Reaction rate theory of radiation exposure: Effects of the dose rate on mutation frequencies

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We develop a kinetic reaction model for the cells having the irradiated DNA molecules due to the ionizing radiation exposure. Our theory simultaneously accounts for the time-dependent reactions of the DNA damage, the DNA mutation, the DNA repair, and the proliferation and apoptosis of cells in a tissue with a minimal set of model parameters. In contrast to the existing theories for the radiation exposition, we do not assume the relationships between the total dose and the induced mutation frequency. We show good agreement between theory and experiment. Importantly, our result shows a new perspective that the key ingredient in the study of the irradiated cells is the rate constants depending on the dose rate. Moreover, we discuss the universal scaling function for mutation frequencies due to the irradiation at low dose rates.

Ionizing and non-ionizing radiation exposures are phenomena that cannot be avoided by all living organisms. While non-ionizing radiation refers to the electromagnetic radiation that does not carry kinetic energy enough to liberate electrons from atoms or molecules, ionizing radiation generated through nuclear reactions naturally or artificially may cause physical damages to the DNA molecules that encode its genome in the living cells by ionizing or breaking the molecular bonds, or by producing harmful free radicals of solvents. In aqueous solution, the overwhelming contribution of cellular DNA damages is caused by hydroxyl radicals arising from the surrounding water molecules.

Of particular interest in radiobiology over the past decades is genetic mutation induced by the irradiation that is a change in the nucleotide sequences of the genome and hence increases the risk of cancers. Muller first studied genetic effects of X-rays on Drosophila, and discovered that the artificial ionizing radiation gives rise to the mutation. The subsequent argument then led to the linear no threshold (LNT) hypothesis that the carcinogenic risk caused by the biological damage due to the ionizing radiation becomes zero at the y-intercept with no dose. That is, there is no safety threshold for the radiation exposure. Russell and Kelly further examined the mutation frequency by studying the frequency of transmitted specific-locus mutations induced in mouse spermatogonial stem cells. Their striking result is that the mutation frequency linearly varies with the total dose of the ionizing radiation within experimental errors, whereas their fitting required two different slopes for chronic and acute dose rates. Since these studies, there has been a vast literature on the subject of radiation exposure and genetic mutation. Specifically, the deviation of the mutation frequency from the linear slope with the total dose is a matter of controversy; however, it is difficult to do justice by just mentioning a few selective examples here. Rather, we refer readers to recent articles for an extensive review of the issues and relevant literature.

On the theoretical side, the target theory in which individual quanta, or photons, of radiation that are assumed to be absorbed at sensitive points (targets) in a cell has been developed originally by Lea, and further developed by Chadwick and Leenhouts to account for the repair of damaged DNA molecules. In the latter theory, the number of mutation is derived from

\[ N_m = \sigma N_n^0 (1 - e^{-\frac{D}{D_0}}), \]

where \( \sigma \) is the proportion of the mutated cells that are not repaired, \( N_m \) is the number of the mutated cells, \( N_n^0 \) is the number of the normal cells before the irradiation, \( D \) is the total dose of the irradiation, and \( D_0 \) is the unit dose to produce one active event. To account for the observed deviation from the classical target theory at high dose rates, Eq. (1) was modified by adding the quadratic term for \( O(D^2) \) to the exponent of the exponential function. It should be noted that at the low dose \( (D \ll D_0) \), Eq. (1) can be expanded into a linear function of \( D \) as \( N_m \sim \sigma N_n^0 D / D_0 \). Thus, the LNT hypothesis is rationalized from the target theory, whereas the dependence of both total dose \( D \) and dose rate on the mutation frequency is still puzzling.

In this paper, we present a new theory for the radiation exposure that accounts for the kinetic reaction of the irradiated DNA molecules. While the study of the molecular dynamics simulation reveals the reaction pathway of the single and double strand breaking of the DNA molecules for picoseconds caused by free hydroxyl radicals due to the ionizing radiation, a reaction theory for longer time scales from hours to days is necessitated to consider the DNA mutation in cell cycles. Our theory shows that the mutation frequency varies with time on account of the irradiation and the environmental stimuli to the DNA molecules, with respect to counteracting effects among the DNA damage and mutation, the DNA repair, and the proliferation and apoptosis of cells. In contrast to the existing theories, the key ingredient in our theory is the dose rate that controls the reaction of the system, without invoking the total dose \( D \) in the theoretical framework.
show that the observed dependence of the dose rate on the DNA mutation frequency in mouse spermatogonial stem cells that cannot be explained by the classical theories falls on the universal scaling function for the low dose rate.

