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A Kinetic Biological Effects Model for Quiescent Cells

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Abstract

Theory and experiment suggest that the initial yield of radiation-induced DNA damage, as well as the subsequent rate of damage repair, is influenced by chromatin structure. To model chromatin structure effects better, a multiple-lesion, multiple-state model is formulated. In essence, this model is a unification and extension of the earlier repair-misrepair (RMR) and lethal and potentially lethal (LPL) models. The proposed model accounts for differences in the initial lesion yield among chromatin types. Because the model also allows the use of a different lesion repair probability (rate) for each chromatin state and type of lesion, the formalism of the model is sufficiently general to account for non-exponential or multi-exponential lesion removal transients, which are sometimes observed experimentally. Limitations of the original RMR and LPL models are illustrated and discussed by reducing the general multiple-lesion, multiple-state formalism to a simpler state-averaged model.

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1.0 Introduction

The repair-misrepair (RMR) model of Tobias *et al.* [To80, To85] and the lethal and potentially lethal (LPL) model of Curtis [Cu82, Cu86] provide useful conceptual and mathematical frameworks for analyzing and interpreting the results of radiobiological experiments. Further, Tobias [Cu85] and Curtis [Cu86] have demonstrated that the RMR and or the LPL models supersede, or are closely related to, most of the major radiobiological models introduced since the pioneering works of Lea [Le46] and Kellerer and Rossi [Ke72, Ke78].

One limitation of the earlier RMR and LPL models is that they may not provide a realistic explanation for the non-exponential or multi-exponential lesion removal transients often observed experimentally [Ba95, Di90, Il91, Ra98, Sc90, St94]. Nor do they account for proximity effects [Sa97] or for differences in the initial lesion yield among chromatin types [Ch82, Lj91, Wa92, Wl92, Ol94]. In this work, a general multiple-lesion, multiple-state kinetic model is formulated to include these effects and to explain better the results of radiobiological experiments.[1] The basic methodology used in the RMR and LPL models to estimate the number of fatal lesions produced in a cell by ionizing radiation is also extended so that the expected number of non-lethal mutations per cell can be estimated. Limitations of the original RMR and LPL models are illustrated and discussed by reducing the more general multiple-lesion, multiple-state formalism to a simpler state-averaged model.

[1] The kinetic model formulated here is an extension of the ideas and models first described in the Ph.D. dissertation "A Theoretical Investigation of Cell Cycle Effects and Interspecies Radiosensitivities" by R.D. Stewart (Department of Mechanical and Nuclear Engineering, Kansas State University, Manhattan, KS, October 1997). Available on-line at URL http://www.pnl.gov/berc/staff/rds/epub/phd/abstract.html

2.0 What is a Lesion?

Most radiobiological models assume, sometimes implicitly, that damaged regions of the DNA separated by undamaged sections of DNA behave as if they are a single coherent entity, *a lesion*, which causes (initiates) harmful biological effects. One of the main goals of a radiobiological model is to provide information about the types, number, and distribution of lesions produced in a group of irradiated cells. To facilitate the estimation of model parameters from experiments or *ab initio* calculations, it is useful to have a precise method of categorizing damaged regions of DNA as a lesion. A simple method to identify a lesion in the DNA, one which is appropriate for use in a radiobiological model, is briefly described below.

2.1 The Basic Unit of DNA Damage

A nucleotide with abnormal alterations in its chemical properties is termed an *elementary damage site* (EDS). To describe regions of DNA damage in a reasonable amount of detail, the following types of EDS are suggested:

1. *Base alteration* (Ba). The chemical properties of a purine or pyrimidine base (A, T, G, or C) are abnormally modified from the form found in undamaged DNA.
2. *Base deletion* (Bd). A purine or pyrimidine base is removed from a nucleotide.
3. *Sugar alteration* (Sa). The chemical properties of a deoxyribose sugar are abnormally modified from the form found in undamaged DNA.
4. *Strand break* (Sb). The covalent bond between the deoxyribose sugar unit and the phosphate group (bridge) is broken. By definition, the nucleotide on the 5´ side of the severed sugar-phosphate bond is arbitrarily designated as the site of the elementary damage site.

Alternate or larger sets of elementary damage sites could also be proposed to provide greater chemical detail in the description of DNA damage; however, the above four fundamental defects serve as a realistic basis for the categorization of DNA damage in radiobiological models.

2.2 Prescription to Identify a Lesion

Elementary damage sites can be said to "act as a single coherent entity" if they are not usually repaired independent of each other. In general, elementary damage sites separated by many base pairs are much more likely to be repaired independently than elementary damage sites separated by a few base pairs. Consequently, a biologically meaningful prescription to identify a lesion in the DNA is as follows:

> A lesion is a section of a DNA molecule that contains a single elementary damage site separated by more than a_0 base pairs from all other elementary damage sites in the DNA – or – a section of a DNA molecule that contains a group of two or more elementary damage sites each of which is within a_0 base pairs of at least one other elementary damage site in the group.

A lesion composed of multiple elementary damage sites is shown schematically in Fig. 2-1. A consideration of biochemical repair pathways (e.g., excision repair) suggests that elementary damage sites separated by more than about 15 or 20 base pairs can usually be repaired independently of each other [Fr95]. Thus, it is reasonable to use a value of 20 base pairs for a_0 in the above prescription.

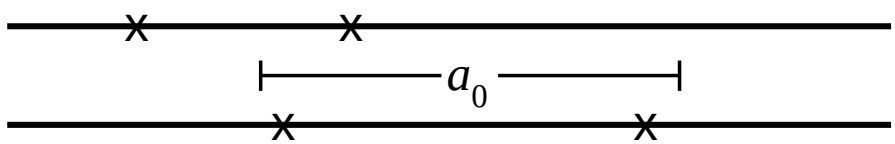

FIG 2-1. An idealized schematic of a group of four elementary damage sites forming a DNA lesion. Elementary damage sites are represented by an X.

A critical issue in the development of quantitative, molecular-level radiobiological models is the estimation of parameters from more fundamental biological and physical processes. The damage classification scheme proposed by the authors provides an unambiguous method of classifying regions of DNA damage into lesions and this facilitates the estimation of model parameters from measured data or *ab initio* calculations. Also, the symbol for an elementary damage site can be used to define a convenient lesion formula

analogous to the chemical formulas used to describe molecules. For example, a lesion composed of a strand break (Sb), two base alterations (Ba), and a base deletion (Bd) can be conveniently denoted by the lesion formula $SbBa_2Bd$. Nevertheless, it is worth noting that a damaged region of DNA classified as a lesion using the present classification scheme is very similar to the regions of damage termed a Locally Multiply Damaged Site (LMDS) by Ward [Wa85, Wa88]. Ward's qualitative description of a LMDS is merely replaced by a more quantitative method of grouping regions of DNA damage into lesions.

Another advantage of the authors' damage classification scheme is that the philosophical underpinnings of the present scheme are more comprehensive than Ward's earlier LMDS concept. Ward developed the LMDS concept from a consideration of the physical and chemical processes associated with the path (track) of a single ionizing particle. Thus, Ward's LMDS concept is limited to the DNA damages created by a single source particle track. In contrast, the above prescription to identify a lesion is motivated by the biological response of a cell to DNA damage regardless of the mechanism of formation. Consequently, the damage classification scheme described in this work can be used to categorize the types of DNA damage produced by agents other than ionizing radiation (or even multiple source particle tracks). This feature of the damage classification scheme is useful because it helps unify radiobiological models with the models used to quantify the biological effects of genotoxic chemicals. Chemical and radiation exposures that produce identical configurations of DNA damages in a cell should produce the same biological effects. Because the same damage classification scheme can be used to categorize the DNA damages produced by chemicals and radiation, the same models can, in principle, be used to quantify the biological effects of genotoxic chemicals and radiation.

3.0 General Formulation of the Balance Equations

Information about the statistical distribution of lesions produced in a group of cells by ionizing radiation can be obtained using a wide variety of stochastic [Al89, Br90, Di90a, Ha98, Ke78, Ki88, Kr92, Sa90] and non-stochastic [Cu86, Go85, Ni90, Os93, To85] models. Non-stochastic models, such as the one described in this work and in the original RMR and LPL models, replace the random variables and probability density functions used in Markov and Monte Carlo models with deterministic quantities and probabilities. In particular, the expected (average) number $\bar{L}(t)$ of lesions in a cell at time *t* is the main quantity used to assess the amount of DNA damage in a cell.

