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Research Article

Molecular Dissection of the UvrC Protein Involved in Nucleotide Excision Repair in *Listeria monocytogenes*

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What do these dates mean?

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Highlights

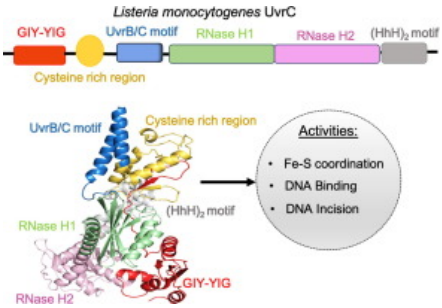
- *Lm*UvrC is a conserved functional homolog of bacterial UvrC proteins.
- Cys-rich region of *Lm*UvrC mediates Fe-S coordination and DNA binding.
- *Lm*UvrC exists predominantly as a monomer in solution.
- RNase H and GIY-YIG domains of *Lm*UvrC act together for efficient DNA incision function.

Abstract

Nucleotide excision repair (NER) is a conserved DNA repair pathway that removes a wide range of DNA lesions and preserves genome integrity. In bacteria, this process is carried out by the UvrABC excinuclease complex, in which UvrC performs dual incisions flanking the damaged sites. Despite its central role in repair, the biochemical properties and mechanistic features of UvrC from several pathogenic bacteria remain poorly defined. Here, we present a biochemical characterization of UvrC from *Listeria monocytogenes* (*Lm*UvrC), a major food-borne pathogen. *Lm*UvrC exhibits appreciable DNA-binding capability and DNA incision activity. Size-exclusion chromatography reveals that *Lm*UvrC predominantly exists as a monomer in solution. In addition, the full-length *Lm*UvrC harbours a cysteine-rich region capable of

coordinating an iron–sulfur cluster, and deletion of this region leads to a complete loss of DNA-binding ability. Systematic deletion of additional domains demonstrates that efficient incision by *Lm*UvrC requires coordinated contributions from both the GIY-YIG and RNase H domains, highlighting the importance of interdomain synergy and UvrB-mediated activation. Together, these findings establish a biochemical framework for UvrC, providing insights into NER in *L. monocytogenes* and revealing conserved mechanistic features of bacterial NER.

Graphical abstract



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Introduction

The nucleotide excision repair (NER) pathway is a universally conserved mechanism across all domains of life that recognizes and removes structurally and chemically diverse DNA lesions arising from both endogenous and exogenous sources. These lesions, which typically distort the DNA helix, trigger an ATP-dependent, multistep NER process involving damage recognition, verification, dual incision, excision, repair synthesis, and ligation [1], [2], [3], [4], [5]. The pathway is initiated by the $UvrA_2B$ or $UvrA_2B_2$ complex, which functions as a damage sensor and scans the genome for helix-distorting lesions [6]. Dual incisions flanking the lesion are catalyzed by UvrC [7], [8], followed by the excision of a short, damage-containing single-stranded DNA (ssDNA) segment by UvrD helicase [9]. DNA polymerase I then fills the resulting gap through repair synthesis, and DNA ligase completes the process by sealing the nick, thereby restoring genomic integrity [10].

UvrA is a member of the ATP-binding cassette (ABC) family of proteins and contains two ATP-binding sites per monomer, termed the proximal and distal centers, relative to the DNA-binding groove [6], [11], [12]. The proximal site comprises the ATP-binding domain I and the signature domain II, which interact with UvrB and DNA, while the distal site comprises the ATP-binding domain II and the signature domain I of UvrA [13], [14]. Previous studies on UvrA have shown that both the proximal and distal ATPase sites can bind and hydrolyse ATP, although stop-flow pre-steady state kinetic analysis has revealed an asymmetry in their affinity for nucleotide binding, with the proximal site exhibiting lower (weaker) affinity and the distal site showing higher (tighter) affinity [15]. This asymmetry in ATP binding and hydrolysis allows UvrA to alternate between open and closed conformations, enabling efficient scanning for DNA lesions [6], [14], [15], [16]. Upon detecting a damaged site, UvrA facilitates the formation of a stable UvrB–DNA pre-incision complex, which verifies the lesion and triggers the release of UvrA [17].

At a broader structural level, comparisons among the available crystal structures of the apo UvrB [18], [19], [20], UvrB-DNA [21], and UvrA-UvrB–DNA complexes [6], [22] reveal that the UvrB subunits are positioned away from the DNA lesion [23], [24]. However, biochemical and structural analyses indicate that UvrB, when bound to the damaged DNA strand, translocates towards the lesion to facilitate recognition and verification [25], [26]. Notably, UvrB contains all the conserved helicase domains characteristic of the SF2 superfamily, enabling it to translocate along ssDNA and unwind double-stranded DNA (dsDNA) through its ATPase-dependent helicase activity [27], [28]. The tight UvrB-DNA pre-incision complex then recruits the UvrC endonuclease, that carries out the initial incision at the 4^{th} or 5^{th} phosphodiester bond on the 3′ side of the lesion, followed by a second incision at the 8^{th} phosphodiester bond on the 5′ side, in a coordinated manner [29]. UvrC has been characterized across a range of bacterial species; however, a high-resolution structure of the full-length protein remains lacking. Structural insights have therefore largely been derived from isolated domains, notably the

N-terminal GIY-YIG nuclease domain and C-terminal regions, which have been independently expressed, purified, and analyzed by crystallographic and NMR approaches [30], [31], [32]. Despite these advances, the mechanistic basis of UvrC-mediated incision, particularly in pathogenic bacteria, remains incompletely understood.

