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The intrinsic ATPase activity of *Mycobacterium tuberculosis* UvrC is crucial for its damage-specific DNA incision function

Manoj Thakur, Ankit Agarwal and Kalappa Muniyappa

Department of Biochemistry, Indian Institute of Science, Bengaluru, India

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Correspondence
K. Muniyappa, Department of Biochemistry, Indian Institute of Science, Bengaluru 560012, India
Tel: +91 80 2293 2235
E-mail: kmbc@iisc.ac.in

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To ensure genome stability, bacteria have evolved a network of DNA repair mechanisms; among them, the UvrABC-dependent nucleotide excision repair (NER) pathway is essential for the incision of a variety of bulky adducts generated by exogenous chemicals, UV radiation and by-products of cellular metabolism. However, very little is known about the enzymatic properties of *Mycobacterium tuberculosis* UvrABC excinuclease complex. Furthermore, the biochemical properties of *Escherichia coli* UvrC (EcUvrC) are not well understood (compared to UvrA and UvrB), perhaps due to its limited availability and/or activity instability *in vitro*. In addition, homology modelling of *M. tuberculosis* UvrC (MtUvrC) revealed the presence of a putative ATP-binding pocket, although its function remains unknown. To elucidate the biochemical properties of UvrC, we constructed and purified wild-type MtUvrC and its eight variants harbouring mutations within the ATP-binding pocket. The data from DNA-binding studies suggest that MtUvrC exhibits high-affinity for duplex DNA containing a bubble or fluorescein-dT moiety, over fluorescein-adducted single-stranded DNA. Most notably, MtUvrC has an intrinsic UvrB-independent ATPase activity, which drives dual incision of the damaged DNA strand. In contrast, EcUvrC is devoid of ATPase activity; however, it retains the ability to bind ATP at levels comparable to that of MtUvrC. The ATPase-deficient variants map to residues lining the MtUvrC ATP-binding pocket. Further analysis of these variants revealed separation of function between ATPase and DNA-binding activities in MtUvrC. Altogether, these findings reveal functional diversity of the bacterial NER machinery and a paradigm for the evolution of a catalytic scaffold in UvrC.

Introduction

All bacteria, archaea and eukaryotic cells experience various types of DNA damage inflicted by endogenous or exogenous agents [1–3]. While some lesions result in structural deformation in the double helix, others lead to abasic sites, base/sugar modifications, inter- or intrastrand cross-links and single- or double-strand DNA breaks [4–10]. Several lines of evidences have established that a network of DNA repair pathways plays pivotal roles in the maintenance of genome stability. These include (a) homology-directed double-strand break repair (HR); (b) base excision repair, which repairs modified bases; (c) mismatch repair, which

Abbreviations
bp, base pair; BSA, bovine serum albumin; DMSO, dimethyl sulfoxide; dsDNA, double-stranded DNA; DTT, dithiothreitol; EcUvrC, *Escherichia coli* UvrC; EMSA, electrophoretic mobility shift assay; MtUvrC, *Mycobacterium tuberculosis* UvrC; ODN, oligonucleotide; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate; ssDNA, single-stranded DNA.