

Report

## Cytogenetic Biodosimetry in Radiation Emergency Medicine: 3. The Basics of Chromosomes for Biodosimetry

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Chromosomes are observed during the mitotic phase of the cell-cycle. As organisms have a species-specific chromosome number and morphology, any changes in the number or morphology can be considered as chromosome aberrations. It is well known that radiation exposure causes chromosome aberrations, and multiple studies have reported on the mechanisms of radiation-induced chromosome aberrations and the effects of radiation dose and quality on chromosome aberrations. Radiation-induced chromosome aberrations can be divided into 2 categories: stable chromosome aberrations which are inherited by daughter cells in cell division (e.g. translocations, inversions, partial deletions and duplications) and unstable chromosome aberrations which cause cell death and eventually disappear (e.g. dicentrics, rings and acentric fragments). In acute external exposure, unstable chromosome aberrations are usually used as indicators for accurate dose assessment to facilitate radiation emergency medical care. On the other hand, stable aberrations are generally used for retrospective dosimetry caused by past exposures. This article summarizes the essential information on chromosomes and chromosome aberrations for cytogenetic biodosimetry.

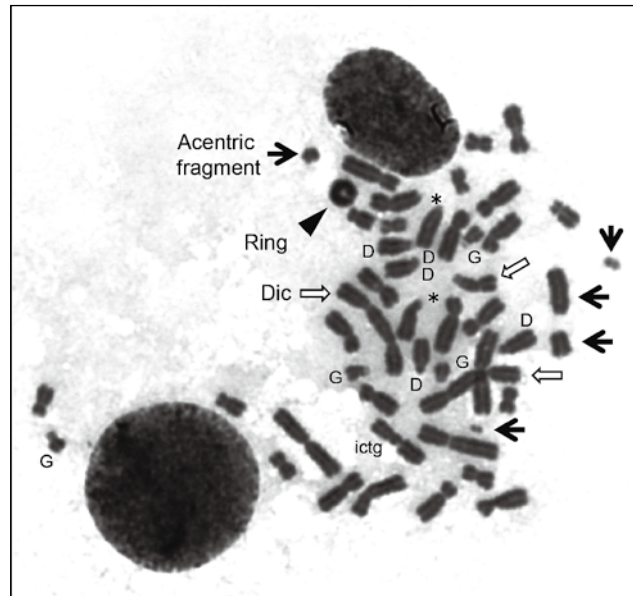
**Key words:** cytogenetic biodosimetry, the morphology of chromosome, chromosome aberration, cell-cycle

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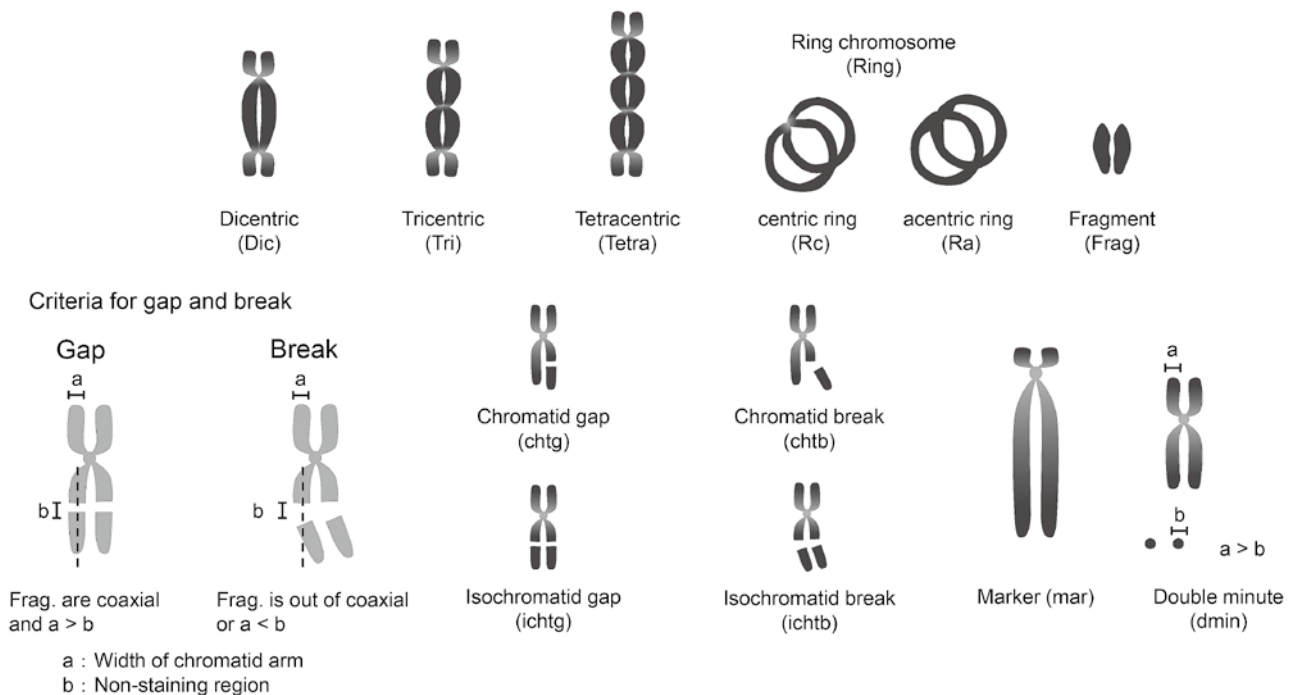
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**Fig. 1.** Representative image of a metaphase spread with diverse radiation-induced chromosome aberrations. Solid arrow: acentric fragment, white arrow: dicentric chromosome, black triangle: ring chromosome, D: Chromosome belonging to group D, G: Chromosome belonging to group G, \*: Marker chromosome (larger chromosome than normal group D chromosome caused by translocation), ictg: Isochromatid gap.



