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A Discrete-Event Model of Carcinogenesis

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ABSTRACT

We describe a new simulation model of carcinogenesis which, in addition to the features of a standard clonal two-stage model (loss of both copies of a tumor suppressor gene by point mutations, cell division and cell death), includes a quantitative description of mitotic recombination, DNA repair, and cell to cell interactions in all stages. The model is implemented as a discrete event process.

We present the results of a sensitivity analysis of the model. The most sensitive paramenters were found to be: the number of normal cells at risk, and the division rate, death rate and DNA repair efficiency for the intermediate stage cells. Accurate information about these parameters is important for a quantitative understanding of carcinogenesis.

The sensitivity of the model to the number of normal cells indicates the importance of understanding the nature of the cells at risk, e.g. stem cells vs. differentiated cells. The model can be used to assess the importance of chromosomal damage such as mitotic recombination and epigenetic mechanisms such as hyperplasia and cytotoxicity in the onset of malignant tumors.

INTRODUCTION

In contrast with the extreme diversity and complexity of the biological observations and theories on cancer developed during the past 30 years, the quantitative description of carcinogenesis is still rudimentary. The conceptualization of carcinogenesis as a probabilistic multistep process led, in the 1950's, to the definition of the multistage model (Armitage and Doll, 1954; Armitage and Doll, 1957; Nordling, 1953). The only assumption made by the multistage model is that, to become fully malignant, cells have to undergo a series of rate-limiting transformation events whose time of occurrence is exponentially distributed. Within such a framework, carcinogens are supposed to act by increasing the probability of transformation from one stage to another. With some methodological refinements (Crump et al., 1977; Crump and Howe, 1984; Krewski et al., 1983; Murdoch and Krewski, 1988; Whittemore and Keller, 1978) this model is still the most widely used.

The discovery and confirmation that the loss of two copies of the retinoblastoma gene could lead to a fully malignant tumor (Benedict et al., 1983; Hethcote and Knudson, 1978; Knudson, 1971; Knudson et al., 1975), and that the clonal expansion of pre-malignant cells was important (Nowell, 1976), led to the formulation of a series of closely related clonal two-stage models for which a considerable literature now exists (Greenfield et al., 1984; Knudson, 1971; Moolgavkar, 1990; Moolgavkar et al., 1988; Moolgavkar and Knudson, 1981; Tan, 1991). Most clonal models developed so far assume that two mutation events are required to form a fully malignant cell. DNA-damaging carcinogens are supposed to act by increasing the probability of these events (Moolgavkar et al., 1990; Portier, 1987), while promoting agents are supposed to induce the proliferation of intermediate stage cells (Cohen and Ellwein, 1990a; Moolgavkar, 1983), therefore increasing the size of the susceptible cell population. A recent extension of the clonal multistage model (Kopp-Schneider et al., 1991; Portier and Kopp-Schneider, 1991) includes the repair of DNA lesions.

Although not included in clonal models developed so far, mitotic recombination (MR) (and aneuploidy in certain cases) is in fact likely to be crucial in the cancer process (Cavenee et al., 1986; Holliday, 1989; Thompson et al., 1989; Vogelstein et al., 1989). Point gene mutations or small deletions have long been known to be involved in cancer, both by activation of oncogenes (Bouchard et al., 1989; Hariharan et al., 1989; Horowitz et al., 1989; van't Veer et al., 1989) and by inactivation of tumor suppressor genes (Solomon et al., 1987; Toguchida et al., 1989; Vogelstein et al., 1989; Weinberg, 1989), but only recently has the importance of MR appeared clearly as a mutational mechanism for the loss of tumor suppressor genes (Cavenee et al., 1986; Gallie and Worton, 1986; Green, 1988; Green, 1989). MR results in reduction to homozygosity for a mutated allele in a cell with one mutated and one intact allele. Although first demonstrated in retinoblastoma, MR has been demonstrated in a variety of cancers (Atkin and Baker, 1989; Baker et al., 1989; Hansen and Cavenee, 1988; James et al., 1989). The effects of MR on the development of cancer have not yet been addressed in any quantitative model of carcinogenesis, nor has the effect of cell to cell interactions, such as cell death prompting the proliferation of neighboring cells. This process of

compensatory growth may have a large impact of the carcinogenicity of many chemicals, particularly at high dose (Cohen and Ellwein, 1990a) because proliferating cells are more at risk of fixing mutations .

