Poster Presentation

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Structural characterization of transient interactions in DNA mismatch repair

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DNA mismatch repair (MMR) is a conserved pathway that safeguards genome integrity by correcting replication errors. The initiation of MMR is orchestrated by two proteins –MutS and MutL. MutS detects replication errors and recruits MutL, a key regulator in coordinating downstream MMR events. The processivity clamp, typically known to tether the replicative polymerase to DNA during DNA synthesis, also has a role in several steps in MMR. We have previously shown that MutL transiently interacts with the clamp and that this complex is important for MMR in vivo. The role of the clamp in eukaryotes and most bacteria is believed to license MutL endonuclease activity. In bacterial organisms where MutL does not have endonuclease activity, such as in Escherichia coli, the clamp also interacts with MutL and this interaction is also important for MMR activity. However, the transient nature of this complex prevents its functional and structural characterization. Here, we develop a method to stabilize the E. coli MutL-clamp complex by engineering a disulfide bond at the known protein complex interface and characterize its structure using small angle X-ray scattering (SAXS). MutL binds the clamp through a consensus motif found in its dimerization domain. Using this domain (MutL-CTD) we monitor complex formation with the clamp. We observe two complexes using SAXS. In one complex the MutL-CTD occupies a single hydrophobic cleft of the clamp, while the other occupies both hydrophobic clefts simultaneously. To identify the physiological complex, we used the full length MutL protein to impose further constraints. Analysis of complex formation suggests that full length MutL binds a single cleft on the clamp. Altogether, our data reveals how MutL interacts with the clamp in the early steps of MMR and this approach could be implemented to structurally characterize other transient complexes, an aspect of structural biology that is largely unexplored.

Keywords: Small angle X-ray scattering, Weak protein complexes, DNA repair