Nucleotide Excision Repair, a Tracking Mechanism in Search of Damage*

Lawrence Grossman‡ and Sam Thiagalingam

From the 3Department of Biochemistry, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland 21205

Nucleotide excision repair (NER) in all organisms, is able to accommodate to virtually any kind of DNA damage unlike many repair systems whose specificity is limited to a single species of damage. As a consequence NER has the advantage of being effective in responding to new types of environmental agents able to damage DNA.

The model repair system under extensive study is the Escherichia coli uvr system consisting of at least 6 structural genes (uvrA, uvrB, uvrC, uvrD, polA, and lig) and 2 regulatory genes (recA and lexA) that control excision repair reactions in the NER. Hence, in vitro, and sequenced and their gene products amplified and purified to accommodate to virtually any kind of DNA damage unlike many repair systems whose specificity is limited to a single species of damage. As a consequence NER has the advantage of being effective in responding to new types of environmental agents able to damage DNA.

**UvrABC Endonuclease**

When UvrA, UvrB, and UvrC interact with damaged DNA in the presence of ATP, dual endonucleolytic breaks are introduced into the DNA, 7 nucleotides 5' and 4 nucleotides 3' to damaged sites, regardless of the nature of the damage and of the primary chemistry of DNA interactions, which can be significantly different from each other (Yeung et al., 1983, 1986a, 1986b; Sancar and Sancar, 1988; Van Houten, 1990). The availability of reagent quantities of these proteins has been instrumental in the biochemical reconstitution of the partial and overall NER enzymatic reactions.

**UvrA Dimerization**

The first step in NER involves the dimerization of UvrA to generate the UvrA dimer, the reactive form of UvrA (Oh et al., 1989).

\[
(ADP-P_i) + 2UvrA \leftrightarrow UvrA_2 + ATP
\]

EAS 1

Significantly, it is the hydrolysis of ATP that drives dissociation of this protein-protein complex, whereas it is nucleotide binding (effected by the poorly hydrolyzable ATPyS) which drives protein-protein associations (Oh et al., 1989). These same principles also hold for protein-nucleic acid interactions in NER. Hence, in nucleotide excision repair, ATP binding and its hydrolysis provide the energy for dissociation-association reactions arising from the ATP \( \leftrightarrow ADP-P_i \) equilibrium, which may provide the basis for sensing damaged sites during translocation along a damaged DNA molecule by the UvrABC protein complex. The ADP–P_\text{i} denotes an intermediate generated by ATP hydrolysis, which is formed prior to release of ADP + P_\text{i} (Fersht, 1977). It is capable of driving association reactions much the same way that non- or poorly hydrolyzable ATP analogs act in this repair system.

(ii, iii) **Nucleoprotein Formation**

It is from kinetic, thermodynamic, structural, and catalytic studies of the interaction between UvrA, UvrB, and DNA that a number of significant molecular principles governing damage recognition, topological allostery, and manifestation of a cryptic ATPase have been revealed.

When comparing the equilibrium association constants for UvrA interactions with undamaged and damaged sites in a 25-mer pair DNA fragment, it was found that the discrimination factor, \( Df (K_u \text{ (damaged)}/K_u \text{ (undamaged)}) \) is only \( 10^3 \) (Mazur and Grossman, 1991). Although the level of damage recognition is too low for the Uvr system to recognize a single damage site per genome by passive diffusion controlled reactions, it is sufficiently discriminating for recognition of damage in short oligonucleotides consisting of higher ratios of damaged to undamaged nucleotides (non-tracking path). It suggested that a more concerted mechanism, such as a tracking model could be involved for damaged site recognition in DNA substrates of greater length (Mazur and Grossman, 1991; Grossman and Yeung, 1990a, 1990b). A tracking mechanism operating in two dimensions with increased local concentrations of the reactive protein subunits could be expected to increase the discrimination factor severalfold as opposed to a simple diffusion-controlled random event.

**Topological Unwinding**

When UvrA reacts with covalently closed circular duplexes of DNA in the presence of ATPyS, and ATP, it leads to a topological distortion, judging from linking number (\( \Delta L \)) changes. The size of the helical distortion is equivalent to a single helical turn, which is the approximate dimension of the dual incision reaction catalyzed by the UvrABC endonuclease complex (Oh and Grossman, 1986).

