Chapter 2

On the Dynamical Approach of Quantitative Radiation Biology

Noriyuki B. Ouchi

Additional information is available at the end of the chapter

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Abstract

A quantitative approach in radiation biology based on the clonogenic method and cell survival curves in various conditions are introduced. The cell survival curves seem to have universality with regard to its functional form; in other words, functional form of survival curve seems to be unchanged under various conditions including different species. Various factors affecting the radiosensitivity have been introduced to find macroscopic nature of living organisms. Mathematical models that describe cell survival curves have been presented for discussing the derivation of the mathematical form based on biological mechanism. Finally, the possibility that the structural change of chromosome affects the repair process is discussed.

Keywords: Cell survival curves, Mathematical model, Target theory, Quantitative radiation biology, DNA repair

1. Introduction

Now, over ten years have passed since initial sequencing and analysis of the human genome [1]. The human genome is thought to contain approximately 20,000 protein-coding genes, which are supposed to drive a human being as a living system. The successful sequencing and analysis of the human genome made a major step forward in the understanding of life. The progress in decoding the human genome reveals aspects of the components of life; however, it is difficult to study dynamical principle of living organisms, which are supposed to emerge via many interactions between many proteins (30,000 or more) in a collective manner.

In fact, a major method in modern molecular biology is each event involved in the corresponding process will be examined by breaking the whole system into small pieces, and biological activity in each small part of the system is explained by the activation of corre-
sponding genes. This is the method so-called reductionism. This kind of reductionism has made great accomplishments in the physical sciences; however, only the knowledge of the breaking elements would not help to understand life, e.g. its origin, evolution, and persistence (Fig. 1). In other words, so-called emergent properties of the system would not be impossible to predict from knowledge of the breaking elements of the system [2]. Therefore, the understanding of life itself requires research technique which handles the whole system.

Figure 1. Karakuri ningyou (Japanese mechanized doll). This is a tea serving mechanized robot. The karakuri ningyou serves a cup of tea, and its sequence is roughly as follows: 1) When a cup of tea is placed on the hands, they start to move toward a guest by moving its feet like walking. 2) It moves, setting distance, and bows its head. 3) When a cup of tea is taken from the hands, it stops and waits for a next action. 4) It turns around and returns to the starting position (host’s place) when an empty cup is placed on the hands. It works by springs. Only the examination of each mechanical gear would not help to understand the entire behavior of the doll. Picture is taken from Wikimedia Commons [3] and converted to gray scale.

There is a well-known logic of research methods that have been used to derive the theory or some formula without breaking the corresponding system. For example, linear response theory is based on the idea that the global behavior of the system is obtained by investigating the various responses of the system against the given perturbation/stimulation. Especially, this kind of research design, i.e., investigating the global response of the system to a spatiotemporally varying perturbation, e.g., electromagnetic field, temperature, etc., for analyzing dynamical properties of the system has been well adopted in nonequilibrium statistical physics [4].
Turning now to the case of biological science, ionizing radiation seems to be a good example of such kind of “perturbation” to examine system’s global behavior from the action to the living organisms. Generally, different types of scientific index of the biological responses to ionizing radiation are used to study the action of ionizing radiation, depending on the corresponding systems. For most cases, cancer incidence is used as the biological response if the corresponding system is a human being. This type of study, known as epidemiology, is basically the statistics.

On the other hand, major advances have been made in the mechanism-based study of molecular radiation biology, and these advances shed light on the relationship between carcinogenesis and radiation-induced DNA damage. Biological studies on the various radiation responses are basically the cellular-scale investigation. By considering ionizing radiation as an example of external stimulus, many types of cellular responses, e.g., induction of chromosomal aberrations, gene mutations, cell apoptosis, cell transformation, and tumorigenesis, are known to occur. In many cases, quantification of these responses can help discuss these issues mathematically. In the next section, a very effective quantification experimental technique in radiation biology is discussed.

2. Quantitative radiation biology

Early in the twentieth century, it is known that the ionizing radiation may cause harmful effects on the biological organisms. Most of the experiments in the early radiation biology research are aimed to study the effects of X-ray irradiation on various types of living organisms: bacteria, virus, and unicellular organism. In short, unicellular organisms were mostly used to study the action of ionizing radiation on living organisms.

