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David K. Orren

University of Kentucky, dkorre2@uky.edu

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The Nobel Prize in Chemistry 2015: Exciting discoveries in DNA repair by Aziz Sancar

David K. Orren

Department of Toxicology and Cancer Biology, University of Kentucky College of Medicine and Markey Cancer Center, University of Kentucky, Lexington, KY 40536-0305, USA

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INTRODUCTION

On October 7, 2015, the Nobel Prize in Chemistry for 2015 was awarded to three deserving scientists for their pioneering research on DNA repair. Tomas Lindahl was recognized for studies that uncovered the inherent instability of DNA as well as the mechanism of the base excision repair pathway, Paul Modrich for characterization of the mismatch repair pathway, and Aziz Sancar for mechanistic elucidation of the nucleotide excision repair (NER) pathway. For me, the announcement of these awards in my area of research was extremely gratifying, particularly so because Aziz was my mentor during my Ph.D. studies that examined the steps of prokaryotic NER. Memorably, I trained in the Sancar laboratory at a time where groundbreaking discoveries were being made in the burgeoning field of DNA repair, and can personally attest to his dedication and keen intellect. The text below primarily addresses Aziz's critical contributions towards our understanding of NER processes in *E. coli* during the early years of study on this pathway. Other researchers have made important contributions in this area that may not be specified or cited here due to emphasis and space constraints of this article.

CONTRIBUTIONS TO UNDERSTANDING PROKARYOTIC NER: BIOCHEMICAL CHARACTERIZATION AND RECONSTITUTION OF NER PROCESSES

Present in both prokaryotic and eukaryotic organisms, NER pathways are distinctive because they recognize and remove a wide spectrum of structurally diverse types of DNA damage, including lesions generated by the ultraviolet (UV) component of sunlight. Unraveling of the mystery that was prokaryotic NER began with isolation of *E. coli* strains that were hypersensitive to killing by UV irradiation (Hill, 1958; Howard-Flanders and Theriot, 1962), followed by the mapping of three gene loci (named *uvrA*, *uvrB*, and *uvrC*) that participated in repair of UV-induced cyclobutane pyrimidine dimers (CPDs) and some other chemically-induced DNA adducts (Howard-Flanders et al., 1966). It was recognized in the 1960's that this pathway of repair of UV-induced damage required nuclease activity on the DNA phosphodiester backbone (Boyce and Howard-Flanders, 1964; Howard-Flanders et al., 1966; Setlow and Carrier, 1964). However, Seeburg and colleagues (Seeburg et al., 1976) in the mid-1970's were the first to prove that this activity required the proteins encoded by the *uvrA*, *uvrB* and *uvrC* genes. During this period, several labs were competing to clone these genes and isolate the individual gene products. As a post-doctoral fellow in Dean Rupp's lab at Yale University, Aziz cloned the *uvrA*, *uvrB*, and *uvrC* separately into multi-copy plasmids and helped devise an approach

email: dkorre2@uky.edu

that allowed the gene products expressed from these plasmids to be partially purified without using a functional assay to track the protein of interest (Kacinski et al., 1981; Sancar et al., 1981a; Sancar et al., 1981b; Sancar et al., 1981c). These breakthroughs revealed the approximate molecular weights for the proteins encoded by the *uvrA*, *uvrB* and *uvrC* genes, making it possible to follow them by this property during subsequent purification efforts. Furthermore, these early UvrA and UvrC protein preparations showed DNA binding activity that was relevant to their function and also could be exploited to enhance purification schemes (Sancar et al., 1981b; Sancar et al., 1981c). Given that molecular biology and cloning technology were relatively new at the time, these were substantial accomplishments done over a short period of time.

