Structure and function of Rad5, the catalyst of error-free bypass

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Cells are constantly exposed to DNA damaging agents. When classical DNA repair mechanisms fail, regions of damaged DNA will persist in the genome. These lesions pose a major problem for replication machinery. 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 catastrophic 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 bypass.

Error-free bypass 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 bypass 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 bypass from a structural perspective. To achieve this goal we are investigating the structure of the helicase domain of the Rad5 protein, the oligomeric state attained by Rad5, and the architecture of the Rad5-DNA complex. Preliminary Size Exclusion Chromatography (SEC) and Dynamic Light Scattering (DLS) suggested that the molecular weight of the helicase domain of Rad5 in solution is approximately four times that of the Rad5 helicase domain monomer. Small Angle X-ray Scattering (SAXS) experiments were carried out, and these data were used to produce envelopes of the helicase domain of Rad5. Homology models of the helicase domain of Rad5 were produced, theoretical SAXS curves of monomeric, dimeric, and tetrameric forms of these models were generated, and the curve generated from the tetrameric model best fit the experimental SAXS data. To investigate the architecture of the Rad5-DNA complex, DNaseI footprinting experiments have been carried out. When the Rad5 helicase domain binds DNA substrates that resemble replication forks, the primer strands (but not the template strands) are affected.

Our data suggest that the Rad5 helicase domain forms a homotetramer and remodels replication fork-like DNA substrates. Further footprinting studies will be carried out to determine where Rad5 sits on various DNA substrates. We will also continue to optimize crystallization conditions to determine the high-resolution structure of the Rad5 helicase domain.