These early experiments have revealed the amount of radiation doses that needed to kill or inactivate the various species. For example, the mean lethal dose, $D_0$ (dose required to reduce the population to the 37% level, i.e., fraction $1/e$) is over 150 Gy for *Chilomonas paramecium* and 400 Gy for virus (Table. 1). Here, Gy is a unit absorbed radiation dose, and 1 Gy=1 J kg$^{-1}$, in SI unit. Therefore, the fact that there is a big difference in mean lethal dose between species, or, in other words, there is a big difference in radiosensitivity between species, has been recognized.

However, irradiation experiment of human cells was needed to study the radiosensitivity of humans. Therefore, experimental technique that possibly cultures the separated cells *in vitro* was needed. In 1907, a new technique, known as cell culture, was successfully introduced by Harrison [6] to study the argument of development process of the nervous system, whether the nervous system was composed of many cells (syncytial theory) or made up of a single seamless, continuous cell (reticular theory) [7]. Nowadays, this simple experiment, cell culture, is becoming a more and more important tool not only for radiation biology but also for other wide varieties of life sciences (Fig. 2). In this way, establishment of the cell culture technique has made possible to study the radiation effects using cultured human cells.
On the Dynamical Approach of Quantitative Radiation Biology

Fig. 2. Two examples of the cell culture system. Left: Flasks containing some growth medium for cell growth. Right: Petri dish containing growth medium, called agar plate. It contains cultured microorganisms. Taken from Wikimedia Commons [8, 9] and converted to grayscale

It was found not to be a true. Then, statistical scientific data taken from the investigation of A-bomb survivors of Hiroshima and Nagasaki and nuclear accident in the United States have revealed a scientific fact that the $D_0$ of unicellular organisms (over 100 Gy) and human beings (1–4 Gy) is quite different. Furthermore, a big difference on lethal dose of human individuals (1–4 Gy) and its constituent cells (over 100 Gy!) was recognized. At that time, this big difference on lethal dose was one of the unresolved questions in radiation biology.

Here, another important aspect of the radiation is discussed. It is important to emphasize that the total absorbed energy by a human body estimated from the lethal radiation dose for human (4 Gy) seems to be extremely small compared to the other harmful sources.

| Species                        | $D_0$ (Gy) |
|-------------------------------|------------|
| *E. coli*                     | 40         |
| T2 bacteriophage              | 400        |
| Newcastle’s disease virus     | 400        |
| Yeast                         | 50–180     |
| *Chilomonas paramecium*       | 150        |
| HeLa cell                     | 1          |

Table 1. Roughly estimated value of $D_0$ for various biological species. After [5]
For a person who has a mass of 60 kg, the total absorbed energy from 4 Gy X-ray is calculated as follows:

\[ 4\text{Gy} \times 60\text{kg} = 240\text{J}, \]

where 240 J = 57 cal. Energy of 1 J equals to the work done by a constant force of 1 N and moves a 1 m displacement. Therefore, 240 J equals to the work that lifts a 60 kg mass to a height of about 40 cm (Fig. 3). Thus, the energy level of lethal radiation dose seems sufficiently small compared to the other harmful sources. Naturally, this scientific fact suggests that there is a small target which controls cell life and death inside the living organisms and viruses, i.e., DNA.

![Figure 3. Lethal radiation dose explanation. Total energy imparted to the 60 kg mass by a 4 Gy radiation, which is the mean lethal dose \( D_0 \) for human, is roughly equivalent to lifting 60 kg mass to a 40 cm height](image)

Now, return the discussion to the problem of the difference in radiosensitivity between human individuals (1–4 Gy) and its constituent cells (over 100 Gy). This problem has been clarified due to the successful development of new experimental technique. More precisely, for the sake of clonogenic assay method developed by Puck and Marcus [5], quantitative study of cellular response of mammalian cells to an external stimulus is established. Thus, the first survival curve, which will be described later, for X-ray irradiated mammalian (HeLa) cells \textit{in vitro} was obtained by the method. The cell culture technique made the observation of proliferation of cells isolated from tissues easier; therefore, cell surviving fraction based on the ability of a single cell to grow into a large colony was successfully conceived (Fig. 4).