Let *L* be the integral number of lesions in a particular cell and $p(L,t)$ be the associated probability density function (pdf) describing the distribution of lesions among a group of identically irradiated cells at time *t*. In other words, the probability one of the cells in the group has exactly *L* lesions in its DNA at time *t* is $p(L,t)$. The expected number of lesions per cell is the first moment of the lesion pdf, i.e.,

$$\bar{L}(t) = \sum_L L\, p(L,t)\,. \tag{3.1}$$

Higher moments of the lesion pdf (i.e.,

$$\overline{L^n}(t) \equiv \sum_L L^n\, p(L,t)\,, \tag{3.2}$$

where $\overline{L^n}$ is the *n*th moment of the lesion pdf) provide additional information about the statistical distribution of lesions expected in a cell.

A non-stochastic, time-dependent model attempts to estimate $\bar{L}(t)$ without first estimating $p(L,t)$. In particular, a lesion balance equation is formulated to describe the net time rate of change in $\bar{L}(t)$. Mathematically, this lesion balance equation is of the form

$$\frac{d\bar{L}}{dt} = \sum_i f_i(\overline{L^1}, \overline{L^2}, \ldots, t)\,, \tag{3.3}$$

where $f_i(\overline{L^1}, \overline{L^2}, \ldots, t)$ is the expected number of lesions produced per cell per unit time at time *t* by the *i*th process. This lesion balance (or lesion conservation) equation is simply a

mathematical expression of the idea that the number of lesions in a cell can be modeled by accounting for all processes that create or remove lesions from the DNA of a cell. When $f_i(\overline{L^1}, \overline{L^2}, \ldots, t) > 0$, lesions are created in the DNA by the *i*th process, and when $f_i(\overline{L^1}, \overline{L^2}, \ldots, t) < 0$, lesions are removed from the DNA by the *i*th process. To simplify the mathematical treatment, Eq. (3.3) is frequently reduced, approximately or exactly, to an equation involving only the expected lesion number, i.e., to the form

$$\frac{d\bar{L}}{dt} = \sum_i f_i(\bar{L}, t). \tag{3.4}$$

In modeling cell killing and carcinogenesis, two distinct classes of lesions are of interest: *repairable lesions* and *unrepairable (or irreversible) lesions*. A repairable lesion is a lesion with a non-zero probability of being correctly repaired or misrepaired by at least one biochemical repair pathway. An irreversible lesion is any type of lesion with no chance of being correctly repaired or misrepaired by any biochemical repair pathway. Irreversible lesions can be further sub-divided into fatal lesions and non-lethal mutations[1]. A fatal lesion is the minimum amount of some type of DNA damage required to kill, in the reproductive sense, a cell.[2] A mutation is a non-lethal alteration in the original (wild-type) base sequence of the DNA. By definition, a mutation that prevents a cell from ever producing viable progeny is a fatal lesion.

Because it is advantageous to write separate lesion balance equations for repairable lesions, non-lethal mutations, and fatal lesions, the following notational conventions will be used throughout the remainder of this report. The lowercase letter *i* or *j* will always indicate a repairable type of lesion, the lowercase letter *k* will indicate a non-lethal mutation, and the

---

[1] A so-called reverse mutation is not, *per se*, biochemical repair so mutations are classified as irreversible lesions. Also, the term *mutation* is used here to mean both point mutations and non-lethal chromosome aberrations.

[2] Regardless of the true molecular identity of the DNA damage responsible for the cell killing effects of ionizing radiation, it is a useful mathematical convenience to attribute these effects to the formation of an irreversible fatal lesion. Attributing cell death to the formation of a non-specific fatal lesion is mathematically advantageous because this abstraction can include the effects of lethal chromosomal aberrations, apoptosis, or even a lethal accumulation of mutations. All that is required to predict the probability a cell survives a dose of radiation is a model to estimate the distribution of fatal lesions produced among a group of irradiated cells. Then, once the distribution of fatal lesions in a group of cells has been determined, the number of cells surviving irradiation is easily computed because all cells with one or more fatal lesions in their DNA die; that is, they are unable to produce viable progeny cells.

lowercase letter *f* will indicate a fatal lesion. Also, the lowercase letter *l* will be used to indicate a repairable lesion, a non-lethal mutation, or a fatal lesion, i.e., $l = i, j, k$, or f. Thus, the term $\bar{L}_i$ (or $\bar{L}_j$) indicates the expected number of the *i*th (*j*th) type repairable lesions in a cell, $\bar{L}_k$ indicates the expected number of the *k*th type of non-lethal mutations in a cell, and the term $\bar{L}_f$ indicates the expected number of fatal lesions in a cell. The quantity $\bar{L}_l$ indicates the expected number of the *l*th type repairable or unrepairable lesions in a cell.

3.1 Balance Equation for Repairable Lesions

Suppose the DNA of a cell can be sub-divided into Q spatially distinct chromatin states (e.g., regions of condensed heterochromatin or unfolded euchromatin). Further, suppose the initial yield of lesions per unit absorbed dose (Gy) per base pair (bp) as well as the rates of damage repair in each state may be different. Let $\bar{L}_i(n,t)$ be the expected number of *i*th type repairable lesions per cell in the *n*th chromatin state at time *t*. Let $P(i,n,t)$ be the expected number of *i*th type repairable lesions produced in undamaged DNA in chromatin state *n* per unit time at time *t* by ionizing radiation or some other agent, and let $R(i,n,t)$ be the expected number of *i*th type repairable lesions in chromatin state *n* correctly repaired per unit time at time *t*.

The same processes responsible for creating lesions in undamaged regions of the DNA can also transform one type of lesion into another. For example, a second source particle can create additional elementary damage sites close enough to an existing lesion in the DNA so that the *i*th type of repairable lesion is converted into an *l*th type of repairable or unrepairable lesion (an inter-track effect). Also, repair processes can convert a repairable lesion into another type of repairable lesion, a mutation, or a fatal lesion. All of these time dependent processes are modeled in the lesion transformation term, $T(i \to l,n,t)$, defined as the expected number of the *i*th type of lesion in the *n*th chromatin state transformed into the *l*th type of (repairable or unrepairable) lesion in chromatin state *n* per unit time at time *t*.

Conceptually and mathematically, it is useful to think of the transformation term $T(i \to l,n,t)$ as an $N \times N$ matrix, where $N \equiv N_r + N_m + 1$, N_r is the number of different types of repairable lesions that can be formed in a cell and N_m is the number of different

types of non-lethal mutations that can be formed in a cell (the +1 accounts for the formation of fatal lesions). Because mutations and fatal lesions are irreversible, many terms in this lesion transformation matrix must be zero. That is, mutations and fatal lesions cannot be transformed into a repairable type of lesion so $T(k \rightarrow i,n,t)$, for all *k* types of mutations, and $T(f \rightarrow i,n,t)$ must equal zero.

A fourth process affecting the number of lesions expected in a chromatin state is the "movement of lesions among states." Lesions move among states because of temporal changes in the structure and organization of a cell's DNA. For example, radiation-induced DNA damage could induce regions of condensed heterochromatin to unfold into less condensed euchromatin. Any lesions present in the heterochromatin when the heterochromatin unfolds into euchromatin will effectively move from a heterochromatin state to a euchromatin state. This does not mean however that the location (position) of a lesion in the DNA will change. In proliferating cells, lesions could also move among states because of cell-directed changes in the structure and organization of the DNA associated with, for example, DNA replication and mitosis. The temporal changes in the structure and organization of the chromatin are modeled in the lesion movement term, $M(i,m \rightarrow n,t)$, defined as the expected number of the *i*th type of lesion in the DNA moved from chromatin state *m* to chromatin state *n* per unit time at time *t*.