**Fig. 2.** Examples of chromosome aberrations.

## 1. Introduction

Depending on the type of radiation exposure, the optimal cytogenetic assay is selected for radiation dose assessment (biodosimetry). Such assays include the dicentric chromosome (Dic) assay<sup>1)</sup> (DCA), cytokinesis-block micronucleus (CBMN) assay<sup>1-4)</sup>, premature

chromosome condensation (PCC) assay<sup>1, 5-9)</sup> and fluorescence *in situ* hybridization based translocation assay<sup>1, 10)</sup>. These assays depend on chromosome aberrations formed during the cell division. In Dic and translocation assays, aberrations are evaluated on chromosomes observed during the mitotic (M) phase of the cell-cycle.

As organisms have a species-specific chromosome

number and morphology, any changes in the number or morphology can be considered as chromosome aberrations and may also induce genetic changes. Radiation is known to cause chromosome aberrations, and multiple studies have reported on the mechanisms of radiation-induced chromosome aberrations and the effects

of radiation dose and quality on chromosome aberrations. As seen in Figure 1 and 2, chromosome aberrations induced can be diverse.

Radiation-induced chromosome aberrations and DNA damage can be caused directly by radiation or indirectly by reactive oxygen species such as hydroxyl radicals ( $\cdot\text{OH}$ ) generated in water molecules by radiation (Fig. 3). During DNA repair of a radiation-induced double-strand break, the broken DNA strand is expected to be correctly repaired with the original DNA strand serving as a template. However, if the double-stranded DNA is damaged more than once and is accidentally bound to different DNA strands, such DNA rejoining can lead to various chromosome aberrations such as a Dic with two centromeres on one chromosome, a ring chromosome with one chromosome broken end joined together to form a doughnut-shaped circular chromosome and translocation, in which parts from the same or different chromosomes are interchanged<sup>1)</sup> (Fig. 4).

Cytogenetics is a field of study that integrates the methods and findings of cytology and genetics, focusing on chromosomes and their behavior in the cell. To date, cytogeneticists have revealed that the frequency of chromosome aberrations correlates with radiation quality, dose and dose-rate<sup>11-15)</sup>. Therefore, exposure dose can be estimated from dose-response curves established in previous studies.