**Catalytic Profile of UvrA\(_B\)C Complex**

When UvrA\(_B\) and UvrB are combined an enhanced DNA-dependent ATPase activity reveals itself that has a turnover number 2 orders of magnitude greater than the UvrA-associates DNA-independent ATPase/GTase (Oh et al., 1988; Thiagalingam and Grossman, 1993). This cryptic ATPase is UvrB-associated and can also be manifested when UvrB is proteolysed specifically at a site 40 amino acids from the COOH-terminus of the protein to generate the truncated form of UvrB referred to as UvrB* (Caron and Grossman, 1988b).

(iv, v) **Tracking Path**

The scope of the ATPase activity accompanying UvrA interaction with UvrB suggested the potential for work by the complex, which proved to be due to the manifestation of a unique 5'→3' helicase which displaces a short (22-mer) single strand fragment with annealed termini (Oh and Grossman, 1987, 1989). It is significant that the gene responsible for the human repair deficiency in xeroderma pigmentosum (complementation group B) has been recently identified as the transcription factor TFIIH (BRC3-3) (Scheaffer et al., 1993). The TFIIH protein has helicase properties that are identical to the E. coli UvrA\(_B\) helicase (Oh and Grossman, 1987, 1989). Because of the increased binding of UvrA to single-stranded regions and the potential of the UvrA\(_B\) helicase activity to function on D-looped DNA, it implies that the helicase activity displaces a short bubble region in an unwinding-reannealing type mode of translocation (Seeley and Grossman, 1990).

(iv) **Supercoiling, A Measure of Simultaneous Tracking**

The responses to damage by the helicase on covalently closed circular DNA substrates and on linear duplex DNAs are enhancement of supercoiling (Koo et al., 1991; Thiagalingam and Gross-
man, 1993) and inhibition of strand displacement, respectively (Oh and Grossman, 1987, 1989). These observations strongly suggest that the damage scanning by helicase is halted by damage encoun-ter as well as the use of high affinity damaged site nucleoprotein complexes as anchor sites effecting more efficient scanning for ad-ditional damaged sites. To further test this hypothesis a UvRA deletion mutant protein unable to recognize damage (Clasensen and Grossman, 1991) was employed. The supercoiling reaction catalyzed by UvRA2B, normally stimulated by damage, is damage-in- sensitive when the ΔC40 UvRA protein is substituted in the UvRA2B complex supporting the notion that the oligomeric UvRA2B recognizes one thymine dimer as an "anchoring" site freeing the other half of the complex to scan for damage (Koo et al., 1991). This apparently accounts for such a robust stimulation of supercoiling suggesting a "skating" type translational supercoiling tracking mechanism.

Translocation terminates at a damaged site resulting in the for-mation of a highly stable nucleoprotein complex that seems to consist of a UvR-DNA complex (Yeung et al., 1986a; Thiagalingam and Grossman, 1993; Orren and Sancar, 1989; Van Houten and Snowden, 1988; Visse et al., 1992). The overall accumulation of UvR2-DNA complexes depends on catalytic rather than stoichiometric amounts of UvRA, suggesting turnover of UvRA at this step (Orren and Sancar, 1989; Visse et al., 1992) (Fig. 1, step v). Furthermore, the molar ratio of nicks to UvRA is as high as 10, suggesting it is not directly involved in the incision reaction.

UvRC is able by binding to this damage-specific complex to carry out a dual incision reaction (Sancar and Rupp, 1983; Yeung et al., 1983). The dual incision event is significantly invariant regardless of the nature of the damage. The nucleotide dimensions are seven nucleotides 5' and three nucleotides 3' to the same damaged site, with the exception of some slight variation in the pattern in DNA containing diadducts (e.g. Pt(NH3)2 (Beck et al., 1988a) and psoralen cross-links (Grossman and Yeung, 1990; Van Houten, 1990). That damage localization by the UvrA-B system goes through such molecular convolutions ostensibly to locate damage suggests its potential capacity to cooperate with those other biological reactions involving supercoiling reactions such as transcription and replication (Koo et al., 1991).

