Mechanism of Rad5-mediated DNA rearrangement in error-free template switching

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Humans are regularly exposed to harmful factors that damage DNA. The handling of damaged DNA, particularly during DNA replication, poses a major obstacle for cells. During replication, the replication fork will stall at sites of DNA damage, putting the cell at risk for chromosomal rearrangements and double stranded breaks that often result in cell death. To prevent such devastating outcomes, the cell utilizes damage-bypass pathways that allow for continued DNA replication through regions of damage. This research focuses on one such pathway, error-free template switching.

Error-free template switching relies upon the activity of the Rad5 protein. Unlike other damage-tolerance pathways, Rad5 rearranges DNA in such a way that regions of damaged DNA are not used as a template during the replication process. As a result, while other damage-bypass pathways inaccurately add nucleotides across from sites of DNA damage (thus introducing disease-causing mutations), error-free template switching always leads to the incorporation of the intended nucleotide, thereby preventing the introduction of mutations.

The goal of this research is to understand the mechanism of Rad5-mediated DNA rearrangement in error-free template switching from biochemical and structural perspectives. The Rad5 protein contains a large structured domain (a helicase domain) and an intrinsically disordered N-terminal tail. The helicase domain of Rad5 is responsible for unwinding and rearranging DNA during template switching, while the flexible N-terminal tail allows Rad5 to remain tethered to the replication fork while it samples the DNA (a process that is important for coordinating the many different proteins that contribute to DNA damage bypass). We have purified the Rad5 protein and shown that it binds DNA and selectively unwinds certain DNA substrates. We also have preliminary Small Angle X-ray Scattering (SAXS) data for full-length Rad5. Moving forward, we will use our SAXS data in conjunction with Brownian Dynamics (BD) simulations to characterize the disordered nature of Rad5.