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CELL TRANSFORMATION

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THE ROLES OF IONIZING RADIATION IN CELL TRANSFORMATION

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Introduction

Significant amounts of quantitative data are now available on a variety of responses of mammalian cells to viruses and to ionizing radiation. Ionizing radiations, including heavy ions, cause lethal effects and at the same time can transform some surviving normal cells to cancer cells. When oncogenic viruses are applied, the cell transformation is enhanced by the additional application of ionizing radiations. Much of this information is summarized in the preceding paper by Yang and Tobias (1984).

We have also developed some mathematical models for the actions of radiations on living cells, with the aim of better understanding the underlying molecular processes. The goal of this paper is to present an hypothesis that both lethal radiation action and cell transformation are results of similar processes: the production of lesions in DNA followed by time dependent repair. Lethal effects of radiation damage and radiation-induced cell transformation both appear to be the results of misrepair. The main feature of viral-induced cell transformation is the integration of viral oncogenes into the DNA of the host and the role of radiations seems to be the introduction of lesions that become the sites of viral integration.

We have earlier described the repair misrepair model, or the RMR-I, which is applicable for radiations of low LET, e. g., X rays and gamma rays (Tobias et al., 1980). RMR-II is described in the paper immediately following this one (Albright and Tobias, 1984). Here we introduce a mathematical modification of the RMR model, RMR-III, which is intended to describe lethal effects caused by heavily ionizing tracks.

Biological Observations of Cell Transformation In Vitro

Information from three kinds of model systems has helped to define the biological problem:

1. The occurrence of spontaneous cell transformation in mammalian cells, grown in monolayer, can on occasion be increased by radiation.
2. Certain specific viruses, those carrying oncogenes, can add new genetic material to the genome of a host, thus causing cell transformation. Ionizing radiation can strongly enhance this process.
3. Bacterial plasmids constructed with specially coded DNA can also cause cell transformation. In plasmid form, one can obtain relatively large amounts of oncogenic DNA, and the plasmids have been used to transfect cells with specific segments of DNA. Ionizing radiation has been shown to enhance transformation events in all three of the biological systems. Some additional background information on each system is necessary.

There are certain classes of "normal" mammalian fibroblastic cells that can be grown in tissue culture medium on sterile glass or specially treated plastic dishes. It was observed more than twenty-five years ago that these normal cells do not multiply well in cell suspension, but prefer to adhere to the surfaces on which they are growing. The cells flatten out on the surface and form a monolayer; as the cells multiply, their number increases until a confluent monolayer is formed. When the cells come in contact with each other, cell division ceases, and this phenomenon is called contact-inhibition.

Abercrombie and Heaysman (1954) and Ambercrombie et al. (1957) noticed that treatment with a noxious substance or environment can cause some cells to lose the property of contact inhibition. These treated cells gave rise to

clones that were not limited to monolayers, but instead formed three-dimensional "transformed" clones containing cells that piled up on top of each other. Working with cells taken from mice and hamsters, these investigators showed that many such transformed cells, when reintroduced back in the bodies of the syngeneic host from which they were originally obtained, produced cancerous tumors; euploid cells that were allowed to grow in monolayers did not produce tumors when reintroduced into the host.

Since that time, quantitative assay systems have been developed for the formation of transformed foci from normal monolayers, and several additional criteria were added to define cell transformation. Borek and Sachs (1968) initially showed that ionizing radiation alone can transform cells. Later it was demonstrated that most, if not all, chemical carcinogens can also transform cells. Some cancer causing viruses under certain conditions can also transform normal monolayer cells. The quantitative relationships obtained in our laboratory with such agents enabled us to begin development of mathematical models to describe the kinetics of this process.

We know today that normal and transformed cells are distinguished by certain characteristics: (1) transformed cells develop three-dimensional colonies in three-dimensional agar medium; normal cells do not; (2) normal cells are contact-inhibited on two-dimensional surfaces; transformed cells are not; (3) if certain compounds are added to the medium, e.g., retinoic acid and related substances, transformed cells can revert and contact-inhibition is re-established. Additional criteria for transformation are detailed in Yang and Tobias (1980).

Cell transformation can be due to mutation or to changes in gene expression; evidence exists for both of these mechanisms. Todaro and Green (1966) have hypothesized the existence of oncogenes, genes that can transform

normal cells into cancerous ones. Several human oncogenes are known (Cooper, 1982); however, the mere presence of the oncogene in the genome of a mammalian cell does not guarantee that the transformation properties are phenotypically expressed. One of the important parameters that relates to expression of the transformation properties is the location of the oncogene in the genome.

Most of the viral work we report here relates to the use of Simian virus, SV40, which was initially isolated from primates. SV40 consists of a single loop of supercoiled, double-stranded, circular DNA, 5,840 base pairs long, which has been completely decoded (Fiers et al., 1978). In typical in vitro cell transformation experiments with rodents, an inactivated form of the virus is used, which can infect cells but does not permit the virus itself to proliferate and will not kill the host cell (Topp et al., 1980).

In previous work with the SV40 virus, it has been shown that the "A" segment of the viral genome is 3218 base pairs long and can produce a specific protein called the T antigen, which has the crucial information for cell transformation (Reddy et al., 1978; Fiers et al., 1978). Botchan et al. (1976) have demonstrated that in the typical process of in vitro cell transformation, several copies of the T antigen-producing-DNA sequence can become incorporated into the host of the genome, most of which are not effective in transforming the host cell. This is one of the reasons why the notion has developed that there is a gene position effect: Apparently, the T region must be integrated into the host genome in certain specific sites and, perhaps, must be adjacent to appropriate promoter regions in order to be effective.

