Gijs Wuite, J Proteomics Bioinform 2017, 10:8(Suppl)
DOI: 10.4172/0974-276X-C1-0100

Gijs Wuite

Vrije Universiteit Amsterdam, The Netherlands

Sliding sleeves of XRCC4–XLF bridge DNA and connect fragments of broken DNA

Non-homologous end joining (NHEJ) is the primary pathway for repairing DNA double-strand breaks (DSBs) in mammalian cells. Such breaks are formed, for example, during gene-segment rearrangements in the adaptive immune system or by cancer therapeutic agents. Although the core components of the NHEJ machinery are known, it has remained difficult to assess the specific roles of these components and the dynamics of bringing and holding the fragments of broken DNA together. The structurally similar XRCC4 and XLF proteins are proposed to assemble as highly dynamic filaments at (or near) DSBs. Here we show, using dual and quadruple-trap optical tweezers combined with fluorescence microscopy, how human XRCC4, XLF and XRCC4–XLF complexes interact with DNA in real time. We find that XLF stimulates the binding of XRCC4 to DNA, forming heteromeric complexes that diffuse swiftly along the DNA. Moreover, we find that XRCC4–XLF complexes robustly bridge two independent DNA molecules and that these bridges are able to slide along the DNA. These observations suggest that XRCC4–XLF complexes form mobile sleeve-like structures around DNA that can reconnect the broken ends very rapidly and hold them together. Understanding the dynamics and regulation of this mechanism will lead to clarification of how NHEJ proteins are involved in generating chromosomal translocations.

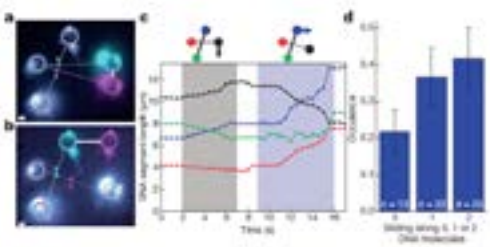

Figure 3 | Mobility of XRCC4–XLF bridges. a, **b**, Overlays of eGFP–XLF logarithmically scaled fluorescence before (cyan) and after (magenta) moving a microsphere (white arrow). DNA molecules were incubated in crossed configuration. Circles denote microsphere positions; dashed lines denote DNA; coloured arrows denote bridge location. Data are representative examples of 13 experiments. **c**, Distance from bridge to bead edges (of complex shown in **a** and **b**) as a function of time. Shaded regions denote bead motion as indicated by the arrows in the cartoons. **d**, Histogram of XRCC4–XLF bridge mobility (both wrapped and crossed configurations). Error bars denote s.e.m.

Biography

Gijs Wuite obtained his PhD in Biophysics in 2000. Since 2001 he leads his own group at the VU University Amsterdam and in 2009 was appointed to full Professor. In his research, he has successfully applied quantitative physical tools to investigate fundamental problems in biology, and to search for the unification of apparently unrelated biological phenomena. Moreover, he has been at the front of recent new and fast developments of biophysical techniques that have enabled visualization, manipulation and control of complex biological reactions. Based on this research work he founded in 2014 a company (LUMICKS) that sell the technology he and his group has developed. His work has appeared in journal such as *Nature, Science, PNAS and Physical Review Letters*. His research has been awarded with the prestigious personal VIDI, VICI and ERC grants. In 2009 Wuite was appointed member of the Young Academy, an independent platform of young top scientists within the Royal Netherlands Academy of Arts and Sciences.

g.j.l.wuite@vu.nl