We now consider a tissue consisting of $N_0(t)$ cells having normal DNA molecules, and $N_m(0)$ cells having the DNA mutation. $N_{\text{max}}$ denotes the maximum number of the cells in the tissue. At $t = 0$, the tissue is artificially irradiated with the dose rate $d(t)$ [Gy/h]. The total dose $D$ during the time $t$ is thus given by $D = \int dtd(t)$. In general, cells experience the proliferation and apoptosis that are the processes of cell reproduction and programmed cells death, respectively. The DNA molecules in cells are also damaged through regular biological processes such as cell cycle and environmental irradiation. The DNA repair process typically responds to the damage in the DNA structure. When the repair of the lesions fails, the DNA mutations can occur. These damage rates may depend on the the ways that the cells are exposed to the radiations arising from the surroundings, or experience the metabolism and hydrolysis. In this paper, however, we do not specify all the biological reactions because we do not wish to include the rate constants that cannot be determined or have large uncertainty. Instead, we write the averaged, effective rate of the DNA mutation due to all these relevant natural reactions in the time-independent form as $d_{eff}$. That is, $d_{eff}$ is the effective reaction rate biologically equivalent to all the other non-ionizing effects that cause the spontaneous mutation, and hence includes the effect of the natural background radiation.

Our reaction model for the low-dose irradiation is given by the following set of kinetic equations,

$$\begin{align*}
\frac{dN_n(t)}{dt} &= f[N_n(t), N_m(t), d_{eff}, d(t)] \\
\frac{dN_m(t)}{dt} &= c_n[N_n(t), d_{eff}, d(t)]N_n(t) - c_m[N_m(t), d_{eff}, d(t)]N_m(t) \\
&+ pN_m(t) - aN_m(t).
\end{align*}$$

(2)

$c_n[N_n, d_{eff}, d(t)]$, $c_m[N_m, d_{eff}, d(t)]$, $p$, and $a$ are the rates of the mutation of the DNA molecules in the normal cells due to all the stimuli, the death of the cells having the mutated DNA molecules, the proliferation of the cells having the mutated DNA molecules, and the apoptosis of the cells having the mutated DNA molecules, respectively. The schematic description of the reaction for the cells is illustrated in Fig. 1.

Note that $c_n[N_n, d_{eff}, d]$ and $c_m[N_m, d_{eff}, d]$ depend on $N_n(t)$ and $N_m(t)$. Importantly, we have also written $c_n[N_n, d_{eff}, d(t)]$ and $c_m[N_m, d_{eff}, d(t)]$ in terms of the rate for the environmental stimuli $d_{eff}$ and the dose rate $d(t)$. While $f$ is a function of $N_n(t)$ and $N_m(t)$ in general [11], [12], for conciseness, we do not assume any reaction model for the normal cells here. Moreover, it should be noticed that a generic theory must account for the chemical reaction between the DNA damage and repair. In the following discussion and supplementary material, however, we show that at low dose rates, the rate equation for the cells having the DNA damage can be cast into the compartment for the kinetic reaction of the normal cells.

We write the thermal energy $\varepsilon$ deposited in a single cell having the mass $m$ [kg] during short time $\Delta t$ as $\varepsilon = m[d_{eff} + d(t)]\Delta t/N_{\text{max}}$. We now introduce $P_n(\varepsilon)$ and $P_m(\varepsilon)$, the probabilities that the DNA mutation in a normal cell and the death of a cell having the DNA mutation occur, respectively. The nature of these probabilities must be rationalized by more microscopic models that account for mitosis, the DNA mutation and repair, metabolism, and all the other reactions in cell cycles. However, at low dose rates, we simply write these probabilities proportional to the energy in the forms of $P_n(\varepsilon) = c_n \varepsilon$ and $P_m(\varepsilon) = c_m \varepsilon$ [11]. We have introduced the constants $c_n$ and $c_m$ that are independent of the number of the cells. Since our model tissue primarily consists of normal cells, we take the approximation, $N_m(t)/N_n(t) \ll 1$ and $N_n(t) \sim N_{\text{max}}$ during the reaction. Our rationale for this treatment is that the experimental data of the mutation in mouse spermatogonial stem cells indicate that $N_m(t)/N_n(t)$ is close to $10^{-5}$. Thus, the number of the normal cells whose DNA molecules are mutated during $\Delta t$ and the number of the cells having the DNA mutation that die during $\Delta t$ are given by $P_n(\varepsilon)N_n(t) \sim c_n m d_{eff} + d(t)]\Delta t$ and $P_m(\varepsilon)N_n(t) \sim c_m m d_{eff} + d(t)]\Delta t$. Thus, the second equation in Eq. (2) can then be cast into