An important area of research is to discover the mechanism whereby the A region of double-stranded DNA becomes integrated into the host genome. This integrative process appears to be somewhat similar to the genetic

recombination process, ubiquitous in nature, that normally includes scission and recombination of double stranded segments of DNA. The normal recombination process usually occurs in the process of producing germ cells and appears to be strongly inhibited in the somatic cells of mammals.

A dose of ionizing radiation, applied at the appropriate time, can enhance the frequency of SV40 induced cell transformation. The fraction of transformed cells can rise dramatically, sometimes to an enhancement ratio of up to 50. (The enhancement ratio is the number of viable cells transformed by the virus with radiation divided by those transformed by the virus alone.)

Cell transformation can also be accomplished without intact viruses. Perez et al. (1984) grew the oncogene portion of the SV40 gene in bacterial plasmids together with a marker gene (thymidine kinase +). The host cells were then exposed to segments of the plasmid DNA. By the processes of transfection, some of the DNA entered the cells and integrated with the cell's genome. The transfection process was significantly enhanced by exposing the host cells to various doses of ionizing radiation. The incorporation of the marker gene and of the oncogene was enhanced in a similar manner, and the enhancement had a similar dose-effect relationship to that obtained in actual viral infection; however, cell transformation was expressed several times less frequently than the expression of the marker gene. We believe these experiments demonstrated that the DNA integration event is a key process in cell transformation and that oncogene integration follows the same steps as the integration of some other DNA segment.

The Repair Misrepair Model: General Features

The RMR model attempts to provide a mathematical framework for cellular responses to injury in genetic material and in the extragenic structures of

cells. In the RMR model, it is important to note that one is usually unable to observe radiation-induced molecular lesions in individual, living cells. The loci of these lesions are so small (on the order of one Angstrom, or a portion of a nucleotide) that we would have to use X rays to observe the lesions. According to the uncertainty principle, these X rays would in all likelihood further injure the genetic material in the course of observation.

It is important not to make deterministic statements about these initial lesions, such as "lesion X kills the cells" or "lesion Y is repaired." The theory should attempt to follow events as they happen in the cell. In the RMR model, this is done in the following manner: (1) Let the number of initial lesions be U_0 lesions, where U stands for uncommitted. (2) U lesions are recognized internally by the cell's informational apparatus. (3) These cells attempt to enzymatically repair U lesions in order to restore the normal properties of genetic material. (4) In the course of attempted repair, U lesions might be modified, enlarged, or diminished, and their numbers will usually decrease until they give rise to an observable expression of radiation injury.

We should be mindful here of the usual methods of observation available to radiobiologists, which fall into three categories:

1. If a population of irradiated cells (e.g., $> 10^6$ cells) is killed and chemically processed, we can obtain information on the mean number of certain lesions produced by radiation using an additional assumption that the chemical assay itself did not introduce new lesions. Thus, we can measure the mean numbers of single and double stranded scissions produced in DNA per cell, the number of nucleotide bases lost or impaired, etc. For these variables, at low doses (D) of X rays: $U_0 = \delta D$, where δ is the lesion yield per cell per unit dose. The δ is usually constant and independent of D; however, the value

of δ depends on the microstructure of the cell nucleus and on the quality of the radiation.

2. Changes in macroscopically expressed quantities, such as metabolism and protein synthesis, can also be measured for an irradiated cell population. Usually such measurements also represent mean values because measurements from a large number of cells are averaged.

3. By appropriate plating techniques after a suitable time interval has elapsed, we can observe in a cell population the dose and time dependent probabilities for survival, mutation, and cell transformation as end results of a set of very complex cellular phenomena. The RMR model was constructed to calculate the mean number of a variety of U lesions in the course of time-dependent repair processes. Combined with statistical calculations, the probabilities of observable effects are calculated.

The RMR-I model for cellular ionizing radiation effects with no defined track structures is summarized in Appendix I. The RMR-I model assumes a linear self-repair mechanism in which a single U lesion is repaired and a quadratic process, in which a pair of DNA lesions interact over the course of repair. Furthermore, we assume that the repair is equivalent to restored DNA. We call this repair <u>eurepair</u> when the restoration produces DNA with its former, pre-irradiation structure. When the repaired DNA is radically different in structure or base sequence from that which existed prior to irradiation, we call the result of the process <u>misrepair</u>. Eurepair and misrepair are the results of statistical processes. The actual processes are probably very complex, and in order to be practical, it is necessary to use simplifying assumptions.

The Physics of Track Structure

In condensed media, many radiations (particularly electrons, neutrons, and fast heavy ions) produce discrete tracks that consist of arrays of ionization and excitation. The tracks extend along the path of the primary particle producing the track and also radially away from the central path. For a homogeneous medium, considerable knowledge exists of the distribution of energy transfer along tracks. A Monte Carlo statistical approach has been used by Paretzke et al. (1978) and Turner et al. (1982). This approach assumes that the initial energy deposition events are very fast (10^{-16} seconds to 10^{-8} seconds) and that secondary electrons (delta rays) are produced in collisions along the primary particle path. The delta rays come to a stop after a more or less torturous path in the medium, and produce further ionizations and excitations. The Paretzke approach has yielded spatial distributions of primary and secondary ionization events.

Mozumder (1969), Chatterjee and Magee, (1980), and Magee and Chatterjee (1980) have realized that the lifetimes of primary ionization products are very short. The primary ionizations are followed by diffusion controlled radical and radical-ion reactions. A large fraction of the initial energy transfer, perhaps 80% to 90%, ends up as thermal energy, diffusing rapidly away from the track (Tobias et al., 1979). The diffusion of chemical products, such as free radicals in aqueous living systems, is slower. To follow these events, a distinction is made between the track core and the surrounding penumbra. The core is limited by the radius at which the primary particle can still cause electronic excitation. The penumbra is much larger, and its size is limited by the maximum range of the more energetic delta rays.

