Radiation-induced chromosome aberrations: insights gained from biophysical modeling

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Summary

Enzymatic misrepair of ionizing-radiation-induced DNA damage can produce large-scale rearrangements of the genome, such as translocations and dicentrics. These and other chromosome exchange aberrations can cause major phenotypic alterations, including cell death, mutation and neoplasia. Exchange formation requires that two (or more) genomic loci come together spatially. Consequently, the surprisingly rich aberration spectra uncovered by recently developed techniques, when combined with biophysically based computer modeling, help characterize large-scale chromatin architecture in the interphase nucleus. Most results are consistent with a picture whereby chromosomes are mainly confined to territories, chromatin motion is limited, and interchromosomal interactions involve mainly territory surfaces. Aberration spectra and modeling also help characterize DNA repair/misrepair mechanisms. Quantitative results for mammalian cells are best described by a breakage-and-reunion model, suggesting that the dominant recombinational mechanism during the G_0/G_1 phase of the cell cycle is non-homologous end-joining of radiogenic DNA double strand breaks. In turn, better mechanistic and quantitative understanding of aberration formation gives new insights into health-related applications. *BioEssays* 24:714–723, 2002. © 2002 Wiley Periodicals, Inc.

Introduction

Ionizing radiation produces rearrangements of the genome. When irradiation occurs during the G_0/G_1 phase of the cell cycle, large-scale rearrangements appear as exchange-type chromosome aberrations at the next mitosis. Such aberrations can alter cellular phenotypes, and are important in various areas of biology:

- Medical and public-health applications include perinatal diagnostics,[1] characterization of specific cancer types,[2,3] carcinogenesis risk estimation,[4–6] radiation biodosimetry,[7–9] and radiotherapeutic treatment planning,[10,11]
- Analyzing chromosome aberrations helps characterize repair/misrepair pathways involved in the processing of DNA damage.[12–17] Ionizing radiation has some unique features as a probe of such pathways. Compared to other genotoxic agents, it produces copious quantities of DNA double strand breaks (DSBs), and its timing can be controlled more accurately. Moreover, initial radiation damage has a discrete, stochastic character that can be modulated by using different kinds of radiation (e.g. α-particles versus x-rays) having different ionization densities.[18,19]
- The spectrum of different radiation-induced chromosome aberrations is informative about the geometry of chromosomes during interphase, and vice versa (reviews: Refs. 14,15).
- Chromosomal instability, in which new aberrations (though often not of the type characteristic of G_0/G_1 damage) continue to arise many generations after irradiation,[20–22] represents a form of genomic instability, and genomic instability is prominent during neoplastic progression.[23,24]

Here we review how data derived using recently developed cytogenetic approaches have been combined with biophysical modeling to elucidate the mechanisms and implications of aberration formation.

A colorful diversity of aberration types

Until rather recently, it was usually assumed that virtually all chromosome exchanges are simple, i.e. involve only two chromosome breaks (Fig. 1). However, chromosome "painting" techniques have now shown that complex aberrations, involving more than two breaks in a single configuration, are common.[25] Fig. 2 (modified from Ref. 26 with permission) gives an example. Many whole-chromosome painting techniques are based on FISH.[27] More recent and sophisticated painting techniques, such as mFISH (Fig. 2) or spectral karyotyping, employ combinatorial hybridization schemes, allow-

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Abbreviations: DSB, DNA double strand break; FISH, fluorescence in situ hybridization; mFISH, multiplex-(multicolor-, multifluor-) FISH; Gy, Gray (a unit of radiation dose; 1 Joule per kilogram).

