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CHROMOSOME ABERRATION MODEL COMBINING RADIATION TRACKS, CHROMATIN STRUCTURE, DSB REPAIR AND CHROMATIN MOBILITY

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The module that simulates the kinetics and yields of radiation-induced chromosome aberrations within the biophysical code PARTRAC is described. Radiation track structures simulated by Monte Carlo methods are overlapped with multi-scale models of DNA and chromatin to assess the resulting DNA damage. Spatial mobility of individual DNA ends from double-strand breaks is modelled simultaneously with their processing by the non-homologous end-joining enzymes. To score diverse types of chromosome aberrations, the joined ends are classified regarding their original chromosomal location, orientation and the involvement of centromeres. A comparison with experimental data on dicentrics induced by gamma and alpha particles shows that their relative dose dependence is predicted correctly, although the absolute yields are overestimated. The critical model assumptions on chromatin mobility and on the initial damage recognition and chromatin remodelling steps and their future refinements to solve this issue are discussed.

INTRODUCTION

Chromosome aberrations represent a traditional but not outdated end point in radiation biology[1]. They link DNA damage induction and repair with cell survival[2, 3] and are associated with cancer[4].

In the breakage-and-reunion theory, chromosome aberrations result from ligating a broken chromosome end with another, inappropriate end, closely resembling the non-homologous end-joining (NHEJ) pathway of double-strand break (DSB) repair. According to the one-hit theory, a single chromosome break may interact with undamaged chromatin and induce an aberration; the homologous recombination repair of DSBs may represent the underlying molecular mechanism.

A number of mathematical models describing the induction of chromosome aberrations by radiation have been proposed[5–14]. They treat DSBs as elementary units of DNA damage. However, most models[6–13] do not directly account for the underlying DNA damage repair. Instead, they employ the concept of the effective DSB misrejoining probability, though with various assumptions on its dependence on the distance between DSB pairs. The models also somewhat differ in the level of details considered on radiation tracks and chromatin structure.

The chromosome aberration module within the PARTRAC biophysical simulation tool aims at providing this missing link between the molecular DNA damage response mechanisms and the induction of chromosome aberrations. Contrary to previous approaches[5–14], the present model explicitly simulates individual free ends of the chromatin fibre, their mobility and enzymatic processing before and after synapsis formation. In this work, the modelling approach and its implications[15] are reviewed, and its future refinements and extensions are discussed.

METHODS

The modelling of chromosome aberrations[15] within the PARTRAC suite of biophysical codes[16] combines the simulation of radiation tracks, chromatin structures, kinetics of DSB repair and chromatin mobility.

Radiation tracks can be simulated in PARTRAC for electrons, photons, protons, alpha particles and heavier ions. The event-by-event method is used, following all relevant interactions of the primary particle as well as all secondary and higher-generation particles. Subsequently, the energy deposition events taking place in the volume of DNA are scored as direct effects, while events outside DNA are passed into the pre-chemical and chemical modules in which water radiolysis is simulated. The formation, diffusion and mutual reactions of reactive species are modelled, and attacks on the DNA scored as indirect effects. The DNA and chromatin target structures, which underlie the assessment of both direct and indirect effects, include atomic models of base pairs, nucleosome, chromatin fibre, euchromatic vs. heterochromatic regions, chromatin loops and domains, and chromosome territories within eukaryotic nuclei. The directly and indirectly induced DNA damages are analysed with respect to damage types including break complexity[16].

The simulated DNA damage is then used as an input for modelling the NHEJ repair of DSBs[16–19], which in turn is the major component of the chromosome

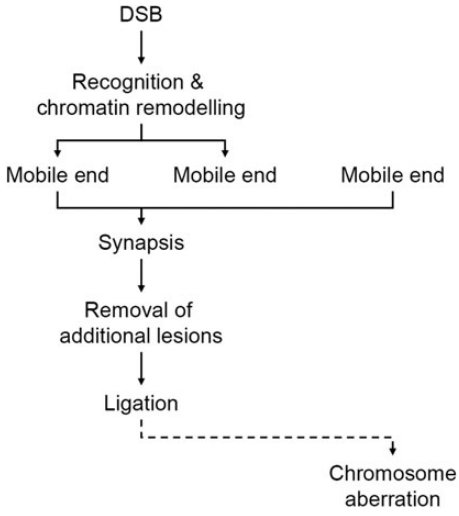

Figure 1. Simplified scheme of the presented chromosome aberration model. DNA damage, in particular DNA DSBs, is assessed by overlapping simulated radiation tracks with multi-scale DNA and chromatin models (not depicted). Following the break recognition, chromatin remodelling takes place. The break opens, and the two induced DNA ends move around independently of each other, yet being limited by corresponding nuclear attachment sites. In parallel, Ku70/80 and DNA-PKcs attach to the ends (not shown). Whenever two ends with these enzymes attached (as indicated not necessarily the original partners) diffuse sufficiently close to each other, a synaptic complex is formed. The ends are processed, and additional strand breaks and base lesions, if present, are removed. Finally, the ends are ligated. If the fragment orientation were reversed or if the ligated ends used to belong to different chromosomes, they are considered in the scoring of chromosome aberrations.

