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Review

'Close-fitting sleeves': DNA damage recognition by the UvrABC nuclease system

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Abstract

DNA damage recognition represents a long-standing problem in the field of protein–DNA interactions. This article reviews our current knowledge of how damage recognition is achieved in bacterial nucleotide excision repair through the concerted action of the UvrA, UvrB, and UvrC proteins.
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Keywords: Protein–DNA interaction; Bacterial nucleotide excision repair; DNA damage recognition

Contents

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Prologue

One of the key aspects of science is problem definition. A few seminal papers in DNA repair and protein–DNA interactions have influenced much of my thinking in DNA damage recognition. In particular, two papers by Hanawalt and coworkers [1,2] have had a lasting impression on me. In 1965, Philip Hanawalt and Robert Haynes outlined an idea for damage recognition that was both elegant and prophetic: After observing that the *Escherichia coli* nucleotide excision repair system could act on a wide variety of DNA adducts, they stated, "The recognition step in the repair mechanism could be formally equivalent to threading the DNA through a close fitting 'sleeve' which gauges the closeness-of-fit to the Watson-Crick structure" [1]. The following review is dedicated to Professor Hanawalt's rich and lasting impact on my career as my laboratory has struggled to understand what I have called the damage recognition problem: how does a protein machine, made up of multiple protein subunits, first identify altered bases in a sea of non-damaged DNA, and secondly, after marking the site for the dual endonuclease activity of UvrC, faithfully remove and replace a damaged stretch of 12–13 nucleotides? Professor Hanawalt has been a shining beacon of inspiration and enthusiasm, and I am greatly indebted to his mentorship throughout my career. This review highlights recent work from my and several laboratories, but is not intended to be comprehensive, and I apologize in advance for any pertinent work that is not cited.

Ben Van Houten

1. Introduction

Structure–function studies using the tools of NMR, X-ray crystallography and biochemistry have helped define the parameters that modulate DNA damage recognition, but large gaps in our knowledge still exist. In this article, we first explore the nature of the substrates that are acted on by the UvrABC system, and then discuss each of the subunits in some detail. We consider how these three components work in concert to identify and remove damage. Finally, we outline what information is still beyond our grasp.

2. Discovery of the genes

In the early days of DNA repair, investigators studied UV-induced mutagenesis and cell death in bacteria. From these studies came the seminal observations that UV-induced DNA lesions were excised from DNA [3,4] and that repair synthesis occurred after excision [5]. In time, the process collectively became known as nucleotide excision repair (NER). The individual bacterial genes responsible for the nucleotide excision repair proteins were later discovered by complementation studies, curiously, in exactly the same order in which they operate in the NER pathway [6].

The NER genes are widely dispersed along the bacterial chromosome. The *uvrA* and *uvrB* genes are under the control of the SOS response (for a review see [7]) while the *uvrC* gene is not. The SOS system governs the up regulation of numerous genes in response to DNA damaging agents. This is significant because the UvrA protein is normally expressed at very low levels, 20–25 copies per cell, while after induction with a DNA damaging agent, UvrA levels rise ten-fold. Likewise, UvrB levels rise four-fold, from 250 copies per cell to 1000 copies (reviewed in [8]). Due to the labile character of UvrA and its low basal level of expression, it required 10 years to go from identification

of the gene to purification and characterization of the protein by Seeberg and Steinum [9].

Nucleotide excision repair, mediated by the UvrABC type proteins, exists in bacteria and archaea, but not in eukaryotic cells. Today, the sequences of more than 200 bacterial *uvrA* genes, 71 *uvrB* genes and 76 *uvrC* genes have been deposited in the Swiss-Protein database. These sequences provide a rich resource for comparative genomics. Thus, the identification of highly conserved residues becomes immediately apparent as functional analysis can be explored by site-directed mutagenesis.

