Proximity effects in chromosome aberration induction by low-LET ionizing radiation

John James Tello Cajiao[a,b,c], Mario Pietro Carante[a,b], Mario Antonio Bernal Rodriguez[c], Francesca Ballarini[a,b,*]

[a] University of Pavia, Physics Department, via Bassi 6, I-27100 Pavia, Italy
[b] INFN-Sezione di Pavia, via Bassi 6, I-27100 Pavia, Italy
[c] Universidade Estadual de Campinas. Cidade Universitária Zeferino Vaz, Campinas, SP, Brazil

A B S T R A C T

Although chromosome aberrations are known to derive from distance-dependent mis-rejoining of chromosome fragments, evaluating whether a certain model describes such "proximity effects" better than another one is complicated by the fact that different approaches have often been tested under different conditions. Herein, a biophysical model ("BIANCA", i.e. BIophysical ANalysis of Cell death and chromosome Aberrations) was upgraded, implementing explicit chromosome-arm domains and two new models for the dependence of the rejoining probability on the fragment initial distance, r. Such probability was described either by an exponential function like $\exp(-r/r_0)$, or by a Gaussian function like $\exp(-r^2/2\sigma^2)$, where r_0 and σ were adjustable parameters. The second, and last, parameters was the yield of "Cluster Lesions" (CL), where "Cluster Lesion" defines a critical DNA damage producing two independent chromosome fragments. The model was applied to low-LET-irradiated lymphocytes (doses: 1–4 Gy) and fibroblasts (1–6.1 Gy). Good agreement with experimental yields of dicentrics and centric rings, and thus their ratio ("F-ratio"), was found by both the exponential model (with $r_0 = 0.8$ μm for lymphocytes and 0.7 μm for fibroblasts) and the Gaussian model (with $\sigma = 1.1$ μm for lymphocytes and 1.3 μm for fibroblasts). While the former also allowed reproducing dose-responses for excess acentric fragments, the latter substantially underestimated the experimental curves. Both models provided G-ratios (ratio of acentric to centric rings) higher than those expected from randomness, although the values calculated by the Gaussian model were lower than those calculated by the exponential one. For lymphocytes the calculated G-ratios were in good agreement with the experimental ones, whereas for fibroblasts both models substantially underestimated the experimental results, which deserves further investigation. This work suggested that, although both models performed better than a step model (which previously allowed reproducing the F-ratio but underestimated the G-ratio), an exponential function describes proximity effects better than a Gaussian one.

1. Introduction

Living cells exposed to ionizing radiation during the G0/G1 phase of the cell cycle can show chromosome aberrations following chromosome breakage and large-scale rearrangement of the fragments, mainly due to Non-Homologous End Joining (e.g. [1,2]). Two chromosome breaks induced in two distinct chromosomes can give rise to a "dicentric", visible in metaphase as a chromosome with two centromeres accompanied by an acentric fragment, or a "reciprocal translocation", where both chromosomes have one centromere. On the contrary if both breaks were induced in the same chromosome, they can produce a "ring"

("centric" or "acentric" depending on the presence of the centromere) or an "inversion" ("pericentric" or "paracentric", respectively). A single, un-rejoined chromosome break will give rise to a "terminal deletion", whereas the expression "interstitial deletion" is used to indicate a small acentric fragment deriving from two chromosome breaks on the same chromosome arm; many, if not most, interstitial deletions are indeed small (acentric) rings. All patterns involving at least three chromosome breaks and two chromosomes are called "complex exchanges". A more detailed classification of the various aberration types can be found in [3].

Besides providing information on the initial DNA damage, the

various repair pathways and the organization of interphase chromatin, chromosome aberrations can influence the fate of the cell. The so-called "asymmetrical aberrations", such as dicentrics and rings, have a high probability of leading to clonogenic cell death [4], which is the main objective of any cancer therapy. On the contrary their symmetrical counterparts, like translocations and inversions, in general do not prevent cell duplication, but they involve genome rearrangements that can initiate carcinogenesis [5]. Furthermore, the scoring of chromosome aberrations accumulated in peripheral blood lymphocytes is applied in biological dosimetry, which can provide useful information following radiation accidents [6] and/or exposure to mixed fields, like those characterizing space radiation exposure [7–12] or heavy-ion cancer therapy [13].