In order to investigate the importance of such events we developed a model of carcinogenesis which, in addition to the features of a clonal two-stage model (cell mutations, death and division), includes a quantitative description of MR, DNA repair, and cell to cell interactions. The model is able to provide a quantitative understanding of the importance of chromosomal damage and epigenetic mechanisms, such as hyperplasia or cytotoxicity, in the onset of malignant tumors. We present here the results of a sensitivity analysis of the model.

MODEL FORMULATION

The model is currently a two-hit clonal expansion model, i.e., two copies of a tumor suppressor gene must be lost for a cell to become malignant. If one copy is lost the cell is refered to as an intermediate cell. Due to the possibility of repair, the model describes the behavior of six types of cells: normal, repairable intermediate (rIN), unrepairable intermediate (IN), twice-repairable malignant (2rMA), once-repairable malignant (1rMA) and unrepairable malignant (MA). For each cell type, one of the following events can eventually take place (see also Figure 1):

Cell death, with probability P_{Death} per unit time, leads to the disappearance of the cell. To maintain a constant number of cells in the tissue, the neighbor of the dying cell is programmed for compensatory division (see division event below).

Cell division, with probability P_{Div} per unit time, leads to the formation of a new cell. If one or more neighbors died previously, compensatory division takes place with probability P_{DivC} (close to 1). If several neighbors die the burden of compensatory division is shared equally between the progenitor and the new cell (i.e., an expanding clone of cells starts replacing the dead ones). The division of normal and MA cells leads to the formation of new cells identical to their progenitors. During the division, all repairable DNA lesions are irreversibly fixed, so the division of 1rMA and 2rMA cells gives two MA cells. The behavior of intermediate cells is somewhat more complex: During the division and DNA damage fixation of rIN cells, either one or both DNA strands can be damaged (with probability P_{2dam} per division). In the former case the division results in one normal cell and one IN cell. When both strands are damaged, division leads to two IN cells, unless MR occurs (see below), in which case one normal and one MA cells are formed. The division of an IN cell leads to a new IN cell, while MR leads to one normal and one MA cell.

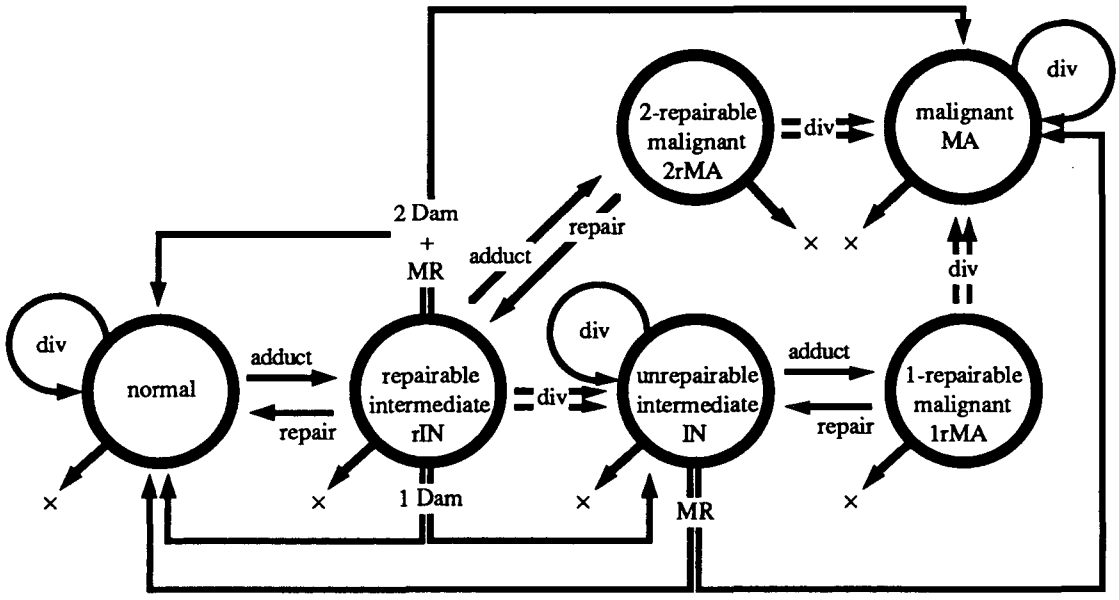

Figure 1: Schematic description of the events leading to the formation of malignant cells, as implemented in the model. Meaning of the symbols: Div: division; ×: death, 1 Dam and 2 Dam: single and double strand damage, respectively (see text); MR: mitotic recombination.

