# CHROMATIN ORGANIZATION REVEALED BY NANOSTRUCTURE OF IRRADIATION INDUCED γH2AX, 53BP1 AND Rad51 FOCI USING STED MIRCOSCOPY

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Ionizing radiation induces double-strand breaks (DSB) with varying local density and complexity with respect to the LET (Linear Energy Transfer). Upcoming evidence indicates that proteins responsible for detection and repair of DSB cluster in different structural and/or functional domains. These domains may be connected to the folding of the chromatin inside the cell nucleus and therefore to the higher order chromatin structure. We studied the size of structures visible in ionizing radiation induced foci (IRIF) using super resolution STED microscopy with a resolution of 105 nm and compared them to models of higher order chromatin structure. Irradiation was performed at the ion microprobe SNAKE using high LET Carbon ions (LET=500 keV/µm) and low LET protons (LET=2.6 keV/µm). For structural size determination the widths extracted from the autocorrelation function were measured.
The analysis of 53BP1 and γH2AX IRIF reveals an IRIF size of (540±40) nm for high LET irradiation and a significantly smaller size for low LET irradiation. The nanostructure size of (129±6) nm was the same throughout the whole LET range. Whereas for Rad51 no nanostructure was visible and the IRIF size was (143±13) nm irrespective of the particle LET, which is the same as the nanostructure size for 53BP1 and γH2AX IRIF. It is very important that the nanostructure of the IRIF does not depend on the LET although macroscopic IRIF distribution and also damage density is dramatically changing with increasing LET. These results substantiate the argument postulated in Reindl et al. [1], that Rad51 and 53BP1 belong to different repair compartments. The structure size of ~135 nm occurring in all analysed IRIF, points to an underlying biological structure at the damaged region. The measured sizes well correspond to the size of the perichromatin region of the chromosome territories/interchromatin model by Cremer et al.[2]. Here, the size of the region where DNA repair and transcription takes place, the perichromatin region, was measured to be 200 nm. In our study the perichromatin region could be experimentally connected to DNA repair for the first time [3].

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