$$\frac{dF_m(t)}{dt} = c_n(p)[d_{eff} + d(t)] - \{c_m(p)[d_{eff} + d(t)] + \mu\} \times F_m(t),$$

(3)

where

$$\mu = a - p.$$  

(4)

Because our primary interest is the mutation frequency $F_m(t)$, we have divided $N_m(t)$ by $N_{\text{max}}$ and have $F_m(t) = N_m(t)/N_{\text{max}}$. $p = m/N_{\text{max}}$ denotes the average weight density of the cells in the tissue. We have written it symbolically as $c_n(p)$ and $c_m(p)$. For conciseness, however, we use the same notations $c_n$ and $c_m$ as their counterparts. In this paper, we consider $d(t) = d\theta(t)$, where $\theta(t)$ is the step function.

Eq. (3) can be analytically solved. For the solutions, we have three classifications with respect to $[c_m(d_{eff} + d) + \mu] > 0$, $[c_m(d_{eff} + d) + \mu] = 0$, and $[c_m(d_{eff} + d) + \mu] < 0$. When the specific condition with $[c_m(d_{eff} + d) + \mu] = 0$ is satisfied,
the solution of Eq. (3) becomes

\[ F_m(D) = c_m(d + d_{\text{eff}})t + F_m(0) = c_m\left(1 + \frac{d_{\text{eff}}}{d}\right)D + F_m(0), \]  

(5)

where we used \( dt = D \). Thus, the LNT hypothesis remains intact with any value of \( D \) only if \( p = c_m d + \alpha \) is exactly satisfied; certainly, this strict condition is unlikely to occur in living organisms and to be externally controlled. When \( c_m(d_{\text{eff}} + d) + \mu \neq 0 \), the solution of Eq. (3) is given by

\[
F_m(D) = \left\{ \frac{c_m(d + d_{\text{eff}})}{c_m(d + d_{\text{eff}}) + \mu} - F_m(0) \right\} \times \left(1 - \exp\left(-\frac{c_m(d + d_{\text{eff}}) + \mu}{d}D\right)\right)
+ F_m(0)
\]

(6)

Again, we have replaced the time \( t \) by \( D/d \) because these variables are more common setup in the experiments.

The steady state solution of Eq. (3), \( F_m \), is derived by setting \( dF_m(t)/dt = 0 \) and becomes \( F_m = c_m(d + d_{\text{eff}}) + \mu \). We then identify the control \( F_m(0) \) with the steady state solution of Eq. (3) without the artificial radiation (i.e., \( d = 0 \)) and hence derive

\[ F_m(0) = c_m d_{\text{eff}}/(c_m d_{\text{eff}} + \mu). \]  

(7)

For the small total dose with \( D \ll d/\{c_m(d + d_{\text{eff}}) + \mu\} \), Eq. (6) is expanded to \( \{c_m(d + d_{\text{eff}})/(c_m(d + d_{\text{eff}}) + \mu) - F_m(0)\}\{c_m(d + d_{\text{eff}}) + \mu\}D/d + F_m(0) + O(D^2) \). Thus, the LNT hypothesis holds only with the small total dose whose condition depends on the dose rate \( d \). In Fig. 2 we have illustrated the possible scenarios with respect to the cases with \( c_m(d + d_{\text{eff}}) + \mu \).

\[ F_m(D) \times 10^{-5} \]

\[ 0 \quad 1 \quad 2 \quad 3 \quad 4 \quad 5 \quad 6 \]

