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Biophysical modelling of proximity effects in chromosome aberration production

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Summary. — Although two chromosome breaks induced in proximity are known to have a higher probability of being (mis-)rejoined, several aspects of these "proximity effects" are still unclear. Herein, proximity effects in human lymphocytes and fibroblasts were investigated by the BIANCA biophysical model, describing the dependence of the rejoining probability on the break initial distance, r, either by an exponential function of the form $\exp(-r/r_0)$, or by a Gaussian function of the form $\exp(-r^2/2\sigma^2)$. The characteristic distance (r_0 or σ) was an adjustable parameter; the only other parameter was the yield of DNA "Cluster Lesions" (CLs), where a CL is defined as a critical damage producing two independent chromosome fragments. The comparison of the simulation outcomes with published experimental and theoretical works showed that an exponential function may describe proximity effects in both the considered cell types, and possibly other cells. Since this exponential behavior has been found to be consistent with confined diffusion of break ends, this also suggests that, at the relatively short times required for chromosome aberration production, (confined) diffusion is preferable to other mechanisms. Furthermore, the results suggested that the ratio of dicentrics to centric rings ("F-ratio") may be a better high-LET fingerprint in lymphocytes, whereas the ratio of acentric to centric rings ("G-ratio") may be a better one in fibroblasts.

1. – Introduction

It is well known that ionizing radiation impinging on living cells during the G0/G1 phase of the cell cycle can produce a variety of chromosome aberrations, that is large-scale

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incorrect rearrangements of chromosome fragments mainly due to the "non-homologous end joining" mechanism. Even before the DNA double helix was discovered, Lea [1] understood that two chromosome fragments involved in a chromosome exchange need to be induced close in space (as well as in time), which is referred to as "proximity effect". The ratios between specific aberration types can help understanding the features of such effect. In particular the "F-ratio", *i.e.*, the ratio of dicentrics to centric rings, is a measure of the existing bias for intra-chromosome, inter-arm exchanges with respect to inter-chromosome exchanges: if the rejoining processes were completely random, and thus independent of the fragment distance, the value of F in diploid human cells would be $\sim$86 [2], whereas values in the range 5–20 are observed experimentally [3]. At the same time, the ratio of acentric rings (including the so-called "interstitial deletions", which mainly consist of small acentric rings where the lumen is not visible) to centric rings, also called "G-ratio", indicates a bias for intra-relative to inter-arm exchanges: while under the hypothesis of complete randomness G should be $\sim$1.2 [4], higher values, up to $\sim$10, are reported in the literature. Both F and G show variations depending on several factors including radiation quality, cell type and dose, which makes non-trivial the data interpretation. Concerning the dependence on radiation quality, some works (*e.g.*, [5,6]) suggest that F decreases with increasing LET and can thus be considered as a "fingerprint" of high-LET exposure, whereas others [7] do not show such dependence. Furthermore, several works report an increase of the G-ratio with LET; however, some other works do not show a significant increase [6].

In addition to experimental works, theoretical approaches can help shedding light on proximity effects. Within their Theory of Dual Radiation Action (TDRA), Kellerer and Rossi [8] proposed a Gaussian function proportional to $\exp(-r^2/2\sigma^2)$ for a rejoining probability slowly decreasing with increasing free-end initial distance, r, or alternatively an exponential function proportional to $\exp(-r/r_0)$ for a faster decrease. Both functions have been applied by other research groups; however, it has not been possible to draw clear-cut conclusions until now, since each group applied one of the two proposed options to a specific scenario. For instance, Sachs and co-workers [9] tested an exponential function against data on human fibroblasts exposed to X-rays, Holley *et al.* [10] applied a Gaussian function to human lymphocytes exposed to low-LET radiation (X- or γ-rays), and Ponomarev *et al.* [11] applied a Gaussian function to human fibroblasts exposed to γ-rays and alpha particles. Other functions, including inverse power laws (*e.g.* [12]), have been also proposed.