Positive identification of these key NER genes and their protein products led to a flurry of subsequent discoveries key to our understanding of the general recognition and excision of DNA lesions removed by NER processes. After designing better purification techniques for each overexpressed protein, Aziz reconstituted damage-specific incision characteristic of NER by adding UvrA, UvrB and UvrC together (collectively known as ABC excinuclease) with UV-irradiated, supercoiled plasmid DNA and monitoring conversion of the supercoiled to relaxed (nicked) plasmids in reactions that also required Mg^{2+} and ATP (Sancar and Rupp, 1983). This immediately led to the finding that, in these reactions, the strand containing the damage was incised both 5' and 3' to the lesion, i.e., the dual incision mechanism that is conserved among all prokaryotic and eukaryotic NER pathways (Sancar and Rupp, 1983). For UV-irradiated DNA, these incisions occurred at the 8th phosphodiester bond 5' to the lesion and between 3 and 5 phosphodiester bonds 3' to the lesion; both incisions resulted in 3'-OH and 5'-PO₄ termini suitable for DNA polymerization and ligation (Sancar and Rupp, 1983). This fundamental dual incision pattern was subsequently shown to be conserved on other types of DNA damage removed by bacterial NER, and the precise positions of the incisions varied only very slightly depending upon the lesion involved and/or sequence contexts (Beck et al., 1985; Sancar and Rupp, 1983; Van Houten et al., 1986a; Yeung et al., 1983). The observation that both incisions were made at a distance from the lesion (as opposed to incision at the damaged site typical of base excision repair) also provided some clues as to why and how NER could remove a wide variety of structurally distinct lesions from DNA. This landmark paper (Sancar and Rupp, 1983) laid the foundation for closer examination of the pre- and post-incision steps of prokaryotic NER, as well as provided mechanistic understanding of the pathway that helped initiate investigation of NER in eukaryotic systems.

Around that time, Aziz and wife Gwen accepted faculty positions at the University of North Carolina in Chapel Hill, then and now an excellent research institution although

more commonly known for its basketball teams. There, research on NER in *E. coli* continued with an emphasis on understanding the finer details of the involved proteins and the overall repair mechanism. Using DNA sequencing techniques developed only several years earlier by Maxam and Gilbert (Maxam and Gilbert, 1977), the Sancar and colleagues published the nucleotide sequences of the *uvrA*, *uvrB*, and *uvrC* genes (Arikan et al., 1986; Husain et al., 1986; Sancar et al., 1982a; Sancar et al., 1982b; Sancar et al., 1984); in parallel, the *uvrC* promoter region and *uvrB* were also sequenced by researchers at the University of Leiden (Backendorf et al., 1986; van Sluis et al., 1983). These studies specified the Uvr A, UvrB and UvrC amino acid sequences and thus their precise molecular weights (103,874, 76,118, and 66,038 daltons, respectively). Sequencing of their promoter regions also confirmed the presence of LexA binding sites in the *uvrA* and *uvrB* promoters, consistent with earlier reports (Fogliano and Schendel, 1981; Kacinski et al., 1981; Kenyon and Walker, 1981) demonstrating inducible expression of these genes through the SOS response that involves up-regulation of many DNA damage response genes by RecA-mediated inactivation of the LexA repressor. The amino acid sequences of UvrA, UvrB, and UvrC also revealed characteristics of each protein that were relevant to the overall NER mechanism. In agreement with the ATP requirement of the incision step, UvrA contained two separate regions with homology to ATP-binding proteins while UvrB also possessed one such region (Arikan et al., 1986; Backendorf et al., 1986; Husain et al., 1986). Additional scrutiny of the UvrA sequence revealed the possible presence of Zn finger motifs that was subsequently confirmed by biophysical and mutagenesis experiments (Doolittle et al., 1986; Myles et al., 1991; Navaratnam et al., 1989). Although some regions of homology were identified between UvrB and UvrC (Arikan et al., 1986; Backendorf et al., 1986), the latter's sequence initially revealed little about its function in NER.

