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Monte Carlo Predictions of DNA Fragment-Size Distributions for Large Sizes after HZE Particle Irradiation

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Abstract

DSBs (double-strand breaks) produced by densely ionizing space radiation are not located randomly in the genome: recent data indicate DSB clustering along chromosomes. DSB clustering at large scales, from > 100 Mbp down to ≈ 2 kbp, is modeled using a Monte-Carlo algorithm. A random-walk model of chromatin is combined with a track model, that predicts the radial distribution of energy from an ion, and the RLC (randomly-located-clusters) formalism, in software called DNAbreak. This model generalizes the random-breakage model, whose broken-stick fragment-size distribution is applicable to low-LET radiation. DSB induction due to track interaction with the DNA volume depends on the radiation quality parameter $\mathbb{2}$. This dose-independent parameter depends only weakly on LET. Multi-track, high-dose effects depend on the cluster intensity parameter λ, proportional to fluence as defined by the RLC formalism. After λ is determined by a numerical experiment, the model reduces to one adjustable parameter $\mathbb{2}$. The best numerical fits to the experimental data, determining $\mathbb{2}$, are obtained. The knowledge of λ and $\mathbb{2}$ allows us to give biophysically based extrapolations of high-dose DNA fragment-size data to low doses or to high LETs.

KEYWORDS: Polymer chromatin model, non-random DNA breakage.

1. Introduction

High-LET[1] space radiation includes energetic, fully ionized heavy ions, whose number, when traversing a certain area, obeys Poisson statistics [1]. The traversal of tissue, however, is modified by atomic/molecular energy-loss processes and nuclear reactions [2]. These high-LET ions are mostly Fe, but other nuclei, such as N, are also present. We focus on HZE particles because they induce non-random breakage in DNA, as the result of the sharp energy profile in a track [3, 4, 5].

Recent pulsed-field gel electrophoresis (PFGE) experiments at LET ≈ 100 keV/μm or more [6, 7, 8] measure the corresponding distributions of DNA fragment sizes, where 'size' is used, here and throughout, to mean DNA content. The ionizations due to one high-LET radiation track are spatially correlated, being predominantly near the line representing the center of the track rather than spread randomly over a whole cell nucleus. Localization of ionizations is determined by the type of incident particles and leads to clustering of DSBs along chromosomes. A determistic model [4] of track structure will be used to calculate the profile of energy imparted to the cell interior.

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[1] Abbreviations: DSB = DNA double-strand break; DNAbreak = computer program based on a Monte Carlo algorithm for chromatin and its breakage by radiation tracks; PFGE = pulsed-field gel electrophoresis; LET = linear energy transfer; kbp = 10^3 base pairs; Mbp = 10^6 base pairs; RLC formalism = randomly-located-clusters formalism; HZE particles = high-LET ions in space environment.

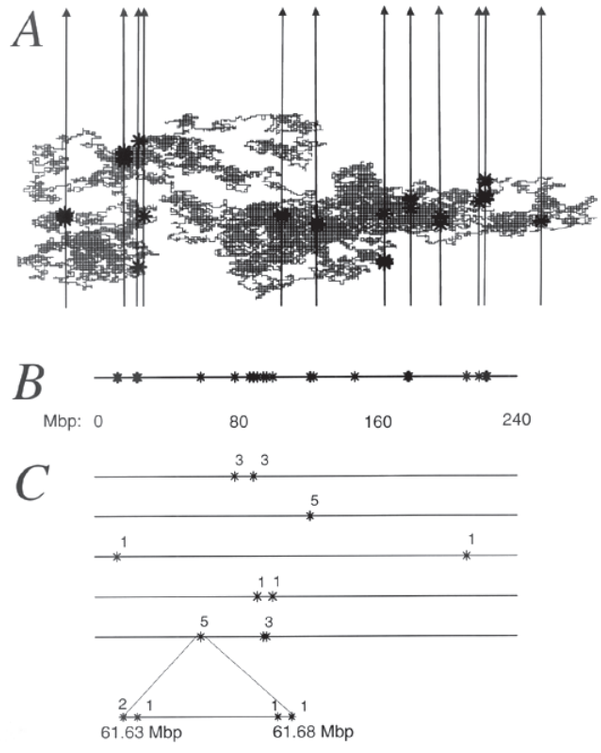

Fig. 1 – DSB clustering along chromosome. Panel A depicts a chromosome represented by a random walk and tracks hitting the DNA volume (tracks that have missed DNA are not shown). Panel B is the locations of DSBs (stars) produced along the chromosome contour, shown as a line. In reality the picture of DSBs on Panel B is the result of juxtaposition of many DSB clusters produced by one track, examples of which are shown on Panel C. Subcluster multiplicities are given by numbers; one of the subclusters is resolved at the bottom of Panel C.