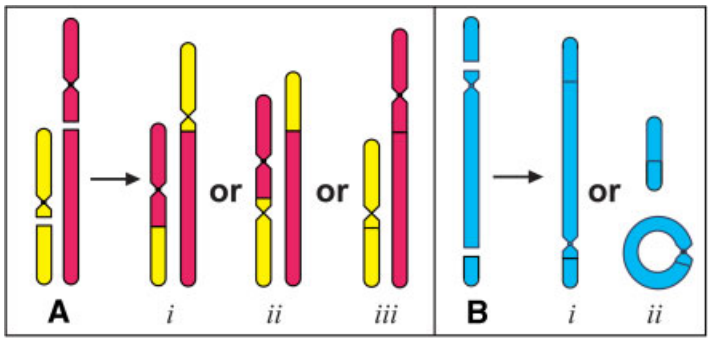

Figure 1. Simple chromosome aberrations. Constrictions represent centromeres; gaps indicate chromosome breaks that are caused either by prompt radiation damage or (in some cases according to some models) by subsequent enzymatic action. Simple aberrations, by definition, involve only two breaks. **A:** Two painted chromosomes, each of which contains a break. The result can be one of the following. (i) A simple (reciprocal) translocation. While a cell harboring this type of aberration usually remains clonogenically viable, the resulting large-scale rearrangement of the genome can lead to phenotypic alterations, including mutation and oncogenic transformation. (ii) A dicentric together with its associated acentric fragment. Dicentrics are usually clonogenically lethal because of segregation problems that arise during anaphase of mitosis. (iii) A double restitution. Restitution is defined as rejoining in the pre-break configuration with, at worst, local alteration of the genome at the nucleotide level, such as a point mutation. **B:** A single chromosome with two breaks. Misrejoining can give simple intrachromosomal aberrations as follows: (i) a (pericentric) inversion or (ii) a centric ring with accompanying acentric fragment. As far as phenotypic changes are concerned, translocations and large inversions are expected to have similar consequences; likewise centric rings and dicentrics are comparable. Because they are often difficult to detect, inversions are among the least well-understood of the simple aberrations. Nevertheless, they are rather common, due to the proximity effects discussed in the text. The remaining types of simple aberrations, acentric rings and paracentric inversions (not shown), are also common but difficult to detect.

ing recognition of most exchanges between heterologous chromosomes.[28–32] Still further extensions of this approach allow better recognition of exchanges between homologous chromosomes, better localization of exchange breakpoints within a chromosome, and better recognition of inversions (Fig. 1Bi).[33–36] The intricate aberration spectra uncovered by chromosome painting give extra information about the mechanisms and geometric aspects of radiation damage.

Aberration formation pathways

For over half a century chromosome aberrations have been extensively studied with regard to the repair/misrepair pathways involved in their formation.[16] The question as to what constitutes the principal underlying mechanism has remained controversial, sometimes contentiously so. Proposed models differ substantially in their predictions for the dose- and dose-rate dependency of aberration frequencies, and differ also with respect to the predicted spectra of aberrations.

Debates have centered around three pathways, though these could be acting in parallel. Updated versions of the pathways are shown schematically in Fig. 3. The breakage-and-reunion pathway (Fig. 3A) corresponds, at the molecular level, to non-homologous end-joining.[37–39] An alternative scenario embraces a "1-hit" paradigm (Fig. 3B), in which a single radiation-induced DSB is sufficient to initiate an exchange with an otherwise undamaged portion of the genome, by interacting with a second, enzymatically induced break.[40,41] This scenario can correspond to homologous DNA repair,[37,38] a correspondence particularly evident if the interaction involves, as has sometimes been observed, limited sequence homology shared between two sites, such as that provided by the abundance of repetitive DNA elements.[42,43] The pathway shown in Fig. 3B has been termed recombinational misrepair (strictly speaking, all three pathways involve recombinational events). The third pathway (Fig. 3C) represents the long-standing concepts of Revell's exchange theory (reviews: Refs. 16,17). Here the initiating lesions are not outright radiogenic breaks that disrupt the continuity of chromosomes, and any breakage that does occur is a consequence of a subsequent enzymatic rejoining/misrejoining process.

