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Physical nature of DNA repair foci revealed by single molecule microscopy

Our genome is constantly damaged by a variety of exogenous and endogenous agents. Among the various forms of DNA damage, double-strand breaks (DSBs) are the most cytotoxic and genotoxic for the cell. Eukaryotic organisms use several mechanisms to repair DSBs among them non-homologous end-joining (NHEJ) and homologous recombination (HR).

Here, **we investigate the molecular mechanisms of HR proteins inside cells at the single molecule level in Saccharomyces cerevisiae yeast**. In response to DSB, repair proteins colocalize from diffuse distribution to repair foci located at the damaged DNA site. An enduring question in the DNA damage field is how do repair proteins find their correct target and accumulate within repair foci: how do they diffuse before DNA damage, during focus formation and inside such a repair focus? Despite their functional importance, the physical nature of repair sub-compartments remains largely unknown.
To answer these questions, we use **single particle tracking** (SPT) and **live PALM** (Photo Activable Localization Microscopy) approaches allowing us to assess the physical properties underlying repair foci formation and decipher the internal structure of these membranless sub-compartments. We found that **Rad52 share many properties characterizing Liquid Liquid Phase Separation** including: sharp change in diffusion coefficient while entering or escaping foci, fusion, existence of a potential attracting molecules to the center of foci. More specifically, Rad52 molecules diffuse an order of magnitude faster than damaged chromatin inside foci indicating that most of the molecules are not bound to damaged chromatin. Instead, they explore the volume of the focus exhibiting a confined motion. In response to multiple DSBs, Rad52 foci fuse into a larger focus inside which Rad52 molecules are shared between the different DSBs. Finally, SPT reveals the existence of an attractive potential maintaining molecules into a focus, but no long-distance collective attraction was observed during the formation of the focus.

Reference:
Judith Miné-Hattab and Angela Taddei, Physical principles and functional consequences of Nuclear compartmentalization in budding yeast. Curr Opin Cell Biol, Jun;58:105-113, 2019.

Camille Clément, Guillermo Orsi, Alberto Gatto, Ekaterina Boyarchuk, Audrey Forest, Bassam Hajj, Judith Miné-Hattab, Mickaël Garnier, Zachary Gurard-Levin, Jean-Pierre Quivy, and Geneviève Almouzni. Nature Communication, 2018 Aug 9;9(1):3181.

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