The net rate of change in $\bar{L}_i(n,t)$ equals (1) the expected number of *i*th type lesions produced in the *n*th chromatin state per unit time at time *t*, minus (2) the expected number of *i*th type lesions in the *n*th chromatin state repaired per unit time at time *t*, plus (3) the net number of the *l*th type lesions transformed into the *i*th type lesions in the *n*th chromatin state per unit time at time *t*, plus (4) the net number of the *i*th type lesions moved from the *m*th chromatin state to the *n*th chromatin state per unit time at time *t*. Expressed mathematically,

$$\frac{d\bar{L}_i(n,t)}{dt} = P(i,n,t) - R(i,n,t) + \sum_{l=1}^{N}\left[T(l \rightarrow i,n,t) - T(i \rightarrow l,n,t)\right] + \sum_{m=1}^{Q}\left[M(i,m \rightarrow n,t) - M(i,n \rightarrow m,t)\right], \tag{3.5}$$

for $i = 1, \ldots, N_r$ and $n = 1, \ldots, Q$. For quiescent (stationary-phase) cells, it seems reasonable to neglect the movement of lesions among states so that Eq. (3.5) simplifies to

$$\frac{d\bar{L}_i(n,t)}{dt} = P(i,n,t) - R(i,n,t) + \sum_{l=1}^{N} \left[T(l \to i, n, t) - T(i \to l, n, t) \right], \qquad (3.6)$$

for $i = 1, \cdots, N_r$ and $n = 1, \cdots, Q$. Mathematical expressions for $M(i, m \to n, t)$ will be formulated and discussed in a later paper when the kinetic model is used to investigate the biological basis for cell cycle effects in proliferating cells.

3.2 Balance Equation for Mutations

Mathematically, mutations can be treated as just another type of lesion. Let $\bar{M}_k(n,t)$ be the expected number of *k*th type mutations in the *n*th chromatin state at time *t* (here, $\bar{M}$ is used instead of $\bar{L}$ to emphasize that a particular type of damage is a non-lethal mutation and not a repairable or fatal lesion). Then, a balance equation for the *k*th type of non-lethal mutation in a stationary-phase cell can be written, for $k = N_r + 1, \cdots, N - 1$ and $n = 1, \cdots, Q$, as

$$\frac{d\bar{M}_k(n,t)}{dt} = P(k,n,t) - R(k,n,t) + \sum_{l=1}^{N} \left[T(l \to k, n, t) - T(k \to l, n, t) \right]. \qquad (3.7)$$

Because fatal lesions and mutations are by definition irreversible (unrepairable), the summation over all *N* types of lesions reduces to a sum over all N_r types of repairable lesions. Also, the repair term $R(k, n, t)$ and the transformation term $T(k \to l, n, t)$ must equal zero. Thus, Eq. (3.7) simplifies to

$$\frac{d\bar{M}_k(n,t)}{dt} = P(k,n,t) + \sum_{l=1}^{N_r} T(l \to k, n, t), \qquad (3.8)$$

for $k = N_r + 1, \cdots, N - 1$ and $n = 1, \cdots, Q$.

The transformation term $T(l \to k, n, t)$ models the time-dependent conversion of repairable lesions into mutations. For example, repair processes might convert a repairable double strand break lesion into a non-lethal point mutation. The production term $P(k,n,t)$ models the instantaneous creation of mutations in the *n*th chromatin state per unit time at time *t* by ionizing radiation or some other agent that damages the DNA. Although it is

conceivable that ionizing radiation could directly create an irreversible form of damage which is non-lethal but mutagenic (e.g., a base pair at the tip of a chromosome could be irreversibly damaged), it is likely that most mutations are formed by the misrepair of DNA damage. Consequently, it is reasonable to assume that $P(k,n,t)$ = 0 so that Eq. (3.8) further simplifies to

$$\frac{d\bar{M}_k(n,t)}{dt} = \sum_{l=1}^{N_r} T(l \to k, n, t), \tag{3.9}$$

for $k = N_r + 1, \cdots, N-1$ and $n = 1, \cdots, Q$.

3.3 Balance Equation for Fatal Lesions

Fatal lesions, like mutations, can be treated mathematically as just another type of lesion. Thus for stationary-phase cells, the time rate of change in the expected number of fatal lesions in the *n*th chromatin state at time *t* can be modeled by

$$\frac{d\bar{L}_f(n,t)}{dt} = P(f,n,t) - R(f,n,t) + \sum_{i=1}^{N_r} \left[T(i \to f, n, t) - T(f \to i, n, t)\right], \quad n = 1, \cdots, Q. \tag{3.10}$$

Because fatal lesions are irreversible, the repair term $R(f,n,t)$ and the transformation term $T(f \to i, n, t)$ are again set to zero so that Eq. (3.10) simplifies to

$$\frac{d\bar{L}_f(n,t)}{dt} = P(f,n,t) + \sum_{i=1}^{N_r} T(i \to f, n, t), \quad n = 1, \cdots, Q. \tag{3.11}$$

The fatal lesion production term, $P(f,n,t)$, accounts for the instantaneous production of fatal lesions in the *n*th type of undamaged chromatin, and $T(i \to f, n, t)$ accounts for the time dependent conversion of repairable lesions into irreversible fatal lesions, for example, by lesion misrepair. Because only the total number $\bar{L}_f(t)$ of fatal lesions per cell is of interest (a cell with a fatal lesion in one chromosome is as dead as a cell with a fatal lesion in a different chromosome), Eq. (3.11) can be summed over all states to yield

$$\frac{d\bar{L}_f(t)}{dt} = \bar{P}(f,t) + \sum_{n=1}^{Q} \sum_{i=1}^{N_r} T(i \to f, n, t). \tag{3.12}$$

4.0 Model for the Production of Lesions

DNA damage is produced by ionizing and non-ionizing radiation, a variety of chemical agents, heat, and "spontaneously" [Fr95]. In this section, a mathematical formalism needed to include a variety of DNA damage mechanisms is briefly outlined. Then, a model for the expected rate of lesion production by ionizing radiation is presented.

Let $P^q(l,n,t)$ be the expected number of the *l*th type of lesion produced in undamaged DNA in the *n*th chromatin state per unit time at time *t* by the *q*th damage production mechanism. Then, the total rate of lesion production $P(l,n,t) = \sum_q P^q(l,n,t)$. When all non-radiation induced lesion production mechanisms are grouped together, $P(l,n,t)$ can be rewritten as

$$P(l,n,t) = P^{rad}(l,n,t) + \sum_{q \neq rad} P^q(l,n,t) \equiv P^{rad}(l,n,t) + P^{nrad}(l,n,t), \qquad (4.1)$$

where $P^{rad}(l,n,t)$ is the expected number of the *l*th type of lesion produced in undamaged DNA in chromatin state *n* per unit time at time *t* by ionizing radiation and $P^{nrad}(l,n,t)$ is the expected number of the *l*th type of lesion produced in undamaged DNA in chromatin state *n* per unit time at time *t* by all other mechanisms. In many radiobiological experiments, $P^{rad}(l,n,t) >> P^{nrad}(l,n,t)$ so that $P(l,n,t) \cong P^{rad}(l,n,t)$.

4.1 Production of Repairable Lesions by Ionizing Radiation

For mammalian cells with a DNA content of about 6000 Mbp per cell, the initial number of double strand break (DSB) lesions per cell increases linearly with dose up to at least 50 Gy [Er95]. In yeast cells, which have a DNA content about 250-fold smaller than mammalian cells [32], the initial DSB yield per cell increases linearly with the dose of radiation up to at least 2400 Gy [Sc81]. The initial yield of other types of DNA damage also increases linearly as the dose of radiation increases (for a review, see [Sc90]). For example, the initial yield of thymine damage in human lung fibroblasts increases linearly up to at least 3000 Gy [Sc90]. These observations imply that, for low dose conditions such as those described above, the expected amount of DNA damage per cell is very small compared to the

amount of undamaged DNA. Consequently, when the cell nucleus is uniformly irradiated by ionizing radiation, it is reasonable to model the expected number of the *i*th type lesions produced in the *n*th chromatin state, per unit time at time *t*, by

$$P^{rad}(i,n,t) = 2\dot{D}(t)Y_n\Sigma_i(n)\,, \tag{4.2}$$

where $\dot{D}(t)$ is the absorbed dose rate in the cell nucleus at time *t*, Y_n is the expected number of base pairs in the *n*th chromatin state, and $\Sigma_i(n)$ is the expected number of the *i*th type lesions produced in the *n*th type of undamaged chromatin per nucleotide per unit absorbed dose.