Chromosome localization and proximity effects

Regardless of the pathway, formation of chromosome exchanges involves having two or more different genomic loci in close proximity (Figs. 1–3). Consequently, chromosome geometry and large-scale chromatin architecture in the interphase nucleus influence aberration spectra. Conversely, aberration spectra can be used to probe chromosome localization and to help estimate the DSB interaction range, dependent on chromosome motion.[18,44]

Some pioneering quantitative analyses of interphase chromosome geometry used radiation-induced chromosome aberrations (e.g. Ref. 45), but until recently comparatively little

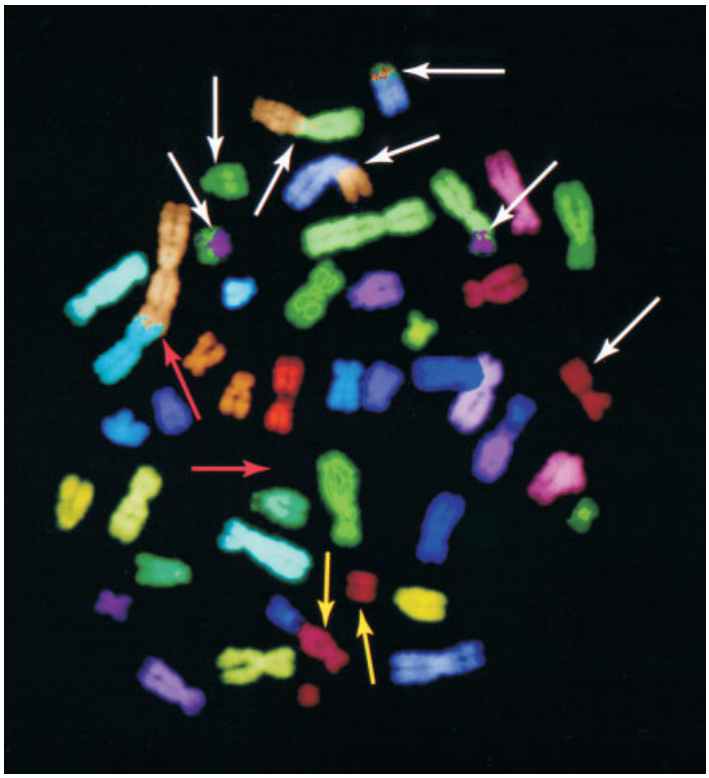

Figure 2. An mFISH image for a chromosome spread containing both simple and complex exchanges. The cell, a human peripheral blood lymphocyte, was irradiated with a 4 Gy dose of gamma rays. In mFISH, chromosomes are assigned colors, corresponding to unique combinatorial hybridization signals that represent the 24 different types of chromosomes in the human genome. Exchange-type aberrations here include a complex rearrangement that simultaneously involves chromosomes 1, 2, 3, 9, 11 and 20 (white arrows). Two simple exchanges were also found in this cell, a dicentric involving chromosomes 1 and X (red arrows; one points to a chromosome near the limit of resolution) and a translocation involving chromosomes 12 and 21 (yellow arrows). Reproduced from Loucas BD, Cornforth MN. Radiation Research 2001;155:660–671 with permission.

direct information was available. The convoluted looping structure of interphase chromosomes defeated attempts to visualize chromatin architecture on the comparatively large scales (>1 Mb) involved in aberration studies. Major progress made over the last decade has now shown that, at any one instant, any one chromosome is predominantly localized to a territory whose volume, in a human cell, is only a few per cent of the volume of the whole nucleus (review: Ref. 46)

The need for close spatial proximity of loci in an aberration-forming interaction, taken together with chromosome localization leads to a number of proximity effects, which can be quantified using computer modeling. Proximity effects include the following:

- Among intrachanges (intra-chromosomal rearrangements, e.g. Fig. 1B) there is a statistical bias for small intrachanges over large intrachanges, compared to expectations based on genomic content.[47]
- As illustrated in Fig. 4, there is also a bias for intrachanges over two-chromosome aberrations.[45,48]
- There is a bias for two-chromosome aberrations (e.g. Fig. 3Ai) over three-chromosome aberrations (e.g. Fig. 3Aii), etc.
- Non-random spatial associations of different genomic loci can be identified by looking for extra exchanges between those loci.[49–51] In some cases, extra exchanges are found, and some are consistent with directly observed spatial correlations.[52,53] However, Brownian motion and other sources of randomness are powerful forces making for variability—both in time and from cell-to-cell—of whole-chromosome spatial associations.