FIG. 2. \( F_m(D) \) vs. \( D \) for the condition of the values of \( c_m(d_{\text{eff}} + d) + \mu \). (1) \( c_m(d_{\text{eff}} + d) + \mu > 0 \) (black solid line) (2) \( c_m(d_{\text{eff}} + d) + \mu = 0 \) (red dashed line) (3) \( c_m(d_{\text{eff}} + d) + \mu < 0 \) (blue dot-dashed line)

In principle, \( c_m \), \( c_m \), and \( \mu \) should be derived from more microscopic models that account for the relevant phenomena such as cell cycles, breaking DNA strands and base pairs due to the irradiation and the chemical reactions in cell cycles, and repairing them. In our kinetic reaction model, however, they are the model parameters determined by experimental data. Using Eqs. (6) and the pronounced experimental data in Ref. [3] for mouse spermatogonial stem cells, we have determined \( c_m = 2.91 \times 10^{-5} \) [1/Gy], \( c_m = 1.00 \times 10^{-1} \) [1/Gy], and \( \mu = 3.13 \times 10^{-3} \) [1/hr] by the least squares fitting [12]. Eq. (7) then leads to

\[ d_{\text{eff}} = \frac{F_m(0) \mu}{c_m - F_m(0) c_m} = 1.11 \times 10^{-3} \text{ [Gy/hr]}. \]  

(8)

We note that this value is significantly larger than \( 2.74 \times 10^{-7} \) [Gy/hr] due to the natural background radiation. Thus, our result indicates that the natural damage of the DNA molecules arises primarily from the chemical reactions in cell cycles. It should also be noted that the value in Eq. (8) is consistent with that for the human (8.4 mGy/hr) due to the double-strand DNA breaks caused by endogenous reactive oxygen species [3].

Our model for mouse spermatogonial stem cells shows that the mutation frequency becomes twice as the control due to the spontaneous mutation when the total dose \( D \) for a year reaches \( \sim 1 \) [Gy]; this value is so-called ‘doubling dose’, the standard concept in radiation biology, and is suggested to be surprisingly similar values among human, mouse, and drosophila that are in the rage of \( 0.11-4.00 \) [Gy] [13] where our result falls on as well. This similarity, together with our result, may also imply that they commonly receive the risk of the spontaneous mutation per gene. However, our kinetic modeling indicates that while the doubling dose is a widely accepted concept in radiation biology, the total dose \( D \) is not a fundamental measure to account for the mutation frequency. Further experiment to clarify the new concept based on the dose rate \( d(t) \) is welcome.

Using the fitted values of the model parameters, the rate of the DNA mutation per normal cell is estimated at \( c_m d_{\text{eff}} = 3.23 \times 10^{-8} \) [1/hr]. However, if we use \( 2.2 \times 10^{-9} \) per base pair per year for the average mammalian genome mutation rate in Ref. [14], the DNA mutation rate per normal cell for mouse must be, at least, \( 8.6 \times 10^{-4} \) [1/hr] with \( 26 \times 10^{-8} \) taken for the number of the base pairs per genome. While there is a large discrepancy in this estimate, it should be noted that the death rate of the cells having the DNA mutation due to the environmental stimuli becomes \( c_m d_{\text{eff}} = 1.11 \times 10^{-4} \) [1/hr] and is hence consistent within the order of the magnitude. This value is also in agreement with that for the mutation rate in a vitro experiment [15]. It is now conceivable that the majority of the cells having the mutated DNA molecules dies.

From the model parameters for mouse spermatogonial stem cells, we find that the primary contribution in the kinetic reaction equation for the DNA mutation [Eq. (3)] is \( \mu \), which leads to the steady state solution \( F_m \). This indicates that the ability of the DNA repair and apoptosis overwhelms the proliferation of the cells having the mutated DNA molecules, and the DNA damage due to the typical environmental stimuli and low-dose irradiation.
We then cast Eq. (6) into
\[ \Phi = \frac{[F_m(\tau) - F_m(0)]}{[F_m - F_m(0)]} = 1 - \exp(-\tau), \]
where we have introduced the scaled time \( \tau = [c_m(d + d_{eff}) + \mu]/\mu \). Importantly, Eq. (9) indicates that in general, mutation frequencies with the low-dose irradiation fall on the universal scaling function \( \Phi \). To illustrate our scaling function, we have used the same experimental data [3] for fitting our model parameters (Fig 3). We note that each experimental data point corresponds to the cases with the different dose rate \( d \). Thus, our theory shows qualitative agreement with the experiments without classifying the dose rate \( d \); we have no parametrization to mimic the LNT hypothesis.