DNA adduct formation, with probability P_{Add} per unit time, introduces one crucial lesion in the DNA of normal, rIN, or IN cells. Because DNA adducts are presumed to interfere with transcription and become *de facto* mutations while in place, the cells become rIN, 2rMA, or 1rMA, respectively.

Repair of DNA adducts can take place with probability P_{Repair} per unit time, as long as the cell has not yet undergone division. If repair occurs the cell bears one less lesion.

Mitotic recombination: MR has an effect only if both DNA strands of the altered allele of a tumor suppressor gene are damaged when division takes place. Therefore, MR can affect either rIN (if both DNA strands are damaged) or IN cells, with probability P_{MR} per division. In both cases the result is the formation of one normal cell and one MA cell. Normal and malignant cells can undergo MR but then do not change stage, we consequently do not keep track of such events.

The behavior of a set of cells (tissue) is followed using discrete events simulations in which the evolution of the population of cells is updated at regular time intervals (Banks and Carson, 1984; Delaney and Erminia, 1989; Dudewicz and Karian, 1985). The time increment, which is also the time unit, was one day, and the cell behavior was followed over 100 days. The tissue is represented by a one-dimensional array (more specifically, a pointed list) of cells, allowing us to keep track of neighboring effects. At any time step (day), the evolution of each cell is computed by randomly selecting an event among the previously described possibilities, with the corresponding probabilities (P_{Div} etc.). We used the pseudo-random number generator described by Park and Miller (1988). To simulate realistic situations we had to keep track of large numbers of cells (between a million and a billion). We reduced the computational burden by using stochastic simula-

tions (Ripley, 1987) for normal and MA cells, whose behavior is not very complex. However, to keep track of neighboring effects, cells adjacent to intermediate cells (repairable or unrepairable) were always simulated individually. For normal and MA cells, the distribution of the number of cells was computed without following the fate of every cell: homogeneous patches of normal or MA cells were treated as groups. For such groups only the number of cells and the net number of previous deaths were updated, using a multinomial random number generator (Devroye, 1986), based itself on a binomial generator (Press et al., 1986). For the multinomial generator the probabilities of individual events were either the set (P_{DivC}, P_{Death}, P_{Add}) with a population size equal to the number of net deaths recorded (assumed to be equal to the number of dead cells neighbors), or the set (P_{Div}, P_{Death}, P_{Add}) for rest of the population of the group. Note that P_{Add} is always zero for MA cells (the ultimate stage). The sampled numbers of divisions and deaths were used to update the group's statistics. For normal cell groups, each DNA-altered cell formed was inserted randomly within the tissue, between two normal cells, therefore subdividing the group in two. At time zero, all simulations were started with one homogeneous group of normal cells. The computer program simulating the process has been coded in C.

INPUT PARAMETER VALUES

The baseline parameter values (probability rates for the various events) were chosen to correspond to the case of mouse liver cells: Table 1 gives the values used for the simulations. The initial number of normal cells corresponds to the whole liver of a mouse. No intermediate or malignant cells were present in the tissue at the start of the simulations. According to Cohen (1990b) the probability of division of normal hepatocytes, in young mice, is approximately 0.005 per cell per day. This is mostly to compensate for normal cell death, so we set the death probability rate to 0.005 and the division probability rate to zero. In absence of precise information, the compensatory division rate was set at a high value, such that the neighbor of a dead cell had no choice but either die, mutate or divide. The same holds for intermediate and malignant cells. The net result of this parameter combination is, as expected, a division rate of about 0.005 and a zero net growth rate for the liver as a whole. The background DNA-adduct formation probability rate was fixed to 10^{-7} per gene per day for both normal and intermediate cells, close to the values used by Cohen (1990b) or reported by Loeb (1991) for humans. Note that these authors use mutation rates that are the net result of DNA damage and repair, which we separate in our model. Using the data presented by Moolgavkar (1990) and Cohen (1990b Figure 2) for mouse liver, a division rate of 0.1 per cell per day was used for intermediate and malignant cells, with a death rate such that the resulting net division rate (division minus death) is 0.005. The repair probability of DNA adducts was set to 0.75 per cell division (Tang et al., 1989) for both intermediate and malignant cells (normal cells do not bear DNA lesions in the critical gene, by definition, and therefore no repair rate is defined for them). The probability of damaging both DNA strands during the irreversible fixation of a DNA adduct, at the time of duplication, was set to 0.2. The probability of MR was set to 2×10^{-5} per division on the basis of human data (Morley, 1991).

