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Interrogating the substrate specificity landscape of UvrC reveals novel insights into its non-canonical function

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ABSTRACT Although it is relatively unexplored, accumulating data highlight the importance of tripartite crosstalk between nucleotide excision repair (NER), DNA replication, and recombination in the maintenance of genome stability; however, elucidating the underlying mechanisms remains challenging. While *Escherichia coli uvrA* and *uvrB* can fully complement *polAΔ* cells in DNA replication, *uvrC* attenuates this alternative DNA replication pathway, but the exact mechanism by which *uvrC* suppresses DNA replication is unknown. Furthermore, the identity of bona fide canonical and non-canonical substrates for UvrCs are undefined. Here, we reveal that *Mycobacterium tuberculosis* UvrC (MtUvrC) strongly binds to, and robustly cleaves, key intermediates of DNA replication/recombination as compared with the model NER substrates. Notably, inactivation of MtUvrC ATPase activity significantly attenuated its endonuclease activity, thus suggesting a causal link between these two functions. We built an in silico model of the interaction of MtUvrC with the Holliday junction (HJ), using a combination of homology modeling, molecular docking, and molecular dynamic simulations. The model predicted residues that were potentially involved in HJ binding. Six of these residues were mutated either singly or in pairs, and the resulting MtUvrC variants were purified and characterized. Among them, residues Glu595 and Arg597 in the helix-hairpin-helix motif were found to be crucial for the interaction between MtUvrC and HJ; consequently, mutations in these residues, or inhibition of ATP hydrolysis, strongly abrogated its DNA-binding and endonuclease activities. Viewed together, these findings expand the substrate specificity landscape of UvrCs and provide crucial mechanistic insights into the interplay between NER and DNA replication/recombination.

SIGNIFICANCE In all cells—from bacteria to humans—nucleotide excision repair (NER) is the major pathway for the repair of a vast repertoire of chemically and structurally different types of DNA lesions. Genetic analysis showed that *Escherichia coli* UvrC suppresses the alternative DNA replication pathway through an undefined mechanism of action. Notably, we observed that MtUvrC strongly binds to, and robustly cleaves, key intermediates of DNA replication/recombination. We also demonstrate that ATPase and DNA endonuclease activities of MtUvrC are tightly coupled. Altogether, these results support the notion that recognition and cleavage of a wide range of branched DNAs by MtUvrC may be of importance in DNA repair and maintenance of genome integrity.

INTRODUCTION

In nearly every cell—from bacteria to humans—nucleotide excision repair (NER) is the major pathway for the repair of a vast repertoire of chemically and structurally distinct DNA lesions, which occur due to DNA damage by UV radiation, chemotherapeutic drugs, and endogenous or exogenous agents (1). Since its discovery in *Escherichia coli*, a combination of genetic, biochemical, and structural analyses has revealed important insights into the molecular mechanisms underlying NER in many organisms, including humans (2–7). Several studies, employing different approaches, have implied that the *E. coli* $UvrA_2B_2$ complex scans the DNA for damaged bases (8–14), although the exact mechanism(s) underlying the search for damaged sites in the context of bacterial chromatin remains unclear. After binding to the damaged site, ATP hydrolysis by UvrA triggers its dissociation from the damage recognition complex (2,8,9,14,15). Subsequently, UvrC is recruited to the UvrB-DNA pre-incision complex, which makes incisions on both sides of the lesion (16–18). Finally, UvrD in

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