Direct evidence indicates little overlap of chromosome territories.[46] Correspondingly, recent results for the dependence of aberration frequency on chromosome DNA content support a picture where interchromosomal interactions occur

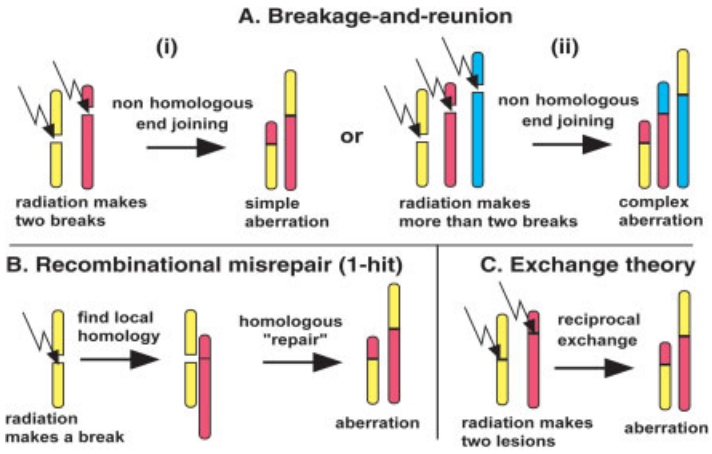

Figure 3. Standard models of exchange formation. The figure depicts radiation-induced DSBs (shown as gaps) and their misrejoining. **A:** A pathway where the two free ends of one break can either misrejoin independently of each other, at different genomic locations (Panel Aii), or act in concert with both free ends going to the same genomic location (Panel Ai). Due to the possibility of independent misrejoining, complex aberrations can arise readily and very complex aberrations can result (compare Fig. 2). **B:** A different pathway. One essential difference is that a single radiation-induced DSB can lead to an aberration, perhaps by enzymatically mediated homologous misrepair as shown. In the usual versions of this type of model, DSB free ends are constrained to act in concert during the recombinational event, as shown in panel B. This constraint leads to model predictions of a much smaller proportion of complex aberrations relative to simple ones than in the breakage-and-reunion case (see text). It also limits the type of aberrations that can arise. For example, in an mFISH experiment, any misrejoining must make either two color junctions between a particular pair of colors or none; consequently, the total number of color junctions between any given pair of colors should be an even number. Metaphases are observed where there are odd numbers of color junctions between some pairs of colors, an observation that favors pathway A over pathway B. Proponents of pathway B have, however, pointed out several confounding factors, including the following: (1) some rearranged chromatin pieces may be too short to observe, which would invalidate the even/odd argument; (2) the two pathways shown in A and B might be acting in parallel, so that some observed rearrangements result from one pathway and some from the other; (3) the constraint of two free ends acting in concert may be an incidental, not essential, feature of a 1-hit model. Because of such arguments, the question remains controversial, and quantitative model predictions of the ratio of complex to simple aberrations, which also bear on the differences between the pathways in A and in B, have drawn added attention. **C:** The Revell-type exchange-theory pathway. As in A, two radiation-induced lesions are required to initiate the exchange process. As in B, free ends of the same break are constrained to act in concert, restricting the type and frequency of complex aberrations.

mainly near the surface of territories.[54–58] However, the formation of multi-chromosome aberrations (e.g. Figs. 2 and 3Aii) suggests, to the contrary, considerable territorial overlap.[15] One possible explanation for this discrepancy is preferential induction of aberrations in special locations outside the main territories, where loops from many different chromosomes may be close.[25] There is some evidence[59] against an alternate explanation,[16] of active transport of damaged chromatin to repair "factories" where DNA on different chromosomes is simultaneously processed. Motion observed on the scale of a whole chromosome territory (~1 μm) is constrained, rather slow, and apparently random,[60–63] though directed motion for a small portion of a chromosome is not decisively precluded.

