, curves A, B, and C as designated in the caption to Fig. 16.

Figure 19. Multiplicity reactivation of UV-irradiated λ phages in the AB1157(uvr+,rec+) and AB2470(uvr+,recB-31) host bacteria--also with data points from the survival of infected complexes in the crosses of λc67 with λc68 at a multiplicity of about 9 phages adsorbed per cell. Otherwise, curves A, B, and C as designated in the caption to Fig. 16.



DBL 696-4723

Figure 18



DBL 696-4724

Figure 19

increase further (Figs. 7 through 10). In the uvr- hosts, the secondary, slower, increases in recombination frequency between the c67 and c68 markers commence at a dose for which there is slight inactivation of the infected complexes (Figs. 8, 10). One cannot completely eliminate the possibility that this secondary increase in recombination between the λc phages is accentuated by a slight selection for recombinant-producing infected complexes, but such an effect is rendered unlikely by the fact that a comparable selection for recombinant-producing complexes does not occur in the uvr+ host strain even at much higher relative doses. The preferred hypothesis, discussed above in section III-A.1, is that this secondary increase in recombination frequencies observed in the uvr- hosts is due instead to the continued stimulation of vegetative phage recombination by unexcised pyrimidine dimers.

It has also been argued (see Appendix I) that the secondary increase in the proportion of λ++ recombinant progeny from crosses of λsusB1 and λsusG9 in the uvrA host (Figs. 7, 9) results from a progressive failure of functional complementation among heavily UV-irradiated phages. That secondary increase in recombinant progeny occurs for the same range of UV doses that cause inactivation of the multiply infected complexes. Since no significant dimunition of the extent of multiplicity reactivation is observed as the dose is increased (Fig. 17), one can conclude that functional complementation does not contribute to the multiplicity reactivation of λ observed here.

From Figs. 16 through 19 it appears that there is much more multiplicity reactivation in the uvr- than in the uvr+ host strains. Let us consider this difference in terms of two parameters, defined

as follows. Survival increase (SI) is the ratio, at a given UV dose, of the experimentally observed survival of multiply infected complexes to the calculated survival expected in the absence of multiplicity reactivation. Dose reduction (DR) is the difference between the UV dose that corresponds to an observed survival of multiply infected complexes and the dose calculated to give that survival in the absence of multiplicity reactivation. These parameters are illustrated in the figures of this section for comparable UV doses--at which the survivals of the infecting phages are similar, the primary UV enhancement of recombination among the infecting phages has been saturated, and the extents of multiplicity reactivation are near the equilibrium values that accompany exponential inactivation of the infected complexes. It is evident that the survival increases are about 50 times greater in the uvr- host strains (Figs. 17 and 18) than in the uvr+ host (Figs. 16 and 19), whereas the extents of dose reduction in the strains differ by no more than around 30%.

These observations may be interpreted in the following way:
1) The dose reductions that can be attained through multiplicity reactivation are similar in the uvr- and uvr+ host strains. This fact suggests that similar numbers of recombination events among the parental phages following infection may effect the neutralization of approximately equal numbers of initial UV lesions in the uvr- and uvr+ strains. Since the multiplicity reactivation is observed at higher dose levels than those necessary to evoke the full primary enhancements of phage recombination, it is likely that most of the recombination associated with multiplicity reactivation is in fact that which has been stimulated by the UV lesions.

2) The survival increases attainable through multiplicity reactivation are much greater in the uvr- host strains than in the uvr+ host. This fact suggests that similar numbers of recombination events can effect the neutralization of more phage-lethal lesions (i.e., potentially lethal lesions that would not otherwise be repaired) in the uvr- strains than in the uvr+ host. Some of the recombination that effects multiplicity reactivation may enter an irreversible stage (though it is conceivable that it need not be consummated) while potentially lethal lesions in the parental phages are still subject to excision-repair. Yet it is obvious that the recombination can occur after the initiation of excision-repair since the recombination-promoting lesions are sensitive to excision. In each infected complex, then, host cell reactivation by excision-repair and multiplicity reactivation through UV-stimulated recombination occur during the same time interval; they are not sequential processes, as has sometimes been presumed. Not only is excision-repair antagonistic to recombination in the sense that it can eliminate recombination-promoting lesions, but the two processes may "compete" for the same class of initial UV lesions.

III-B.2 Other UV-Sensitive Host Bacteria

On the basis of evidence presented in the preceding section, it has been concluded that excision-repair and multiplicity reactivation of λ phage in *E. coli* K12 host strains are not sequential phenomena. From observations on the multiplicity reactivation of T1 phage in *E. coli* B (HCR+) and B_{s-1} (HCR-) host strains, Sauerbier has drawn precisely the opposite conclusion (187). His data show that, for comparable UV doses to the infecting T1 phage, similar survival increases are observed

in the B and B_{s-1} hosts but the dose reduction is about five-fold less in the B_{s-1} strain.

The source of this contradiction could rest either in a difference in the physiology of λ and T1 phages or in the difference in the genetic constitutions of strain B_{s-1} relative to B on the one hand and the K12 uvr- strains relative to the uvr+ strain on the other. The latter alternative seems, a priori, more likely; it is now known that B_{s-1} is a double mutant of strain B (155), whereas the K12 uvr- strains are single mutants of the control uvr+ strain (106). The B_{s-1} strain carries both an hcr- mutation, analogous in map location and phenotype to the uvrB mutation in E. coli K12, and an exr- mutation to X-ray sensitivity that may correspond to the K12 lex-1 mutation (see section III-A.3). On the other hand, λ and T1 have similar phenotypes with respect to recombination, multiplicity reactivation, and host cell reactivation in wild type host strains (206)--although they do differ in their behavior on recA mutant host bacteria (134).

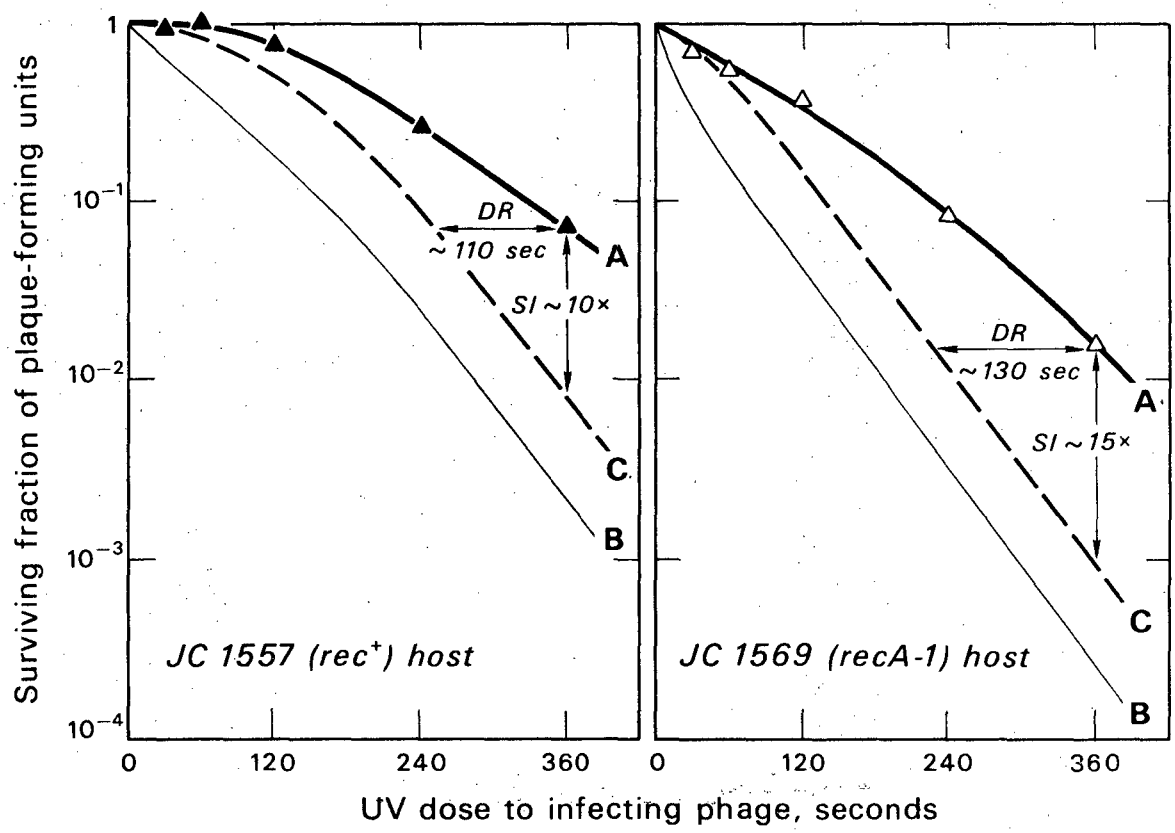
In view of the similar dose reductions observed for multiplicity reactivation of λ in HCR+ and HCR- host strains, the diminished dose reduction for T1 in the hcr-exr- host B_{s-1} could conceivably indicate an effect of the exr- mutation to impair multiplicity reactivation by suppressing the UV stimulation of phage recombination. One would then expect to observe little or no UV enhancement of recombination frequency among T1 phages in an exr- bacterial host. The possibility that an analogous effect might be observed in the λ-K12 system was briefly investigated by performing crosses of λ phages in the AB2494(lex-1) host strain. As discussed in section III-A.3 above, a UV-enhancement of phage recombination frequencies does occur in this host, and a slight multiplicity

reactivation of UV-irradiated phages is also observed in the lex-1 strain. From an experiment in which AB2494(lex-1) bacteria were infected with an average of 6 λc phages each, a multiplicity reactivation plot analogous to those shown in Figs. 16 and 19 for the AB1157 host indicates a dose reduction of approximately 60 seconds and a survival increase of 3×.

Although further experiments utilizing a uvr-lex- double mutant host might be of value in confirming these results, it can be tentatively concluded that the lex mutation does not significantly affect the UV stimulation of recombination or multiplicity reactivation of λ. The explanation of the apparent contradiction between the findings reported here and those of Sauerbier then probably involves either a difference between the physiology of λ and T1 or a distinction between the functions of the exr and lex loci.

The multiplicity reactivation of UV-irradiated λc phages in recB and recA mutant bacteria is shown in Figs. 19 and 20, in comparison with the results in the rec+ host strains. Both Rec- host strains support efficient multiplicity reactivation. The slightly greater dose reduction apparent for the recB host (Fig. 19) correlates with the slightly higher plateau level of UV-enhanced phage recombination in that strain (Fig. 13); these minor effects may be attributable to the restrained degradation of UV-irradiated DNA in the recB bacteria (104, 57).

It is evident in Fig. 20 that the survival of the multiply infected recA complexes declines even for low UV doses to the phages (though the experimental survival at low doses is not less than that predicted by the multi-target calculation). This observation is



DBL 696-4725

Figure 20. Multiplicity reactivation of UV-irradiated λ phages in the JC1557(rec+) (left) and JC1569(recA-1) (right) host strains. The data are from the relative survival of infected complexes in the crosses of λc67 with λc68 at a multiplicity of about 4 phages adsorbed per cell. In other respects the curves A, B, C are as designated in the caption to Fig. 16.

consistent with the notion that the intact, overlapping Red function of the phage supports repair at full efficiency in only a subpopulation of the infected cells (section III-A.2). Part of the difference in survivals of the λred+ phage for single and multiple infections of the recA host may be due to a gene dosage effect. Moreover, there could be a slight artificial enhancement of recombination frequencies from crosses of UV-irradiated phages in the recA host (Fig. 14) due to the selection for progeny of the active complexes (section III-A.2).

IV. RECOMBINATION OF UV-IRRADIATED PARENTAL LAMBDA PHAGE GENOMES

IV-A. Recombination of Unirradiated HCM-labeled Parental Phages

The effect of UV-irradiation upon recombination among parental λ bacteriophage chromosomes was investigated by performing genetic crosses in which the DNA strands of the irradiated infecting phages were labeled by means of host-controlled modification (HCM). Before proceeding to the rationale and results of these experiments, in sections B through D of this chapter, it is necessary to briefly review the molecular basis of host-controlled modification and to consider in detail the properties and potential significance of the HCM label in recombination studies, as demonstrated in crosses of unirradiated phages.

IV-A.1 Host-Controlled Modification

Many bacterial strains impart a biological specificity, or modification, to their own DNA and to that of the dependent λ bacteriophage (19, 6). This specificity is inseparably associated with the physical structure of the modified DNA strands (52), but it is not replicated with the genetic information and so is non-heritable. The chemical basis of the modification is thought to consist in a pattern of base methylation at discrete and specific sites, applied to the DNA after synthesis (7, 93, 74, 72).

DNA derived from cells conferring a particular type of host-controlled modification and introduced into a different host strain may be either accepted, its biological activity unimpaired, or restricted, in which case the alien DNA is rapidly degraded and its genetic

potential destroyed (51, 161). The specificity of the HCM is thus dependent upon two distinct functions: modification of the DNA within the cell conferring specificity, and restriction of inappropriately modified DNA within the challenged host. Mutants that are deficient in either restriction (R-) or both restriction and modification (R-M-) have been isolated (71, 245). At present, it appears that three genetic characters--r, m, and s--affect, respectively, restriction, modification, and the specificity that characterizes each of these processes (147, 25); it is uncertain whether these functions are all exercised by the same enzyme complex. The effects of host-controlled modification and restriction upon the viability of λ bacteriophages in several different strains of *E. coli* are illustrated in Table V.

When λ phages carrying a particular HCM label are grown on a host that neither restricts them nor confers the parental type HCM upon newly synthesized DNA, progeny phage particles that conserve the parental HCM specificity are recovered at a low frequency. Experiments in which the parental DNA has also been labeled with isotopes indicate that the parental HCM specificity is associated with conserved and semi-conserved parental DNA, although the label can be transmitted by somewhat less than an entire parental strand (8). The HCM specificity is serially transmissible with either of the DNA strands of a parental phage, indicating that both strands are modified at each site and that modification in either strand suffices to forestall degradative attack (9, 161).

The K12-K12(P1) system for host-controlled modification of phages (Table V) (8, 6) was utilized in the experiments reported below. P1 bacteriophage carries its own genes that confer modification and

Phage type	Efficiency of plating on E.coli host strains			
	K12 (R_K+M_K+)	K12(P1) (R_K+M_K+ R_P+M_P+)	C (R-M-)	B (R_B+M_B+)
λ·K12 (= λ·K)	1	$2x10^{-5}$	1	10^{-4}
λ·K12(P1) (= λ·P)	1	1	1	10^{-4}
λ·C	$4x10^{-4}$	$4x10^{-7}$	1	$2x10^{-4}$
λ·B	$4x10^{-4}$	$7x10^{-7}$	1	1

Table V - The host-controlled modification and restriction of λ bacteriophages on several E. coli host strains (8, 6). Each phage type is defined by a dot and letter indicating the host strain in which the phage DNA was synthesized. Relevant restriction and modification phenotypes of the host strains are denoted by the R and M symbols (245, 71). The solid box encloses the HCM system utilized in the present study.

restriction characteristics in addition to those of the host cell (8, 71). λ bacteriophages grown in an E. coli K12(P1) host (λ·P) possess both K12- and P1-mediated modifications, and they plate with 100% efficiency on both K12(P1) and non-lysogenic K12 bacterial strains. By contrast, phage synthesized in K12 strains non-lysogenic for P1 (λ·K) plate with 100% efficiency upon other non-lysogenic K12 hosts but at an efficiency of only 2×10^{-5} on K12(P1) strains. In the non-restricting K12 hosts, both λ·K and λ·P participate in normal vegetative multiplication and recombination (8).

The HCM label can be utilized as a marker to distinguish the genomes containing strands of predominantly parental DNA among the progeny from crosses of λ phages. Thus the progeny of a cross of λ·P phages in a non-lysogenic K12 host include many phages with newly synthesized λ·K chromosomes and a few percent λ·P* phages, which retain the parental HCM specificity because they contain conserved or semi-conserved parental DNA. The frequency and genetic constitution of these progeny λ·P phages can be examined by assaying the cross lysate on

---

* In the text that follows, phage are designated as λ·P (or λ·C, etc.) by either of two quite distinct criteria--i.e., because they were grown in a K12(P1) host or because they plate on a K12(P1) indicator. The λ·P phages used to infect host bacteria in phage crosses were synthesized in K12(P1) host strains; their chromosomes were thus composed entirely of DNA carrying the type P host-controlled modification, and they were naturally viable on a K12(P1) indicator strain. The progeny phages, from crosses of λ·P parental phages in non-lysogenic K12 host bacteria, that plated on a K12(P1) indicator are also referred to as λ·P because they retained the parental HCM specificity, but with the implicit reservation that in order to transmit the HCM label they need only have retained somewhat less than a single continuous strand of parental λ·P DNA (or, more rigorously, a modification in either DNA strand at each site of type P HCM along the chromosome). The meaning of the λ·P designation in the text will be clear from the qualification "parental" (infecting) or "progeny." Attention is sometimes directed by quotation marks ("λ·P") to the fact that a particular λ·P progeny phage type presumably contains some DNA synthesized in a type K host cell over a portion of the phage chromosome.

appropriate K12(P1) indicator strains, which restrict the growth of the more numerous λ•K progeny of the infection.

IV-A.2 Recombination by Breakage and Reunion of Parental DNA Strands

Among the progeny of genetic crosses involving unirradiated λ•P parental phages in a non-restricting E. coli K12 or C host strain, genetic recombinants can be detected that retain the parental λ•P HCM specificity (8, 111). For example, when λhc•P is crossed with λh+c+•P in E. coli C, the frequency of λhc+ recombinants among the progeny that plate on a K12(P1) indicator is 25% as great as that among the total progeny population which plates on a strain C indicator (111). In evaluating the significance of HCM-labeled recombination, one must consider both the mechanism by which the parental HCM recombinants are formed and the criteria by which the frequency of these recombinants can be interpreted as an index of recombination among the infecting phage chromosomes.

The occurrence of intact recombinant chromosomes that retain a parental HCM label--as of recombinants that contain segments of an isotopically labeled parental chromosome (160, 131)--indicates that material from mating parental DNA strands is incorporated into recombinant molecules. The quantity of DNA from a labeled parental phage that is contained in a recombinant chromosome is proportional to the amount of genetic information contributed by that parent (160, 131). HCM-labeled progeny phages that are recombinant in the central region of the chromosome are formed at a significant frequency only if both parental phage types carry the HCM label (111). Since progeny phages that retain the

HCM label are found to contain, at a minimum, nearly a complete strand of the parental modified DNA, these facts imply that at least one strand of an HCM-labeled recombinant molecule is formed by breakage and reunion of modified parental DNA strands. Experiments in which only one of the parental phage types was isotopically labeled have shown that both strands of a parental chromosome can be integrated into a recombinant molecule (160). One would therefore infer that natural formation of HCM-labeled recombinants proceeds by a breakage and reunion of chromosomes comprised of conserved and/or semi-conserved parental DNA.

This conclusion has been confirmed by Meselson's direct demonstration of recombination among λ phages by breakage and reunion of double-stranded DNA molecules (157, 120). Crosses were performed in which all parental phages were labeled with heavy isotopes, and the density distribution of the recombinant particles was determined. Some recombinants were composed almost entirely of heavy parental DNA; these recombinants could have arisen only by breakage and reunion among the infecting phage chromosomes. The densities of all recombinants were consistent with breakage and reunion among conserved, semi-conserved, and newly synthesized chromosomes. Recombinants with density labels corresponding to semi-conserved to conserved DNA also retained a parental HCM label. While incidentally confirming that HCM-labeled recombinant progeny are formed by breakage and reunion of chromosomes that each contain at least one parental DNA strand, these results imply that breakage and reunion of double-stranded chromosomes is the general mechanism of natural phage recombination. This conclusion is strongly supported by the studies of Tomizawa and Anraku with T^4 phages (cf., section I-B.3).

IV-A.3 Properties of the HCM label on parental recombination: conditions for retention of the HCM specificity

The recombination frequency observed among the HCM-labeled progeny of a cross provides an index of the amount of recombination involving parental DNA strands; however, parental HCM-labeled recombinants need not necessarily originate in breakage and reunion exchanges between conserved parental--or even two parental--genomes.

Because the parental HCM specificity can be transmitted to a progeny phage by modified sites in only one strand of the chromosome, an HCM-labeled recombinant may be formed by breakage and reunion of any pair of conserved and/or semi-conserved parental chromosomes. Most progeny phages, both recombinant and non-recombinant, that retain a parental HCM label contain semi-conserved parental DNA (8, 157). In order to determine whether HCM-labeled recombinants formed in a particular instance, e.g., in crosses of UV-irradiated phages, result from recombination among conserved infecting chromosomes (or even to confirm that they are formed by breakage and reunion of both strands of each mating molecule), it is necessary to ascertain whether unreplicated recombinants contain conserved parental DNA or whether the recombination occurs prior to any DNA replication.

Furthermore, some types of HCM-labeled recombinants may be formed by recombination between a chromosome containing modified parental DNA and a chromosome composed wholly of newly synthesized and/or unmodified DNA (8, Appendix II of this report). Several lines of evidence bear on the amount and location of unmodified DNA that may be acceptable in such a "λ·P" recombinant molecule, and hence upon the extent to which the frequency of HCM-labeled recombinant progeny of a cross may be

representative of recombination among chromosomes containing only parental DNA strands.

Genetic observations imply that a recombinant chromosome retaining a parental type P HCM specificity may include on its right end a segment of unmodified DNA that is on the order of 10% the total molecular length (8, Appendix II-1). "λ·P" singly recombinant progeny are formed in crosses of λ·P phage carrying a selected marker distal to the right end of the chromosome with λ·K phage carrying a selected marker proximal to and within about 10% of the right end of the chromosome (Figs. C and D in Appendix II-1). For crosses of unirradiated λsus·P phages in a K12 host, more than half of the λsusP3+susQ73+"·P" recombinant progeny and nearly all of the λsusQ73+susR5+"·P" progeny are formed by single recombination of chromosomes containing parental λ·P DNA with newly synthesized λ·K chromosomes. However, single recombinants for two markers that are situated toward the interior of the chromosome--such as sus029 and susP3--retain the λ·P specificity only if both parental phages carry the type P HCM label (111, Appendix II-1).

A segment of λ·K DNA can also be integrated, by multiple recombination, into the interior of a λ·P chromosome without destruction of the type P HCM specificity. In mixed infections of a restricting K12(P1) host with λ·K and λ·P phages, marker rescue of interior segments of the restricted λ·K genome competes with degradation of the restricted DNA (51, 216); the length of the rescued segments can be on the order of 10% of the genetic map. Moreover, detectable numbers of recombinant "λ·P" progeny are recovered from crosses in non-restricting host cells of λ·K with λ·P phages for markers located toward the interior of the chromosomes or so situated that the λ·P specificity is not retained by single recombinants (Appendix II-2).

These genetic observations are obviously related to the number, activity, and location on the λ chromosome of the modified sites that constitute the HCM label, a topic concerning which there is little specific information at present. Most generally, it can be concluded that there are intervals within the λ chromosome, of significant length, that either do not contain relevant sites of modification or that contain sites whose activity is dispensable to preservation of the HCM label.

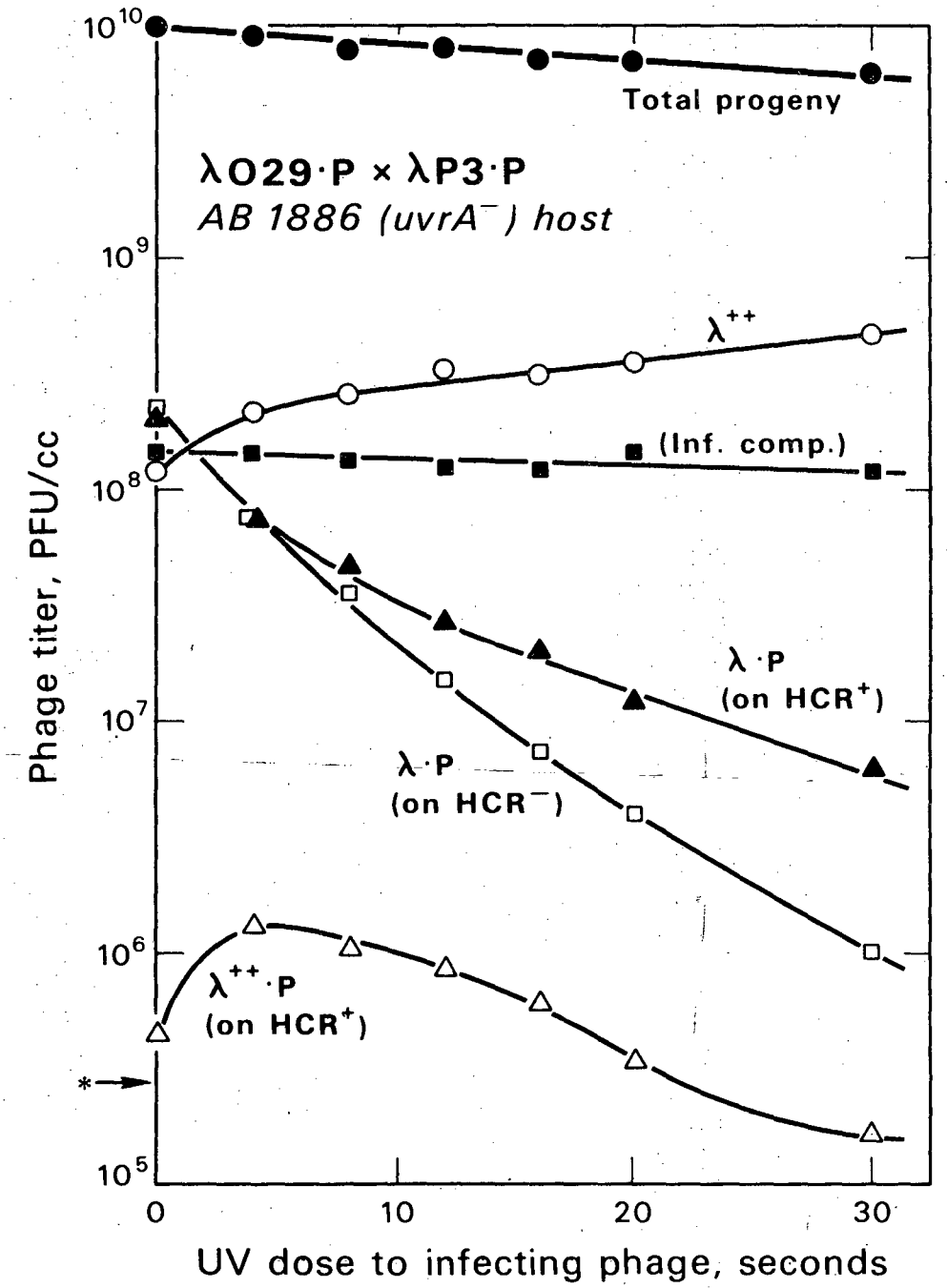
It has been suggested that modification and the primary events of restriction may occur at the same specific sites on the chromosome, possibly as alternate activities of the same enzyme complex (6, 25); *in vitro* evidence concerning modification and restriction of the small coliphage *fd* support this view (148, 147). If this also holds true for λ, some indication as to the number of HCM sites may be had from *in vitro* studies of restriction in the K12-C HCM system (Table V). Yuan and Meselson (161) have found that chromosomes of λ·C phages, which are restricted to an EOP of about 10^{-4} on a K12 indicator strain, are degraded to fragments on the order of 1/5 the intact molecular weight in a limit digest with a purified extract of the K12 restriction enzyme. This suggests that there may be about five sites of type *K* HCM on the λ chromosome. These authors report that a similar restriction enzyme has been isolated from P1 lysogens. One would expect that λ·K phages, which are restricted to an EOP of about 2×10^{-5} on a K12(P1) indicator, lack between 5 and 10 sites of P1-mediated modification. That the right 10% and certain interior segments of the λ·P chromosome are unnecessary to retention of the HCM label would then be attributable to the absence of modification sites in these intervals.

IV-B. Enhancement of Recombination among HCM-labeled Parental λ Phages; UV-Induced Recombination by Breakage and Reunion

The results of an experiment to characterize the effects of UV-irradiation upon recombination involving HCM-labeled parental phages are shown in Fig. 21. A series of crosses was performed by infecting bacteria of the *E. coli* K12 host strain AB1886(uvrA) with a mixture of λsusO29·P and λsusP3·P phages that had been exposed to various doses of UV; the progeny phages from each cross were characterized on restricting and non-restricting indicators as described in the figure legend and in section II-I.

Such experiments provide--in addition to the types of dose-response curves for UV enhancement of overall recombination frequencies and for multiplicity reactivation discussed in Chapter III--three kinds of information concerning the particular fate of the UV-irradiated HCM-labeled infecting phages: a) the relative recovery as a function of UV dose of λ·P progeny phages; b) the frequency of genetic recombinants among the progeny phages that retain the parental λ·P HCM specificity; and c) the relative viability on HCR+ and HCR- indicators of the λ·P progeny. The frequency of genetic recombinants among HCM-labeled progeny phages from different types of genetic crosses will be considered in this section; the relative recovery and viability of HCM-labeled progeny phages from crosses of UV-irradiated phages will be examined in section IV-C.

Figure 21. The complete set of data from an experiment in which λc71sus029·P and λc71susP3·P phages irradiated with various UV doses were crossed in K12 strain AB1886(uvrA) host bacteria. The crosses were performed using the identical culture of host cells and the lysates were stored for later titration--as described in Materials and Methods. The various phage types among the progeny population were assayed as follows: total progeny = lysate titer on permissive non-restricting indicator AB1157; λ++ recombinants = lysate titer on non-permissive non-restricting indicator W3350; total λ·P on HCR+ and HCR- hosts = lysate titers on permissive restricting AB1157(P1) and AB1886(P1) indicators, respectively; λ++·P recombinants = lysate titer on non-permissive restricting (HCR+) indicator W3350(P1). (The infected complexes were assayed on AB1886 indicator. Lysate titers are calculated relative to the concentration of infected complexes in the original infection mixture, prior to dilutions for incubation, etc.) This assay system for distinguishing parental HCM-labeled phages is explained further in Table III, section II-I. The asterisk on the ordinate indicates the approximate titer in the lysates of infecting λsus·P phages unadsorbed to the host bacteria and not neutralized by anti-λ serum treatment--as determined in mock crosses. Because the procedure used in these experiments would not permit accurate titration of phage types present at a concentration much lower than that indicated for λ++·P on the HCR+ indicator, it was not feasible to assay the λ++·P recombinant progeny on an HCR- indicator strain. The data points shown in this figure represent the average of the results determined for two replicate crosses.



DBL 696-4726

Figure 21

The solid dose-response curves in Fig. 22 describe the UV enhancement of recombination frequencies in the total and λ·P progeny populations from crosses of λsus029·P with λsusP3·P. It is clear from these curves that the initial UV enhancement of recombinant frequency among all progeny accompanies a quite similar increment in the frequency of recombinants among progeny containing parental λ·P DNA strands. Thus while a 12-second UV dose to the infecting phages increases the recombination frequency among all progeny threefold, from 1.2% to 4.1%, a fifteenfold increase from 0.2% to 3.1% is induced among the progeny that retain the parental HCM label.

The UV-induced HCM-labeled recombinants are attributable to recombination of λ·P genomes early in the phage infection, leading to the enhancement of recombination frequency observed among all progeny phages. The fairly uniform difference between the λ·P and total recombination frequencies at low to moderate UV doses is attributable to a constant increment in recombinants formed by natural vegetative recombination among newly synthesized chromosomes during later phases of the infection. (The divergence of the dose-response curves at higher UV doses will receive note presently.) These results are consistent with the demonstration by Jacob and Wollman (115) that the UV-induced recombination occurs early in the latent period. Of particular significance here is that HCM-labeled parental DNA is incorporated into UV-induced recombinant genomes; this finding implies that the UV-stimulated recombination proceeds by a breakage and reunion, or closely related, mechanism.

Confirmation and elaboration of these simple conclusions require that the potential limitations of the HCM label on recombination be taken into account--e.g., by examining to what extent UV-induced

Figure 22. Dose-response curves for the UV enhancement of recombinant frequencies among all progeny and among HCM-labeled λ·P progeny in the crosses of λc71sus029·P with λc71susP3·P (Fig. 21) and in crosses of λc71sus029·K with λc71susP3·P.

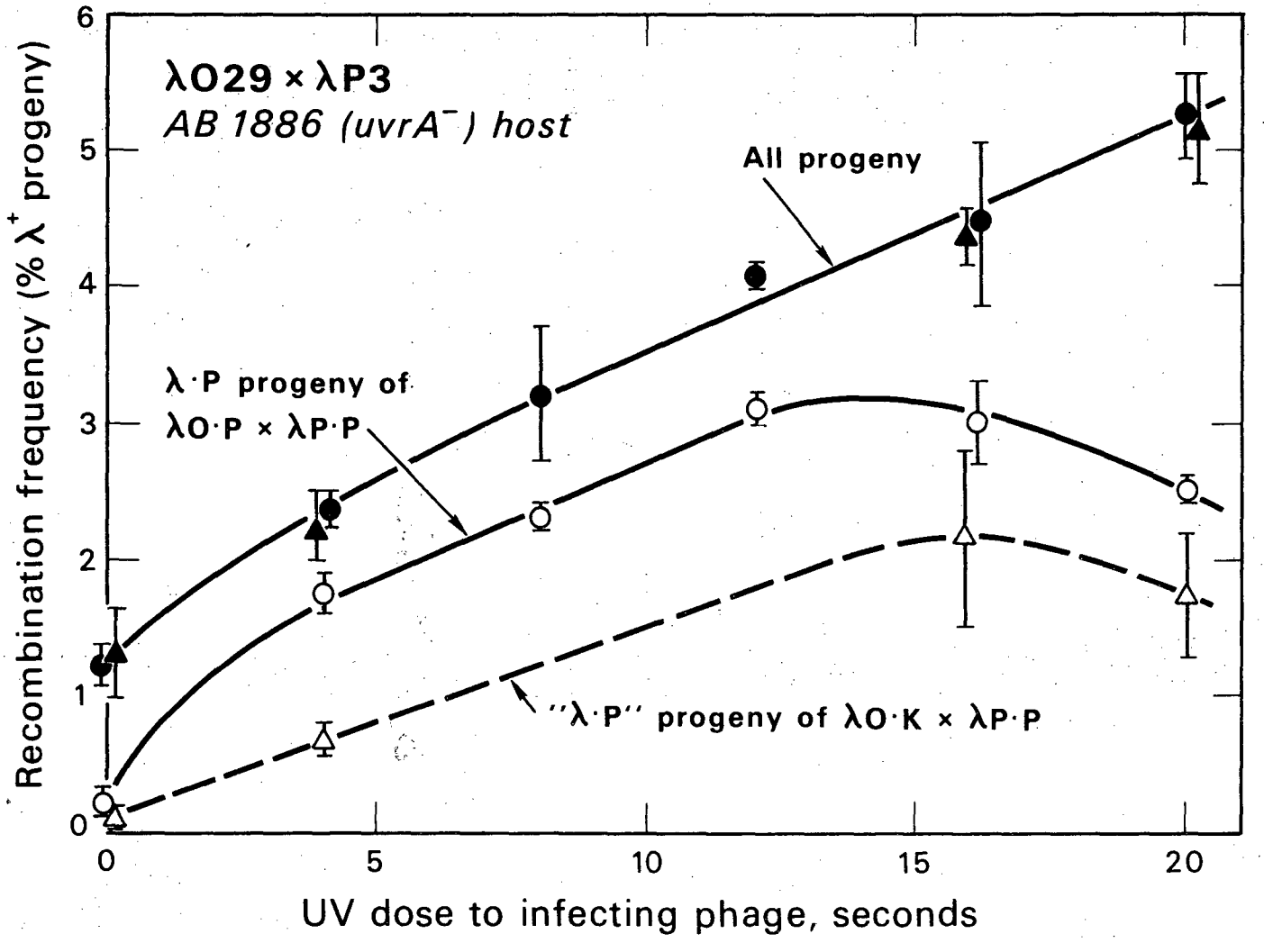


Figure 22

DBL 696-4727

recombinant λ·P phages might derive from matings of parental λ·P chromosomes with newly synthesized λ·K chromosomes. The observations discussed below support the conclusion that the UV-stimulated HCM-labeled recombination does in fact occur by breakage and reunion of parental DNA strands, and most likely of both conserved and semi-conserved infecting phage chromosomes (section IV-C).

