SINGLE MOLECULE LOCALIZATION MICROSCOPY REVEALING STRUCTURE AND MECHANISMS OF DNA DOUBLE STRAND BREAK REPAIR PROTEIN COMPLEXES

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KEY WORDS: Single Molecule, DNA Repair, Homologous Recombination, PALM

Superresolution techniques made it possible to visualise cellular structures at much higher resolution than with conventional fluorescence microscopes. One example of such small structures are the Double Strand Break foci. These foci, which are only a few hundred nanometers in size, appear at the sites of DNA damage inside the nucleus. The proteins we study are involved in the Homologous Recombination (HR) pathway which during replication repairs the DNA using the sister chromatid as homologous template. While other proteins detect DSBs and resect DNA at the damage, RAD51, known as the key protein during invasion of the homologous template, binds to the ssDNA at the break and forms protein filaments around it. One of the proteins facilitating RAD51 is the ATPase, RAD54, a protein which is known to have many functions in HR.

In this study we use Single Molecule Localization Microscopy (SMLM) to create a nanoscopic road map of DSB proteins inside the double strand break focus, aiming to relate protein distribution to function. We pairwise image DSB proteins labelled with photoswitchable fluorescent proteins (PS-FP) and imaged alone or in pairs in fixed cells. Using near-TIRF illumination we acquire SMLM movies which are analysed to detect the single molecules.

Preliminary results show that irradiated cells containing RAD54 proteins labelled with the PS-FP mEos2, contain ~100 RAD54 molecules per focus of about 500 nm, and foci are often somewhat elongated structures. Preliminary results using dual colour SMLM showed (Figure 1) the possibility to study the distribution of two proteins relative to each other.

Figure 1 Image displaying individual localized molecules of RAD51-PSCFP2 (black) and RAD52-PsmOrange (white). The size of the circles relate to the localization precision of that molecule. In the background a reconstructed image of the focus.