After about 10^{-7} seconds have passed, macromolecular lesions exist in the irradiated cells. These lesions can be further modified by the presence of radiation sensitizers or protectors (Klayman and Copeland, 1975; Stradford, 1982), but after about 10^{-3} seconds have passed, we may regard many of the macromolecular U lesions in DNA and other cellular macromolecules as having stabilized. The enzymatic apparatus of mammalian cells appears to take about 10^{-3} seconds or longer to recognize lesions in its fabric and to start repairing them.

The number of U lesions per cell nucleus is much smaller than the number of initial energy transfer events. In a single human cell, we estimate perhaps 10^5 to 10^6 primary enery transfer events, about 2,000 to 4,000 DNA single-strand break events per cell nucleus, and perhaps up to 120 DNA double-strand scissions per cell from a dose that can inhibit the reproductive integrity of 50% of the cells. Most of the single strand breaks eurepair; it is likely that many of the U lesions that relate to the production of lethal effects are misrepaired double strand breaks.

The yield of U lesions is a function of LET (linear energy transfer); it also depends on the radial structure of tracks, which is a function of the particle velocity and charge. The yield also depends on the distribution of chromatin in the cell nucleus. It is not likely that a track can produce a greater number of U lesions than the number of times it crosses chromatin fibers. Microscopic evaluation suggests that the structural distribution of chromatin is uneven in the cell nucleus; this distribution is different in various physiological states.

The Track Survival Equation (RMR-III)

Assume that a uniform cell population is exposed to a parallel stream of particles of linear energy transfer L, and that the particles produce a distribution of U lesions along their tracks. We know that the energy transferred to a thin slab of matter follows the Landau-Vavilov distribution (Landau, 1944; Symon, 1948; Vavilov, 1957); however, we simplify the treatment by assuming Poisson statistics. In the first part of this calculation we assume that the mean number of lesions per particle is n(t). At a given particle fluence F, let the mean number of particles that crosses a cell nucleus be $\bar{m}$. Since the particles each arrive independently, $\bar{m}$ represents the mean of a Poisson distribution:

$$\bar{m} = \sigma \cdot F \qquad (1)$$

neglecting the radial dimensions of tracks, σ is the geometrical cross section of the region represented by DNA in the cell nucleus. Following the RMR model, we assume that two kinds of repair processes take place in DNA. A linear "self-repair" process is characterized by rates λ_o and λ. In quadratic processes, interactions between two different lesions take place in the course of repair to produce misrejoinings. If these quadratic repair events occur between lesions produced by the <u>same track</u> (intratrack repair), let their rate be k_I; when these occur between lesions produced by different tracks (intertrack repair), let their rate be k_{II}.

When m particles cross the cell nucleus at t = 0, then the time rate of change of the number of lesions due to repair can be written as:

$$\frac{d(mn)}{dt} = \underbrace{-\lambda_0 mn}_{\text{self-repair}} \underbrace{- k_I mn(n-1)}_{\substack{\text{intratrack}\\ \text{coop repair}}} \underbrace{- k_{II} m(m-1)n^2}_{\substack{\text{intertrack}\\ \text{coop repair}}} \qquad (2)$$

This equation is analogous to equation (2) of Appendix I, except that there are two cooperative terms, corresponding to the idea that cooperative repair occurs at a higher rate for lesions within the same track than between lesions of separate tracks. Since m is not a function of time, we obtain the differential equations for lesions along a single track:

$$\frac{dn}{dt} = -\lambda n - k(m)n^2;$$

$$\text{where} \quad \lambda = \lambda_0 - k_I \text{ and usually } \lambda >> k_I \qquad (3)$$

The term k(m) is a function of the number of particles crossing the cell nucleus:

$$k(m) = k_I + (m-1)k_{II} \qquad (4)$$

If $n(\infty) = 0$, then for an initial number of n_0 lesions per track we obtain:

$$n(t) = \frac{\lambda\, n_0\, e^{-\lambda t}}{\lambda + n_0 k(m)\,(1-e^{-\lambda t})} \qquad (5)$$

If all linear self repair is eurepair, and all quadratic repair is lethal misrepair, we obtain for the survival probability S (m):

$$S(m) = e^{-mn_o} \left[1 + \frac{n_o (1-e^{-\lambda t})}{\varepsilon(m)} \right]^{m\varepsilon(m)} \tag{6}$$

where the repair ratio $\varepsilon(m)$ is given by:

$$\varepsilon(m) = \frac{\lambda}{k_I + (m-1)K_{II}} \tag{7}$$

when densely ionizing tracks are present, usually $k_I >> k_{II}$. If a single particle track produces so many lesions and has so much intratrack interaction that they can kill a cell, and if intertrack interaction is absent, then k_{II} = 0 and:

$$\varepsilon(m) = \varepsilon(1) = \lambda/k_I = \varepsilon_I \tag{8}$$

On the other hand, when we deal with low LET radiation, and there are no special intratrack effects, then $k_I = k_{II}$ and:

$$m\varepsilon(m) = \lambda/k_{II} = \varepsilon_{II} \tag{9}$$

In this case, for simpler notation, let the time factor be denoted as T, where $T = 1-e^{-\lambda t}$:

$$S(m) = e^{-\delta D} \cdot \left[1 + \frac{\delta DT}{\varepsilon_{II}} \right]^{\varepsilon^{II}} \tag{10}$$

In this equation, we substituted for the number of lesions, mn_0, the more conventional expression δD, where δ is the number of lesions produced per cell nucleus by a unit dose.

