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Investigation of bacterial nucleotide excision repair using single-molecule techniques☆

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ABSTRACT

Despite three decades of biochemical and structural analysis of the prokaryotic nucleotide excision repair (NER) system, many intriguing questions remain with regard to how the UvrA, UvrB, and UvrC proteins detect, verify and remove a wide range of DNA lesions. Single-molecule techniques have begun to allow more detailed understanding of the kinetics and action mechanism of this complex process. This article reviews how atomic force microscopy and fluorescence microscopy have captured new glimpses of how these proteins work together to mediate NER.

1. Introduction

1.1. Action mechanism of the bacterial UvrABC NER system: formation and disassembly of the machinery on DNA

Prokaryotic nucleotide excision repair (NER) was reconstituted with six highly purified proteins in 1985 by the Grossman and Sancar laboratories [1,2]. Since that time a huge wealth of functional and structural information has accumulated on this system, reviewed in [3–6]. Bacterial NER is initiated in two ways: (i) during transcription when RNA polymerase encounters a progress blocking lesion in a process termed transcription-coupled repair (TCR); or (ii) when the $UvrA_2UvrB_2$ complex encounters a region of DNA which is distorted by the presence of a DNA lesion unconnected with transcription, this process is known as global genome repair (GGR). During the former, the TCR factor (Mfd) pushes RNA polymerase off from the lesion and recruits $UvrA_2$ to the damaged site. Both GGR and TCR then proceed in a similar manner. In a step not well understood, the $UvrA_2$ dimer passes the damaged region of DNA to UvrB, which uses a beta-hairpin to verify the damaged nucleotide on one of the two DNA strands [7,8]. Engagement of UvrB at the damage site facilitates $UvrA_2$ dissociation and serves as a landing site for UvrC. UvrC is a dual nuclease which incises the damaged strand 3′ to the lesion using its N-terminal nuclease domain, and 5′ to the lesion using its C-terminal nuclease domain [9,10]. This post-incision UvrBC–DNA complex and an oligonucleotide containing the damage are dissociated by the dual action of UvrD and DNA polymerase I. DNA pol I fills in the excised region, and the repair patch is sealed by the action of DNA ligase [1,2] (see Fig. 1).

Despite almost three decades of research many fundamental questions remain unanswered regarding how the components of the prokaryotic NER machinery assemble at sites of damage [11]. These include: (i) how do DNA repair proteins, at levels of 100–1000 per bacterial cell, efficiently sort through a multi-million base pair genome for rare DNA lesions?; (ii) what are the dynamics of Mfd recruitment to a stalled RNA polymerase at a damaged site, and how/when are UvrA and UvrB subsequently recruited?; (iii) how and when is the lesion passed from UvrA to UvrB?; (iv) how is ATP binding is coupled to domain movement within UvrA and UvrB during damage engagement and verification?; (v) how does UvrD bind to the 5′ nick of the post-incision complex to allow dissociation of UvrC?; (vi) how is UvrB removed with the damaged oligonucleotides by the dual action of UvrD and DNA pol I?; and (vii) how is DNA ligase I recruited to the repair patch to seal the nick created by the action of DNA pol I? This review discusses how single-molecule techniques are being used to address these unanswered questions