Around the same time, Ben van Houten in the Sancar lab developed DNA substrates containing single psoralen lesions at defined sites; these were incised efficiently by ABC excinuclease and also facilitated investigation of the pre- and post-incision steps of NER (Van Houten et al., 1986b; Van Houten et al., 1987) as well as the involvement of NER in repair of interstrand crosslinks (Cheng et al., 1988; Van Houten et al., 1986b). Hydrodynamic studies that I performed in Aziz's lab showed that UvrB and UvrC in isolation were monomeric, while UvrA alone formed dimers (Orren and Sancar, 1989). Mixtures of the proteins in the absence of DNA revealed a (UvrA)₂UvrB complex, and UvrC did not associate with this complex or with UvrA or UvrB alone (Orren and Sancar, 1989). UvrA was shown to be instrumental in DNA damage recognition, with higher affinity to damaged than to undamaged DNA, a difference that was further stimulated by ATP; UvrA bound to a 33 bp region surrounding a psoralen adduct (Seeberg and Steinum,

1982; Van Houten et al., 1987; Yeung et al., 1986). In contrast, UvrC showed equal affinity for damaged and undamaged DNA while UvrB alone did not bind DNA (Kacinski and Rupp, 1981; Orren and Sancar, 1989; Sancar et al., 1981b; Yeung et al., 1983). However, combining UvrA, UvrB, ATP and damaged DNA caused remarkable changes. Under these conditions, UvrB did bind specifically to damaged DNA in a manner dependent upon UvrA and ATP hydrolysis and the resulting DNA-protein complexes were more abundant and stable than those formed with UvrA alone (Kacinski and Rupp, 1981; Van Houten et al., 1988; Van Houten et al., 1987; Yeung et al., 1983). Initially, these complexes were believed to contain DNA and both proteins, but experiments I performed clearly demonstrated that UvrA could catalytically deliver UvrB to the DNA damage and then dissociate, leaving behind UvrB-DNA complexes that were precisely and stably bound at lesion sites (Orren and Sancar, 1989, 1990). These UvrB-DNA complexes were apparently also more compact than those made with UvrA, covering 19 bp surrounding a psoralen adduct (Van Houten et al., 1987). Importantly, addition of UvrC to isolated UvrB-damaged DNA complexes triggered damage-specific incision events (Orren and Sancar, 1989, 1990). While it was previously known that all three proteins were required to achieve dual incision surrounding the lesion, these results demonstrated that both the 5' and 3' incision activities were contained within UvrB and/or UvrC, while UvrA was not involved in making these incisions. While UvrB's presence in the incision complex is required, subsequent experiments clarified that the 5' and 3' incisions were catalyzed by separate domains within UvrC (Lin and Sancar, 1992; Verhoeven et al., 2000). Together, these findings suggested that the dual incision events characteristic of NER in *E. coli* occurred through an ordered series of steps; theoretically, this sequential mechanism ensures specificity for initiating removal of DNA adducts while minimizing incision of undamaged DNA. It is notable that eukaryotic NER is widely believed to take place by a similar sequential mechanism, even though many more proteins are involved to achieve dual incision of the damaged strand.

While the experiments above focused on the events leading up to the incisions of the damaged strand, other studies in Aziz's lab examined the post-incision events of prokaryotic NER. Evidence had indicated that, even after dual incisions occurred, Uvr proteins and the oligomer containing the damage were still associated with the remaining DNA (Caron et al., 1985; Husain et al., 1985; Sancar and Rupp, 1983; Van Houten et al., 1987; Yeung et al., 1983). Earlier research had also predicted the involvement of helicase II (the *uvrD* gene product) and DNA polymerase I (pol I) in NER processes, the latter responsible for DNA synthesis activity associated with NER (Boyle et al., 1970; Cooper and Hanawalt, 1972; Kuemmerle and Masker, 1980, 1983; Rothman and Clark, 1977; Van Sluis et al., 1974; Youngs and Smith, 1973), so they were natural candidates to use in