The release of the damaged fragment from the UvrBC-incised DNA and the turnover of the UvrBC subunits bound to the damage are dependent on the coordinated excision-resynthesis reaction by UvrD and DNA polymerase in the presence of deoxynucleoside triphosphates (Caron et al., 1986). The damaged DNA containing DNA fragment is released as a 12-13-nucleotide-long oligonucleotide. The ligation reaction that restores the integrity of the re-paired DNA strands is dependent on the prior reactions of UvrD and DNA polymerase I.

**Intragenomic Heterogeneity and Transcriptional Modulation**

When UV survival and removal of pyrimidine dimers are studied in Chinese hamster ovary (CHO) cells and human cells in culture, it was noted that although both survive UV exposure equally well, the removal of pyrimidine dimers was only 15% in CHO cells, while 80% were removed in human cells during a 24-h period (Bohr et al., 1986). The efficient removal of dimers from active genes in CHO cells was thought to be a possible explanation for this paradox. Further, a dramatic difference in the removal of pyrimidine dimers from transcribed versus non-transcribed strands has been reported both in eukaryotes and prokaryotes (Mellon et al., 1987; Mellon and Hanawalt, 1989). The dimers were removed more efficiently from the transcribed strand of the induced operon, whereas little repair was detected at this time in the non-transcribed strand.

**Sources of Energy**

**ATP Binding Site of Uvr—The anatomy of the individual uvr genes provides, in some cases, valuable clues as to the potential physiological role of the gene product, which is only proved when the molecular phenotype is established. For example, the putative Walker-type sequence motifs at amino acid positions 40–50 intu-tively inferred a role for ATP in the translocational process, thus providing for a rational approach for examining the consequences of ATP hydrolysis by directed mutagenesis of such a site (Seeley and Grossman, 1989, 1990).**

The ATP binding region of UvR was mapped from amino acid 39 to 57, and the consensus lysine 45 was converted to an alanine to produce the UvrB(K45A) protein that lacks the cryptic ATPase activity. UvrB(K45A) is also unable to support the helicase activity although it is able to bind irreversibly to UvrA to form UvrAZ- UvrB(K45A)-DNA complexes, which, as a consequence, act as clamps at an undamaged region because of its inability to translo-cate (Seeley and Grossman, 1989, 1990). This was the first experi-mental proof supporting the genetic and thermodynamic conclu-sions (Mazur and Grossman, 1991) that binding to undamaged regions is a requisite precursor to locating damaged sites. Lacking the ATPase and the machinery to translocate, the UvrB(K45A) irreversibly binds to undamaged DNA sites.

Significantly, the amino acid region downstream from the con-sensus lysine is genetically silent. Further, there is no tryptophan, a fluorescent amino acid, in UvrB that facilitated the introduction of this amino acid into the active site of the ATPase motif as a reporter amino acid (Hildebrand and Grossman, 1993). There is, however, only a single cysteine residue in the middle of the protein. Cysteine can be derivatized with IAEsDANS, a fluorescent probe. Measurement of fluorescence energy transfer between these two residues is providing useful insights for measuring the conforma-tional changes of UvrB during catalysis by employing steady state and picosecond time-resolved fluorescence spectroscopic techniques (Hildebrand and Grossman, 1993).

**Functional Domains of Uvr—**This extremely complicated gene (Fig. 2) containing tandem ATP binding and zinc finger sites and a single helix-turn-helix motif was initially studied by deletion mutagenesis in which regions starting from the COOH terminus region (Δ44 to Δ716 amino acids) were selectively deleted and in-serted into a ΔuvrA mutant in which the entire ΔuvrA gene was deleted for biological phenotyping. The representative mutant ΔuvrA genes were amplified and the respective truncated UvrA deletion mutant proteins isolated by unfolding and refolding from inclusion bodies and characterized (Clasensen et al., 1991). From such studies we learned that zinc was absolutely required during the refolding process for catalytic activity in a stoichiometric ratio of 2 mol of zinc to 1 mol of UvrA.