Recent high-LET PFGE data on mammalian cells include results on larger fragment sizes. There are 'globally' multiply damaged sites. The DNA is multiply folded during interphase, but even on large scales DNA loci which are separated by fewer base pairs along the DNA contour tend, at least on average and neglecting fine-structure, to be closer [9]. With this kind of geometry, a bias for ionizations close to each other in space, at high LET, produces a bias for DSBs close to each other along the DNA (Fig. 1).

2. Theoretical Background

The intuitive ideas underlying the RLC formalism are that one-track action can make a stochastic cluster of DSBs along a chromosome; that different clusters on a chromosome, due to different tracks, are independent; and that the location of clusters in the genome, unlike the non-random location of DSBs within one cluster, is random (hence the term randomly-located-clusters, RLC).

The main purpose of the RLC formalism is relating low-dose, one-track effects to high-dose, multitrack effects. The basic equations use one-track DNA fragment-size distributions to determine dose-dependent multi-track DSB clustering patterns. Fragment-size frequencies are not linear in dose because of the juxtaposition of DSBs from different tracks on one chromosome. The formalism facilitates extrapolations of the high-LET PFGE data, usually obtained using large doses (> 100 Gy), to the much smaller doses (10-500 mGy) relevant to risk estimation during space flights.

The main result of the RLC formalism [10] is given by the formula:

$$\Phi = -\left[\left[s\lambda(1-\tau s)\left[M\left(1-F(s)\right)+F(s)\right]+1\right] \times \exp\left[-\lambda\left[s+(M-1)SE(s)\right]\right]\right]_1^2 \quad (1)$$

where the DNA fraction (by size, i.e. by DNA content) Φ is evaluated for those DNA fragments that have sizes in the range from S_1 to S_2. The brackets are to be evaluated at $s = S_1$ and $s = S_2$.

Other parameters: λ is the cluster intensity (in the numerical model described here, it is one of the adjustable parameters); τ is a reciprocal of the chromosome size; M is the cluster multiplicity; F is the cumulative fragment-size distribution function of one DBS cluster; E is the remaining-size distribution function of one DSB cluster.

Because the high-dose multi-track quantity Φ is expressed through the low-dose one-track quantities F and E, only a numerical simulation of the one-track action will be carried out. The test of the RLC formalism was done [11, 12] previously. Here the RLC formalism is used to speed up the simulations.

3. Methods

Previous work on the DNAbreak model was done with a simplistic track structure that allowed one to simulate only α-particles [11], whose tracks have only small radial distribution of energy (< 50 nm). In this article a more general track structure is introduced by means of incorporating the radial energy distribution code [4]. The code uses a deterministic model of an ion track, that takes into account average energies imparted to DNA by the ion itself and secondary electrons.

The profile of energy imparted to DNA is thus a function of E, the energy of an ion, A, its atomic weight, and Z, its charge, and the distance t from the track core. DNA will be represented by a polymer, whose monomers occupy lattice sites [11]. The radial energy profile will be used to calculate the probabilities of having a DSB on a monomer, given the monomer location t, which is a distance between a monomer and the track center in the XY plane (that is in the plane perpendicular to the direction of radiation). This probability is given by

$$\psi = 1 - e^{-\mathcal{Q}D(E,A,Z,t)} \quad (2)$$

where $D\ (E, A, Z, t)$ is the energy imparted to DNA at a site $t = t_i$ (i is an index for a specific lattice site that has a monomer); and $\mathcal{Q}$ is a radiation quality parameter, which controls the probability ψ to create at least one DSB on a monomer given the amount of energy $D\ (E, A, Z, t)$ at a monomer. In this model a monomer can have at most one DSB, neglecting more detailed pattern of damage on scales below one monomer. Thus the lowest limit of resolution in this model is about 2 kbp. The units of $\mathcal{Q}$ are Gy^{-1}, and it is an adjustable parameter in the DNAbreak algorithm, to be determined from numerical fits to the PGFE data. All radiochemistry involved in creation of DSBs by ionizing radiation is encapsulated in $\mathcal{Q}$.

4. Results

We discuss results for Fe and N ions at two different doses. The cluster intensity λ is determined from a multi-track simulation run at the experimental fluence. The adjustable parameter $\mathcal{Q}$ is determined from a numerical fit obtained at fixed λ. The one-track contribution to DNA breakage will be shown in one case. The dependence of the fragment-size distribution function on LET will be also shown in one case. Finally, the behavior of the adjustable parameters under various experimental conditions is analyzed in application to the predictions of DNA damage by various ions and at various LETs.

