Influence of the chromatin remodeler ACF1 on the dynamic behaviour of 53BP1 foci after heavy ion irradiation

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By exposure to heavy ion irradiation, a cell nucleus accumulates multiple double strand breaks (DSBs) along the ion traversal. DNA repair proteins accumulate at these lesions and can be detected as irradiation induced foci (IRIF) by fluorescence microscopy (Fig.1a). As it is still an open question how the outcome of DNA repair is influenced by the dynamic behavior of damaged chromatin, we investigated foci movement after irradiation with charged particles. By tracking of 53BP1-GFP foci over two hours using live cell microscopy we observed a random walk behavior in wt cells which leads to a mobility of damaged sites well below 1 µm per hour (Fig.1b) [1].

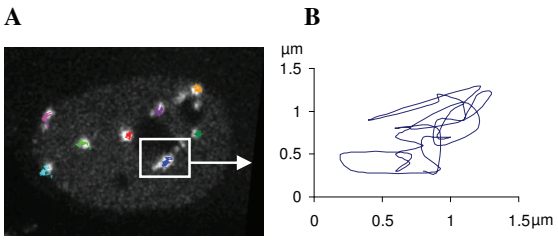

Figure 1: (a) U2OS cell expressing 53BP1-GFP 2 hours after irradiation with Cr ions (LET 2630 keV/µm). Movement of occurring foci is tracked and marked in different colours. (b) Motional trajectory of a 53BP1 foci over 2 hours in 2D.

To investigate if the motion of IRIFs is an active process relying on energy consumption, we depleted ATP 30 min before irradiation with heavy ions and tracked the motion of occurring 53BP1 foci. A strong reduction in mobility from 0.6 µm^2/h in wild type cells to a msd of 0.3 µm^2/h could be observed (Fig 2). This demonstrates a strong energy dependence of chromatin dynamics. However, ATP depletion might also influence the chromatin structure and compaction.

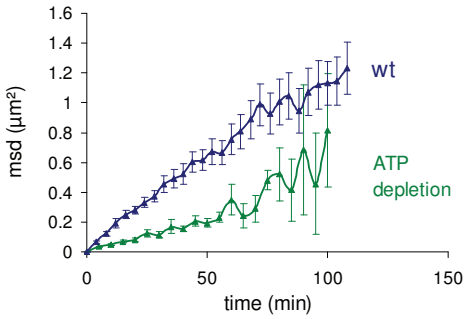

Figure 2: Mean square displacement (msd) of 53BP1 foci after irradiation with Cr ions in ATP depleted and wt U2OS cells.

To investigate the influence of changes in chromatin structure in more detail we knocked down the chromatin remodeler ACF1 which is involved in DNA repair, especially NHEJ, and contributes to chromatin relaxation [2]. By siRNA mediated knockdown of this remodeler, local decondensation should be diminished and thus DNA repair by NHEJ suppressed. As a result of the reduced decondensation, leading to a less opened chromatin network, we expected a more confined mobility. The msd plot of IRIFs after ACF1 knockdown as well as in wt cells is shown in Fig. 3.

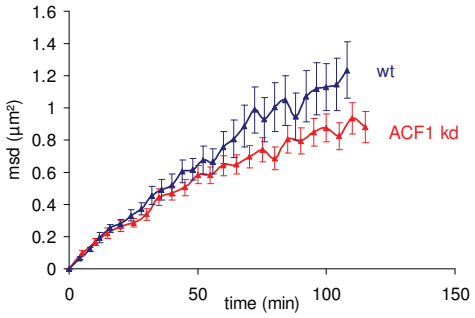

Figure 3: Mean square displacement (msd) of 53BP1 foci after irradiation with Cr ions (LET 2630 keV/µm) in ACF1 knockdown and mock treated U2OS cells.

A trend of a slight reduction in mobility can be observed in ACF1 knockdown cells. However this is presently not statistically significant. As there are many remodelers working together and in part redundantly way, it is highly probable, that a single knockdown does not create major differences in chromatin structure. In addition, chromatin alterations by the remodeler might be restricted to a smaller scale of only few nucleosomes, which would not influence the mobility of the whole IRIF significantly. Further experiments generating more pronounced changes in the chromatin are needed to solve the still open question in how far chromatin structure influences the foci mobility and if this mobility can be linked to the formation of chromosomal aberrations.

References

[1] B. Jakob et al., PNAS (2009)
[2] L. Lan et al., Mol. Cell (2010)

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