MODEL OUTPUT

The various quantities currently computed by the model are:

- total number of cells.
- number of cells of any type.
- total number of intermediate or malignant clones formed. By definition, a clone occurs as soon as a IN or MA cell is formed other than by division of a cell of the same type.
- time of appearance of the first malignant clone.

Each simulation of the tissue evolution, from time zero to 100 days, produces a unique configuration of values for the model output variables, which are in fact random variables. To obtain the distribution of values for each variable it is necessary to repeat the simulations. The output variable distributions can be computed with arbitrary accuracy, provided that enough repetitions are performed: in all cases, we performed 1000 repetitions of the simulations.

Table 1: Model parameter values for the baseline behavior (to which the references apply) and modified values used for sensitivity analysis. The parameter values are for mice.

Parameter	Baseline Value	Modified Value	Reference
Normal Cells			
N. Init.	1.5×10^{8}	1.35×10^{8}	(Altman and Dittmer Katz, 1976)
P_{Death_0}	0.005	0.0055	(Cohen and Ellwein, 1990b)
P_{Div_0}	0.0	0.001	(Cohen and Ellwein, 1990b)
P_{DivC_0}	0.9949999	0.9	- *a*
P_{Add_0}	1.0×10^{-7}	0.9×10^{-7}	(Loeb, 1991)
Intermediate Cells			
P_{Death_1}	0.095	0.105	(Moolgavkar et al., 1990)
P_{Div_1}	0.1	0.09	(Cohen and Ellwein, 1990b)
P_{DivC_1}	0.9049999	0.8	- *a*
P_{2dam}	0.2	0.18	- *b*
P_{Add_1}	1.0×10^{-7}	0.9×10^{-7}	(Loeb, 1991)
P_{Repair_1}	0.75	0.825	(Tang et al., 1989)
P_{MR_1}	2.0×10^{-5}	1.8×10^{-5}	(Morley, 1991)
Malignant Cells			
P_{Death_2}	0.095	0.105	(Moolgavkar et al., 1990)
P_{Div_2}	0.1	0.09	(Cohen and Ellwein, 1990b)
P_{DivC_2}	0.905	0.805	- *a*
P_{Repair_2}	0.75	0.825	(Tang et al., 1989)

[a] values for this parameter were fixed by us so that for a given stage the sum of P_{Death}, P_{DivC}, and P_{Add} probabilities was equal to 1

[b] values for this parameter were fixed by us to a plausible value.

SENSITIVITY ANALYSIS

For an analysis of the model's sensitivity to variations in its parameter values we used a standard differential analysis (Iman and Helton, 1988). Each parameter was varied by a fixed amount (about 10%) (see Table 1, modified values). The sensitivity coefficient, $S_{i,j}$, of each observed variable of the model, with respect to each parameter is:

$$S_{i,j} = \frac{(\bar{y}_{i,j} - \bar{y}_{io,j})}{2 \cdot (x_j - x_{jo})/(x_j + x_{jo})} \tag{1}$$

where $\bar{y}_{i,j}$ is the mean of the 1000 values of the ith output variable obtained during the repetitions, with the jth parameter being modified; $\bar{y}_{io,j}$ is the mean of 1000 values of the ith output variable obtained during the baseline simulations; x_j and x_{jo} are the modified and baseline values of the jth parameter, respectively. The denominator of the fraction measures the difference between the modified and baseline parameter values, relative to their average. This standardizes the sensitivity coefficients for a given output variable. Note that the difference in output variable values is not standardized.

The values of the sensitivity coefficients are function of the averages $\bar{y}_{i,j}$ and $\bar{y}_{io,j}$ of the output variables. These averages have a variance, since only 1000 repetitions were made. To assess the variability of the sensitivity coefficients we drew 50 bootstrap samples (Efron, 1990; Efron and Tibshirani, 1991) from the set of 1000 of repetitions. The sensitivity coefficients were computed for each bootstrap sample, and their standard deviation formed (N=50).