In previous works performed by the BIANCA (Biophysical ANalysis of Cell death and chromosome Aberrations) model, the possibility of applying either an exponential function or a Gaussian function has been implemented and tested on normal human lymphocytes and fibroblasts exposed to different radiation qualities [13, 14]. Herein, the comparisons between simulations and data were extended and further discussed, also considering in more detail analogous modelling works available in the literature, especially that by Sachs *et al.* [9]. This allowed extending and reviewing the previous results interpretation, with more focus on the biophysical mechanisms governing DNA damage repair. The main methodological aspects will be described in sect. 2, whereas in sect. 3 the results will be presented and discussed.

2. – Materials and methods

The BIANCA model/code, which initially was specific for chromosome aberrations (*e.g.*, [15,16]) and later has been extended to cell death [17-26], is based on the idea that

some DNA damage types lead to chromosome aberrations via distance-dependent mis-rejoining (or un-rejoining) of chromosome fragments, and some aberration types lead to clonogenic cell death. More specifically, it is assumed that: 1) ionizing radiation induces DNA critical damages called "cluster lesions" (CLs), where by definition a CL is any critical damage that produces two independent chromosome fragments; 2) distance-dependent mis-rejoining (or un-rejoining) of chromosome fragments leads to chromosomal aberrations; 3) dicentrics, rings and large deletions (where "large" means visible under Giemsa staining, as explained below) lead to clonogenic cell death. A discussion on the rationale of these assumptions, as well as their limitations, can be found in the previous works quoted above.

The yield of induced CLs (mean number of CLs per Gy and per dalton, which can be easily converted into the mean number of CLs per Gy and per cell) is the first adjustable parameter. Previous studies have shown that the CL yield, which tends to increase with LET before the over-killing region, mainly depends on radiation quality, but is also modulated by the target cell features: more radiosensitive cells, like lymphocytes, require higher CL yields, whereas more radioresistant cells, like fibroblasts, require lower CL yields. The various CLs are distributed within the cell nucleus according to the radiation track-structure. In old chromosome-aberration works, as well as in works focused on cell death, the distance dependence of chromosome fragment end-joining has been modelled by a step function. Recently [13, 14], an exponential function of the form $\exp(-r/r_0)$ and a Gaussian function of the form $\exp(-r^2/2\sigma^2)$ have been also implemented; in this case, the rejoining characteristic distance (r_0 or σ) is the second, and last, adjustable parameter.

In the present work, the modelling of the cell nucleus and its interphase chromosomes, as well as the simulation of nucleus irradiation by photons and charged particles, the association of each CL to a specific chromosome and chromosome arm, the distance-dependent process of chromosome-fragment rejoining, and the scoring of Giemsa aberrations, was performed like in [13, 14]. Similarly to previous works, chromosome fragments with a DNA content smaller than 3 Mega base pairs (Mbp) were neglected during the scoring procedure, because they are not visible in metaphase. A detailed description of these aspects can be found in [13, 14].

3. – Results and discussion

3˙1. *Lymphocytes.* – In [13], dose-response curves for different aberration types (dicentrics, centric rings and excess acentric fragments) in human lymphocytes exposed to gamma rays have been simulated by applying either an exponential or a Gaussian function. The model parameters (*i.e.*, the rejoining characteristic distance r_0 or σ, which mainly controls the aberration ratios, and the CL yield, which mainly controls the absolute aberration yields) have been adjusted to the experimental data reported in [7]. Concerning the exponential model, with $r_0 = 0.8\,\mu$m and $3.1\,\mathrm{CL}\cdot\mathrm{Gy}^{-1}\cdot\mathrm{cell}^{-1}$ the simulated yields were within the experimental error bars for each considered dose and aberration category, with the only exception of acentrics at 4 Gy. Interestingly, $0.8\,\mu$m is the value found by Sachs *et al.* [9] for X-irradiated human fibroblasts; this issue will be discussed in sect. 3.2. The Gaussian model allowed to obtain good agreement for dicentrics and centric rings (with $\sigma = 1.1\,\mu$m and $2.3\,\mathrm{CL}\cdot\mathrm{Gy}^{-1}\cdot\mathrm{cell}^{-1}$) but underestimated the yields of acentric fragments, showing that for lymphocytes exposed to low-LET radiation the exponential model provides a better description with respect to the Gaussian one.

