Characterization of the N-terminal arms of the polymerase manager protein UmuD

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Abstract

Escherichia coli cells that are exposed to exogenous or endogenous DNA damaging agents induce the SOS response that involves expression of the umuD genes, among more than 50 others. Full-length UmuD is expressed as a 139-amino-acid protein, which eventually cleaves its N-terminal 24 amino acids to form UmuD'. Both UmuD and UmuD' exist alone as homodimers, but can also exchange to form UmuD'/UmuD heterodimers. The N-terminus of UmuD exist as conformationally-dynamic arms, and contain a number of recognition sites for partner proteins. Cleavage of UmuD to UmuD' dramatically affects the function of the protein, and activates UmuC for translesion synthesis (TLS). Recently, we have constructed additional N-terminal truncations of UmuD, specifically UmuDΔ (UmuD-Ala-7) and UmuDΔ (UmuD-Ala-17). We are probing the conformation of the N-terminal arms, effect on cleavage as well as the effect on protein-protein interactions using these truncation variants. We found that the loss of just the N-terminal seven amino acids of UmuD results in significant changes in conformation of the N-terminal arms. UmuDΔ is cleaved as efficiently as full-length UmuD in vitro and in vivo, but expression of plasmid-borne UmuDΔ makes cells hypersensitive to UV irradiation. UmuDΔ does not cleave to form UmuD', but confers resistance to UV irradiation. We used site-directed spin labeling and electron paramagnetic resonance spectroscopy to identify increased local motion of the N-terminal arms of the UmuD truncations. This work will lead to understanding the mechanism by which UmuDΔ causes UV hypersensitivity.

Introduction

DNA damage invokes SOS response

Accumulation of RecA/ssDNA facilitates self-cleavage of UmuD to UmuD'

UmuD proteins contain many recognition sites for protein-protein interactions

Pol V is responsible for UV-induced mutagenesis

Results

UmuDΔ causes UV hypersensitivity

UmuDΔ cleaves as efficiently as full-length UmuD

Protease deletions do not affect steady-state UmuD levels

UmuD arm truncations change melting profile

UmuDΔ and Δ18 cross-linking similar to UmuD3A

References


Acknowledgements

Northeastern University