RESULTS

At time 100 days, the averages (± standard deviation) of the number of rIN, IN, and MA cells were 19 (±4), 6700 (± 3900), 1.2 (± 4.7) respectively, with the baseline parameter values reported in Table 1. No repairable malignant cells (r1MA and r2MA) were observed at time 100. On average 47 (SD: 6.8) and 0.3 (SD: 0.59) intermediate and malignant clones were formed, respectively. Malignant clones were formed in 27% of the repetitions. Generally no more than one clone was formed since the average number of clones is 0.3.

Table 2 gives the sensitivity coefficients and their bootstrapped standard deviations, for each output variable, with respect to each parameter. Given the standardization by parameters only, comparisons should be made between rows (for a given output variable), and not between columns (for a given parameter). For a given output variable, the distributions of the sensitivity coefficients tend to overlap, in particular for insensitive parameters (indicated by low absolute values of the sensitivity coefficients). Figure 2 illustrate these distributions for the number of malignant clones formed. The box plots give the 10th percentile (lower bar), the 25th percentile (bottom of the box), the median (middle bar), the 75th percentile (top of the box)

and the 90th percentile (upper bar) of the distributions. Simulation results falling in the lowest or the highest 10% of the distribution are displayed individually with circles. The parameters tend to fall into groups of similar influence, and the 4 or 5 most influential parameters are fairly well defined for the majority of output variables. Table 3 gives for each model output variable the ranking of the four most important parameters. Rankings for the 1rMA and 2rMA cells are not presented because of the very low occurrence of these cells and the corresponding lack of confidence in the values of their sensitivity coefficients (see Table 2). The ranking of parameters for the time to tumor is also subject to caution given the variability of the corresponding sensitivity coefficients.