An important special case of equation (2) is m = 1, when only a single ionizing track crosses the cell nucleus. The single track survival curve is S(1):

$$S(1) = e^{-n_0} \cdot \left[1 + \frac{n_0 T}{\varepsilon_I} \right]^{\varepsilon_I} \tag{11}$$

If there is no intertrack interaction ($k_{II} = 0$), then the survival curve for m tracks is exponential:

$$S(m) = S(1)^m \tag{12}$$

The slope of the exponential "lesion" curve, the first term in equation (6), is different from the slope of the actual survival curve, which is modified by the second term of equation (6). If there is no repair, or if all repair is lethal misrepair, $\varepsilon_I = 0$ and the first term of equation (6) expresses the probability of survival. The single track survival equation S(1) is important for characterizing the biological effects of densely ionizing particulate radiations. In order for equation (6) to be useful, we should determine the dependence of n_0 on the linear energy transfer and on the

track structure. These depend on the atomic number and velocity of the ionizing particles. We have made an experimental study of this problem using cell cultures of human Ataxia telangiectasia cells, the AT-2SF cell line, and also human T-1 cells (Tobias et al., 1984). We find that at low values of the linear energy transfer L, n_0 is proportional to L, whereas at medium L values, n_0 increases approximately as $L^{2.2}$. The amount and structural configuration of DNA in the cell nucleus is also important. At very high L values, n_0 saturates due to the limited amount of DNA in the path of the particles. Figure 1 shows S(1) as function of L for T-1 cells exposed to neon ions.

The particles arrive independently of each other, so that the probability P(m) of m particles crossing the cell nucleus is:

$$P(m) = \frac{\bar{m}^{\,m}}{m!} e^{-\bar{m}} \tag{13}$$

If we use equations (2),(6), (8), (9), and equation (12) of Appendix 1, the survival probability S(m) with T = 1 is:

$$S(\bar{m}) = e^{-\sigma F} \cdot \left[1 + S(1)\sigma F + S(2) \frac{\sigma^2 F^2}{2!} + \ldots + S(m) \frac{(\sigma F)^m}{m!} + \ldots \right] \tag{14}$$

Instead of equation (14), we usually employ a simpler, approximate RMR survival expression usually identical with equation (13) of Appendix I. The coefficient of fidelity is the same as the single particle survival probability $\Phi = S(1)$. Equation (13) of Appendix I is usually sufficiently accurate to represent radiobiological survival curves.

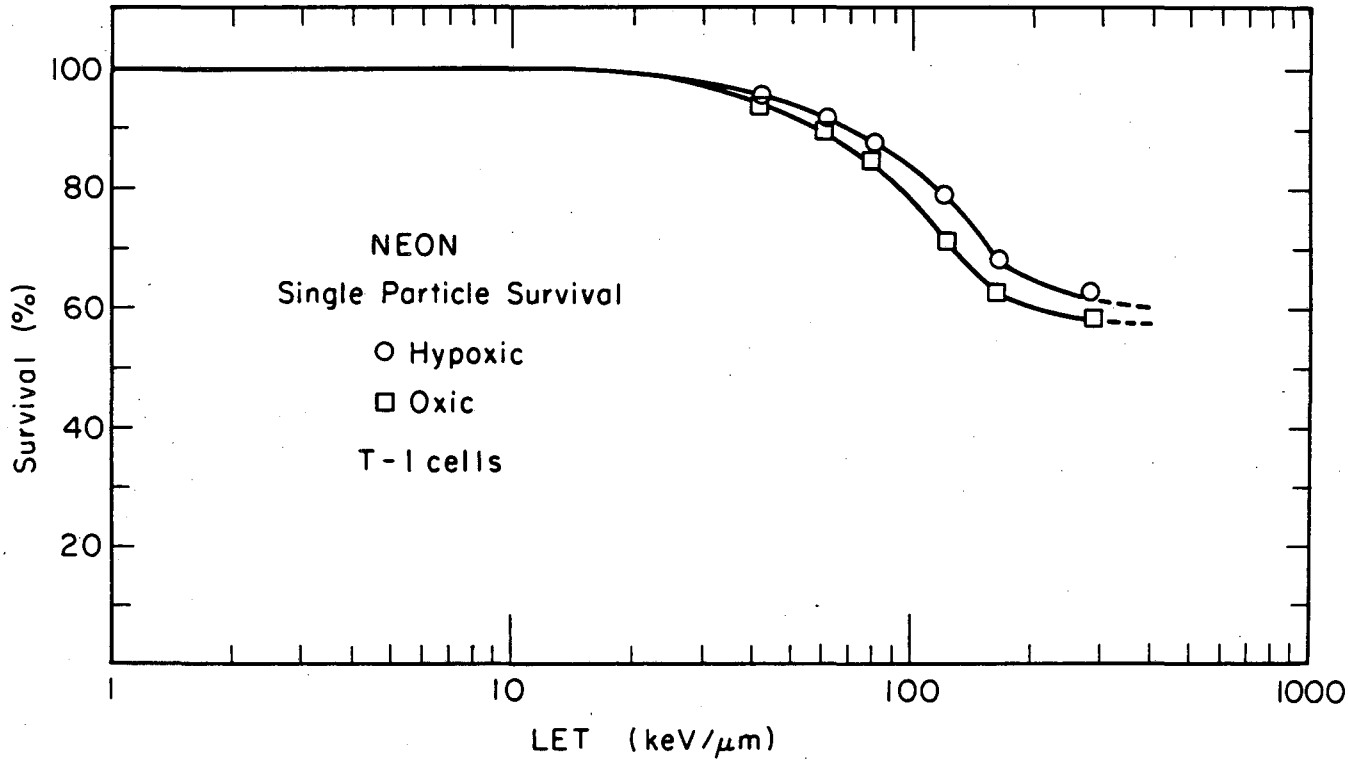

Figure 1. The probability of cell surival as function of LET when a single neon particle passes through the nucleus of a human T-1 cell in culture. (XBL 843-7618).