Modeling aberration formation mechanisms quantitatively

In order to quantify proximity effects or other aspects of repair/misrepair mechanisms, and to make sense of the complicated data sets, computer modeling of aberration formation has become common.[15,64] Modern models of chromatin structure[46,65] can be integrated with simple models of DSB motion and misrejoining as well as with previously developed, sophisticated radiation track codes describing physical and radiochemical aspects of radiation.[14,17,66,67]

Such aberration modeling is probabilistic, implemented by what are called Monte Carlo techniques, where the computer in effect "rolls dice" to give extremely detailed output. The time course of aberration formation is simulated in each cell. For example, suppose it is known that on average 1.5 DSBs are produced in any homologue of chromosome 1 in a given experiment. The computer first selects whether the number of DSBs for a copy of chromosome 1 in the first cell is 0, 1, 2, etc., either by using a random number generator together with an appropriate probability distribution or by using a probabilistic radiation-track code together with a polymer, random-walk geometric model of the chromosome. The specific location of the simulated DSB(s) on the chromosome is found. The other 45 chromosomes are then treated similarly, taking into

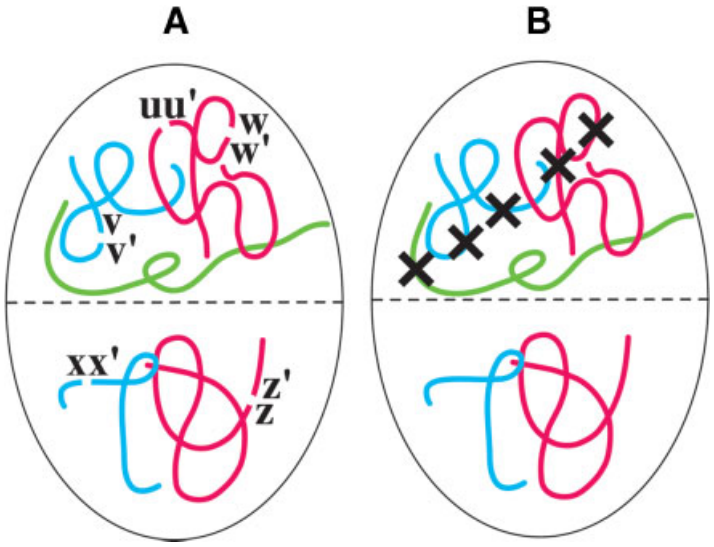

Figure 4. Chromatin architecture, in relation to aberration spectra and radiation track structure. One way in which territory/proximity effects are seen is in a statistical bias for aberrations involving a single chromosome as compared to aberrations involving two different chromosomes. **A:** The basic phenomenon in simplified form. The panel schematically shows five G_1 phase chromosomes in an interphase cell nucleus, localized to territories, with five DSBs. For sparsely ionizing radiation, such as gamma-rays, all five DSBs will usually (though not always) come from different radiation tracks, and usually be scattered at random throughout the nucleus. DSB free ends can interact only over a limited range, so proximity effects influence misrejoining. In the figure, proximity effects can be visualized if we imagine that a free end can interact only with a free end in its own half of the nucleus, as indicated schematically by the dotted line. For example the free end u of DSB uu′ can restitute with u′ or misrejoin with v, v′, w, or w′, but not misrejoin with x, x′, z, or z′. In general, there will be a bias, relative to expectations based on randomness with all five DSBs capable of interacting, for forming a ring or inversion (Fig. 1B) compared to forming a translocation or a dicentric (Fig. 1A), i.e. a bias for one-chromosome aberrations as compared to two-chromosome aberrations. Thus in the diagram some reactions that can form dicentrics or translocations are allowed (e.g. the reaction xz, x′z′, forming a translocation or dicentric, while the other three DSBs restitute as uu′,vv′, ww′; or the reaction vw, v′w′, with restitutions uu′, xx′, zz′; etc.). But territory/proximity effects prohibit some dicentrics or translocations that could otherwise occur (e.g. ux, u′x′). In this diagram, territory/proximity effects have no effect on reactions capable of forming rings (i.e. u′w′). Overall, therefore, the ratio of dicentrics plus translocations to rings is smaller than it would be if territory/proximity effects were absent. **B:** For the case of densely ionizing radiations, such as alpha particles, the five DSBs would typically be near the track of a single alpha particle, as indicated by the crosses, instead of being randomly located in the nucleus as in A. This difference in spatial DSB patterns leads to a different aberration spectrum, as described in the text.

account their geometry and DNA content. Repair/misrepair for all the DSBs is next simulated, as what is called a discrete-time Markov process, by an algorithm probabilistically favoring misrejoining of those DSB free ends that are spatially close to each other. The result is a simulated configuration of rearranged chromosomes in the first cell. Simulating the scoring system (such as mFISH) used in the experiment then gives the observable aberration pattern for that simulated cell; a simple example might be that the cell contains just one dicentric, involving chromosomes 4 and X. Iterating, thousands or millions of individual cells are simulated one by one, each with its own aberration pattern. The results are then compared to experimentally observed aberration spectra and to dose–response relationships for aberration frequencies.