The separate truncated proteins were mapped for their ability to bind DNA, their ATPase activities, and their ability to interact with UvrA as a helicase. These studies identified, as a minimal require-ment, the first 230 amino acids for UvrB interaction, 680 amino acids as the dimerization interface, 900 amino acids as the DNA binding domain, and the COOH-terminal site required for damage

---

**TABLE I**

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Protein size, aa</th>
<th>No./cell</th>
<th>Functional motifs</th>
<th>Role in NER</th>
</tr>
</thead>
<tbody>
<tr>
<td>UvR</td>
<td>940 (107 kDa)</td>
<td>20</td>
<td>ATP binding (3)</td>
<td>Damage sensing</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Zn fingers (2)</td>
<td>UvrA binding</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Helix-turn-helix</td>
<td>Helicase subunit</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polybriple</td>
<td>UvrB delivery to damaged site</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ATP binding</td>
<td>Helicase subunit</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ADA site</td>
<td>Binds damaged site after UvrA delivery</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nuclease (?)</td>
<td>Damage-specific nuclease with UvrA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nuclease</td>
<td>Damage-specific nuclease with UvrB</td>
</tr>
</tbody>
</table>

---

**Zinc fingers (2)**

**Polyriple**

**ATP binding**

**ADA site**

**Nuclease (?)**

**Nuclease**
The COOH terminus of UvrA results in a functional asymmetry to hinge region (Fig. 1). Recognition. The most important observation from these studies is the localization of the site required for damage recognition stabilization in a novel motif spanning the last 44 amino acids at the COOH-terminal end of UvrA. This region is a glycine-rich poly-hinge region (Fig. 3) (Claessen and Grossman, 1991) in which the spectrum of damage specificity is being mapped, and the properties of the synthetic peptides are under investigation by NMR spectroscopy and scanning microcalorimetry. The location of such a motif at the COOH terminus of UvrA results in a functional asymmetry to a protein with an already apparent functional symmetry contributing, perhaps, to the directionality of the translocational process.

Because glycine and proline residues allow for more accessible conformations (Matthews et al., 1987), it suggests that the Uvr system can accommodate to virtually any kind of damage through a flexible induced fit mechanism on the part of the protein, thereby accommodating itself to conformations unique to damaged nucleotide regions of DNA.

Role of Tandem ATP Binding Sites of UvrA—With two tandemly arranged ATP binding motifs UvrA is one of a few unique proteins with this apparent duplication of ATP binding motifs (Thiagalingam and Grossman, 1991, 1993). For example, the role of tandem ATP binding sites (Fig. 2) has been implicated in transport of ions by the human cysst fibrosis factor (Anderson et al., 1991) and in the transport of proteins across the E. coli inner membrane by the SecA protein (Oliver, 1990). Each of the putative UvrA ATP binding sites was mutagenized independently or in combination. The separate genes were screened in uvrA mutant host strains, isolated, and the respective protein products purified and their molecular phenotypes characterized (Thiagalingam and Grossman, 1991, 1993). The complete loss of ATPase activity in the K37A,K646A UvrA double ATPase mutant and the retention of ATPase activity in the single mutants with differing kinetic parameters indicated that the individual ATPase sites could function independently. Surprisingly but consistent with the role of ATP in the repair process is the irreversible binding of the COOH-terminal and double ATPase mutant proteins to DNA. These findings provide firm evidence for the role of ATP hydrolysis in the nucleoprotein dissociation reactions. Furthermore, these studies also provide direct proof for the UvrB-associated cryptic ATPase activity, as well as the requirement for ATPase in addition to the UvrB ATPase requirement for the UvrA,B-helicase supercoiling activity (Thiagalingam and Grossman, 1993).

The Helix-Turn-Helix and Zinc Finger Motifs of UvrA—Random-directed mutagenesis was used to analyze the putative helix-turn-helix and zinc finger motifs of UvrA (Wang and Grossman, 1993). Those proteins mutated in the helix-turn-helix motif led to significant conformational changes, which are reflected in their circular dichroic spectra. The phenotypic properties affected by such mutations include damage discrimination and specificity. Zinc finger motifs, however, may play a less specific role in the intermolecular avidity required for protein-protein and nucleoprotein interactions.

