Poster Presentation

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Inner Workings of the UvrA•UvrB DNA Damage Sensor during Bacterial Nucleotide Excision Repair

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Efficient elimination of DNA lesions by the nucleotide excision repair (NER) pathway is critical for all organisms. In bacteria, the NER pathway is implemented by the successive action of three proteins, UvrA, UvrB and UvrC via a series of large and dynamic multi-protein complexes. A large number of studies have defined three major stages associated with the early steps of the NER pathway. In stage 1, a large (300-400 kDa) complex of the UvrA and UvrB proteins (AB) scans the genome to identify lesion-containing DNA. This process requires rapid binding and release of DNA; moreover, damage must be specifically recognized, and distinguished from native DNA, despite the fact that the relevant lesions induce widely different DNA structures. Once lesion-containing DNA has been located, it is stably bound by a dimeric form of UvrA within the AB complex (Stage 2). A major reorganization then occurs in which UvrA is lost from the ensemble, and concomitantly, UvrB becomes localized at the site of damage (Stage 3). Following these early stages, additional events lead to excision of the damage on one strand, and repair of the resulting single-stranded gap. Over the past few years, we have determined three structures of UvrA and the UvrA•UvrB complex. Our first structure of isolated UvrA revealed its overall architecture, its DNA binding surface, and the arrangement of its four-nucleotide binding sites. In the structure of the complete UvrA•UvrB damage sensor, a central UvrA dimer is flanked by two UvrB molecules, all linearly arrayed along a DNA path predicted by biochemical studies. DNA is predicted to bind to UvrA in the complex within a narrow and deep groove that is compatible with native duplex DNA only. In contrast, the shape of the corresponding surface in our prior structure of UvrA is wide and shallow, and appears compatible with various types of lesion-deformed DNA. These differences point to conformation switching between the two forms as a component of the genome-scanning phase of damage sensing. We also show that the highly conserved signature domain II of UvrA, which is adjacent to the proximal nucleotide-binding site, mediates a critical nexus of contacts to UvrB and to DNA. Moreover, in the novel UvrA conformer, the disposition of this domain is altered such that association with either UvrB or DNA is precluded. Concomitantly, nucleotide is uniquely absent from the proximal binding site. Thus, the signature domain II is implicated in an ATP-hydrolysis-dependent conformational change that detaches UvrA from both UvrB and DNA after initial damage recognition. The disposition and number of UvrB molecules in the AB complex, both unanticipated, suggest that once UvrA departs, UvrB localizes to the site of damage by helicase-mediated tracking along the DNA. Together these results permit a high-resolution model for the dynamics of early stages in NER.

Keywords: nucleotide excision repair, UvrA, UvrB, DNA damage repair