In addition to the results from crosses of λ·P phage types, data from several crosses of UV-irradiated λsus029·K with λsusP3·P phages are shown in Fig. 22. The recombination frequency among the λ·P progeny of these crosses seems to increase linearly with UV dose (dashed curve) but to be significantly lower than the frequency observed if both infecting phage types carry the type P HCM label. The control experiments presented in Appendix II imply that a recombinant formed by breakage and reunion of λ·P and λ·K phages in the sus029-susP3 interval can retain the λ·P specificity only if a second recombination occurs in an adjacent interval so that the sus+·K allele is enclosed in a λ·P genome. The results shown in Fig. 22 for crosses of UV-irradiated λsusP3·P with λsus029·P and λsus029·K are consistent with the view that the same conditions apply for retention of the λ·P specificity in UV-induced recombination over this interval, but that the UV-stimulated recombination is characterized by a great increase in the relative frequency of the double crossovers that permit formation of "λ+·P" recombinants of the λ·P and λ·K parental phages. The latter point is analogous to the finding of Jacob and Wollman (115) that UV enhances to comparable extents the frequencies of single and double recombinants in the overall progeny population. That a higher frequency of UV-stimulated λ+·P recombinants is observed if both infecting phage types carry the λ·P HCM label implies that most of the UV-

induced HCM-labeled recombinants do contain DNA derived from both infecting phage types and are formed by breakage and reunion of parental DNA strands.

Recombination between λ•P and newly synthesized λ•K chromosomes that occurs in crosses involving UV-irradiated λ•P infecting phages in the HCR- AB1886(uvrA) host would necessarily entail mating of a molecule containing parental DNA with unexcised UV lesions and a molecule free of such lesions. Jacob and Wollman (115) have established that in crosses of an unirradiated with a UV-irradiated parental phage type the irradiated parent acts as a donor of genetic segments, e.g., preferentially contributing the median marker in double recombinants from three factor crosses. (This could be understood as due to the stimulation of further recombination by unrepaired UV lesions in the portion of a recombinant molecule composed of irradiated parental DNA. The observed result corresponds to marker rescue of selected alleles from the irradiated parental phage type.) One would then anticipate that, in the present experiments, matings between λ•P phage containing unexcised UV lesions and newly synthesized λ•K phage would generally lead to fragmentation of the λ•P genome by recombination and destruction of the HCM label. The polarity of the UV-stimulated recombination would inhibit formation of "λ•P" recombinants containing a selected λ•K allele; the UV-induced increases in the frequencies of λ+•P progeny shown in the crosses of Fig. 22 would then be attributable exclusively to UV-stimulated recombination among conserved and/or semi-conserved parental phages.

This inference may be verified by considering the UV enhancement of recombination frequencies in the λ•P and overall progeny populations

from crosses of λsusP3·P with λsusQ73·P and of λsusQ73·P with λsusR5·P. Because a segment of type K DNA can be incorporated on the right end of a recombinant molecule that retains the type P HCM specificity, from crosses of unirradiated λ·P phages around 90% of the λ·P progeny recombinant in the susQ73-susR5 interval and about 60% of the λ·P progeny recombinant in the susP3-susQ73 interval result from single recombinations between λ·P and newly synthesized λ·K molecules (Appendix II). The argument of the preceding paragraph predicts that in crosses of UV-irradiated λ·P phages there will be a dimunition in the number of "λ·P"recombinant progeny accruing from matings of λ·P with newly synthesized λ·K phages at the same time that the recombination among the λ·P phages containing parental DNA is stimulated. The dose-response curves for the UV enhancement of λ·P recombination frequencies in the crosses λsusP3·P × λsusQ73·P and λsusQ73·P × λsusR5·P should reflect a superposition of these antagonistic effects.

The results shown in Figures 23 and 24, for crosses of λsusP3·P with λsusQ73·P and of λsusQ73·P with λsusR5·P,* respectively, are consistent with this expectation; in these crosses the observed UV enhancement of recombination frequencies among λ·P progeny is less efficient, relative to the enhancement among all progeny phages, than that observed for crosses of λsus029·P with λsusP3·P (Fig. 22). For increasing doses of UV to the infecting phages there is a clear absolute increase in the frequency of λ+·P recombinant progeny, but in the moderate dose range the slopes of the dose-response curves for λ·P recombination in the susP3-susQ73 and susQ73-susR5 intervals are only about 1/2 and 1/3,

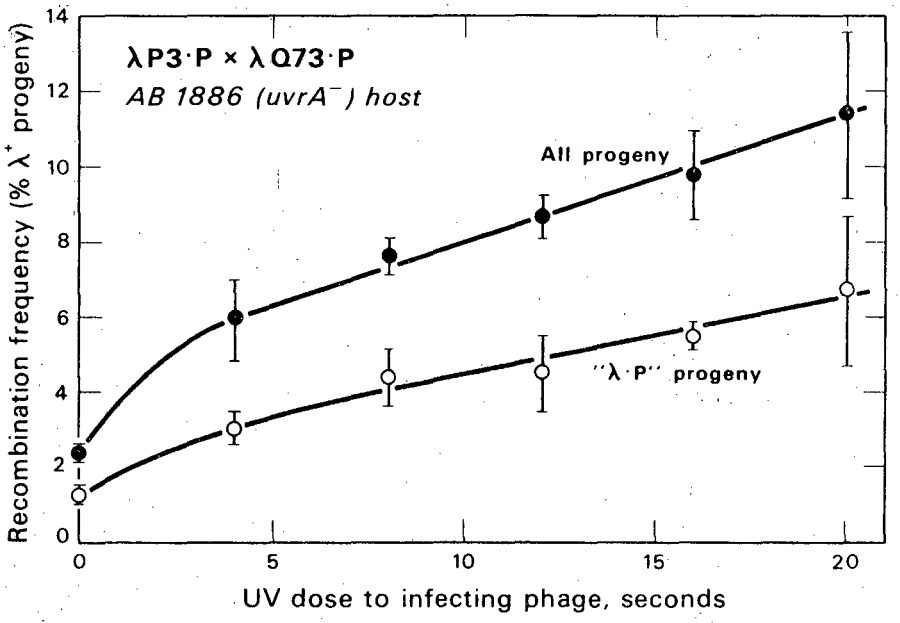
---

* The data in Figures 22, 23, and 24 also provide measures of the relative UV stimulation of overall recombination in intervals characterized by differing ratios of physical to genetic length, and are considered from this viewpoint in section IV-D and Fig. 29.

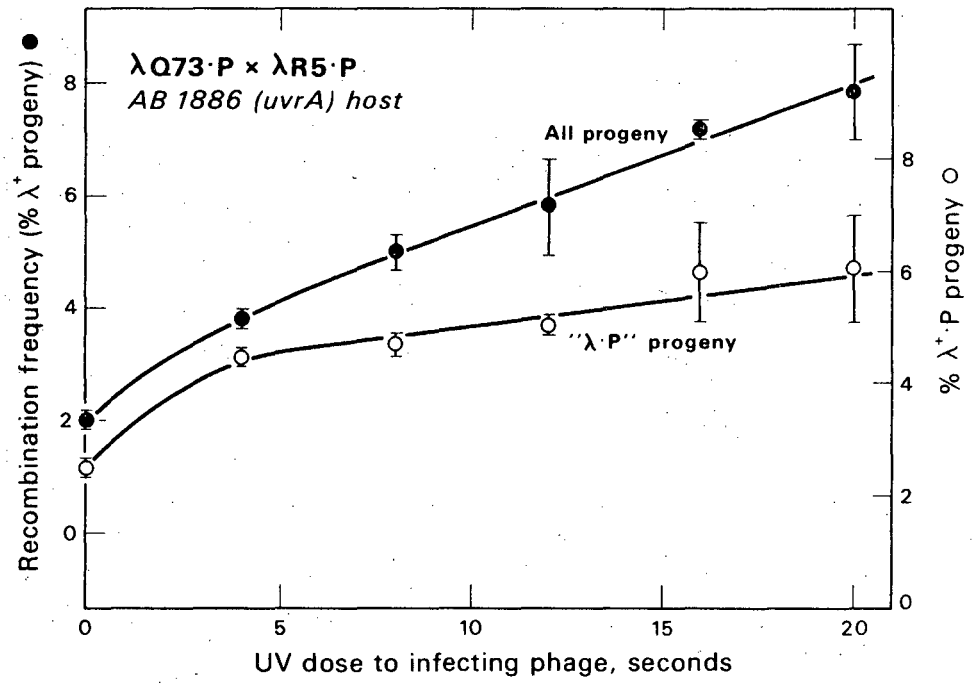
Figure 23 (top). Dose-response curves for the UV enhancement of recombinant frequencies among all progeny and among λ•P progeny in crosses of λc71susP3•P with λc71susQ73•P in the K12 strain AB1886. (This experiment was performed concurrently with that described in Figs. 21 and 22, utilizing the same cultures of host bacteria for the crosses and for the titrations.)

Figure 24 (bottom). Dose-response curves for the UV enhancement of recombinant frequencies among all progeny and among HCM-labeled λ•P progeny in crosses of λc71susQ73•P with λc71susR5•P in the K12 strain AB1886, from an experiment analogous to those described in Figs. 21-22, and 23.

Figure 23



DBL 696-4728



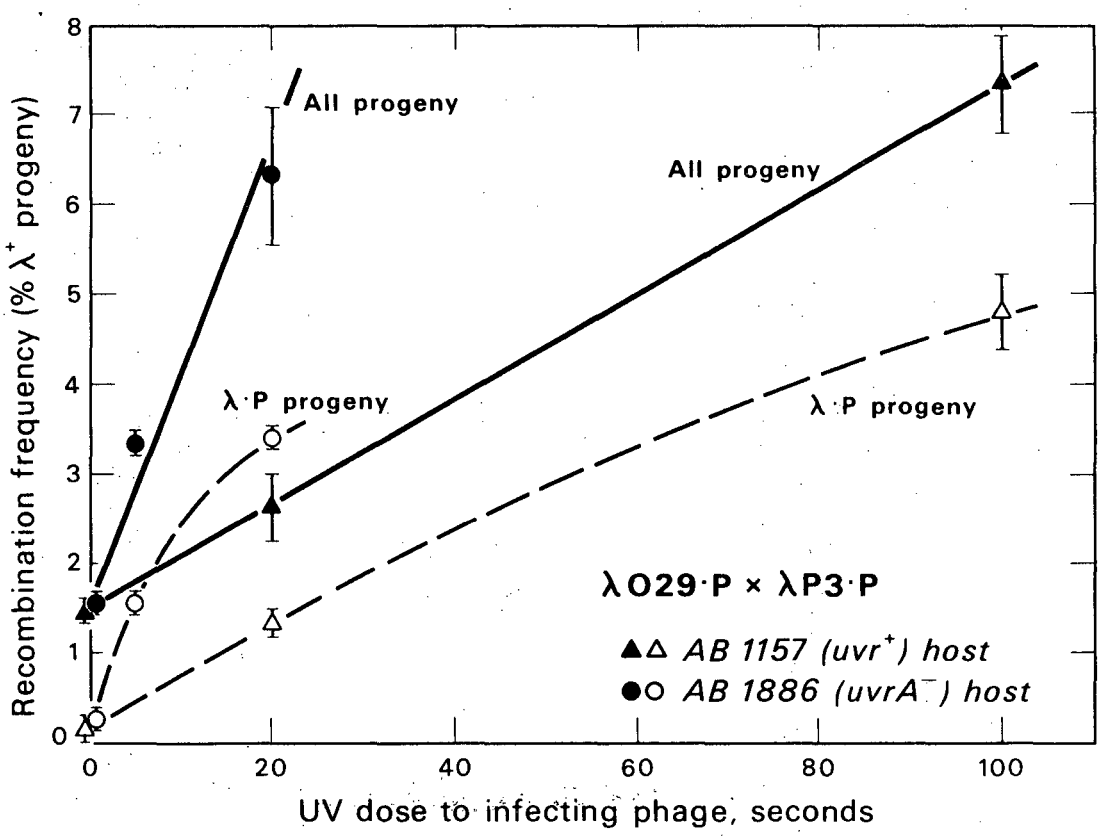
DBL 696-4729

Figure 24

respectively, as great as the slopes of the curves describing the enhancement of recombination among all progeny. By contrast, for the crosses of λsus029·P with λsusP3·P the UV-induced increases in recombination frequencies among λ·P progeny and among all progeny are nearly the same over this dose range. There is thus an inverse relation between the observed UV enhancements of λ·P recombination and the extents to which recombination of λ·P and newly synthesized λ·K genomes contributes "λ·P" recombinant progeny in the crosses of unirradiated phages over these three intervals.

This evidence supports the conclusion that UV-induced HCM-labeled recombinants are not formed by eccentric recombination of molecules containing parental λ·P DNA with newly synthesized λ·K molecules; rather, matings of such chromosomes in crosses of UV-irradiated phages are even more likely to result in destruction of the λ·P HCM label than in crosses of unirradiated phages (regardless of the interval of recombination examined). The UV enhancement of recombination frequencies among the λ·P progeny from crosses of λsus029·P with λsusP3·P and λsus029·K with λsusP3·P (Fig. 22) is then attributable exclusively to recombination of conserved and/or semi-conserved parental genomes, and the UV-stimulated recombination must proceed by breakage and reunion of parental DNA strands. Conversely, divergence of the dose-response curves for enhancement of recombination among λ·P progeny and among all progeny (at moderate UV doses in Figures 23 and 24 and at higher doses in Fig. 22) may be attributed to the increasing frequency, with increasing dose, of UV-stimulated recombinations between conserved or semi-conserved parental chromosomes and newly synthesized chromosomes; the recombinant products of such events do not retain the λ·P HCM label.

The excision-defective AB1886(uvrA) host was used in the experiments reported above in order to determine the UV-stimulated parental recombination for a calculable number of unexcised UV-induced pyrimidine dimers, to facilitate interpretation of any indications of recombination between parental and newly synthesized phage chromosomes, and to permit assay of any UV lesions remaining in the λ·P progeny recovered from the crosses (cf., section V-D.1 below). An analogous stimulation of recombination among parental DNA strands occurs in an HCR+ strain. The curves in Fig. 25 furnish a comparison of the UV stimulations of recombination frequencies in the λ·P and total progeny populations from crosses of λsus029·P with λsusP3·P in the AB1886(uvrA) and AB1157(uvr+) host strains. The slope of the dose-response curves is about 1/5 as great for the HCR+ AB1157 host, consistent with excision-repair of the recombination-promoting lesions in this strain as discussed in Chapter III.



DBL 696-4730

Figure 25. The UV enhancement of total and λ·P recombination frequencies for crosses of λc71susO29·P with λc71susP3·P in the HCR+ and HCR- host strains AB1157(uvr+) and AB1886(uvrA).

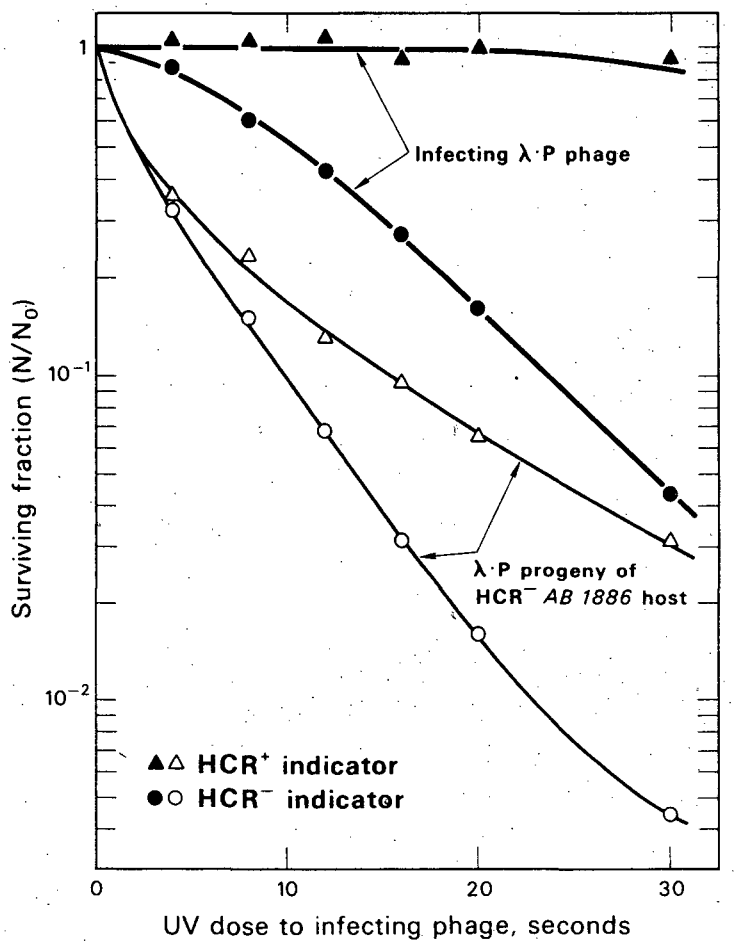
IV-C. Recovery and Viability of UV-irradiated HCM-labeled Parental λ Phages

UV-irradiation of the infecting phages causes a marked reduction in the frequency with which progeny phages retaining a parental λ•P HCM specificity are recovered from crosses in an E. coli K12 host. The survival curves in Fig. 26 show the decrease in recovery on HCR+ and HCR- K12(P1) indicators of total λ•P progeny from the crosses of λsus029•P with λsusP3•P in AB1886(uvrA) host bacteria, in comparison with the survivals of the infecting phages on the HCR+ and HCR- indicators. Comparable results were obtained in the crosses of λsusP3•P with λsusQ73•P and of λsusQ73•P with λsusR5•P. From these data it is clear not only that the absolute recovery of λ•P progeny on the HCR+ indicator falls sharply with increasing UV dose, but also that the λ•P progeny recovered still retain potentially lethal lesions which reduce their viability on the HCR- indicator. The analogous data from the several crosses in AB1157 (uvr+) host bacteria, plotted in Fig. 27, show that UV-irradiation of the infecting phages results in a similar decreased recovery of λ•P progeny from crosses in this strain; in this case, however, the viability of the HCM-labeled progeny phages is identical on HCR+ and HCR- indicator strains.

This information regarding the recovery and viability of λ•P progeny phages permits further characterization of the HCM-labeled progeny populations and furnishes several significant clues pertaining to the nature of UV-stimulated recombination and of concomitant repair processes. In particular, having established that HCM-labeled parental DNA strands are incorporated into UV-induced recombinant λ•P molecules, one may further inquire into: 1) whether the λ•P progeny which are recovered from the

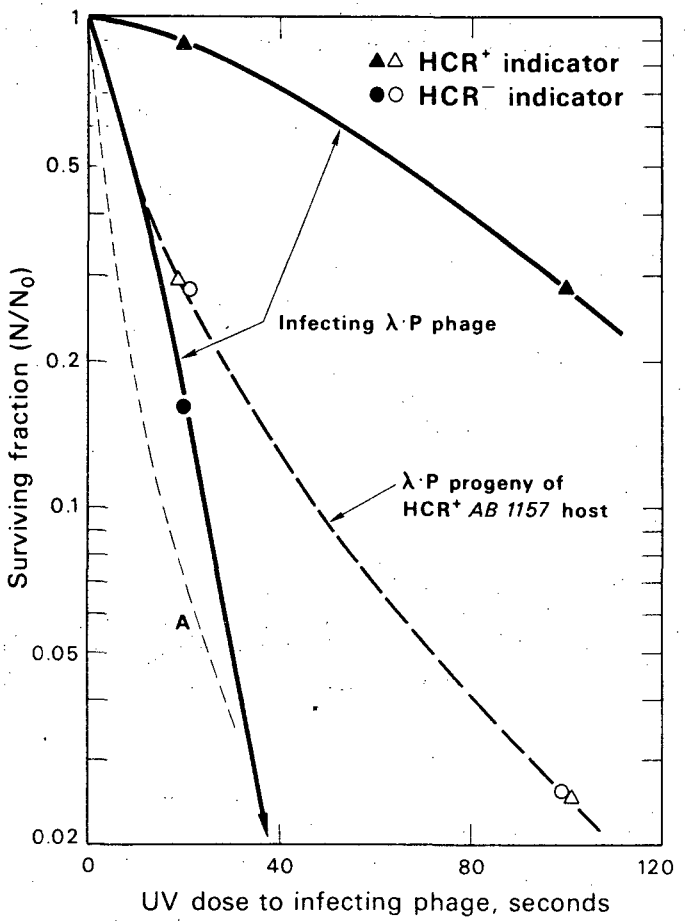
Figure 26 (left). The recovery of HCM-labeled λ·P progeny from crosses of λ·P phages (λc71sus029·P × λc71susP3·P) in the K12 strain AB1886(uvrA), as a function of UV dose to the infecting phages. The open symbols indicate the relative concentration of λ·P progeny phages in the cross lysates (cf., Fig. 21), assayed on the restricting HCR+ indicator AB1157(P1) and on the restricting HCR- indicator AB1886(P1). The UV survivals of the parental phages in single infections of the HCR+ and HCR- indicators are shown for comparison (solid symbols). All these data were obtained using the same titration procedure, with preadsorption of the diluted phage samples to the indicator bacteria.

Figure 27 (right). The recovery of HCM-labeled λ·P progeny phages from crosses of λ·P phages (λc71sus029·P × λc71susP3·P) in the HCR+ K12 strain AB1157(uvr+), presented according to the format of Fig. 26. The dashed line labeled "A" represents the relative position of the curve for recovery (on the HCR+ restricting indicator) of λ·P from the HCR- K12 host AB1886 (uvrA) under comparable experimental conditions.



DBL 696-4731

Figure 26



DBL 696-4732

Figure 27

excision-defective uvrA host bacteria, and which retain potentially lethal UV lesions, are composed mainly of conserved or of semi-conserved parental DNA--and hence whether the UV lesions might induce recombination by simple breakage and reunion of irradiated phage chromosomes prior to their replication; and 2) whether the reduced recovery of HCM-labeled progeny from infections with UV-irradiated parental phages is associated with UV-induced recombination and/or originates in a UV-induced selection against recovery of a particular class of phage chromosomes with parental DNA.

IV-C.1 Relative viability of HCM-labeled parental λ phages recovered from HCR+ and HCR- host bacteria

The data presented in Figures 26 and 27 are replotted in Fig. 28 to illustrate, as a function of UV dose, the ratios of the viable titers on HCR- and HCR+ indicators of the λ·P infecting phages and the λ·P progeny from crosses in the uvrA and uvr+ host strains. The purpose of this plot is to cancel the effect of the UV-induced decrease in absolute recovery of λ·P progeny, as measured on the restricting HCR+ indicator, so that the relative survivals on the HCR- indicator of the infecting phages and the λ·P progeny phages can be compared directly. For simplicity, the ratio of an HCR- titer to the corresponding HCR+ titer--plotted on the ordinate in Fig. 28--will hereafter be referred to as the "viability index," and the curve in Fig. 28 for the infecting phages will sometimes be termed the "reference curve."

For each curve in Fig. 28, the extent of the decrease in viability index with increasing UV dose is a linear measure of the accumulation in the phages of potentially lethal lesions which are reactivable

in the HCR+ indicator bacteria but not in the HCR- indicator. The slope of the curve for the λ·P progeny of the uvr+ host strain is zero; no host cell reactivable lesions remain in the HCM-labeled progeny from this strain. The curve for the λ·P progeny from the uvrA strain has a slight shoulder and lies somewhat above the curve for the infecting phages, but for UV doses from 5 to 20 seconds these two curves fall at approximately the same rate. The results from several different experiments indicate that over the moderate dose range the slope of the curve for λ·P progeny of the uvrA host is at least 80% and probably 100% as great as the slope of the reference curve for the infecting phages. For technical reasons, it was not possible to assay the viability index of the uvrA progeny reliably for higher UV doses.

The data presented in Fig. 28 show that the progeny phages retaining a parental HCM label from multiple infections of the AB1886(uvrA) host contain nearly as many potentially lethal, host cell reactivable UV lesions as the infecting phages, whereas HCM-labeled progeny phages from the AB1157(uvr+) host do not retain any host cell reactivable lesions. The uvrA strain is known to be defective in the excision of UV-induced pyrimidine dimers (105, 23b); the results reported here may be viewed as a direct "biological" demonstration that the differences in UV survival and UV-induced recombination of λ phages in the uvr+ (HCR+) and uvrA (HCR-) hosts are attributable to the failure of the latter strain to eliminate potentially lethal UV-induced lesions in the infecting phage DNA. Furthermore, the fact that the infecting λ·P phages and the λ·P progeny phages from the uvrA host bacteria accumulate potentially lethal UV lesions at about the same rate with increasing UV dose suggests that most of the HCM-labeled progeny from these crosses of UV-irradiated λ phages contain <u>conserved</u> parental chromosomes.

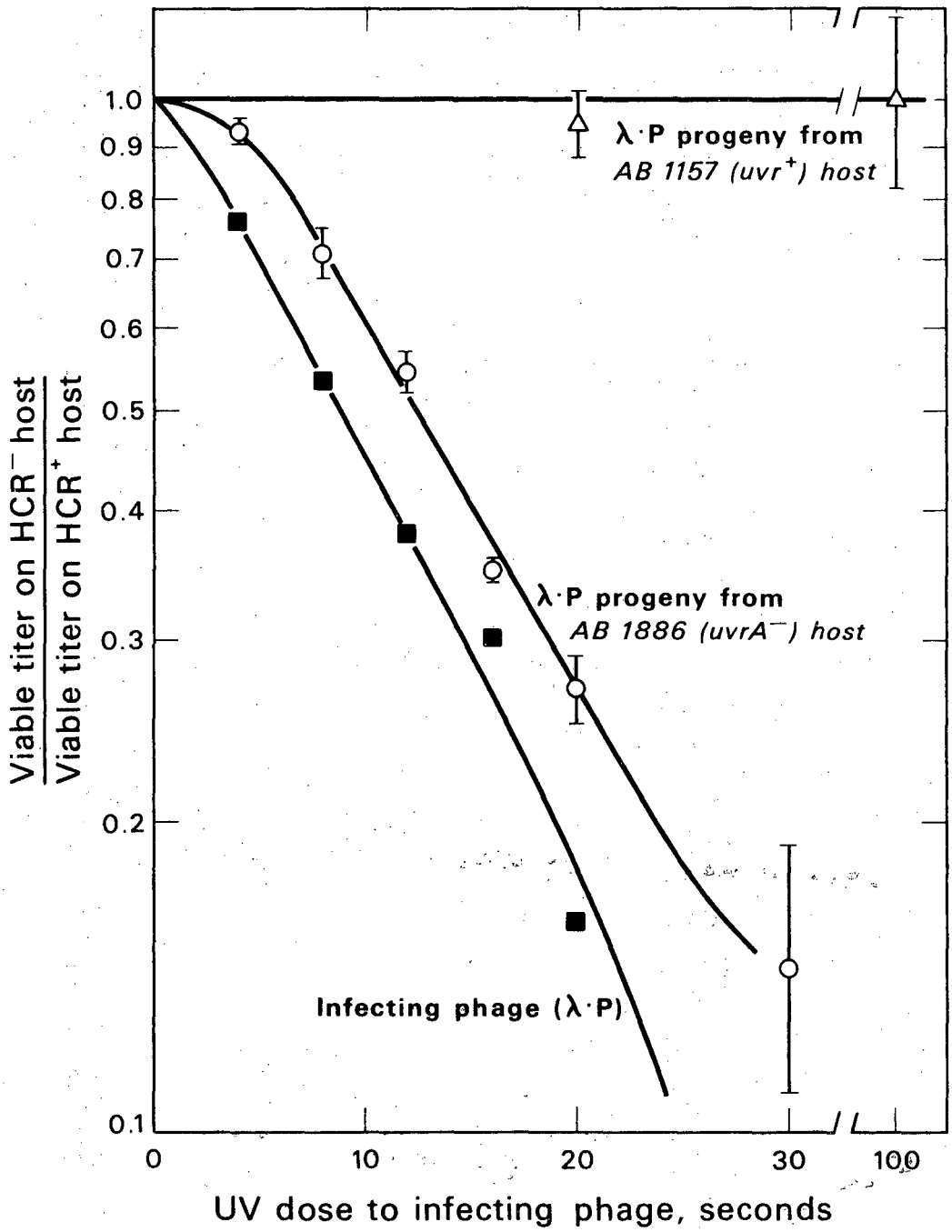


Figure 28. DBL 696-4733

The viabilities on the HCR- indicator relative to those on the HCR+ indicator (i.e., the "viability indices") of the infecting λ·P phages and the λ·P progeny from crosses in the K12 strains AB1886(uvrA) and AB1157(uvr+). The data for infecting phages and progeny from the AB1886 host refer to the same experiment described in Figs. 21, 22, and 26. Brackets denote the range of values determined for duplicate crosses.

Estimation from Fig. 28 of the proportions of conserved and semi-conserved parental DNA contained in the λ·P progeny from the uvrA host hinges upon the relative UV survivals envisioned for intact semi-conserved and conserved parental chromosomes on the HCR- indicator. Inactivating UV damage is now thought to result from unexcised photoproducts (primarily pyrimidine dimers) in the genomes that either block DNA replication or cause defects in the daughter chromosomes that are not healed by post-replication repair processes (88, 193, 102)--and perhaps sometimes from interference between separate repair events or repair modes (85, Appendix III). Any natural mechanisms for bypass and post-replication repair in UV-irradiated λ genomes are presumably intact in the crosses performed here,* so it must be assumed that unexcised UV photoproducts could be present in a parental λ·P DNA strand opposite a newly synthesized and intact complementary strand in a λ·P progeny phage chromosome.

As between conserved and intact semi-conserved parental chromosomes, the probability of inactivation on the HCR- indicator of a λ·P progeny phage chromosome that carries 2N UV photoproducts randomly distributed between the two DNA strands should be at least twice as great as the probability of inactivation of a chromosome with N photoproducts confined in a single strand--particularly for low UV doses where the lesions would rarely be concentrated within the molecule. The viability index of pure semi-conserved λ·P progeny from the uvrA host should then decline with increasing UV dose at a rate at most half that characteristic of the reference index for the UV-irradiated infecting phages. Furthermore, should successful initial replication of a lightly irradiated

---

* See discussion of this point in section III-A.2.

parental genome suffice to insure that further replication of a semi-conserved parental molecule will usually yield at least one viable daughter chromosome--whether because the successfully replicated parental DNA strand contains no unexcised UV photoproducts or because all of the remaining UV lesions can be bypassed--the slope of the curve for λ·P progeny with exclusively semi-conserved parental chromosomes would be zero.*

Some of the λ·P progeny may contain recombinant genomes with segments of conserved and semi-conserved parental DNA in different regions, and these would exhibit an intermediate viability index. But since the observed rate of decline in viability index of λ·P progeny from the uvrA host is about as great as that for the infecting phages over the moderate UV dose range (Fig. 28), it seems clear that the λ·P progeny contain mostly conserved parental DNA.

---

* Unfortunately, there is no direct experimental evidence, concerning the viability of "heteroduplex" λ DNA molecules with UV photoproducts in only one strand, by which to support this reasoning. However, the plausibility of the conclusion may be argued from the facts that the single-stranded DNA phages ∅x174 and S13 are not susceptible to HCR when UV-irradiated as extracellular particles, although when irradiated in their double-stranded replicative form (RF) they are repairable by HCR (182, 249). This finding has been interpreted as indicating that successful excision-repair requires a double-stranded substrate. It probably also reflects that the viability of RF containing UV lesions in only one DNA strand is not significantly affected by excision-repair of those lesions; the facts that UV inactivates ∅x174 viability at a rate fivefold greater than it reduces initial synthesis of RF (151) and that UV enhancement of recombination is observed for these phages (219, 185) suggest that UV photoproducts are successfully bypassed at an appreciable frequency during generation of the initial RF (202) from an irradiated single-stranded chromosome. Perhaps a single undamaged template strand in the initial RF insures that semi-conservative replication will produce at least one viable daughter RF. An analogous situation might well obtain for the double-stranded λ phage, so that the viability of a chromosome containing semi-conserved parental DNA from a UV-irradiated infecting phage would also be little affected by HCR. In the present case, λ·P progeny phages from the uvrA host which contain semi-conserved parental DNA would exhibit viability indices near 1.0--in marked contrast to the experimentally observed result (Fig. 28).

Two factors could contribute to the small shoulder, the fairly uniform lateral displacement, and any slight divergence of the uvrA curve from the reference curve in Fig. 28. First, there is convincing evidence that a small number of initial UV lesions per infected complex can be repaired by residual HCR (80, 84) in excision-defective host bacteria. The results of experiments to be described in sections V-B, D (Figs. 31-b and 33-b), in which residual excision of UV lesions in the phage DNA was competitively inhibited by prior UV-irradiation of the uvrA host cells themselves, imply that about 70% - 80% of the potentially lethal and recombination-promoting lesions induced in phages by a 5-second UV dose are excised from the phages in the multiply infected unirradiated uvrA host bacteria. This extent of repair may represent the maximum capacity of the residual HCR; the corresponding shift in viability indices would account virtually quantitatively for the gap between the uvrA curve and the reference curve in Fig. 28.* Analogous

---

* The displacement of the uvrA curve from the reference curve in Fig. 28 corresponds to excision-repair of the number of initial UV lesions induced by a dose of around 4 seconds, or about 3 pyrimidine dimers (cf., p. 77) per infecting phage chromosome, independent of the dose to the multiply infecting phages in the 5 to 20 second UV dose range. The fairly uniform extent of this "dose-reduction" could reflect a saturation of the residual excision activity of the uvrA host cells or could indicate that this particular uvr- mutation (uvrA6) has an effect upon the excision-repair function that mimics non-competitive, rather than competitive, inhibition of HCR.

