TARGETED IRRADIATION USING ACCELERATED CARBON IONS TO DETECT THE ACCUMULATION OF PROTEINS INVOLVED IN NHEJ REPAIR PATHWAY*

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The live cell imaging facility at the ion microprobe SNAKE allows online studies of fluorescence-tagged repair protein accumulation in living cells after heavy ion irradiation [1, 2]. Several proteins involved in the Non-Homologous Repair (NHEJ) pathway have been reported to accumulate to visible foci after laser irradiation at the sites of DNA damage [3, 4], however accumulation Ku70-GFP and XRCC4-GFP could not be detected after either sparsely ionizing radiation nor single carbon ion irradiation. Assuming the DNA damage load after ionizing radiation is not sufficient to visualize the recruitment of these proteins to the DNA double-strand breaks (DSB), we made use of the possibility of SNAKE to apply a defined number of carbon ions per point to increase the dose per point, and thus locally the number of DSB induced. To speed up the irradiation procedure single cells were targeted irradiated with a five-point cross-like pattern instead of performing irradiation of the total area of the cell sample with e.g. a matrix like pattern.

U2OS cells expressing a GFP-tagged version of the protein XRCC4 were irradiated with 1000, 300, 100 or 30 carbon ions per point. Protein recruitment was only detected at doses higher than 100 carbon ions per point; 30 ions per point were not sufficient for foci formation (see Fig. 1).

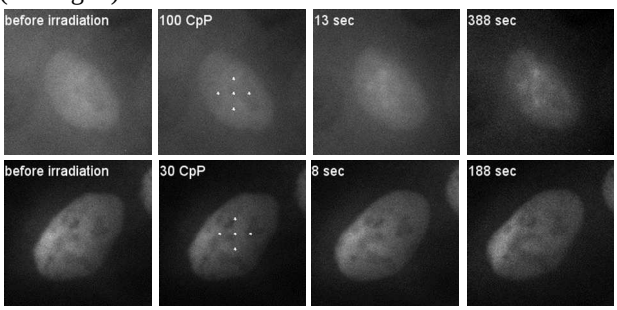

Figure 1: U2OS cells stably transfected with XRCC4-GFP were irradiated with the indicated number of carbon ions per point in a five point cross-like pattern. The accumulation of XRCC4-GFP at the sites of irradiation can be noticed in the brighter parts of the nucleus compared to the nucleus before irradiation.

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The XRCC4 foci form within a minute to the maximum intensity and persist for several minutes.

HT-1080 cells stably transfected with an expression vector for Ku70-GFP and a non-tagged version of Ku80 were irradiated with 300 CpP (see Fig. 2).

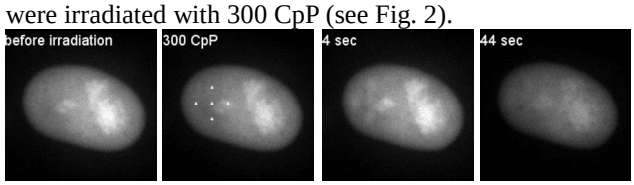

Figure 2: Focus formation of HT-1080 cells expressing Ku70-GFP irradiated with 300 Carbon ions per point. Focus formation and disassembly of Ku70 appears very rapidly.

Ku70 foci show a very fast association and disassembly kinetics, thus monitoring the recruitment of Ku70-GFP is challenging. The protein is probably recruited to the sites of DSB within less than one second and dissociates with a high rate from the foci.

Using the technique of targeted single cell irradiation in combination with the ability of SNAKE to deliver a defined number of ions on a single point, we show that the reported visible accumulation of the proteins XRCC4 and Ku70 at sites of laser induced DNA damage is due to a tremendous amount of DNA lesions.

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