RADIATION-INDUCED CHROMOSOME DAMAGE IN MAN

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Preface

There is increasing concern in our time over adverse effects of the environment upon our human genetic heritage. Chromosome mutation constitutes one type of genetic damage which can be readily recognized and quantitatively evaluated. Moreover, with the addition to our knowledge in these exciting days of molecular genetics, it has become a matter of general agreement that the induction of chromosome mutation is intimately related to other types of mutations, such as subvisible or point mutation and mutation toward cancer.

Since 1940, much has been learned about the mechanisms of induction of chromosome aberrations by ionizing radiations and their biological significance. In the 1960's, technical advances made it possible to study radiation-induced chromosome damage in humans and permitted estimation of hazards to humans from exposures to radiations. This field has been called human radiation cytogenetics and antedated the cytogenetic dissection for the rapid rise in awareness of the potential mutagenic effects upon humans afforded by chemical exposures. The need for knowledge regarding the effects of chemicals upon human chromosomes has attracted a number of radiation cytogeneticists, as well as infusing new researchers into the fields of cancer studies, environmental mutagenesis, and toxicology. As it is so for ionizing radiations, chromosome damage by chemicals is a consequence of a reaction of cells to induce and repair lesions in their DNA. The biological processes, including the metabolic alteration of the chemical itself, are much more complicated and diverse for chemicals than for radiation. And, thus in one sense, the need for studies on the mechanisms of chromosome aberration formation by ionizing radiation, and their subsequent biological significance, has been evoked and again has received a considerable impetus. Also, there is growing current interest in the harmful effects of low-level radiations. However, there is an array of technical problems in assessing the hazards to humans from exposure to low-level radiations. Detailed and better understanding on the submicroscopic structure of radiation energy deposition, mechanisms underlying the formation of chromosome damage and its biological significance, as well as the extrapolation from experimental animals to man, is thus particularly important and studies along this line are to be greatly encouraged.

This volume presents a number of different approaches to such investigations. These include recent progress and topics in (1) the origin and nature of radiation-induced chromosome aberrations, (2) the chemical and biological modifications of chromosome aberration formation, (3) chromosome damage in relation to other biological consequences, (4) chromosome aberrations in germ-line cells, (5) chromosome aberrations in humans exposed to radiations and (6) chromosome aberrations and risk assessment. Each chapter presented in this volume updates our current

knowledge in the field of radiation cytogenetics and advances our bridge-building to future approaches to the understanding of the origin, nature, and biological consequences of radiation damage to human chromosomes.

We wish to thank Dr. Avery A. Sandberg, the series editor, for his ceaseless efforts, encouragements, and valuable suggestions in bringing this volume into existence. We are much indebted to members of the Production Department of Alan R. Liss, Inc., particularly Mr. Kieran Murphy, for his expert editing of the manuscripts. We are grateful to Mr. Alan R. Liss, the publisher, for his interest and help in many ways.

Takaaki Ishihara
Masao S. Sasaki

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1

Microdosimetric Aspects of the Induction of Chromosome Aberrations

Manfred Bauchinger

The data of numerous irradiation experiments on the formation of chromosome aberrations in eukaryotic cells reveal that the biological effectiveness of ionizing radiations can be only inadequately explained by the physical quantity absorbed dose. According to the statistical fluctuations of energy absorption, which is greatest in very small regions, at low doses, and with densely ionizing radiations, the spatial distribution of absorbed energy is of main importance. The concepts of microdosimetry accounts for the microscopic patterns of energy deposition in critical regions of micrometer or nanometer dimension, *e.g.*, the cell nucleus and the chromosomes. It is demonstrated how the formation of chromosome aberrations can be interpreted in terms of the quantities and concepts of microdosimetry.