This quantitative, probabilistic approach requires making explicit the basic assumptions (e.g. which aberration formation pathway is being considered and what geometric model is being used for chromosomes). It systematically emphasizes dominant processes and likely outcomes, appropriately discounting, without completely ignoring, minor contributory pathways and a large number of possible but highly unlikely aberration patterns. The approach uses randomness assumptions[68] on DSB distribution in the genome and on misrejoining to test mechanistic models quantitatively, using a minimum number of adjustable parameters. Randomness holds to reasonable approximation (reviews:Refs. 48,69), though there are some reports of "hot spots" or other deviations from randomness (e.g. Refs. 20,54,55).

Based on such biophysical modeling, on analyzing aberration dose–response (discussed below), and on evidence concerning likely molecular mechanisms, we believe that breakage-and-reunion is the dominant pathway for aberrations produced in mammalian cells following exposure to ionizing radiation during G_0/G_1.[12,13,29] One main reason is that in the simulations the other mechanisms described in Fig. 3 are unable to reproduce the full richness in aberration

spectra that are observed experimentally, especially the frequency and extent of complex aberrations. However, the modeling does not preclude some admixture of the other pathways, and the situation remains controversial.[17,41]

An excess of very complex aberrations?

If one accepts the breakage-and-reunion model and assumes that all reactions are complete (*i.e.* that no DSB free ends are left over at the end), one can prove that any aberration-producing reaction can be uniquely decomposed into irreducible reactions called cycles. (e.g. Refs. 29,70,71) The order of a cycle quantifies aberration complexity. For example Figs. 1Ai–iii, 1Bi, 1Bii, and Fig. 3Ai all correspond to cycles of order 2; Fig. 1Aiii is the result of two cycles of order 1 (restitutions); Fig 3Aii shows a cycle of order 3; and analysis of Fig. 2 indicates that one of the reactions was a cycle of order 6. mFISH data show cycles of unexpectedly high order and cells with unusually large numbers of different chromosomes taking part in aberrations.[26,30] Biophysical modeling suggests that an additional mechanism may be operating, to generate the most complex rearrangements seen.[15] This could perhaps be an early onset of chromsomal instability.

From mechanisms to predicted dose and dose-rate dependencies

Apart from insights into nuclear architecture and into DNA repair/misrepair, mechanistic understanding of chromosome aberration formation is useful in various applications of radiobiology. Almost always, the key questions in applications are how radiation effects depend on dose and on dose rate. Understanding aberration formation mechanisms helps answer these two questions. Experiments, and models using ordinary differential equations and/or stochastic-process theory, have over the years uncovered some rather general rules about dose and dose-rate dependence for different mechanisms (reviews: Refs. 72,73). The rules are based primarily on a distinction between "1-track action" and "2-track action" (Fig. 5), where 2-track action involves the interaction of uncorrelated damage from two different primary radiation tracks (such as two different x-ray photons), so that its dose and dose-rate dependencies are more complicated than for 1-track (i.e. intra-track) action. Despite some exceptions, and the fact that they are approximations rather than exact statements, the rules cover a large variety of cases. They often hold to good approximation, not only for simple chromosome aberrations, as illustrated in Fig. 5, but also for many other kinds of ionizing radiation damage. The rules are the following.