The most important concept in the development of clonogenic assay is the definition of cell death. In other words, the cell survival is defined as whether the cell has a proliferation ability or not. In this situation, "dead cell" means the cell which loses its reproductive integrity, and this kind of cell death is called "reproductive cell death." The cells which cause reproductive cell death may still be present physically and morphologically intact, may even be able to make proteins or synthesize DNA, and may even be able to progress a few cell cycles and a few divisions may still occur. Generally, a dose of over 100 Gy is required to destroy basic cell function, in contrast to the 1–4 Gy for causing reproductive cell death. Quantifying the pure cell survival number by employing single-cell-based culturing technique has enabled to
distinguish the reproductive cell death from the cell death in the narrow sense. Thus, clonogenic assay method may clarify the problem of the difference in radiosensitivity between human individuals and its constituent cells. Human individuals and its constituent cells may have almost the same value of mean lethal dose. Establishment of their work is often considered to mark the beginning of quantitative cellular radiation biology [10].

Generally, cell survival curves are used for the quantitative representation of biological cellular responses. Cell survival curves, or in short, survival curves, are defined by the proportion of surviving cells \( S \) as a function of radiation dose \( D \), as in Fig. 5, and known to be different, depending on corresponding biological systems (mammalian cells, virus, yeast, bacteria, etc.), both in terms of shape and absolute value of \( S \) at a given dose. In this sense, "slope" of each survival curve implies degree of radiation sensitivity of corresponding biological systems. Interestingly, its slope is known to vary with DNA content [11].

Until now, many experiments to measure radiation survival curves have been performed for various species, including human cells. As a consequence of these experiments, many new findings have been revealed. For example, radiosensitivities for the corresponding species A, mammalian cells; B, \( E. coli \); C, \( E. coli \) B/r; D, yeast; E, phage staph E; F, \( Bacillus megaterium \); G, potato virus; and H, \( M. radiodurans \), are comparable in the slope of the cell survival curves, and they have the relation in radiosensitivity:

\[
A > B > C > D > E > F > G > H.
\] (2)

Thus, the mammalian cells are most radiosensitive than other species. The differences of the radiosensitivity of various species are said to be correlated with its DNA content and efficiency of the DNA repair system [12].
Generally, differences on the biological or experimental conditions make the radiosensitivity change, even among the same type of cells. A lot of experiments have been performed to study various factors that affect the radiosensitivity until now; some of the major factors are described in the following. Here, various factors affecting the shape of survival curves (radiosensitivity) are summarized.

The radiosensitivity is known to vary with various conditions, for example:

1. species [11]
2. type of radiation [13]
3. dose rate [14, 15]
4. oxygen concentration [16]
5. extent of apoptosis [12]
6. AT cell lines [17]
7. type of mutation [18]
8. cell cycle (cell time) [19, 20]

An affecting factor regarding the difference of species can be interpreted as the dependence of DNA content, and that of the radiation type and oxygen concentration can be interpreted as the dependence of the linear energy transfer (LET) values, i.e., average energy deposition per unit length. Here, the definition of the LET is described later. Clearly, these factors (2–4) are the external factors not relating to the biological activities inside the cell, and the following factors (5–8) seem to be the intracellular factors depending on the biological activities.
The extent of apoptosis seems to have been associated with the abrogation of p53 function. It is well known that the gene p53 mediates cellular responses to DNA damage, and results show that radiosensitivity is inversely correlated with the amount of DNA double-strand breaks (DSBs). In this case, the amount of DSBs should be the important feature to elucidate the affecting factor on cellular radiosensitivity. Moreover, cell lines derived from subjects with ataxia telangiectasia (AT) are three or four times sensitive to radiation than the normal cell lines, and no or very small shoulders appeared in survival curves for AT cell lines [17]. The genetic disorder responsible for the AT subject is the mutation of ATM gene, which is involved in the cellular response to DNA damage, especially in the DSB damage. Results of the E. coli mutant experiment clearly show that the rec- mutants have a higher radiosensitivity than that of rec+. The rec gene is involved in genetic recombination and DNA repair. These experimental facts also imply that the ability or capacity of DNA repair in specific cellular system plays an important role in radiosensitivity of cell.