Because the overall structure of chromatin is similar among all eukaryotes [Ho89], the lesion formation probability $\Sigma_i(n)$ is most likely similar among all eukaryotes. However, the number of base pairs per cell, the amount of condensed heterochromatin, and the amount of unfolded euchromatin in a cell may vary widely among cell types and at different positions in the cell cycle. The fraction of transcriptionally active DNA in a cell also varies widely between the lower and higher eukaryotes [Ca85]. Most of these effects can be included in the lesion formation model by adjusting the parameter Y_n (the number of base pairs in the *n*th chromatin state). To account for dynamic (temporal) changes in the rate of damage production during, for example, the cell cycle, the lesion production model [Eq. (4.2)] can be coupled to a model that describes how Y_n changes in time and with position in the cell cycle [i.e., $Y_n \rightarrow Y_n(t)$]. Because the main objective of the present work is to develop a radiobiological model for quiescent cells, time and cell-cycle dependent changes in the structure and organization of the chromatin are neglected here.

Summation of Eq. (4.2) over all *n* states gives the expected number of *i*th type lesions produced in a cell per unit time at time *t*, $\bar{P}(i,t)$, by ionizing radiation, i.e.,

$$\bar{P}(i,t) = 2\dot{D}(t)\sum_{n=1}^{Q} Y_n\,\Sigma_i(n) = 2\dot{D}(t)Y\bar{\Sigma}_i\,, \tag{4.3}$$

where *Y* is the total number of base pairs in the cell nucleus and $\bar{\Sigma}_i \equiv \sum_n Y_n\,\Sigma_i(n)/Y$. The parameter h_{PL} in the LPL model and the parameter δ in the RMR model are equivalent to $2Y\bar{\Sigma}_i$ in Eq. (4.3).

4.2 Production of Fatal Lesions by Ionizing Radiation

The balance equation for fatal lesions [Eq. (3.12)] includes a term for the direct instantaneous formation of fatal lesions by ionizing radiation. Physically, this direct fatal lesion production term should be interpreted as the production of some type of DNA damage that is so severe that it has *no chance* of ever being repaired by a cell. In addition, this DNA damage must be in a region of the DNA critical to cell viability. This hypothesis seems unlikely, even for high-LET radiation. Instead, it seems more likely that most or all of the lesions initially formed by ionizing radiation in an undamaged region of DNA have at least a small chance of being repaired by a cell. In other words, most of the radiation-induced DNA damage initially created in a cell is repairable, and it is likely that additional processes must alter further the chemical properties of a damaged section of DNA before the lesion becomes truly irreversible and lethal. The time-dependent conversion of a repairable lesion into an irreversible fatal lesion is described by the lesion transformation term $T(i \to f, n, t)$.

Nevertheless, the lethal and potentially lethal (LPL) model includes a mechanism for the direct instantaneous production of fatal lesions by ionizing radiation, and the formalism described in this work can easily include one too. Let $\Sigma_f(n)$ be the expected number of fatal lesions produced in the *n*th type of undamaged chromatin per nucleotide per unit absorbed dose. Then for low dose conditions when most of the DNA in a cell is undamaged, the expected number of fatal lesions produced in the *n*th chromatin, state per unit time at time *t*, can be estimated from

$$P(f, n, t) = 2\dot{D}(t) Y_n \Sigma_f(n) . \tag{4.4}$$

When Eq. (4.4) is summed over all states, the expected number of fatal lesions produced in a cell per unit time at time *t*, $\bar{P}(f, t)$, becomes

$$\bar{P}(f, t) = 2\dot{D}(t) \sum_{n=1}^{Q} Y_n \Sigma_f(n) = 2\dot{D}(t) Y \bar{\Sigma}_f , \tag{4.5}$$

where $\bar{\Sigma}_f \equiv \sum_n Y_n \Sigma_f(n) / Y$. The h_L parameter in the LPL model [Cu86] is equivalent to $2Y\bar{\Sigma}_f$ in the model described here.

5.0 Model for Lesion Repair

Cells employ a multitude of biochemical mechanisms to repair DNA damage. Some mechanisms repair specific types of DNA damage, while others, such as the nucleotide excision repair pathway, remove many types of DNA damage [Fr95]. Thus it is not surprising that some types of DNA damage are repaired more quickly than others. Lesion repair rates may also be affected by a large number of factors other than the type of DNA damage. Lesion repair rates are affected by the availability of repair enzymes (i.e., repair saturation [Di93, Wh87, Wh91]), inducible repair mechanisms [Fr95], preferential repair of transcriptionally active DNA [Fr96, Ha94, Me89, Sm90], chromatin structure [Fr95, Ol84, We97], and the modulation of repair pathways associated with, for example, apoptosis and cell cycle checkpoints [Fr95, Sa95, Le97].

Suppose repair saturation, inducible repair mechanisms, and repair pathway modulation can be neglected in stationary-phase cells so that the rate of lesion repair only depends on the chromatin state, the type of lesion, and the type of repair mechanism. Let $l^{q}(i|n)$ be the conditional probability the *q*th mechanism attempts to repair the *i*th type of lesion in the *n*th chromatin state, per infinitesimal unit of time. It is frequently convenient to relate this repair probability $l^{q}(i|n)$ to the expected lesion repair half-time $T_h^q(i|n)$. This latter quantity is the expected amount of time required for a cell to remove (repair or misrepair) half of the *i*th type of lesion in the *n*th chromatin state produced by an acute dose of radiation. For so-called first-order unsaturated repair kinetics,[1] $l^{q}(i|n)$ is related to $T_h^q(i|n)$ by $l^{q}(i|n) = \ln 2 / T_h^q(i|n)$.

The product $l^{q}(i|n)\ \bar{L}_i(n,t)$ yields the expected number of repair attempts by the *q*th mechanism on an *i*th type of lesion in the *n*th chromatin state at time *t*. Suppose the fidelity, or accuracy, of lesion repair is independent of the chromatin state (i.e., the lesion repair fidelity only depends on the type of lesion and the type of repair mechanism). Let the repair

[1] An exponential decrease in the expected number of lesions per cell as a function of time implies that that the expected rate of lesion repair is proportional to the number of lesions in a cell, i.e., so-called unsaturated first-order repair kinetics. Many radiobiological models [To85, Cu86, Ke78, Al89, Br90, Di90a, Ni90, Ha98] include a term to account for first-order, unsaturated repair kinetics.

fidelity $a^q(i)$ be the probability per repair attempt on the *i*th type of lesion that the *q*th mechanism correctly restores the structure and base sequence of the DNA. Then, $a^q(i)\ l^q(i|n)\ \bar{L}_i(n,t)$ is the expected number of the *i*th type of lesion in the *n*th chromatin state correctly repaired, per infinitesimal unit of time at time *t*, by the *q*th mechanism. To obtain the total number of correct repairs expected per infinitesimal unit of time at time *t*, $a^q(i)\ l^q(i|n)\ \bar{L}_i(n,t)$ is summed over all mechanisms to yield

$$R(i,n,t) = \bar{L}_i(n,t)\sum_q a^q(i)\, l^q(i\,|n) = \bar{a}_i(n)\, l_i(n)\bar{L}_i(n,t)\,, \tag{5.1}$$

where the total repair probability $l_i(n) \equiv \sum_q l^q(i|n)$ and the effective repair fidelity $\bar{a}_i(n) \equiv \sum_q a^q(i)\, l^q(i|n) / l_i(n)$.