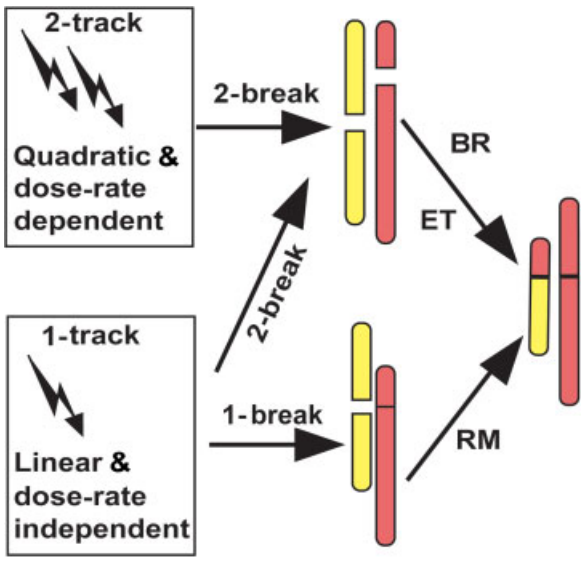

Figure 5. Dose and dose-rate dependence of aberration formation. The figure shows different pathways for producing the simple chromosome aberration on the right. It summarizes basic rules for the way in which the yield of simple aberrations depends on dose and dose rate.

- 2-track action (Fig. 5, top rectangle) usually produces an approximately quadratic yield (proportional to the dose squared). The intuitive reason is that the number of tracks is linearly proportional to dose, so that the number of track pairs is approximately proportional to dose squared. Moreover 2-track action usually produces a smaller effect when a given dose is prolonged, i.e. decreasing dose rate decreases yield. The intuitive reason is that if the dose is spread out in time, repair can take place between the time of the first track and the time of the second.
- 1-track action (Fig. 5, bottom rectangle), on the contrary, usually produces a yield that is linearly proportional to dose and independent of dose rate.

Applying these rules to the chromosome aberration formation pathways discussed earlier, one sees from Fig. 5 that the breakage-and-reunion (BR) and the Revell-type exchange theory (ET) mechanisms, both of which require two different radiation-induced breaks to initiate an exchange (Fig. 3), lead to a mixture of linear and quadratic dose dependence. The linear component occurs because a single track sometimes produces two or more DSBs (Fig. 4). In this linear-quadratic response, the linear term dominates at low doses, and the quadratic term dominates at higher doses.[17] One also sees from the figure that the recombinational misrepair (RM) mechanism (Fig. 3) usually leads to a linear dose dependence that is independent of dose rate.

For brevity, some complications are omitted from Fig. 5, including the following.

- By postulating specific chemical kinetic mechanisms involving saturation of repair enzyme action and competition between several repair/misrepair pathways, it is possible to

construct models in which 1-break action shows a non-linear dose-dependence and does depend on dose rate.[41]

- Dose-rate dependence, comprising either increase or decrease of radiation effects if a given dose is prolonged, can also result from a variety of biological processes not directly related to whether the initial radiation damage is 1-track or 2-track (review: Ref. 72).
- At quite high doses of sparsely ionizing radiation (say above ~5 Gy), 2-track action does not necessarily produce an approximately quadratic response. There are two main reasons: (1) an incremental dose, instead of making added aberrations of a particular type (e.g. dicentrics), may instead turn some already produced aberrations of that type into more complex aberrations, leading to a less rapid increase of yield with increasing dose, and (2) *saturation*: i.e., when, in a competition between restitution and misrejoining, the dose becomes so high that misrejoining starts to dominate, then misrejoining cannot grow faster than linearly with dose.[74,75]

Medical and public health aspects

Despite these complications, the rules of Fig. 5 have important applications to biodosimetry, risk estimation, and radiotherapy treatment planning.

In applications to biodosimetry, the goal is usually to infer dose retrospectively from the level of chromosome aberrations in an individual's peripheral blood lymphocytes. This procedure requires knowing how aberration frequencies vary with dose for the particular radiation type and exposure conditions involved. Under the breakage-and-reunion scenario described above, radiation-induced dicentric or translocation frequency is expected to have, for sparsely ionizing radiation in the relevant dose range, a linear-quadratic dependence on dose (Fig. 5). In vitro, this expected linear-quadratic dependence is in fact observed.[8,17] Densely ionizing radiation, in contrast, operates almost exclusively via intra-track action (Fig. 4) over the relevant dose range, so near-linearity in dose is expected (Fig. 5), and is observed.[8,17]

In addition, because of the interplay between chromatin geometry and radiation track structure (Fig. 4), the spectrum of aberration *types* is expected to be different for densely ionizing radiation.[76] A different spectrum is indeed observed in vitro: there are higher frequencies of aberrations involving several exchange breakpoints within the same chromosome arm, compared to interchromosomal interactions; at low doses, there is a higher frequency of complex aberrations compared to simple ones.[77–80] Because of such tell-tale differences, retrospective biodosimetery should eventually be able to identify the type of radiation as well as the dose received.

