

A NOVEL X-RAY MICROIRRADIATION SYSTEM FOR REAL-TIME IMAGING OF THE KINETICS OF DNA DOUBLE-STRAND BREAK REPAIR RESPONSES

Emilie C.B. Desclos¹, Jakub A. Kochan¹, Jan Stap¹, Lianne E.M. Vriend¹, Meindert Rijpkema², Barbara Steurer⁴, Jacob A. Aten¹, Jan Verhoeven³, Carel van Oven¹, Ron A. Hoebe¹, Jurgen A. Marteijs⁴, Wim Vermeulen⁴ and Przemek M. Krawczyk¹

¹Van Leeuwenhoek Center for Advanced Microscopy, ²Cluster 3 workshop, Academic Medical Center, University of Amsterdam, The Netherlands

³Kamerlingh Omnes Laboratory, Leiden Institute of Physics, University of Leiden, Leiden, The Netherlands

⁴Dept. Of Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands

E-mail: p.krawczyk@amc.uva.nl

DNA double-strand breaks (DSBs) are among the most dangerous DNA lesions and cellular DSB repair (DSBR) responses are relevant for understanding the mechanisms of tumorigenesis, as well as for cancer diagnostics and treatment. “Localized” DNA damage -- induced in a restricted area of the cell nucleus -- is an important tool in DSBR research because signaling and repair responses triggered by the localized DNA damage can be studied in great detail using microscopical techniques. Induction of DSBs using focused UV laser beams is a commonly applied technique, but there is a growing concern about the poor characterization of the DNA lesions produced by these clinically-irrelevant methods. This is an important issue, since various repair processes are known to be involved in repair of different DNA lesions. To overcome this drawback, we constructed a novel instrument, coined the X-ray multi-microbeam microscope (XM³). XM³ uses ultra-soft X-rays to locally induce DSBs and allows real-time imaging of the ensuing cellular responses. Using this instrument we studied the kinetics of accumulation of various DSBR factors at X-ray-damaged chromatin. We also analyzed the influence of DNA sensitizer Hoechst on accumulation kinetics and compared accumulation upon X-ray microirradiation and UV-A laser microirradiation. Our results hint at the temporal sequence of some DSBR events and demonstrate that Hoechst does not likely influence DSBR responses. However, we find that the accumulation of some DSBR factors may depend on whether the damage is induced by a UV laser or ionizing radiation, striking a cautionary note for DSBR investigations performed with help of laser-microirradiation. Further, we used our system to study the spatial organization of DSBR at super-resolution using the Leica SR GSD 3D microscope. Our results deepen the understanding of cellular responses to clinically-relevant DNA damage.

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