biochemical experiments using purified proteins. Neither helicase II nor pol I was required to achieve incision of damaged DNA. However, together helicase II and pol I stimulated the levels of incision mediated by the UvrA, UvrB and UvrC proteins, apparently by displacing limiting amounts of Uvr proteins from the damaged site after the incisions are made and thereby allowing them to catalyze multiple rounds of damage recognition and incision (Caron et al., 1985; Husain et al., 1985). Further studies (Caron et al., 1985; Orren et al., 1992) demonstrated that helicase II had two specific functions in NER that occurred subsequent to the dual incision step. First, it unwound and thus released the single-stranded oligomer containing the DNA lesion from the remainder of the undamaged double-stranded DNA, leaving a gap to be subsequently filled by pol I. In addition, helicase II displaces UvrC from the damaged site. Pol I concomitantly resynthesizes the short (12-13 nt) patch of DNA and displaces UvrB from the gapped DNA. Thus, helicase II and pol I not only perform the excision and resynthesis steps of NER, respectively, but they are also necessary for catalytic turnover of UvrB and UvrC. The NER process is completed by DNA ligase, which joins the resynthesized patch to the original DNA strand. Thus, the basic mechanism of NER in *E. coli* was essentially clear (Figure 1). In separate studies, key genetic and molecular details regarding the efficient repair of transcribed DNA (known as transcription-coupled repair) by NER processes in *E. coli* were uncovered by Chris Selby in the Sancar lab, specifying the mechanism by which the NER process detailed above was linked to transcription (Selby and Sancar, 1991, 1993; Selby et al., 1991).

ADDITIONAL CONTRIBUTIONS TO DNA REPAIR AND CIRCADIAN RHYTHMS

In addition to the research on bacterial NER detailed above, Aziz has made other important contributions to our understanding of DNA damage and its repair as well as circadian rhythms. While a group including myself examined NER processes in *E. coli*, several colleagues in the Sancar lab were working feverishly to develop methods to investigate mammalian NER. Their efforts laid the groundwork for subsequent, important discoveries that have greatly advanced our understanding of mammalian NER. Those findings from the Sancar lab have helped clarify the mechanism of NER in higher eukaryotes and the roles of the proteins involved. As observed for the bacterial pathway, mammalian NER occurs through dual incision of the damaged strand at some distances from the damaged site, although the 5' incision site is much further away (20-25 nt) from the lesion (Huang et al., 1992; Petit and Sancar, 1999). It is also noteworthy that hereditary defects of certain human NER factors cause the sun-sensitive, skin cancer-prone disease xeroderma pigmentosum, linking the inability to repair UV-induced DNA damage directly to dramatically in