In cytogenetic biodosimetry, Dic and ring chromosomes

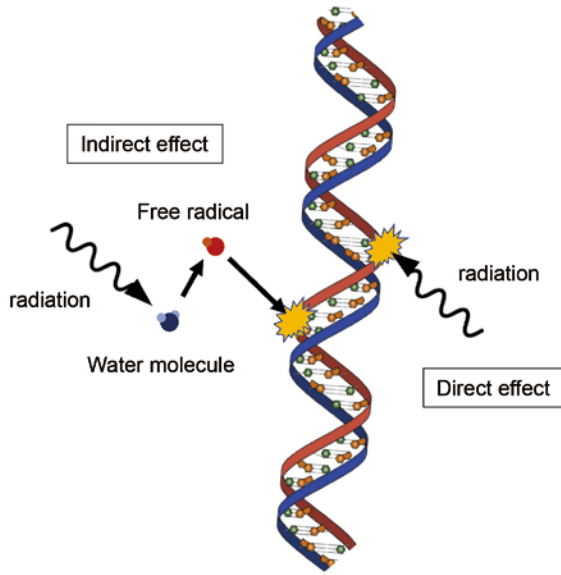


Fig. 3. Direct and indirect effects of radiation on DNA.

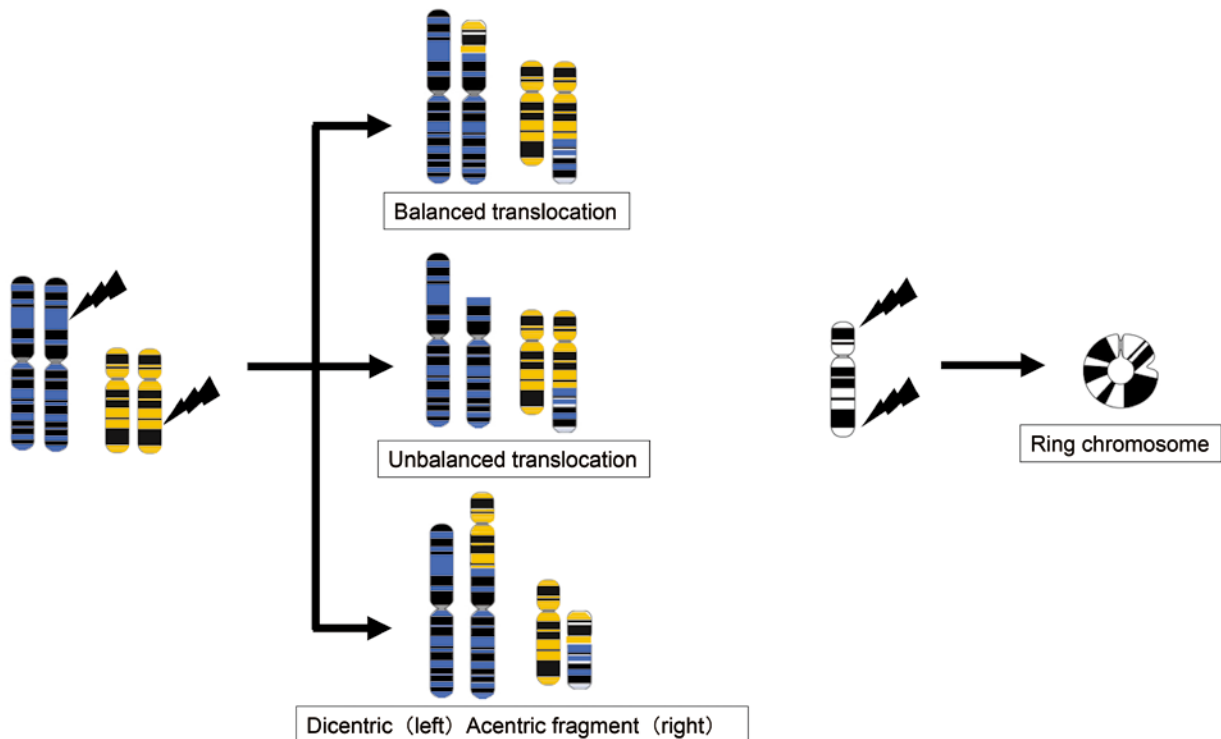
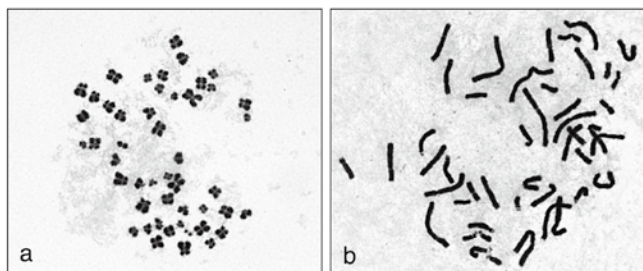
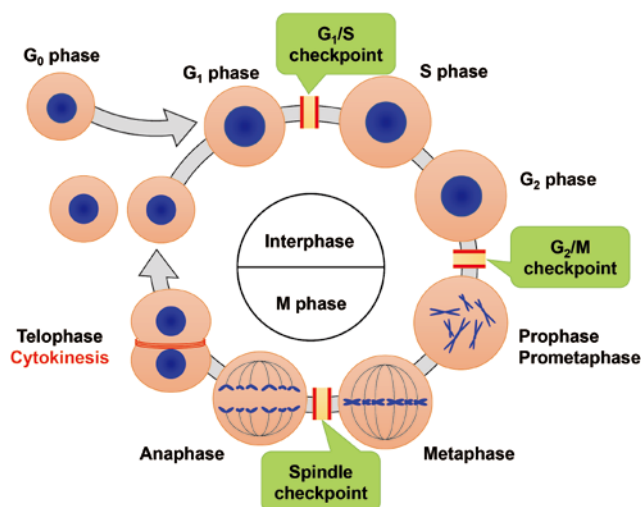