The Role of Radiation in DNA Integration

When radiation is used in conjunction with specific oncogenic viruses, it enhances the viral transformation process. Recent evidence has been obtained in our laboratory to support the hypothesis that a key step in cell transformation is the integration of oncogenic segments of DNA. These experiments also indicate that the role of DNA viruses is primarily to assure propagation of the oncogenes independently of host cells and to provide a convenient entry of the transforming DNA into the cells of the host.

The question of how foreign DNA is integrated into a mammalian genome after its entry into the cells remains unknown. Links must be provided between both strands of the host DNA and of the viral DNA. We do not know at present whether the steps in this process either involve initially linking of single-stranded DNA (strand invasion), or whether severed double-stranded breaks rejoin, or whether the intermediate linking steps include other molecules, for example, RNA chains. For this discussion, we assume that the process involves rejoinings between completely scissioned double-stranded DNA. This approach seems fruitful in the RMR model, where misrepair occurs when mismatched DNA endings join. Whereas above, in discussing RMR-III, we assumed that all misrepair is lethal, we now modify that assumption, admitting that a small fraction of misrepair events may not necessarily be lethal. The evidence for this is that chromosomal translocations are known to occur in cells that were exposed to deleterious agents but remained viable. Thus, the incorporation of viral DNA into mammalian cell DNA according to this model is a misrepair step. The incorporation of a linear segment of DNA would require at least two nonlethal misrepair steps. If there are more than two misrepair steps, the probability for a lethal effect increases.

Assume that in the course of integration a quantity of r scissions per cell are made in the DNA by ionizing radiation. We can calculate the probability $Q(x,r-x|r)$ that a cell which sustained r lesions at t = 0 will, in the course of the repair process, develop x quadratic misrepairs and (r-x) eurepairs. In the calculation, the repair process was viewed as a Markov chain, as represented in Appendix I. Without presenting details, we propose that the probability of dual misrepairs may be expressed as:

For x = 2:

if $r < 3$ then $Q(2, r-2|r) = 0$

$$\text{if } r > 3 \text{ then } Q(2,r-2|r) = Q(0, r|r) \left[\sum_{q=1}^{r-2} \frac{q}{r-q} \cdot \sum_{l=q+1}^{r-1} \frac{l}{r-l} \right] \quad (15)$$

If one follows the reasoning according to equation (12) in Appendix I, the overall probability M(x) that a fluence of F particles will produce cells with two misrepairs is:

$$M(F,2) = \sum_r e^{-\sigma F} \cdot \left[s(0|r)\ Q(2,(r-2)|r)\ \frac{(\sigma F)^r}{r!} \right] \quad (16)$$

Assume that viruses alone, in the absence of radiation, can transform a fraction ν of the isolated cells (usually $10^{-4} > \nu > 10^{-7}$). Consider further the subpopulation of cells that have suffered x = 2 misrepairs in the postirradiation period. This represents cells with chromosome deletions and symmetric or asymmetric translocations. Most of these cells die, but a small portion, γ_s, might survive (we estimate $10^{-1} > \gamma_s > 10^{-3}$). Let γ_ν be the probability that survivors express the transformation property. With these factors in mind we can write an expression for the transformation enhancement ratio ψ:

$$\psi = \frac{\nu + \gamma_s \gamma_\nu \frac{M(F,2)}{S(F)}}{\nu} \tag{17}$$

S(F) is the overall probability of survival, including individual cells that survived DNA and chromosome rearrangements.

Our approach has tacitly assumed that the oncogene has been integrated into the host cell DNA between the two misrepairs. There are many missing details of the biological process that must be incorporated into the model at some future time, but equation (17), with appropriately adjusted constants, can be made to fit the experimental data. In Figure 2, a set of X-ray and neon ion cell transformation data (Yang et al., 1980) are superimposed on a set of curves representing equation (17). Cell survival data following irradiation by the same ions is given in Figure 3, where the continuous lines represent the RMR survival curve, using the same values for the coefficients as used in Figure 2. Figure 4 indicates the probability of cell transformation as a function of cell survival for cell irradiated with X rays.

The calculations, as presented here, are obviously incomplete, and are only useful if the rate limiting steps in the integration process are the misrepair steps. Further experimental data are needed to clarify the mode of entry of the oncogenic virus and of plasmid DNA into the cell and its nucleus, and the biochemical steps leading to integration. The use of labeled plasmid DNA may be helpful in this regard. We are, in fact, engaged in a set of new experiments (Perez and Tobias, 1984) using plasmid recombination technology, which provides a powerful tool to answer these questions.