The least-square fit was done varying $\mathcal{Q}$ for four datasets: N ions with LET=97 keV/μm at doses 70 Gy and 189 Gy; Fe ions with LET=151 keV/μm at doses 80 Gy and 189 Gy [6]. The results are summarized in Tables I, II and III.

Table I - Experimental Fluences.

Fluences, mm^{-2}	N	Fe
High Dose	12.2	7.81
Low Dose	4.51	3.31

Table II - Cluster Intensity

λ, Mbp^{-1}	N	Fe
High Dose	0.275	0.174
Low Dose	0.120	0.0745

Table III - Radiation Quality Parameter

$\mathcal{Q}$, Gy^{-1}	N	Fe
High Dose	1.33×10^{-4}	4.94×10^{-4}
Low Dose	1.29×10^{-4}	5.00×10^{-4}

Using LETs and doses from the experimental data the fluences can be calculated (Table I). Then, a multi-track simulation calculates the number of hits on average that DNA receives. This determines λ's (Table II). The results of the numerical fits are summarized in the following table for the parameter $\mathcal{Q}$ (Table III). One observes only a weak dependence of $\mathcal{Q}$ on LET, and no dependence on dose.

4.1. *One-track contribution and low-dose extrapolation*

For both low-dose and high-LET extrapolations we will focus on Fe ions acting on human fibroblasts [6] at the dose 189 Gy, LET=151 keV/μm and fluence = 7.81 μm^{-2} (Fig. 2). The DNAbreak algorithm allows one to show explicitly the one-track contribution to the fragment-size distribution function produced by Fe ions. This allows an extrapolation to low doses when one-track action dominates. This result is very useful, as the PFGE experiments were done at very high doses. At low doses a chromosome is either not hit, or hit by one ion in the majority of events. In the latter case only one DSB cluster is present, and the results shown in Figure 2 are applicable.

4.2. *Prediction for high LETs*

DNAbreak also allows one to extrapolate results to high LETs for the same ions. The parameters $\mathcal{Q}$ and λ are determined from the best numerical fit (Fig. 2). The parameter $\mathcal{Q}$ may weakly depend on LET: this will be checked in more detail in the future work. LET is controlled by an ion energy E in MeV/u, which the dose in one track depends on. This leads to higher D and a different fragment-size distribution function as shown on Figure 2. For comparison, a case with a smaller LET is also shown.

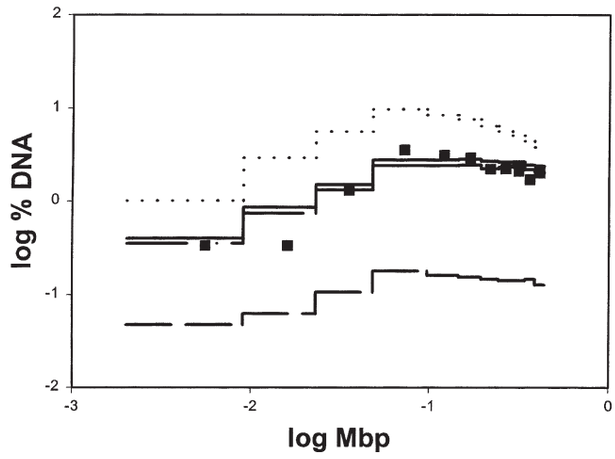

Fig. 2 – Least square fit to the Fe ion data. We plot DNA mass fraction vs. genomic content in Mbp in a log-log scale. The solid line is a least square numerical fit to the PFGE data (boxes) with the values of the adjustable parameters determined as $\mathcal{Q} = 4.94 \times 10^{-4}$ Gy^{-1} and $\lambda = 0.174$ Mbp^{-1}. The dashed line shows the predicted one-ion contribution to DNA breakage as it would happen at a low dose (that is, the same LET, but very low fluence). The dotted line is the predicted result of DNA breakage at higher LET as predicted by the model: LET = 495 keV/μm with $\mathcal{Q}$ and λ the same as for solid line. For comparison, a predicted low-LET (LET = 139 keV/μm) case is shown as a dot-dashed line.

5. Conclusion

In addition to locally and regionally multiply damaged DNA sites, globally multiply damaged sites also occur at high LETs. They can be analyzed by assuming a random-walk model of chromatin. This leads to a coarse-grained approach, not useful for small DNA fragment sizes but capable of dealing with size scales spanning more than four orders of magnitude, including scales at least three orders of magnitude larger than previously considered. The analysis gives the patterns for one-track DSB clusters and a systematic way to see what happens when different DSB clusters, from different tracks, overlap or come close to overlapping. It relates the high-dose experimental data to the one-track action of primary interest in such applications as radiotherapy, biodosimetry, or risk estimation for carcinogenesis. We have here shown that extrapolations in LET may also be feasible.

Acknowledgments

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