Even before the discovery of the DNA double-helix, Lea hypothesized that the probability of chromosome fragment (mis-)rejoining is distance-dependent, i.e. the involved fragments have to be close in space [14]. Different functions have been proposed to describe such distance-dependence [15]. However, evaluating whether a certain function can model the rejoining process better than another one is not trivial, because in general different models have been implemented in different simulation codes and tested under different conditions in terms of considered cell types, dose values, radiation quality etc. In previous works modelling the induction of chromosome aberrations [10,16–18] and cell death [19–24], we adopted a step-like function, assuming that the rejoining probability for two chromosome fragments was 1 below a threshold distance *d*, and 0 above the threshold. Basing on the hypothesis that DNA repair mainly takes place at the boundaries of chromosomal and sub-chromosomal domains [25], and inspired by the CAS model developed by Sachs and co-workers [26,27], who assumed that two DSB can interact only if they are in the same "interaction site", the value of *d* was set equal to the mean distance between two adjacent chromosome territories, which resulted to be about 1.5 μm in human lymphocyte nuclei (modeled as 3-μm-radius spheres), 3.0 μm in AG1522 human fibroblast nuclei (modeled as cylinders of elliptical base, with 4-μm height and axes of 20 and 10 μm), and 3.6 μm in V79 Chinese hamster fibroblast nuclei (modeled as cylinders of circular base, with 6-μm height and 6-μm radius). For all considered cell types, simulated dose-response curves for dicentrics and centric rings induced by different radiation qualities showed good agreement with experimental data taken from the literature. Introducing the further assumption that each chromosome fragment has a certain probability of remaining un-rejoined, good agreement was also found with yields of "excess acentric fragments", that is acentric fragments associated neither to a dicentric nor to a centric ring, which are given by the sum of interstitial and terminal deletions. However, interstitial deletions were underestimated, whereas terminal deletions were overestimated. This was a consequence of assuming a step-like distance dependence of the rejoining probability with threshold distance in the order of the linear dimensions of interphase chromosome territories. This approach allowed reproducing the observed bias of centric rings to dicentrics (or, more generally, inter-arm intrachanges to interchanges) with respect to randomness, but provided very similar yields of centric and acentric rings, which is what one should expect in case of randomness: more specifically, in [28] it was calculated that, in case of randomness, the G-ratio should be about 1.2. On the contrary, many experimental works indicate that the ratio between interstitial deletions (or, more generally, intra-arm exchanges) and centric rings (more generally, inter-arm exchanges), also called G-ratio, is substantially larger than 1. The observed values are likely to depend on several factors including cell type, radiation quality and dose: while for lymphocytes exposed to low-LET radiation values around 2.5 were reported [29], much higher values were reported for fibroblasts; for instance, in [30] the ratio of inversions (paracentric plus pericentric) to centric rings was found to be about 7, which assuming equivalence between rings and inversions would correspond to a G-ratio of about 6. Furthermore, this ratio may increase with radiation LET, and may decrease with decreasing dose.

Overall, these findings suggest that a step-like function with cut-off distance in the order of the dimensions of interphase chromosome territories can discriminate between inter-chromosome exchanges and inter-arm intra-changes (e.g., between dicentrics and centric rings). However, it is not adequate to discriminate between inter- and intra-arm intra-changes (e.g., between centric and acentric rings), for which a function that decreases monotonically with increasing distance seems to be more appropriate. On this subject, it is worth mentioning that recent data from the new GHA array technique suggest a possible excess of rings with size smaller than 20 kbp, below the detection limit of tradiational cytogenetic techniques [31]. According to an interpretation reported in [31], these small deletions might be induced as a byproduct of repair in cases where the chromosome loop structure was disrupted; the breaks would appear as properly repaired, but would actually involve a small deleted sequence. This is not necessarily inconsistent with our model (see below), which deals with chromosome aberrations originating from DNA "Cluster Lesions": since by definition each CL breaks a chromosome into two independent fragments, a CL is likely to consist of a series of clustered, multiple DSBs possibly implying the production of such small interstitial deletions.

In their generalized Theory of Dual Radiation Action (TDRA), Kellerer and Rossi [32] suggested a function proportional to $\exp(-r/a)^2$ for an interaction probability varying slowly with the distance r, or alternatively a function proportional to $\exp(-r/b)$. The latter was adopted in [33], where the authors applied it to human fibroblasts exposed to low-LET radiation in their CAS2 (Chromosome Aberration Simulator) model. More specifically, at each step the probability of mis-rejoining between two DNA free-ends with initial distance r was taken to be

$$P = A\Sigma\exp(-r/r_0)/[N + A\Sigma\exp(-r/r_0)] \qquad (1)$$

r_0 and A were both adjustable parameters, the former related to the mean DSB interaction distance and the latter related to the chance that two very nearby DSB will undergo mis-rejoining rather than restitution. At each simulation step, the sum extended over all pairs of free-ends still in play (excluding pairs belonging to the same DSB), and N was the number of DSB with both free ends still in play. In case of mis-rejoining, the two involved free-ends were chosen according to the exponential distribution, whereas in case of restitution, one of the N DSB was chosen at random. The mean chromosome-territory intersection factor, Ω, defined as the volume that two territories have in common, summed over all chromosome pairs and divided by the cell nucleus volume, was the third and last adjustable parameter. Simulated yields of different aberration categories (dicentrics, translocations, centric rings, acentric rings and 8 different types of complex exchanges) were compared with FISH data on human fibroblasts exposed to 2, 4 and 6 Gy of X-rays. The model parameters, chosen by trial and error, were $r_0 = 0.8$ μm, A = 0.0095 and Ω = 1.1. On the whole, a good correspondence between simulations and data was found. The main exception was represented by acentric rings, which were underestimated. Such underestimation could not be interpreted quantitatively because simulated acentric rings were compared with the experimental category of "breaks", which also included terminal deletions in addition to interstitial ones. Apart this underestimation, the model developed in [33] predicted acentric rings to be much more frequent (about four times) than centric rings, in line with the evidence that the G-ratio is substantially higher than the 1.2 value expected from randomness. Among the possible drawbacks of this approach, the authors mentioned the fact that three parameters were used (whereas their previous CAS model used two parameters) and that the simulated dose-response, especially for complex aberrations, was steeper than the experimental one. Furthermore, the approach was not extended to other cell lines and/or other radiation qualities.

The slowly-varying function proposed in [32] was adopted in other works, including [31] and [34]. In the former work, applied to human