The most complicated factor is the cell cycle. Generally, radiosensitivity is cyclically changed during cell cycle (Fig. 6), depending on the position of cell-cycle stages. To put it more precisely, radiation sensitivity is minimal when cells are irradiated in the early postmitotic (G1) and the premitotic (G2) phases of the cell-cycle and maximal in the mitotic (M) phase and late G1 or early synthesis (S) phases [19]. However, the pattern of the response cycle may vary depending on cell lines, especially on the length of G1 period [20]; typically, it is known to indicate a bimodal pattern. Generally, the origin of cell-cycle-dependent radiation sensitivity is supposed to be a consequence of some intracellular dynamics, e.g., the regulation mechanism of cell-cycle checkpoint, repair ability of DNA damage, and higher-order structure of chromosomes; no explicit theoretical explanation exists presently for this cyclic response.

![Figure 6](image-url)

*Figure 6.* Plot of the surviving fraction of synchronized HeLa cells irradiated by X-ray. The horizontal axis "cell time" shows irradiated time of cell cycle. Time zero represents mitosis (M in figure), and G1, S, and G2 represent corresponding cell-cycle stages. Adapted from [19]
Finally, the affecting factors will be summarized using quantitative indices (Table 2). It should be noted that the term "repair ability" in Table 2 seems not to be a quantitative index, but rather a qualitative index, it is thought that it can be measured, although not directly, but through other quantities, e.g., the DSB yield. Mathematical methods to analyze these experimental data and many attempts to understand the biological mechanisms involved in the variation of radiosensitivity under a variety of conditions are introduced in the next section.

| Factor                | Radiosensitivity |
|-----------------------|------------------|
| DNA content           | ↗                |
| LET                   | ↗                |
| Dose rate             | ↗                |
| Repair ability        | ↘                |
| Cell cycle (cell time)| ↘↗↘↗             |

Table 2. Summary of the factors that affect the radiosensitivity. Arrows inside the table show variation of the radiosensitivity. Radiosensitivity dependence has a linear relation with respect to the corresponding affecting quantities, except for the cell-cycle dependence, which has a cyclic relation

3. Mathematical representations

Many efforts have been done to understand a priori the actions of radiations on the living organisms from the early 1920s, and most of these types of studies have been done by physicists. The logical structure of this theory is basically based on the simple idea, that is, studying the behavior of the living organisms as the "response" to the radiation as the "action"; actually, it is a so-called dose-response relationship in today's radiation biology. Most of experiments in such studies have mainly used mortality or survival as the corresponding biological "effects." The surviving fraction of the various living organisms, including bacteria, virus, and drosophila egg, have been measured by irradiation experiments. It should be noted that the inactivation is used for the survival endpoint of virus or drosophila egg.

In a radiation biology, a plot of the surviving fraction of cells vs radiation dose is called survival curve. Generally, radiation dose is plotted along the horizontal axis (logarithm in some cases), and surviving fraction is plotted along the vertical axis. This kind of dose-response relationship obtained by measuring cell surviving fraction is a momentous indicator of the radiosensitivity of corresponding living organisms, up to now.

As functional forms of survival curves, two general types of functions are well known to represent various survival curves, exponential and sigmoid (Fig. 7). However, as described later, there is no theory that can clearly explain the difference of the functional form of these survival curves from biological mechanisms.

In such a situation, the most famous mathematical model to derive the function that has a good fit with the experimentally obtained survival data is the target theory that has been summar-
Figure 7. Two examples of survival curves. "E" means exponential and "S" means sigmoid

ized by Lea [21]. Target theory assumes that the "hit" of the discrete radiation to the radiation-sensitive site called "target" may induce cell death in a broad sense; moreover, the radiation hit is assumed a random process which has a Poisson probability distribution. (Fig. 8).

Figure 8. Schematic explanation of the target theory. Target theory assumes that each cell has some targets and radiation hits of all target may induce cell death. Left: Single radiation hit to the single target kills cell. Right: Single radiation hit to all of three targets kills cell

Let \( P(n) \) be the probability of \( n \) hits with expected value of hits \( m \); then

\[
P(n) = e^{-m}m^n/n!
\] (3)

Let \( V \) be a target volume, and suppose that \( p \) denotes hit number per unit volume; then the expected value of hit number is \( m = VP \). Then, the hit number \( p \) may be proportional to the radiation dose \( D \); thus, \( p = \alpha D \), where \( \alpha \) is a proportional coefficient. Therefore, the expected hit number is written as

\[
m = V\alpha D
\] (4)
There are several model types in target theory corresponding to the combination of required target and hit number for cell death; for simplicity, single-hit-single-target model and single-hit-multi-target model are described.