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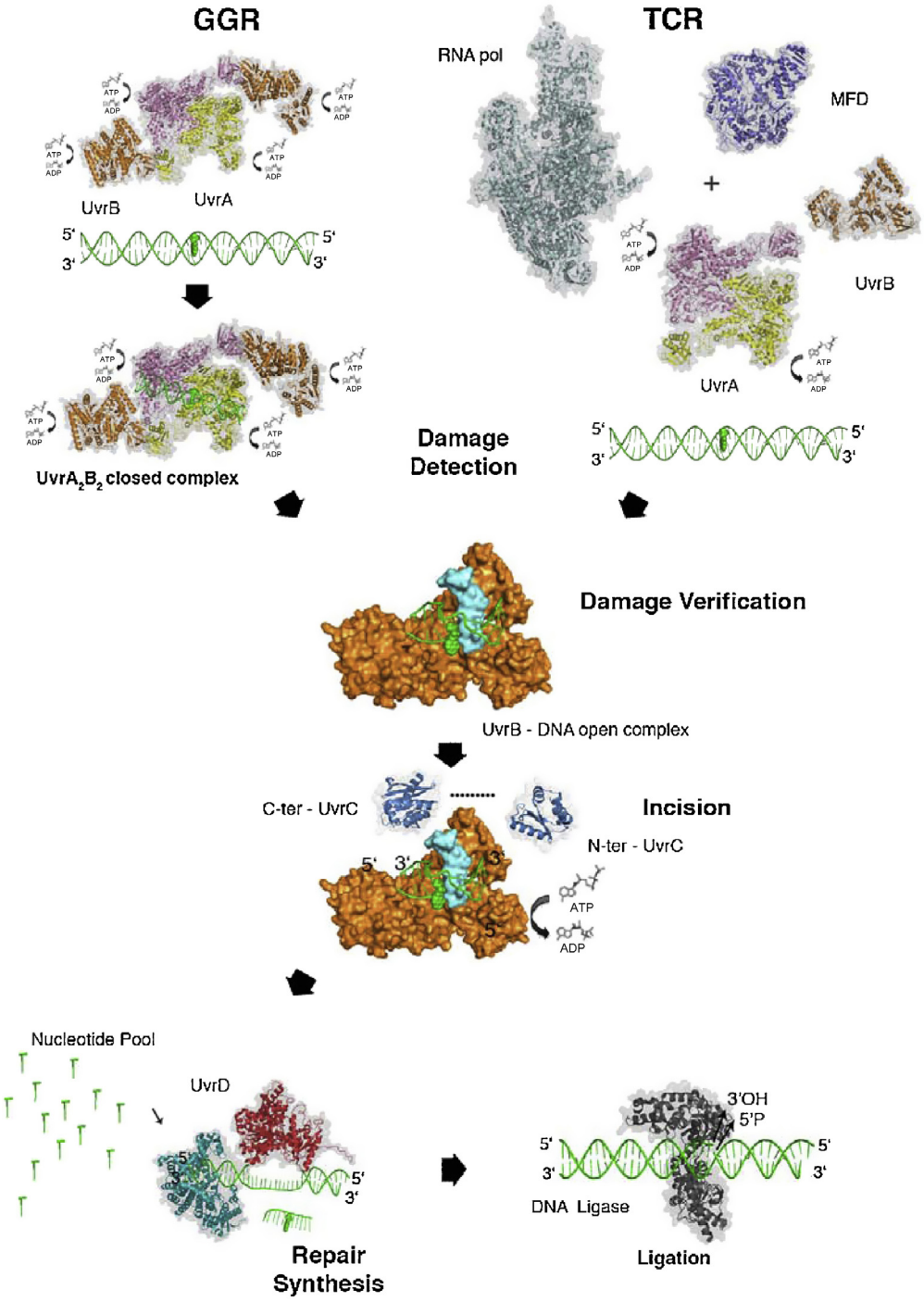

Fig. 1. Prokaryotic nucleotide excision repair. Structural model of prokaryotic NER showing the key protein and steps in global genomic repair (GGR) and transcription coupled repair (TCR). TCR damage recognition is initiated by a stalled RNAP (PDB ID: 4LJZ) that recruits MFD (PDB ID: 2EYQ). MFD displaces RNAP and brings UvrA to the damaged site. In GGR, the $UvrA_2B_2$ complex (PDB ID: 3UWX) for the contact interface: 3FPN first searches for the distortion along the DNA caused by the lesion. Both pathways converge after the initial recognition steps. UvrA then transfers the damaged DNA to UvrB for damage verification. The dimeric UvrA protein (PDB ID: 2R6F) hydrolyzes both ATP and GTP. It also forms a complex with UvrB (PDB ID: 2FDC) and activates the ATPase activity of UvrB. During damage verification, the β-hairpin of UvrB (shown in *turquoise*) inserts between the two strands of DNA and forms a stable pre-incision complex, which is believed to activate UvrB's ATPase. Binding and hydrolysis of ATP by UvrB is essential for recruitment of UvrC. The N-terminal endonuclease domain of UvrC (PDB ID: 1YCZ) initiates the cut 4–5 nucleotides 3′ to the damaged site followed by the 5′ cut by C-terminal endonuclease domain of UvrC (PDB ID: 2NRR) eight nucleotides away from the lesion. UvrD (PDB ID: 2IS1) unwinds the DNA and releases the oligonucleotide containing the lesion. Simultaneously, DNA polymerase I (PDB ID: 2HHQ) synthesizes the missing strand. Finally, DNA ligase I (PDB ID: 1DGS) seals the repair patch. All protein structures in this figure, with the exception of UvrB, are shown with a transparent surface and in ribbon presentation. UvrB is shown with its surface in orange for domains 1 to 3, and the β-hairpin is shown in cyan. C-ter, carboxy terminal; N-ter, amino terminal. From [6] with permission.

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