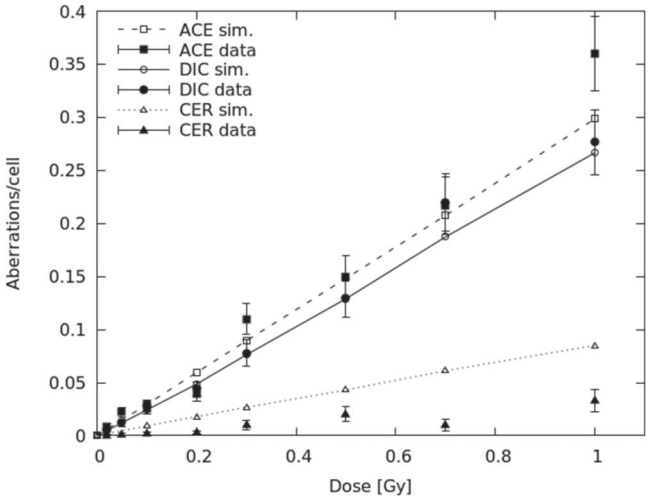

Fig. 1. – Dicentrics (DIC), centric rings (CER) and excess acentric fragments (ACE) in human lymphocytes exposed to 150 keV/μm alpha particles. The open symbols, connected by lines to guide the eye, represent simulation outcomes obtained by applying the Gaussian model, whereas the closed symbols are experimental data taken from [7].

Subsequently [14], the approach has been extended to higher LET values, comparing the results with proton and alpha-particle data reported in the same paper used for gamma rays, and using the same characteristic distance used at low LET, since this parameter should be independent of radiation quality. The proton results (at 3.5, 5.3 and 19.0 keV/μm) showed that, in lymphocytes, an exponential function provides a good description of the fragment rejoining distance dependence also at intermediate LET.

Concerning high-LET radiation, the results obtained by the exponential model, which can be found in [14], showed a good agreement for dicentrics and acentrics (with 1.06 CL/μm), but overestimated the centric ring data reported in [7] for 150 keV/μm alpha particles. The simulations were then repeated by applying the Gaussian model; the results, which are reported in fig. 1, were similar to those obtained by the exponential model, *i.e.*, provided good agreement for dicentrics and acentrics (with 0.95 CL/μm) but overestimated the yields of centric rings. This discrepancy will be discussed below.

Since for the dose-response curves the exponential model provided a better description than the Gaussian one, the analysis of the F- and G-ratio was mainly performed by the exponential model. Concerning the F-ratio, a comparison with the data reported in [7], which can be found in [14], showed very good agreement at low and intermediate LET, whereas it outlined an underestimation of the data at high LET, due to the aforementioned over estimation of centric rings.

To investigate this issue, the F values obtained at high LET (150 keV/μm alpha particles) were compared with other high-LET (lymphocyte) data, *i.e.*, those reported in [6] (150 keV/μm alpha particles), [27] (plutonium workers) and [28] (Thorotrast patients). As shown in table I, if compared with these other data the underestimation of F becomes much less pronounced (even not significant if one considers the data in [27]), suggesting that, in lymphocytes, an exponential function may provide a good description also at high LET. Interestingly Mestres *et al.* [6], who applied a pan-telomeric probe, noted that

TABLE I. – *F-ratio in human lymphocytes exposed to alpha particles.*

Simulation	data [7]	data [6]	data [27]	data [28]
3.0	10.1 ± 1.9	5.47 ± 0.36	4.5 ± 2.0	5.0 ± 0.3

TABLE II. – *G-ratio in human lymphocytes exposed to low-LET radiation.*

Simulation	data [7]	data [29]	data [30]
1.6	0.9 ± 0.1	2.84 ± 0.61	~ 2

when tri-centrics and tetra-centrics were considered as dicentric equivalents and included in the F-ratio calculation, their ratio increased from 5.47 to 7.16, suggesting that the inclusion of higher-order multicentrics may explain, at least in part, the higher values reported in some works. The fact that F was found to decrease with increasing LET (except for [7]) supported the hypothesis that F is a "good" fingerprint of high-LET exposure.