Another potential application involves estimating cancer risks from radiation exposure. A long-standing problem has been that the doses of primary interest are too small to produce quantifiable—or, often, even detectable—biological effects, either experimentally or even epidemiologically. And yet, there are serious concerns over the effects of such low doses when acting on very large populations. Major uncertainties in low-dose and low dose-rate experimental estimates, together with the major health and economic issues involved, have made this area highly contentious. Biophysical, mechanistic models of radiation damage, such as the models outlined above, though themselves controversial, are one of the few hopes for extrapolating measurable risks appropriately to lower doses. This approach to risk estimation is valid only to the extent that dose and dose-rate dependence of radiation carcinogenesis parallel those of aberration formation. Currently risk estimate extrapolations from higher doses, with consideration given to effects of dose rate, are based on the linear-quadratic model (Fig. 5), in part motivated by results on chromosome aberrations.[4,5] On one hand, there is also good evidence for a causal link between translocations and certain cancers, especially leukemias; on the other hand, the radiogenesis of some solid tumors may be more closely related to other forms of radiation damage, having different dose- and dose-rate dependency.[6]

A third application concerns treatment of tumors with radiation. Much of the tumor cell killing, and the undesired side effect of killing surrounding normal cells, probably comes from production of dicentrics and centric rings (Fig. 1).[81,82] A linear-quadratic dose-response together with a dose-rate effect, suggested by chromosome aberration results and modeling (Fig. 5), is confirmed by more direct clinical data and forms the basis of modern, biologically based treatment planning for tumor radiotherapy.[83,84] In addition, aberration-based predictive assays for sensitivity of normal tissues surrounding a tumor offer promise of individualized treatment.[10,11]

Conclusions and prospects

Recent technological advances such as mFISH and spectral karyotyping have led to an explosive increase in cytogenetic data, which, together with computer-assisted modeling, allow new insights into the formation of radiation-induced chromosome aberrations. For mammalian cells, the weight of the evidence—on complexity of aberrations, on underlying molecular mechanisms, and on dose-response/dose-rate relationships—favors a breakage-and-reunion mechanism during G_0/G_1, involving non-homologous end-joining. The chromosome geometry picture emerging, from radiobiological and other data, is one of localized chromosomes, overlapping and interacting with each other mainly at territory surfaces or via loops protruding far from the home territories. For the most part, whole-chromosome territories seem to be almost randomly located with respect to each other. The randomness is modulated by some more systematic associations, but these are typically weak, transient, variable from cell to cell, or highly localized in the genome rather than being firm whole-chromosome associations, so they show up in aberration

experiments as a statistical bias for extra exchanges, set against a backdrop where any chromosome can undergo exchanges with any other.

Better quantitative characterizations of DNA repair/misrepair mechanisms and of chromosome geometry will surely emerge from currently ongoing aberration work, but some other areas call for new initiatives. One issue that has not to date received as much attention as it deserves is the interrelation between aberrations analyzed cytogenetically and mutations identified by using selection for specific missing gene products. There is a considerable consensus that most "large" mutations (e.g. total deletions of the *HPRT* gene) are formed by essentially the same pairwise misrepair mechanism(s) as intrachromosomal exchange aberrations (review: Ref. 85), but more systematic attempts to interrelate aberrations and mutations quantitatively are needed. As well, more experimental evidence and quantitative modeling on the relation between gene expression microarray data and aberration data in radiobiology are needed. Applications of aberrations to retrospective biodosimetry and tumor radiotherapy will no doubt benefit from continued technical improvements in cytogentic techniques, but for applications to radiation risk estimation technical improvements by themselves do not hold out as much promise. Probably nothing less than a conceptual breakthrough as regards radiation carcinogenesis can lead to credible low-dose risk estimates.

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