IMAGING RECRUITMENT OF CHROMATIN ASSOCIATED PROTEINS TO THE SITES OF DNA DAMAGE INDUCED BY LASER MICROIRRADIATION

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Living organisms are constantly under toxic endogeneous and exogeneous stresses to their genomic stability. When not faithfully repaired, these damages can lead to genetic mutations and ultimately to cancer and apoptosis. Therefore DNA Damage Responses (DDR) are crucial mechanisms and have evolved into complex redundant pathways involving, among others, chromatin remodeling and DNA repair.

To better understand these responses, we are focusing our investigations onto the *in vivo* recruitment of pairs of DDR proteins that occurs during the first minutes of DDR activation following the induction of DNA damage. To characterize the recruitment kinetics of 3 known interdependent DDR actors PCNA, PARG and PARP1 [1], we have developed a combined approach of two different fluorescence microscopy methods and use a focused pulsed UV laser to induce local DNA damage in living mammalian cells.

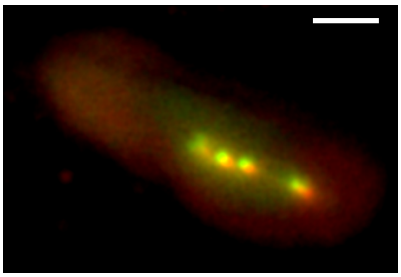

Figure 1. Parallel widefield acquisition at 2 fps of protein recruitment along the stripe of DNA damage induced with a 355nm laser. U2OS, GFP: PARG, RFP: PCNA. Scale bar : 5µm

We first set up a simultaneous 2-color widefield acquisition using an Optosplit image splitter to ensure the exact simultaneity of the fast kinetic recording of pairs of proteins (Fig.1). In opposition to previous studies, our setup allows us to evaluate protein interdependency at the single cell level rather than comparing average behaviors acquired for different samples, hence dimishing cell to cell biological variability. A second system, based on FLIM-FRET imaging with *in situ* nanodissection, provides insight into the type of recruitment (bound or individual proteins). This experimental approach has been combined with a phasor analysis in order to minimize the required photon count.

Taken together, we developed a combination of optical systems to gain access to a more local, rich and dynamic information of the DDR using live cell imaging and localized DNA damage. Our results underscore the importance of the binding capacities of PCNA and PARG and the interplay of the different DNA damage recruitment pathways.

[1] Mortusewicz, O., Fouquerel, E., Amé, J.-C., Leonhardt, H. & Schreiber, V., “PARG is recruited to DNA damage sites through poly(ADP-ribose)- and PCNA-dependent mechanisms”. *Nucleic Acids Res.* 39, 5045–5056 (2011).