Fig. 4. Various radiation-induced chromosome aberrations formed after DNA repair and chromosome rejoining at different sites.



**Fig. 5.** Different levels of chromosome condensation affected by humidity and temperature during slide preparation. (a) Overly condensed and (b) elongated chromosomes.



**Fig. 6.** Phases of the cell-cycle.

are commonly used as endpoints because they can be easily distinguished from other normal chromosomes based on their morphological features. However, even for trained and highly skilled scorers, Dic and ring identification can be quite challenging as the detection is generally based on morphological analysis of chromosome images using a single-color stain with Giemsa. In addition, other factors such as colcemid treatment for metaphase arrest during cell culture<sup>16, 17</sup>, hypotonic treatment<sup>18, 19</sup> during cell harvest and the temperature and humidity during slide preparation<sup>20</sup> also affect the optimal chromosome morphology for easy aberration scoring (Fig. 5). Thus, scorers must have considerable experience in preparing chromosome specimens and identifying chromosome morphology with metaphase images or under a microscope. Moreover, several guidelines have been published to facilitate Dic identification<sup>21, 22</sup> and prepare appropriate chromosome specimens<sup>23, 24</sup>. These guidelines can greatly facilitate the observation of chromosomes in metaphase spreads by helping scientists with limited experience to understand

chromosome morphology and the types of chromosome aberrations.

In this article, we review the fundamentals of chromosomes and chromosome aberrations as the understanding of chromosomes is required when performing cytogenetic biodosimetry.

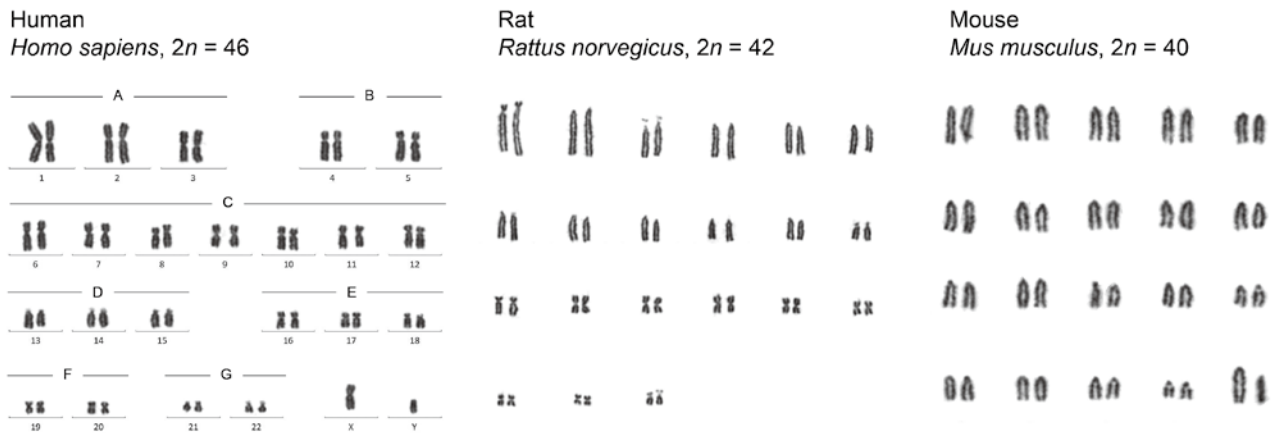
## 2. Chromosomes in the cell-cycle

Figure 6 depicts the different cell-cycle phases. Many cell types are usually in  $G_0$  phase, which is a resting state as no cell division is taking place. When a cell divides and proliferates under the appropriate stimulation, the cycle from the initial cell division to the next division is known as the cell-cycle. In the cell-cycle, the period during which chromosomes are observed is called M phase, and the period between mitosis and the next mitosis is called interphase. Cells then enter the cell-cycle as needed and will divide and proliferate through interphase and mitosis.

