Subcellular Processes in Plants and Animal Cells

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Interaction of heavy ions with nuclear chromatin: Spatiotemporal investigations of biological responses in a cellular environment

B. Jakob[1], M. Beuke[1], I. Alber[2], J. Engelhardt[3], S. Hell[3], M. Durante[1,4], G. Taucher-Scholz[1,5]

[1]GSI Helmholtzzentrum für Schwerionenforschung, Biophysik, Darmstadt, Germany
[2]GSI Helmholtzzentrum für Schwerionenforschung, Materialforschung, Darmstadt, Germany
[3]DKFZ, High Resolution Optical Microscopy, Heidelberg, Germany
[4]Technische Universität Darmstadt, Festkörperphysik, Darmstadt, Germany
[5]Technische Universität Darmstadt, Biologie, Darmstadt, Germany

b.jakob@gsi.de

Ion beams offer the possibility to generate strictly localized DNA lesions within subregions of a cell nucleus. The distribution of the ion induced damage can be indirectly visualized by immunocytochemical detection or, in living cells, by GFP-protein constructs of repair related proteins in the form of ionizing radiation induced foci (IRIF). The observed inhomogeneous spatial pattern of lesions depends mainly on the radial dose profile of the traversing particle, but biological properties of the target like chromatin distribution and/or chromatin movement can also affect the obtained images. Introducing an irradiation geometry characterized by a small angle between the plane of the cellular monolayer and the incoming ion-beam allows the spatial analysis of protein accumulations along linear ion trajectories, revealing an unexpected clustering. To study the chromatin and protein dynamics during and after irradiation, a remote controlled microscope device was used at the accelerator facility of GSI. The system enables the acquisition of high-resolution fluorescence images of stained living cells during ion irradiation. This allows us to study early radiation effects without the time lag of minutes presently conditional on limitations of access to the irradiation device.

Time-lapse images of GFP-coupled proteins during irradiation proved accumulations within seconds at sites of ion hits indicating a very fast recognition of DNA damage in combination with a quite stable location of damage processing. The number of observed radiation-induced foci along the ion tracks did not match the expected amounts of DSBs. To gain further insight in the distribution of lesions along the ion path, we applied high resolution 4Pi and STED microscopy revealing a central focus of repair proteins Mre11 or RPA representing multiple DSBs embedded in a speckled cloud of γ-H2AX or 53BP1 visualizing the response on megabase-pair domains. In addition, first attempts to image the DNA damage and IRIF along the ion tracks using scanning electron microscopy (SEM) in combination with immunogold-labeling under conditions preserving the 3D topology of the chromatin are shown.