aberration simulations[15] reviewed here. A simplified scheme of the model is shown in Figure 1. First, a DSB recognition and chromatin remodelling step[20] is considered. After this step, the DSB is assumed to open, i.e. form two mobile DNA ends (chromatin broken ends)[21]. The two ends are followed separately in the code. They are allowed to diffuse around, being limited by the corresponding nuclear attachment sites (per 1 Mbp spherical chromatin domain[16] typically 10 anchorage points where the chromatin loop structure approaches the domain's centre). By this, semi-confined diffusion mobility of DNA ends is assumed, but the presence of chromatin fibres hindering the ends' mobility is not considered explicitly. In parallel with diffusive mobility of the ends, also the association of repair enzymes is considered. The ring-like Ku70/80 heterodimer has to bind to an end first, followed by the DNA-PKcs subunit, forming together the functional DNA-PK enzyme. When two ends with DNA-PK attached happen to appear in close vicinity, they form a synaptic complex. The two DNA-PK molecules cross phosphorylate each other, and further repair proteins bind to the site and perform their tasks. In particular, end processing and removal of additional strand breaks and/or base lesions take place before the final ligation of the two fragments in synapsis.

It may happen that not the original partners, i.e. the two DNA ends formed by a DSB, but another pair of ends get ligated. This often happens when a free end diffusing around with attached DNA-PK finds an incorrect partner ready for joining, e.g. because its original partner, though in proximity, lacks DNA-PK and cannot be ligated yet. Whenever such incorrect joining occurs, the fragments' orientation is tested (to assess inversions), and information is recalled on the chromosomes that the joined fragments used to belong to and whether centromere regions are involved. The output files listing the kinetics of chromosome aberration induction are updated accordingly.

Further details on the methods, specific assumptions and parameters used can be found in Ref.[15].

RESULTS

The yields of dicentrics predicted by the chromosome aberration module in PARTRAC[15] have been compared with measurements for AG1522 primary human skin fibroblasts irradiated by 0–6.1 Gy of ^{137}Cs γ rays or 0–2.2 Gy of ^{238}Pu α particles[22]. The simulations provide a linear-quadratic dose dependence for γ rays and a linear one for α particles, as observed in the experiments. Aberrations induced by α particles thus arise from misrejoining of DNA ends formed by single particles, whereas for γ rays the inter-track misrejoining represents a significant contribution. The data are reproduced in absolute terms when the simulated yields are reduced by *ad hoc* factors[15] of five for γ rays and two for α particles (Figure 2).

The model is also capable of assessing the size distributions of aberrant chromosomes or even of individual fragments contributing to aberrations[15]. Importantly, by combining spatial mobility of DNA ends with the kinetics of DSB rejoining, the model makes predictions on the DSB misrejoining probability. This probability, used as an input in existing chromosome aberration models, is predicted to decrease exponentially with increasing original distance between the two DNA ends. Incorrect joining within a chromosome (intrachromosomal misrejoining) dominates at short scales; for α particles, 50 % of such events happen within 80 nm initial separation of the ligated ends. Incorrect joining of fragments from different chromosomes appears over longer scales, with 50 % events within 280 nm[15].

DISCUSSION

The chromosome aberration module in PARTRAC represents to the authors' knowledge the first detailed

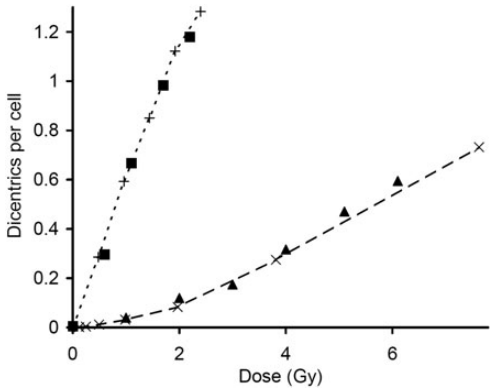

Figure 2. Predicted absolute yields of dicentrics[15] scaled down by factors of 5 for γ rays (×) and 2 for α particles (+) compared with data (γ rays: ▲, α particles: ■) gathered by Cornforth *et al.*[22]. Lines connecting the scaled simulation results are included to guide the eye only.

mechanistic bottom-up model that combines into a unique simulation framework the information on radiation track structures, multi-scale DNA and chromatin structures, kinetics of enzyme attachment and DSB repair, and chromatin mobility. Naturally, this systems-based approach may serve as a tool for testing specific hypotheses on the mechanisms of radiation action on biological systems.