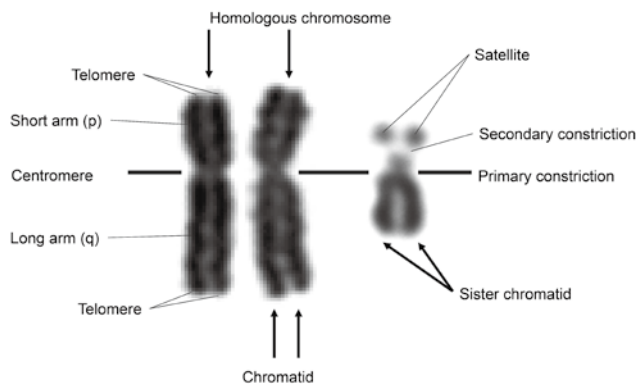
The interphase of the cell-cycle is composed of  $G_1$ , S, and  $G_2$  phases, in which DNA is replicated for cell division in S phase. Chromatin is found in the cell nucleus during interphase, which then condenses into chromosomes that can be easily observed with a light microscope during M phase. The M phase in human lymphocytes occupies a short period, about 30 minutes to an hour within the entire cell-cycle of about 24 hours<sup>25</sup>.

The M phase can then be subdivided into prophase, prometaphase, metaphase, anaphase and telophase. Chromosome morphology and its arrangement in the nucleus differ in each phase. During prophase to prometaphase, the nuclear envelope breaks down and a chromosome composing of two sister chromatids emerges. In addition, spindle microtubules from each centriole that have moved to each cell pole of the cell extend into the center of the cell and attach to the centromeres at the kinetochore (spindle assembly). During metaphase, the spindle-associated chromosomes align along the equator planes (metaphase plate) with equal tension from both poles. In anaphase, each pair of sister chromatids separates and begins to move towards opposite poles of the cell. From late anaphase to telophase, the separated daughter chromosomes migrate to the pole of the cell, during which two daughter cells are formed by cytokinesis, a process in which the cytoplasm is divided into two parts. Finally, in telophase, the nuclear envelope reforms and the condensed daughter chromosomes then decondense and return to the interphase state.

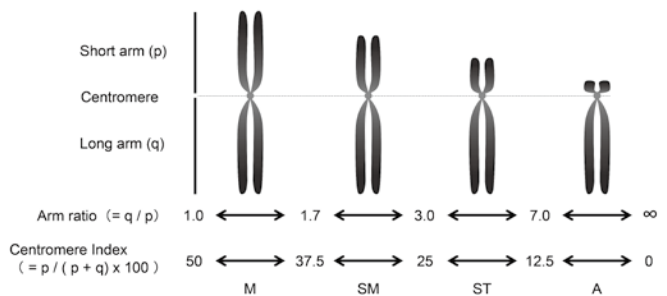
In order to transmit accurate genetic information to daughter cells after cell division, cells have cell-cycle checkpoints where various cell conditions are assessed<sup>26, 27</sup>. If any defects are found, the cell-cycle is arrested until the damage is repaired. The  $G_1/S$  checkpoint is necessary to prevent replication of damaged DNA. The  $G_2/M$



**Fig. 7.** Human, rat and mouse chromosome karyotypes.



**Fig. 8.** Structural characteristics of a chromosome.



**Fig. 9.** Classification of chromosomes based on centromere position. M: metacentric, SM: submetacentric, ST: Subtelocentric, and A: acrocentric chromosomes.

checkpoint ensures that DNA replication is complete and prevents damaged DNA from entering mitosis. In the spindle checkpoint, all chromosomes must attach with microtubules and align on the middle of the cell in metaphase in order to move on to anaphase (Fig. 6).