UV-irradiation of the uvrA host bacteria does cause a slight shift in the viability indices of the λ·P progeny which corresponds roughly to the gap in Fig. 28 between the uvrA and reference curves. In a separate experiment from that described in Fig. 28, λ·P progeny from multiple infections of uvrA cells with λ·P phages UV-irradiated for 16 seconds were found to exhibit a viability index of 0.26 ± 0.01 when the host cells had themselves been UV-irradiated for 210 seconds (about 2100 ergs/mm^2), compared to an index of 0.41 ± 0.06 for progeny from unirradiated cells. However, this particular effect, although necessary, is not sufficient evidence in itself for attributing to residual HCR the enhanced viability indices of λ·P progeny from the uvrA host bacteria; UV-irradiation of the host bacteria not only inhibits HCR but also evidently blocks the replication of some infecting phages (sections V-B, C) and so would also reduce the relative frequency of HCM-labeled progeny phages containing semi-conserved parental DNA.

results have been reported by Harm (80) for the UV survival of λ phages in single infection of the "K12S hcr-" strain. He found that the UV survival curves of λ phages on unirradiated indicator cells and on indicator bacteria heavily UV-irradiated themselves lie in relative positions analogous to those of the uvrA curve and the reference curve, respectively, in Fig. 28.

Second, of course, a subpopulation of λ·P progeny phages that contain semi-conserved parental DNA would also contribute to enhanced viability indices. However, because of the magnitude of the effect attributable to residual HCR and the fact that the slopes of the uvrA and reference curves are quite similar over the moderate UV dose range, the amount of semi-conserved parental DNA in the progeny λ·P genomes must either be small or diminish with increasing UV dose to the infecting phages. For crosses in the uvrA host of phages UV-irradiated for 20 seconds, a reasonable estimate is that the HCM-labeled progeny phages which plate on an HCR+ indicator strain contain no more than 20%--and conceivably not any--semi-conserved parental DNA.

Straightforward experiments by several other investigators (8, 157, 120, 128)--with procedures and multiplicities of infection comparable to those for the work reported here--clearly indicate that from infections with unirradiated λ phages about 70% - 90% of the progeny that retain a parental HCM label contain semi-conserved parental chromosomes. In those studies, host bacteria were infected with phages that carried both a density and an HCM label, and the density distribution of the HCM-labeled progeny population was determined; it would be of interest to ascertain quantitatively by this method the association of

a parental HCM label with conserved and semi-conserved parental DNA as a function of UV dose to the multiply infecting phages. If the λ•P progeny from crosses of UV-irradiated λ•P in the AB1886(uvrA) host contain mainly conserved parental DNA, as suggested by the foregoing analysis of the data in Fig. 28, there is evidently a selection with increasing UV dose against the appearance of semi-conserved parental DNA in progeny phage particles that retain the parental HCM specificity.

A decrease in the relative frequency of semi-conserved parental chromosomes would be expected, nay predicted, if the significant factor in the lethality of UV-induced photoproducts in λ were their potential to irreversibly block the complete replication of the irradiated infecting phage genome. If, on the other hand, there exists an efficient mechanism for replication-bypass of UV-induced photoproducts in λ phage DNA (with any necessary post-replication repair of defects in the daughter chromosomes), a selection against recovery of semi-conserved HCM-labeled parental DNA could also reflect a preferential destruction within the host cell--by nuclease action or by UV-induced recombination--of replicated infecting genomes or of their parental HCM specificity. The characteristics of the decrease in total recovery of λ•P progeny from the crosses of UV-irradiated phages are also consistent with an elimination of semi-conserved parental chromosomes from the HCM-labeled progeny population; the matter is considered further from that standpoint in the following section (IV-C.2).

For crosses in the AB1886(uvrA) host of λ•P phages UV-irradiated with doses up to at least 12 seconds, the enhancement of recombination frequency among the λ•P progeny corresponds quantitatively to that

observed among the overall progeny population (section IV-B, Fig. 22). The HCM-labeled progeny from these crosses appear to contain mostly conserved parental DNA; if some of the UV-induced recombinant λ·P progeny contain only conserved parental DNA, it would follow that the UV-stimulated recombination can be induced and executed between unreplicated irradiated phage genomes.

On the basis of the evidence considered above, one cannot categorically exclude the possibility that all of the recombinant λ·P progeny induced by UV do contain at least some semi-conserved parental DNA, but comprise a sufficiently small or sufficiently inviable subpopulation of the λ·P progeny that they do not markedly influence the values of the viability indices noted in Fig. 28. Such an interpretation would be required, for example, by the application to this experimental system of the hypothesis that UV-stimulated recombination in bacteria might be primed by defects resulting from replication-bypass of UV photoproducts and does not occur between two unreplicated irradiated genomes (209, 240). However, in light of all available evidence (see sections I-B, III-A.2, IV-D, VI-C), such an interpretation appears to be unwarranted. The simplest possible conclusion consistent with the results presented above is also probably the correct one: UV-induced recombination in λ can occur by straightforward breakage and reunion of the irradiated infecting phage chromosomes prior to their replication.*

---

* This conclusion might be tested by the following experiment.

Immediately after infection of host cells with UV-irradiated λ phages in a genetic cross, the infected complexes could be held for a period in the presence of KCN in order to inhibit replication of the infecting phage chromosomes, then submitted to an optimal dose of photoreactivating light in order to remove recombination-promoting UV lesions, and finally incubated in the absence of KCN to lysis. Comparison of the recombination frequency observed among the progeny phages with those

IV-C.2 UV-induced decrease in the recovery of parental HCM-labeled phages

From the evidence obtained in the present experiments it is not possible to conclusively identify the causes of the reduced total recovery of λ•P progeny from crosses of UV-irradiated λ•P phages in an *E. coli* K12 host (Figs. 26 and 27). However, it seems likely that at least a component of the decrease in λ•P recovery is directly associated with the degradation which has been observed by others (127, 50, 183) of UV-irradiated λ phage DNA within the host cells. The data are consistent with the notion that the reduced λ•P recovery corresponds in part to the virtual elimination of semi-conserved λ•P parental chromosomes from the HCM-labeled progeny population. The observations discussed below suggest that this effect is neither trivial nor simple and that it might be more pertinent to an understanding of the UV inactivation of λ phage viability (*vide infra*, Appendix III)than to characterization of the UV-induced phage recombination.

No significant component of the decreases in recovery of HCM-labeled progeny, as measured on the HCR+ indicator strain in Fig. 26 and on both the HCR+ and HCR- indicators in Fig. 27, could be ascribed to originally induced UV photoproducts that are retained in the DNA of the

---

observed in the appropriate controls would indicate whether UV-stimulated recombination can enter an irreversible phase in the absence of an energy source and without replication of the infecting genomes. If this experiment were carried out utilizing an excision-defective host strain and HCM-labeled parental phages, increased resolution of UV-stimulated recombination could be obtained by considering the relative enhancements of recombination frequencies among HCM-labeled progeny phages, and the effectiveness of the photoreactivation could be monitored by examining the viability indices of the HCM-labeled progeny phages. A preliminary attempt to perform such an experiment in this laboratory was unsuccessful, apparently because of interference by KCN with phage injection.

λ·P progeny phage particles. Such potentially inactivating UV lesions in the λ·P progeny of the uvrA host would be completely repaired in the HCR+ indicator, since in the relevant UV dose range the survival on the HCR+ indicator of the infecting λ·P phages is essentially 100% (Fig. 26). The λ·P progeny from crosses in the uvr+ host do not retain host cell-reactivable UV lesions even at a UV dose which inactivates an appreciable fraction of the parental phages in single infection (Fig. 27). The assayed recoveries of λ·P progeny on the HCR+ restricting indicator are thus reliable indices of the decreases, with increasing UV doses to the infecting phages, in the yields of all intact HCM-labeled progeny phages from the crosses.

For crosses of the more heavily irradiated infecting phages, the relative frequency of λ·P phages among the progeny falls well below both the levels of conserved parental genomes usually recovered from multiple infections and of semi-conserved HCM-labeled genomes usually recovered from single infections with unirradiated phages (8, 9, 157, 120), which would both correspond to surviving fractions on the order of 0.1 to 0.3 in Figs. 26 and 27. This fact implies that there is a reduced recovery of both conserved and semi-conserved parental HCM-labeled DNA with increasing UV dose.

The decreased recovery of λ·P progeny is found for crosses of UV-irradiated phages in both HCR+ and HCR- host strains, but the available data suggest that the reduction in λ·P recovery may not be quite the same function of UV survival of the infecting phages in the two hosts. The rate of decline in λ·P recovery with increasing UV dose seems to be about threefold greater for infections of the uvrA strain than of the uvr+ host (Fig. 27), whereas the viability of the infecting

phages is fivefold to sixfold more sensitive to UV for single infection of the uvrA strain than of the uvr+ host (Figs. 27, 5, 6). That excision-repair of the infecting phage chromosomes does not contribute significantly to destruction of the HCM label is confirmed by control experiments, which show the UV-sensitivity of λ·P phages to be essentially the same on restricting and non-restricting HCR+, as well as HCR-, indicators (Table VI); but the excision activity may render the parental DNA or its HCM specificity more susceptible to destruction by another process or in semi-conserved parental DNA.

Other investigators have reported a rapid degradation of UV-irradiated λ phage DNA within E. coli K12 host bacteria (127, 50, 183). The observation that for a given UV dose the degradation of isotope-labeled UV-irradiated λ DNA to acid soluble material is half as extensive in an HCR- host strain as in HCR+ host bacteria suggests that much of the breakdown of UV-irradiated DNA may be independent of excision-repair processes (50, 183). It is reasonable to presume that the UV-induced decrease in λ·P recovery is due in part to the digestion by cellular nucleases of UV-irradiated parental phage DNA fragmented in UV-induced recombination or rendered inviable in abortive replication or repair events. There is probably little indiscriminate digestion of infecting phage DNA (vide infra).

The dose-response curves that describe the UV-induced decrease in overall recovery of λ·P progeny (Figs. 26, 27) are concave upward. These kinetics do not exclude the possibility that the reduced recovery of HCM-labeled progeny phages is for the most part a consequence of UV-induced recombination, exponentially dependent upon the number of genetic exchanges. When plotted against the square root of the UV dose to the

UV dose to infecting λ·P (sec)	Surviving Fraction of Phage ($N/N_{D=0}$) (single infection)	
	HCR- Indicator Bacteria	
	AB1886	AB1886(P1)
0	1.0	1.0
20	0.16	0.21
30	0.017	0.024
40	0.0036	0.0045
50	0.0003	0.0004
	HCR+ Indicator Bacteria	
	AB1157	AB1157(P1)
0	1.0	1.0
40	0.56	0.54
120	0.11	0.15
160	0.034	0.061
240	0.008	0.008

Table VI - The UV survivals of λc71sus029·P phage in single infection of non-restricting and restricting AB1886(uvrA) and AB1157(uvr+) host bacteria. Each of the indicated surviving fractions is based upon a single assay. From the fact that the phage survivals on the HCR+ AB1157 and AB1157(P1) strains are essentially the same it may be concluded that excision-repair of the infecting λ·P phage does not significantly affect its HCM specificity.

infecting phages the inactivation of λ·P recovery from the uvrA host is virtually exponential (with a slight shoulder at very low UV dose), while the UV-induced increase in recombination of parental phage chromosomes in the uvrA host is approximately linear with the square root of the dose (Fig. 30-section IV-D below). On the other hand, the concave shape of the dose-response curve for the reduction in λ·P recovery could simply reflect a population heterogeneity among the host bacteria or among the λ·P genomes, quite possibly a difference in the susceptibility of diverse λ·P chromosomes within the same host cell to the inactivating factor(s). This latter interpretation is strongly supported by the apparent selection, with increasing UV dose to the infecting phages, against recovery of HCM-labeled progeny phages with semi-conserved parental DNA (section IV-C.1 above).

In evaluating the possible causes of the UV-induced decrease in absolute recovery of HCM-labeled progeny from crosses of UV-irradiated λ phages one must weigh an interlocking series of alternative factors: destruction of the HCM specificity of parental phage DNA vs. fragmentation and nuclease digestion of the HCM-labeled DNA itself, loss of semi-conserved parental DNA vs. loss of conserved parental genomes, destruction of the HCM-labeled progeny as a consequence of UV-inactivation or repair processes vs. loss of the HCM-labeled chromosomes as a result of UV-induced recombination...While at present there is insufficient supporting evidence to justify a firm conclusion, the author is inclined to the view that the primary inactivation of λ·P recovery which is effected for quite low UV doses to the infecting phages is due to a nuclease degradation of HCM-labeled semi-conserved parental DNA (keyed by defects resulting from replication-bypass of UV photoproducts) and

is not associated with UV-induced recombination. The secondary, seemingly exponential, decrease in λ•P recovery observed for further UV exposure of the infecting phages may reflect the loss of parental HCM-labeled chromosomes in UV-induced non-reciprocal recombinations or in matings with newly synthesized λ•K chromosomes. (Also, with increasing UV inactivation of the infecting phages an increasing proportion of parental phage genomes in the multiple infections may be replicated, rendering an increasing fraction of the HCM-labeled DNA vulnerable to degradation in semi-conserved parental chromosomes.)

The remainder of the present section and Appendix III are devoted to exposition of reasoning which underlies this view, its implications relevant to the nature of UV-induced recombination and a conceivable mechanism for UV-inactivation of λ phages.

It is possible that the reduced recovery of HCM-labeled progeny from crosses of UV-irradiated phages is associated to some extent with the UV-stimulated recombination of the infecting λ•P phage genomes. Some perspective on this point is provided by the data in Table VII, which indicate that for a given UV dose to the infecting phages the relative recovery of λ•P progeny phages is quite similar from crosses in the uvrA host of equal numbers of λ•K and λ•P parental phages and from crosses in which all parental phages carry the type P HCM label. When considered in conjunction with the requirements envisioned for retention of the HCM label (section IV-A.3) and the λ•P recombination frequencies shown in Fig. 22 from crosses of λ•P with λ•P and with λ•K phages (for the sus029 and susP3 markers), the results in Table VII suggest two conclusions.

First, it seems likely that a sizable fraction of the λ•P parental genomes do not engage in the recombination induced <u>among</u> the

UV dose to infecting phages (seconds)	Relative titer of λ·P progeny ($N/N_{D=0}$) (from AB1886 host, MOI = 10)	
	λ·P x λ·P	λ·K x λ·P
0	1.0	1.0
8	0.29 ± 0.02	0.25 ± 0.05
12	0.18 ± 0.01	0.15 ± 0.02
20	0.070 ± 0.005	0.067 ± 0.008
30	0.024 ± 0.002	0.029 ± 0.001

Table VII - The relative recoveries of HCM-labeled λ·P progeny phages from crosses in the K12 host AB1886(uvrA) of UV-irradiated λc71susQ73·P and λc71susR5·P (center column) and of λc71susQ73·K and λc71susR5·P (right column), as assayed on the HCR+ restricting indicator. All irradiations, crosses, and titrations were performed at the same times under identical conditions; the indicated errors refer to the range of values assayed from duplicate crosses.

irradiated infecting phages. In the 0 to 20 second UV dose range, the additional decrease in overall λ·P recovery observed when but one of the infecting phage types carries the λ·P specificity is very small (Table VII); there is a greater relative decrease in the recovery of recombinant λ·P progeny, which is reflected in the reductions of 30% - 60% in the frequencies of HCM-labeled progeny recombinant in the sus029-susP3 interval (Fig. 22).* Thus a significant portion of the λ·P chromosome population within the host cell is probably not vulnerable to destruction of the HCM specificity in UV-induced recombination between the conserved and/or semi-conserved parental genomes.

It is noteworthy that over the low UV dose range, for which the total recovery of λ·P progeny phages declines sharply (Fig. 26), there is no evidence of any significant preferential loss of intact λ·P chromosomes that are genetically recombinant. For the crosses of λsus029·P with λsusP3·P phages UV-irradiated for up to 12 seconds there are similar increments in the total and λ·P recombination frequencies (Fig. 22), according to the simplest expectation if virtually all the UV-stimulated recombination were attributable to breakage and reunion of parental phage chromosomes early in the infection. Hence the UV-induced recombination between conserved and/or semi-conserved parental phage genomes does not lead to a reduced recovery of λ·P progeny due to destruction of the λ·P specificity (e.g., by any replacement of critical

---

* Analogous reductions in the λ·P recombination frequencies, compared to those observed when both parental phage types carry the type P HCM label (Figs. 23, 24), were found for crosses of UV-irradiated λsusP3·P with λsusQ73·K and λsusQ73·K with λsusR5·P. The latter result confirms that the difference in the relative decreases in recovery of total λ·P and of λ·P recombinant in the sus029-susP3 interval when but one of the infecting phage types is HCM-labeled could not be accounted for as due simply to retention of the type P HCM specificity by phages recombinant only toward the extremities of the chromosome (cf., section IV-A.3 and Appendix II).

HCM sites in the parental DNA strands during tailoring and repair of the recombinant structures--cf., section I-B.3). On the other hand, the decrease in the λ·P recombination frequency at higher UV doses in Fig. 22 may reflect a preferential loss of HCM-labeled phages that are recombinant, perhaps as a result of further recombination with newly synthesized λ·K phages (vide infra).

Second, in order to reconcile the various observations cited above it is necessary to suppose that most of the UV-stimulated recombinations among the irradiated parental phage genomes occur in the course of single mating events. From Fig. 22, one can estimate that the λ·P progeny recovered from a cross of λ·P phages UV-irradiated for 12 seconds contain an average of around four points of recombination each.* Yet the recovery of HCM-labeled recombinant progeny is reduced by only around 50% if one of the irradiated parental phage types carries the λ·K specificity rather than the λ·P HCM label, despite the fact that single recombinants of λ·P and λ·K phages at a locus toward the interior of the chromosome cannot retain the λ·P HCM specificity. This result would be difficult to understand if single exchanges among the parental phages occurred in random and successive pairwise mating events. However, if multiple exchanges--perhaps clustered somewhat within the chromosomes--proceeded in a single UV-induced mating, recoveries of λ·P

---

* For a 12-second UV dose to the infecting phages, the λ·P recombination frequency observed for crosses of λsus029·P with λsusP3·P in the AB1886 host strain was about 3% (Fig. 22). Taking into account both the λsus+·P and the reciprocal λsus029susP3·P recombinant genotypes, about 6% of the λ·P progeny from these crosses were genetically recombinant in the sus029-susP3 interval, which can be estimated to comprise about 3% of the genetic map (Fig. 4) (2, 120, 235). Allowing also for incestuous matings, the average λ·P progeny phage thus contained about four sites of physical recombination over the entire length of the chromosome.

recombinant and non-recombinant progeny such as those indicated in Fig. 22 and Table VII would be possible and even consistent with involvement of a majority of the λ·P parental phage genomes in the UV-stimulated recombination. This interpretation accords with the suggestion of Jacob and Wollman (115), on the basis of genetic evidence from three factor genetic crosses of irradiated λ(·K) phages, that a great majority of UV-induced double recombinants are formed in single mating events.

In the context of these observations, there are two ways in which destruction of the parental HCM label or of parental genomes might be a straightforward consequence of UV-stimulated recombination of the conserved and/or semi-conserved infecting phage chromosomes:

(a) Non-reciprocal recombination would lead to loss of parental HCM-labeled phage genomes. Reciprocal recombinant genotypes are not recovered with equal frequency among the progeny from single host bacteria in crosses of either unirradiated or UV-irradiated λ phages (122, 115), but it is not clear whether this fact reflects a significant probability that only one intact, recombinant molecule issues from a mating of two phage genomes or the role of chance in fixing the relative frequencies of replication and maturation of the reciprocal recombinants within the host cell (cf., 207, 87).* UV-induced non-reciprocal recombination by breakage and reunion could lead to fragmentation, and probably subsequent nuclease digestion, of some of the

---

* Curtiss (41) was unable to detect reciprocal products of UV-stimulated recombination between an exogenote and endogenote in partially diploid E. coli bacteria, but Meselson (159) has observed reciprocal recombination between prophage genes in unirradiated partially diploid bacteria, and Roman (180) has reported that UV-stimulated mitotic recombination in yeast is reciprocal.

mating λ phage chromosomes; the prospects that HCM-labeled phage genomes would survive intact to be replicated and/or included among the viable progeny of the infection would dim as the extent of their involvement in UV-stimulated recombination increases.

A high probability of non-reciprocal recombination by breakage and reunion does not seem compatible with other characteristics inferred for the UV-induced phage recombination, such as frequent formation of λ·P recombinants of irradiated λ·P and λ·K parental phages by multiple recombinations in a single mating and efficient generation of intact, highly recombinant genomes in multiplicity reactivation. Nor is it necessary to postulate non-reciprocal recombination in order to rationalize the UV-induced decrease in λ·P recovery. There is no direct evidence regarding the extent to which the UV-induced recombination among λ phages may be reciprocal or non-reciprocal, however, and it must be borne in mind that loss of intact parental genomes due to a significant probability of non-reciprocal recombination could contribute to the reduced recovery of HCM-labeled progeny.

(b) As discussed at length above, recombination between parental λ·P genomes and newly synthesized λ·K chromosomes usually results in destruction of the type P HCM specificity. In fact, it can be calculated that recombination between λ·P and λ·K genomes could account quantitatively for the fact that only a few progeny phages per cell retain the parental HCM specificity from multiple infections of *E. coli* K12 bacteria with unirradiated λ·P phages. With increasing UV dose to the infecting phages, the HCM specificity of an increasing fraction of conserved and semi-conserved parental chromosomes containing recombinagenic UV lesions would be lost in recombination with newly synthesized genomes, as the number of

such matings increases and the polarity of the recombination shifts further toward destruction of the HCM label (cf., section IV-B).

The most tangible indication that recombination between parental and newly synthesized phage genomes may not be the principal cause of the observed decrease in λ·P recovery comes from the observation that, relative to the survival of the infecting phages, the loss of HCM-labeled progeny is even greater in the uvr+ host than in the uvrA strain (Fig. 27). In the uvr+ bacteria, the recombination-promoting lesions in the parental phage DNA are subject to continuing excision-repair throughout the latent period, and the λ·P progeny that are recovered contain no host cell reactivable lesions (Fig. 28). In the uvrA host bacteria, by contrast, UV-induced lesions are retained in the parental phage DNA; UV-stimulated recombination between parental and newly synthesized chromosomes can proceed throughout the phage infection, and is probably reflected explicitly in the secondary increase in overall recombination frequency at high UV doses to the infecting phages (Fig. 10, section III-A.1). One might expect a decrease in λ·P recovery due to UV-induced recombination between parental and newly synthesized genomes to be greater, relative to the UV survival of the infecting phages, for crosses in the uvrA host than in the uvr+ strain, and perhaps more pronounced at high than at low UV doses. Such an effect could only be a secondary factor in the results shown in Figs. 26 and 27 if the primary causes of the reduced recoveries of HCM-labeled progeny from the uvr+ and uvrA hosts are presumed to be the same.

The results discussed in section IV-C.1 (Fig. 28) strongly suggest that the HCM-labeled progeny from crosses of UV-irradiated λ·P

phages in the uvrA host contain mainly conserved parental DNA, from which one might infer that there is a selection with increasing UV dose to the infecting phages against recovery of HCM-labeled progeny phages with semi-conserved parental DNA. The marked decrease in recovery of λ·P progeny phages which is observed for low UV doses to the infecting phages (Figs. 26, 27) could be due to an elimination from the HCM-labeled progeny populations of chromosomes with semi-conserved parental DNA. The more gradual, seemingly exponential, decrease in λ·P recovery that occurs with further UV exposure of the infecting phages (Fig. 26) could indicate a relatively lower susceptibility of conserved parental genomes to the processes that cause loss of the semi-conserved λ·P DNA or a secondary source(s) of inactivation that does affect HCM-labeled molecules with conserved parental DNA.

According to this interpretation, the curve in Fig. 26 for recovery (on the HCR+ indicator) of λ·P progeny from crosses in the uvrA host would imply that the selection against semi-conserved parental genomes in the λ·P progeny populations is fully effected for UV doses of about 10 seconds or more to the infecting phages. The selection against semi-conserved parental DNA might not be complete, but the viability indices of the HCM-labeled progeny support the view that for UV doses of about 5 seconds or more these phages do contain mostly conserved parental DNA (section IV-C.1). The approximately linear portion of the curve in Fig. 26 for λ·P recovery on the HCR+ indicator, for doses from 12 to 30 seconds, extrapolates back to intercept the ordinate at a surviving fraction of about 0.3 (as does the linear portion of the curve for recovery on the HCR- indicator if allowance is made for the enhanced viability of the progeny thought to result from residual HCR).

This value might be taken as a maximum estimate of the fraction of λ•P progeny from the crosses of unirradiated phages which contain conserved parental chromosomes; it compares well with the values of 0.1 - 0.3 indicated for this parameter by the direct experiments with unirradiated, density-labeled λ phages performed elsewhere (8, 157, 120, 136--cf., p. 88 above).

A selection against HCM-labeled semi-conserved parental DNA in progeny from crosses of UV-irradiated λ phages might be caused by: a) a reduced formation of semi-conserved parental genomes; b) a preferential degradation of semi-conserved parental DNA; and/or c) a preferential destruction of the HCM specificity of semi-conserved parental chromosomes. It is worthwhile to examine these possibilities.

If a major factor in the lethality of UV photoproducts is their potential to irreversibly block the complete replication of the infecting chromosome, with increasing UV dose to the parental phages fewer semi-conserved parental genomes would be generated per viable multiply-infected complex. However, it is not certain that the sharp decrease in the recovery of λ•P progeny for low UV doses could be totally explained on this basis. Until recently it was thought that a few, perhaps but one, unrepaired photoproducts in the irradiated chromosome might be able to efficiently block DNA replication (88, 214). Because of the recognition of frequent replication-bypass of UV photoproducts in the chromosomes of (singly mutant) excision-defective E. coli strains (184, 26, 109), this notion is no longer tenable with reference to the UV-inactivation of bacteria. A replication-bypass of UV-induced photoproducts in λ phage DNA may also be possible, but at present there is only indirect evidence to support this view (section III-A.2). This question is crucial,

because the inactivation of λ·P recovery from the uvrA host observed for low UV doses may be caused by just a single unexcised pyrimidine dimer per infecting λ·P chromosome.* Obviously, such an efficient suppression of the recovery of semi-conserved parental DNA could be reasonably ascribed to UV-induced blocks to replication only if the potentially lethal photoproducts in the phage DNA cannot be bypassed at an appreciable frequency.

Note also that for low UV doses the relative recovery of λ·P progeny falls substantially below the UV survival of the parental phages, which defines the minimum fraction of infecting phages that can complete replication in single infections, for both the uvrA and the uvr+ bacterial host strains (Figs. 26 and 27). It is conceivable that a smaller proportion of the parental phages may be capable of completing the initial replication in the multiply infected cells than in singly infected cells because of a competitive inhibition of excision-repair, but one might expect that the limited residual HCR activity in the uvrA strain would be more susceptible to competitive inhibition in this dose range than the wild type HCR activity in the uvr+ host. Alternatively, some factor in addition to UV-induced blocks to replication might be involved in a sharply reduced recovery of semi-conserved HCM-labeled progeny phages.

---

* A UV dose of 5 seconds to the infecting λ·P phages reduces the relative recovery of all λ·P progeny from the multiply infected uvrA host bacteria by about a natural logarithm (Fig. 26, see also Table VII). This dose induces an estimated 4 pyrimidine dimers in each parental phage chromosome (see p. 77), of which 70%-80% can eventually be repaired by residual HCR activity (sections IV-C.1, V-B, D). The sharp inactivation of λ·P recovery at low UV doses might thus be effected with a D_{37} of around one unexcised pyrimidine dimer per λ·P chromosome.

If replication-bypass of UV-induced photoproducts in the infecting λ phage chromosomes does occur at a significant frequency, the most distinctive physical feature of the daughter chromosomes which could serve as a basis for selection against semi-conserved parental DNA would be the presence of unexcised photoproducts in the parental DNA strand apposed to abnormalities caused by replication-bypass in the newly synthesized strand. Following the hypothesis of Rupp and Howard-Flanders concerning the replication of bacterial DNA in UV-irradiated uvr- strains (184, 109), one may suppose that bypass of the unexcised UV lesions in the λ phage chromosome results in gaps in the newly synthesized DNA strands opposite the pyrimidine dimers in the template strand, necessitating a post-replication repair of the defective regions in the daughter chromosomes. A UV-induced decrease in λ·P recovery could follow from replication-bypass and/or post-replication repair in the irradiated parental phage DNA.

Howard-Flanders et al. have also proposed that post-replication repair of bypass-induced defects in the replication products of UV-irradiated parental chromosomes might proceed by "sister-strand" exchanges among the daughter chromosomes, stimulated by the bypass-induced defects themselves (184, 109, 240). According to that hypothesis, since UV-induced inter-molecular recombination and post-replication repair would likely be manifestations of the same processes, a decreased recovery of HCM-labeled semi-conserved parental chromosomes might be attributed directly to UV-stimulated exchanges which are non-reciprocal or which occur between a molecule with semi-conserved parental DNA and a molecule composed of newly synthesized DNA--as discussed above.

If the notion of post-replication repair by a strictly recombinational process is discounted, one may conjecture that a preferential loss of HCM-labeled semi-conserved parental DNA might follow from a competition between repair processes and degradative nucleases at the sites of double-strand defects resulting from bypass in the replicated irradiated DNA. This explanation is preferred here. It is enticingly consistent with an hypothesis that would relate a UV-induced selection against the recovery of semi-conserved parental DNA to the inactivation of phage viability that can be partially reversed in the phenomenon of UV-reactivation (230, 183) (where the survival of UV-irradiated λ phages is enhanced within lightly UV-irradiated host bacteria) while specifying the nature of a major UV-induced lethal defect in semi-conserved irradiated DNA. This hypothesis is outlined in Appendix III.

IV-D. The Kinetics of UV Enhancement of λ Phage Recombination

In order to understand the nature of the stimulus which UV-induced lesions provide to genetic recombination, it is worthwhile to examine whether the UV enhancement of recombination in various intervals of the chromosome is a function of the physical or of the genetic length of those intervals and to consider the closely related question of whether the recombinagenic effect of the UV lesions is localized or diffused within the DNA molecule. The experimental observations reported above include assays of the relative UV enhancements of phage recombination frequencies in intervals of the λ chromosome that differ significantly in the ratios of their physical to their genetic length; the results indicate that recombination is stimulated according to the genetic rather than the physical length of each interval. The data also suggest that with increasing UV dose to the infecting phages the likelihood of UV-induced recombination in any particular interval increases more slowly than the number of functional recombination-promoting UV lesions in the phage DNA, as might be expected if the recombinagenic effect of the UV lesions is diffused along the irradiated DNA. These results are consistent with the view that the UV lesions simply facilitate the natural process of mating among DNA molecules rather than providing a singular stimulus to the initiation of a recombination event. They are perhaps best discussed in the context of an explicit statement of the dose-response kinetics of the UV enhancement of λ phage recombination.

The "classically" described kinetics for UV stimulation of overall λ phage recombination frequencies (115) are exemplified by the

curves in Figs. 7 and 8 for crosses of UV-irradiated phages infecting the AB1157(uvr+) host strain. The UV-induced increases in recombination frequency seem to be approximately linearly dependent on both the natural recombination frequency among unirradiated phages and the UV dose to the infecting phages, until a plateau of maximally enhanced recombination is attained at a dose which inactivates about 90% of the phages in single infection. (Following Jacob and Wollman (115), this dose will be referred to as the "optimal dose"--d_{opt}.) These kinetics can be qualitatively described by the discontinuous equations:

$$\nu_T = \nu_o + \nu_d = \nu_o + K\nu_o d \ , \qquad d \leq d_{opt} \ , \tag{1a}$$

$$= \nu_o + \nu_{d,max} = \nu_o + K'\nu_o \ , \qquad d > d_{opt} \ , \tag{1b}$$

where d = the UV dose to the infecting phages, ν_T = the recombination frequency observed among all progeny for any two markers x and y, ν_o = the natural recombination frequency for the markers x and y in crosses of unirradiated phages, ν_d = the increment in the total observed recombination frequency that is attributable to recombination induced by the UV dose d to the infecting phages, and K and $K'=Kd_{opt}$ are constants defining the slope of the linear dose-response and the maximum factor of enhancement, respectively. (It is assumed here that ν_o is sufficiently small that ν_d is not limited by attainment of genetic equilibrium--ν=0.5--for UV doses less than d_{opt}.) From Fig. 8 it is clear that equation (1b) does not hold for the enhancement of phage recombination in the excision-defective HCR- host strains, presumably because of a supplementary UV-stimulation of recombination between unrepaired parental DNA and newly synthesized phage chromosomes which would become significant in these hosts at higher UV doses. The

following discussion is principally concerned with the relations implied in equation (1a).

The Visconti-Delbruck theory of natural recombination kinetics among bacteriophages (227, 206) relates the observed recombination frequency for two genetic markers x and y to the probability of genetic recombination in the x - y interval, P(R), by an equation of the form:

$$\nu = K''(1 - e^{-P(R)}) \cong K''P(R) \quad \text{for } P(R) \ll 1.^{*} \tag{2}$$

It seems reasonable to apply this general relation to the UV-induced phage recombination. Then for low UV doses and low recombination frequencies, the relations implied in equation (1a) may be recast as:

$$\nu_T = K''\ P_o(R) + K''\ P_d(R) = K''\ P_o(R) + K\ K''\ d\ P_o(R).$$

In particular,

$$\nu_d = K''\ P_d(R) = K''K\ \ d\ \ P_o(R)\ , \qquad d < d_{opt}\ \&\ P(R) \ll 1, \tag{3}$$

---

* This relation supposes equal input of the parental genotypes, recombination between paired phage chromosomes random with respect to time and partners, and takes into account the effect of multiple crossovers on the recombination frequency. The constant K'' takes on a value of $\frac{1}{2}$ for reciprocal recombination and $\frac{1}{4}$ for non-reciprocal recombination; the function P(R) is resolved as $P(R) = p_{xy}m$, where p_{xy} = the probability of natural recombination between the x and y loci per mating, and m = the number of matings in the lineage of the average progeny phage genome (227, 206). These assumptions are consistent with the utilization of equation (2) for a description of UV-induced recombination, but the Visconti-Delbruck parameters are not directly relevant to the questions of primary interest in this section.

Because of the experimental error in determination of recombination frequencies, the approximation $\nu = K''\ P_o(R)$ is acceptable in practice for frequencies up to at least 0.10 (for which the imprecision is 10%).

which is simply an explicit statement of the intuition that the probability per progeny phage of a UV-stimulated genetic recombination between the x and y loci seems to vary directly as the probability of natural recombination in that interval and the UV dose to the infecting phages. These are the dependences to be scrutinized.

Over most of the λ chromosome, there is an approximately constant relation of the genetic map distances between various loci to the corresponding physical distances along the molecule (120). Although the probabilities of observed genetic recombination in intervals of differing length are not directly proportional to the genetic map distances (2, 206), the rough approximation $P_o(R_{x-y}) \alpha D_{x-y}$, where D_{x-y} is a measure of the physical length of the x - y interval, is adequate within the reliability of the experimental observations upon which equation (3) is founded. Thus an important question concerning the relation in equation (3) is whether the variation of the probability of UV-induced recombination, $P_d(R)$, with $P_o(R)$ is in essence a dependence upon the probability of natural recombination in the interval or upon the physical length of the phage genome that is included in the interval between the markers. The significance of this distinction to the nature of the recombinagenic stimulus attributed to the UV-induced lesions is apparent from the following simple model.