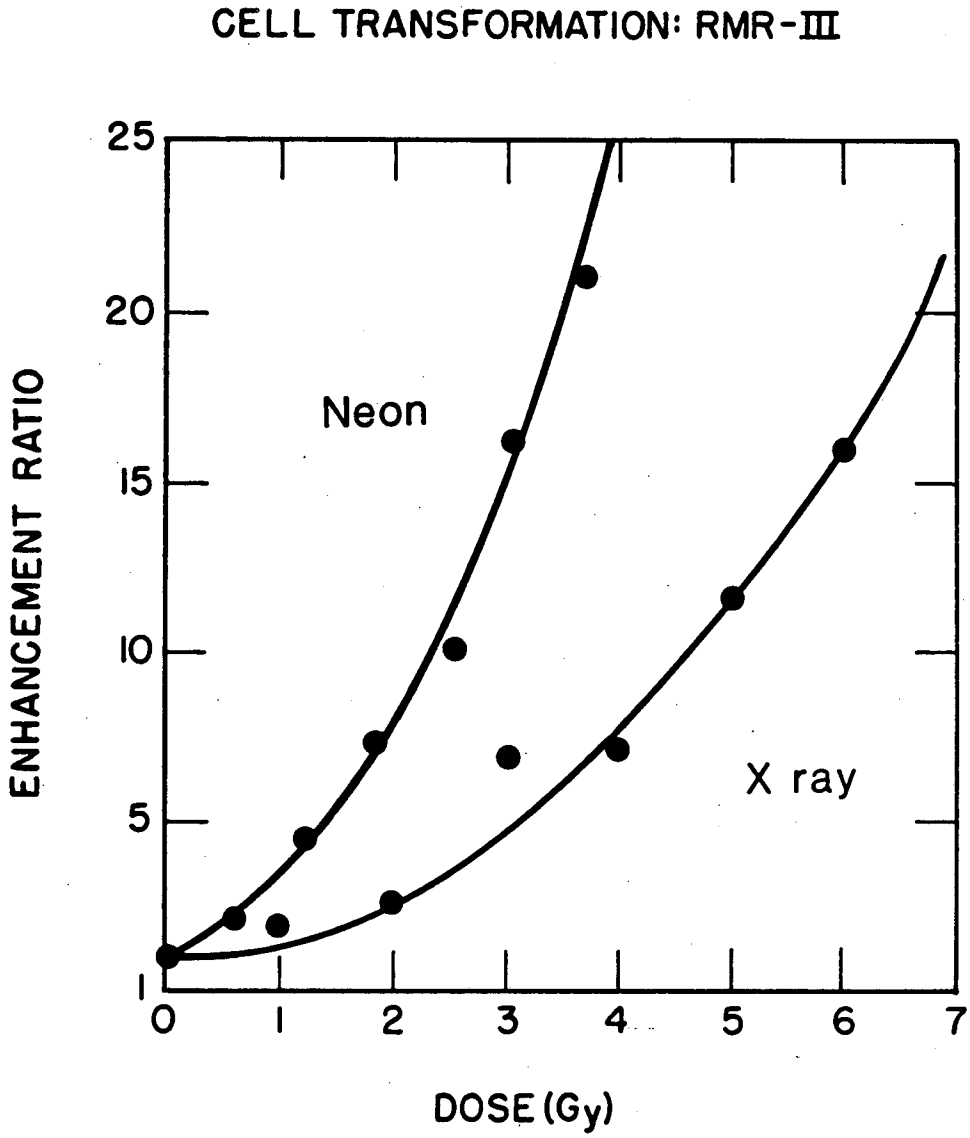

Figure 2. Data on the dose dependence of the enhancement ratio of transformation of viable C3H10T1/2 cells by SV40 virus augmented by exposure to X rays or to neon ions of 1.4 cm residual range. Solid lines represent calculations based on the RMR model as described in the test. The RMR coefficients were determined to fit the survival curves shown in Figure 3. Additional ajustable constants γ_s and γ_v were used from equation (17). In the case of neon, the entire effect apears to be due to intratrack lesion repair. (XBL 843-7636).