At first, suppose each cell contains only one single target and single radiation hit kills (inactivates) the cell, then the cell survival probability $S$, i.e., non-hit ($n = 0$) probability, is

$$S = P(0) = e^{-m} = e^{-V\alpha D}$$

Let $D_0$ be a radiation dose that has an expected hit number $m = V\alpha D_0 = 1$; thus,

$$V\alpha = 1/D_0$$

therefore,

$$S = e^{-D/D_0}$$

This survival curve equation (7) has a good fit with exponential-shaped curve (denoted as "E") in Fig. 7. Consequently, the probability of cell death for single-target cell is

$$1 - S = 1 - e^{-D/D_0}.$$ (8)

Next, suppose each cell contains two targets, and single radiation hit of both targets kills (inactivates) the cell. Moreover, suppose these two hit events are stochastically independent, then the cell death probability of two targets case $D_2$ equals the product of cell death probability of single target case, equation (8), therefore

$$D_2 = (1 - S)(1 - S) = \left(1 - e^{-D/D_0}\right)^2$$

Thus, the cell survival probability of two targets case, $S_2$, is

$$S_2 = 1 - \left(1 - e^{-D/D_0}\right)^2.$$
consequently, cell survival probability of $k$ targets case, $S_k$, is

$$S_k = 1 - \left(1 - e^{-D/D_0}\right)^k$$  \hspace{1cm} (11)$$

Alternatively, this survival curve, equation (11), has a good fit with sigmoid-shaped survival curve (denoted as “S”) in Fig. 7.

Studying the validity of target theory, various inactivation experiments of viruses by radiation have been done and examined [21]. Various measurement results have shown that there is a strong correlation between virus size (diameter) and inactivation dose (of X- or γ-rays); moreover, in some cases, target volume $V$ equals the size of the virus itself. It should be noted that the word "target" used in the theory does not give a specific object; it is just a concept. Today, many experimental evidences suggest the DNA as the primary target for radiation.

Although the target theory can derive the functions to fit well with the survival curves of various experimental data, it is known that target theory gives higher surviving probability in very low-dose region than the real experimental data [22]. That is to say, actually, many cell deaths have occurred than expected from target theory in the very low-dose region. Moreover, it has been found that survival function obtained by target theory do not fit well with the experimental data not only in the low-dose region but in low-dose-rate irradiation.

In target theory, $D_0$ is generally called "mean lethal dose"; in a sense, one hit will kill the living organisms; however, it is just a parameter that can be estimated from fitting of experimental data to the target theory. On the contrary, by tuning this $D_0$ parameter, target theory can derive any functions to fit well with almost all experimental data. It is clear that this kind of high possibility of application to fit experimental data represents the arbitrariness of the model; thus, the model is not supposed to capture the essential dynamics of phenomenon.

Here, another mathematical model which will explain the shape of cell survival curves is introduced. The so-called linear-quadratic model (L-Q model) has been proposed for the candidate expression of the cell survival curves,

$$S = e^{-\alpha D - \beta D^2}$$  \hspace{1cm} (12)$$

where $S$ is the surviving fraction for radiation dose $D$ and $\alpha$ and $\beta$ are proportionality constants. This kind of mathematical expression of the dose-response relationship can be found in many articles, e.g., [23, 24, 25, 26, 27].

L-Q model is often used to explain dose-response relationship of unstable chromosomal aberrations, e.g., dicentrics and rings ((b) and (d) in Fig. 9), which is thought to be the major cause of cell death. Rings and dicentrics can be seen and counted under the light microscope, and about 3 years of the half-life of lymphocytes carrying these aberrations have made it possible to estimate the accumulated radiation dose during a long period. Generally, this
What is the phenomenological meaning of this function? It has been shown that the frequency of the chromosomal aberrations caused only by one break or damage has a linear dose dependence, $\alpha D$; however, that of induced by two chromosome breaks has a quadratic dose dependence, $\beta D^2$ (Fig. 10). In other words, frequency of the chromosome aberrations made from one break (one hit event) has a linear dose-response relationship, and then, frequency of the two-break (requires two hit)-induced aberrations has a quadratic dose-response relationship. Consequently, frequency of the chromosome aberrations per cell $X$ may have the dose-response relationship

