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New insights into DNA repair

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DNA within the nucleus of cells can be damaged by normal metabolic processes and environmental factors, such as exposure to UV light, other forms of radiation, and certain environmental chemicals. DNA repair processes exist within cells to repair DNA damage and maintain the integrity of the genome. DNA breaks can be repaired by homologous recombination, where DNA can be exchanged between two similar molecules of DNA. RecA is an important protein in this repair process, which has a role in identifying strand breaks and searching for similar DNA sequences for repair. Exactly how RecA does this is largely unknown. Forget and Kowalczykowski investigated the mechanisms underlying RecA mediated DNA repair using optical trapping to manipulate DNA and single molecule fluorescence to observe DNA pairing.

Forget AL, Kowalczykowski SC. Single-molecule imaging of DNA pairing by RecA reveals a three-dimensional homology search. Nature. 2012 Feb 8;482(7385):423-7. doi: 10.1038/nature10782.

It is known that following a double stranded DNA (ds DNA) break, sections of the five prime ends are cut away to produce single stranded DNA (ssDNA). RecA assembles on the single stranded DNA formed following a double stranded DNA break and searches for homologous sequences in dsDNA for repair. Forget and Kowalczykowski demonstrate that both the conformation of the dsDNA target and the length of the RecA-ssDNA strand are important factors in the homology search. Longer target DNA molecules constrain available 3D conformations and slow the rate of homologous pairing while longer ssDNA strands result in faster homologous pairing. The authors propose an 'intersegmental contact sampling' process for homology searches, where RecA forms weak, transients contacts in 3D for DNA recognition. Imaging was performed using an Eclipse TE2000-U inverted microscope with a total internal reflection fluorescence (TIRF) attachment, using a CFI Plan Apo TIRF 100× 1.45 NA oil-immersion objective.

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