Conclusions for Role of ATP in Nucleotide Excision Repair

The source of energy driving macromolecular associations in the E. coli system is derived from the binding of ATP. The function of ATP to UvrA and to UvrB induces allosteric transitions to a conformation engendering a high affinity protein-protein or nucleoprotein state. The induction of such a state is essential for two molecular events which require the input of free energy. These two events include (a) the formation of stable nucleoprotein subunits (ii) the topological unwinding of duplex DNA. Both of these events encourage a molecular interchange between different structural conformations (Table II).

Macromolecular dissociation requires ATP hydrolysis, which
does two things. ATP hydrolysis results in the loss of ATP as an effector molecule, and second, it produces ADP which when protein bound probably induces a UvrA conformational change that results in a low affinity DNA binding state, thereby promoting dissociation. The induction of a low affinity state is important for: (a) nucleoprotein dissociations, (b) translocation from an undamaged to a damaged site, (c) the directionality during translocation, and (d) turnover of protein subunits following dual incision.

ATP, in therefore, required for all preincision steps in NER, and once the UvrA•B complex is delivered to a damaged site, ATP is no longer required for incision by UvrC (Caron and Grossman, 1988a).

Sensing Mechanisms

It is implied that the ability of the UvrA•B helicase to be able to detect the presence of damage along the DNA chain is a consequence of the ATP → ADP-P equilibrium leading to micro-DNA association-dissociation reactions coupled to unidirectional translocation (Fig. 4). The direction of that equilibriu should be governed by the 3' steps of magnitude discrimination of damaged versus undamaged sites. The energy required for the unidirectional translocation and damage sensing is apparently derived from ATP binding and hydrolysis by both the UvrA and UvrB subunits of the helicase (Thiagalingam and Grossman, 1993). During translocation, UvrA can sense DNA damage by the combined conformational influences of the helix-turn-helix and polyhinge regions (Claassen and Grossman, 1991; Wang and Grossman, 1993) utilizing the conformational flexibility inherent in these motifs driven by the ATP association-dissociation states of the protein. The enhancement of supercoiling in the presence of damage on DNA (Koo et al., 1991) suggests that UvrA can anchor at a damaged site and allow for detection of additional damaged sites on opposite duplexes. The potential for an anchored damage-scanning mechanism is interpreted as due to the ability of UvrB to link two UvrA molecules by protein-protein interactions and to nucleotide binding leading to conformational flexibility of the damage-sensing helix-turn-helix and polyhinge motifs in the dual UvrA molecules.

These concepts are supported by the following experimental observations: (i) The UvrA protein by itself exhibits enhanced binding to damaged DNA in the presence of ATP (Seeberg and Steinum, 1982; Thiagalingam and Grossman, 1991); (ii) the UvrA and UvrB subunits when together not only exhibit enhanced ATPase activity associated with the helicase in the presence of either damaged or undamaged DNA, but the complex binds to damaged sites with greater avidity (Yeung et al., 1986a; Oh et al., 1986; Thiagalingam and Grossman, 1993); (iii) the COOH-terminal and double ATPase mutants of UvrA form stable nucleoprotein complexes in a damage-independent fashion with no apparent effect on the stability by UvrB (Thiagalingam and Grossman, 1991, 1993); (iv) the deletion of the COOH-terminal 44-amino acid polyhinge region of UvrA leads to an apparent loss of damage discrimination (Claassen and Grossman, 1991); and (v) the random directed mutations in the helix-turn-helix motif of UvrA result in the disappearance of damage-enhanced UvrA binding (Wang and Grossman, 1993).

REFERENCES


TABLE II

<table>
<thead>
<tr>
<th>ATP Binding, Hydrolysis</th>
<th>ATP Binding</th>
<th>Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. UvrA dimerization</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>b. (UvrA) monomerization</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>c. (UvrA)•DNA association</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>d. (UvrA)•DNA dissociation</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>e. (UvrA)•B assembly</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>f. (UvrA)•B•DNA association</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>g. (UvrA)•B•DNA dissociation</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>h. (UvrA)•B•helix-supercoiling reaction</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>i. UvrB-damaged site complex formation</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>j. UvrBC damage-specific incision</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>k. UvrABC endonuclease overall dual incision</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>