

Experimental and modeling analysis of γ H2AX dose response curves in the framework of the GLOBLE model*

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Among the spectrum of DNA lesions induced by ionizing radiation, the Double Strand Breaks (DSB) are generally recognized as the type of DNA damage more directly related to cell killing. Nowadays, the γ H2AX assay represents one of the methods of choice for the study of DSB induction and processing [1]. The method is based on the phosphorylation of histone H2AX in presence of a DSB. Importantly, the phosphorylation extends over a large chromatin region in the order of the Mbp, reaching its maximum in about 30-60 min after irradiation, and then slowly decaying as the damage is processed [2]. The phosphorylated molecules can be tagged with a fluorescent antibody, giving rise to the so-called γ H2AX foci, which can then be observed and/or analyzed with different techniques. Consequently, γ H2AX foci are considered as a marker for DSB presence.

The Giant LOop Binary LEsion (GLOBLE) model is a radiobiological model developed in order to predict biological effects resulting from photon irradiation [3]. The cell nucleus where the genome is stored is identified with the critical target. The assumption is made, that the higher-order chromatin organization can be described by means of Giant Loop structures, each one involving about 2 Mbp of genome [4]. The simulated nucleus is thus divided into cubic domains of about 500 nm edge length resembling the DNA organization into loop structures. For a given dose, DSB are distributed among nuclear domains, assuming that the level of DSB clustering at the micrometre scale defines the severity of the induced damage.

Our aim here is to compare GLOBLE predictions in terms of DSB induction patterns with measurements of integral γ H2AX fluorescence obtained with flow cytometry. This is based on the hypothesis, supported by the literature, that a direct relation exists among γ H2AX foci and chromatin domains as defined in the model [1,5]. Specifically, we make the hypothesis that in presence of at least one DSB the whole loop is phosphorylated, and that the presence of additional DSB does not lead to increased fluorescence. After defining as *hit domain* a domain where at least one DSB is induced, we can compare the measured signal with predicted numbers of hit domains.

Fig. 1 shows a typical example of model predictions. The curves are calculated combining the standard domain size of 2 Mbp, or an enlarged one of 8 Mbp, with a DSB in-

duction yield of 30 DSB per Gy and cell nucleus. At high doses the tendency to saturation is observed, reflecting that the majority of available domains are hit. Intuitively, the domain size will influence the dose at which saturation is reached. It thus emerges that this analysis could give indirect indications concerning the actual dimension of chromatin loops.

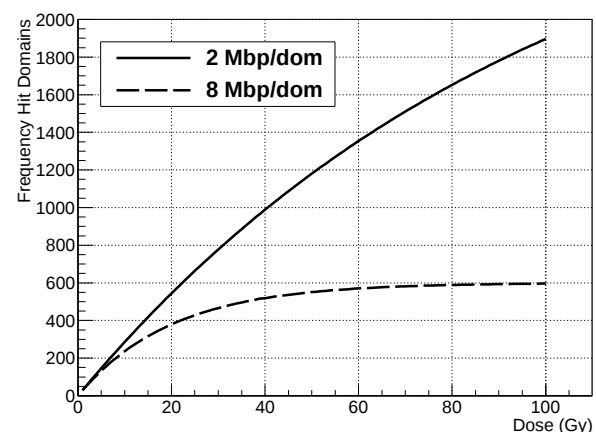


Figure 1: simulated dose response curves calculated assuming a standard DSB induction yield of 30 DSB/Gy and a domain size of 2 Mbp and of 8 Mbp.

Experiments will involve the use of mammalian cell lines, which will be irradiated in an extended dose range. The comparison will allow testing the validity of basic modeling assumptions. The influence of the values assigned to different model parameters (e.g. DSB induction rate, domain size) will be investigated. Importantly, this will be relevant also for the model extension to heavy ion irradiation, which is currently under development in our group. At the same time, the systematic study of γ H2AX phosphorylation over a large dose range in different cell lines could contribute to a better understanding of the relation existing between DSB and γ H2AX foci, as well as of the phosphorylation process itself.

References

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* This work was supported by a DFG-funded Graduiertenkolleg (GRK 1657). The work is part of HGS-HIRe.