### 3. Structural characteristics of the chromosome

Chromosomes were initially discovered as filamentous structures that appear during cell division and were thought to be just one of the many structures in the cell. However, it took additional years of research to realize that chromosomes also serve as genetic materials. W. S. Sutton<sup>28)</sup> and T. H. Boveri<sup>29)</sup> proposed for the first time in 1902 and 1904, respectively, that the mode of inheritance is due to the nature of the chromosome. The “chromosome theory of inheritance”, in which genes reside on the chromosome, was not yet readily accepted. This theory was ultimately confirmed by T. H. Morgan and colleagues, who found that genes are arranged in each chromosome<sup>30)</sup>.

As the genetic material was subsequently shown to exist within chromosomes, the relationship between chromosome structure<sup>31, 32)</sup>, species and chromosome number<sup>19, 33, 34)</sup>, and cancer and chromosome aberrations<sup>35, 36)</sup> has become increasingly clear.

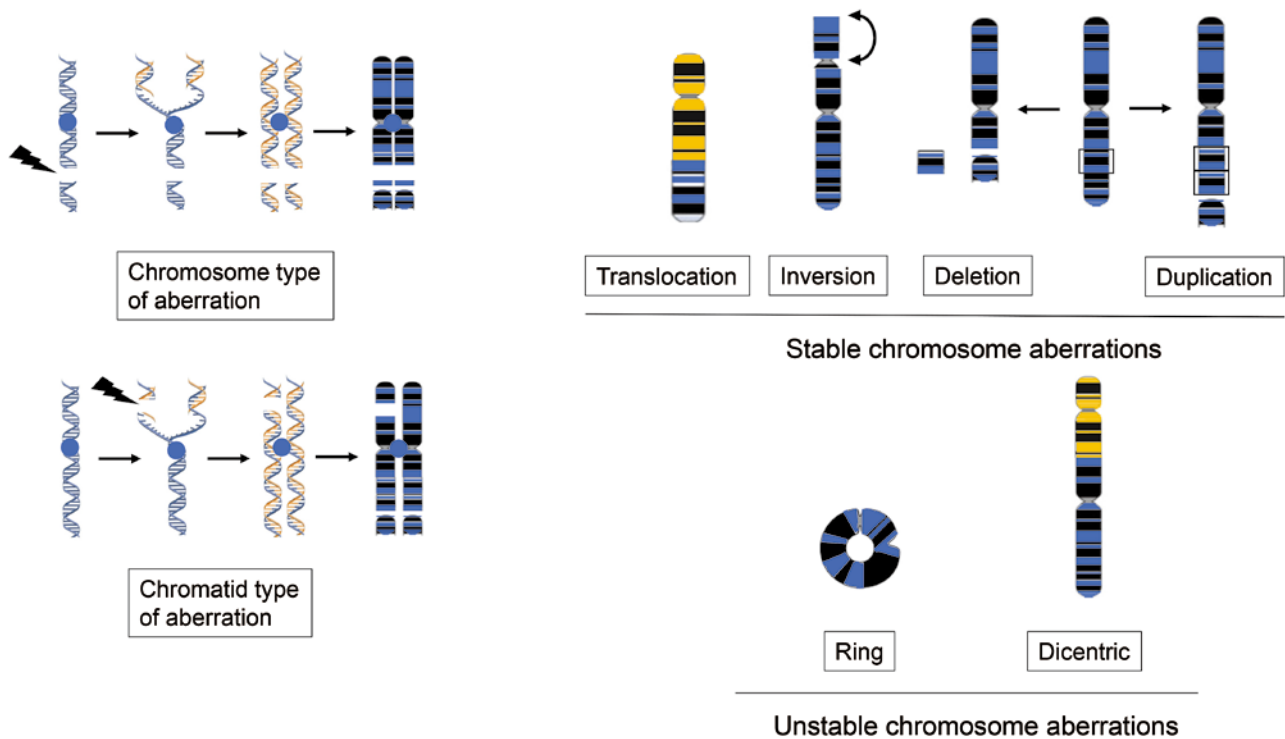
Eukaryotes have a species-specific chromosome formula (karyotype), which are sorted and ordered based on the differences in chromosome numbers (Fig. 7). The chromosomes are symmetrical in shape on the longitudinal axis and have a central constriction dividing the entire chromosome arm length to a short arm (petite: p arm) and a long arm (queue: q arm). The centromere is located at the central/primary constriction while telomeres occupy the chromosome ends (Fig. 8).

