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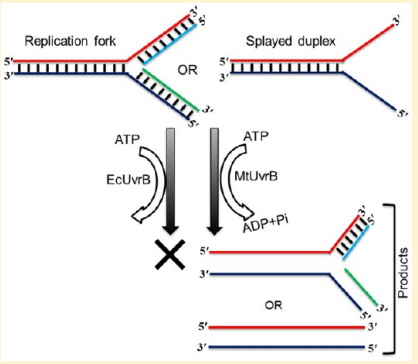
Mycobacterium tuberculosis UvrB Is a Robust DNA-Stimulated ATPase That Also Possesses Structure-Specific ATP-Dependent DNA Helicase Activity

Manoj Thakur, Mohan B. J. Kumar, and K. Muniyappa*

Department of Biochemistry, Indian Institute of Science, Bangalore 560012, India

Supporting Information

ABSTRACT: Much is known about the *Escherichia coli* nucleotide excision repair (NER) pathway; however, very little is understood about the proteins involved and the molecular mechanism of NER in mycobacteria. In this study, we show that *Mycobacterium tuberculosis* UvrB (MtUvrB), which exists in solution as a monomer, binds to DNA in a structure-dependent manner. A systematic examination of MtUvrB substrate specificity reveals that it associates preferentially with single-stranded DNA, duplexes with 3′ or 5′ overhangs, and linear duplex DNA with splayed arms. Whereas *E. coli* UvrB (EcUvrB) binds weakly to undamaged DNA and has no ATPase activity, MtUvrB possesses intrinsic ATPase activity that is greatly stimulated by both single- and double-stranded DNA. Strikingly, we found that MtUvrB, but not EcUvrB, possesses the DNA unwinding activity characteristic of an ATP-dependent DNA helicase. The helicase activity of MtUvrB proceeds in the 3′ to 5′ direction and is strongly modulated by a nontranslocating 5′ single-stranded tail, indicating that in addition to the translocating strand it also interacts with the 5′ end of the substrate. The fraction of DNA unwound by MtUvrB decreases significantly as the length of the duplex increases: it fails to unwind duplexes longer than 70 bp. These results, on one hand, reveal significant mechanistic differences between MtUvrB and EcUvrB and, on the other, support an alternative role for UvrB in the processing of key DNA replication intermediates. Altogether, our findings provide insights into the catalytic functions of UvrB and lay the foundation for further understanding of the NER pathway in *M. tuberculosis*.



Studies over the past four decades have uncovered distinct DNA repair pathways and myriad types of biochemical reactions, and these studies were recognized with Nobel Prizes for T. Lindahl, P. Modrich, and A. Sancar in 2015.[1] Among the many forms of genomic damage, "bulky" DNA lesions that cause helical distortion in the double helix can lead to mutations and ultimately cancer.[2−5] The bulky chemical adducts and interstrand cross-links between adjacent base residues are induced by UV irradiation and chemical modification in all organisms.[2−5] In all three domains of life, various proteins and/or enzymes recognize DNA modifications and either repair them by direct reversal of the chemical alteration or target them for removal by a variety of different DNA repair pathways.[2−5] One of the most studied and best understood DNA repair pathways is nucleotide excision repair in *Escherichia coli*.[1−3] The nucleotide excision repair (NER) pathway was reconstituted with highly purified proteins in the mid-1980s.[1−3,6,7] Since that time, a wealth of functional and structural knowledge about the components of the NER pathway has been published.[8,9]

The NER pathway in eubacteria and some archaea has the unique ability to remove a broad range of chemically and structurally unrelated DNA lesions, including UV-induced photoproducts (cyclobutane dimers, 6-4 photoproducts, and thymine glycol), bulky adducts, apurinic or apyrimidinic (AP) sites, and cross-links, albeit with variable efficiencies.[2,4,9] In *E. coli*, NER is a multistep, ATP-dependent process catalyzed by UvrABC endonuclease, encoded by *uvrA*, *uvrB*, and *uvrC* genes.[10] UvrABC endonuclease, a heterotrimeric enzyme complex, recognizes the damage and excises a short oligonucleotide-containing lesion by the successive action of its constituent subunits.[2,4,9] The mechanism of NER is essentially conserved across evolution, although it is considerably more complex in eukaryotic cells than in bacteria.[2,3,5] The general working model of *E. coli* NER envisages a symmetric complex of $UvrA_2B_2$ that scans DNA for the modified sites.[2,4,8] Specifically, UvrA recognizes damage-induced structural distortions in the DNA,[11−15] and then the damage is passed from the dimeric form of UvrA to UvrB, resulting in a tight UvrB−DNA preincision complex at the site of the damage.[16−20] During these events, UvrB plays a central role in the functioning both in damage recognition and repair and by direct interaction with all the other components in the pathway: UvrA, UvrC, UvrD, and DNA pol I.[21−25] In addition, the UvrA2·B2 symmetric complex unwinds duplex DNA, engages UvrB at the damage site, and facilitates the eviction of UvrA.[17−19,22−30] During the formation of the preincision complex, UvrB uses a β-hairpin to verify the modified nucleotide and inserts itself between the two strands and clamps the substrate.[31−33] Concomitant with the dissocia-

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