3. General mechanism

A hypothetical scheme for key steps in the UvrABC damage recognition and incision reaction is shown (Fig. 1). In solution, UvrA dimer formation is driven by ATP [10]. While UvrA can recognize damaged DNA independent of UvrB, we believe it is the complex of UvrA and UvrB together that provide this function in vivo. UvrB interacts with the $UvrA_2$ dimer in solution, creating an $UvrA_2B$ or $UvrA_2B_2$ complex. Based on X-ray crystallography results, UvrB appears to be a monomer [11–13]; however, solution cross-linking studies and atomic force microscopy studies have suggested that UvrB can form a dimer in solution and on DNA ([14], H. Wang and Van Houten, unpublished observation). A heterotetramer consisting of two molecules each of UvrA and UvrB provides an attractive model for damage scanning in which UvrA, in association with one molecule of UvrB could scan each strand in a search for DNA adducts [14,15]. Once a damaged strand has been recognized, it is hypothesized that the DNA is bent and wrapped around one molecule of UvrB [14,16]. The other UvrB molecule would dissociate, allowing for the binding of UvrC to mediate the dual incision reactions. However, more definitive studies using analytical centrifugation and gel filtration chromatography are required to determine the exact oligomeric state of UvrB that is necessary for damage engagement. Thus, for the sake of simplicity, we refer to UvrB as a monomer, and due to the controversy regarding the stoichiometry of the proteins when they are in complex with each other, we have chosen to depict UvrA and UvrB together as the UvrAB complex. Within this UvrAB complex, UvrA initiates contact with the DNA and then facilitates the transfer of DNA to UvrB's DNA binding domain [17]. The cryptic ATPase activity of UvrB (the red nodule on UvrB, see Fig. 1) is activated in the context of the UvrAB:DNA complex and is required for damage verification. It is believed that UvrA hydrolyzes ATP, and affords its self-dissociation from the recognition complex leaving a salt resistant UvrB:DNA complex [18]. Before 3′ incision by UvrC, UvrB must be in its ATP bound conformation [19]. UvrC catalyzes both incisions with the first incision four-phosphodiester bonds 3′ to the lesion, and the second, eight-phosphodiester bonds away from the DNA lesion on the 5′ side. The dual incisions create a 12-nucleotide stretch of DNA containing the lesion [20–23]. Following incision, DNA helicase II (UvrD) is required to release UvrC and the incised oligonucleotide, while DNA polymerase I is thought to remove UvrB from the non-damaged DNA strand during the repair synthesis [24,25]. DNA ligase I, encoded by the *LigA* gene, joins the newly synthesized DNA to the parent DNA, thus completing the NER pathway.

4. Grasping the substrates: structures of some key DNA adducts

Long before the genes were cloned and the proteins purified, it was recognized that UvrABC nucleotide excision repair removes a large number of structurally and chemically diverse set of DNA adducts [1]. These include: UV-induced pyrimidine dimers and 6-4 photoproducts, polycyclic aromatic hydrocarbon adducts, anticancer agents, interstrand adducts involving both strands and most recently, even protein–DNA cross-links in vitro though possibly not in vivo ([26] and see Table 1 with references cited therein).

Several attempts have been made to correlate the structure and conformation of the DNA adducts with the rate of incision by the UvrABC nuclease complex [8]. It was recognized early on that the 6-4 photoproduct is more distorting than the cyclobutane pyrimidine dimer (TT pyrimidine dimer), and that the incision rate of 6-4 photoproducts is higher than that for TT pyrimidine dimers. These in vitro data nicely recapitulate the rate of removal of these adducts in vivo [122]. In another example, Van Houten and Snowden [32] determined that while a ring closed abasic site (AP) is very poorly incised, UvrABC incised the AP < ring-