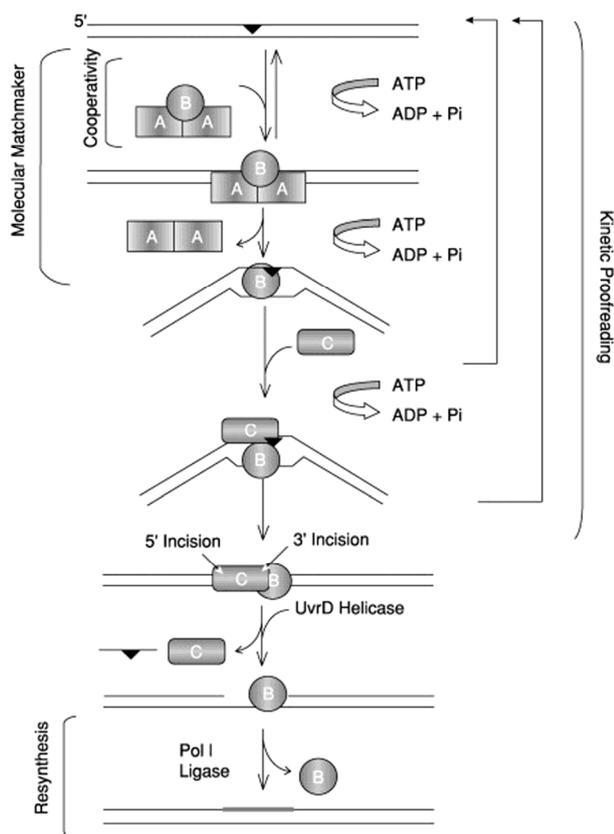


Figure 1 The mechanism of NER in *E. coli*. The pre- and post-incision steps of NER are shown. The DNA lesion is depicted as a black triangle, while shaded A, B, and C represent UvrA, UvrB and UvrC proteins, respectively; UvrD helicase is synonymous with helicase II (Reproduced from Reardon and Sancar (2005), with permission from Elsevier).

creased skin cancer susceptibility.

In another line of research that occurred concurrently with these NER studies, Aziz and colleagues also studied photolyases, DNA repair enzymes that catalyze the light-dependent reversal of UV-induced CPDs. This work (reviewed in Sancar (1994)) demonstrated that photolyases bind specifically to CPDs and utilize their two chromophore co-factors to absorb and transfer light energy to the enzyme's active site. This energy acts to destabilize and eliminate the aberrant cyclobutane ring that covalently links adjacent pyrimidines within CPDs. Furthermore, Aziz's interest in photolyases directly led to his subsequent investigations on the structurally-related, chromophore-containing CRY1 and CRY2 proteins, and their roles in circadian rhythms in higher eukaryotes. Circadian rhythms govern the process by which organisms set and maintain their 24-hour "biological clock" that responds to light (day) and dark (night) cycles. This research (reviewed in Sancar (2000)) has contributed to our current knowledge of how these CRY proteins present in retinal tissue absorb light to regulate circadian rhythms certainly in mice and humans and probably in most higher eukaryotes. Intriguingly, recent studies in this area from the Sancar lab (reviewed in Sancar et al.

(2015); Sancar et al. (2010)) indicate that circadian rhythms regulate nucleotide excision repair efficiency, checkpoint pathways and apoptosis. Importantly, these findings may potentially influence how cancer chemotherapies that target DNA are delivered in the future. Although results from these research efforts are not described in detail here, you should appreciate that Aziz's contributions to science go far beyond the molecular characterization of bacterial NER.

PERSONAL REFLECTIONS

In essentially one decade, the stepwise mechanism of NER in *E. coli* was entirely elucidated and, with only minor modifications, reflects our current understanding of this process. It is worthwhile to mention that, during the time these studies took place, molecular biology tools and computer technologies were rudimentary and thus research in general was significantly more labor-intensive. For example, purifications of overexpressed but untagged proteins were accomplished using multiple (slow) liquid chromatography columns and therefore were performed around the clock. DNA sequencing of each gene was painstakingly done bit by bit in the lab without using PCR techniques, automated sequencing technologies, or commercial assistance readily available today. Despite these limitations, for me the palpable energy from Aziz and others in the lab and the excitement of discovery more than compensated for the sometimes long hours at work. Aziz was so dedicated to moving the research forward, consistently there to ask the right questions and help logically derive the answers through carefully designed and controlled experimentation. During that period, the rapid and constant progress not only in our specific field but also in molecular biology in general made it seem that biological and biomedical research could and would solve many human health problems. While progress in addressing human diseases sometimes seems slower than we all wish for, tremendous strides have been made in our understanding of biology and disease processes. For the contemporary generation of new scientists, I hope that graduate students and post-doctoral fellows today still feel the excitement that goes along with new scientific discoveries like I felt when I was in Aziz's lab. To paraphrase an important Hindu philosophy described in the Gita, the work itself is virtuous without expectation of recognition or reward. However, if you are truly dedicated and insightful like Aziz, you might be fortunate enough to be recognized for that work!

Compliance and ethics The author(s) declare that they have no conflict of interest.

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