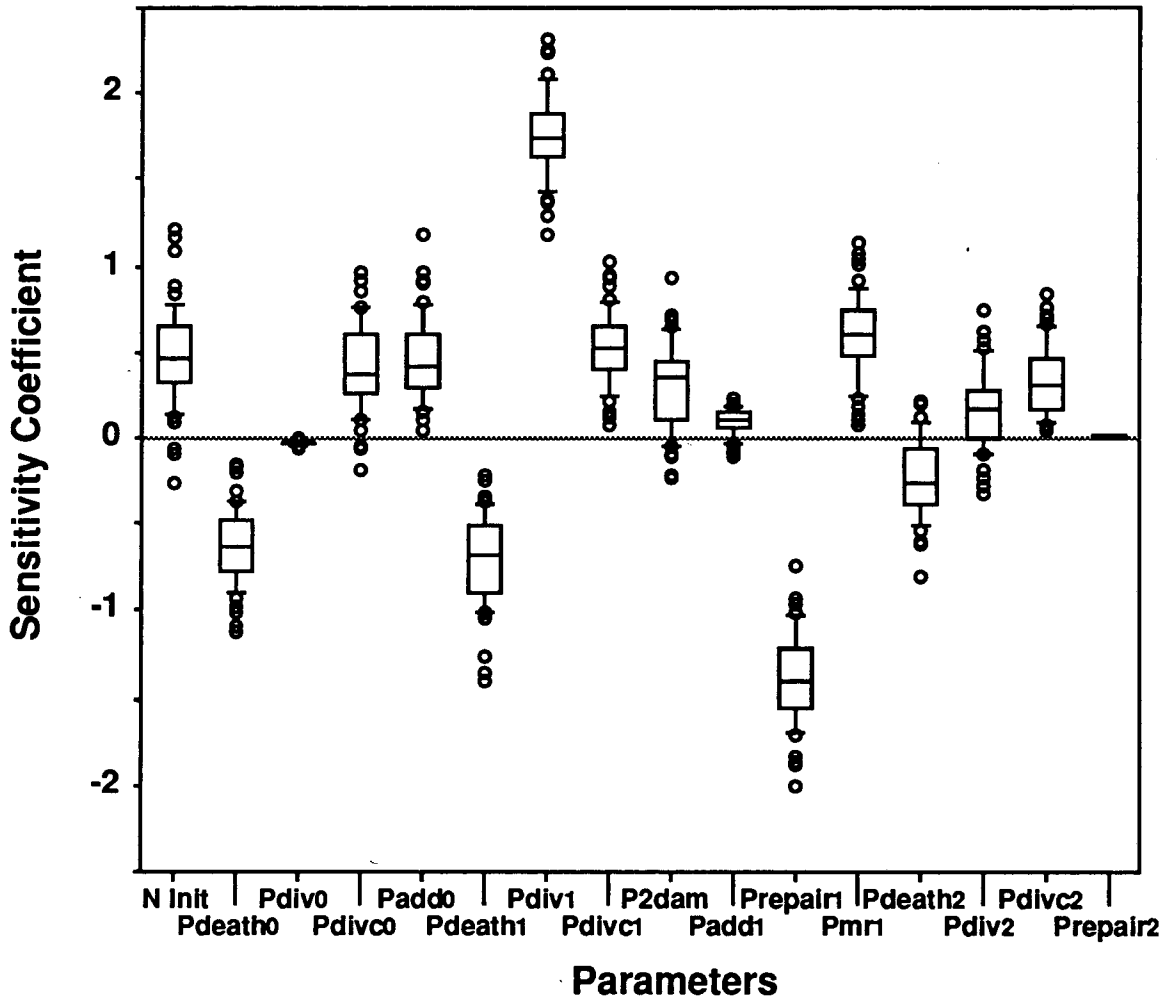

Figure 2: Sensitivity analysis results for the number of malignant clones formed in 100 days. The box plots (see text) represent the distribution of sensitivity coefficient values for the various model parameters. The symbols used for the parameters are given in the Model Formulation Section.

Table 2: Sensitivity coefficients for the model output variables, mean and (standard deviation)

Parameter	Normal Cells	Repairable Intermediate	Unrepairable Intermediate	Intermediate Clones	Unrepairable Malignant	Malignant Clones	Time to Tumor
N. Init.	1.42×10^8 (480)	22.9 (1.9)	7630 (1700)	42.5 (3.0)	2.39 (2.0)	0.437 (0.29)	1.09 (9.8)
P_{Death_0}	-8470 (520)	-6.20 (2.2)	976 (2500)	3.27 (3.2)	-1.41 (2.5)	-0.567 (0.22)	-11.7 (11)
P_{Div_0}	74700 (22)	-0.096 (0.11)	10.4 (110)	0.318 (0.17)	-0.086 (0.12)	-0.023 (0.014)	-0.257 (0.63)
P_{DivC_0}	789000 (450)	5.08 (1.6)	354 (2100)	-4.30 (3.0)	0.219 (2.3)	0.419 (0.26)	7.53 (9.8)
P_{Add_0}	5720 (400)	22.1 (1.5)	6850 (1900)	45.8 (3.0)	1.24 (2.1)	0.437 (0.24)	9.65 (10)
P_{Death_1}	-458 (400)	-3.64 (1.8)	-18500 (1800)	-0.52 (2.6)	-2.16 (2.1)	-0.67 (0.26)	-15.5 (11)
P_{Div_1}	45.0 (440)	3.21 (1.8)	36800 (1700)	41.1 (2.6)	5.98 (1.9)	1.76 (0.24)	3.31 (11)
P_{DivC_1}	383 (360)	4.28 (1.2)	8310 (1700)	-3.78 (2.7)	1.06 (1.8)	0.496 (0.21)	7.42 (10.5)
P_{2dam}	223 (420)	4.15 (1.7)	3470 (1900)	-2.79 (2.7)	0.447 (2.1)	0.276 (0.26)	6.29 (9.9)
P_{Add_1}	314 (150)	0.741 (0.50)	-407 (540)	0.257 (1.1)	0.0475 (0.47)	0.095 (0.083)	2.10 (3.1)
P_{Repair_1}	-353 (430)	-18.0 (2.1)	-23800 (2100)	-169 (3.4)	-5.89 (2.2)	-1.35 (0.26)	4.89 (12)
P_{MR_1}	85.5 (430)	1.07 (1.6)	-2280 (1800)	-2.14 (2.8)	0.846 (2.4)	0.551 (0.24)	7.77 (11)
P_{Death_2}	-394 (370)	-3.42 (2.0)	-542 (1800)	1.95 (2.6)	-1.43 (2.1)	-0.22 (0.24)	-7.01 (13)
P_{Div_2}	111 (410)	2.06 (2.0)	2430 (1900)	-0.665 (2.6)	0.494 (2.1)	0.133 (0.24)	1.97 (9.8)
P_{DivC_2}	28.2 (360)	2.31 (1.7)	-424 (1600)	-5.46 (2.9)	1.31 (2.0)	0.308 (0.20)	3.19 (9.3)
P_{Repair_2}	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Table 3: Ranking of the model parameters according to the sensitivity of each model output variable.