![Scaling function \( \Phi \) vs. scaled time \( \tau \). Solid line and triangular points with errors indicate theory and experiment [3], respectively.](image)

In summary, we performed a kinetic reaction modeling for the proliferation and apoptosis of cells, the DNA damage and mutation due to the environmental stimuli and the irradiation, and the DNA repair. Our theory, for the first time, accounts for the effect of the dose rate that leads to the antagonistic regulation on the time evolution of the number of cells having the mutated DNA molecules. The key features in our theory are that our kinetic rate equations include the dose rate \( d(\tau) \) in the rate constants and the rate equations for the normal cells and the cells having the damaged DNA molecules due to the low dose irradiation are decoupled from that for the cells having the mutated DNA molecules. Despite the simplicity of the equations, we are able to qualitatively explain the puzzling behavior in the pronounced experiment for the mega-mouse projects [3] in which two linear slopes for the mutation frequency vs. the total dose rate exist with respect to the acute and chronic irradiations. Thus, we conclude that the total dose \( D \) is not a fundamental measure to study the irradiation, as deduced from the systematic relationships for the dose rate vs. the induced mutation frequency [18, 19]. Depending on the rate constants, the number of the cells having the mutated DNA molecules may continue to monotonically increase. While this phenomenon is certainly unwished clinically, a lesson demonstrated in this article indicates the importance of the accurate control of the dose rate in the study of mutation frequencies and presumably, cancer risks. Importantly, our theory predicts that all the experimental data of mouse spermatogonial stem cells with low-dose rates would fall on the universal scaling function \( \Phi \) with the scaled time \( \tau \) [Fig 3]. Because this experiment necessitated seven million mice for sampling, the similar data cannot be obtained by the current code of ethics in experiments. Thus, the validation of this prediction for the universal scaling awaits further challenge and wisdom in experiments.

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[1] C. v. Sonntag, *Free-radical-induced DNA damage and its repair: a chemical perspective* (Springer, 2006).
[2] H. J. Muller, *Science* 66, 84 (1927).
[3] W. L. Russell and E. M. Kelly, Proc Natl Acad Sci U S A 79, 542 (1982).
[4] M. Tubiana, L. E. Feinendegen, C. Yang, and J. M. Kaminski, Radiology 251, 13 (2009).
[5] A. M. Kellerer and H. H. Rossi, Radiation Research 75, 471 (1978).
[6] D. E. Lea, *Actions of radiations on living cells*, 2nd ed. (University Press, 1955).
[7] K. H. Chadwick and Leenhou, *Hp, Physics in Medicine and Biology* 18, 78 (1973).
[8] R. M. Abolfath, A. C. T. van Duin, and T. Brabec, J. Phys. Chem. A 115, 11045 (2011).
[9] Y. Manabe, K. Ichikawa, and M. Bando, Journal of the Physical Society of Japan 81, 104004 (2012).
[10] Y. Manabe and M. Bando, Journal of the Physical Society of Japan 82, 094004 (2013).
[11] In the limit of very high dose rate, we expect that the DNA molecules are always damaged. Thus, these probabilities should
be unity per unit time, regardless the deposited energy.

[12] In Ref. [3], we found that one data point does not appear to be classified by the chronic and acute radiations that the authors defined. While we have eliminated this data point, there is no bias or prejudice in our selecting the data for fitting.

[13] J. V. Neel, Teratology 59, 216 (1999).
[14] S. Kumar and S. Subramanian, Proceedings of the National Academy of Sciences of the United States of America 99, 803 (2002).
[15] B. Dubertret, S. M. Liu, Q. Ouyang, and A. Libchaber, Phys. Rev. Lett. 86, 6022 (2001).
[16] R. Peto, F. J. C. Roe, P. N. Lee, L. Levy, and J. Clack, Br. J. Cancer 32, 411 (1975).
[17] H. S. Kaplan and L. E. Moses, Science 145, 21 (1964).
[18] M. M. Vilenchik and A. G. Knudson, Proc. Natl. Acad. Sci. U. S. A. 97, 5381 (2000).
[19] M. M. Vilenchik and A. G. Knudson, Proc. Natl. Acad. Sci. U. S. A. 103, 17874 (2006).