Spatiotemporal Mapping of DNA Double Strand Break Repair Using Super Resolution Microscopy

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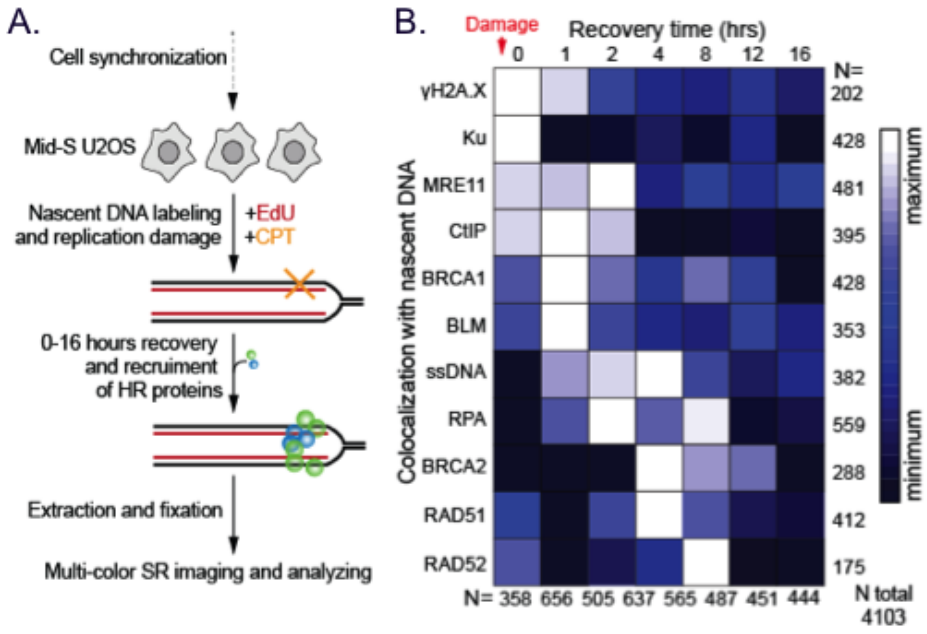
Imaging the spatiotemporal details of the DNA damage response (DDR) in cells is difficult due to the dense and varied nature of the nuclear environment, and the inherent complexities, redundancies, and crosstalk of DDR pathways. What we currently understand about these important processes has been elucidated over the course of several decades by combining biochemical and biophysical techniques[1,2]. This knowledge forms the cornerstone for both understanding cancer and developing ways of treating it. Here, we have successfully used single molecular localization super resolution (SR) imaging[3] to circumvent the diffraction limit of light and capture spatially and temporally resolved snapshots of double strand break (DSB) repair in cells. Moreover, we specifically generated individual one-sided DSBs similar to those encountered endogenously, by collapsing single replication forks. The resulting repair foci could be visualized in three color SR by labelling nascent DNA via modified base incorporation and click chemistry, DSBs via the TUNEL assay or direct ligation, single stranded DNA via BrdU incorporation, and proteins via immunolabelling[4]. High throughput imaging and novel analyses have allowed us to examine thousands of cells, each with several thousand DNA and protein foci. From this, we have been able to quantify the changing degree of colocalization between proteins and naDNA following damage and generate a temporal map of the arrivals, occupancies and departures of specific proteins at repair foci[5]. Further analyses using center-of-mass distance calculations and Monte Carlo randomizations yielded insights into the inter-relationships between proteins during DDR, as well as the internal organization of repair foci. This data was generally in good agreement with current models of the repair process, demonstrating the overall success of the multiplexed SR imaging and analysis approach. However, the enhanced spatial and temporal resolutions and the singular nature of the DSBs themselves revealed several exciting and novel insights including the dynamic interactions of proteins such as Ku, MRE11 and RAD51 at the DSB, the redundant role of RAD52 in repair, and a critical in vivo BRCA2 dependence on BRCA1. Parallel to this research we have also developed *in vitro* methods for imaging chromosomes and chromatin, as well as double-stranded DNA. These approaches allowed us to reconstitute systems in their simplest form and examine them using single molecule imaging techniques like Forster resonance energy transfer. I will present these findings within the context of their importance to the genomic integrity research community, as well as the broader novelty and applicability of the various replication/transcription/damage SR assays we have developed.



A. Experimental scheme used to generate and visualize DSBs and their repair. B. A heatmap describing protein arrival, occupancy and departure.

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