For a given UV dose, the probability $P_d(R)$ of UV-induced recombination in a particular interval x - y could be considered *a priori* to consist of two component terms: $P_d(L)$ = the probability at dose d that a UV-induced lesion(s) is appropriately situated to stimulate recombination between the x and y loci, and $P_d(R:L)$ = the conditional

probability at dose d of a UV-induced recombination in the x - y interval given the location of a suitably placed recombination-promoting lesion(s), where

$$P_d(R) = P_d(L) \quad P_d(R:L) \tag{4}$$

The relation in equation (3) indicates that for different intervals within the irradiated λ phage genomes the probability of UV-induced recombination $P_d(R)$ varies with the first power of the probability of recombination between unirradiated genomes $P_o(R)$. In light of equation (4), a simple interpretation is that at constant dose d either $P_d(L)$ or $P_d(R:L)$ varies directly with $P_o(R)$ for the different intervals while the other component term is a constant.

A variation of $P_d(L)$ with $P_o(R)$ would presumably reflect an actual dependence of the term $P_d(L)$ upon D_{x-y} ; the probability that a randomly placed UV lesion in the phage DNA would be properly located to stimulate recombination between the x and y loci would depend upon the length of the DNA molecule between those loci, implying that the site of the recombination-promoting lesion itself defines within narrow limits the point of the induced recombination --i.e., the point at which the genetic linkage of the recombining chromosome is disrupted. $P_d(R:L)$, the other component term of the probability of UV-induced recombination, would then be a constant, implying that the recombination-promoting lesion induces a crossover adjacent to its site with constant probability. This interpretation of equations (3) and (4) ascribes a local and constant recombinagenic effect to the UV-induced lesions. It is implicit in the early hypothesis that UV-induced recombination might reflect lesion-provoked switchovers in a copy-choice recombination scheme (115, 117)

and in the recent suggestion that the decreased linkages in UV-stimulated bacterial recombination are the result of gaps in semi-conserved irradiated DNA caused by replication-bypass of photoproducts (109, 240).

Conversely, a variation of the component term $P_d(R:L)$, in equation (4), with $P_o(R)$ would imply that the UV-induced lesions serve simply to enhance the probabilities of natural mating and recombination phenomena in the various intervals and that the recombinagenic effect of the lesions might be diffused somewhat along the irradiated chromosomes. The term $P_d(L)$ would increase with increasing UV dose but would be constant at each UV dose for intervals of varying genetic length, from which it could be inferred that a UV lesion need not be located closely adjacent to a site at which it induces recombination.* A

---

* There is an inherent ambiguity in the conception of a "local" recombination by breakage and reunion in that the recombining chromosomal segments are very likely joined by overlapping complementary DNA strands from the two parental molecules (Fig. 3). A local recombinagenic lesion could presumably determine the site of one terminus of the heterozygous region, which may have a typical length on the order of 3% of the genetic map (130, 2). If the heterozygous region of the joint molecule contains the information for different alleles of one or more genetic markers on the complementary DNA strands, repair or segregation of the heterologous loci could lead to an observed genetic recombination up to around 3% of the genetic map from the actual site of the lesion.

This point should not be allowed to confuse the distinction drawn in the text between local and diffuse recombinagenic effects, which is based on the criterion of whether the recombinagenic lesion need be located within the genetic span of the interval between the recombining markers. Recombinations involving the inclusion of loci within heterozygous regions of joint molecules are subsumed in the category of exchanges within the interval between the genetic markers, since for each interval they contribute to the recombination observed among unirradiated phages and are implicitly taken into account in the determination of the genetic length of the interval. Moreover, a comparable argument can be formulated to cover the contingencies of single strand exchanges or gene conversion by informational transfer (61). Thus uncertainty as to the site of an observed recombination due to the generation of internally heterozygous regions in the recombinant molecule could not be a source of any "diffuse" character of the recombinagenic stimulus.

finite range of action for each lesion would insure that over the population of irradiated genomes the probability of recombination in each interval would be enhanced by a constant factor dependent only upon the density of the UV-induced lesions along the molecule. This interpretation of the recombinagenic character of the UV-induced lesions is compatible, for example, with the hypothesis that by facilitating formation of regions of local denaturation within the DNA (153) the photoproducts stimulate the pairing of DNA molecules precedent to recombination (190, 41).

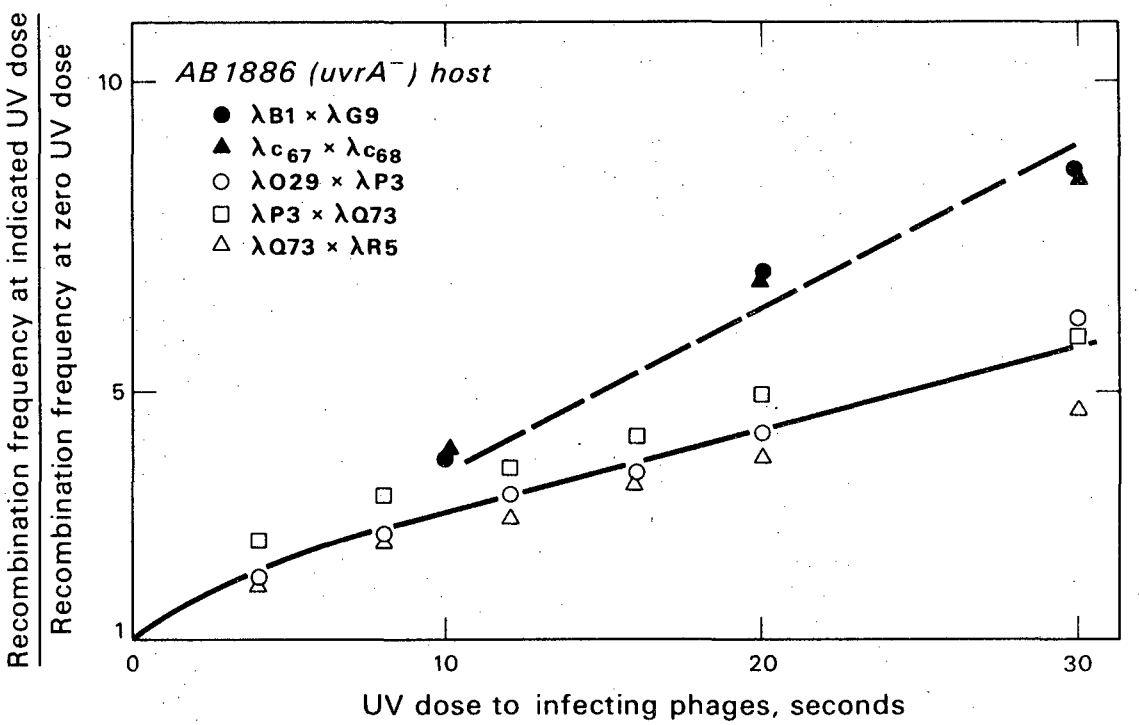
These alternative interpretations, according to which the recombinagenic effect of the UV-induced lesions is either local or diffuse, might be distinguishable on two points with regard to the kinetics of the UV stimulation of recombination. First, they lead one to expect different results for the relative UV enhancements of recombination in intervals that differ in the ratios of their physical to their genetic lengths. For a diffuse recombinagenic effect (with $P_d(L)$ = constant), the recombination frequencies must be increased by comparable factors in the different intervals. For a local recombinagenic effect (where $P_d(L) \alpha D_{x-y}$) the increment of recombination in each interval could be proportional to the ratio of its physical to its genetic length. Second, the model of a local recombinagenic effect implies that with increasing UV dose the enhancement of phage recombination should be a linear function of the number of functional recombination-promoting lesions in the DNA, whereas the model for a diffuse recombinagenic effect would be consistent with dependence on a lower power of the effective UV dose.

The crosses of UV-irradiated λsusO29 with λsusP3 (Fig. 22), λsusP3 with λsusQ73 (Fig. 23), and λsusQ73 with λsusR5 (Fig. 24)

furnish a determination of whether the relative UV enhancement of recombination frequencies varies with the physical or with the genetic lengths of the intervals. Jordan and Meselson have found in studies of the density distribution of progeny from crosses of density-labeled parental phages that there is an excess of progeny physically recombinant in the interval from susQ73 to the right end of the chromosome (see Fig. 4) compared to the susP3-susQ73 interval (120). They ascribe this result to a ratio of physical to genetic length for the susP3-susQ73 interval which is around an order of magnitude greater than that characteristic of the rest of the chromosome, including the adjacent c-susP3 and susQ73-susR54 intervals.

The data for the UV enhancement of overall recombination frequencies in the susO29-susP3, susP3-susQ73, and susQ73-susR5 intervals (Figs. 22, 23, and 24) are replotted in Fig. 29 to show the relative increments in the recombination frequencies with increasing UV doses. For each UV dose the natural frequency of recombinants is increased by essentially the same factor in each of the three intervals. The UV stimulation of recombination is a function of the genetic rather than the physical length of the intervals. This result indicates that either the UV stimulus to recombination is diffused along the irradiated genome or a local recombinagenic effect of the UV lesions is suppressed in the susP3-susQ73 region by the same unknown factor which reduces the frequency of natural recombination there. Either conclusion implies that the UV-induced lesions do not provide a singular stimulus to the initiation of a recombination event.

Also plotted in Fig. 29 are the relative enhancements of recombination frequencies in the susB1-susG9 and c67-c68 intervals, from the data in Figs. 7 and 8. The stimulation of recombination seems



DBL 696-4734

Figure 29. The relative factors by which UV-irradiation of the infecting phages enhances overall recombination frequencies in five different intervals of the λ chromosome. The data points are replotted from the results shown in Figs. 7, 8, 22, 23, and 24 for crosses in the AB1886 host strain, omitting the brackets for simplicity. (The positions of the markers on the genetic map are indicated in Fig. 4.)

to be about 50% more efficient in these intervals than in the sus029-susR5 region--a difference which is probably significant. Similar instances of a slightly heterogeneous topography of UV-induced recombination, unrelated to the genetic lengths of the intervals, have been reported for T4 phage (62).

From the curves in Figs. 7 and 8 it would seem that for crosses in the HCR- (uvrA, uvrB, and uvrC) hosts as well as in the HCR+ (uvr+) strains the UV stimulation of recombination is directly proportional to the dose administered to the infecting phages, as in equations (1a) or (3)--or, if it were presumed that at each dose a constant fraction of the initially induced photoproducts in the parental phage DNA is repaired, to the effective number of unrepaired UV lesions in the parental phage DNA during the period of recombination. However, the more detailed data considered in the present chapter for crosses in the uvrA host indicate that the situation is probably not so simple.

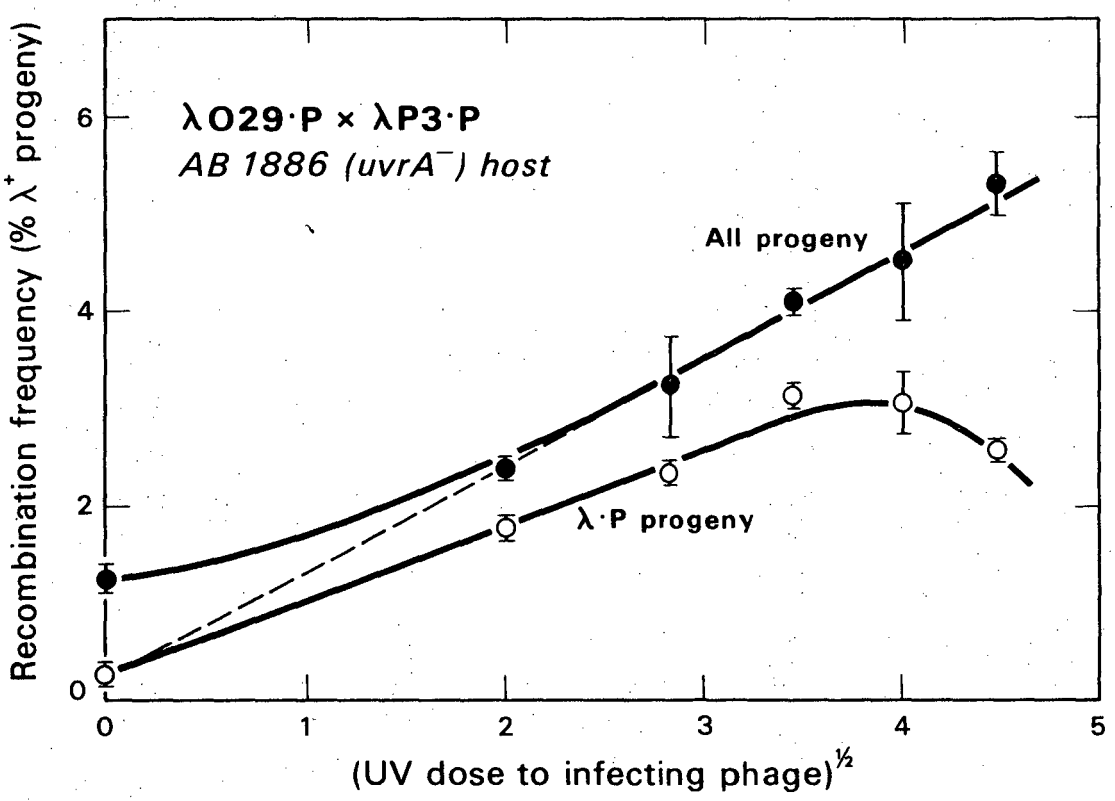
First, there are prominent shoulders on both the curve in Fig. 26 for the survival of the infecting phages on the uvrA strain and the curve in Fig. 28 for the viability indices of the λ·P progeny from the crosses in the uvrA host. These curves, in particular the latter, imply that over the dose range where the primary enhancement of phage recombination occurs the number of unexcised potentially lethal and recombination-promoting UV lesions in the phage DNA increases somewhat faster than the actual UV dose to the infecting phages, due to the saturation of residual HCR in the uvrA host. The enhancement of recombination frequencies in the uvrA host is less linear with respect to the number of unrepaired UV lesions in the parental phage DNA than with respect to the UV dose to the infecting phages.

Second, from Figs. 22 through 24 it is evident that for very low doses the enhancement of recombination frequencies in the uvrA host is actually not directly proportional to the UV exposure of the infecting phages. Perhaps the best example is seen in Fig. 22 for the crosses of λsus029•P with λsusP3•P, where the increment in the frequency of recombinants among the λ•P progeny is half as great for a dose of 4 seconds to the parental phages (from 0.2% to about 1.8%) as for a UV dose of 12 seconds (from 0.2% to 3.1%). (The non-linearity of the enhancement at very low UV doses is probably more apparent in the curve for the λ•P progeny than in that for all progeny because the factor by which a UV exposure enhances the pertinent parental recombination is not obscured by experimental variation in the frequency of recombinants that accrue from natural vegetative exchanges among the newly synthesized phage chromosomes.) Similar curvatures at low UV doses are apparent in the dose-response curves for enhancement of recombination in crosses of λsusP3 with λsusQ73 and of λsusQ73 with λsusR5, in Figs. 23 and 24.

These two indications of non-linearity in the dose-response are additive. Taken together, they imply that the probability of UV-induced recombination cannot be directly proportional to the number of unexcised recombination-promoting UV lesions in the phage DNA. The essence of this argument can be crudely illustrated by simply plotting the enhancement of recombination frequencies in the uvrA host as a function of the square root of the dose to the infecting phages, as has been done in Fig. 30 for the data from the crosses of λsus029•P with λsusP3•P (Fig. 22).* In this plot the initial increase in the frequency of λ•P

---

* The choice of a dose parameter for plots such as that in Fig. 30 is problematical, because of uncertainty as to the relative number of functional (i.e., the average number of unexcised) recombination-promoting



DBL 696-4735

Figure 30. The UV enhancement of recombination frequencies among all progeny and among HCM-labeled λ·P progeny for the crosses λc71susO29·P × λc71susP3·P (Figs. 21, 22)--plotted as a function of the square root of the UV dose to the infecting phages.

recombinants is approximately linear. The UV-enhanced recombination frequencies among all progeny phages fall on a biphasic curve that is linear with respect to the square root of the dose for doses greater than 4 seconds but extrapolates back to intercept the ordinate near the λ·P recombination frequency at zero dose. The divergence of the linear segments of the curves for overall and λ·P recombination may be taken as representing the occurrence, except at very low doses, of UV-induced matings of parental and newly synthesized genomes, the recombinant products from which would very seldom retain the parental HCM label.

Whatever the limitations with regard to the indicated dose parameter of the plot in Fig. 30, it does serve to point out that the UV enhancement of phage recombination in the uvrA host is not a linear function of the number of initially induced UV photoproducts (much less functional recombination-promoting lesions) in the phage DNA. Moreover, it emphasizes that the UV-induced recombinants originate in matings of the HCM-labeled parental phages--for the most part with each other but at higher doses to a significant extent also with newly synthesized genomes. Thus for the crosses in the uvrA host equation (3) could be

---

lesions in the phage DNA during the period of parental phage recombination for each UV dose. In Fig. 30 the recombination frequencies have been plotted against the square root of a dose proportional to the number of initially induced lesions. Curves quite similar to those in Fig. 30 result if the recombination frequencies are plotted against the parameter $d_c^{0.3}$, where d_c is a corrected dose proportional to the number of potentially lethal lesions retained in the HCM-labeled progeny from these crosses in uvrA host bacteria and can be ascertained directly from the data shown in Fig. 28.

The UV enhancements of recombination frequencies from crosses in the uvrA host of λsusP3·P with λsusQ73·P (Fig. 23) and of λsusQ73·P with λsusR5·P (Fig. 24) are also linear with respect to the square root of the dose and consistent with the interpretation presented in the text; but in these cases the argument is obscured by variation with increasing dose in the sector of HCM-labeled recombinants formed in matings of λ·P with newly synthesized λ·K genomes (section IV-B).

empirically reformulated as:

$$\nu_d = K''P_d(R) \cong K''K''' \left[d^{\frac{1}{2}} P_{p/p,o}(R) + \frac{(d-4)^{\frac{1}{2}}}{3} \frac{P_{p/k,o}(R)}{10} \right], \quad d \leq d_{opt}, \tag{5a}$$

where $P_{p/p,o}(R)$ and $P_{p/k,o}(R)$ refer to the natural probabilities of recombination of a parental ($\lambda \cdot P$) phage genome with another parental ($\lambda \cdot P$) chromosome and with a newly synthesized ($\lambda \cdot K$) genome, respectively, and it is assumed that $P_{p/p,o}(R) \sim \frac{P_{p/k,o}(R)}{10}$ (Appendix II). The second term within the bracket in equation (5a) would give rise to the secondary increase in phage recombination at high UV doses in the excision-defective host strains (section III-A.1, Fig. 8):

$$\nu_d = K''P_d(R) \cong K''K''' \left[\underbrace{d_{opt}^{\frac{1}{2}} P_{p/p,o}(R)}_{\text{(constant)}} + \frac{(d-4)^{\frac{1}{2}}}{3} \frac{P_{p/k,o}(R)}{10} \right], \quad d > d_{opt}. \tag{5b}$$

The dose dependence of the secondary enhancement of recombination, between parental $\lambda \cdot P$ and newly synthesized $\lambda \cdot K$ genomes, represented in these equations is consonant with several conclusions from section IV-C above. The effective UV dose "(d - 4)" corresponds to the number of UV photoproducts which are not excised by residual HCR in the uvrA host (section IV-C.1: Fig. 28), but would remain in the parental DNA strands to induce recombination with newly synthesized chromosomes. (In an HCR+ host, continuing excision of recombinagenic photoproducts would forestall the occurrence of such a secondary enhancement of recombination.) The rate of the secondary enhancement of recombination frequency

with increasing effective dose appears to be only about 1/30th that for the primary enhancement of recombination between λ·P genomes; this accords with the notion that UV-induced recombination may be curtailed during the later part of the infection because of a destruction of chromosomes containing semi-conserved parental DNA (section IV-C.2: Fig. 26, and Appendix III).*

If the recombinagenic effect of the UV lesions in the phage DNA were strictly local, it would be necessary for a lesion to be situated within or closely adjacent to the interval in which it might induce a recombination. Since for the uvrA host it seems that the probability for each parental phage particle that an unexcised recombination-promoting lesion is located in a particular genetic interval increases somewhat faster than the UV dose, while the enhancement of recombination among the phages varies with a root power of the UV dose, one may infer that the recombination-promoting lesion need <u>not</u> be situated within the interval of the induced genetic recombination. Studies by Krieg on the UV-inactivation of gene function and cross reactivation in T4 phage also support this conclusion (136, 137).

As UV lesions accumulate in the chromosome with increasing dose there is apparently an increasing degree of redundancy in their stimulation of recombination, as might be expected if the recombinagenic effect were diffused along the molecule. (An alternative interpretation is

---

* <u>A priori</u>, the UV-irradiation might be expected to stimulate recombination of λ·P with newly synthesized λ·K genomes at an efficiency on the order of $\frac{1}{2}$ to $\frac{1}{4}$ that for the stimulation of recombination between λ·P genomes--allowing for the facts that in the former case only one of the genomes would contain recombinagenic lesions (115) and the λ·P chromosome may more often contain semi-conserved parental DNA, and assuming that the natural and UV-induced recombination proceed by comparable mechanisms.

that there is a positive interference in the UV-enhanced recombination, so that each additional recombination induced in the irradiated molecule requires a greater initiating stimulus. However, since natural recombination among λ phages is characterized by a low generalized negative interference over the entire chromosome (122, 2), and since there are indications that multiple UV-induced recombinations often occur in a single mating (115, sections IV-B, C above), this interpretation does not seem plausible.) The achievement of complete redundancy of recombination-promoting lesions, or the stimulation of the maximum frequency of recombination events which can be accommodated among the parental phage genomes, for the UV dose d_{opt} would account for the saturation of the primary UV enhancement of recombination. In the former case, from the fact that the primary UV enhancement of phage recombination in the HCR- hosts occurs for UV doses which leave up to about 15 unexcised pyrimidine dimers per phage chromosome (section III-A.1), one could estimate a standard effective range of the recombinagenic lesion as no less than 6% - 7% of the phage genome.

In the light of the results discussed above, it would be reasonable to reformulate equation (4) for the probability $P_d(R)$ of UV-induced recombination in a particular interval $\underline{x}$ - $\underline{y}$ as:

$$P_d(R) = \sum_{\underline{x}-\underline{y}} \int_z P_d(R:L_z)\, dP_d(L_z) \leq P_{d,max}(R) = kP_o(R)\,, \qquad (6)$$

where $P_d(L_z)$ refers to the probability of a recombination-promoting lesion at a distance z along the molecule from a contemplated site of recombination. The probability $P_d(L_z)$ would be a function not only of the number of UV photoproducts initially induced in the phage DNA but also of the relative efficiencies with which the recombinagenic lesions

are excised in the host bacteria for the various UV doses (88). The function $P_d(R:L_z)$ would be proportional to the natural frequency of recombination $P_o(R)$ and would vary inversely and non-linearly with the parameter z, up to a maximum value.

Notwithstanding the evidence cited above to support the contention that the probability $P_d(R)$ does not increase linearly with the number of functional recombination-promoting UV lesions in the phage DNA, the data in Fig. 7 and for the few crosses described in Fig. 25 suggest that in the HCR+ host bacteria the UV enhancement of recombination may indeed be directly proportional to the UV dose--i.e., to the number of initially induced recombinagenic photoproducts. This linearity of the dose-response curves for crosses in HCR+ host bacteria may well be fortuitous; with increasing UV dose a decline in the efficacy of repair of typical recombinagenic photoproducts (due, for example, to an increase in the average lag time preceding excision) may be superimposed upon the enhancement of recombination in the dose-response assay.

V. INDIRECT STIMULATION OF LAMBDA PHAGE RECOMBINATION BY UV-IRRADIATION OF HCR+ AND HCR- HOST BACTERIA

V-A. The Context

Recombination frequencies from crosses of unirradiated λ phages in HCR+ host bacteria can be increased severalfold by exposure of the host cells to high doses of UV radiation prior to infection (129, 130). The mechanism of this recombination enhancement is unknown; the stimulation of phage recombination occurs in the context of varied and complex effects of UV-irradiation of the host bacteria upon λ phage growth. (The early work in this field has been reviewed by Stent: 206.)

The "capacity" of HCR+ bacteria to host infections of unirradiated λ phages is considerably more sensitive to UV-irradiation than the capacity for autonomous T-even phages, as a consequence of the requirement for functions provided by the bacterial genome throughout a productive λ infection (129, 208, 206). With increasing UV dose to the cells, the latent period of the λ phage infection increases while the number of progeny phages produced per active host cell decreases (129, 130). The relative number of bacteria of an HCR+ host strain that are capable of supporting λ phage growth declines rapidly for UV doses to the cells on the order of 3,000 ergs/mm^2 at 2537 Å (129); the capacity of HCR- host bacteria is more resistant to UV-irradiation (45).

Although the proportions of HCR+ host cells that are able to support unirradiated λ phages in single and in multiple infections, respectively, begin to decrease at about the same UV dose, the capacity to sustain multiple infections thereafter declines at a lesser rate

than does the capacity for single infections (129). Thus there appears to be "multiplicity reactivation" of a UV-inactivated factor of the host capacity, in that part of the effect of UV-irradiation upon the host capacity can be offset by the presence of an increased number of infecting phage genomes. The relation of this effect to the increased phage recombination that occurs in these multiple infections of UV-irradiated host cells is not clear.

The survival of λ phages that have themselves been UV-irradiated is enhanced by low UV doses, in the range of 500 ergs/mm^2, to HCR+ host bacteria (UV-reactivation) (230, 183). But at higher UV doses to the cells the capacity to host UV-irradiated dependent phages is reduced at a much faster rate than their capacity for unirradiated phages (67, 206). This effect is due to a UV-inhibition of host cell reactivation (and indeed provided the first indication that such a process existed). Since the kinetics of the inhibition imply that it is an "all or none" phenomenon for each host cell, it is probably caused by inactivation of a host gene function rather than the competition of the UV-induced lesions in the bacterial DNA for HCR enzymes (183). This view is consistent with recent evidence suggesting that UV doses to HCR+ host bacteria of about 5,000 ergs/mm^2 are necessary to competitively inhibit excision of heterozygous base pairs in heteroduplex λ DNA (94, 47). By contrast, the residual HCR activity in excision-defective host bacteria can be competitively inhibited at reasonably low UV doses to the host cells (80).

***

The enhancement of λ phage recombination frequencies caused by UV-irradiation of the host cells has been investigated in the present study by performing crosses of unirradiated and lightly UV-irradiated

($N/N_o \sim 0.8$) λsus029•P and λsusP3•P phages in UV-irradiated AB1157 (uvr+) and AB1886(uvrA) host bacteria. For each case, the effects of increasing UV dose upon the relative survivals of the multiply infected complexes and the frequencies of recombinants among all progeny phages and among parental HCM-labeled λ•P progeny (cf., sections IV-A, B) were determined.

These experiments focus on four principal points:

a) The relative UV-sensitivities of the capacities of uvr+ and uvrA host bacteria to support phage growth following multiple infections with unirradiated and with lightly UV-irradiated λ phages provide information concerning the nature of the inactivation of host capacity. The results suggest that there is a failure of multiplicity reactivation as well as host cell reactivation of UV-irradiated λ phages in heavily irradiated host bacteria.

b) The relative increases in recombination frequencies among all progeny and among λ•P progeny from crosses in irradiated host cells imply that there may be an increased frequency of physical exchanges, apparently by breakage and reunion, among all phage genomes throughout the latent period in irradiated bacteria; however, the appearance of genetically recombinant progeny is suppressed at high UV doses to the cells.

c) Comparison of the dose-response curves for the enhancement of phage recombination by UV-irradiation of uvr+ and uvrA host strains indicate that the damage that stimulates this recombination is susceptible to excision-repair, and hence that the recombination is promoted by unexcised UV lesions in the bacterial genome.

d) Comparison of the recombination of unirradiated and lightly UV-

irradiated phages in irradiated host bacteria suggests that the enhancements caused by UV-irradiation of the host and of the infecting phages are cumulative. The inhibition of HCR by irradiation of uvr+ host cells does not markedly affect the recombination enhancement due to UV-induced lesions in the infecting phage DNA; however, competitive inhibition of residual HCR by UV-irradiation of uvrA host bacteria does increase the number of effective recombination-promoting lesions in irradiated infecting phage DNA.

In addition to restricting the range of conceivable mechanisms by which UV-irradiation of the host bacteria could cause λ phage recombination, the results of these experiments permit qualitative comparison of the stimulations of phage recombination evoked by UV-irradiation of the host cells and by irradiation of the infecting phages themselves, which are seen to differ in a number of aspects.

V-B. UV-Sensitivities of the Host Capacities of HCR+ and HCR- Bacteria

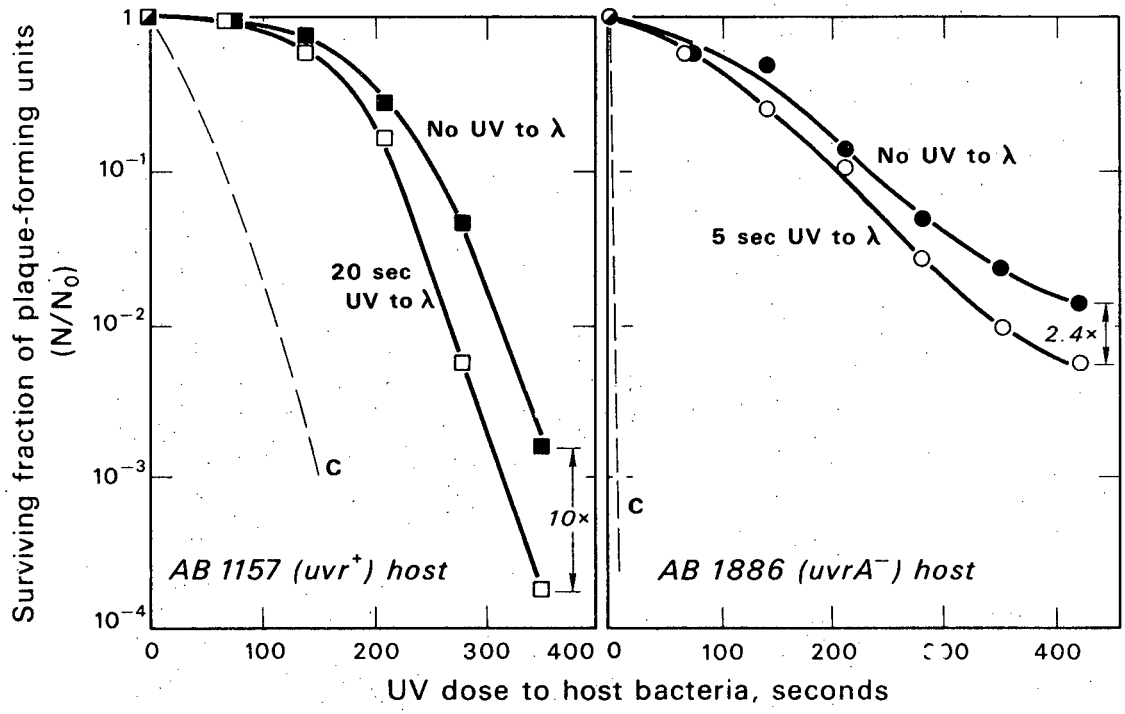
The curves in Fig. 31 show the inactivation as plaque-forming units, with increasing UV dose to the cells before infection, of AB1157(uvr+) and AB1886(uvrA) host bacteria multiply infected with unirradiated or lightly UV-irradiated ($N/N_o \sim 0.8$) λ phages. For low UV doses to the cells the curves are shouldered to varying extents; in the higher dose range, from about 2,000 to 4,000 ergs/mm^2 (200 to 400 sec. UV), the capacity to support growth of the infecting phages falls off exponentially in each case.

There are two differences between the results for the UV-irradiated uvr+ and uvrA host bacteria. First, for both unirradiated and irradiated infecting phages, the slopes of the curves describing inactivation of the uvr+ host capacity are steeper in the high dose range than those for the uvrA host capacity. If the sensitivities of the hosts' capacities are viewed in relation to the UV-sensitivities of their colony-forming ability (represented by the dashed curves labeled "C" in Fig. 31) rather than in relation to the absolute UV doses, the capacity of the uvrA host is enormously more resistant to UV than that of the uvr+ strain. This result agrees with the findings of Devoret and Coquerelle concerning the UV-sensitivities of HCR+ and HCR- host capacities to support the growth of unirradiated λ phages in single infection, from which they have concluded that excision-repair processes themselves are primarily responsible for inactivation of the HCR+ host capacity (45).*

---

* The following can be offered as a possible explanation for the UV-sensitivities of the uvr+ and uvrA capacities which is consistent with the available experimental evidence, including several findings presented in this report.

The fact that the capacity of the host cells to support growth of autonomous T-even phages is impaired only at UV doses to the bacteria



DBL 696-4736

Figure 31. UV-inactivation of the capacity of AB1157(uvr+) (a, left) and of AB1886(uvrA) (b, right) bacteria to host multiple infections of unirradiated and of lightly UV-irradiated λ phages. For each host strain, the UV dose to the irradiated infecting phages reduced the phage survival to about 0.8 in single infection of unirradiated cells of that strain. (The dashed lines labeled "C" denote the approximate UV-inactivation curve for the colony-forming ability of each host strain.) These data were obtained by assaying the relative survival of the infected complexes in crosses of λc71sus029•P with λc71susP3•P (MOI = 10) as a function of UV dose to the cells prior to infection. The results were reproducible in several different experiments. The recombination frequencies among the progeny phages from the active infected complexes are described in Figs. 32 (a/b) and 33 (a/b).

Second, UV exposures of the infecting phages that reduce their survival in single infection of unirradiated host cells to ~ 0.8 (20 seconds and 5 seconds, respectively, for the AB1157(uvr+) and AB1886(uvrA) hosts) had a greater effect on the survival as plaque-

---

that are several times greater than those considered here (206) illustrates that the inactivation of the capacities for λ phage is not attributable to unacceptable damage to basic cellular apparatus involved in energy metabolism and protein synthesis. A fundamental contribution of the host cell to λ phage growth, however, consists in required functions encoded in the radiation-sensitive host genome that are apparently non-constitutive (208) (and/or for which the gene product itself might be susceptible to UV-inhibition, conceivably by dint of its possible association with cellular DNA in a radiation-sensitive complex). The recently discovered repI bacterial function (167), which is necessary to λ phage replication, is probably an example of such a required host factor.

The UV-inactivation of the uvrA host capacity for λ could be due to inhibition of messenger RNA transcription (173, 162a) that limits the expression of these required host functions. Experimental results described in section III-A.1 and Appendix I, concerning crosses of heavily UV-irradiated λsusB1 and λsusG9 phages (Figs. 7, 9) suggest that complementation among infecting phage genomes is significantly inhibited in the uvrA host by UV doses to the phages on the order of 100 seconds. It seems likely that the failure of complementation is due primarily to an obstruction of transcription of the relevant phage genes by unexcised UV-induced lesions in the phage DNA. UV doses to the uvrA host bacteria of similar magnitude should induce a comparable density of transcription-inhibiting lesions in the bacterial DNA, and for such doses there is significant inactivation of the uvrA host capacity for λ (Fig. 31).