Equation (5.1) implies that, because $\bar{a}_i(n)$ is determined by a weighted sum of the repair fidelities for all *q* mechanisms, modulation of damage repair pathways, inducible repair, and repair saturation phenomena have the potential to increase *or decrease* the effective rate of correct lesion repair. In other words, an increase (decrease) in total repair probability $l_i(n)$ does not necessarily mean the rate of correct lesion repair will also increase (decrease). Suppose, for example, that a small dose of ionizing radiation causes a cell to temporarily increase the production of a critical enzyme involved in the *q*th damage repair pathway so that $l^q(i|n)$ increases. If the repair fidelity $a^q(i)$ is less than the repair fidelity of the other pathways that repair the *i*th type of lesion, the total repair probability $l_i(n)$ could increase while the effective repair fidelity $\bar{a}_i(n)$ decreases. Also, an increase in the total repair probability could decrease the overall yield of misrepaired lesions while still increasing the yield of non-lethal mutations. That is, an increased rate of damage repair could decrease the yield of fatal lesions and increase the yield of non-lethal mutations in such a way that the total yield of irreversible damage decreases. It can be concluded that the existence of inducible repair mechanisms [Fr95] is not *sufficient evidence* to prove that a small dose of ionizing radiation which increases the rate of damage repair [increases $l_i(n)$] will automatically reduce the radiation-induced yield of mutations produced by subsequent doses (a beneficial hormesis effect). Inducible repair mechanisms could enhance the probability a cell survives a dose of radiation (reduce the yield of fatal lesions) while

simultaneously increasing the yield of radiation-induced cancer promoting (initiating) mutations.

The total (state-averaged) rate of correct lesion repair expected in a cell is obtained by summing Eq. (5.1) over all *n* states, i.e.,

$$\bar{R}(i,t) = \sum_{n=1}^{Q} \bar{a}_i(n)\, l_i(n) \bar{L}_i(n,t) = \bar{\Lambda}_i(t) \bar{L}_i(t)\,, \tag{5.2}$$

where $\bar{\Lambda}_i(t) \equiv \sum_n \bar{a}_i(n)\, l_i(n) \bar{L}_i(n,t) / \bar{L}_i(t)$ and $\bar{L}_i(t) = \sum_n \bar{L}_i(n,t)$. Although Eq. (5.1) indicates that the rate of lesion repair in the *n*th chromatin state follows first-order unsaturated repair kinetics regardless of the number of mechanisms repairing the *i*th type of lesion, the total state-averaged lesion repair rate for the *i*th type of lesion in a cell, i.e., Eq. (5.2), most likely follows higher-order repair kinetics because the effective rate of correct lesion repair in a cell, $\bar{\Lambda}_i(t)$, is a function of time. The formalism of Eq. (5.1) is general enough to account for the non-exponential or multi-exponential lesion removal transients observed experimentally [Ba95, Di90, Il91, Ra98, Sc90, St94] without invoking additional higher-order lesion repair processes.[2] In the RMR and LPL models, a time-independent repair probability is used to model the repair or misrepair of individual damages [i.e., $\bar{\Lambda}_i(t) \rightarrow \bar{\Lambda}_i$].

[2] For a review and discussion on the implications of second-order (binary misrepair or quadratic) lesion removal kinetics, see Fowler [Fo99].

6.0 Models for the Transformation of Lesions

Suppose that repairable lesions are converted into other types of lesions by a variety of time-dependent processes. Let $T^q(i \to l, n, t)$ be the expected number of *i*th type repairable lesions in the *n*th chromatin state converted by the *q*th mechanism into type *l* lesions, per unit time at time *t*. Then, the expected number of repairable lesions of type *i* in the *n*th chromatin state transformed by all mechanisms into lesions of type *l*, per unit time at time *t*, is the sum of $T^q(i \to l, n, t)$ over all transformation mechanisms, i.e.,

$$T(i \to l, n, t) = \sum_q T^q(i \to l, n, t). \tag{6.1}$$

In the present work, three mechanisms for converting repairable lesions into other types of lesions are formulated: (1) lesion misrepair, (2), lesion fixation, and (3) pairwise lesion interaction. Lesion misrepair is the conversion (transformation) of a repairable lesion into another type of lesion by a repair process. For example, rejoining of the break-ends associated with a DSB might produce a lethal or non-lethal genetic alteration. Alternatively, the excision repair of a strand break with base damage in the opposite strand might produce a point mutation.

Lesion fixation is the conversion of a repairable lesion into another type of lesion by non-repair related processes. The "sticky ends" of a double strand break lesion might interact irreversibly with proteins or undamaged regions of DNA and form a simple chromosome break. Or, post-irradiation treatment of a cell with an alkylating agent might produce additional damage sites near an existing radiation-induced lesion and transform the lesion into another type of lesion. The third lesion transformation mechanism considered in this work is pairwise lesion interaction. In the pairwise lesion interaction process, the DNA associated with two different repairable lesions interact to form a mutation, a compound lesion[1], or a fatal lesion. For example, the sticky ends associated with two different double

[1] A *compound lesion* is a configuration of DNA damage in which one or more damaged nucleotides associated with different lesions interact with each other chemically to form a more complex type of repairable damage. For example, two damaged regions of DNA might interact chemically to form a DNA cross link (most likely still a repairable type of damage). Lesion interactions can be denoted symbolically in much the same way as chemical reactions. For example, a lesion-lesion interaction might be written symbolically as $SbBa_2$ + BdBa =

strand break lesions might recombine to form a lethal or non-lethal exchange-type chromosome aberration.

In this work, Eq. (6.1) thus simplifies to

$$T(i \to l, n, t) = T^{lmr}(i \to l, n, t) + T^{fix}(i \to l, n, t) + T^{pli}(i \to l, n, t), \tag{6.2}$$

where the superscripts *lmr*, *fix*, and *pli* denote the lesion misrepair, lesion fixation, and pairwise lesion interaction processes, respectively. The mathematical formulation of each of these lesion transformation processes is described below.

6.1 Lesion Misrepair (or Linear Misrepair)

An attempt at lesion repair results in either a correct or incorrect restoration of the original base sequence and structure of a damaged section of the DNA. Because $a^q(i)$ is the probability, per repair attempt on an *i*th type lesion, that the *q*th mechanism correctly restores the structure and base sequence of the DNA, it follows that [1-$a^q(i)$] must be the probability, per repair attempt on an *i*th type lesion, that the *q*th mechanism does not correctly restore the structure and base sequence of a damaged section. In other words, [1-$a^q(i)$] is the probability, per repair attempt, that an *i*th type lesion is *misrepaired* by the *q*th mechanism.

Let $b^q(i \to l)$ be the probability per misrepair that the *q*th mechanism transforms an *i*th type lesion into a *l*th type lesion. The product $[1-a^q(i)]\ b^q(i \to l)\ l^q(i|n)\ \bar{L}_i(n,t)$ is thus the expected number of *i*th type lesions in the *n*th chromatin state transformed by the *q*th mechanism into lesions of type *l*, per unit time at time *t*. Summation of the quantity $[1-a^q(i)]\ b^q(i \to l)\ l^q(i|n)\ \bar{L}_i(n,t)$ over all *q* independent repair mechanisms yields then the expected number of *i*th type lesions in the *n*th chromatin state transformed into *l*th type lesions, per unit time at time *t*. That is,

$$T^{lmr}(i \to l, n, t) = \bar{L}_i(n,t) \sum_q [1-a^q(i)]\, b^q(i \to l)\, l^q(i|n)\,. \tag{6.3}$$

Let the effective lesion repair transformation probability $\bar{b}_{i \to l}(n)$ be defined as

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$SbBa_3Bd$ or $BdBa$-$SbBa_2$ (the dash - indicates that the strand break interacts chemically with the base alteration).

$$\bar{b}_{i\to l}(n) \equiv \frac{1}{\left[1-\bar{a}_i(n)\right] l_i(n)} \sum_q [1-a^q(i)]\, b^{\,q}(i\to l)\, l^{\,q}(i|n)\,. \tag{6.4}$$

Then, Eq. (6.3) simplifies notationally to

$$T^{lmr}(i\to l,n,t) = l_i(n)\left[1-\bar{a}_i(n)\right]\bar{b}_{i\to l}(n)\bar{L}_i(n,t)\,. \tag{6.5}$$

Summation of Eq. (6.5) over all n chromatin states yields the expected number of ith type lesions, per cell, transformed into lth type lesions per unit time at time t, i.e.,

$$T^{lmr}(i\to l,t) = \sum_{n=1}^{Q} l_i(n)\left[1-\bar{a}_i(n)\right]\bar{b}_{i\to l}(n)\bar{L}_i(n,t)\,. \tag{6.6}$$

If the repair probability for an ith type lesion by the qth mechanism is independent of the chromatin state [i.e., $l^{\,q}(i|n) \to l^{\,q}(i)$], then $l_i(n) \to l_i$, $\bar{a}_i(n) \to \bar{a}_i$, and $\bar{b}_{i\to l}(n) \to \bar{b}_{i\to l}$. Under these conditions, Eq. (6.6) simplifies to

$$\bar{T}^{lmr}(i\to l,t) = l_i\left[1-\bar{a}_i\right]\bar{b}_{i\to l}\bar{L}_i(t)\,. \tag{6.7}$$

The RMR model of Tobias [To85] includes a lesion misrepair mechanism equivalent to the one modeled by Eq. (6.7). However because this result is only valid under the unrealistic condition that $l^{\,q}(i|n) = l^{\,q}(i)$, the lesion misrepair mechanism in the RMR model may be too simplistic.