Over the past five decades, the NER pathway has remained unexplored in *Listeria monocytogenes*, the pathogen responsible for listeriosis—a disease that typically presents as meningitis, encephalitis, sepsis, spontaneous abortion, or gastroenteritis, and primarily affects immunocompromised individuals [33]. For *L. monocytogenes* to establish infection through contaminated food, it must endure harsh conditions in the gastrointestinal tract, including stomach acidity and high bile concentrations in the small intestine, and subsequently cross the intestinal barrier [34]. Interestingly, UvrA has been implicated in the acid and bile tolerance of *L. monocytogenes* [35]. Studies in other organisms indicate that exposure to acid and bile can cause DNA damage, suggesting a potential role for NER genes in the *L. monocytogenes* genome in conferring resistance under these conditions [36], [37]. Additionally, in *L. monocytogenes*, *uvrA* is part of the SOS response regulon, a pathway essential for DNA repair and the recovery of stalled or collapsed replication forks [35], [36], [38]. Moreover, the mutations in the *uvrB* gene result in increased tolerance to benzalkonium chloride, one of the most commonly used sanitisers to control the growth and spread of *L. monocytogenes* in food-processing facilities [39]. Collectively, these studies indicate that a deeper mechanistic understanding of NER subunits may inform the development of targeted strategies against *Listeria* infection.

Here, we describe the isolation and purification of the *L. monocytogenes* UvrC (*Lm*UvrC) and *L. monocytogenes* UvrB (*Lm*UvrB) proteins, overexpressed in *Escherichia coli*. The purification and characterization of several *Lm*UvrC variants, alongside the wild-type protein, are also reported. Our results reveal that *Lm*UvrC exists as a monomer, exhibits robust DNA-binding and incision activities, and can coordinate an iron-sulfur cluster. Furthermore, domain truncation analyses demonstrate that efficient incision by *Lm*UvrC requires coordinated contributions from both the GIY-YIG and RNase H domains, highlighting the importance of interdomain synergy and UvrB-mediated activation. Overall, these observations contribute to our understanding of the biochemical properties of UvrC from *L. monocytogenes* and its functional role within the NER pathway.

Section snippets

UvrC of *Listeria monocytogenes* contains conserved domains similar to the prototype UvrC from *Escherichia coli*

The full-length *Lm*UvrC protein sequence was utilized as a query in the protein database to identify homologous sequences across different species of *Listeria*, as well as other bacterial and archaeal species, including *E. coli* (Figure S1). The search yielded several potential UvrC homologs within various *Listeria* species and other bacterial lineages. These homologous amino acid sequences exhibited a similar overall domain composition to that of *E. coli* UvrC (*Ec*UvrC) (Figure 1A). *Lm*UvrC contains …

Discussion

After the damage is recognized by the $UvrA_2$-$UvrB_2$ complex, followed by the formation of the UvrB-DNA preincision complex with concomitant release of UvrA, NER proceeds to repair damage sites by recruitment of UvrC. UvrC is a multidomain nuclease composed of several structural domains/motifs that perform distinct functions during NER; however, how these domains/motifs coordinate to execute the dual incision reaction remains incompletely understood. Secondly, the structures of truncated UvrC …

Biochemicals, restriction endonucleases, oligonucleotides, antibodies, bacterial strains, and plasmids

All chemicals used in this study were molecular biology or analytical reagent grade and purchased from Merck or Himedia Laboratories. The HiPrep™ 16/60 Sephacryl™ S-200 HR and Superose 6 10/300 GL FPLC columns were obtained from GE Healthcare Life Sciences. The polymerase chain reaction (PCR) purification kit, plasmid Miniprep kit, DNA ladders, and unstained protein molecular weight markers were procured from Thermo Fisher Scientific. The restriction endonucleases, T4 ligase, Q5 High-Fidelity …

Author contributions

All the three authors conceptualized the study: PCS contributed to the methodology and investigations; PCS and MT performed the formal analysis and validated the data. MT wrote the original draft of the manuscript. KNP and MT reviewed and edited the manuscript; KNP provided resources; KNP and MT supervised the study. All authors read and approved the manuscript. …

CRediT authorship contribution statement

Preetha C. Sivadasan: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Manoj Thakur:** Writing – review & editing, Validation, Software, Resources, Formal analysis, Conceptualization. **K. Neelakanteshwar Patil:** Writing – review & editing, Supervision, Software, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. …

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. …

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