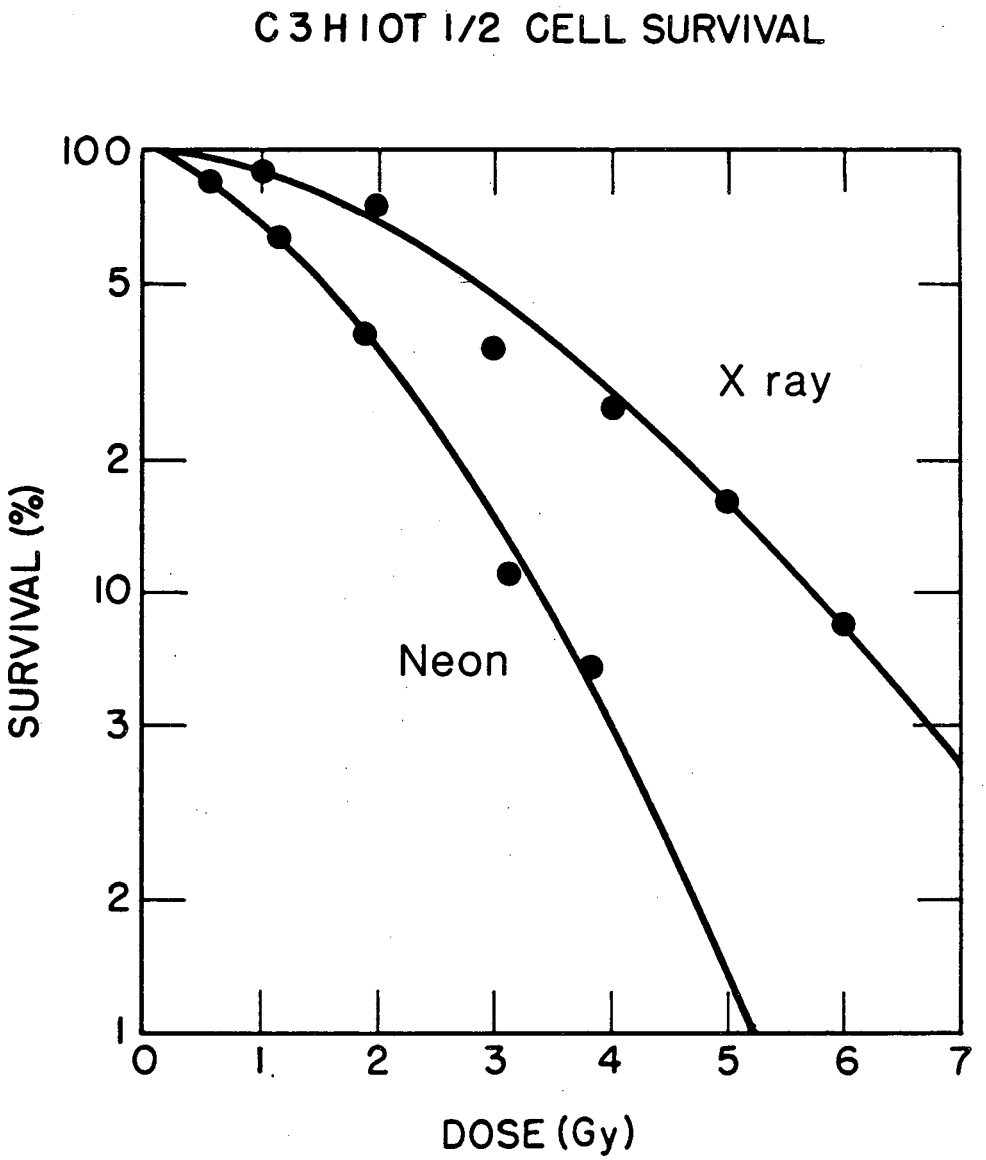

Figure 3. Survival curves of C3H10T1/2 cells following administration of X rays or of neon ions of 1.4 cm residual range. The solid lines are drawn by the use of the RMR model. (XBL 843-7637).

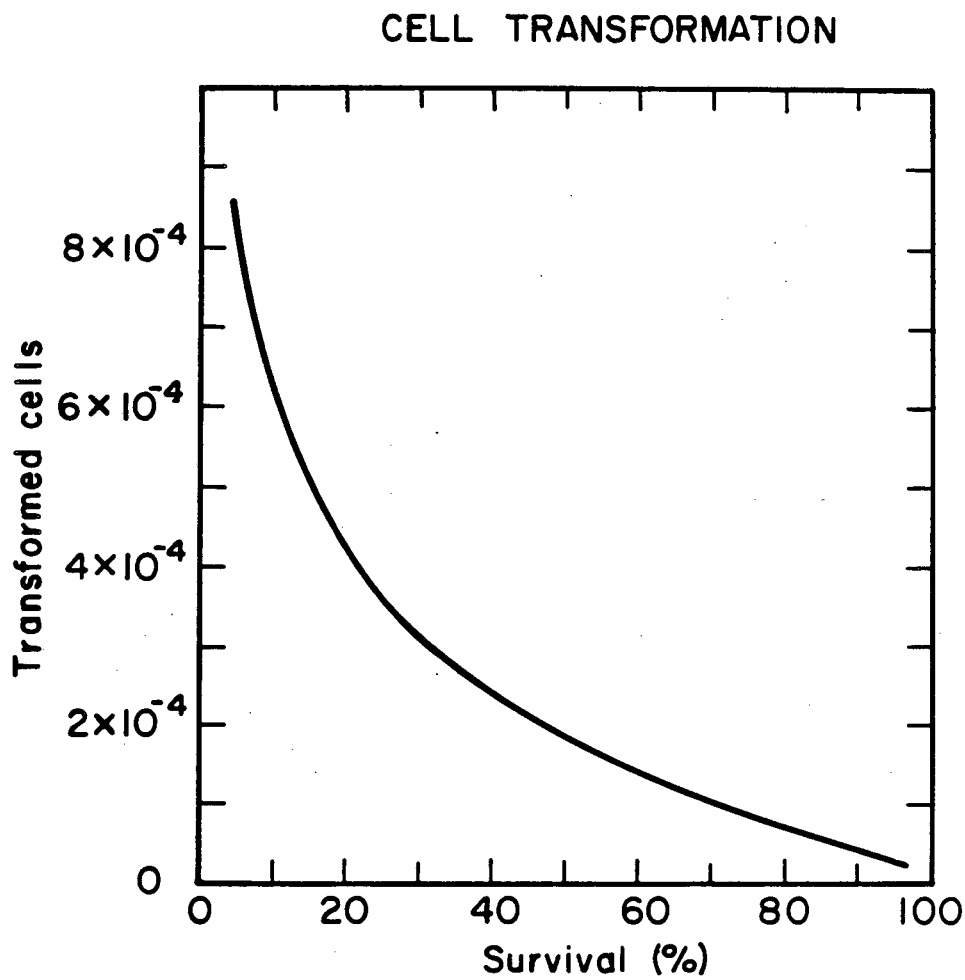

Figure 4. Relationship between cell survival and cell transformation frequency of viable C3H10T1/2 cells irradiated with X rays. The line is based on the RMR expression for dual misrepair. (XBL 843-7611).

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APPENDIX I: DESCRIPTION OF THE RMR-I MODEL

Let the yield of a population of uncommitted U lesions be proportional to dose, D. At $t = 0$:

$$U_0 = \delta D(1)$$

U(t) represents the mean number of uncommitted lesions per cell at time t.

The cells recognize the U lesions, and attempt to repair them. The time rate of self-repair of individual lesions is λ. In the course of repair, cooperative processes with a rate k participate with pairs of U lesions.

The dose-dependent production of U lesions is not coupled with the time-dependent repair processes. The rate of repair is expressed by the RMR equation, which we assume is valid for the mean of the U distribution:

$$dU/dt = -\lambda U - kU^2 \qquad (2)$$

Initially there are U_0 lesions and $U(t)_{t\to\infty} = 0$. This leads to:

$$U(t) = \frac{[U_0 e^{-\lambda t}]}{1 + [U_0/\varepsilon\ (1-e^{-\lambda t})]} \qquad (3)$$

where ε is the repair ratio: $\varepsilon = \lambda/k$.

The repair of each lesion may result either in <u>eurepair</u>, perfect reestablishment of DNA structure and coding, or in <u>misrepair</u>, where there are

structural and/or coding defects. Examples of misrepair are chromosome fragments and mis-rejoinings often found in cells exposed to radiation.

Equation (3) often describes the mean values of the quantity of DNA strand breaks that can be chemically assayed in the postirradiation period.