Concerning the G-ratio, *i.e.*, the ratio between intra- and inter-arm exchanges, a comparison with the data reported in [7], which can be found in [14], showed very good agreement at intermediate and high LET, whereas it outlined an overestimation of the data at low LET. However, the value reported in [7] (0.9 ± 0.1) is even smaller than the value expected assuming randomness, which is ~ 1.2 [4]. Table II reports comparisons with other published data [29, 30], which are consistent with the simulated value. This suggests that, in lymphocytes, an exponential model provides a good description of the bias for intra-relative to inter-arm exchanges also at low LET. Concerning the dependence of G on the radiation quality, the simulations and the data by Bauchinger and Schmid [7] showed an increase with LET, but this difference was not found by Benkhaled *et al.* [29], nor Deng *et al.* [30].

3˙2. *Fibroblasts.* – The approach described in sect. 3.1 has been extended to human fibroblasts exposed to low- and high-LET radiation. In [13], the results obtained at low LET have been compared with the data on γ-irradiated AG1522 fibroblasts reported in [31]. The exponential model, which showed a good agreement with dicentrics and rings and a reasonable agreement with (total) acentrics (with $r_0 = 0.7\,\mu$m and $4.0\,\mathrm{CL} \cdot \mathrm{Gy}^{-1} \cdot \mathrm{cell}^{-1}$), described the data better than the Gaussian one, which provided good agreement for dicentrics and rings (with $\sigma = 1.3\,\mu$m and $2.0\,\mathrm{CL} \cdot \mathrm{Gy}^{-1} \cdot \mathrm{cell}^{-1}$) but substantially underestimated acentrics.

Concerning high-LET radiation, in [14] the exponential model has been tested against alpha-particle data reported in the same experimental paper [31]. The results compared reasonably well with the data on dicentrics and acentrics (with $r_0 = 0.7\,\mu$m, *i.e.*, the same value used for photons, and $0.47\,\mathrm{CL}/\mu\mathrm{m}$); however, the rings were overestimated. The simulations were then repeated by applying the Gaussian model; the results obtained with $\sigma = 1.3\,\mu$m (*i.e.*, the value used for gamma rays) and $0.32\,\mathrm{CL}/\mu\mathrm{m}$, which are reported in fig. 2, showed good agreement with dicentrics and an overestimation of rings (analogous to the exponential model), but also an underestimation of total acentrics. This indicates that the exponential model describes the data better than the Gaussian one also in fibroblasts.

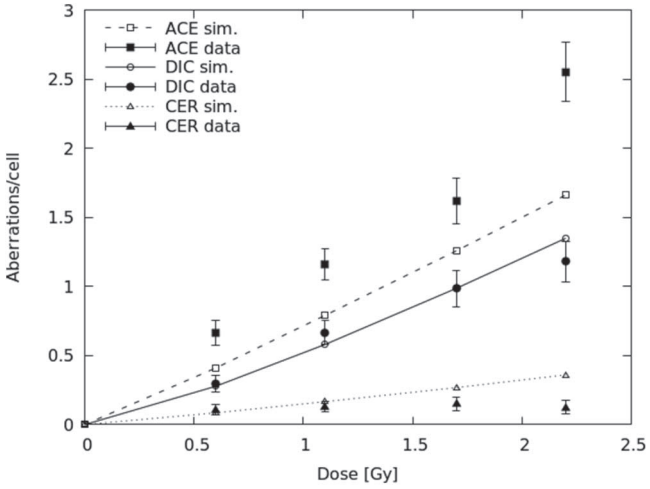

Fig. 2. – Dicentrics (DIC), centric rings (CER) and excess acentric fragments (ACE) in human fibroblasts exposed to 116 keV/μm alpha particles. The open symbols, connected by lines to guide the eye, represent simulation outcomes obtained by the Gaussian model (with $\sigma = 1.3\,\mu$m and $0.32\,\mathrm{CL}/\mu$m), whereas the closed symbols are experimental data taken from [31].