One would expect the initial UV lesions that inhibit transcription to be susceptible to excision-repair in the uvr+ host (173). Indeed, complementation among heavily irradiated λsusB1 and λsusG9 genomes is not inhibited in the uvr+ host at doses fivefold greater than those that affect complementation in the uvrA host (Figs. 7, 9); this result probably reflects continuing HCR of damage in susB1+ and susG9+ alleles of inviable infecting phages, since these functions are expressed relatively late in the phage infection ((232). However, it is conceivable that the disruption by excision-repair of bacterial genes whose functions are required early in a successful λ infection might be as effective in preventing their timely transcription as the presence of unexcised UV lesions. Alternatively, the host cell repair processes and phage replication processes in the uvr+ host might be in competition for enzymes (e.g., ligase), metabolic precursors, energy sources, etc., necessary to both processes (45). In such cases the UV-sensitivity of the capacity to host λ infections would be considerably greater, relative to the sensitivity of the colony-forming ability, for uvr+ bacteria than for uvrA cells--as is observed experimentally (45, Fig. 31).

forming units of multiply infected, UV-irradiated uvr+ complexes than on that of uvrA complexes. For the highest UV doses to the cells assayed, the survivals of the uvr+ complexes infected with unirradiated and with irradiated phages differed by a factor of ten, whereas the survival of uvrA complexes infected with irradiated phages differed only by a factor of around 2.4. The different results for the two host strains can be attributed to the inhibition of host cell reactivation by UV-irradiation of the host cells (67), which has a much greater impact on the viability of UV-irradiated phages in the HCR+ bacteria than in the excision-defective uvrA bacteria with only residual HCR activity.

The data shown in Fig. 31 also imply that at high UV doses to the host bacteria the phage infection is propagated by a single infecting λ phage genome in most of the complexes that are active. This point is clear if one compares, for the infections of heavily UV-irradiated cells, the frequencies of viable infecting phages with the observed survivals of the multiply infected complexes. For example, in single infection of unirradiated uvr+ host bacteria the survival of the λ phages UV-irradiated for 20 seconds was about 0.84 relative to the titer of unirradiated phages, corresponding to 0.2 lethal "hits" per phage. Assuming that in the experiment described in Fig. 31-a an essentially complete inhibition of HCR (and "residual HCR") was attained for the high UV doses to the uvr+ bacteria, a typical irradiated phage infecting a heavily irradiated uvr+ cell carried about 2 effectively lethal "hits," corresponding to a survival in single infection of around 0.15 relative to unirradiated phages.* Since the host bacteria were infected with 10 phages per

---

* This estimate is based on the results in Figs. 5 and 6 concerning the conventional HCR and on the data in Figs. 28, 31-b, and 33-b--which are internally consistent--regarding the residual HCR.

cell, however, the uvr+ bacteria irradiated for 280 and for 350 seconds received an average of about 1.5 viable phage genomes each. Assuming a Poisson distribution of the viable infecting phages among the cells, around 80% of the infected complexes received at least one viable phage genome. Yet it is evident from Fig. 31-a that the survival of the heavily irradiated uvr+ complexes infected with irradiated phages was only 10% the survival of those infected with unirradiated phages--as if the success of the infection hinged upon a single, randomly selected, infecting phage genome. An analogous examination of the results for the uvrA host strain, shown in Fig. 31-b, leads to the same conclusion--while implying that about 80% of the potentially lethal lesions induced in the infecting phages by the 5-second UV dose were repaired by residual HCR in unirradiated uvrA host cells.

The above analysis indicates that the multiplicity reactivation of UV-irradiated λ phages observed in unirradiated HCR+ and, in particular, HCR- host bacteria (cf., section III-B.1) is supplanted in heavily UV-irradiated host cells by an apparent multiplicity deactivation; the relative survival as plaque-forming units of the complexes formed of heavily irradiated cells infected with irradiated phages is substantially lower than that predicted by the simple probability that they receive a viable infecting phage. Such an effect clearly need not be related to the extent of recombination among the parental phage genomes, but only to a reduced probability that a viable chromosome within a cell multiply infected with viable and inviable phages be able to express its potential for successful replication and propagate the infection. In the present case, the potential of the viable phages was diluted in proportion to

their frequency among the infecting phages, presumably because of competition among the parental molecules for a host factor necessary to replication.

These findings cannot be attributed to failure of phage adsorption (which control assays confirmed to be unaffected by UV-irradiation of the host cells), injection, or nuclease degradation of infecting phage DNA within the irradiated cells, since the yields of progeny phages that carried a parental λ•P HCM label from active complexes of irradiated bacteria were comparable to those from unirradiated cells. Rather, the observations imply that with increasing UV dose to either HCR+ or HCR- host bacteria the initial replication of an increasing fraction of the infecting phage genomes is blocked--an effect probably identical to the UV-inactivation of "phage-synthesizing centers" previously observed for phages p8 and T2 (58). This effect could be a consequence of damage to host gene functions--e.g., the repI factor (167)--and a principal element in the UV-inactivation of the host capacity for λ phage.

V-C. Enhancement of Recombination Frequencies for Unirradiated Infecting λ Phages

The data in Fig. 32 show the frequencies of genetic recombinants observed among all progeny and among λ•P progeny phages from crosses of unirradiated λsus029•P with λsusP3•P phages in UV-irradiated AB1157 (uvr+) and AB1886(uvrA) host bacteria. In each instance, the enhancement of recombination frequency with increasing UV dose to the host cells was small compared to the enhancement evoked by light UV-irradiation of the infecting phages themselves (compare Fig. 32 with Fig. 25). (One may draw assurance as to the qualitative significance of the variations of recombination frequencies with dose in Fig. 32 and Fig. 33 below) from the facts that all of the crosses for each host were performed and titrated using the same preparations of host bacteria, that the variations were reproducible in duplicate crosses--note brackets on the experimental points--and that there is a consistency in the trends of the curves with changing dose.)

In these experiments, the total recombination frequencies increased to maxima at values 150% - 200% the natural level, peaking at a dose of about 200 seconds to uvr+ host bacteria* (Fig. 32-a) and at a dose of 70 seconds or less to uvrA host cells (Fig. 32-b). With further UV

---

* Over the 0-200 second UV dose range, the curve in Fig. 32-a for the stimulation of total recombination by irradiation of HCR+ host bacteria corresponds qualitatively to the results reported by Kellenberger et al. (130); these authors observed twofold and fourfold increases in phage recombination frequencies in crosses over the co_1-mi and c-mi intervals, respectively. For crosses of λsus029 with λsusP3, Weigle (231) found an enhancement of the recombination frequency from a usual value of around 0.5% in unirradiated cells to about 3.7% in heavily UV-irradiated HCR+ bacteria.

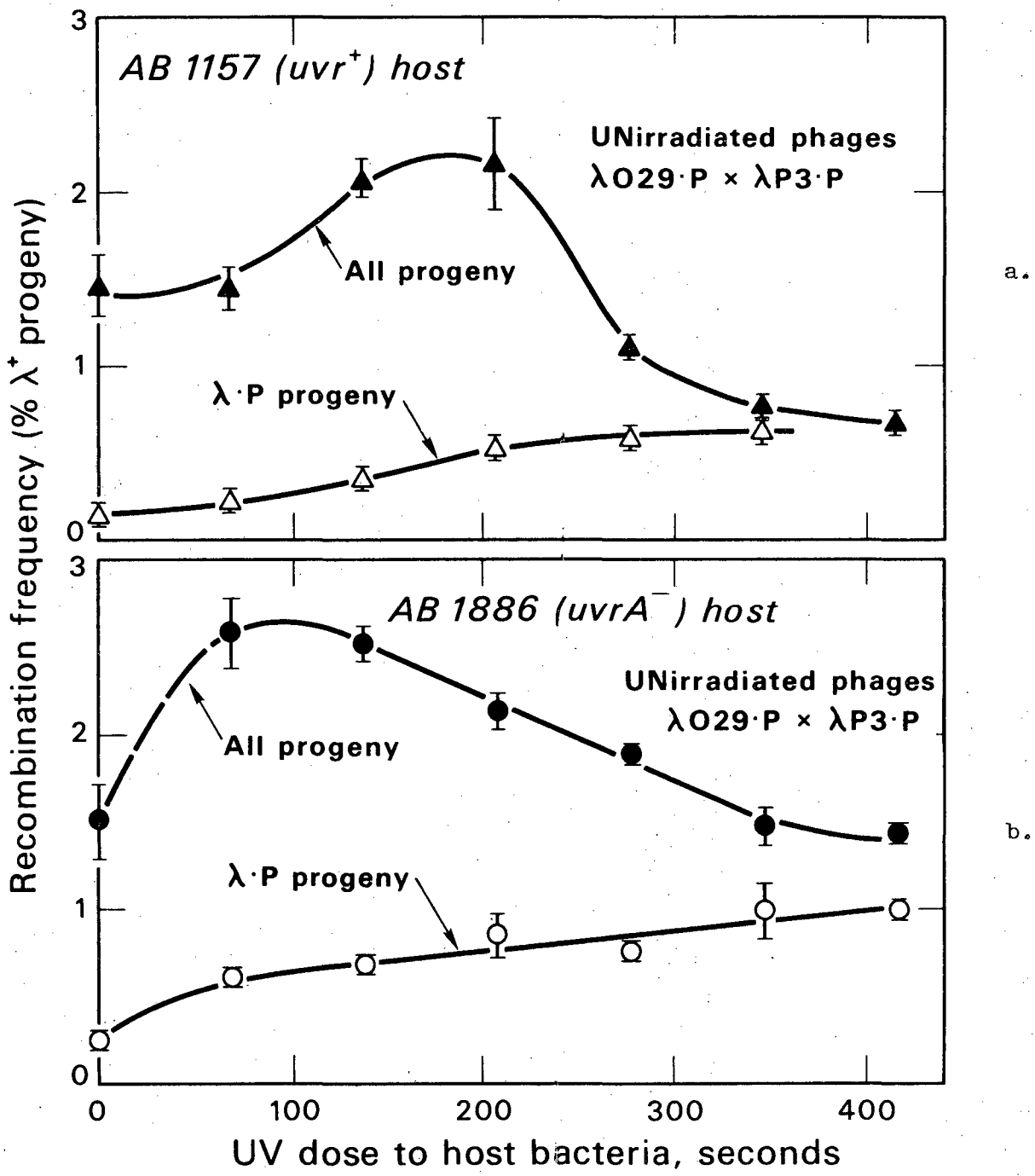


Figure 32. DBL 696-4737

Recombination frequencies among total and among HCM-labeled λ•P progeny from crosses of unirradiated λc71susO29•P and λc71susP3•P as a function of UV dose to K12 strain host cells prior to infection. a. (top): AB1157(uvr+) host bacteria. b. (bottom): AB1886(uvrA) host bacteria.

exposure of the cells the overall recombination frequencies declined, more abruptly in the uvr+ host bacteria than in the uvrA cells, to levels 50% - 100% that observed among the progeny of unirradiated cells. The fact that the peak of recombination enhancement occurs for lower UV doses to uvrA host bacteria implies that the stimulation of phage recombination is caused at least in part by repairable but unexcised UV lesions in the bacterial DNA.

Among the parental-HCM-labeled λ·P progeny of these crosses there was a fairly steady increase in the frequency of recombinants in both uvr+ and uvrA host strains, although evident at lower doses in the latter host. At UV doses of 350-400 seconds to the bacteria the λ·P recombination frequencies were only slightly less than the corresponding total recombination frequencies and about 400% the λ·P frequencies observed for crosses in unirradiated cells. However, from the relative differences between corresponding overall and λ·P recombination frequencies at various UV doses to the bacteria (Fig. 32-b in particular), it is evident that the variations in total phage recombination frequency were due in the main to an increase in genetic recombination among newly synthesized λ·K genomes at low to moderate doses and a decrease in such recombination at high UV doses. This result contrasts sharply with that for the enhancement of phage recombination by UV-irradiation of the infecting phages. The latter is characterized by similar absolute increments in the total and λ·P recombination frequencies that reflect increased mating among parental HCM-labeled phage genomes early in the infection (Figs. 22, 25 - section IV-B). On the other hand, the fact that HCM-labeled parental DNA strands were included in recombinants formed in response to UV-irradiation of the host implies

that this type of UV-stimulated recombination also proceeds by a mechanism of breakage and reunion of the mating chromosomes.

In these experiments UV-irradiation of the host bacteria caused an increase in the duration of the latent periods of the phage infection and a decrease in the numbers of progeny phages produced per active host cell, as has been observed previously by others (130, 45).* However, the decreases in overall phage recombination frequency for high UV doses to the host bacteria (Fig. 32) were not simply the result of reduced phage DNA synthesis--i.e., of an absence of recombination among λ·K genomes because few progeny phage were synthesized in the irradiated cells. The active host cells produced substantial bursts of newly synthesized phages, as attested not only by the relative titers of assayed infected complexes and of total free phages in the cross lysates, but also by the yields of progeny phages that did not exhibit the parental λ·P HCM specificity. With increasing UV dose to the bacteria, the proportion of λ·P among the progeny phages only increased from about 2% for crosses in unirradiated host cells to 5% - 10% for crosses in heavily UV-irradiated cells, in rough correspondence with a reduction in the calculated burst sizes from around 100-200 phages per active unirradiated

---

* Kellenberger et al.(130) observed that following multiple infection of heavily UV-irradiated E. coli K12 HCR+ host cells with unirradiated genetically marked λ phages little phage DNA synthesis occurred even in those cells that retained the capacity to sustain the infection, and the active host cells each produced only a few progeny phage particles. In this way they obtained lysates with an enhanced frequency of internally heterozygous recombinant phages, since destruction of heterozygotes by replication was minimized (and excision-repair of the heterozygous base sequences may have been less efficient than usual due to the inhibition of HCR). No such drastic reduction in the burst sizes from active infected complexes was observed at comparable UV doses in the experiments reported here (cf., text), nor was it observed by Devoret and Coquerelle (45) in similar experiments with singly infected UV-irradiated host cells.

host cell to around 25-50 progeny phages per active heavily irradiated host cell. It can be concluded that even from heavily irradiated host cells around 90% of the progeny phages contained newly synthesized λ·K chromosomes.

A reasonable interpretation of the results in Fig. 32 is that UV-irradiation of the host bacteria stimulates normal λ phage recombination by breakage and reunion, involving both parental and newly synthesized chromosomes, by the same factor throughout the latent period, but that with progressively higher UV doses to the host the correlation between formation of physically recombinant chromosomes and appearance of genetically recombinant λsus+ progeny if preserved only for mating events involving parental λ·P genomes. Recall the inference from the results in Fig. 31 that UV-irradiation of the host cells leads also to a reduction in their capacity for initial replication of parental phage genomes. It is possible that few (if any) of the infecting phages whose replication is forestalled early in the infection participate in the later multiplication of phage chromosomes (58). In this event, with increasing UV dose to the host cells in an increasing fraction of the active infected complexes only one of the parental genotypes in the phage cross would be represented among the newly synthesized λ·K chromosomes. A simple consequence would be a decline in the observed frequency of λsus+ genetic recombinants from matings among λ·K genomes.* Then in Fig. 32,

---

* In this connection, it is interesting to note that both the UV-inactivation of the host capacity (Fig. 31) and the depression of λ·K recombination (Fig. 32) seem to be more gradual in the uvrA host bacteria than in the uvr+ cells, consistent with the notion that the two effects are related manifestations of the same events.

This hypothesis could easily be tested by examining the HCM specificities and genotypes of progeny phages from individual infected complexes in single burst experiments.

the curves for recombination frequency among progeny phages that retain the parental λ·P HCM label should perhaps be regarded as real indices of the amount of phage recombination stimulated by UV-irradiation of the host bacteria, while the curves for recombination frequency among all progeny may represent a superposition of severely antagonistic effects.

Implicit in this scheme is the presumption that most of the λ·P recombinants that were induced by UV-irradiation of the host bacteria in the crosses described here had their origin in matings of parental λ·P with newly synthesized λ·K genomes. From control crosses of unirradiated λ·P and λ·K phages in unirradiated host cells--discussed in Appendix II--it has been inferred that, whereas recombinants formed from two λ·P phages probably retain the λ·P HCM label with 100% efficiency, recombinants formed by a crossover between a λ·P and a λ·K chromosome in the sus029-susP3 interval may preserve the λ·P HCM label at a frequency on the order of 5%. Since over the course of the phage infection a λ·P chromosome is much less likely to mate with another λ·P than with a newly synthesized λ·K genome, events of the latter type can account for most (ca. 75%) of the λsus+·P recombinant progeny from crosses of λsus029·P with λsusP3·P. If all types of vegetative phage recombination were stimulated to the same extent within UV-irradiated host bacteria, most of the λ·P recombinant progeny induced would still be formed in exchanges between parental λ·P and newly synthesized λ·K chromosomes. Furthermore, if the appearance of λsus+ genetic recombinants from exchanges between newly synthesized λ·K genomes were suppressed with increasing UV exposure of the host bacteria, as the overall recombination frequency declines a progressively larger proportion of the λsus+·K recombinant progeny from these crosses would also have their origin in matings of λ·K with λ·P chromosomes.

On the basis of this interpretation the relative values of the total and λ·P recombination frequencies observed for quite high UV doses to the host bacteria can be simply rationalized. Thus for the crosses in heavily irradiated cells, in which perhaps but one of the infecting phages is replicated, few λsus+ recombinants may be generated either in the relatively rare matings among heteroallelic λ·P chromosomes or in the relatively frequent matings among isoallelic λ·K chromosomes. By analogy to the control crosses in unirradiated host cells, one may suppose that of the λsus+ genetic recombinants issuing from matings of heteroallelic λ·P and λ·K chromosomes, about 5% retain the λ·P HCM label and about 95% exhibit a λ·K HCM specificity. Since the λ·P progeny population comprises about 5% - 10% of the total progeny population from the crosses in heavily irradiated bacteria, it is understandable that the total and λ·P recombination frequencies should converge to quite similar values for the very high UV doses to the host cells--as indicated in Fig. 32 for both uvr+ and uvrA bacteria.

The results shown in Fig. 32 seem to rule out previously conceived explanations for this indirect enhancement of recombination. The hypothesis that viable phage recombinants might often be formed in UV-induced exchanges of short segments of the phage and bacterial genomes (206, 129) cannot account for the observation for high UV doses to the host of an increasing recombination frequency among unirradiated HCM-labeled parental genomes accompanied by a decreasing frequency of recombinants among all progeny. Incorporation into newly synthesized phage DNA of (recombinagenic) UV photoproducts from excised or degraded segments of the bacterial genome (cf., 45) is excluded by the facts that the indirect enhancement occurs for lower doses to excision-defective hosts and that for any dose the

relative increment in recombination is at least as great among parental as among newly synthesized phage genomes. Finally, it seems unlikely that the increased frequency of recombinants could be attributed solely to prolonged matings of the phages in the absence of DNA synthesis (130). Most of the active irradiated complexes yielded a moderate number of progeny, and for lower UV doses there is a clear enhancement of genetic recombination among newly synthesized phage chromosomes.

V-D. Enhancement of Recombination Frequencies for UV-Irradiated Infecting λ Phages

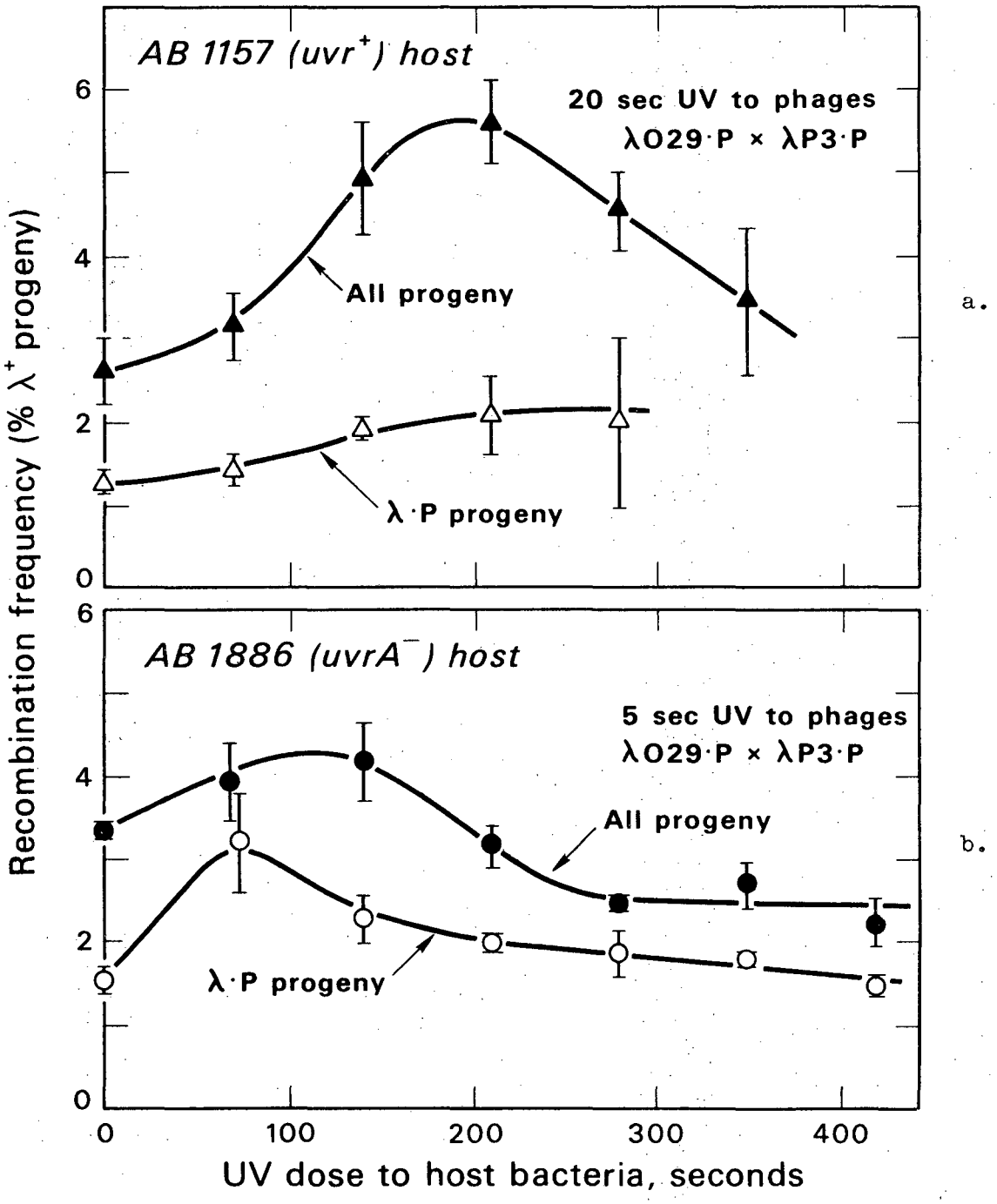
The curves in Fig. 33 describe the enhancement of phage recombination frequencies from crosses of lightly UV-irradiated phages by UV-irradiation of the AB1157(uvr+) and AB1886(uvrA) host bacteria. The frequencies of recombinants among all progeny and among λ·P progeny at zero dose to the host cells were higher for the crosses of irradiated phages (Fig. 33) than for those of unirradiated phages (Fig. 32) because of the recombination among parental chromosomes promoted by UV-induced lesions in the infecting phage DNA (Fig. 25). The results shown in Fig. 33 derive from the effects of UV-irradiation of the host bacteria not only upon replication of the infecting phages and natural phage recombination, as discussed above, but also upon host cell reactivation, and conceivably upon processes involved in the enhancement of recombination induced by UV lesions in the parental phage DNA; they must be viewed with some caution in light of the potentially complex interactions and the limited resolution of the date.

From a comparison of Figures 32-a and 33-a, it is evident that the patterns according to which total and λ·P phage recombination frequencies were affected by UV exposures of the uvr+ host bacteria are comparable for the crosses of unirradiated and UV-irradiated infecting phages. These results support the view that UV-irradiation of the host bacteria stimulates recombination of both parental λ·P and newly synthesized λ·K genomes, while light UV-irradiation of the infecting phages independently enhances recombination of parental λ·P phage chromosomes.

In addition to stimulating recombination in its own right, UV-irradiation of the host bacteria inhibits host cell reactivation of

Figure 33. Recombination frequencies among all progeny and among λ•P progeny from crosses of very lightly UV-irradiated ($N/N_o \sim 0.8$) λc71sus029•P and λc71susP3•P as a function of UV dose to K12 host bacteria prior to infection. a. (top): AB1157(uvr+) host cells. b. (bottom): AB1886 (uvrA) host strain.

The data shown in Figs. 31-a, 32-a, and 33-a and in Figs 31-b, 32-b, and 33-b were obtained in the same two experiments. For each of the host strains all crosses were performed and titrated under the identical experimental conditions, using the same preparations of bacterial cells.



DBL 696-4738

Figure 33

UV-induced lesions in the infecting phage DNA, presumably including those lesions that cause increased recombination among the λ•P parental genomes. For the crosses of irradiated phages in irradiated uvr+ host cells (Fig. 33-a), however, the increase in recombination among HCM-labeled parental DNA strands which accompanied suppression of HCR with increasing UV dose to the host does not appear to be greater than that observed for crosses of unirradiated infecting phages (Fig. 32-a). These data suggest that excision--which is probably not significantly affected in HCR+ host cells at these UV dose levels (94, 47)--of the UV-induced lesions in the phage DNA suffices to neutralize their recombination-promoting activity whether or not the physical integrity of the chromosome is restored.

As can be seen in Fig. 33-b, UV-irradiation of the excision-defective uvrA host bacteria had different effects upon recombination frequencies of lightly UV-irradiated infecting phages. At relatively low UV dose to the host cells, there was an approximately twofold increase in the λ•P recombination frequency accompanied by a similar absolute increment in the overall recombination frequency. Since in this case the UV doses to the host bacterial were sufficient to cause a competitive inhibition of any residual HCR, this result probably reflects the elimination of residual excision of recombination-promoting lesions in the parental phage DNA strands, with a consequent magnification of the enhancement induced by a constant UV dose to the infecting phages. A twofold increase in λ•P recombination frequency for doses of 70 seconds to the uvrA host bacteria, considered in conjunction with the dose-response curve in Fig. 22, suggests that around 70% of the recombination-promoting lesions induced in the infecting phages by the 5-second UV

exposure are normally repaired by residual HCR in unirradiated uvrA host cells. This figure is in good agreement with the estimate of residual HCR of potentially lethal lesions from the data in Fig. 31-b (and also from Fig. 28, section IV-C.1), consistent with the view that the recombination-promoting and potentially lethal classes of UV photoproducts are equally susceptible to HCR and probably identical.

The data in Fig. 33-b for the crosses of irradiated phages show no increases in recombination frequencies that might be identified as due solely to UV-irradiation of the uvrA host bacteria. The curves in Figs. 32-a/b and 33-a lead one to expect that such an enhancement would be manifested at low UV doses to the host by an increase in total recombination frequency accompanied by a smaller increment in the λ·P frequency, and/or with higher UV doses to the cells by a steady increase in the λ·P recombination frequency as the overall frequency decreases. In the present case, any enhancement that might have occurred at low doses may be obscured in Fig. 33-b by the increase in recombination among parental genomes which is attributed to the inhibition of residual HCR. With further UV exposure of the uvrA host bacteria, the λ·P recombination frequencies from the crosses of irradiated phages decline gradually to the level observed for unirradiated host cells, rather than increasing as in the other cases examined.

UV-irradiation of the uvrA host with doses greater than that necessary to inhibit residual HCR does not further enhance, but reduces somewhat, the recombination observed <u>among</u> irradiated <u>parental</u> λ·P genomes. Because of the enhancement of recombination involving newly synthesized phages observed for crosses of irradiated phages in the uvr+ host (Fig. 33-a), however, one might presume that exchanges <u>among newly synthesized</u>

λ·K phages are stimulated by irradiation of the uvrA host cells also, regardless of whether the infecting phages are irradiated or not. It is not clear whether the frequency of exchanges that involve only one newly synthesized genome and a parental chromosome with irradiated DNA might also be increased by irradiation of the uvrA host. In comparing the results in Figs. 32-b and 33-b for λ·P recombination at higher UV doses to the host bacteria, one should bear in mind not only that most of the λ·P recombinants induced by UV-irradiation of the host in crosses of unirradiated phages (Fig. 32-b) were probably formed in matings between parental λ·P and newly synthesized λ·K chromosomes, but also that from the uvrA host (but not the uvr+) such recombinants would be recovered somewhat more rarely for crosses of irradiated than of unirradiated phages; the polarity of the recombination toward destruction of the λ·P HCM label would be increased if the parental genomes contained unexcised UV lesions (cf., section IV-B).

The decline in λ·P recombination frequencies shown in Fig. 33-b for higher UV doses to the uvrA cells may have been due solely to an impairment of processes that facilitate the recombination stimulated by irradiation of the infecting phages. For example, it is conceivable that the recombination-promoting lesions are more effective in semi-conserved chromosomes that also contain lesions resulting from replication-bypass of UV photoproducts, while irradiation of the host inhibits replication of most of the infecting genomes. It is also conceivable that recombination among parental λ·P and newly synthesized λ·K phages induced by irradiation of the host contributed to the decline in λ·P recombination frequency. With increasing UV dose to the host, the increases in recombination between λ·P and λ·K chromosomes might have

led to the formation of fewer λ+"·P" double recombinants than the number of pre-existing HCM-labeled recombinants (formed in prior matings among the irradiated infecting genomes) of which the λ·P HCM specificity was destroyed; a similar effect seems to obtain for high UV doses to λ·P phages infecting unirradiated uvrA host cells (cf., for example, Fig. 22--section IV-B).

The relative survivals as plaque-forming units of the infected complexes from crosses of unirradiated and of irradiated λ phages in very heavily UV-irradiated host bacteria (Fig. 31), and the variations in overall and λ·P recombination frequencies of unirradiated phages with increasing UV dose to the host (Fig. 32), suggest that perhaps but a single infecting phage genome may have been replicated in most active infected complexes of heavily irradiated host cells. In the experiment described in Figs. 31/32/33-b, for the highest UV doses to the uvrA host bacteria both the overall and the λ·P recombination frequencies are from 50% (at 420 sec.) to 80% (at 350 sec.) greater for the crosses of irradiated infecting phages (Fig. 33-b) than for the crosses of unirradiated phages (Fig. 32-b). These differences are significant, as the recombination frequencies were reproducible within about 10% of the mean in duplicate crosses and/or titrations. Other points of detailed interpretation aside, these differences in recombination frequencies support the view that UV-irradiation of the infecting λ phages can induce recombination of unreplicated parental phage chromosomes. (The results from the crosses of unirradiated and irradiated phages in heavily UV-irradiated uvr+ host bacteria (Figs. 31/32/33-a) are also consistent with this conclusion, although in this case it was not possible to accurately ascertain the recombination frequencies of irradiated phages for very high UV doses

to the cells because of the severe inactivation of the infected complexes.)

In sum, the crosses described in Fig. 33 suggest that the enhancements of λ phage recombination induced by UV-irradiation of the host cells and of the infecting phages are "cumulative" in the sense that the induced recombinants apparently originate in differentiable categories of mating events and both phenomena may be exhibited in the same phage crosses, in the uvr+ if not the uvrA host, but that irradiation of the host further increases the probability of recombination among irradiated phage genomes only insofar as it inhibits the excision of recombination-promoting UV lesions from the λ phage DNA.

VI. SOME GENERAL CONCLUSIONS

VI-A. The Mechanism of UV-Induced Recombination

The experiments utilizing HCM-labeled parental phages suggest that UV-induced recombination among λ phages proceeds by a mechanism of breakage and reunion of DNA molecules analogous to that observed for recombination of unirradiated λ (111, 157) and T4 (222) phage chromosomes.

For crosses of lightly UV-irradiated infecting phages, there are comparable absolute increases in the frequencies of recombinants among all progeny phages and among progeny which contain HCM-labeled parental DNA strands (Fig. 22). While affirming that the primary direct UV enhancement of phage recombination occurs early in the infection (115), this result proves that the UV-induced recombination always involves at least one strand of the irradiated parental DNA and that at least one strand of the recombinant molecule can be composed of predominantly parental DNA. The frequency of UV-induced recombinants among the HCM-labeled progeny is significantly reduced if only one of the irradiated parental phage types carries the HCM label, implying that DNA from both parental phage types can be incorporated into the recombinant molecules by breakage and reunion of parental DNA strands of conserved and/or semi-conserved infecting phage chromosomes. UV-induced exchanges between parental and newly synthesized molecules do not contribute to the enhanced recombination frequency among HCM-labeled genomes.

From assays of the relative numbers of host cell reactivable UV lesions retained in the DNA of HCM-labeled progeny from crosses in excision-defective HCR- host bacteria, it appears that the HCM-labeled

progeny phages contain mostly conserved parental DNA (Fig. 28). Some of the UV-induced recombinants may contain only conserved parental DNA; in conjunction with the results noted above, this would indicate that the UV-induced recombination can occur by breakage and reunion of infecting phage chromosomes prior to their replication. This view is supported by the observation that UV-irradiation of the infecting phages enhances recombination of the HCM-labeled chromosomes in host bacteria which have themselves been heavily UV-irradiated, in which perhaps but a single infecting phage genome is replicated.

Although the results reported here do not categorically exclude the possibility of UV-induced recombination by single-strand exchanges or even some variety of semi-conservative copy choice, they are incompatible with a mechanism involving informational transfer unaccompanied by exchanges of physical substance. The experiments can be simply interpreted only in terms of a model by which the UV photoproducts in the λ phage DNA induce simple breakage and reunion of the double-stranded chromosomes.

VI-B. Relevance of DNA Repair to UV-Induced Recombination

Comparative studies of the UV enhancement of λ phage recombination in wild type and various repair-deficient host bacteria show that the UV lesions which promote recombination can be repaired by host cell reactivation. These experiments did not reveal any association of UV-induced recombination with DNA repair processes mediated by the host bacteria.

The UV-induced increases in phage recombination frequencies, among all progeny phages and among progeny which contain HCM-labeled parental DNA strands, are even greater for crosses in excision-defective HCR- host bacteria than in wild type HCR+ hosts. (Similar results with regard to the UV enhancement of overall recombinant frequencies have been reported for bacterial recombination (104) and for mitotic recombination in yeast (204).) Furthermore, the recombination-promoting and potentially lethal UV lesions in the λ phage DNA have the same sensitivity to repair by host cell reactivation. It is clear that excision is not involved in either the stimulation or execution of the UV-induced recombination event; rather, the UV stimulation of recombination is caused by unexcised but host cell reactivable UV lesions in the parental phage DNA. This conclusion is underscored by the occurrence in excision-defective HCR- hosts of a secondary enhancement of recombination that is attributable to exchanges between newly synthesized genomes and parental DNA with unexcised UV photoproducts, whereas in HCR+ host bacteria the continuing excision of recombinagenic lesions apparently curtails UV-induced recombination during the latter part of the phage infection.

The dose-response for UV enhancement of λ(red+) phage recombination is essentially the same in REC+, recA mutant, and recB mutant host

bacteria. The UV-induced phage recombination, like that among unirradiated λ phages (53, 201), does not require the host recA or recB functions. The recA-1 allele of the host bacteria does have some effect upon the viability of UV-irradiated λ phages.

The findings reported here are in general incompatible with the suggestion that UV-induced recombination might be equated with a specific post-replication repair mode that restores information lost due to replication bypass of UV photoproducts (109, 100, 240). Not only are UV-induced phage recombination and phage survival affected differently by the recA gene of the host, thought to be necessary to post-replication repair of the bacterial DNA, but the recombinagenic effect in λ appears to be neither local nor restricted to semi-conserved irradiated DNA. However, it is certainly possible that closely related enzymic functions are involved in the UV-induced recombination and a post-replication or excision repair mode. And it seems likely that recombination induced by unexcised photoproducts in semi-conserved irradiated DNA could contribute to survival of the irradiated genome by effecting rearrangements of inactivating lesions among the daughter chromosomes.

