Structural Basis of DNA Double-Strand Break Repair by NHEJ

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DNA double-strand breaks, one of the most cytotoxic forms of DNA damage, can be detected and repaired by the fast-responding and tightly regulated non-homologous end-joining (NHEJ) machinery [1, 2]. NHEJ factors are targets for the development of cancer therapeutics and are essential for the generation of antibody and antigen receptor diversity in immune cells [3, 4]. Core NHEJ factors (Ku70/80 heterodimer (Ku), catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs), DNA ligase IV (LigIV), XRCC4 and XLF) form an initial long-range (LR) synaptic complex that transitions into a DNA-PKcs free, short-range (SR) state to align and repair the DSB ends [5]. Using single-particle Cryo-Election Microscopy (Cryo-EM), we have visualized three additional key NHEJ complexes representing different transition states, with DNA-PKcs adopting distinct dimeric conformations within each of them. Integrated modeling with both experimental reconstruction and in silico structural prediction reveals how an accessory NHEJ scaffolding factor, PAXX, stabilizes the LR complex during ATP-dependent DNA-PKcs signaling. Upon DNA-PKcs autophosphorylation, the LR complex undergoes a substantial conformational change, with both Ku and DNA-PKcs rotating outward to promote DNA break exposure and DNA-PKcs dissociation. In addition, we captured a dimeric state of catalytically inactive DNA-PKcs, which resembles structures of other PIKK family kinases, revealing a model of the full regulatory cycle of DNA-PKcs during NHEJ.

References:

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