Like for lymphocytes, also for fibroblasts the analysis on F and G was mainly performed by the exponential model. A comparison with the results reported in [31], which has been performed in [14] and is reported in table III, showed an underestimation of G at low and high LET, as well as an underestimation of F at high LET. Even lower G values were obtained by the Gaussian model; reducing r_0 in the exponential model provided higher values for G but lower values for F. This would suggest that, in fibroblasts, an exponential function of the form $\exp(-r/r_0)$ underestimates the bias for intra- to inter-arm exchanges, which in fibroblasts is more pronounced than in lymphocytes. Analogous conclusions can be drawn if the simulations are compared with the data reported by Muhlmann-Diaz and Bedford [32], who found a G-ratio of about 6 for human fibroblasts exposed to gamma rays.

However, applying an exponential model to X-irradiated human fibroblasts, Sachs *et al.* [9] found a G-ratio of about 4, as well as very good agreement with experimental yields of dicentrics, translocations, centric rings and eight different types of complex exchanges (with $r_0 = 0.8\,\mu$m). This suggests that the exponential model may work not only in lymphocytes but also in fibroblasts, and possibly other cell types like epithelial cells, for which Durante *et al.* [33] found $G \sim 6$ for X-rays and $G \sim 10$ for alpha particles.

This would imply that, independently of radiation quality (and possibly of cell type, if this is confirmed for other cells), the dependence of the rejoining probability on the (initial) break distance can be described by a (negative) exponential law. Interestingly, Friedland and Kundrát [34] found that such exponential decrease is consistent with a mechanism of (semi-confined) break-end diffusion, as suggested by several experimental works (*e.g.*, [35]). According to Friedland and Kundrát [34], the confinement is determined by the presence of nuclear attachment sites between two subsequent chromatin loops. Assuming, for instance, that a loop size is $\sim$100 kbp and that each 1.2 kpb structure corresponds to 10 nm, one would obtain that the maximum distance that a free end can travel is 0.8 μm, consistent with the characteristic distance for the rejoining

TABLE III. – *F-ratio and G-ratio in AG1522 human fibroblasts (data from* [31]*).*

Radiation	F-simulation	F-data	G-simulation	G-data
γ-rays	5.1	5.3 ± 0.6	2.2	6.8 ± 0.8
α-particles	3.2	5.7 ± 1.0	5.5	10.3 ± 1.8

probability found in this and other works. Possible differences in the characteristic distance between different cell types would reflect differences in chromatin mobility and/or in the loop size.

Concerning the dependence of F and G on the radiation quality, G increased with LET both in the simulations and in the data, supporting its use as a fingerprint of high-LET exposure. On the contrary F decreased with increasing LET in the simulations, but not in the data. This suggests that F may be a better high-LET fingerprint in lymphocytes and possibly other small cells (which implies a small number of nucleus traversals and thus a small inter-track effect), whereas G may be a better fingerprint in fibroblasts and possibly other larger cells like epithelial cells.

4. – Conclusions

By means of the BIANCA biophysical model, a previous study on proximity effects in human lymphocytes and fibroblasts exposed to different radiation qualities was extended, performing further comparisons with experimental and theoretical works available in the literature. We therefore concluded that the dependence of the rejoining probability on the initial distance between two break ends can be described by an exponential function of the form $\exp(-r/r_0)$ (with r_0 in the order of $\sim 1\,\mu$m) in both cell types, although some aspects (*e.g.*, the underestimation of the G-ratio in fibroblasts by the BIANCA model) need for further investigation. Since such exponential dependence has been shown to be consistent with a mechanism of break-end (confined) diffusion, this supports the hypothesis that, at the relatively short times required for chromosome aberration production, the mobility of the various break ends, which, in turn, may be related to the size of chromatin loops, is mainly governed by diffusion. Of course this does not exclude that other mechanisms, including active transport by motor proteins, may play a role at longer times. Concerning the use of specific aberration ratios as fingerprints of high-LET exposure, this work suggests that F may be a better fingerprint for lymphocytes (and possibly other cells with similar nucleus geometry, *i.e.*, shape, dimensions, 3D chromatin organization...), whereas G may be a better fingerprint for fibroblasts and other cells with similar geometry.

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