VI-C. The Mode of UV Stimulation of λ Phage Recombination

Three findings from the experiments reported above are particularly pertinent to understanding how UV-induced lesions in the chromosomes act to promote their genetic recombination:

a) The UV-stimulated recombination occurs by breakage and reunion of parental phage DNA strands--most likely of conserved and semi-conserved infecting phage chromosomes. The UV-induced recombination can probably proceed prior to the replication of the irradiated genomes.

b) The recombination is promoted by unexcised but host cell reactivable--as well as photoreactivable (115)--UV lesions in the phage DNA. It is not an artifact of multiplicity reactivation.

c) The UV enhancement of λ phage recombination is apparently proportional to the genetic rather than the physical length of the interval between markers on the chromosome. The probability of UV-induced recombination varies with a root power of the number of functional recombinagenic lesions in the phage DNA, suggesting that the stimulus to recombination can be diffused along the irradiated genome. The primary UV enhancement of recombination is saturated for doses which leave about 15 unexcised pyrimidine dimers per infecting phage chromosome.

If the UV-induced recombination can occur prior to the replication of the infecting genomes and is not associated directly with any DNA repair mode, one must infer that the alterations in the secondary structure of the DNA which accompany the formation of UV photoproducts are intrinsically conducive to recombination. No difference has been discerned between the fundamental mechanisms of the UV-induced and natural recombination events; the most plausible hypothesis to account

for the enhancement--as suggested by Setlow (190) and Curtiss (39)--is that the recombinagenic UV lesions function primarily to increase the efficiency of pairing of homologous DNA molecules which is prerequisite to recombination.

The pairing of homologous DNA molecules prior to recombination is thought to be accomplished through the annealing of complementary base sequences of single DNA strands from the mating molecules (87, 211), which could occur before the introduction of single-strand breaks into the recombining molecules (cf., Fig. 3). The "stable minimum length" for annealing of complementary nucleotide sequences may be estimated at 10 to 20 contiguous nucleotides (221), and the "minimum recognition length" for the alignment of homologous DNA sequences prior to recombination may be on the order of 10 to 100 nucleotides (221, 14). The denaturation of DNA, the separation of the complementary single strands, is known to be facilitated by the presence of UV photoproducts--as attested by a dose-dependent reduction in the thermal denaturation temperature (T_m), enhancement of reactivity with formaldehyde, increased susceptibility to acid denaturation, and increased buoyant density of heavily UV-irradiated DNA (153). The breakage of hydrogen bonds and distortions of the secondary structure due to UV photoproducts weaken the DNA duplex; there is probably an increased frequency of local denaturation (15, 112) in all regions of the irradiated molecules for low as well as high UV doses. UV photoproducts in the DNA, by increasing the frequency of local denaturation within each molecule and thereby promoting the annealing of undamaged bases of the parental chromosomes, should significantly increase the efficiency of pairing between different molecules.

Following the establishment of a minimum stable configuration of annealed homologous sequences from the mating molecules, the region of continuous pairing might extend to a length of around 1000 to 3000 nucleotides (2% to 6% of the λ chromosome)--comparable to the span of internally heterozygous regions (130) and clusters of exchanges from high, localized negative interference (2) in recombinant λ genomes. This would accord well with the observation that the primary UV enhancement of λ recombination saturates for doses which leave about 15 unexcised pyrimidine dimers per infecting phage chromosome. The site of a UV-induced structural defect need not define the site of recombination; moreover, the regions of pairing are probably of variable length and might tend to migrate along the chromosome from the intervals of initial recognition. For low UV doses the recombinagenic stimulus would be diffused along the chromosome.

This model is clearly consistent with UV-induced recombination by a mechanism essentially identical to that which prevails for natural recombination among unirradiated genomes. It can also account simply for several singular characteristics of the UV-induced recombination. First, the increased frequency with which irradiated genomes undergo multiple recombinations in a single mating event could be ascribed to UV-induced pairing of the parental molecules in a number of separate regions along their length, where the stabilization of an initial pairing might quite frequently lead to the establishment of secondary sites of recognition and recombination between the apposed and locally denatured molecules. The UV photoproducts would thus introduce a generalized negative interference into the recombination. Second, some variation in the topography of UV-induced recombination within the chromosome (62, Fig.

29) would follow from differences in the guanine-cytosine (GC) vs. adenine-thymine (AT) composition of different segments of the molecule (203). Regions of the chromosome of different composition would differ in their susceptibility to UV-induced pairing--the AT-rich segments being more easily denatured (153, 112)--as well as in their characteristic content of various types of photoproducts.

From the observations that the UV enhancement of recombination is subject to almost complete photoreactivation (115) (as well as excision-repair) and that the principal photoreactivable UV lesions are pyrimidine dimers (247, 190), one may infer that the primary recombinagenic photoproducts are pyrimidine dimers in the irradiated DNA. Other UV photoproducts in addition to pyrimidine dimers may contribute to the increased denaturability of UV-irradiated DNA. At present there is insufficient photochemical evidence to assess the relative importance to initial disruption of hydrogen bonding of pyrimidine dimers as opposed, for example, to UV-induced hydrates (228) of cytosine residues (239, 194, 191). However, an effect of the pyrimidine dimers either to initiate or to stabilize regions of local denaturation in the DNA would be consistent with the observed enhancement of recombination.

An alternative hypothesis to account for the UV stimulation of recombination is that the increased number of genetic exchanges is a direct or indirect consequence of the inhibition of DNA replication by structural defects in the DNA (95,100). (For example, a delay in DNA synthesis might facilitate the pairing and recombination of chromosomes in a favorable topological state (98). The experiments reported

here provide no unambiguous evidence by which to evaluate this possibility, although the results can be most simply interpreted according to the view that the mere excision of UV photoproducts from DNA suffices to nullify their recombinagenic effect whether or not the integrity of the damaged nucleotide sequence is restored so that normal replication can resume. This point might be clarified by examining the UV enhancement of λ recombination in an HCR- excision-proficient host strain--such as the uvrD mutant (Table I)--which is deficient in repair of UV-damaged phage DNA at a step subsequent to excision of the photoproducts.

Also relevant to this point are the results of experiments to be reported elsewhere in which the enhancement of λ phage recombination by nitrogen mustard (HN2) has been examined (10, 13b). The survival of HN2 treated λ phages is only slightly higher on a wild type AB1157(uvr+) host strain than on excision-defective AB1886(uvrA) bacteria, yet a significant HN2 enhancement of phage recombination is observed in the uvrA host but not in the uvr+ strain. The principal lethal and recombination-promoting HN2 lesions are not identical. The primary lesions induced in DNA by HN2 are inter- and intra-strand guanine-guanine crosslinks (Fig. 1-b) (138, 149). At present there is some uncertainty as to the relative lethality of the inter- and intra-strand crosslinks (139b; Low-Beer, A., Ph.D. thesis, Univ. of Calif., in preparation). However, the most reasonable interpretation of the results for HN2 enhancement of λ recombination is that the primary lethal lesion in λ phage is an inter-strand crosslink while the HN2 lesions that stimulate recombination are intra-strand crosslinks normally removed from the DNA by excision-repair in HCR+ bacteria. Since HN2 is known to inhibit DNA synthesis even in repair-proficient bacteria (139a) and since the formation of intra-

strand HN2 crosslinks probably involves considerable distortion of the secondary structure of the DNA helix (139b), it might be concluded that the salient property of the recombinagenic lesion is the nature of the structural alteration in DNA rather than its affect upon DNA replication.

The results for indirect UV stimulation of λ phage recombination by heavy irradiation of the host bacteria offer an interesting contrast to those for the direct stimulation of exchanges by UV-irradiation of the infecting phages themselves. The data are consistent with an interpretation that all phage recombination--by breakage and reunion of both HCM-labeled parental and newly synthesized genomes--is enhanced during the infection of the UV-irradiated host. At high UV doses the appearance of genetic recombinants is limited by failure of the host to replicate most of the infecting chromosomes, which leads to an unequal multiplicity of the infecting phage types in each active complex. The fact that the indirect enhancement occurs for lower doses to an excision-defective HCR- host than to a wild type HCR+ host argues that the recombination-promoting lesions are susceptible to excision-repair in the bacterial genome, and hence that they consist in unexcised UV photoproducts in this instance also.

It is conceivable that the indirect enhancement of recombination could be due to an impairment of phage DNA synthesis throughout the infection as a result of UV damage to required genes of the bacterial host. On the other hand, the indirect enhancement of recombination might be explicable in terms of a model similar to that advocated for the direct stimulation of phage recombination. There is substantial sequence complementarity between the DNA of λ phage and the E. coli host (132). Denaturation of the host DNA due to UV photoproducts could well

lead to increased pairing and annealing of strands from the phage and host genomes. If the usual lengths of the phage-host hybrids were to exceed the minimum stable length for annealing but fall short of the minimum extent of pairing necessary to avail recombination, the enhanced pairing would seldom lead to successful genetic exchanges between the phage and bacterial genomes. However, by increasing the frequency of unpaired single-stranded regions among the phage genomes this effect could result in an enhanced efficiency of mating and recombination between different λ chromosomes.

ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. Robert H. Haynes for his encouragement, support, and advice through the course of this work; to Dr. David Freifelder, who with Dr. Haynes suggested this area of research to me and who called my attention to the HCM label on recombination; to Dr. Mark Gurevitch for his example of humanity and intellectual integrity; to my fellow graduate students, above all Gary E. Jones for invaluable stimulation and comradeship; to Susan Daniels and Patricia Mason for technical assistance with many of these experiments; and to Drs. A. J. Clark, R. K. Mortimer, J. A. Heddle, and H. Echols for their assistance at critical moments. I am grateful to the U.S. Public Health Service for financial support during my graduate career, through Biophysics Training Grant No. 5T1-GM-829 during the early years and later through a pre-doctoral fellowship No. 1-F1-GM-30,952, and to the Lawrence Radiation Laboratory for laboratory facilities and for six months' support as a research assistant. My deepest thanks to Juliet, Sam, and Schmug Baker for their understanding and endurance.

To the memory of Gina.

(The author's current address is: Dept. of Medical Biophysics, University of Toronto, 500 Sherbourne St., Toronto 5, Ont., Canada.)

Appendix I: The Origin of an Anomalous Secondary UV Enhancement of Recombination Frequency among λsus Phages in HCR- Host Bacteria

The data in Figs. 7 and 9 of the text (section III-A.1) show that for relatively high UV doses to λsusB1 and λsusG9 phages infecting the HCR- (uvrA) host bacteria the frequency of λ+ recombinant progeny increases beyond 25%--the level which might be expected to represent equilibrium recombination between equally abundant parental and recombinant genotypes. However, the assignment of a 25% frequency of λsus+ recombinant progeny as the level of genetic equilibrium for such crosses rests upon the assumption that none of the phage genotypes has a selective advantage in mixed infection. This assumption may well not hold for crosses of some heavily UV-irradiated λsus mutants in an HCR- host, since the λsus mutants were originally selected as small plaque-formers and in general yield relatively small bursts of progeny from single vegetative infections (32). As higher and higher UV doses to the λsus phages gradually reduce to 1. the number of viable parental phage genomes per active, multiply infected complex and then significantly impair functional complementation (but not UV-induced recombination) among the viable and inviable phage chromosomes (136, 173), the larger bursts from those complexes containing a viable λsus+ recombinant genome might result in an artificially high frequency of this phage type in the lysate. In other words, if most of the infected cells contain but a single functional parental phage genome and 25% of these are of the λsus+ recombinant genotype, the frequency of λsus+ progeny in the overall lysate could be substantially greater than the 25% which might be anticipated *a priori* for genetic equilibrium.

The results of a simple reconstruction experiment illustrating this point with reference to mixed infections with λsusB1 and λsusG9 are shown in Table A. Host cells were infected with equal numbers of unirradiated λsusB1, λsusG9, λsusB1susG9, and λsus+ phages at total multiplicities of 12 and 0.012 phages per cell, and the lysates were assayed for the frequency of λsus+ progeny. When the infected cells contained more than one phage of each type (experiments A, A', and B), the frequency of λsus+ in the lysates was essentially the same as that in the infecting phages--recombination among the unirradiated phages being negligible. When the multiplicity of infection was reduced (experiments C and C'), so that virtually all infected complexes received but one phage, of whatever type, the frequency of λsus+ in the lysates was about twice that in the infecting phages.

In view of these results, the secondary UV enhancement of the frequency of λ+ recombinants for crosses in the uvrA host of λsusB1 and λsusG9 phages irradiated to very low survival (text Figs. 7, 9) might be interpreted as due not to the stimulation of recombination past the level of genetic equilibrium, but rather to a shift in the observed level of genetic equilibrium because of a progressive UV-inactivation of functional complementation at the B1 and G9 loci. This conclusion is supported by the results of complementation tests on the progeny of a cross in the uvrA host of λc71susB1 and λc71susG9 irradiated to a survival of 10^{-5} in a single infection. The cross lysate, which included the bursts from about 10^4 active infected complexes, contained 40% λsus+ recombinant progeny as assayed by the usual titrations on permissive and nonpermissive indicators. Of 30 plaques picked at random from plates of this lysate on a permissive host and characterized by complementation tests,

Expt.	Host Bacteria	Infecting Phages	Multiplicity of Infection		% λ++ Phage	
			each phage	total	among inf. phage	among progeny
A	AB1886 (uvrA)	λsusB1, λsusG9, λsusB1susG9, and λ++.	3	12	25	22±2
A'	AB1157 (uvr+)	"	"	"	25	20±0
B	AB1886 (uvrA)	λsusB1susG9 and λ++.	3	6	50	50±1
C	AB1886 (uvrA)	λsusB1, λsusG9, λsusB1susG9, and λ++.	0.003	0.012	25	47±1
C'	AB1157 (uvr+)	"	"	"	25	55±7

Table A - An experiment to assay the selective advantage of wild type λsus+ over mutant λsusB1 and λsusG9 phages in single and in multiple infections of AB1157 or AB1886 host bacteria. Infections were performed according to the same procedure and used for the λsusB1 × λsusG9 phage crosses described in Figs. 7 and 9 of the text, except that all phage types here carried a c71 clear-plaque mutation. The fractions of infecting and of progeny phages that plated on the non-permissive indicator were determined in order to ascertain any change in the frequency of the λsus+ genotype attributable to a selective growth advantage.

15 were λsus+, 6 λsusB1, 5 λsusG9, and only 4 λsusB1susG9. Although genetic exchanges to form the reciprocal λsus+ and λsusB1susG9 recombinant genotypes were presumably induced at comparable frequencies, the average frequencies of these recombinants in the lysate differed by a factor of around four. This result confirms that for these crosses of heavily irradiated phages the λsus+ recombinant does indeed have a selective advantage in the multiply infected HCR- bacteria.

The absence of a comparable secondary UV enhancement of recombination frequency for crosses in the HCR+ host strain (text Figs. 7, 9) implies that functional complementation of the B and G cistrons is more resistant to potentially inactivating UV lesions in the HCR+ strain than in the HCR- host. This resistance would be understandable if repair of transcription-inhibiting lesions (173) in inviable, but potentially complementing, parental phage genomes were to continue during early vegetative development of a viable genome, since the B and G cistrons provide late phage functions (232).

Appendix II: Control Crosses of Unirradiated HCM-labeled λ Phages--concerning the incorporation of λ•K DNA into λ•P recombinant genomes

Two factor genetic crosses with different configurations of the selected markers in unirradiated λ•P and λ•K parental phages were performed in the non-restricting, E. coli K12 strain AB1886. Table B shows the frequencies of λ+ and λ+•P recombinant progeny* that were observed for crosses over the adjacent sus029-susP3, susP3-susQ73, and susQ73-susR5 intervals on the right arm of the λ chromosome (Fig. 4). These crosses of HCM-labeled infecting phages are unirradiated controls on the crosses of UV-irradiated HCM-labeled phages presented in section IV-B of the text. Also shown in Table B for comparison are recombinant frequencies within the h-c interval for crosses involving λ•P and λ•C parental phage types in an E. coli C host, calculated from the data of Ihler and Meselson (111). The results in Table B demonstrate the labeling by host-controlled modification of recombinant chromosomes that contain parental DNA (section IV-A.2); in particular, as discussed in the

---

* The assays for λ+•P recombinant progeny are described generally in section II-I and Table III of the text; a more detailed technical note here will further clarify the resolution of these assays for the interested reader. The controls discussed below--concerning the background of unadsorbed infecting phages, restriction, and complementation--imply that even the lowest recombination frequencies among λ•P progeny shown in Table B are reliable indices of the formation of λ+•P progeny phages in the crosses rather than a reflection of background "noise" in the assay system. The arguments advanced hold with slight modification for the λ•P recombination frequencies determined from crosses of UV-irradiated infecting phages and presented in section IV-B of the text.

A) The minimum observed titers of λ+•P recombinant progeny were well over an order of magnitude higher than the levels at which the more numerous λ+•K progeny in the lysates could plate on the restricting, non-permissive (pm-) K12(P1) indicator strain. Only 1% - 5% of any particular λ+•P titer on the pm- K12(P1) indicator was attributable to λ+•K phages that escaped restriction. For example, the lysates from crosses of λ λsus029 with λsusP3 (Table B) assayed as approximately 97% λsus•K, 1% - 1.5% λ+•K, 1% - 2% λsus•P, and 0.001% - 0.005% λ+•P; although the ratio

Legend to Table B -

The frequencies of recombinants among all progeny phages and of λ·P recombinants among progeny containing parental DNA strands, for crosses over adjacent intervals on the right arm of the chromosome. The relative positions of the markers on the genetic map are shown in Fig. 4. The configurations of the markers crossed in λ·P and λ·K (or λ·C) infecting phages are lettered in accordance with the schematic model in Fig. C.

The λ·P recombination frequencies (column E) are the assayed titer of λsus+·P or λhc+·P recombinant progeny divided by the total number of progeny containing parental DNA strands; the latter quantity is defined for the a and for the b or c configurations as one and two times, respectively, the assayed titer of all λ·P progeny. The ratios averaged in column F were calculated for each cross independently of the average frequencies in columns D and E; the indicated errors are the calculated standard deviations.

All crosses of λsus mutants were performed in the K12 host strain AB1886, in a total of four separate experiments with no more than two crosses of a particular configuration being performed on the same day. (The parental λsus phages also carried a c71 clear-plaque mutation.) The results shown for crosses of λh+c with λhc26, in an E. coli C host, were calculated from the data of Ihler and Meselson (111); the frequencies for the two crosses of λ·P with λ·C phages, cases b and c, are based on counts of a small number of recombinant plaques. The absolute values of the recombination frequencies for the h-c crosses and the sus-sus crosses are not comparable because of the differing experimental conditions. Amati and Meselson (2) report recombination frequencies for λh+c+ × λhc and λsus029 × λsusP3 of 3.3% λhc+ and 0.25% λsus+ recombinant progeny, respectively, for crosses under similar conditions.

CROSS	Configuration of markers γ		β	# crosses	Recombination Frequency (% λsus+ recombinants) Total: among all progeny - D -	λ·P: among parental DNA strands - E -	Relative Freq. of λ·P Recombinants (E÷D) - F -
λsusQ73 X λsusR5							
	a) λQ·P	X	λR·P	(3)	1.9 ± 0.1	2.2 ± 0.4	1.2 ± 0.2
	b) λQ·P	X	λR·K	(3)	2.0 ± 0.1	0.24 ± 0.04	0.12 ± 0.03
	c) λQ·K	X	λR·P	(3)	2.0 ± 0.2	2.3 ± 0.2	1.2 ± 0.1
λsusP3 X λsusQ73							
	a) λP·P	X	λQ·P	(3)	2.4 ± 0.1	1.1 ± 0.2	0.47 ± 0.11
	b) λP·P	X	λQ·K	(3)	2.7 ± 0.2	0.066 ± 0.011	0.026 ± 0.002
	c) λP·K	X	λQ·P	(3)	2.5 ± 0.2	0.69 ± 0.03	0.28 ± 0.01
λsusO29 X λsusP3							
	a) λO·P	X	λP·P	(3)	1.4 ± 0.3	0.21 ± 0.03	0.16 ± 0.05
	b) λO·P	X	λP·K	(3)	1.2 ± 0.3	0.085 ± 0.010	0.077 ± 0.025
	c) λO·K	X	λP·P	(4)	1.2 ± 0.3	0.052 ± 0.010	0.044 ± 0.011
λh+c+ X λhc26 - from (111) -					(% λhc+ recombinants)		
	a) λ++·P	X	λhc·P	(3)	2.8 ± 0.5	0.66 ± 0.20	0.25 ± 0.12
	b) λ++·P	X	λhc·C	(1)	2.8	0.02	-
	c) λ++·C	X	λhc·P	(1)	2.8	0.01	-

Table B

following pages, they indicate the extent to which unlabeled (λ·K) DNA can be incorporated into chromosomes that are recombinant in these intervals and retain a parental λ·P HCM specificity (section IV-A.3).

1) HCM-labeled single recombinants

To date, two genetic observations have been reported that relate to the length and location of a segment of unlabeled DNA that could occur in a singly recombinant chromosome retaining a parental λ·P HCM label. Arber and Dussoix (8) found that for a cross of λc+mi·K with density labeled λcmi+·P in an *E. coli* K12 host the frequency of λcmi recombinants among the λ·P progeny was 40% that among the total progeny population and twentyfold greater than the frequency of the reciprocal λc+mi+·P recombinant. The densities of the λcmi·P recombinant progeny from this cross corresponded closely to those of phages with semi-conserved or conserved λcmi+·P parental chromosomes,

---

(λ+·P titer):(λ+·K titer) was on the order of 10^{-3}, the efficiency of plating (EOP) of λ+·K on the restricting pm- K12(P1) indicator, as monitored in control assays, was $2\text{-}4 \times 10^{-5}$. (This same argument can obviously be applied *a forteriori* with reference to the assays for total λ·K and λ·P progeny phages.)

B) While complementation and marker rescue among different λsus·P mutants, and to a lesser extent among λsus·P and λsus·K phages (51, 216), does occur in the restricting pm- indicator used to assay the λ+·P recombinant progeny, the lower recombination frequencies assayed among λ·P progeny phages were still more than 100-fold greater than could be accounted for on the basis of such artifacts in the titrations. This point is illustrated by the following control assays.

For one of the preparations of infecting phages used in the crosses shown in Table B, a mixture was made of equal numbers of λsusP3·P phages that had an EOP of 3×10^{-7} on the pm- indicator strains and λsus029·K phages, which had an EOP of 4×10^{-6} on the pm- K12 indicator and a (theoretical) EOP of 10^{-11} on the pm- K12(P1) strain. This mixture of λsusP3·P and λsus029·K plated at an efficiency of 3×10^{-6} on the restricting pm- K12(P1) indicator, tenfold greater than the EOP of the non-restricted λsusP3·P phages; this result confirms that some interaction among these restricted and non-restricted mutants can occur in multiply infected cells on the assay plate. The mixture of λsusP3·P and λsus029·K had an EOP of 10^{-4} on the pm- K12 indicator, due to complementation

indicating that the unlabeled DNA contributing the mi allele constituted only a small portion of the "λ·P" recombinant DNA strands. By contrast, for crosses of λ·P parental phages in an E. coli C host, the frequency of recombinants in the h-c interval is 25% as high among the λ·P progeny as among the entire progeny population but the frequency of λ·P recombinants is reduced more than 30-fold if either of the infecting phage types does not carry the type P HCM label (111) (Table B).

Important factors in an interpretation of these results are the relative positions of the h, c, and mi loci on the λ genetic map (Fig. 4) and the fact that most naturally occurring λ recombinants are formed by single crossovers (122) involving breakage and reunion of the mating DNA molecules (157). The observations suggest that particular "λ·P" recombinants can be formed at high frequency from labeled λ·P and unlabeled chromosomes only if the minimum amount of unlabeled DNA integrated into the selected recombinant type by a single breakage and reunion (i.e., the length of DNA from the selected unlabeled marker to

---

among the mutants in the absence of restriction; similarly, a mixture of λsus029·P phages (that plated at $3x10^{-5}$ on the pm- indicators) with equal numbers of the λsusP3·P phages had an EOP of 10^{-4} on both pm- K12 and pm- K12(P1) indicators. However, among the λsus·P progeny from the crosses of λsus029·K with λsusP3·P and λsus029·P with λsusP3·P, the frequencies of λ+·P recombinants assayed on the pm- K12(P1) strain were 0.1% - 0.2%. Moreover, the concentration of the critical phage types on the assay plates was lower in the actual cross titrations than in the controls cited (ca. 10 λ+·P plaques per plate in the former case), so that in fact less than 1% of any λ+·P titer was attributable to complementation in multiply infected indicator cells. Comparable results were obtained for the stocks of λsusQ73 and of λsusR5 phages and for mixed preparations and crosses of λsusP3 with λsusQ73 and of λsusQ73 with λsusR5.

C) Finally, neither λsus·P nor λsus+·P revertants present in the cross lysates as unadsorbed infecting phages could have perceptibly affected the assayed λ·P recombination frequencies. Before incubation of the infected cells to lysis in the course of a cross, the level of free unadsorbed phages was reduced by treatment with anti-λ serum to a titer (confirmed by mock crosses) less than 1% that of the λ·P progeny phages in the cross lysate.

the end of the chromosome distal to the selected λ·P marker) does not exceed a certain critical length. This critical length may be regarded as defining an interval at the extremity of the chromosome that either contains HCM sites whose activity is dispensable or lacks altogether sites of modification that are relevant* to retention of the type P HCM label.

This interpretation is illustrated in Fig. C, which shows--for various configurations of two markers in λ·P and λ·K chromosomes--the λ+ recombinant products of a breakage and reunion in an interval on the right arm of the chromosome. Chromosomes formed by recombination of two λ·P molecules (Fig. C-a) would retain the type P HCM specificity, whereas if the selected marker distal to the end of the chromosome were contributed by a λ·K parent (Fig. C-b) the recombinant would contain primarily type K DNA and would be restricted on a K12(P1) indicator. In the event that the marker proximal to the end of the chromosome were contributed by a λ·K parent (Fig. C-c), the retention of the type P HCM label would depend on whether the length of unlabeled DNA ("L_K") were within the limit necessary to avoid restriction.

The model presented in Fig. C is supported by the more complete data obtained in the present study and presented in Table B. From these results it is obvious that within each interval examined the overall recombination frequencies (Table B, column D) are the same regardless of the configuration of HCM specificities in the infecting phage types, but the λ·P recombination frequencies (column E) vary

---

* I.e., a site of P1-mediated HCM for crosses of λ·P and λ·K phages in a K12 host, a site of P1- or K12-mediated HCM for crosses of λ·P and λ·C phages in a strain C host.

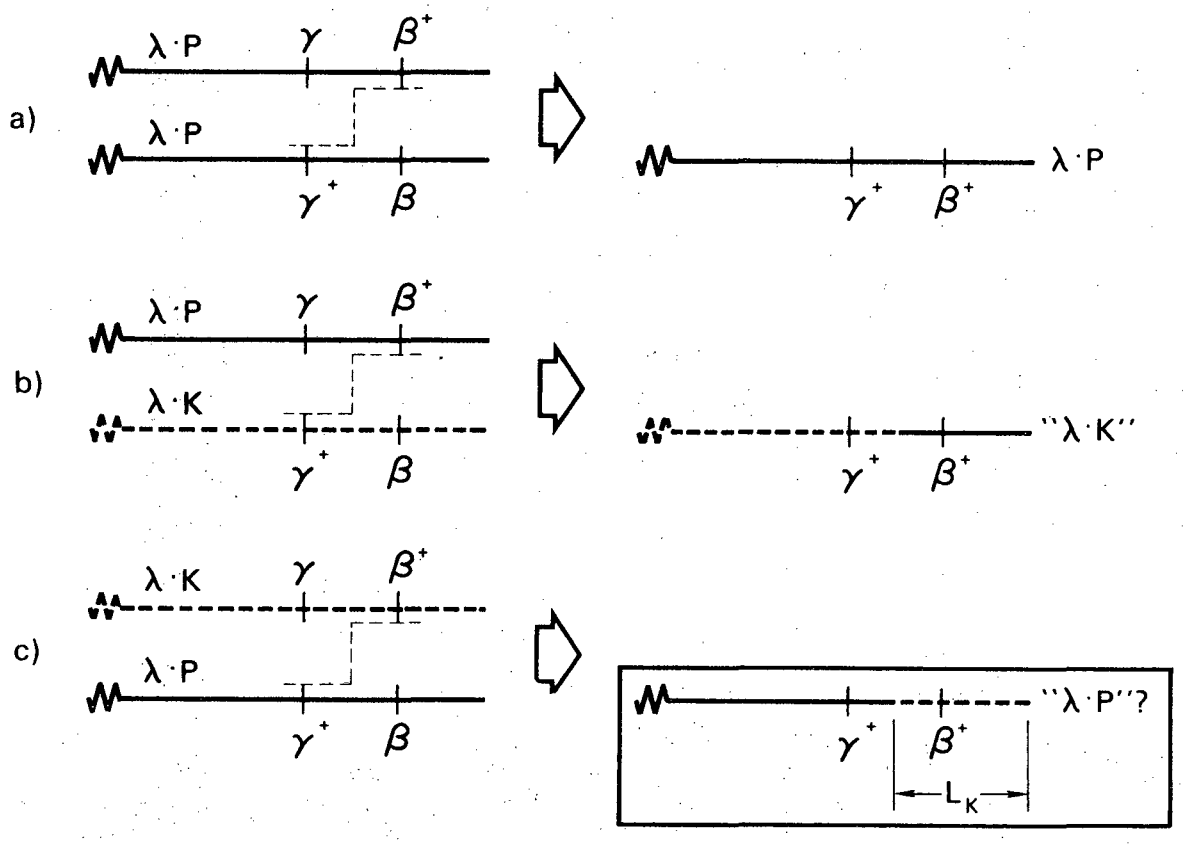


Figure C. DBL 696-4746

Different types of recombinant molecules that could result from a single crossover by breakage and reunion between γ and β markers, on the right arm of the λ chromosome, for different configurations of the markers in λ·P and/or λ·K parental genomes. The three cases depicted in this figure are drawn and lettered to correspond to the three configurations of parental markers for each cross in Table B.
a) γ+β+ recombinants formed from two λ·P chromosomes would be expected to retain the type P HCM specificity.

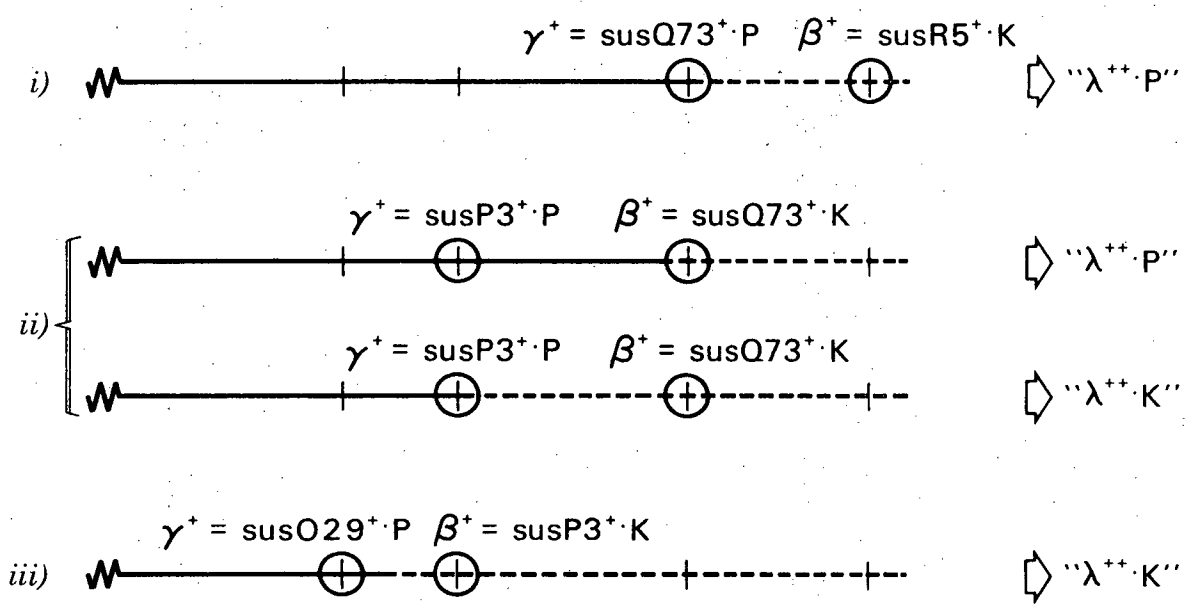
b) Recombinants of a λ·P and a λ·K chromosome in which only the right terminal segments of the λ·P molecule is retained would exhibit a λ·K specificity because they lack critical sites of type P HCM.

c) Recombinants of a λ·K and a λ·P chromosome in which only the terminal segment of the λ·K genome is incorporated might retain the type P HCM label if the extent of the λ·K DNA does not exceed a critical length "L_K."

widely and the pattern is not the same in the different intervals. For comparing the numbers of λ·P progeny recombinants formed in the various cases, the most convenient index is the ratio of the λ·P recombination frequency to the total recombination frequency (the "relative frequency" shown in column F).

The maximum possible recovery of λ·P recombinants in each interval is defined by the results of the crosses in which both selected markers were contained in λ·P parental phages (cases a in Table B and Fig. C). By contrast, the relative frequency of λ·P recombinants is quite low when the selected marker distal to the end of the chromosome is provided by a λ·K parental phage (cases b in Table B and Fig. C). When the selected marker proximal to the end of the chromosome is contributed by a λ·K parent (cases c in Table B and Fig. C), the type P HCM label is well preserved by recombinants in the susQ73-susR5 interval near the end of the chromosome, but there are fewer labeled progeny recombinant in the adjacent susP3-susQ73 interval, and there is as little formation of λ·P recombinant in the sus029-susP3 interval as when the HCM specificities of the infecting phage types are reversed. The results in the latter case are in essence the same as those for the h-c interval, which brackets the center of the chromosome.

A principal conclusion to be drawn from Table B is summarized schematically in Fig. D, which represents the inferred composition and HCM specificities of the selected recombinants from the crosses of λ·P and λ·K typical of case c in Fig. C. The susP3 and susQ73 loci bracket the internal terminus of the segments of λ·K DNA that can be accepted on the right end of a singly recombinant molecule retaining the type P HCM label. Recombination between either b or c configuration of λ·P



DBL 696-4747

Figure D. The HCM specificities inferred (from the data in Table B) for the singly recombinant genomes typical of case c in Fig. C that are formed in crosses over three adjacent intervals on the right arm of the λ chromosome. The relative frequencies of HCM-labeled λγ+β+•P recombinants in the different intervals imply that the interior limit of the critical length L_K (Fig. C, text) lies between the susP3 and susQ73 loci.

and λ·K molecules produces a low absolute frequency of "λ·P" recombinant in the sus029-susP3 interval, which is attributable to double crossovers (cf., section AII-2 below).