The cumulative value of linear repairs, $R_L(t)$ is:

$$R_L(t) = \lambda \int_0^t U(t)dt \qquad (4)$$

The value of quadratic repairs is $R_Q(t)$:

$$R_Q(t) = k \int_0^t U^2(t)dt \qquad (5)$$

The initial distribution of the number of U lesions per cell caused by a homogeneous radiation field, X rays, for example, is assumed to be a Poisson distribution. As repair progresses, the distributions of U, R_L, and R_Q deviate from a Poisson distribution. A conservation condition:

$$U(t) + R_L(t) + R_Q(t) = U_o \qquad (6)$$

assures that in the course of time the sum of the distributions remains Poisson. From equation 6, at time t the mean lesions per cell are: $U(t) + R_Q(t)$, because U(t) represents uncommitted lesions and $R_Q(t)$ lethally misrepaired lesions.

If survival is determined at time t, and the condition of survival is that cells have no lesions at all, then the probability S(t) of survival is:

$$S(t) = \exp(-R_Q(t) - U(t)) = e^{-U_o\left[1 + \frac{U_o(1-e^{-\lambda t}}{\varepsilon}\right]^{\varepsilon}} \qquad (7)$$

We know that the distribution of $(R_Q(t)+U(t))$ is not Poisson, but in deriving equation (7) we assumed that the first term of the distribution is identical with the Poisson expression, an assumption that is verified below.
The first term in equation (7) represents the probability that no U lesions are present in the cell initially; the second term represents the probability for eurepair. The time factor is also important: t is the time when the lethal effect is expressed; it is often the time when the irradiated cells divide.

The RMR process can also be described as a result of a statistical branching process. Let P(J) be the probability that a cell has J lesions of the U type. Initially:

$$P(J) = \frac{U_o^{\,J}}{J!} e^{-U_o} \qquad \text{where } U_o = \sum_{0}^{\infty} JP(J) \qquad (8)$$

We are interested in how the probability P(J) varies in time as the U lesions are repaired at a rate of $du/dt = \overset{\circ}{U}$; differentiating equation (8) we obtain:

$$\frac{dP(J)}{dt} = \dot{U}\left[\frac{U^{J-1}}{(J-1)!} e^{-U} - \frac{U^J}{J!}\right] e^{-U} \qquad (9)$$

substituting for $\dot{U}$ the right side of equation (1) and rearranging, we obtain:

$$\frac{dP(J)}{dt} = \lambda \left[(J+1)\ P(J+1) - JP(J)\right] + k\left[(J+2)(J+1)P(J+2) - (J+1)JP(J+1)\right] \qquad (10)$$

The first term corresponds to linear self-repair, and the second term corresponds to quadratic misrepair. By analyzing the meaning of equation (10) for all J, we realize that it represents a branching process.

If a given cell has initially r lesions with probability P(r), we can use equation (10) for describing the repair processes. The rate of repair of lesions in that cell is described by a set of equations:

$$\frac{dP(r)}{dt} = -\lambda r P(r)$$

$$\frac{dP(r-1)}{dt} = \lambda r P(r) - \lambda(r-1)P(r-1) - r(r-1)P(r)$$

$$\frac{dP(r-2)}{dt} = \lambda(r-1)P(r-1) - \lambda(r-2)P(r-2) + kr(r-1)P(r) - k(r-1)(r-2)P(r-2) \qquad (11)$$

We can integrate the set of equations (11) and obtain for $t \to \infty$ the probability of all eurepair. In the process of integration, we assume that once a quadratic misrepair event developed (terms with k), it is slated to die

and will not contribute further to the probability of survival, which implies that each of the successive repair steps must be linear eurepair. For cells with r initial lesions, the survival probability $S(0|r)$, expressed for the simple case where λ/k is an integer:

$$S(0|r) = \frac{\lambda(\lambda-k)(\lambda-2k) \cdot \cdot \cdot \lambda-(r-1)k}{\lambda^r} \qquad (12)$$

and where $S(0|r)$ is the probability that a cell has performed in succession r eurepairs in the course of time. The total survival probability S, when $t \to \infty$, is:

$$S = e^{-U_0} \cdot \sum_{r=0}^{\infty} \frac{S(0|r)\ U_0^r}{r!} \qquad (13)$$

Equation (13), with the value of $S(0|r)$ from equation (12), is identical to equation (7) if the repair term of equation (8) is represented by a Taylor expansion.

There are many applications of the RMR-I model (Tobias et al., 1980; Pirruccello and Tobias, 1980). Perhaps the most interesting applications relate to experiments in which the radiation is delivered in successive dose fractions, separated by time. The function U(t) can also be termed as a measure of "remnant" lesions, representing the memory of the cell for previous radiation injuries. New U lesions can be added to remmant U lesions, and the RMR process as described here can predict the final outcome.

A frequent occurrence is that the linear self-repair does not have perfect fidelity, meaning that some linear repairs are misrepairs. We can then introduce a coefficient Φ for the fidelity of linear repair: $0 \leq \Phi \leq 1$. In this case we introduce the eurepair ratio, μ, with $\mu = \Phi\ \varepsilon$: equation (7) then modifies to:

$$S = e^{-U_o} \cdot \left[1 + \frac{U_o \Phi\ (1-e^{-\lambda t})}{\mu} \right]^{\mu} \qquad (14)$$

Equation (14) describes very well the shape of most experimental cellular radiation survival curves.

Perhaps the most important contribution of the RMR-I model was the introduction of a method for quantitating eurepair and misrepair. Recently, several modifications have been suggested. For example, Curtis described the "LPL" model (1983). The next article by Albright and Tobias (1984) introduces RMR-II, a model based entirely on a Markov description. In the main body of this report, we extended the RMR model to describe radiation effects of heavy ion tracks. We have named this track version of the model: RMR-III.

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