Chromosome can be classified morphologically based on the arm ratio, which is the ratio of the length of the short arm and the length of long arm, and the centromere index (CI), which is presented as the ratio of the length of the short arm to the total length of the chromosome<sup>37)</sup>. According to the centromere position as defined by these values, chromosomes can be categorized into metacentric



**Table 1.** Classification of human chromosomes based on morphology using Giemsa staining

Group	Chromosomes	Type	Description
A	1-3	metacentric and submetacentric	Chromosomes 1 and 3 are metacentric. Chromosome 3 is slightly smaller than 1. Chromosome 1 has a secondary constriction at the proximal region of long arm.
B	4, 5	submetacentric	Chromosome 4 is slightly longer.
C	6 ~ 12, X	submetacentric	Chromosome 9 has a secondary constriction at the proximal region of long arm.
D	13 ~ 15	telocentric	These chromosomes have satellites at the short arms.
E	16 ~ 18	metacentric and submetacentric	Chromosome 16 has a secondary constriction at the proximal region of long arm.
F	19, 20	metacentric	
G	21, 22, Y	telocentric	Chromosomes 21 and 22 have satellites at the short arm.

**Fig. 10.** Different types of radiation-induced chromosome aberrations.

(nearly median centromere), submetacentric (submedian centromere), subtelocentric (subterminal centromere) and acrocentric (terminal centromere) (Fig. 9).

The centromeres contain specialized regions called kinetochores that attach chromosomes to spindle fibers, which are also often referred to as the centromere in chromosome analysis. One chromosome in mitosis consists of two chromatids. These two chromatids are replicated in the S phase of the cell-cycle and are genetically identical, thus are known as sister chromatids. Telomeres protect the end of the chromosome, and chromosome fragments with missing telomeres are more likely to form Dic and ring chromosomes because their broken ends are easily fused with other breaks. In some cases, regions of the chromosome arms may

become thinner and constricted in regions other than the centromere. This constriction is termed secondary constriction (SCT) and is observed as a satellite at the chromosome tips. Such chromosomes are not chromosome aberrations and should be noted (Fig. 8).

In human somatic cells including lymphocytes, there are 46 chromosomes composing of 22 pairs of autosomes and two sex chromosomes (XX or XY). They are then classified into seven groups based on chromosome morphology (Fig. 7) and the characteristics of each chromosome are shown in Table 1. In particular for Dic assay, if groups D and G chromosomes are involved in the formation of the dicentric chromosome, it is important to verify the number of chromosomes in groups D and G to ensure a reliable analysis<sup>38</sup>.

#### 4. Chromosome aberrations formed due to misrepair during interphase

In general, most cells are in G<sub>0</sub> phase and the duration of mitosis is also short in mitotic cells. Thus, radiation-induced damage is thought to occur in interphase and most chromosome aberrations generated are likely the result of DNA misrepair. When radiation-induced DNA double-strand breaks (DSBs) occur during G<sub>1</sub>, this usually triggers cell-cycle checkpoints and induce cell-cycle arrest. If incomplete G<sub>1</sub>/S checkpoints allow incorrect genome replication at S phase, chromosome-type aberrations are seen at metaphase as DNA breaks and exchanges occur at the same locations of duplicated chromatids (Fig. 10). On the other hand, when DSBs arise from late S to G<sub>2</sub> phase after DNA replication, chromatid-type aberrations with DNA breaks or exchanges in only one chromatid are instead observed (Fig. 10). Therefore, depending on the cell-cycle phase in which radiation exposure occurs, the chromosome aberration seen in damaged cells is either a chromosome or chromatid-type aberration. For example, as peripheral blood lymphocytes at the time of exposure are in G<sub>0</sub> phase, chromosome-type aberrations are usually seen.

#### 5. Chromosome aberrations in human peripheral blood lymphocytes

To prepare chromosome specimens from G<sub>0</sub> peripheral blood lymphocytes for cytogenetic biodosimetry, it is necessary to stimulate cell division in peripheral blood lymphocytes with a mitogen, such as phytohemagglutinin (PHA), a type of lectin<sup>39-41</sup>. Cells entering the cell-cycle are then treated with microtubule polymerization inhibitors, such as colcemid, which limits microtubule formation and inactivates the spindle fiber and leads to activation of the spindle checkpoint and metaphase arrest in mitosis. As a result, a large number of metaphase cells can be acquired for chromosome aberration analysis.