6.2 Lesion Fixation

Consider a lesion fixation process in which non-repair related processes transform repairable lesions into other types of lesions. Let $e^{\,q}_{i\to l}(n,t)$ be the probability, per lesion per infinitesimal unit of time, that an ith type lesion in the nth chromatin state is transformed into a lth type lesion by the qth fixation process at time t. The lesion fixation probability depends on time because temporal changes in the physical or chemical environment of a cell may influence the probability a lesion is transformed into another type of damage. The fixation probability depends on the chromatin state because the chance a chemically reactive (damaged) nucleotide that is part of a lesion will interact with a histone protein (or other DNA-bound molecule) should depend strongly on the density of proteins near the lesion. Summation of $e^{\,q}_{i\to l}(n,t)\,\bar{L}_i(n,t)$ over all q fixation processes yields the expected number of

*i*th type lesions in the *n*th chromatin state that are transformed by lesion fixation into *l*th type lesions, per unit time at time *t*, i.e.,

$$T^{fix}(i \to l, n, t) = \sum_q e^{q}_{i \to l}(n,t)\bar{L}_i(n,t) \equiv \bar{e}_{i \to l}(n,t)\bar{L}_i(n,t). \tag{6.8}$$

Because the rate of lesion fixation and the rate of lesion misrepair are both proportional to $\bar{L}_i(n,t)$, the dose and dose rate effects produced by these two mechanisms will be similar but not necessarily identical. For example, repair saturation should affect the rate of lesion misrepair but not the rate of lesion fixation. Also, a mutation which alters the rate of lesion repair will also alter the rate of lesion misrepair but not the rate of lesion fixation. On the other hand, it is easy to imagine situations in which physical processes or chemicals introduced into the nucleus alter the chemical properties of a damaged section of DNA without directly affecting damage repair. For example, chemical agents or spontaneous DNA damaging events might produce additional damage sites near an existing lesion and transform one type of lesion into another.

6.3 Pairwise Lesion Interaction

The pairwise lesion interaction hypothesis states that the probability two lesions interact to form another type of lesion is proportional to the square of the number of unrepaired lesions in a cell [To85, Cu86]. Suppose that the random distribution of the number of *i*th type lesions in chromatin state *n* at time *t* is $p\left(L_i(n),t\right)$, and the random distribution of number of *j*th type of lesions in chromatin state *m* at time *t* is $p\left(L_j(m),t\right)$. The expected number of independent ways an *i*th and *j*th type lesion ($i \neq j$) can interact in pairwise fashion is

$$\begin{aligned}\left\langle L_i(n)L_j(m)\right\rangle &= \sum_{L_i(n)}\sum_{L_j(m)} L_i(n)L_j(m)\, p\left(L_i(n),t\right) p\left(L_j(m),t\right) \\ &= \bar{L}_i(n,t)\bar{L}_j(m,t).\end{aligned} \tag{6.9}$$

Similarly, the expected number of independent ways an *i*th type lesion in chromatin state *n* can interact with an *i*th type lesion in chromatin state *m* ($n \neq m$) is

$$\begin{aligned}\langle L_i(n)L_i(m)\rangle &= \sum_{L_i(n)}\sum_{L_i(m)} L_i(n)L_i(m)\, p\big(L_i(n),t\big)\, p\big(L_i(m),t\big) \\ &= \bar{L}_i(n,t)\bar{L}_i(m,t).\end{aligned} \tag{6.10}$$

However because a lesion cannot interact with itself in pairwise fashion, the expected number of independent ways an *i*th type lesion can interact with another *i*th type lesion in the same chromatin state is proportional to $L_i(n)\big[L_i(n)-1\big]$. Thus,

$$\begin{aligned}\big\langle L_i(n)\big[L_i(n)-1\big]\big\rangle &= \sum_{L_i(n)} L_i(n)\big[L_i(n)-1\big]\, p\big(L_i(n),t\big) \\ &= \overline{L_i^2}(n,t) - \bar{L}_i(n,t).\end{aligned} \tag{6.11}$$

Equation (6.11) implies that the pairwise lesion interaction process cannot be rigorously formulated by a first-order, non-stochastic model, except under special circumstances such as those discussed below.

In the formulation of the RMR and LPL models, it is assumed that the number of repairable lesions in a cell can be accurately described by a Poisson distribution. For this distribution, the number of independent ways an *i*th type of repairable lesion can interact with another *i*th type lesion, in the same cell, equals $\bar{L}_i(t)\ \bar{L}_i(t)$ [Cu86]. The validity of this "Poisson assumption" as it applies to the LPL and RMR models has been investigated by others [Al89, Cu88, Sa90] who found that the neglect of higher-order statistical aspects of the pairwise lesion interaction process generally introduces a systematic bias into these models, especially for large doses and high-LET radiation.

Nevertheless, the Poisson assumption is appropriate for low-LET radiation [Sa97a], and for lack of contrary models we also assume that the distribution of lesions in the *n*th chromatin state at time *t* can be reasonably modeled by a Poisson distribution. Thus, it follows that the expected number of independent ways an *i*th type lesion in the *n*th chromatin state can interact with another *i*th type lesion in the same chromatin state is

$$\langle L_i(n)(L_i(n)-1)\rangle = \big[\bar{L}_i(n,t)\big]^2. \tag{6.12}$$

From Eqs. (6.9), (6.10), and (6.11), it can be concluded that the expected number of independent ways two lesions can interact is proportional to $\bar{L}_i(n,t)\bar{L}_j(m,t)$ for all types of repairable lesions and all states.

Two types of pairwise lesion interactions can be envisioned: one mediated by a repair mechanism (so-called "binary misrepair") and one in which the lesions interact directly without a biochemical (repair) intermediary to form a compound lesion that may or may not be repairable. In this section, a model for the binary misrepair interaction process is developed. The derivation of a mathematical model for direct (chemical) lesion-lesion interaction is the same, but the model parameters have a slightly different biophysical interpretation.

