CORRELATIVE LIGHT AND ELECTRON MICROSCOPY ANALYSIS OF CHROMATIN REORGANIZATION UPON CLUSTERED DNA DAMAGE INDUCED BY CHARGED PARTICLE IRRADIATION

Susanne Tonnemacher[1], Mikhail Eltsov[2], Gisela Taucher-Scholz, Burkhard Jakob
GSI Helmholtzzentrum für Schwerionenforschung, Dept. Biophysik
Planckstr. 1, 64291 Darmstadt, Germany
E-mail: b.jakob@gsi.de

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Chromatin architecture plays major roles in gene regulation as well as in the repair of DNA damaged by endogenous or exogenous factors, such as after radiation. Opening up the chromatin might provide the necessary accessibility for the recruitment and binding of repair factors, thus facilitating timely and correct repair. Charged particle irradiation provides a tool for the generation of locally confined clustered DNA lesions often consisting of multiple DSBs in close vicinity but, compared to frequently used laser micro-irradiation, offers the advantage of representing ionising radiation with a predictable dose deposition in the cell nuclei [1]. The generally observed formation of ionizing radiation-induced foci (IRIF) of repair factors, such as 53BP1, or the histone modification γH2AX upon induction of DNA double-strand breaks has been recently linked to local chromatin decompaction [2,3]. Here we applied correlative light and electron microscopy (CLEM) in combination with DNA-specific contrasting for transmission electron microscopy (Fig. 1) or tomography (3D). We were able to show that at the ultrastructural level, these DNA damage domains induced by irradiation with carbon or iron ions reveal a chromatin compaction and organization not distinguishable from regular euchromatin of the same nucleus [4]. Low Density Areas (LDAs) at sites of particle-induced DNA damage, as observed previously [3] and as well in this study after unspecific uranyl acetate (UA)-staining, are thus unlikely to represent pure chromatin decompaction. RNA-specific terbium-citrate (Tb) staining suggests rather a reduced RNA density contributing to the LDA phenotype at sites of particle traversals.

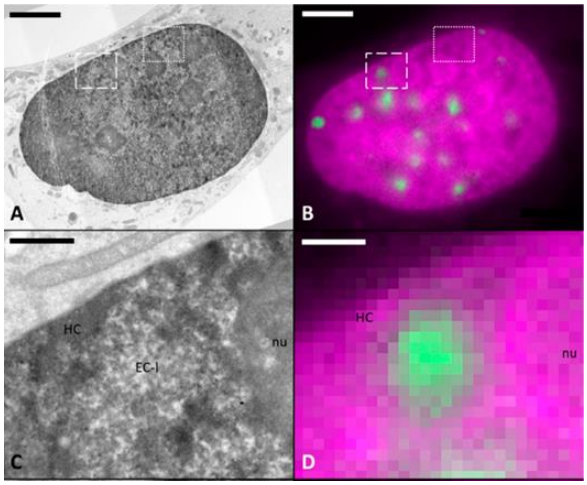

Figure1: CLEM at sites of IRIF in U2OS cells. DNA specific ChromEMT image (A) and corresponding fluorescence image (B) showing DNA (DRAQ5, magenta) and 53BP1 (GFP, green); bar 5µm. (C)&(D) magnification; bar 1µm. Images from [4].

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Present addresses: [1]Paul-Ehrlich-Institut, Langen, Germany; [2]University of Strasbourg, Illkirch, France