Live cell observation of chromatin decondensation after heavy ion irradiation at the microbeam*

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Introduction

The inaccurate repair of DNA double strand breaks (DSBs), as generated by heavy ion irradiation, can result in chromosome aberrations, cell death and various forms of cancer. To keep genomic stability the cell uses different repair mechanisms with diverse signalling factors and repair proteins assembling at the lesion in a highly ordered spatial and time-dependent manner. Within this orchestrated response local as well as global chromatin decondensation has been found at various stages, thought to enable the access of repair factors to the damage site [1]. However the direct visualisation of these relaxation steps is difficult and usually only global decondensation, via indirect methods, is detectable. Recently we described local chromatin decondensation visible as a depletion of DNA staining within the highly compacted chromocenters in fixed mouse embryonic fibroblast, and due to low hitting probability at the UNILAC broad beam, also in a small numbers of living cells [2].

Results and Conclusions

To increase the number of live cell observations and to study the decondensation process within chromocenter after heavy ion irradiation in more detail we used the GSI microbeam, allowing the defined and targeted irradiation of subnuclear compartments [3].

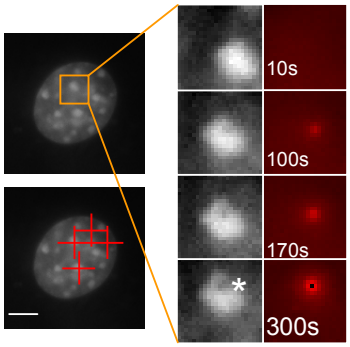

Figure 1: Chromatin decondensation within chromocenters of living cells at the microbeam. DNA of NIH3T3 cells were stained with Hoechst 33342 (left). Chromocenters, which are visible as areas of more intensive DNA staining, were targeted using the in-situ microscope (see red crosses). Cells were irradiated with gold ions (LET 12.800 keV/µm) and a time series was recorded with one picture every 10s for at least 5 min. Zoomed in pictures at the indicated timepoints (right) show a single hit chromocenter verified by the accumulation of XRCC1-RFP (red) an early repair protein. Decondensation is visible as an emerging, less stained area (white asterisk) within the chromocenter. (Scale bar 5µm)

Chromatin decondensation at the microbeam-hit sites within chromocenters is visible as an appearing darker area at sites of damage (Figure 1). Single line measurements of DNA staining intensities over time verify that depletion is a continuous process with the fluorescence decreasing over time leading to the typical dip formation (Figure 2) attesting to the assumptions made before [2] that dip formation is a printout of decondensation.

While little is known about the chromatin decondensation mechanism within compacted chromocenters after ion irradiation, the establishment of live cell observations of this process at the microbeam is a breakthrough that will help to elucidate the pathways behind.

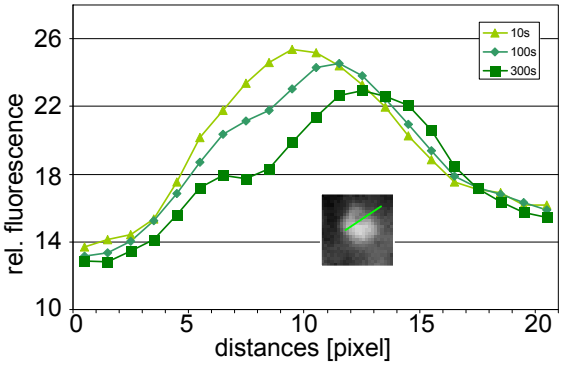

Figure 1: Dip formation as the printout for chromatin decondensation. Recorded sequences of live cell observation (Figure 1) were corrected for bleaching and cell movement and normalised to 1. Plot shows the DNA fluorescence intensities measured at the depicted single green line within the picture at the specific timepoints after irradiation. The staining intensities at the damage sites decrease, leading to the formation of a dip.

References

[1] Misteli and Soutoglou (2009), *Nat Rev Mol Cell Biol* 10, 243-254

[2] Jakob et al, Nucleic acids research (2011) 39, 6489-6499

[3] Heiss et al (2006), *Radiat Res* 165, 231-239.

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