FRAP measurements after targeted irradiation of heterochromatin and euchromatin at the GSI microbeam*

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Introduction

The GSI microbeam is routinely employed for targeted irradiation of living cells in culture [1]. Over the last years the custom-build epifluorescence microscope was improved, and a laser system was integrated [2].

Now the microscope has a temporal and spatial resolution to image fast repair protein kinetics after irradiation with heavy ions. This gives an insight into the kinetics of repair proteins recruiting at the damage sites (foci) [3].

By a laser system the fluorescent markers at these foci can be locally bleached and the fluorescence recovery after photobleaching (FRAP) can be recorded. This yields information about the protein exchange at the damage sites [4].

Here we use the microbeam to irradiate heterochromatic (HC) and euchromatic (EC) regions followed by FRAP analysis to see if there is a difference in protein exchange for high (HC) and low (EC) chromatin density [2].

Experiment

In the mouse embryonic fibroblast cell line NIH3T3 HC and EC can be easily visualized by a DNA staining (Hoechst 33342) (Figure 1 a). After targeted irradiation of these sub-cellular compartments by single gold ions (LET of 12900 keV/u) (Figure 1 at red crosses) the DNA repair is starting by accumulation of repair proteins to the damage sites. These cells are in addition transfected with an green fluorescent protein (GFP) fused to repair protein Ku80, a important protein for the non-homologous end joining repair pathway. Figure 1 b shows foci of Ku80-GFP one minute after ion irradiation, which act as targets for bleaching by the laser system (Figure 1 c green crosses) [2]. Afterwards these targets are bleached and time-lapse images are recorded. Figure 2 shows typical FRAP curves of HC and EC measured in the cell depicted in Figure 1. Evaluation of several of these curves yields FRAP times of $t_{1/2} = 4.2 \pm 0.4$ s (N=35) for HC and $t_{1/2} = 2.8 \pm 0.5$ s (N=38) for EC and resulting diffusion coefficients of $D_{HC} = 0.24 \pm 0.02$ $\mu m^2 s^{-1}$ and $D_{EC} = 0.36 \pm 0.06$ $\mu m^2 s^{-1}$ [2]. The diffusion coefficient reported in HeLa cells for Ku80 is ~ 0.35 $\mu m^2 s^{-1}$ [5] and in excellent agreement with our measurements in EC. In addition a significant difference in protein exchange of Ku80 in EC and HC is visible.

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*Work supported by HGS-HIRe.

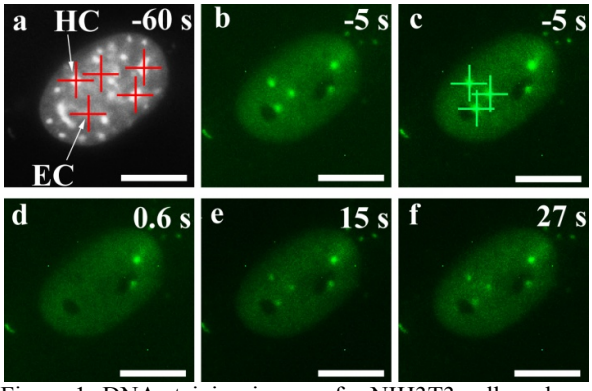

Figure 1: DNA staining image of a NIH3T3 cell nucleus before targeted irradiation with 4.8 MeV/u gold ions (a). After accumulation of repair protein Ku80-GFP to the DNA damage (b) targets to bleach are selected (c) and a fluorescence recovery is imaged (d-f). Scale bar 5 µm.

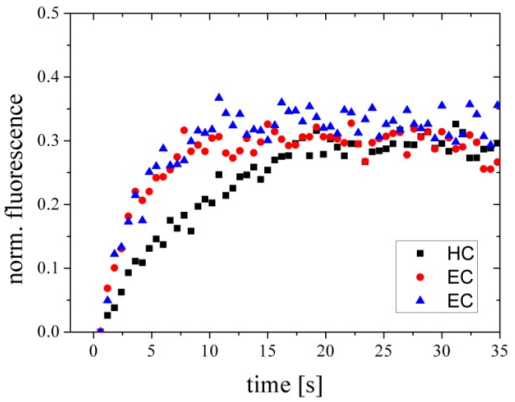

Figure 2: FRAP curves of Ku80-GFP in one cell nucleus. Significant difference of HC and EC curves is visible. Normalized to pre-bleach intensity.

References

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