Rank	Normal Cells	Repairable Intermediate	Unrepairable Intermediate	Intermediate Clones	Unrepairable Malignant	Malignant Clones	Time to Tumor
1	N. Init.	N. Init.	P_{Div_1}	P_{Repair_1}	P_{Div_1}	P_{Div_1}	P_{Death_1}
2	P_{DivC_0}	P_{Add_0}	P_{Repair_1}	P_{Add_0}	P_{Repair_1}	P_{Repair_1}	P_{Death_0}
3	P_{Div_0}	P_{Repair_1}	P_{Death_1}	N. Init.	N. Init.	P_{Death_1}	P_{Add_0}
4	P_{Death_0}	P_{Death_0}	P_{DivC_1}	P_{Div_1}	P_{Death_1}	P_{Death_0}	P_{MR_1}

DISCUSSION

MODEL FORMULATION

Our formulation of the model as a discrete event process has several advantages over an analytical treatment. When attempting to describe the complexity of carcinogenesis, analytical derivations face mathematical intractability. The level of detail with which the cancer process is known to biologists, however unsatisfying it may seem, is well beyond what current mathematics can describe. Ambitious attempts at treating analytically mildly complex models often have to rely on simplifying assumptions, some of them being arbitrary. This has been illustrated and discussed recently by Kopp-Schneider et al. (1991), who mentioned the potential advantage of performing discrete event simulations. The forced simplicity of purely mathematical models, notwithstanding its aesthetic appeal, is usually met with the disdain, if not the exasperation, of many biologists. This is unfortunate since quantitative models are unique tools for bringing together separate pieces of information and performing a rigorous check of hypotheses (Bois et al., 1991). Discrete simulations, as used in our model, allow us to model processes that are intractable analytically. Our model includes for example cell to cell interactions and mitotic recombination, events so far left out of cancer models if not by ignorance at least by necessity. Such a computational treatment has also the advantage of flexibility: biologists, including ourselves, have rarely the same conception, from one month to the next, of what a cancer model should be. We plan for example to add to the model the description of oncogene activation. It is also a powerful approach for obtaining the entire statistical distribution of the model output variables, therefore allowing a better design of experiments or statistical analyses.

However detailed our model may seem, is still a large simplification of reality. This is because two limits remain: computability and data availability. Discrete events simulations are a computer intensive method, and we are limited by our current computer hardware (Sun™ SPARC or Apollo™ RISC workstations). Our simulations of 100 days in the life of a mouse take approximately 10 minutes on a Sun 4. Given that we have to repeat the simulations to obtain the distribution of output variables, rather than just a random outcome, it would be unrealistic to perform the computations with a minute as unit time. We have not yet attempted to measure the improvement in accuracy brought by a finer time scale. Another problem is the limited amount of data available to identify realistic values for the model parameters. For example, rates may vary from tissue to tissue, but we often have data from limited cell types, such as lymphocytes or established cell lines. In the tissue we chose, the liver, the number of initial cells and the division or death rate are relatively well known. Yet, specific mutation rates are not known for the liver since primary hepatocytes cannot at present be cultured. We have not yet fit the model to *in vivo* data to obtain better parameter values; however, the parameter values used here are realistic.

SENSITIVITY ANALYSIS

The sensitivity analysis results show that, besides the number of normal cells at risk, division rate, death rate and DNA repair efficiency for the intermediate cells are the most sensitive parameters. This analysis has some limitations. We used a classical "point" sensitivity analysis, the results of which depend in part on the model parameter values. The analysis was also performed over a short period of time, i.e., 100 days. A longer simulation time might have given more precise results for the influence of the various parameters on the time to tumor. A global sensitivity analysis, such as performed by Monte Carlo simulations, would be preferable, but we decided to first use a less cumbersome method.

The sensitivity of the model to the number of normal cells indicates the importance of understanding the nature of the cell population at risk. For example, it could be that only the undifferentiated cells of a tissue (i.e., the stem cells) are susceptible to become cancerous. The importance of DNA repair also points to the need for more accurate data for this process. The efficiency of adduct repair is known to vary at least between bulky and small adducts (Jones et al., 1991). Chemical specific data is therefore needed. Repair is also known to vary with the position of the adduct and the three-dimensional structure of DNA in the adduct vicinity (Bohr, 1991). In the current version of the model we have used values valid for exogenous DNA adducts, such as aminofluorene adducts. Endogenous adducts, caused by oxidative damage are thought to occur at much higher rate, about 10^{-5} or 10^{-4} per gene per day (Ames, 1989), but their repair is also thought to be much more efficient.