INTRODUCTION

The biological effect of ionizing radiation results from physical processes of energy loss and radiochemical mechanisms. Biophysical argumentation and models of radiation effects have been utilized for the interpretation of various endpoints, such as cell reproductive death, cell transformation, mutations, and chromosome aberrations. One approach is microdosimetry, which deals with the microscopic energy distribution in critical regions, *e.g.*, the cell or the cell nucleus. The first attempts for a description of actual patterns of energy deposition are documented in the pioneering monograph "The Actions of Radiations on Living Cells" by D. Lea [1]. However, Lea's data were still based on the concepts of linear energy transfer (LET), introduced by Zirkle [2,3], which has certain limitations, if one deals with very small volumes of micrometer or nanometer scale [4–9].

The beginning of microdosimetry in its true sense dates back to 1955 when Rossi and Rosenzweig [10,11] developed a gas-filled, tissue-equivalent ioni-

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zation chamber for the measurement of dose as a function of specific ionization. It then became apparent that the stochastic nature of the interaction of charged particles with matter requires a statistical treatment of energy deposition. Such a treatment must be given for various radiation qualities if an adequate interpretation of the biological effect is attempted [12].

The stochastic quantities, energy imparted, specific energy, and lineal energy, as well as their probability distributions, have been introduced in a framework of microdosimetric concepts [5, 6, 13, 14] which was the basis for the "theory of dual radiation action" [15]. Applying detailed track structure analyses in the generalized formulation of this theory, the notion of the distance distribution of energy transfers has been included [16]. In the present article essential considerations on the microdosimetric aspects of the induction of chromosome aberrations will be illustrated with selected examples of experimental cytogenetic data. For that purpose, it is neither necessary to deduce the entire mathematical formalism nor to enter into details of physical processes, such as the track structure analysis of ionizing particles.

INTERACTION OF RADIATION WITH MATTER

Types of Ionizing Radiations

Ionizing radiations [7, 17] consist of directly or indirectly ionizing particles or a mixture of both. Directly ionizing particles are charged particles (electrons, or heavy charged particles, such as protons, α-particles) with sufficient kinetic energy to cause excitations or ionizations by collision. Indirectly ionizing particles are uncharged particles which can liberate directly ionizing particles. Energetic photons, such as x-rays and γ-rays, produce secondary electrons of various energies by the photoelectric effect, the Compton effect, or by pair formation; the secondary electrons then transfer their energy by excitations and ionizations. Energetic neutrons impart their energy mainly by elastic collisions to hydrogen nuclei; the recoil protons then lose their energy by excitations and ionizations. Neutrons with energies below a few keV and above tens of MeV transfer their energy through inelastic nuclear reactions which produce heavy particles and γ-radiation.

Pattern of Energy Deposition

Although the primary events of energy deposition (excitation and ionization) along the tracks of different types of radiations are essentially equal, such radiations reveal, nevertheless, distinct differences in their biological effectiveness. This can be explained by the different microscopic patterns of energy depositions, *i.e.*, by the fact that the energy transfers occur with

different spatial concentrations. According to the ionization density along their tracks, sparsely and densely ionizing radiatons can be distinguished. Energy transfer may occur localized as single ionization in the track; larger energy transfer to orbital electrons can lead to the formation of ion clusters of several (about 2–4) ion pairs, still larger numbers of ionizations occur in separate short electron tracks called δ-rays. The range of the most energetic δ-rays formed by fast electrons can be of a magnitude similar to that of the primary particle. The spacing between successive primary collisions is, for the sparsely ionizing electrons, often larger than the range of the majority of the δ-rays. Whereas the tracks of slow primary electrons and δ-rays are tortuous and branched, the tracks of heavy charged particles are essentially straight. The theory of track structure distinguishes two regions, the core and the penumbra. Within the core, energy is mainly deposited through "glancing collisions" that result in very densely spaced successive ionizations and ion clusters. The core is surrounded by the penumbra, an area composed of the track of "knock-on electrons." These secondary electrons (δ-rays) are created in the core of the primary particle and move away from the core in tortuous trajectories. Different concepts have been developed to characterize the energy transfer in irradiated media, one of these is that of linear energy transfer (LET).

