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UvrA and UvrC subunits of the *Mycobacterium tuberculosis* UvrABC excinuclease interact independently of UvrB and DNA

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The UvrABC excinuclease plays a vital role in bacterial nucleotide excision repair. While UvrA and UvrB subunits associate to form a $UvrA_2B_2$ complex, interaction between UvrA and UvrC has not been demonstrated or quantified in any bacterial species. Here, using *Mycobacterium tuberculosis* UvrA (MtUvrA), UvrB (MtUvrB) and UvrC (MtUvrC) subunits, we show that MtUvrA binds to MtUvrB and equally well to MtUvrC with submicromolar affinity. Furthermore, MtUvrA forms a complex with MtUvrC both *in vivo* and *in vitro*, independently of DNA and UvrB. Collectively, these findings reveal new insights into the pairwise relationships between the subunits of the UvrABC incision complex.

Keywords: *M. tuberculosis*; nucleotide excision repair; protein-protein interaction; UvrABC excinuclease; UvrA-UvrC complex

In bacteria, nucleotide excision repair (NER) is a vital DNA repair pathway that acts on a wide variety of bulky DNA adducts such as cyclobutane pyrimidine dimers, alkylation adducts and cisplatin interstrand cross-links [1,2]. In the well-studied *Escherichia coli* paradigm, the multistep NER pathway begins with the binding of $UvrA_2B_2$ heterotetramer to the damaged region [3–11]. Subsequently, the dissociation of $UvrA_2$ from $UvrB_2$ enables the latter to perform damage verification, recruit UvrC and assemble the pre-incision complex [3,11,12]. The ATP hydrolysis by $UvrA_2$ facilitates the release of UvrA dimer from DNA [13,14]. After incision at either side flanking the DNA lesion by UvrC within the UvrB-UvrC-DNA complex, the postincision complex comprising of 12- to 13-mer oligonucleotide and UvrB:UvrC is removed by UvrD and Pol I [15–20]. The resulting gap is filled in by DNA polymerase I, and the broken strands are sealed by DNA ligase [21].

Although NER is one of the most extensively studied DNA repair processes in *E. coli*, much remains unknown about the formation and disassembly of NER repair machinery [1,2,22]. Additionally, the mechanisms underlying the coordination between the various proteins/enzymes involved in the NER pathway are not fully understood [1,2]. The translation of *in vitro* models of the *E. coli* NER pathway to an actual *in vivo* context remains challenging [10,11]. Furthermore, to what extent the findings from the *E. coli* NER pathway can be extrapolated and applied to other strains and species, and during different physiological conditions, remains to be shown. While the enzymes of the NER pathway exist in *Mycobacterium tuberculosis* [23], very little is known about their catalytic and regulatory mechanisms. Notwithstanding,

Abbreviations
MtUvrA, *M. tuberculosis* UvrA; MtUvrB, *M. tuberculosis* UvrB; MtUvrC, *M. tuberculosis* UvrC; NER, nucleotide excision repair; RU, response units; SEC, size-exclusion chromatography; SPR, surface plasmon resonance; ssDNA, single-stranded DNA; Y2H, yeast two-hybrid system.