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Analysis of DNA fragment size distribution following rejoining of radiation – induced DNA double-strand breaks

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The repair of DNA double-strand breaks is commonly quantified by measuring the time-dependent decrease of the mass-fraction of fragmented DNA that is able to enter electrophoresis gels. When converted into an equivalent smaller dose (by comparison with an appropriate sample without repair), this value is reported as fraction of residual breakage and the derived kinetics typically exhibits a rapid initial component and a decreasing rate at longer repair intervals. This formalism, however, assumes that the spatial distribution of unrejoined breakage follows the prediction of randomness, such as found for initial DSBs when resolved by conventional pulsed-field gel electrophoresis (PFGE). Giving this presumption, no data have been available explicitly testing this relationship for residual breakage, except for very recent observations with transformed cells where a disagreement was elicited [Gauter *et al*. 2002]. Therefore, analogous experiments with non-transformed cells were performed in order to characterise the respective phenomenon in more general manner.

Human embryonic lung fibroblast (MRC-5) and osteoblasts in primary culture were irradiated with different doses (10-120 Gy) or were incubated for repair for up to 6 h after a single dose of 90 Gy (MRC-5) or 80 or 120 Gy (osteoblast) before PFGE measurement. The analysis of DNA-DSB induction was performed using the RBM formalism. Fragment length distributions were calculated by convolution of the PFGE profiles with an appropriately generated size calibration function.

The integral formulation of the random breakage model was fitted to the experimental data by using upper DNA size limits of 4.6 Mbp, 3.5 Mbp and 2.2 Mbp (such as represented by chromosomal size standards) and with the DSB induction rate as adjustable parameter. This yielded values of 0.0076 Mbp^{-1} Gy^{-1} for MRC-5 and
0.0078 Mbp^{-1} Gy^{-1} for osteoblast cells, respectively. The excellent agreement of the measured dose-dependencies with the RBM prediction underscores its applicability for the fragment length range considered. It was shown that the DSB induction rate increases strictly linear with dose. The investigated cell types show negligible difference in the average induction rate, as expected.

The time-dependent amount of residual breakage was calculated for different fragment size ranges by application of the dose equivalent approach. Similar to the recent observations with transformed cells, the fraction of calculated residual breakage was dependent on the selected size ranges. The fragment profiles after different repair intervals exhibited a clear deviation from RBM-like distributions (overdispersion with an increased relative abundance of fragments detected in the 2 - 3 Mbp size range). But for extended repair times, also this persisting fragmentation was removed.
The present observations demonstrate non-randomness of fragment distributions after incubation for repair for non-transformed cells and confirm the earlier suggestion of a proximity effect for DSB rejoining [Gauter *et al*. 2002]. Interestingly, it appeared that with the non-transformed cells larger fragments were included in the phenomenon of a relative reduced rate of rejoining.

As an important methodological aspect, the possible accumulation of irradiated cells in S-phase during incubation for repair was tested. Such perturbed cell cycle distributions can seriously confound PFGE measurements, particularly, when cell

have deregulated cell cycle control functions (i.e. the transformed cell systems of the previous study [Gauter *et al*. 2002]). Measurements using flow cytometry and with a reirradiation protocol, however, did not indicate any S-phase accumulation during incubation for repair. Another aspect is the mobility inversion effect that is a frequently observed complication in the analysis of PFGE experiments. The excellent fit of the RBM curves to the experimental data, however, strongly indicates that no inversion of mobility had occurred in the potentially affected size range of the *S. pombe* chromosomal markers.

In consequence, the time-dependent decrease of dose-equivalent values calculated from data on the fraction-released assay may not directly reflect DSB rejoining rates. The present findings are compatible with an earlier suggestion of slow rejoining of breaks when such breaks had been induced (by chance) as multiples in large chromosomal structures or loops of several Mbp size. The mechanism behind this proximity effect is presently unknown but could reflect a functional link between nuclear chromatin organization and the efficacy of radiation-induced DSB removal. An elucidation of the respective molecular process(es) will significantly increase our understanding of a fundamental aspect of the cellular response to initial radiation damage.