$$X = \alpha' D + \beta' D^2$$

where $D$ is the radiation dose and $\alpha'$ and $\beta'$ being parameters. Moreover, it has been found that the relationship between chromosome aberrations per cell $X$ and surviving fraction $S$ has a relation [30]:

$$S = e^{-\gamma X}$$
therefore,

\[ S = e^{-\gamma \alpha' D} e^{-\gamma \beta' D^2} = e^{-\alpha D} e^{-\beta D^2} \]  \hspace{1cm} \text{(15)}

where \( \alpha = \gamma \alpha' \), \( \beta = \gamma \beta ' \).

**Figure 10.** Dose effect curves for the different types of chromosome aberrations (gap and exchange) induced by X-ray in the *Vicia faba*. Clearly, gap shows a linear dose dependence while the exchange shows quadratic. Adapted from[29]

As stated above, the dose-response relationship between chromosome aberrations and radiation dose can be well explained from the relationship between the number of chromosome breaks and radiation dose using L-Q model. However, it may be said that L-Q model is rather descriptive than the dynamical.

Finally, one more interesting experimental data corresponding to the relation between radiation quality and dose is shown in Fig. 11. Here, each type of radiation has a different radiation quantity, linear energy transfer (LET) which is defined as the linear density of imparted energy. Therefore, LET is written mathematically as

\[ \text{LET} = \frac{dE}{dl} \]  \hspace{1cm} \text{(16)}

where \( dE \) is the averaged energy deposition to the medium by charged particles in traversing a total length \( dl \). In short, it is a quantity that represents averaged energy deposition around the tracks of charged particles (Fig. 12). Some examples of the LET values for different types of radiation are shown in Table 3. Moreover, Fig. 11 clearly shows that quality of the radiation determines the number of aberrations. It should be noted that the LET is not an actual, experimentally obtained value but just a practical, stochastically estimated value.
The effects of ionizing radiation on the living organisms have been studied in many years to understand three principal physical aspects: 1) quantity of radiation, 2) temporal distribution of radiation, and 3) radiation quality [32]. Studies on the dose-response relationship including cell survival curves are considered to belong to the research field of quantity of radiation, and studies on the dose rate and fractionation [33] are considered to belong to the research field of temporal distribution.

Historically, the concept of radiation quality has been established to study the biological effectiveness of ionizing radiation depending not only on the amount of absorbed radiation dose but also on the spatial distribution of energy deposition [34]. It is thought that the beginning of such study may have been closely related to the discovery of scientific evidence; i.e., DNA is the principal target for the biological radiation responses. For the sake of the presence of a small target in the cell, scientific knowledge of ionizing radiation, not only the macroscopic property (amount of absorbed radiation dose) but also detailed track structure (spatial distribution of energy deposition), has been required to study biological radiation responses.

As mentioned above, requirement of the detailed information concerning radiation quality leads to plenty of studies that correspond to radiation track structure [35]; moreover, many studies on the dynamical approach of quantitative radiation biology have been performed. For example, Figure 11 shows dose-response curves for dicentric chromosome aberration induction in human lymphocytes with various LET radiations. Adapted from [31].

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| Radiation          | LET keV/μm |
|--------------------|------------|
| Cobalt-60 γ-rays   | 0.2        |
| -kV X-rays         | 2.0        |
| -MeV protons       | 4.7        |
| -MeV protons       | 0.5        |
| 5 MeV α-particles  | 166        |
| -GeV Fe ions       | 1000       |

Table 3. Typical values of LET. Values taken from [12]
In other words, a detailed information on how much damage on the DNA are produced by the different types of radiations with the same amount of dose has been necessary to estimate the radiation effects. Estimation or calculation of LET is one of such studies.

As mentioned above, requirement of the detailed information concerning radiation quality leads to plenty of studies that correspond to radiation track structure [35]; moreover, many studies regarding radiation action that has a more broad timescale including very early physical ($10^{-18}$-$10^{-12}$ sec) and chemical ($10^{-12}$-$10^{-9}$ sec) process have been performed [36].