For each interval, the potential for inclusion of unlabeled DNA in a selected λ·P recombinant naturally affects the relative λ·P recombination frequency observed for crosses of two λ·P parental phage types (cases a in Table B and Fig. C), since it determines the extent to which recombination of parental λ·P and newly synthesized λ·K phages will contribute to the assay for recombinants that retain the parental HCM specificity. Indeed, the data in Table B furnish indirect proof that parental DNA strands participate in vegetative recombination throughout the latent period. The relative frequencies of λ·P recombinants (column F) for the case a and case c crosses of λsusQ73 with λsusR5 imply that the frequency of progeny that contain predominantly conserved or semi-conserved parental DNA and are recombinant in this interval is about 1.2 times the frequency of these recombinants among the entire progeny population. Assuming that this relation holds throughout the genetic map, the relative frequencies of λ·P recombinants observed in the other intervals examined in Table B indicate that no more than 10% - 20% of the recombinations of λ·P phage are with a λ·P partner, the remainder occurring between a λ·P and a newly synthesized phage.

The average length of the DNA segments from a λ·K molecule that may be incorporated on the right end of a "λ·P" recombinant chromosome can be estimated if one assumes that the probability of a single crossover is uniform through the susP3-susQ73 interval.*

---

* This assumption is most likely an oversimplification of the true case, since for as yet unknown reasons the susP3-susQ73 interval exhibits an unusually high ratio of physical:genetic lengths (120).

From Table B, the frequency of "λ+·P" recombinant progeny formed in crosses of λsusP3·K with λsusQ73·P (case c) is 0.7% while the total frequency of λ·P parental phage that recombine for these markers is about 1.2 x 2.5 = 3%. According to the mapping function for λ (2), the former recombination frequency corresponds to an interval about 1/7 the absolute genetic length (in crossover units) of the susP3-susQ73 interval. This interval is presumably that portion of the susP3-susQ73 interval proximal to the chromosome end, within which λ·K DNA may be incorporated without loss of λ·P HCM specificity (Fig. D). The susP3 and susQ73 loci have been estimated to be about 36% and 7%, respectively, of the length of the molecule from the right end of the chromosome (120). Therefore the average total length of the λ·K DNA segment that may be incorporated on the right end of the λ·P chromosome is on the order of (36-7)/7 + 7 = 11% of the chromosome.* If this segment of λ·K DNA is permissible in a chromosome retaining the type P specificity only because there are no sites of P1-mediated HCM in this interval, this calculation may be regarded as a crude mapping of the location of that site of modification nearest to the right end of the molecule.

2) HCM-labeled double recombinants

The resolution of the assays for λ·P phage types is such that even the lower λ·P recombination frequencies reported here in Table B are indicative of a real formation of λ+·P recombinants in the

---

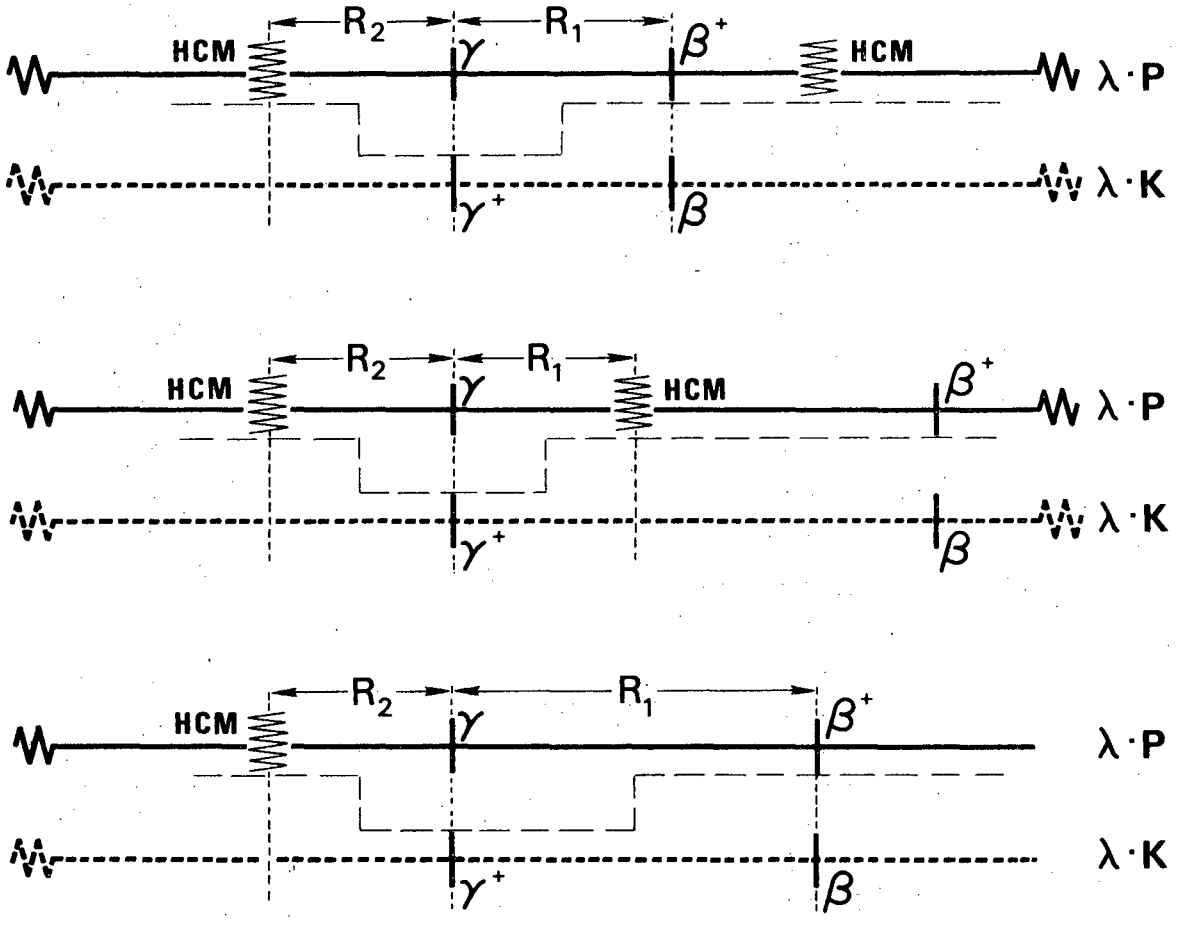
* An analogous estimate utilizing the data of Arber and Dussoix (8) for a single cross over the c-mi interval yields a similar value. These approximations of the length of the permissible λ·K DNA segment may be high, as more recent and direct physical mapping of the λ chromosome (235) indicates that the cited values for the physical distances from the susP3 and susQ73 loci to the end of the chromosome are probably overestimated by as much as 100%.

crosses rather than a reflection of background noise in the assay system.* The λ+·P recombinants detected in the case b crosses (and in the case c crosses of λsus029 with λsusP3) are probably attributable to the incorporation by multiple recombination of a segment of λ·K DNA into the interior of a λ·P chromosome. The assay for λ·P recombinant progeny from these crosses of λ·P with λ·K parental phages is a selection for phages that are doubly recombinant, with crossovers in adjacent intervals on each side of the selected λ·K locus, so that the selected marker from the λ·K phage type is included within a chromosome that retains the critical sites of P1-mediated HCM from a parental λ·P strand as well as the selected allele from the λ·P phage type. This interpretation is illustrated schematically in Fig. E.

There are several indications that most of these HCM-labeled double recombinants of λ·P with λ·K phages are formed in the course of a single mating event. Even over very long intervals of the λ chromosome, a threefold excess of double recombinants is observed over that expected if single recombinations in two independent matings were necessary to generate a double crossover (122, 2). In the present case, an upper bound on the combined lengths of the intervals in which the two crossovers must occur is set by the locations of the nearest required sites of P1-mediated modification adjacent on each side to the locus of the selected λ·K marker. If there are 5-10 critical sites of HCM more or less evenly distributed along the chromosome, the maximum length of a λ·K segment that could be integrated into a λ·P chromosome

---

* See Table III (section II-I.) in text and footnote on the first pages of this appendix.



DBL 696-4748

Figure E. A schematic illustration of different ways in which segments of unmodified DNA including particular markets (γ+) might be integrated by double recombination into the interior of an HCM-labeled (β+) chromosome. The lengths of the two intervals R_1 and R_2 in which crossovers must occur to generate an HCM-labeled recombinant genome would vary according to the relative dispositions of the selected γ+ and β+ markers and adjacent sites of HCM in the labeled DNA molecule. These factors would determine the frequency of HCM-labeled double recombinants relative to the usual recombination frequency between the genetic markers.

would be 10% - 20% of the molecular length. Most double crossovers within such short intervals of the λ chromosome are due to the clustering of genetic exchanges in a single mating, i.e., the generalized negative interference is augmented by a high, localized negative interference (2).

This inference can be verified directly from the data in Table B. Recall that the frequency of progeny containing a strand of predominantly parental DNA that are genetically recombinant for the markers crossed--regardless of whether the recombinants have retained a λ·P HCM label--is about 1.2 times the recombination frequency among the entire progeny population (Appendix II-1 above). Then for the relevant crosses the relative frequency in column F divided by 1.2 indicates the freguency with which a crossover in the interval between the genetic markers is correlated with a second crossover outside the selected λ·K allele to preserve the λ·P specificity of the recombinant genome. For crosses of λsusQ73·K with λsusR5·P (case b), recombination in the susQ73-susR5 interval is correlated with recombination in the interval between the susQ73 locus and the adjacent HCM site to the left in about (0.12/1.2)x100 = 10% of the cases. From the λ·P recombination frequency for crosses of λsusP3·K with λsusQ73·P (case c), the frequency of single recombinants from λ·P and λ·K phages in the interval between the susQ73 locus and this HCM site can be estimated as about 0.7% (cf., Appendix II-1), which would be the expected frequency of correlation in the absence of interference. Since the generalized negative interference should enhance this correlation by only about a factor of three (122, 2), a further fivefold excess of double crossovers is attributable to high, localized negative interference.

It should be remarked that during most of the latent period of the phage infection, chromosomes carrying a parental λ·P HCM label are a minority species within the K12 host cells because of the accumulation of newly synthesized λ·K genomes. A polarity is thus inherent in the recombination of λ·P with λ·K phages in a K12 host, since any particular genome will be more likely to mate with a λ·K chromosome than with a λ·P genome. For example, it has been noted that no more than 10% - 20% of the recombinations of λ·P chromosomes involve a λ·P partner, the balance occurring between λ·P and λ·K partners. This natural polarity is antagonistic to the formation of HCM-labeled double recombinants of λ·P with λ·K phage types by independent crossovers, which would require the participation of a single λ·K genome in each of two successive matings with different λ·P chromosomes.

The sketches in Fig. E illustrate that, for a particular cross, the apparent efficiency with which HCM-labeled recombinants are formed by double crossovers between labeled and unlabeled genomes may depend on the length of the interval between the genetic markers examined, the disposition of required modification sites adjacent to the locus of the selected marker from the unlabeled (λ·K) chromosome, and the length in relation to these intervals of the clusters of genetic exchanges that give rise to double crossovers. Variations in these factors no doubt account for the different frequencies with which such doubly recombinant progeny are formed in the different crosses.

For intervals toward the <u>center</u> of the chromosome, the fraction of λ·P recombinant progeny from crosses of two λ·P parental phage types that is attributable to double crossovers between λ·P and newly synthesized λ·K genomes is indicated by the λ·P recombination frequencies

for case b plus case c crosses relative to case a crosses. The results in Table B imply that for crosses of λsus029·P with λsusP3·P approximately 75% of the λ+·P recombinant progeny are formed in recombination events that involve only one λ·P chromosome. Although recombinants formed from two λ·P phages may preserve the HCM label with 100% efficiency while recombinants formed by crossovers between λ·P and λ·K phages only retain the HCM label with an efficiency on the order of 5%, events of the latter type occur much more frequently within the host cell, so that in this instance they contribute a majority of the λ+·P recombinant progeny.

Appendix III: An Hypothesis Concerning the Nature of UV-Inactivation and UV-Reactivation of Bacteriophage λ--A lethal defect which may occur in semi-conserved UV-irradiated genomes

Experimental results shown in Figs. 26 and 27 and discussed in section IV-C of the text indicate that with increasing UV dose to λ·P phages multiply infecting E. coli K12 bacteria there is a marked decrease in the relative recovery of HCM-labeled λ·P progeny phages. Assays of the relative viability on HCR+ and HCR- indicators of those λ·P progeny which are recovered from excision-defective uvrA host cells suggest that the HCM-labeled progeny contain mainly conserved parental DNA (section IV-C.1). In contrast, direct measurements in other laboratories (8, 157, 120) indicate that most of the HCM-labeled progeny from comparable multiple infections with unirradiated phages contain semi-conserved parentl DNA.

These findings may be viewed in light of circumstantial evidence that UV photoproducts can be bypassed during the replication of irradiated λ phage DNA (section III-A.2) and of observations that UV-irradiated λ DNA is subject to nuclease degradation within the host bacteria which is associated only in part with excision-repair processes (127, 50, 183). A primary component of the UV-induced decrease in λ·P recovery could be due to a selection against recovery of semi-conserved parental DNA that derives from susceptibility to degradative nuclease attack of defects caused by replication-bypass of UV photoproducts (section IV-C.2). Although other explanations certainly cannot be excluded, this interpretation has the merit of suggesting a more general hypothesis which would delineate the nature of a major lethal defect that may occur

in semi-conserved UV-irradiated DNA and would account for the phenomenon known as UV-reactivation and perhaps for certain aspects of UV mutagenesis. Before detailing this hypothesis further, it is worthwhile to briefly review some pertinent characteristics of UV-reactivation.

The term UV-reactivation (UV-R) denotes the phenomenon in which light UV-irradiation of the host bacteria enhances the survival of UV-irradiated dependent coliphages (230, 129, 183). Under optimum conditions (ca. 500 ergs/mm^2 to HCR+ host cells), on the order of 50% of lethal UV damage to λ phages can be neutralized by this effect. On the other hand, high UV doses to the host bacteria cause a marked decrease in the relative survival of irradiated dependent phages (67, 129, 154), presumably because the depression of phage survival caused by UV damage to the HCR capacity of the host cells exceeds any enhancement of survival effected by UV-R. Thus dose-response curves for UV-R, in which the survival in single infection of phages irradiated with a constant UV dose is plotted against the UV dose to the host bacteria, are typically bell-shaped and are characterized by a maximal UV-reactivation effect for a relatively low, optimum dose to the host.

The processes of UV-reactivation and host cell reactivation are not independent of each other (80). Typically, the optimum dose for UV-R is substantially lower, and the maximum reactivation effect is significantly less, in excision-defective HCR- host bacteria than in HCR+ host strains (80)--although there is some phenotypic variation among the different excision-defective mutants (154, 182). In view of the apparent correlation between occurrence of HCR and UV-R and the observations concerning nuclease degradation of UV-irradiated λ DNA,

Harm has advanced the proposal that UV-reactivation is due to perturbation of an equilibrium in unirradiated host bacteria between antagonistic processes of HCR and nuclease degradation of the irradiated phage DNA (80, 183); under the appropriate conditions, UV photoproducts in the bacterial (or other heterologous) DNA might facilitate survival of an irradiated dependent phage genome by preferentially inhibiting nuclease degradation, relative to HCR, of the phage DNA. There are several difficulties with this hypothesis, but evidence has recently become available to permit its further elaboration with regard to the UV-reactivation of λ bacteriophage.

First, HCR- bacterial strains mutant at the uvrD locus (Table I), which are not defective in excision but are unable to perform a later step of HCR, exhibit characteristics similar to those of uvr+ bacteria with respect to the UV-reactivation of λ (168). This implies that it is the excision function rather than the overall process of HCR (excision-repair) which plays a role in the mechanism of UV-reactivation.

Second, UV-reactivation of λ is not observed in recA mutant host bacteria (168, 134), implying that a process dependent on the recA allele of the cell is also an essential element in the mechanism for UV-reactivation of λ phage. The different characteristics for UV-reactivation of λ in recA and uvrD mutant hosts supports the view (section III-A.2) that the recA allele affects a DNA repair mode in λ other than excision-repair. As discussed in the text (cf., sections I-B.2, 3), it has been proposed that certain REC- mutations prevent successful post-replication repair in the bacterial DNA of defects--thought to be single-strand gaps in a newly synthesized DNA strand, apposed to unexcised pyrimidine dimers in the template strand--which

result from replication-bypass of UV photoproducts (184, 109, 108); and there is some evidence for a similar replication-bypass/post-replication repair mode, partially dependent on the recA allele of the host bacteria, in UV-irradiated λ phage DNA (section III-A.2).

These observations suggest that the equilibrium between antagonistic processes which is perturbed in UV-reactivation is not between host cell reactivation and nuclease degradation of the irradiated phage DNA, but between excision and degradative functions on the one hand and post-replication repair processes involving the recA gene on the other, and that part of the UV-induced decrease in recovery of HCM-labeled progeny phages is a reflection of the degradative activity in unirradiated host bacteria. A way in which these processes might interact is described below.

Genomes containing DNA with gaps caused by replication-bypass of UV photoproducts would exhibit a marked susceptibility to inactivation by fragmentation and/or nuclease degradation, even in bacteria proficient in post-replication repair, if healing of the gaps could be delayed or prevented by the intervention of a third factor -- to wit, the occasional binding of excision endonuclease to a bypassed photoproduct in the parental DNA strand opposite an unrepaired gap in the newly synthesized complementary DNA strand. While the excision enzyme could not act to correct the photoproduct in the single-stranded substrate (182, 249, 109), its presence might inhibit the post-replication repair processes from closing the apposed gap before the onset of irreversible nuclease degradation, or it might occasionally cut the single intact strand (172b) and thereby cause a double-strand scission of the genome.

A competition between post-replication repair processes on the one hand and excision and degradative functions on the other might thus influence the fate of the double-stranded defect resulting from replication-bypass. Whereas there could be a successful closing of the gap followed by excision-repair of the bypassed photoproduct in the parental DNA strand, the binding of excision enzyme to a photoproduct prior to healing of the apposite gap would sometimes preclude the realization of either repair operation. The sequence would influence whether both elements of the double-stranded defect could be corrected or whether interference among repair processes would render the molecule inviable and vulnerable to nuclease degradation proceeding from an attack upon the "stabilized" gap or following a double-strand scission.

The frustration of post-replication repair of <u>one</u> or more such double-strand defects in each of the daughter chromosomes of an irradiated genome would cause it to be inviable even though it had been able to complete an initial round of replication past the unexcised UV photoproducts. Any perturbation that facilitates post-replication repair of just the <u>initial</u> replicas of an irradiated λ phage genome, so that a further round of replication will result in at least one intact and viable chromosome composed entirely of newly synthesized DNA, would increase the assayed survival of the irradiated infecting phages. Thus light UV-irradiation of the host bacteria might at least temporarily effect a competitive inhibition of the binding of excision enzyme to bypassed photoproducts and of nuclease activity; for relatively low UV doses to the cells, the consequent enhancement of phage survival could more than offset the effect of UV damage to the overall HCR and post-replication repair capacities of the host bacteria. Such a phenomenon

would correspond precisely to the observed UV-reactivation of λ phages.

The details of this model must of course be viewed as speculative, since at present little is known concerning the exact enzymatic reactions which effect excision of photoproducts and healing of bypass gaps; however, this scheme is perfectly consistent with the experimental observations noted above:

(a) The UV-R of λ phage is smaller and occurs at lower UV doses to the host for excision-defective HCR- (uvrA, uvrB, and uvrC) host bacteria than for excision-proficient HCR+ and uvrD host strains (80, 182, 168). This can be understood if unexcised photoproducts in the host DNA competitively inhibit binding of excision enzyme to lesions in the phage, while the sector of potentially lethal damage in the phage that is susceptible to UV-reactivation is dependent upon the activity of the host excision function. The exact UV-R phenotype of various excision-defective mutant hosts would vary according to the ability retained by the mutant enzyme to bind, and perhaps to cut, the irradiated DNA.

(b) UV-R of λ phage does not occur in recA host bacteria (168, 134), which may be defective in cellular post-replication repair processes in any case (184, 109). The relevance to UV-R of any component of post-replication repair mediated by the red genes of the phage is not clear; apparently the UV-R of λred mutant phages has not yet been investigated.

(c) The hypothesis predicts that, due to nuclease attack at the sites of "stabilized" double-strand defects, there would be a substantially lower recovery of semi-conserved parental DNA in the progeny from infections with UV-irradiated than with unirradiated phages, consistent with the UV-induced decrease in recovery of HCM-labeled progeny phages indicated by the results in Figs. 26-28 and discussed in section IV-C.

Furthermore, the degradation of irradiated DNA in both HCR+ and HCR- cells can be suppressed by UV-irradiation of the host bacteria (50).

The details of the selection against semi-conserved irradiated DNA would differ somewhat in the multiple infections of the wild type uvr+ and the excision-defective uvrA host strains (section IV-C). Assuming for simplicity that at similar survivals of the infecting phages in the two host strains comparable numbers of UV photoproducts are bypassed during initial replications of the parental genomes, the efficiency of inhibition of post-replication repair events in the initial replicas would presumably be lower in the uvrA host, which retains only residual excision activity, than in the uvr+ host. On the other hand, in the uvr+ host strain photoproducts in the parental DNA strands not eliminated prior to the initial replication, and not trapped in stabilized double-strand defects in the initial replicas, would ultimately be removed from the parental DNA by continuing HCR; in the uvrA strain, the repeated replication of parental DNA strands with unexcised pyrimidine dimers would amplify the opportunity for inhibition of post-replication repair and/or nuclease action at the sites of bypass gaps. Thus, relative to the UV survivals of the infecting phages, the overall loss of semi-conserved parental DNA in progeny phages need not be much more pronounced in the uvr+ host than in the uvrA host strain--consistent with the kinetics that characterize the UV-induced decreases in recovery of λ·P progeny shown in Figs. 26 and 27.

The general hypothesis outlined above is also pleasingly compatible with several other key experimental findings;

(d) The UV-reactivation effect observed for the single-stranded DNA coliphages S13 and ØX174 is distinctly different from the bell-shaped dose-response found for double-stranded dependent phages; with

increasing UV dose to wild type host bacteria the survival of the single-stranded phages extracellularly irradiated with a constant UV dose rises to a plateau (217, 183). The extracellularly irradiated single-stranded genomes are not repairable by HCR (182, 249), but it seems likely that UV photoproducts can be "bypassed" during synthesis of the initial double-stranded replicative form (RF) on an irradiated template since the ØX174 viability is inactivated at a rate fivefold greater than RF formation (151) and these phages exhibit UV-enhanced recombination (219, 185); from these facts one might infer that the viability of a genome with UV photoproducts in only one strand is not enhanced by excision-repair of those lesions. Irradiation of the host would inhibit the stabilization of double-strand defects in the initial RF due to binding of excision enzyme, but at high UV doses to the cells the UV damage to the HCR capacity of the host would not significantly affect phage survival. Hence the UV-R curves for S13 and ØX174 would be expected to rise to a plateau with increasing UV dose to the host, as is observed experimentally.

(e) It has recently been proposed that UV-induced mutations in the bacterial genome may be a consequence of errors in post-replication healing of gaps that result in newly synthesized DNA strands from the replication-bypass of photoproducts in the template strand (243a, 28). This idea is apparently supported by the findings that UV-induced mutations do not occur in E. coli B exr- cells (242, 28), E. coli K12 recA bacteria (163, 243b), or in λ phage infecting recA host cells (163)--where these bacterial mutants are thought to be deficient in post-replication repair processes. One might then anticipate that a perturbation which would enhance the efficiency of post-replication

repair in λ phage would also increase the frequency of mutation in the irradiated phage DNA. Consistent with this expectation and the mechanism for UV-R suggested above, light UV-irradiation of the host bacteria enhances the frequency of mutations induced by UV-irradiation of the infecting λ phages (230, 129, 163) just as it simultaneously enhances the phage viability.

(f) Finally, also consistent with the general model is the effect of caffeine upon each of the component phenomena considered above. Treatment of the λ infected complexes with caffeine causes a suppression of HCR (84), a decrease in the optimum dose to the host cells for UV-R (183), an enhancement of UV-induced mutation--as assayed in bacteria--(145), and a virtually complete inhibition of nuclease degradation of UV-irradiated DNA (50). In the context of the hypothesis outlined above, all of these results are explicable on the basis of a caffeine inhibition of the binding of excision enzyme to UV photoproducts in the UV-irradiated DNA.

The magnitude of the UV-reactivation effect for UV-irradiated λ phages in wild type host bacteria (230, 80) implies that 50% or more of the lethal UV hits to λ phage infecting unirradiated host bacteria may be attributable to stabilized double-strand defects of the type envisioned above, in which binding of excision endonuclease to a bypassed photoproduct in semi-conserved parental DNA forestalls the post-replication healing of a gap at the site of bypass and invites nuclease degradation of the genome. An impressive characteristic of this model is the implication that the UV-induced decrease in recovery of HCM-labeled infecting phage DNA, UV-reactivation, and the efficiency of UV

mutagenesis may all be related to a single complex interaction of excision-repair functions, the recA dependent λ repair mode, and degradative action on λ phage DNA that has been replicated following UV-irradiation.

The general model proposed here might also account for a portion of the lethal damage to UV-irradiated bacteria (for which there is no opportunity to observe such phenomena as UV-reactivation). From experiments concerning the dependence upon dose rate of the UV-inactivation of E. coli, Harm has shown that excision-repair of a lesion while the irradiated cells are held in buffer may be inhibited by the presence of additional repairable lesions and inferred that many of the lethal hits to bacteria irradiated at high dose rates may be attributable to interference between individual excision-repair events (85). Such interference might derive from interactions closely analogous to those suggested above. It is possible that interference between excision- and post-replication repair processes might account for the sector of lethal hits which Harm observed to be independent of dose following the achievement of an equilibrium between inactivation and repair at low dose rates.

These ideas must remain speculative at least until further information is available concerning the intracellular enzymatic reactions which are involved in excision and, in particular, post-replication repair. But it would indeed be ironic if a principal source of inactivating UV damage in microbial organisms were lesions occasioned by interference between DNA repair processes striving to preserve the integrity of the genome.

REFERENCES

1. Adams, M. H., Bacteriophages, Interscience Publishers, Inc., New York (1959).

2. Amati, P., and M. Meselson, Localized negative interference in bacteriophage lambda. Genetics 51, 369-379 (1965).

3. Anraku, N., and J. Tomizawa, Molecular mechanisms of genetic recombination in bacteriophage, III. Joining of parental polynucleotides of phage T4 in the presence of 5-fluorodeoxyuridine. J. Mol. Biol. 11, 501-508 (1965).

4. Anraku, N., and J. Tomizawa, Molecular mechanisms of genetic recombination of bacteriophage, V. Two kinds of joining of parental DNA molecules. J. Mol. Biol. 12, 805-815 (1965).

5. Appleyard, R. K., Segregation of new lysogenic types during growth of the doubly lysogenic strain derived from E. coli K-12. Genetics 39, 440-452 (1954).

6. Arber, W., Host-controlled modification of bacteriophage. In Annual Review of Microbiology, vol. 19, 365-378 (1965).

7. Arber, W., Host specificity of DNA produced by E. coli, V. The role of methionine in the production of host specificity. J. Mol. Biol. 11, 247-256 (1965).

8. Arber, W., and D. Dussoix, Host specificity of DNA produced by E. coli, I. Host controlled modification of bacteriophage lambda. J. Mol. Biol. 5, 18-36 (1962).

9. Arber, W., S. Hattman, and D. Dussoix, On the host-controlled modification of bacteriophage lambda. Virology 21, 30-35 (1963).

10. Baker, R. M., unpublished data.

11. Baker, R. M. and R. H. Haynes, UV-induced enhancement of recombination among lambda bacteriophages in UV-sensitive host bacteria. Molec. Gen. Genetics 100, 166-177 (1967).

12. Baker, R. M., R. H. Haynes, and D. M. Freifelder, UV-induced enhancement of recombination among lambda bacteriophages in UV-sensitive host cells (abstract). Radiation Res. 31, 625 (1967).

13a. Baker, R. M., G. E. Jones, and R. H. Haynes, Recombination and reactivation of lambda phages treated with UV and nitrogen mustard (abstract). Biophysical J. 8A, 56 (1968).

13b. Baker, R. M., G. E. Jones, and R. H. Haynes, in preparation.

14. Bautz, F. A., and E. K. F. Bautz, Transformation in phage T4: minimal recognition length between donor and recipient DNA. Genetics 57, 887-895 (1967).

15. Beers, W., A. Cerami, and E. Reich: An experimental model for internal denaturation of linear DNA molecules. Proc. Nat'l. Acad. Sci., U.S., 58, 1624-1631 (1967).

16. Belling, J., Chromomeres of lillaceous plants. Univ. Calif. Publs. Botany, 16, 153 (1931).

17. Bernstein, H. and M. W. Baldy, Genes affecting recombination and repair in phage T4. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

18. Bertani, G., Lysogeny. In Advances in Virus Research, vol. 5, 151-193 (1958).

19. Bertani, G. and J. J. Weigle, Host controlled variation in bacterial viruses. J. Bacteriol. 65, 113-121 (1953).

20. Bode, V. C. and A. D. Kaiser, Changes in the structure and activity of lambda DNA in a super-infected immune bacterium. J. Mol. Biol. 14, 399-417 (1965).

21. Bodmer, W. F., Recombination and integration in Bacillus subtilis transformation: involvement of DNA synthesis. J. Mol. Biol. 14, 534-557 (1965).

22. Bollum, F. and R. Setlow, Ultraviolet inactivation of DNA primer activity. I. Effects of different wavelengths and doses. Biochim. Biophys. Acta 68, 599-607 (1963).

23a. Boyce, R. P. and J. W. Farley, Production of single-strand breaks in covalent circular lambda phage DNA in superinfected lysogens by monoalkylating agents and the joining of broken DNA strands. Virology 35, 601-609 (1968).

23b. Boyce, R. P. and P. Howard-Flanders, Release of ultraviolet light-induced thymine dimers from DNA in E. coli K12. Proc. Nat'l. Acad. Sci, U.S., 51, 293-300 (1964).

24a. Boyce, R. and P. Howard-Flanders, Genetic control of DNA breakdown and repair in E. coli treated with mitomycin C or ultraviolet light. Z. Vererbungsl. 95, 345-350 (1964).

24b. Boyce, R. P. and M. Tepper, X-ray-induced single-strand breaks and joining of broken strands in superinfecting lambda DNA in E. coli lysogenic for lambda. Virology 34, 344-351 (1968).

25. Boyer, H. W. and D. Roulland-Dussoix, A complementation analysis of the restriction and modification of DNA in E. coli. J. Mol. Biol. 41, 459-472 (1969).

26. Bridges, B. A., and R. J. Munson, The persistence through several replication cycles of mutation producing pyrimidine dimers in a strain of E. coli deficient in excision-repair. Biochem. biophys. Res. Commun. 30, 620-624 (1968).

27. Bridges, B. A., M. J. Ashwood-Smith, and R. J. Munson, Susceptibility of mild thermal and ionizing radiation damage to the same recovery mechanisms in E. coli. Biophys. Biochem. Res. Commun. 35, 193-196 (1969).

28. Bridges, B. A., J. Law, and R. J. Munson, Mutagenesis in E. coli II. Evidence for a common pathway for mutagenesis by ultraviolet light, ionizing radiation, and thymine deprivation. Molec. Gen. Genetics 103, 266-273 (1968).

29. Brooks, K., Studies in the physiological genetics of some suppressor-sensitive mutants of bacteriophage lambda. Virology, 26, 489-499 (1965).

30. Brooks, K. and A. J. Clark, Behavior of lambda bacteriophage in a recombination-deficient strain of E. coli. J. Virology 1, 283-293 (1967).

31. Brown, A. and W. Arber, Temperature-sensitive mutants of coliphage lambda. Virology 24, 237-239 (1964).

32. Campbell, A., Sensitive mutants of bacteriophage lambda. Virology, 14, 22-32 (1961).

33. Campbell, A., Episomes. In Advances in Genetics, vol. 11, 101-145 (1962).

34. Campbell, A. and A. del Campillo-Campbell, Mutant of lambda bacteriophage producing a thermolabile endolysin. J. Bacteriol. 85, 1202-1207 (1963).

35. Caro, L. D., The molecular weight of lambda DNA. Virology 25, 226-236 (1965).

36. Carrier, W. and R. Setlow, Excision of pyrimidine dimers from irradiated deoxyribonucleic acid in vitro. Biochem. Biophys. Acta 129, 318-325 (1966).

37. Clark, A. J., The beginning of a genetic analysis of recombination proficiency. J. Cell. Physiology 70, Suppl. 1 part II, 165-180 (1967).

38. Clark, A. J. and A. D. Margulies, Isolation and characterization of recombination-deficient mutants of E. coli K12. Proc. Nat'l Acad. Sci. U.S., 53, 451-459 (1965).

39. Clark, A. J., M. Chamberlin, R. P. Boyce, and P. Howard-Flanders, Abnormal metabolic response to ultraviolet light of a recombination-deficient mutant of E. coli K12. J. Mol. Biol. 19, 442-454 (1966).

40. Cummings, D. J. and L. Mondale, Thymineless death in E. coli: strain specificity. J. Bacteriol. 93, 1917-1924 (1967).

41. Curtiss, R., III, Ultraviolet-induced genetic recombination in a partially diploid strain of E. coli. Genetics 58, 9-54 (1968).

42. Darlington, C. D., The time, place and action of crossing over. J. Genet. 31, 185 (1935).

43. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman, A proposal for a uniform nomenclature in bacterial genetics. Genetics 54, 61-76 (1966).

44. Denhardt, D. T., D. H. Dressler, and A. Hathaway, The abortive replication of ØX174 DNA in a recombination-deficient mutant of E. coli. Proc. Nat'l. Acad. Sci., U.S., 57, 813-820 (1967).

45. Devoret, R. and T. Coquerelle, Multiplication of phage lambda in bacteria unable to repair ultraviolet lesions. In Proceedings of the Conference on Genetical Aspects of Radiation Sensitivity: Mechanisms of Repair, pp. 89-95, International Atomic Energy Agency, Vienna (1966).

46. Devoret, R. and J. George, Induction indirecte du prophage lambda par le rayonnement ultraviolet. Mutation Res. 4, 713-734 (1967).

47. Doerfler, W. and D. S. Hogness, Gene orientation in bacteriophage lambda as determined from the genetic activities of heteroduplex DNA formed in vitro. J. Mol. Biol. 33, 661-678 (1968).

48. Dove, W., Action of the lambda chromosome, I. Control of functions late in bacteriophage development. J. Mol. Biol., 19, 187-201 (1966).

49. Dove, W. F., The genetics of lambdoid phages. In Annual Review of Genetics, vol. 2, 305-340 (1968).

50. Dussoix, D. Roulland, Degradation par la cellule hote du DNA du bacteriophage lambda irradie par le rayonnement ultraviolet. Mutation Res. 4, 241-252 (1967).

51. Dussoix, D. and W. Arber, Host specificity of DNA produced by E. coli, II. Control over acceptance of DNA from infecting phage lambda. J. Mol. Biol. 5, 37-49 (1962).

52. Dussoix, D., and W. Arber, Host specificity of DNA produced by E. coli, IV. Host specificity of infectious DNA from bacteriophage lambda. J. Mol. Biol. 11, 238-246 (1965).

53. Echols, H. and R. Gingery, Mutants of bacteriophage lambda defective in vegetative genetic recombination. J. Mol. Biol. 34, 239-249 (1968).