Let $h^q(i \to l,n;\, j,m)$ be the probability, per *j*th lesion per infinitesimal unit of time, that an *i*th type lesion in the *n*th chromatin state undergoes the *q*th type of binary misrepair interaction (i.e., a pairwise lesion interaction mediated by the *q*th repair mechanism) with a *j*th type lesion in the *m*th chromatin state to form an *l*th type lesion in the *n*th chromatin state. The product $h^q(i \to l,n;\, j,m)\ \bar{L}_j(m,t)\ \bar{L}_i(n,t)$ is thus the expected number of *i*th type lesions in the *n*th chromatin state that undergo the *q*th type of binary misrepair interaction with a *j*th type lesion in the *m*th chromatin state to form an *l*th type lesion in the *n*th chromatin state, per unit time at time *t*. Summation of $h^q(i \to l,n;\, j,m)\ \bar{L}_j(m,t)\ \bar{L}_i(n,t)$ over all *q* independent repair mechanisms, *m* states, and *j*th repairable lesion types yields the expected number of *i*th type lesions in the *n*th chromatin state transformed into *l*th type lesions in chromatin state *n*, per unit time at time *t*, i.e.,

$$\begin{aligned} T^{pli}(i \to l,n,t) &= \sum_{m=1}^{Q}\sum_{j=1}^{N_r}\sum_{q} h^q(i \to l,n;\, j,m)\bar{L}_j(m,t)\bar{L}_i(n,t) \\ &= \bar{L}_i(n,t)\sum_{m=1}^{Q}\sum_{j=1}^{N_r} h_{i\to l}(n;\, j,m)\bar{L}_j(m,t). \end{aligned} \tag{6.13}$$

Equation (6.13) suggests that the binary misrepair interaction rate in a cell is a function of the distribution of DNA lesions among states. Because the binary misrepair probability $h_{i\to l}(n;\, j,m)$ for lesions in the same state can be different than the interaction

probability for lesions in different states, the pairwise lesion interaction model formulated in this work can account for proximity effects. That is, the interaction probability for lesions in the same state can be larger than the interaction probability for lesions in different states. Because the same biochemical processes that are involved in lesion repair [Eq. (5.1)] must also be involved in the binary misrepair interaction process, the interaction probability $h_{i\to l}(n;j,m)$ is most likely affected by phenomena such as repair saturation and inducible repair. Equation (6.13) also suggests that modulation of damage repair pathways and differences in chromatin organization among cell types most likely influence the binary misrepair interaction rate in a cell.

Suppose an *l*th type lesion can be formed only by the pairwise interaction of a single type (family) of lesion (e.g., $j = i$). Summation of Eq. (6.13) over all n states yields the expected number of the *l*th type of lesion produced in a cell, per unit time at time t, by the pairwise interaction of the *i*th type of lesion, i.e.,

$$T^{pli}(i\to l,t)=\sum_{n=1}^{Q}\bar{L}_i(n,t)\sum_{m=1}^{Q}h_{i\to l}(n;i,m)\bar{L}_i(m,t)=\bar{h}_{i\to l}(t)\bar{L}_i(t)\bar{L}_i(t)\,, \qquad (6.14)$$

where $\bar{h}_{i\to l}(t)\equiv\sum_n\bar{L}_i(n,t)\sum_m h_{i\to l}(n;i,m)\bar{L}_i(m,t)/\bar{L}_i(t)\bar{L}_i(t)$. In the earlier RMR and LPL models, the interaction probability $\bar{h}_{i\to f}(t)$ is replaced by a state-averaged, time-independent, interaction probability denoted by e_{2PL} in the LPL model [Cu86] or by k in the RMR model [To85]. It is this non-linear, binary misrepair term that produces dose-rate effects on cell killing in the RMR and LPL models. The binary misrepair term in these models also add a second-order term to the lesion removal process as discussed by Fowler [Fo99]. Similarly, the state-dependent binary misrepair interaction term, Eq. (6.13), adds a second-order lesion removal process to the model and produces a dose-rate effect. However, the multiple-lesion, multiple-state, binary-misrepair interaction process formulated in this work is more comprehensive than those included in the earlier RMR and LPL models because it can account for both proximity effects and pairwise lesion interaction processes among the different types of lesions.

7.0 Model Summary and Discussion

Ionizing radiation initially creates a plethora of different types of lesions, some of which may be immediately lethal to a cell. Then, a variety of mechanisms compete with each other so that eventually all of the potentially repairable damage initially formed by ionizing radiation is either repaired correctly or transformed into an irreversible mutation or fatal lesion. In model developed in this report, these time dependent processes are modeled in stationary phase cells by set of non-linear, coupled differential equations summarized below.

7.1 Repairable Lesions

To obtain the repairable lesion balance equation, first substitute Eqs. (4.2), (5.1), and (6.2) into Eq. (3.6). Then, substitute Eqs. (6.5), (6.8), and (6.13) into this equation for the transformation terms $T^{lmr}(i \to l,n,t)$, $T^{fix}(i \to l,n,t)$, and $T^{pli}(i \to l,n,t)$, respectively. After rearranging terms, the repairable lesion balance equation can be written, for $i = 1,\cdots,N_r$ and $n = 1,\cdots,Q$, as

$$\begin{aligned}\frac{d\bar{L}_i(n,t)}{dt} &= 2\dot{D}(t)Y_n\,\Sigma_i(n) - \left\{\bar{a}_i(n) + \left[1-\bar{a}_i(n)\right]\bar{b}_i(n)\right\} l_i(n)\bar{L}_i(n,t) - \bar{e}_i(n,t)\bar{L}_i(n,t) \\ &\quad + \sum_{l=1}^{N_r}\bar{L}_l(n,t)\left\{\left[1-\bar{a}_l(n)\right]\bar{b}_{l\to i}(n)\,l_l(n) + \sum_{m=1}^{Q}\sum_{j=1}^{N_r} h_{l\to i}(n;j,m)\bar{L}_j(m,t)\right\} \\ &\quad - \bar{L}_i(n,t)\sum_{l=1}^{N}\sum_{m=1}^{Q}\sum_{j=1}^{N_r} h_{i\to l}(n;j,m)\bar{L}_j(m,t) + \sum_{l=1}^{N_r}\bar{e}_{l\to i}(n,t)\bar{L}_l(n,t).\end{aligned} \tag{7.1}$$

where $\bar{e}_i(n,t) \equiv \sum_l \bar{e}_{i\to l}(n,t)$ and $\bar{b}_i(n) \equiv \sum_l \bar{b}_{i\to l}(n)$.

In Eq. (7.1), the terms $\bar{b}_{i\to i}(n)$, $\bar{e}_{i\to i}(n,t)$, and $h_{i\to i}(n;j,m)$ are zero for all *i* and *n* because the misrepair or fixation of a lesion must, by definition, transform an *i*th type of repairable lesion into some other type of lesion. Because the misrepair of a lesion must result in the transformation of a repairable lesion into some other type of lesion, $\bar{b}_i(n)$ must also equal unity. Thus, Eq. (7.1) simplifies to

$$\frac{d\bar{L}_i(n,t)}{dt} = 2\dot{D}(t)Y_n\,\Sigma_i(n) - \left\{ l_i(n) + \bar{e}_i(n,t) + \sum_{l=1}^{N}\sum_{m=1}^{Q}\sum_{j=1}^{N_r} h_{i\to l}(n;j,m)\bar{L}_j(m,t) \right\}\bar{L}_i(n,t)$$
$$+ \sum_{l=1}^{N_r}\bar{L}_l(n,t)\left\{ \left[1-\bar{a}_l(n)\right]\bar{b}_{l\to i}(n)\,l_l(n) + \sum_{m=1}^{Q}\sum_{j=1}^{N_r} h_{l\to i}(n;j,m)\bar{L}_j(m,t) \right\} \quad (7.2)$$
$$+ \sum_{l=1}^{N_r}\bar{e}_{l\to i}(n,t)\bar{L}_l(n,t).$$

7.2 Mutations

Substitution of Eqs. (6.7), (6.8) and (6.13) into Eq. (6.2) gives the expected number of *i*th type repairable lesions in the *n*th state that are transformed into the *k*th type of mutation in the *n*th state, per unit time at time *t*. The balance equation for the *k*th type of mutation is then obtained by summing this equation over all types of repairable lesions, i.e., for $k = N_r + 1, \cdots, N-1$ and $n = 1, \cdots, Q$,

$$\frac{d\bar{M}_k(n,t)}{dt} = \sum_{i=1}^{N_r}\bar{L}_i(n,t)\left\{ l_i(n)\left[1-\bar{a}_i(n)\right]\bar{b}_{i\to k}(n) + \sum_{m=1}^{Q}\sum_{j=1}^{N_r} h_{i\to k}(n;j,m)\bar{L}_j(m,t) \right\}$$
$$+ \sum_{i=1}^{N_r}\bar{e}_{i\to k}(n,t)\bar{L}_i(n,t). \quad (7.3)$$

Because it is difficult to imagine a fixation process that will directly transform a repairable lesion into an irreversible but non-lethal mutation, it is reasonable to set $\bar{e}_{i\to k}(n,t) = 0$ for all mutation types so that, for $k = N_r + 1, \cdots, N-1$ and $n = 1, \cdots, Q$, Eq. (7.3) reduces to

$$\frac{d\bar{M}_k(n,t)}{dt} = \sum_{i=1}^{N_r}\bar{L}_i(n,t)\left\{ l_i(n)\left[1-\bar{a}_i(n)\right]\bar{b}_{i\to k}(n) + \sum_{m=1}^{Q}\sum_{j=1}^{N_r} h_{i\to k}(n;j,m)\bar{L}_j(m,t) \right\}. \quad (7.4)$$

For large doses and dose-rates, $\bar{L}_j(m,t)$ will be large. Under these conditions, Eq. (7.4) indicates that most mutations will be created by binary misrepair interaction processes. Conversely, the importance of binary misrepair in the creation of mutations will decrease as

the dose and dose-rate decrease. For very small doses and dose-rates [i.e., when $\bar{L}_j(m,t)$ is small], Eq. (7.4) suggests that most mutations will be formed by (linear) lesion misrepair.