![Figure 12. Schematic explanation of the variation of the ionization density. LET is increased in the order from A to C (top to bottom). Black circles show positions of ionization.](image)

**4. Analyzing the various survival curves based on the knowledge of modern molecular biology**

Survival curves of cells obtained under various conditions and mathematical expressions describing the survival curves have been introduced until now. It seems that the radiosensitivity and most of affecting factors have a simple proportional relationship; however, the interpretation of the cell-cycle-dependent nature of the radiosensitivity seems to be the most difficult. Can such a radiosensitivity really be explained by the mathematical model mentioned above? Here, the cell-cycle-dependent cellular response has been briefly discussed from the viewpoint of mathematical model and expected intracellular affecting factors.

At first, factors that can affect the shape of cell survival curve are discussed from the target theory, equations (7) and (11). Clearly, these equations show that the radiation dose $D$, required target number to cell death $k$, and target volume $V$ are all the affecting factors; moreover, radiosensitivity is inversely proportional to a required target number $k$ and in proportion to the target volume $V$ and radiation dose $D$. In order to determine a target volume and number, the information concerning the substance of the target should be needed. What is the actual target of radiation? Generally, it is believed that DNA is the principal target of radiation.

In case of cell-cycle-dependent radiosensitivity, the radiation dose $D$ is not the control parameter, and the required target number $k$ for cell death (reproductive cell death) seems to
be unchanged, because one chromosome aberration may induce reproductive cell death. Thus, the required target number to cell death $k$ and target volume $V$ are the factors that need to be considered now. The volume of the target seems to have almost the same value from the M-phase of the cell cycle to the early S-phase; occupying the volume of all chromosomes for the most condensed stage (M-phase) is $\sim 91.6 \mu m^3$ [37], whereas that of the interphase is estimated as the volume of cell nucleus; in case of $5.4 \mu m$ diameter, interphase volume is almost the same as that of metaphase. On the contrary, target volume (DNA content) is doubled at the late S phase. By the interpretation from a target theory, increase in target volume causes increase in cell death. From Fig. 6, cell death seems to decrease during S-phase; this is not consistent with the analysis from the target theory.

As seen in the above discussion, time variation in the target number and volume is not recognized to have a bimodal change in the cell cycle. In other words, it is difficult to discuss a cell-cycle-dependent cyclic change of radiosensitivity by using a target theory. Then, does any kind of intracellular biochemical molecule which is involved in the DNA repair vary periodically during cell cycle?

There are not so many quantitative time-series data about the intracellular density change of proteins involved in DNA repair; however, the experimental data for cyclin B1 [38] shows that the variations in radiosensitivity (several tens fold in range) during the cell cycle cannot be satisfactorily explained by the temporal variation in cellular molecules; the functional form is completely different from a radiosensitivity curve during cell cycle (Fig. 6). Then, what kind of dynamics will be related with this characteristic bimodal periodic variation? One of the possible reasons for the radiosensitivity variation during cell cycle is that some kind of biological changes as a consequence of the structural change of the chromosome may affect the radiosensitivity variation.

Chromosomes are known to change their structure and volume corresponding to cell cycle stages. Generally, higher-order chromosome structure is important for many intracellular dynamics including DNA repair [39, 40]. Regarding spatial structure, a part of the aspects are not yet understood, but it is thought to have roughly four hierarchical levels, i.e., nucleosome, 30 nm chromatin fiber, lambrush structure, and chromosomal structure. These structures are also known to exhibit cell-cycle-dependent structural changes. The shortest human autosome, chromosome 21, contains $48 \times 10^6$ base pairs (bps) of DNA, and its extended length is about $16 \mu m$; however, its length is about $1 \mu m$ during the metaphase, the most condensed state. Comparing the length of metaphase chromosomes to linear DNA, the packing ratio of DNA in metaphase chromosome is about 10,000.

