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Research paper

Deciphering the essentiality and function of SxSx motif in *Mycobacterium tuberculosis* UvrB

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ABSTRACT

The UvrB subunit is a central component of the UvrABC incision complex and plays a pivotal role in damage recognition, strand excision and repair synthesis. A conserved structural motif (the SxSx motif) present in UvrB is analogous to a similar motif (TxGx) in the helicases of superfamily 2, whose function is not fully understood. To elucidate the significance of the SxSx (Ser143-Val144-Ser145-Cys146) motif in *Mycobacterium tuberculosis* UvrB (MtUvrB), different variants of MtUvrB subunit were constructed and characterized. The SxSx motif indeed was found to be essential for MtUvrB function: while Ser143 and Cys146 residues within this motif were crucial for MtUvrB function, Ser145 plays an important but less essential role. The SxSx motif-deleted mutant was drastically attenuated and three single (S143A, S145A and C146A) mutants and a double (S143A/S145A) mutant exhibited various degrees of severity in their DNA-binding, DNA helicase and ATPase activities. Taken together, these results highlight a hitherto unrecognized role for SxSx motif in the catalytic activities of UvrB.

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1. Introduction

In all living organisms, robust biochemical and network-level mechanisms exist to ensure the integrity of their genomes. The nucleotide excision repair (NER) pathway is a frontline defense mechanism in all cells for repair of bulky DNA lesions that destabilises the DNA structure [1–3]. In bacteria, NER is an important DNA repair pathway that removes different types of structurally unrelated DNA adducts such as cyclobutane pyrimidine dimers, alkylation adducts, chemotherapeutic adducts as well as cisplatin interstrand crosslinks [3–5]. The process of NER involves the concerted action of six proteins, namely UvrA, UvrB, UvrC, DNA helicase II (UvrD), Pol I and DNA ligase, which orchestrate the sequential steps in the process of damage recognition, unwinding of DNA around the lesion, dual excision flanking the damaged site and finally, repair resynthesis and DNA ligation [4–7]. Based on numerous genetic, biochemical and X-ray crystallographic studies, three major stages associated with the early steps of the NER pathway have been defined in the *Escherichia coli* paradigm [3–12]. Notwithstanding the early uncertainties, a growing body of evidence suggests that the $UvrA_2B_2$ hetero-tetrameric complex is responsible for damage recognition [10–15]. The ATP hydrolysis by $(UvrA)_2$ renders its dissociation from the lesion complex, thereby allowing $UvrB_2$ to recruit UvrC to form a pre-incision complex [3–6,16–21]. The next step involves dual incisions by UvrC at sites flanking the DNA lesion, four/five nucleotides 3′ from the lesion and eight nucleotides from the 5′ side [3–6,22–25]. The post-incision complex comprising of 12 to 13-mer single-stranded DNA and UvrBC subunits is removed by UvrD and Pol I [3–6,25–28]. Finally, the gap is filled by DNA polymerase and the broken strands are sealed by DNA ligase [3–6,28].

The UvrB protein, which belongs to the superfamily 2 (SF2) of helicases, plays a central role in the NER pathway. A hallmark of the SF2 superfamily of helicases is the presence of seven conserved helicase motifs (motifs I, Ia, II, III, IV, V and VI), and some possess auxiliary domains [29,30]. These motifs are spread over two RecA-like domains that together constitute the helicase core, which by themselves couple ATP hydrolysis to DNA/RNA unwinding [29,30].

Abbreviations: BcUvrB, *Bacillus caldotenax* UvrB; bed vol, bed volume; bp, base pair; CBB, Coomassie brilliant blue; dsDNA, double-stranded DNA; DTT, dithiothreitol; EDTA, ethylene diamine tetraacetic acid; EMSA, electrophoretic mobility shift assay; FPLC, fast performance liquid chromatography; IPTG, isopropyl-1-thio-β-D galactopyranoside; MtUvrB, *Mycobacterium tuberculosis* UvrB; NER, nucleotide excision repair; ODN, oligonucleotide; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; ssDNA, single-stranded DNA; TLC, Thin layer chromatography.

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