Concept of Linear Energy Transfer (LET)

LET or stopping power [1–3] is a linear average of the rate of energy loss of a charged particle. The common unity is keV/μm. A detailed description and a rigorous definition is given by the International Commission on Radiation Units and Measurements (ICRU) [7].

Since irradiation creates particles with different energies and different LET, each type of ionizing radiation is always characterized by a full spectrum of LET. In order to account for energy transfer which generate δ-rays with selected cut-off limits, *e.g.* 100 eV, the concept of restricted LET has been introduced and for a specified cut-off energy either a track average LET, $\bar{L}_T$, or an absorbed dose average LET, $\bar{L}_D$, can be determined. These averages can also be derived for unrestricted LET.

The ICRU report on LET [7] refers to certain basic limitations of the LET concept. Kellerer and Chmelevsky [8] have quantitatively assessed the criteria for the applicability of LET. In large regions the finite range of the primary particle and the change in LET as the particle traverses this region has to be considered. In very small regions, energy loss straggling leads to substantial fluctuations of energy loss of the charged particles and the dissipation of energy by δ-rays that escape the volume of interest can also be critical.

LET is, therefore, only one of the factors which determine energy deposition in microscopic regions. It was demonstrated [8] that for protons and other heavy ions a substantial interval of diameters of such regions and of particle energies exists for which the LET concept is appropriate. In contrast, no such interval exists for electrons and, consequently, also for photons. The interpretation of the biological effectiveness of different radiations requires, therefore, the consideration of the actual configuration of particle tracks. To deal with this situation in small volumes, the local distribution of energy deposition must be accounted for. This can be achieved in terms of the quantities and concepts of microdosimetry.

Concepts of Microdosimetry

Microdosimetry deals with the statistical fluctuations of energy absorption in small volumes of irradiated matter.

For a meaningful interpretation of the biological effects of radiations, one has to know the actual amounts of energy deposited in a sensitive region, *e.g.* the cell nucleus, rather than its mean or expectation value. For this reason the stochastic quantities ϵ, z, y and the nonstochastic quantity D are distinguished. Detailed definitions of these microdosimetric quantities and considerations of their theoretical properties can be found elsewhere [5, 6, 13, 14, 17–19].

Energy imparted, ϵ, is defined as the difference of radiation energy entering into and leaving the reference volume. The specific energy, z, is the quotient of energy imparted, ϵ, to the mass of the reference region. At a certain macroscopic dose, D, one always deals with statistical fluctuation of the specific energy, z, *i.e.*, D is only an expectation value of z. In very small regions, at very low doses, and with densely ionizing radiations, the deviations of z from D are greatest.

A further microdosimetric quantity is the lineal energy, y, *i.e.*, the energy imparted by only a single particle track to the volume divided by its mean chord length. As for LET, a frequency average, $\bar{y}_F$, and a dose average, $\bar{y}_D$, of lineal energy can be determined, and the common unit is keV/μm.

If the various limitations of LET are disregarded, the mean values $\bar{y}_F$ and $\bar{y}_D$ are equal to $\bar{L}_T$ and $\bar{L}_D$. The averages of specific energy, z, produced in individual events are, in the same approximation, linked to the averages of LET, but the relations are more complicated and contain the diameter, d, of the volume of interest:

$$\bar{z}_D = \frac{22.9\ \bar{L}_D}{d^2} \quad \text{and} \quad \bar{z}_F = \frac{20.4\ \bar{L}_T}{d^2} \tag{1}$$

The units are assumed to be rads, keV/μm, and μm. In microdosimetric terminology $\bar{z}_D$ is often denoted as ζ, the energy mean of the increments of specific energy, z, produced in single events.

Specific-energy density distributions from single-event spectra can be measured with spherical proportional counters filled with tissue-equivalent gas at low pressures, and tissue spheres with < 1 μm can be simulated [20, 21].