Radiation-induced chromosome-type aberrations can be classified into stable chromosome aberrations that are inherited by daughter cells in cell division (e.g. translocations, inversions, partial deletions and duplications) (Fig. 7) and unstable chromosome aberrations that cause cell death and eventually disappear with each cell-cycle (e.g. Dic, ring, and acentric chromosome fragments). Thus, aberration stability is dependent on the presence of centromeres and telomeres as chromosomes require one centromere for equal distribution during cell division and telomeres for structural maintenance at the ends of both arms.

Acentric ring chromosome and chromosome fragments that do not have centromeres are unable to migrate to either cell pole during mitosis and therefore likely to

disappear during anaphase<sup>42, 43</sup>. Dic, on the other hand, forms a chromosome bridge when the two centromeres segregate to the opposite poles at anaphase, which either inhibits cell division or physically cleaves the chromosome on itself<sup>44</sup>. Cells harboring unstable chromosome aberrations without centromeres and telomeres often die during cell division. Even if they can divide, they may be impaired in further cell division. Therefore, the half-life of cells with unstable chromosome aberrations *in vivo* is relatively short, and it has been reported from 4 months to 1 year and a half<sup>45-47</sup>. In contrast, as cells carrying stable chromosome aberrations can slip through cell-cycle checkpoints, the half-lives of cells with stable chromosome aberrations are usually much longer, from 2 years to even beyond the average human life span<sup>47-49</sup>.

Based on the above, unstable chromosome aberrations (e.g. Dic, rings) are scored for dose assessment to facilitate radiation emergency care after acute external exposure, while stable aberrations (e.g. translocations) are generally used for retrospective dosimetry caused by past exposures.

#### 6. Summary

When chromosome aberrations are used for dose assessment, it is very important to prepare optimal chromosome specimens which can easily distinguish chromosome aberrations. The chromosome quality is affected by a variety of factors, including the number of metaphases in the sample, the degree of chromosome condensation, the extent of metaphase spreading and the condition of dye staining. In addition, the effects of reagents such as mitogens and colcemid could vary among individuals, depending on the different genetic backgrounds caused by the age and gender. Therefore, it is necessary to routinely check the standardized protocol for chromosome preparation and evaluate scoring criteria of chromosome aberrations in order to reduce individual differences among scorers and reduce the evaluation errors such that more accurate biodosimetry can be performed.

Chromosome analysis is often regarded as a highly specialized skill due to the difficulty of experimental techniques and morphological observations. However, in recent years, manuals of chromosome analysis have been developed which facilitates the sharing of various information. As chromosome aberration classifications, test protocols, and results criteria can subtly differ from each cytogenetic laboratory, the use of international standardized protocols has been recommended.

It is our hope that this article will serve as a concise summary of chromosomes and their structure to facilitate medical professionals involved in radiation emergency medicine in understanding cytogenetic biodosimetry.

**Point 1. Introduction of a chromosome**

- A chromosome is a structure observed under light microscopy during the mitotic phase of the cell-cycle.
- As chromosomes contain DNA and other genetic material, DNA misrepair induces chromosome aberrations.

**Point 2. Chromosome structure and morphology**

- Structural characteristics of a chromosome consists of a centromere at central/primary constriction, and telomeres at the chromosome ends. The short arm is the p arm while the long arm is the q arm.
- Chromosomes can be subdivided into different morphological groups according to the centromere position: metacentric (nearly median centromere), submetacentric (submedian centromere), subtelocentric (subterminal centromere) and acrocentric (terminal centromere).

**Point 3. Classification of chromosome aberrations**

- Peripheral blood lymphocytes at the time of exposure in biodosimetry are in the G<sub>0</sub> phase, thus chromosome-type aberrations are usually formed.
- Radiation-induced chromosome-type aberrations can be classified into stable chromosome aberrations that are inherited by daughter cells in cell division (e.g. translocations, inversions, partial deletions, duplications) and unstable chromosome aberrations that cause cell death and eventually disappear (e.g. Dic, ring, acentric fragments).
- For acute external exposure, unstable chromosome aberrations (e.g. Dic, ring) are used as indicators for accurate dose assessment to facilitate the radiation emergency medical care. On the other hand, stable aberrations (e.g. translocations) are used for retrospective dosimetry caused by past exposures.

**Conflict of Interest**

The authors declare that they have no conflict of interests.

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