As the first level of higher order compaction, 147 bps of DNA wraps 1.67 times around the histone octamer and makes unique unit structure called nucleosome core particle, and these units are connected by small amount of DNA, linker DNA. This basic repeating structure consists of nucleosome core particle and linker DNA is called "nucleosome," and it looks like a "beads-on-a-string" structure that corresponds to the state of interphase chromosome and the most basic unit structure of DNA packaging. Moreover, nucleosome is coiled into a 30 nm diameter helical structure known as the 30 nm chromatin fiber; however, their detailed packing manner is still unknown, and this is one of the expected basic structures in interphase. The
most condensed metaphase chromosome is expected to have further two-step compaction from this 30 nm chromatin fiber, i.e., 300 nm "loop-like" (lampbrush) structure and 700 nm coiled structure (chromatid). Interphase chromosomes are known to occupy cell nucleus in spatially organized manner, i.e., chromosome territories (CTs), and on the other hand, their internal organized structures are poorly understood [41]. CTs have irregular shapes and occupy discrete compartments with little overlap; so interphase chromosomes are clearly separated inside a cell nucleus, respectively. Through the process of condensation, chromatin fiber increases its number of linkages and makes a condensed state, i.e., M-phase chromosome [42]. This process is very similar to the kind of phase transition from liquid to solid, freezing.

In addition to its unique structural properties, chromosome is known to have extraordinary physical properties. Observations of stretching in the region of DSB using partially broken X-irradiated chromosomes led to the proposal of a governing equation for chromosome movement by Nicklas [43] as follows:

\[
F = Kl + \Gamma \frac{dl}{dt}
\]

(17)

where \(F\) is the force; \(K\), the elastic proportionality constant between the force and stretch \(l\); and \(\Gamma\), the proportionality constant between force and velocity. Figure 13 is a schematic description of the structural changes of chromosome during cell cycle. From this figure, structural parameters of chromosome, \(L\) and \(R\) are found to change periodically. Similarly, elastic coefficient of chromosome \(K\) in eq. (17) is also expected to change during cell cycle.

\[\begin{array}{c|c|c|c|c|c|c}
\text{M} & \text{G1} & \text{S} & \text{G2} & \text{NEB} \\
\hline
L & L_M & L_I & L_{30nm} & 2L_I & L_M \\
R & R_M & R_I & R_{15nm} & R_I & 2R_M \\
\end{array}\]

Figure 13. Schematic explanation of the structural change of chromosome during cell cycle. M, G1, S, G2, and NEB show phases of cell cycle. "NEB" is an abbreviation for "nuclear envelope breakdown." L and R are the "length" and "radius (thickness)" of chromosome, respectively. The subscripts M and I for the L and R show length and radius for mitosis and interphase, respectively, and \(L_{30nm}\) shows a length of 30 nm chromatin fiber and \(R_{15nm}\) shows that of radius.

In the recent years, many simulations using molecular dynamics (MD) on the DNA damage sites induced by ionizing radiation have been performed to study the detailed mechanics of the process of DNA damage and repair. These MD simulations have found some detailed states...
of the damage sites, though it is a limited condition, and one of the impressive findings is the very fast separating movement of the atoms that constitute damaged sites [44, 45]. Generally, the structure of the DNA damage sites is expected to be important for the repair probability which determines its ability. Thus, the dynamical aspects of the damaged sites accompanied by the structural transition of chromosome during cell cycle may affect the cell survival through a process of repair.

5. Conclusion

Some quantitative approaches in radiation biology based on the clonogenic method and obtained cell survival curves as a dose-response relationship are introduced. Cell survival curves seem to have a universality of its function, i.e., functional form of survival curves seems to be unchanged under various conditions including different species. Generally, the functional form of cell survival curve, especially by its slope, represents a quantity of radiosensitivity. Various factors affecting the radiosensitivity have been investigated to find macroscopic nature of living organisms, not to divide the system but to investigate the whole of it. Many mathematical models that describe cell survival curves have been presented; however, functional form of cell survival curves derived from, based on biological mechanism, does not yet exist. Mathematical or theoretical derivation of the functional form of cell survival may lead to understand the general theory of cellular responses to ionizing radiation, especially for the low-dose region.

Finally, the possibility that the structural change of chromosome affects the repair process is discussed. The survival curves that have a universality with respect to the dose-response relationship of cell should be studied further, to derive more analytically with the biological mechanism, also in order to progress the research on the secret of life.

Author details

Noriyuki B. Ouchi

Address all correspondence to: ouchi.noriyuki@jaea.go.jp

Research Group for Radiation and Biomolecular Science, Radiation Biology Research Division, Quantum Beam Science Center, JAEA, Japan

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