In Figure 1 the dose-related distribution, d (y), is demonstrated for different radiation qualities at a simulated diameter of 1 μm [15]. It is evident that in small volumes the event spectra of low LET radiations overlap in the range of about 1.0–10.0 keV/μm with the spectra of neutrons and that comparable effects may be produced by these radiations and by energetic recoil protons. However, the main difference is that, for sparsely ionizing radiations, high energy events occur with far lower probability.

On the basis of the microdosimetric concepts, the "dual radiation action theory" was developed for the interpretation of biological radiation effects [15]. It was stated that elementary lesions result from a combination of pairs of sublesions and that the number of elementary lesions produced in sites of micrometer dimension is proportional to z^2. However, it was found that the determination of probability distributions of z or y was insufficient for an adequate quantitative explanation of radiation effects. Therefore, in the generalized form of theory [16], the site concept was eliminated and an essential parameter was introduced which accounts for the distances between sublesions. The "dual radiation theory" will be treated in more detail in connection with the quantitative interpretation of the induction of chromosome aberrations.

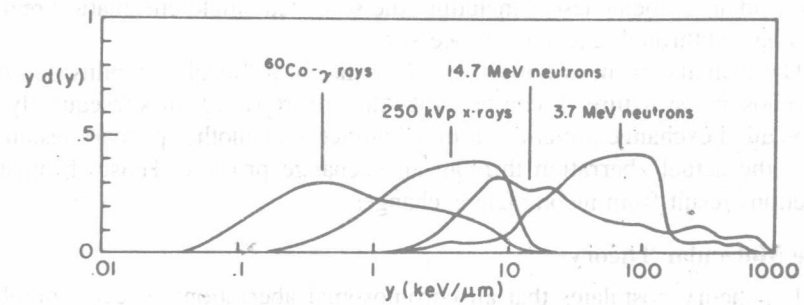

Fig. 1. Distribution of lineal energy for different types of radiation in simulated spherical regions of 1-μm diameter [15].

INTERPRETATION OF THE FORMATION OF RADIATION-INDUCED CHROMOSOME ABERRATIONS

The formation of structural chromosome aberrations is generally explained by two fundamental hypotheses. The "breakage-first hypothesis" was originally proposed by Stadler [22]. As an alternative to this classic hypothesis, Revell [23–26] has developed the "exchange hypothesis."

The results of various irradiation experiments could be either interpreted in terms of the breakage-first model or in terms of the exchange model. On the other hand, both hypotheses were questioned under certain assumptions. Recently, Chadwick and Leenhouts [27–29] postulated that neither the classical nor the exchange hypothesis is correct and introduced the "molecular theory" of radiation-induced chromosomal aberrations.

In the following the basic propositions of the three interpretations are briefly summarized.

The Breakage-First Hypothesis

From experiments with x-rays and *Tradescantia* microspores of Sax [30–33] it was concluded that primary breaks should result at the passage of an ionizing particle, nearby or through the continuous interphase chromosome. The breaks may restitute to the original chromosome configuration, they may also interact and rejoin to form exchange aberrations (two-break aberrations), or they may remain open, resulting in terminal deletions.

The Exchange Hypothesis

This hypothesis essentially explains radiation-induced chromatid aberrations in *Vicia faba*. It is postulated that all aberrations, observed in first postirradiation metaphases, including the so-called single chromatid breaks, are induced through exchange processes.

The primary event is not an actual break but a "local instability" in the chromosome structure. It can be either directly repaired, or subsequently to a so-called exchange initiation, it may interact with another primary lesion to form the actual aberration through an exchange process. Thus, chromatid deletions result from incomplete exchanges.

The Molecular Theory

The theory postulates that all chromosomal aberrations (except complex forms) at first postirradiation mitoses can be explained on the basis of one radiation-induced DNA double-strand break in the backbone of the unineme chromatid (one chromatid-arm break). Thus, terminal deletions are the result of unrepaired DNA double strand breaks. Exchange-type aberrations need