7.3 Fatal Lesions

The fatal lesion balance equation is obtained by substituting Eqs. (4.5) and (6.2) into Eq. (3.12). After substitution of Eqs. (6.6), (6.8), and (6.13) for $T^{lmr}(i \to f,n,t)$, $T^{fix}(i \to f,n,t)$, and $T^{pli}(i \to f,n,t)$, respectively, the fatal lesion balance equation becomes

$$\frac{d\bar{L}_f(t)}{dt} = 2\dot{D}(t)Y\bar{\Sigma}_f + \sum_{n=1}^{Q}\sum_{i=1}^{N_r}\bar{L}_i(n,t)\left\{l_i(n)\left[1-\bar{a}_i(n)\right]\bar{b}_{i\to f}(n)+\bar{e}_{i\to f}(n,t)\right\} + \sum_{n=1}^{Q}\sum_{i=1}^{N_r}\bar{L}_i(n,t)\sum_{m=1}^{Q}\sum_{j=1}^{N_r}h_{i\to f}(n;j,m)\bar{L}_j(m,t). \tag{7.5}$$

Except, perhaps, for very high-LET radiation, it seems likely that most or all of the lesions initially formed by ionizing radiation have at least a small chance of being repaired (or misrepaired) by a cell. In other words, the quantity $2\dot{D}(t)Y\bar{\Sigma}_f$ is most likely negligibly small compared to other fatal lesion formation mechanisms. It is thus reasonable to set $\bar{\Sigma}_f = 0$, especially for low-LET radiations, so that Eq. (7.5) simplifies to

$$\frac{d\bar{L}_f(t)}{dt} = \sum_{n=1}^{Q}\sum_{i=1}^{N_r}\bar{L}_i(n,t)\left\{l_i(n)\left[1-\bar{a}_i(n)\right]\bar{b}_{i\to f}(n)+\bar{e}_{i\to f}(n,t)\right\} + \sum_{n=1}^{Q}\sum_{i=1}^{N_r}\bar{L}_i(n,t)\sum_{m=1}^{Q}\sum_{j=1}^{N_r}h_{i\to f}(n;j,m)\bar{L}_j(m,t). \tag{7.6}$$

For large doses and dose-rates [i.e., when $\bar{L}_j(m,t)$ is large], Eq. (7.6) indicates that most fatal lesions will be created by the binary misrepair interaction process. Conversely, most fatal lesions will be created by either (linear) lesion misrepair or lesion fixation when cells are exposed to small doses and dose-rates.

7.4 Linear Energy Transfer (LET) Effects

The kinetic model proposed in this work explicitly includes dose and dose-rate effects. However, LET effects are implicit in the formulation. The fatal lesion formation probability $\Sigma_f(n)$ increases as the particle LET increases. In addition, low- and high-LET radiation can create groups of spatially correlated lesions because of the higher-order folding of chromatin [Ho96, Ry96]. But because high-LET radiation produces spatially correlated groups of lesions more frequently than low-LET radiation, the binary misrepair interaction probability $h_{i\to l}(n; j,n)$ should increase as the particle LET increases (a proximity effect). The interaction probability for lesions in different states $h_{i\to l}(n; j,n)$, $n \neq m$, may or may not increase with particle LET.

LET effects could also arise because the lesion formation probability $\Sigma_i(n)$ increases for complex lesions more rapidly with particle LET than does the formation probability for simple lesions (a "hardening" of the radiation-induced lesion spectrum). In other words, the average number of elementary damage sites per lesion (the lesion complexity) increases as the particle LET increases and causes, on the average, the rate of correct lesion repair to decrease, i.e., $\sum_i \sum_n \bar{a}_i(n)\, l_i(n) \bar{L}_i(n,t)$ decreases as the particle LET increases. However, it is important to realize that the repair probability $l_i(n)$, the average repair fidelity $\bar{a}_i(n)$, the effective repair transformation probability $\bar{b}_{i\to l}(n)$, and the lesion fixation probability $e_{i\to l}(n,t)$ are most likely independent, or almost independent, of particle LET.

8.0 Conclusions

In proliferating cells, the structure and organization of the chromatin changes dramatically through the cell cycle. Even the quantity of DNA in the nucleus changes during the cell cycle. All of these phenomena most likely play a role in determining the radiosensitivity of cells, tissues, and organisms. Also, the pervasive role of repair processes in the model suggests that modulation of repair pathways during the cell cycle could be a principal cause of cell cycle effects. Because all of the model parameters associated with the biological characteristics of a cell are independent of time and cell cycle position, the balance equations formulated in this work, i.e., Eqs. (7.1) to (7.6), are more appropriate for quiescent (stationary-phase) cells than for proliferating cells. The same limitation is true for many other models [Al89, Br90, Go85, Ha98, Ke78, Kr92, Ni90, Os93, Sa90], including the original RMR and LPL models [To85, Cu86].

Measurements [Ba95, Di90, Il91, Ra98, Sc90, St94] indicate that the rate of rejoining double strand breaks in a cell is described better by higher-order repair kinetics than by first-order repair kinetics. For example, the rate of DSB rejoining is often described by a sum of two exponential components (i.e., biphasic repair) instead of a single (first-order) exponential component. One reasonable explanation for the observed higher-order DSB rejoining kinetics is that they result from the heterogeneous formation and repair of double strand breaks among chromatin states. An alternative explanation [e.g., see [Ra98]) is that the damage detected experimentally as a double strand break is composed of several types of double strand breaks, each of which has a different characteristic total repair probability $l_{dsb}(n)$. A third possibility is that the observed DSB rejoining kinetics are due to a second-order (binary misrepair) lesion removal process [Fo99]. Regardless, the formalism of the balance equation for repairable DNA lesions, Eq. (7.1), is general enough to include all of these effects and account for non-exponential or multi-exponential lesion removal transients.

The kinetic model proposed in this report contains a large number of model parameters and rigorous testing of all aspects of the model may be impractical. Nevertheless, all of the parameters are motivated through a consideration of fundamental physical and

biological processes that can, at least in principle, be measured or estimated from *ab initio* calculations. A more comprehensive parameterization of the intrinsic biological factors and processes involved in the repair and misrepair of DNA damage can help identify the potential strengths and weakness of simpler models (e.g., the earlier LPL and RMR models). Moreover, the multiple-lesion, multiple-state kinetic model can be used to guide the development of practical, but more refined, models to explain and quantify the biochemical processing of genotoxic damage. That is, guide the development of, for example, a two-lesion, two-state kinetic model that better describes chromatin and proximity effects, lesion removal transients, and the creation of lethal and non-lethal genetic alterations.

In 1993, the National Council on Radiation Protection and Measurements (NCRP) issued recommendations outlining the research needs of the radiation protection community [NC93]. The NCRP states "… additional research should be devoted to establishing stronger links among [radiobiological] studies conducted in different species of animals, and among studies conducted at the tissue, cellular and molecular [DNA] levels." Knowledge (modeling) of the cell killing effects of ionizing radiation is also important for continuing efforts to predict the effectiveness of external and internal radiotherapy protocols for the treatment of cancer. The kinetic model described in this work takes us a step closer to formulating a comprehensive conceptual and mathematical basis for explaining dose and dose-rate effects, LET effects, and for understanding the biochemical processing of genotoxic damage.

9.0 References

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