The present chromosome aberration simulations build in particular on the following hypotheses. First, radiation-induced DSBs are supposed to lead to pairs of free ends of DNA molecule and to manifest also at the level of the chromatin fibre, i.e. to produce chromosome fragments. This hypothesis is supported by the sophisticated DNA end monitoring experiments by Soutoglou *et al.*[21]. By a specific restriction endonuclease, the authors induced a single DSB in living cells at a defined genomic site. Then, they followed in real time the fate of the two DNA ends that had been labelled for fluorescence beforehand. The local separation of the two tags labelling the ends more than doubled after the DSB induction. The local separation of the ends was especially high in cells lacking Ku but not in cells deficient in other repair factors, providing further evidence for the mechanisms assumed in the present DSB repair and chromosome aberration model.

The second set of assumptions concerns the mobility of chromatin ends. This has been modelled by a diffusion process confined by nuclear attachment sites where chromatin fibres are anchored. Synaptic complexes have been also allowed to diffuse, yet with a 10-fold reduced diffusive coefficient to reflect reduced mobility of synaptic complexes that typically belong to chromatin loops anchored at two attachment sites. This diffusion model has reproduced[15] the effectively subdiffusive mobility over several hours scale, observed for diverse chromosomal loci including those containing DSBs[23]. However, the fact that the absolute aberration yields constantly overestimate the measured data (Figure 2) indicates that the long-range mobility and the number of free chromatin ends at later repair times might have been overestimated.

Third, the present modelling scheme supposes that the speed of DSB rejoining kinetics can be traced back to lesion complexity. The distinctive steps slowing down the DSB repair kinetics in the model are the post-synaptic steps in which additional lesions on dirty ends from complex DSBs are removed. The pre-synaptic processing of clean and dirty ends is almost identical[15]. Damage complexity may in principle affect the repair outcome and aberration formation indirectly through prolonged post-synaptic processing of dirty ends, which provides more chance for reverse processes to break the synaptic complex[16]; however, such processes have been neglected in the aberration modelling so far[15]. Taken together, the simulated yields and kinetics of chromosome aberrations are governed by DSB proximity rather than by lesion complexity. Most chromosome aberration models are based on this hypothesis, although the postulated distance dependence of DSB misrejoining varies[6–9, 11–14]. A contrary assumption has been used by Ballarini *et al.*[10], who postulated that only complex lesions are responsible for chromosome aberrations, but the misrejoining probability is uniform over micrometre ranges.

Finally, the damage recognition and chromatin remodelling processes have been modelled so far by a single step[15, 16], which likely oversimplifies the underlying biological mechanisms[20, 24]. The involved processes and their extent are not identical in hetero- and euchromatin and likely may vary with damage complexity too.

CONCLUSION

The mechanistic bottom-up approach to chromosome aberration modelling in PARTRAC combines radiation track structures, multi-scale DNA and chromatin models, kinetics of enzyme attachment and DSB repair, and chromatin mobility. Contrary to other model approaches, chromosome aberrations are not assumed to result from pairwise misrejoining of DSBs but of chromatin free ends that are tracked individually. With the presently used parameters, the model provides correct relative but too high absolute dose-dependent yields of dicentrics for both γ rays and α particles, indicating the need for further refinements. Nevertheless, the module and the whole PARTRAC suite possess a solid mechanistic basis and as such enable alternative hypotheses on the mechanisms of radiation action on biological systems to be tested in quantitative terms. If, for example, the assumption were implemented that the two chromatin fibre ends

induced by a DSB stay tethered and diffuse together until potentially meeting another damage site and forming an aberration, the present approach would represent a detailed analogue of the existing aberration models. If complemented with chromatin models for S/G2 phase cells, the present model could predict also chromatid-type aberrations originating from NHEJ repair. Likewise, the model could be used to simulate chromosome aberrations in cells deficient in pre- or post-synaptic NHEJ enzymes.

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