54. Echols, H. and A. Joyner, The temperate phage. In The Molecular Basis of Virology (H. Frankel-Conrat, ed.), pp. 532-575, Reinhold, New York (1968).

55. Echols, H., R. Gingery, and L. Moore, Integrative recombination function of bacteriophage lambda: evidence for a site-specific recombination enzyme. J. Mol. Biol. 34, 251-260 (1968).

56. Ellison, S. A., R. R. Feiner, and R. F. Hill, A host effect on bacteriophage survival after ultraviolet irradiation. Virology, 11, 294-296 (1960).

57. Emmerson, P. T., Recombination-deficient mutants of E. coli K12 that map between thyA and argA. Genetics 60, 19-30 (1968).

58. Epstein, H. T., Studies of phage growth in irradiated cells. Arch. Biochem. Biophys. 86, 286-293 (1959).

59. Epstein, R. H., A study of multiplicity-reactivation in the bacteriophage T4, I. Genetic and functional analysis of T4D-K12(λ) complexes. Virology 6, 382-404 (1958).

60. Esposito, R. E. and R. Holliday, The effect of 5-fluorodeoxyuridine on genetic replication and mitotic crossing over in synchronized cultures of Ustilago maydis. Genetics 50, 1009-1017 (1964).

61. Fogel, S. and R. K. Mortimer, Informational transfer in meiotic gene conversion. Proc. Nat'l. Acad. Sci., U.S., 62, 96-103 (1969).

62. Folsome, C. E., Topographical effects of ultraviolet irradiation upon recombination in phage T4. Z. Vererbungsl. 93, 404-416 (1962).

63. Fox, M. S. and M. K. Allen, On the mechanism of deoxyribonucleate integration in pneumococcal transformation. Proc. Nat'l Acad. Sci., U.S., 52, 412-419 (1964).

64. Fry, B. A., Conditions for the infection of E. coli with lambda phage and for the establishment of lysogeny. J. Gen. Microbiol. 21, 676-684 (1959).

65. Gallant, J. and T. Spottswood, The recombinogenic effect of thymidylate starvation in E. coli merodiploids. Genetics 52, 107-118 (1965).

66. Ganesan, A. K., and K. C. Smith, Recovery of recombination deficient mutants of E. coli K-12 from ultraviolet radiation. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

67. Garen, A. and N. D. Zinder, Radiological evidence for partial genetic homology between bacteriophage and host bacteria. Virology 1, 347-376 (1955).

68. Geissler, E., Reactivation of photodynamically inactivated lambda phages. Molec. Gen. Genetics 103, 233-237 (1968).

69. Gellert, M., Formation of covalent circles of lambda DNA by E. coli extracts. Proc. Nat'l. Acad. Sci., U.S., 57, 148-155 (1967).

70. Gellert, M., J. W. Little, C. K. Oshinsky, and S. B. Zimmerman, Joining of DNA strands by the DNA ligase of E. coli. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

71. Glover, S. W., J. Schell, N. Symonds, and K. A. Stacey, The control of host-induced modification by phage P1. Genet. Res., Camb. 4, 480-482 (1963).

72. Gold, M., M. Gefter, R. Hausmann, and J. Hurwitz, Methylation of DNA. J. Gen. Physiol. 49, No. 6 part 2, 5-28 (1966).

73. Gottesman, M. E. and M. B. Yarmolinsky, Integration-negative mutants of bacteriophage lambda. J. Mol. Biol. 31, 487-505 (1968).

74. Gough, M. and S. Lederberg, Methylated bases in the host-modified deoxyribonucleic acid of E. coli and bacteriophage lambda. J. Bacteriol. 91, 1460-1468 (1966).

75. Grossman, L., J. Kaplan, S. Kushner, and I. Mahler, Early enzymatic steps in repair of UV-irradiated DNA. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

76. Hanawalt, P. and R. Haynes, Repair replication of DNA in bacteria: irrelevance of chemical nature of base defect. Biochem. Biophys. Res. Commun. 19, 462-467 (1965).

77. Hanawalt, P. C., D. E. Pettijohn, E. C. Pauling, C. F. Brunk, D. W. Smith, L. C. Kanner, and J. L. Couch: Repair Replication of DNA in vivo. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

78. Harm, W., Multiplicity reactivation, marker rescue, and genetic recombination in phage T4 following X-ray inactivation. Virology 5, 337-361 (1958).

79. Harm, W., Mutants of phage T4 with increased sensitivity to ultraviolet. Virology 19, 66-71 (1963).

80. Harm, W., On the relationship between host-cell reactivation and UV-reactivation in UV-inactivated phages. Z. Vererbungsl. 94, 67-79 (1963).

81. Harm, W., On the control of UV-sensitivity of phage T4 by the gene x. Mutation Res. 1, 344-354 (1964).

82. Harm, W., Host-cell reactivation of non-lethal ultraviolet-effects. Photochem. Photobiol. 4, 575-585 (1965).

83. Harm, W., The role of host-cell repair in liquid-holding recovery of UV-irradiated E. coli. Photochem. Photobiol. 5, 747-760 (1966).

84. Harm, W., Differential effects of acriflavine and caffeine on various ultraviolet-irradiated E. coli strains and T1 phage. Mutation Res. 4, 93-110 (1967).

85. Harm, W., Effects of dose fractionation on ultraviolet survival of E. coli. Photochem. Photobiol. 7, 73-86 (1968).

86. Harm, W., Dark repair of acridine dye-sensitized photoeffects in E. coli cells and bacteriophage. Biochem. Biophys. Res. Commun. 32, 350-358 (1968).

87. Hayes, W. H., The Genetics of Bacteria and Their Viruses, John Wiley & Sons, Inc., New York (1964).

88. Haynes, R. H., The interpretation of microbial inactivation and recovery phenomena. Radiation Res., Suppl. 6, 1-29 (1966).

89. Haynes, R. H., R. M. Baker, and G. E. Jones, Genetic implications of DNA repair. In Energetics and Mechanisms in Radiation Biology (ed. G. Phillips), pp. 425-465, Academic Press, New York (1968).

90. Hershey, A. D., and R. Rotman, Genetic recombination between host-range and plaque-type mutants of bacteriophage in single bacterial cells. Genetics 34, 44-71 (1949).

91. Hershey, A. D., and M. Chase, Genetic recombination and heterozygosis in bacteriophage. Cold Spring Harbor Symp. Quant. Biol., 16, 471-479 (1951).

92. Hershey, A. D., E. Burgi, and L. Ingraham, Cohesion of DNA molecules isolated from phage lambda. Proc. Nat'l. Acad. Sci., U.S., 49, 748-755 (1963).

93. Hirsch-Kauffmann, M. and W. Sauerbier, Inhibition of modification and restriction for phages lambda and T1 by co-infecting T3. Molec. Gen. Genetics 102, 89-94 (1968).

94. Hogness, D. S., The structure and function of the DNA from bacteriophage lambda. J. Gen. Physiol. 49, No. 6, part 2, 29-57 (1966).

95. Holliday, R., The induction of mitotic recombination by mitomycin C in Ustilago and Saccharomyces. Genetics 50, 323-335 (1964).

96. Holliday, R., A mechanism for gene conversion in fungi. Genet. Res. Camb. 5, 282-304 (1964).

97. Holliday, R., Induced mitotic crossing over in relation to genetic replication in synchronously dividing cells of Ustilago maydis. Genet. Res. Camb. 6, 104-120 (1965).

98. Holliday, R., Studies on mitotic gene conversion in Ustilago. Genet. Res. Camb. 8, 323-337 (1966).

99. Holliday, R., Altered recombination frequencies in radiation sensitive strains of Ustilago. Mutation Res. 4, 275-288 (1967).

100. Holliday, R., Genetic recombination in fungi. In Replication and Recombination of Genetic Material (ed. W. J. Peacock), Australian Acad. Sci. (1968).

101. Horii, Z. I., and K. Suzuki, Degradation of the DNA of E. coli K12 Rec- (JC1569b) after irradiation with ultraviolet light. Photochem. Photobiol. 8, 93-105 (1968).

102. Howard-Flanders, P., Genes that control DNA repair and genetic recombination in E. coli. In Advances in Biological and Medical Physics, vol. 12, 299-317 (1968).

103. Howard-Flanders, P. and R. P. Boyce, A biochemical pathway in the repair of DNA after UV-irradiation that may have steps in common with those of genetic recombination by breakage-reunion (abstract). Genetics 50, 256-257 (1964).

104. Howard-Flanders, P. and R. P. Boyce, DNA repair and genetic recombination - studies on mutants of E. coli defective in these processes. Radiation Res., Suppl. 6, 156-184 (1966).

105. Howard-Flanders, P., R. P. Boyce, E. Simson, and L. Theriot, A genetic locus in E. coli K12 that controls the reactivation of UV-photoproducts associated with thymine in DNA. Proc. Nat'l. Acad. Sci., U.S., 48, 2109-2115 (1962).

106. Howard-Flanders, P., R. P. Boyce, and L. Theriot, Three loci in E. coli K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics 53, 1119-1136 (1966).

107. Howard-Flanders, P., and Lee Theriot, Mutants of E. coli K12 defective in DNA repair and in genetic recombination. Genetics 53, 1137-1150 (1966).

108. Howard-Flanders, P., L. Theriot, and J. B. Stedeford, Some properties of excision-defective recombination-deficient mutants of E. coli K-12. J. Bacteriol. 97, 1134-1141 (1969).

109. Howard-Flanders, P., W. D. Rupp, B. M. Wilkins, and R. S. Cole, DNA replication and recombination after UV-irradiation. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

110a. Howard-Flanders, P., personal communication (1968).

110b. Huskey, R. J., Multiplicity reactivation as a test for recombination function. Science 164, 319-320 (1969).

111. Ihler, G., and M. Meselson, Genetic recombination in bacteriophage lambda by breakage and joining of DNA molecules. Virology 21, 7-10 (1963).

112. Inman, R. B., A denaturation map of the lambda phage DNA molecule determined by electron microscopy. J. Mol. Biol. 18, 464-476 (1966).

113. Jacob, F. and J. Monod, Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. 3, 318-356 (1961).

114. Jacob, F. and E. L. Wollman, Etude genetique d'un bacteriophage tempere d'Escherichia coli, I. Le systeme genetique du bacteriophage. Ann. Inst. Pasteur 87, 653-673 (1954).

115. Jacob, F. and E. L. Wollman, Etude genetizue d'un bacteriophage tempere d'Escherichia coli, III. Effet du rayonnement ultraviolet sur la recombinaison genetique. Ann. Inst. Pasteur 88, 724-749 (1955).

116. Jacob, F. and E. L. Wollman, Lysogeny. In The Viruses, vol. 2 (ed. Burnet and Stanley), pp. 319-351, Academic Press, New York (1959).

117. Jacob, F. and E. L. Wollman, Sexuality and the Genetics of Bacteria, Chapter XIII: "Genetic Recombination," pp. 223-248, Academic Press, New York (1961).

118. Jagger, J., Introduction to Research in Ultraviolet Photobiology, Prentice-Hall, Inc., Englewood Cliffs, N.J. (1967).

119. James, A. P., and B. Lee-Whiting, Radiation-induced genetic segregations in vegetative cells of yeast. Genetics 40, 826-831 (1955).

120. Jordan, E., and M. Meselson, A discrepancy between genetic and physical lengths on the chromosome of bacteriophage lambda. Genetics 51, 77-86 (1965).

121. Joyner, A., L. N. Isaacs, and H. Echols, DNA replication and messenger RNA production after induction of wild-type lambda bacteriophage and lambda mutants. J. Mol. Biol. 19, 174-186 (1966).

122. Kaiser, A. D., A genetic study of the temperate coliphage λ. Virology, 1, 424-443 (1955).

123. Kaiser, A. D., Mutations in a temperate bacteriophage affecting its ability to lysogenize E. coli. Virology 3, 42-61 (1957).

124. Kaplan, J. C., S. R. Kushner, and L. Grossman, Enzymatic repair of DNA, 1. purification of two enzymes involved in the excision of thymine dimers from ultraviolet-irradiated DNA. Proc. Nat'l. Acad. Sci., U.S., 63, 144-151 (1969).

125. Kato, T. and K. Kondo, Two types of X-ray sensitive mutants of E. coli B: their phenotypic characters compared with UV-sensitive mutants. Mutation Res. 4, 253-263 (1967).

126. Kellenberger, E., Vegetative bacteriophage and the maturation of virus particles. In Advances in Virus Research, 8, 1-61 (1962).

127. Kellenberger, G., W. Arber, and E. Kellenberger, Eigenschaften UV-bestrahlter λ-phagen. Z. Naturforsch. 14b, 615-629 (1959).

128. Kellenberger, G., N. Symonds, and W. Arber, Host specificity of DNA produced by E. coli, 8. Its acquisition by phage lambda and its persistence through consecutive growth cycles. Z. Vererbungsl. 98, 247-256 (1966).

129. Kellenberger, G. and J. Weigle, Etude au moyen des rayons ultraviolets de l'interaction entre bacteriophage tempere et bacterie hote. Biochim. Biophys. Acta 30, 112-124 (1958).

130. Kellenberger, G., M. L. Zichichi, and H. T. Epstein, Heterozygosis and recombination of bacteriophage lambda. Virology 17, 44-55 (1962).

131. Kellenberger, G., M. L. Zichichi, and J. J. Weigle, Exchange of DNA in the recombination of bacteriophage lambda. Proc. Nat'l. Acad. Sci., U.S. 47, 869-878 (1961).

132. Kiger, J. and M. H. Green, Thermal dissociation of synthetic lambda RNA-E. coli DNA hybrids. Virology 22, 602-607 (1964).

133. Kiger, J. A., E. T. Young, and R. L. Sinsheimer, Purification and properties of intracellular lambda DNA rings. J. Mol. Biol. 33, 395-413 (1968).

134. Kneser, H., Relationship between K-reactivation and UV-reactivation of bacteriophage lambda. Virology 36, 303-305 (1968).

135. Kornberg, A., T. Jovin, M. Goulian, P. Englund, and M. Deutscher, Recent studies on structure and function of DNA polymerase. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

136. Krieg, D. R., A study of gene action in ultraviolet-irradiated bacteriophage T4. Virology 8, 80-98 (1959).

137. Krieg, D. R., in discussion following Doermann, A. H., The analysis of ultraviolet lesions in bacteriophage T4 by cross reactivation. J. Cell. Comp. Physiol. 58, Suppl. 1, 79-93 (1961).

138. Lawley, P. D., Effects of some chemical mutagens and carcinogens on nucleic acids. In Prog. in Nucleic Acid Research and Molecular Biology, vol. 5, 89-131, Academic Press, New York (1966).

139a. Lawley, P. and R. Brookes, Molecular mechanism of the cytotoxic action of difunctional alkylating agents and of resistance to this action. Nature 206, 480-483 (1965).

139b. Lawley, P. D., J. H. Lethbridge, P. A. Edwards, and K. V. Shooter, Inactivation of bacteriophage T7 by mono- and difunctional sulphur mustards in relation to cross-linking and depurination of bacteriophage DNA. J. Mol. Biol. 39, 181-198 (1969).

140. Lederberg, S. and M. Meselson, Degradation of non-replicating bacteriophage DNA in non-accepting cells. J. Mol. Biol. 8, 623-628 (1964).

141. Lehman, I. R., B. M. Olivera, and Y. Anraku, Enzymatic joining of DNA. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

142. Lennox, E. S., Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1, 190-206 (1955).

143. Levinthal, C., Recombination in phage T2: relationship to heterozygosis and growth. Genetics 39, 169-184 (1954).

144. Levinthal, C., Bacteriophage genetics. In The Viruses (ed. Burnet and Stanley), vol. 2, pp. 281-317, Academic Press, New York (1959).

145. Lieb, M., Enhancement of UV-induced mutation in bacteria by caffeine. Z. Vererbungsl. 92, 416-429 (1961).

146. Lindegren, C. C., Gene conversion in saccharomyces. J. Genet. 51, 625-637 (1953).

147. Linn, S., personal communication (1969).

148. Linn, S. and W. Arber, Host specificity of DNA produced by E. coli, X. In vitro restriction of phage fd replicative form. Proc. Nat'l Acad. Sci., U.S., 59, 1300-1306 (1968).

149. Loveless, A., Genetic and Allied Effects of Alkylating Agents, The Pennsylvania State University Press, University Park (1966).

150. Luria, S. E., and R. Dulbecco, Genetic recombination leading to production of active bacteriophage from ultraviolet inactivated bacteriophage particles. Genetics 34, 93-125 (1949).

151. Lytle, C. D., and W. Ginoza, Intracellular development of bacteriophage ØX174 inactivated by γ-ray, ultraviolet light, or nitrous acid. Virology 38, 152-165 (1969).

152. Manney, T. R., and R. K. Mortimer, Allelic mapping in yeast by X-ray-induced mitotic reversion. Science 143, 581-583 (1964).

153. Marmur, J., W. F. Anderson, L. Mathews, K. Berns, E. Gajewska, D. Lane, and P. Doty, The effects of ultraviolet light on the biological and physical chemical properties of deoxyribonucleic acids. J. Cell. Comp. Physiol. 58, Suppl. 1, 33-55 (1961).

154. Mattern, I. E., M. P. van Winden, and A. Rorsch, The range of action of genes controlling radiation sensitivity in E. coli. Mutation Res. 2, 111-131 (1965).

155. Mattern, I. E., H. Zwenk, and A. Rorsch, The genetic constitution of the radiation-sensitive mutant E. coli B_{s-1}. Mutation Res. 3, 374-380 (1966).

156. McGrath, R. and R. Williams, Reconstruction in vivo of irradiated E. coli deoxyribonucleic acid; the rejoining of broken pieces. Nature 212, 534-535 (1966).

157. Meselson, M., On the mechanism of genetic recombination between DNA molecules. J. Mol. Biol. 9, 734-745 (1964).

158. Meselson, M., The molecular basis of genetic recombination. In Heritage from Mendel (ed. R. A. Brink), pp. 81-104. University of Wisconsin Press, Madison (1967).

159. Meselson, M., Reciprocal recombination in prophage lambda. J. Cell. Physiology 70, Suppl. 1 part II, 113-118 (1967).

160. Meselson, M., and J. J. Weigle, Chromosome breakage accompanying genetic recombination in bacteriophage. Proc. Nat'l Acad. Sci., U.S., 47, 857-868 (1961).

161. Meselson, M., and R. Yuan, DNA restriction enzyme from E. coli. Nature 217, 1110-1114 (1968).

162a. Michalke, H. and H. Bremer, RNA synthesis in E. coli after irradiation with ultraviolet light. J. Mol. Biol. 41, 1-23 (1969).

162b. Mitchell, M. B., Aberrant recombination of pyridoxine mutants of Neurospora. Proc. Nat'l Acad. Sci., U.S., 41, 215-220 (1955).

163. Miura, A. and J. Tomizawa, Studies on radiation-sensitive mutants of E. coli, III. Participation of the Rec system in induction of mutation by ultraviolet irradiation. Molec. Gen. Genetics 103, 1-10 (1968).

164. Morgan, T. H., Random segregation versus coupling in Mendelian inheritance. Science 34, 384 (1911).

165. Morpurgo, G., Induction of mitotic crossing-over in Aspergillus nidulans by bifunctional alkylating agents. Genetics 48, 1259-1263 (1963).

166. Muhhamed, A. and C. M. Steinberg, Action spectrum for cross-reactivation with various amber mutants of bacteriophage T4D. J. Mol. Biol. 21, 395-397 (1966).

167. Naha, P. M., Bacterial control of lambda replication I. Initial characterization of a rep mutation of E. coli K12. Virology 36, 434-441 (1968).

168. Ogawa, H., K. Shimada, and J. Tomizawa, Studies on radiation-sensitive mutants of E. coli I. Mutants defective in the repair synthesis. Molec. Gen. Genetics 101, 227-244 (1968).

169. Ogawa, H. and J. Tomizawa, Bacteriophage lambda DNA with different structures found in infected cells. J. Mol. Biol. 23, 265-276 (1967).

170. Ogawa, H. and J. Tomizawa, Breakage of polynucleotide strands by disintegration of radiophosphorous atoms in DNA molecules and their repair. J. Mol. Biol. 30, 1-15 (1967).

171. Ogawa, T., J. Tomizawa, and M. Fuke, Replication of bacteriophage DNA, II. structure of replicating DNA of phage lambda. Proc. Nat'l Acad. Sci., U.S., 60, 861-865 (1968).

172a. Okubo, S. and W. R. Romig, Impaired transformability of B. subtilis mutant sensitive to mitomycin C and ultraviolet radiation. J. Mol. Biol. 15, 440-454 (1966).

172b. Ono, J. and Y. Shimazu, In vivo cleavage of a circular, single-stranded DNA of bacteriophage ØR irradiated with ultraviolet light. J. Mol. Biol. 24, 491-495 (1967).

173. Pardee, A. B. and L. S. Prestidge, Ultraviolet-sensitive targets in the enzyme-synthesizing apparatus of E. coli. J. Bacteriol. 93, 1210-1219 (1967).

174. Pauling, C. and L. Hamm, Properties of a temperature-sensitive radiation-sensitive mutant of E. coli. Proc. Nat'l Acad. Sci., U.S., 60, 1495-1502 (1968).

175. Pauling, C. and P. C. Hanawalt, personal communication via R. H. Haynes (1966).

176. Pettijohn, D. and P. Hanawalt, Evidence for repair replication of ultraviolet damaged DNA in bacteria. J. Mol. Biol. 9, 395-410 (1964).

177. Ptashne, M. and N. Hopkins, The operators controlled by the lambda phage repressor. Proc. Nat'l Acad. Sci., U.S., 60, 1282-1287 (1968).

178. Richardson, C., R. Inman, and A. Kornberg, Enzymatic synthesis of deoxyribonucleic acid. XVIII. The repair of partially single-stranded DNA templates by DNA polymerase. J. Mol. Biol. 9, 46-69 (1964).

179. Richardson, C. C., G. C. Fareed, A. Jacquemin-Sablon, T. R. Live, Y. Masasume, and B. Weiss, Studies on the joining of DNA by polynucleotide ligase of phage T4. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

180. Roman, H. L., discussion remarks following Meselson: reciprocal recombination in prophage lambda. J. Cell Physiol. 70, Suppl. 1, 113-118 (1967).

181. Roman, H. and F. Jacob, A comparison of spontaneous and ultraviolet-induced allelic recombination with reference to the recombination of outside markers. Cold Spring Harbor Symp. Quant. Biol., 23, 155-160 (1958).

182. Rorsch, A., P. van de Putte, I. E. Mattern, and H. Zwenk, Genetic and enzymic control of radiation sensitivity in E. coli. In Proceedings of the Conference on Genetical Aspects of Radiation Sensitivity: Mechanisms of Repair, pp. 105-129, International Atomic Energy Agency, Vienna (1966).

183. Rupert, C. S. and W. Harm, Reactivation after photobiological damage. In Adv. in Radiation Biology, vol. 2, 1-81, Academic Press, New York (1966).

184. Rupp, W. D. and P. Howard-Flanders, Discontinuities in the DNA synthesized in an excision-defective strain of E. coli following ultraviolet irradiation. J. Mol. Biol. 31, 291-304 (1968).

185. Rush, M. G. and R. C. Warner, Molecular recombination in a circular genome. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

186. Sadowski, P., A. Yudelevich, B. Ginsberg, L. Feiner, and J. Hurwitz, Enzymatic studies on the repair and breakage of DNA. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

187. Sauerbier, W., The bacterial mechanism reactivating UV-irradiated phage in the dark (host-cell reactivation). Z. Vererbungsl. 93, 220-228 (1962).

188. Sauerbier, W. Ultraviolet sensitivity and thymine dimerization in mutants of bacteriophage T4. Biochim. Biophys. Acta 87, 356-358 (1964).

189. Sechaud, J., G. Streisinger, J. Emrich, J. Newton, H. Laniford, H. Reinhold, and M. Stahl, Chromosome structure in phage T4, II. Terminal redundancy and heterozygosis. Proc. Nat'l Acad. Sci., U.S., 54, 1333-1339 (1965).

190. Setlow, J. K., The molecular basis of biological effects of ultraviolet radiation and photoreactivation. In Current Topics in Radiation Research, vol. II, 195-248, North Holland Publishing Co., Amsterdam (1966).

191. Setlow, R., Cyclobutane-type pyrimidine dimers in polynucleotides. Science 153, 379-386 (1966).

192. Setlow, R. B., Repair of DNA. In Regulation of Nucleic Acid and Protein Biosynthesis (ed. Koningsberger and Bosch), pp. 51-62, Elsevier Publishing Co., Amsterdam (1967).

193. Setlow, R. B., The photochemistry, photobiology,and repair of polynucleotides. In Prog. in Nucleic Acid Research and Molecular Biology, vol. 8, 257-295, Academic Press, New York (1968).

194. Setlow, R. B. and W. L. Carrier, Identification of ultraviolet-induced thymine dimers in DNA by absorbance measurements. Photochem. Photobiol. 2, 49-57 (1963).

195. Setlow, R. B. and W. L. Carrier, The disappearance of thymine dimers from DNA: an error-correcting mechanism. Proc. Nat'l Acad. Sci., U.S., 51, 226-231 (1964).

196. Setlow, R. B. and W. L. Carrier, Pyrimidine dimers in ultraviolet-irradiated DNA's. J. Mol. Biol. 17, 237-254 (1966).

197. K. Shimada, H. Ogawa, and J. Tomizawa, Studies on radiation-sensitive mutants of E. coli II. Breakage and repair of ultraviolet irradiated intracellular DNA of phage lambda. Molec. Gen. Genetics 101, 245-256 (1968).

198. Signer, E. R., personal communication (1967).

199. Signer, E. R., Lysogeny: the integration problem. In Annual Review of Microbiology, vol. 22, 451-488 (1968).

200. Signer, E., H. Echols, J. Weil, C. Radding, M. Shulman, L. Moore, and K. Manly, The general recombination system of bacteriophage lambda. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

201. Signer, E. R. and J. Weil, Recombination in bacteriophage lambda, I. Mutants deficient in general recombination. J. Mol. Biol. 34, 261-271 (1968).

202. Sinsheimer, R. L., Bacteriophage ØX174 and related viruses. In Prog. Nucleic Acid Research and Molecular Biology, vol. 8, 115-169, Academic Press, New York (1968).

203. Skalka, A., E. Burgi, and A. D. Hershey, Segmental distribution of nucleotides in the DNA of bacteriophage lambda. J. Mol. Biol. 34, 1-16 (1968).

204. Snow, R., Increased mitotic recombination induced by ultraviolet light in UV sensitive strains of yeast (abstract). Genetics 56, No. 3, pt. 2, 591-592 (1967).

205. Stahl, F. W., One way to think about gene conversion. Genetics 61, No. 1, pt. 2 (Suppl.), 1-13 (1969).

206. Stent, G. S., Mating in the reproduction of bacterial viruses. In Advances in Virus Research, vol. V, 95-149, Academic Press, New York (1958).

207. Stent, G. S., Molecular Biology of Bacterial Viruses, W. H. Freeman and Company, San Francisco (1963).

208. Stent, G. S. and C. R. Fuerst, Decay of incorporated radioactive phosphorous during development of a temperate bacteriophage. Virology 2, 737-752 (1956).

209. Strauss, B., DNA repair mechanisms and their relation to mutation and recombination. In Current Topics in Microbiology and Immunology, Springer-Verlag, New York (1968).

210. Strauss, B., Differential destruction of the transforming activity of damaged deoxyribonucleic acid by a bacterial enzyme. Proc. Nat'l Acad. Sci., U.S., 48, 1670-1675 (1962).

211. Strauss, B., Alkylation damage and its repair. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

212. Sturtevant, A., A History of Genetics, Harper and Row, New York (1965).

213. Swartz, M. N., T. A. Trautner, and A. Kornberg, Enzymatic synthesis of deoxyribonucleic acid- XI. Further studies on nearest neighbor base sequences in deoxyribonucleic acids. J. Biol. Chem. 237, 1961-1967 (1962).

214. Swenson, P. A. and R. B. Setlow, Effects of ultraviolet radiation on macromolecular synthesis in E. coli. J. Mol. Biol. 15, 201-219 (1966).

215. Takagi, Y., H. Nakayama, S. Okubo, K. Shimada, and M. Sekiguchi, Nucleases specific for UV-irradiated DNA and their possible role in dark repair. Cold Spring Harbor Symp. Quant. Biol., 33 (1968).

216. Terzi, M., Physiology of bacteriophage lambda in the restrictive host. J. Mol. Biol. 34, 165-173 (1968).

217. Tessman, E. S., and T. Ozaki, The interaction of phage S13 with ultraviolet-irradiated host cells and properties of the ultraviolet-irradiated phage. Virology 12, 431-449 (1960).

218. Tessman, I., Genetic recombination of phage S13 in a recombination-deficient mutant of E. coli K12. Biochem. Biophys. Res. Commun. 22, 169-174 (1966).

219. Tessman, I., Selective stimulation of one of the mechanisms for genetic recombination of bacteriophage S13. Science 161, 481-482 (1968).

220. Tessman, I. and T. Ozaki, Multiplicity reactivation of bacteriophage T1. Virology 4, 315-327 (1957).

221. Thomas, C. A., Jr., Recombination of DNA molecules. In Prog. Nucleic Acid Research and Molecular Biology, vol. 5, 315-337, Academic Press, New York (1966).

222. Tomizawa, J., Molecular mechanisms of genetic recombination in bacteriophage: joint molecules and their conversion to recombinant molecules. J. Cell Physiol. 70, Suppl. 1 part II, 201-214 (1967).

223. Tomizawa, J. and N. Anraku, Molecular mechanisms of genetic recombination in bacteriophage, I. Effect of KCN on genetic recombination of phage T4. J. Mol. Biol. 8, 508-515 (1964).

224. Tomizawa, J. and N. Anraku, Molecular mechanisms of genetic recombination in bacteriophage, II. Joining of parental DNA molecules of phage T4. J. Mol. Biol. 8, 516-540 (1964).

225. Van de Putte, P., C. A. van Sluis, J. van Dillewijn, and A. Rorsch, The location of genes controlling radiation sensitivity in E. coli. Mutation Res. 2, 97-110 (1965).

226. Van de Putte, P. H. Zwenk, and A. Rorsch, Properties of four mutants of E. coli defective in genetic recombination. Mutation Res. 3, 381-392 (1966).

227. Visconti, N. and M. Delbruck, The mechanism of genetic recombination in phage. Genetics 38, 5-33 (1953).

228. Wacker, A., Molecular mechanisms of radiation effects. In Prog. Nucleic Acid Research and Molecular Biology, vol. 1, 369-399, Academic Press, New York (1963).

229. Waites, W. M. and B. A. Fry, Effect of infection with phage lambda on the synthesis of protein, RNA, and DNA in E. coli. J. Gen. Microbiol. 34, 418-426 (1964).

230. Weigle, J. J., Induction of mutations in a bacterial virus. Proc. Nat'l Acad. Sci., U.S., 39, 628-636 (1953).

231. Weigle, J. J., The structure of heterozygotes of phage lambda. In Biology Annual Report, 1965, Calif. Inst. Technology, p. 41.

232. Weigle, J., Assembly of phage lambda in vitro. Proc. Nat'l Acad. Sci., U.S., 55, 1462-1466 (1966).

233. Weigle, J. J. and W. Bode, Heterozygotes and UV repair mechanisms. In Biology Annual Report, 1965, Calif. Inst. Technology, p. 42.

234. Weissbach, A. and L. A. Salzman, The biosynthesis of phage lambda DNA: the structure of the first intermediate. Proc. Nat'l Acad. Sci., U.S., 58, 1096-1101 (1967).

235. Westmoreland, B. C., W. Szybalski, and H. Ris, Mapping of deletions and substitutions in heteroduplex DNA molecules of bacteriophage lambda by electron microscopy. Science 163, 1343-1348 (1969).

236. Whitehouse, H. L. K., A theory of crossing-over by means of hybrid deoxyribonucleic acid. Nature 199, 1034-1040 (1963).

237. Whitehouse, H. L. K., Crossing over. Sci. Prog. 53, 285-296 (1965).

238. Whitehouse, H. L. K., An operator model of crossing-over. Nature 211, 708-713 (1966).

239. Wierzchowski, K. L., in discussion following Marmur, J. et al., The effects of ultraviolet light on the biological and physical chemical properties of deoxyribonucleic acids. J. Cell. Comp. Physiol. 58, Suppl. 1, 33-55 (1961).

240. Wilkins, B. M. and P. Howard-Flanders, The genetic properties of DNA transferred from ultraviolet-irradiated Hfr cells of E. coli K-12 during mating. Genetics 60, 243-255 (1968).

241. Willetts, N. S., A. J. Clark, and B. Low, Genetic location of certain mutations conferring recombination deficiency in E. coli. J. Bacteriol. 97, 244-249 (1969).

242. Witkin, E. M., Mutation-proof and mutation-prone modes of survival in derivatives of E. coli B differing in sensitivity to ultraviolet light.. Brookhaven Symposia in Biology, vol. 20, 17-55 (1967).

243a. Witkin, E. M., The role of repair and recombination in mutagenesis. Proc. 12th Intern. Congr. Genetics, III (1968).

243b. Witkin, E. M., The mutability toward ultraviolet light of recombination-deficient strains of E. coli. Mutation Res. 8, 9-14 (1969).

244. Wollman, E. L. and F. Jacob, Etude genetique d'un bacteriophage tempere d'Escherichia coli, II. Mecanisme de la recombinaison genetique. Ann. Inst. Pasteur 87, 674-690 (1954).

245. Wood, W. B., Host specificity of DNA produced by E. coli: bacterial mutations affecting the restriction and modification of DNA. J. Mol. Biol. 16, 118-133 (1966).

246. Wulff, D. L., The role of thymine dimer in the photoinactivation of the bacteriophage $T4v_1$. J. Mol. Biol. 7, 431-441 (1963).

247. Wulff, D. L. and C. S. Rupert, Disappearance of thymine photodimer in ultraviolet irradiated DNA upon treatment with a photoreactivating enzyme from baker's yeast. Biochem. Biophys. Res. Commun. 7, 237-240 (1962).

248. Yamamoto, N., Recombination: damage and repair of bacteriophage genome. Biochem. Biophys. Res. Commun. 27, 263-269 (1967).

249. Yarus, M. and R. L. Sinsheimer, The UV-resistance of double-stranded ØX174 DNA. J. Mol. Biol. 8, 614-615 (1964).

250. Young, E. T., II, and R. L. Sinsheimer, Vegetative lambda DNA III. Pulse-labeled components. J. Mol. Biol. 33, 49-59 (1968).

251. Zimmermann, F. K. and R. Schwaier, Induction of mitotic gene conversion with nitrous acid, 1-methyl-3-nitro-1-nitrosoguanidine and other alkylating agents in Saccharomyces cerevisiae. Molec. Gen. Genetics 100, 63-76 (1967).

252. Zimmermann, F. K., R. Schwaier, and U. v. Laer, Mitotic recombination induced in Saccharomyces cerevisiae with nitrous acid, diethylsulfate and carcinogenic alkylating nitrosamides. Z. Verebungsl. 98, 230-246 (1966).

253. "General Discussion" (led by Max Delbruck), in Radiation Res., Suppl. 6, Structural defects in DNA and their repair in microorganisms, 227-234 (1966).

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

TECHNICAL INFORMATION DIVISION
LAWRENCE RADIATION LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720