Cell to cell interactions, in this version of our model, are limited to division triggered by the death of a neighboring cell. This is important for modeling the effect of cytotoxicity, but other interactions, such as the suppression of growth by contact inhibition have not yet been included in the model. It is interesting that compensatory division, as implemented in the model, renders intermediate or malignant clones much more resilient to cell death than isolated cells. In a clone, the dying cells are replaced by identical cells, while isolated cells are replaced by cells of the neighbor's type (generally less malignant). This translates to a quite different set of influential parameters for the repairable intermediate cells, which are usually isolated, and the non-repairable intermediate cells, which occur in clones. For the former, normal cell parameters are important, while for the latter endogenous parameters dominate. Endogenous parameters are mostly division and death rates. These rates appear more often, and with higher sensitivity coefficients (in particular for the malignant stage) than the rates of adduct formation or MR.

Mitotic recombination is an important mechanism for the reduction to homozygosity in a number of human tumors (James et al., 1989). A recent article has shown that MR is significant in the loss of heterozygosity of chromosome 7 in chemically-induced skin tumors in the mouse (Bianchi et al., 1991). However, the significance of MR in mouse liver is not known. The ability of a particular gene to undergo reduction to homozygosity (RH) by MR probably varies according to its location. In humans RH in the retinoblastoma

gene on chromosome 13 occurs primarily by non-disjunction of the whole chromosome (Cavenee et al., 1986), where for HLA-A, on chromosome 6, and for chromosome 17 in astrocytomas, MR is the major mechanism (James et al., 1989; Morley, 1991). MR is obviously qualitatively different from point mutations and deletions: it is linked to cell division, may naturally occur at high rate, and usually affects a large part of the chromosome. MR is one of the ways by which cell division may increase the formation of tumors and some "non-genotoxic" carcinogens may act by increasing MR (Schiestl, 1989). Unfortunately, few chemicals have been tested for their ability to induce MR in mammals. In our simulations, MR is responsible for the formation of all the malignant clones in the baseline case (326 clones were formed in the 1000 repetitions) and is therefore a major determinant in the formation of malignant clones. This effect is strongly correlated to cell division which takes precedence in the sensitivity analysis and masks the importance of MR. It is interesting that in the case of radon-induced lung cancer the animal data are sufficiently informative to suggest, after fitting a simple two-stage model, that the second mutation event is qualitatively different from the first one (Moolgavkar et al., 1990). We hypothesize that the second mutation event involved could be a mitotic recombination, leading to the loss of the intact copy of a tumor-suppressor gene.

The increased risk of cancer observed after high exposure to chemical carcinogens has been attributed to the compensation of cell death by subsequent cell proliferation (Ames and Gold, 1990; Cohen and Ellwein, 1990a). Ames and Gold (1990) incriminate endogenous oxidative damage as the main promutagenic lesion, converted to fixed mutations after cell division. Although in our simulations adduct formation rates are more typical of exogenous chemical exposure, our results emphasize the importance of cell proliferation in the carcinogenic process. This effect could be due to an increased target cell population size, increased fixation of DNA lesions or MR. Further analyses will help understand the mechanism most contributing to this effect.

This work points to several directions for future research. It seems necessary to better understand the influence of DNA repair and mitotic recombination and their interactions with cell division. A global sensitivity analysis, using ranges of values for the corresponding parameters, should give more insight into this complex process. It would also be useful to parametrize the model for different tissues, such as the mouse skin, or the hematopoietic system in humans. In the later case, human rates of adduct formation, repair, mutation and mitotic recombination, which are often obtained from blood cells, would be directly usable. The model structure itself could be modified and we plan to extend it to describe the involvement of more than one gene, and in particular the action of oncogenes. In most cases, it is unlikely that a single gene is involved in carcinogenesis. While retinoblastoma appears to be an exception, Vogelstein *et al.* (1989) have shown that many genes are involved in colon carcinogenesis. The suppression of cell division by contact inhibition is also potentially important aspect of carcinogenesis, given importance of division/death events highlighted by our results. The flexibility of a discrete event model should easily accommodate new approaches and experimental data.

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