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Probing for Conformational Changes in the Repair Enzyme Mfd Using Mutant Protein Constructs

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Abstract
DNA repair is essential for survival, as damage to the genome can interrupt the precarious balance of cell functions, causing further mutations and possibly leading to cancer. The bacterial transcription repair coupling factor, Mfd, is capable of recognizing a stalled RNA polymerase at a site of DNA damage. The Mfd works both to remove the RNA polymerase through its motor function (utilizing the energy of ATP to translocate along DNA), and to recruit the DNA repair complex UvrA/B/C. To study conformational changes in the protein, we are creating multiple mutants of the full length Mfd protein. My approach is to use a cleavable mutant of full-length Mfd as a template for further mutations. This will allow us to probe for conformational changes by changing interactions at the interface of the two halves of Mfd, and then using the ability to cut with TEV protease as a sensor to identify and characterize the open state of the protein. By introducing this TEV protease cut site at residue 450 in the protein linker region between the N (amino-) and C (carboxy-) terminal domains, we can then assess the conformational changes Mfd must undergo to obtain activity. We can study the effect of further

mutations on the full length and cut versions of the protein. Another approach attempted in this study involves using cysteine modification of the full length Mfd protein as a sensor for these conformational changes. Mfd acts as a model system for studying the DNA repair mechanisms found in humans, and the elucidation of functional and conformational changes in Mfd contributes to studying disease phenotypes resulting from aberrant transcription coupled repair.

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