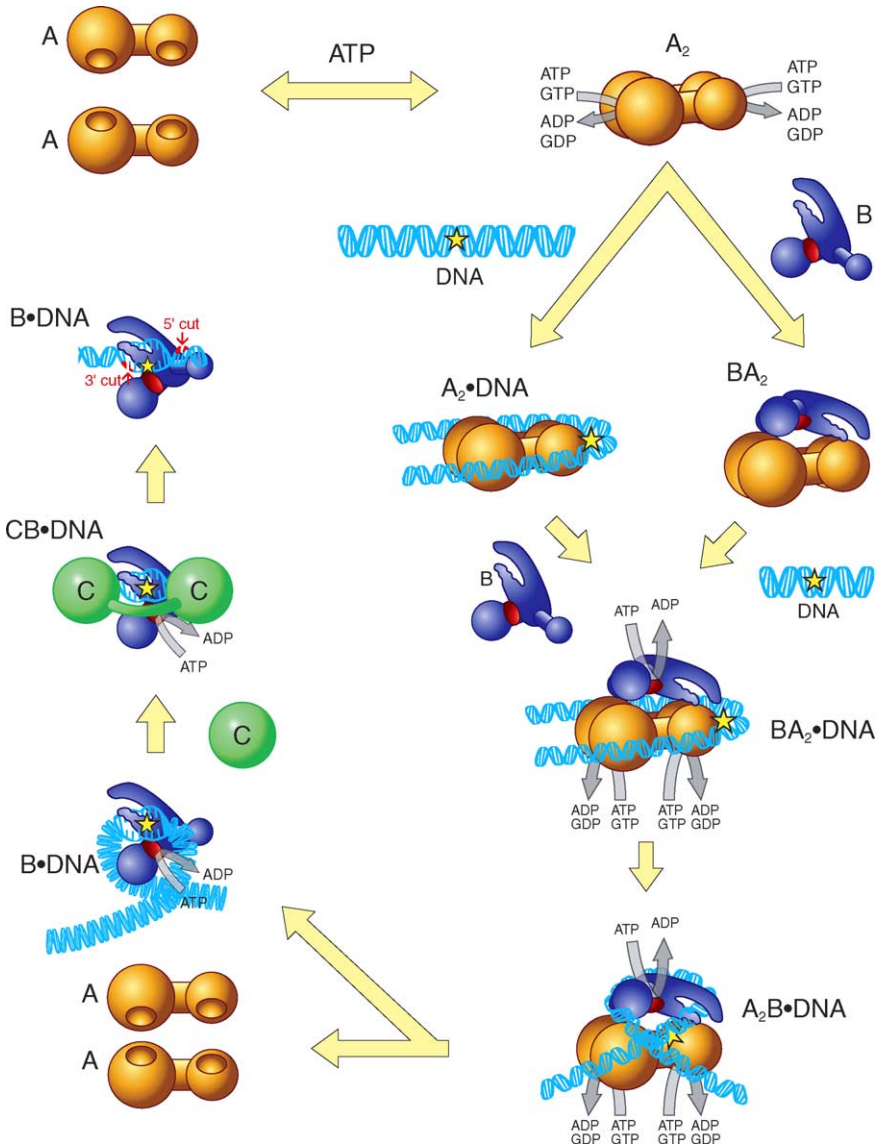

Fig. 1. Graphic representation of catalytic mechanism. A hypothetical scheme for the key steps in the mechanism is shown; see the text for references and a more complete description. In solution, two molecules of UvrA form a dimer, presumably between the ABC ATPase modules and ATP binding drives dimer formation. The $UvrA_2$ complex possesses ATP/GTPase activity. UvrB can interact with this $UvrA_2$ dimer in solution or on DNA, creating the $UvrA_2B$ complex. Upon binding to DNA, the $UvrA_2B$:DNA complex undergoes conformational changes. The DNA lesion remains in close contact with UvrA and then it is transferred to UvrB. UvrB is endowed with a cryptic ATPase activity (the red nodule on UvrB) that is activated in the context of $UvrA_2B$:DNA. In this complex, the DNA is unwound around the site of the lesion because UvrB has inserted its β-hairpin structure between the two strands of the DNA to facilitate damage verification. The DNA is also wrapped around UvrB. The UvrA molecules hydrolyze ATP and dissociate from the complex, thereby creating a stable UvrB:DNA complex. UvrC recognizes this UvrB:DNA complex. We have depicted one UvrC molecule with two catalytic sites. Before UvrC can make the 3′ incision, UvrB must bind ATP, but not hydrolyze it. After the 3′ incision is generated, a second incision event on the 5′ side of the DNA lesion is produced; thus, UvrC forms a dual incision approximately twelve nucleotides apart. After the incision events, the DNA remains stably bound to UvrB until UvrD, DNA pol I and ligase perform the repair synthesis reaction.

Table 1
DNA damage recognized by UvrABC

Category	Damaging agent	Lesion or adduct description	Repair by UvrABC	References
I. Single base modifications	4-Nitroquinoline-1-oxide	4NQO-purine adducts	+	[27–29]
	Apurinic/apyrimidinic sites	Abasic sites, reduced apurinic sites (ring opened)	+	[30–33]
	Aflatoxin-B1	Purine adducts, N^7-guanine, formamidopyrimidine	++	[34–38]
	Alkoxyamine modified AP sites	AP analog	++	[39,40]
	Anthramycin	N^2-Guanine	+++	[41–43]
	Cholesterol	Synthetically prepared cholesterol adducted base	+++	[44]
	Fluorescein	Synthetically prepared fluorescein adducted thymine	+++	[17]
	Ionizing radiation	Dihydrothymine, *N*-glycoside-β-ureido iodobutyric acid Urea residues/thymine glycol HO-C^5, C^6-thymine	Not repaired/++	[30,45,46]
	Menthol	Synthetically prepared menthol adducted base	+	[47]
	Multi-functional alkylating agents	O^4-Alkyl thymine, O^6-methyl guanine, N^6-methyl adenine	Not repaired/+	[48–52]
	N-Acetoxy-2-acetylaminofluorene (AAF), *N*-hydroxy-aminofluorene (AF)	C^8-Guanine	++	[53–60]
	Polycyclic aromatic hydrocarbons (PAHs)	N^2-Guanine, bezno[*a*]pyrene diol epoxide, methylchrysene/C^8-guanine, 1-nitropyrene	+++/++	[37,60–69]
	Psoralen	Mono-adduct (e.g. 8-methoxypsoralen (8-MOP) and 4,5′,8-trimethylpsoralen (TMP).	+++	[20,55,70–74]
II. Intra- and Inter-strand Cross links	CC-1065	N^3-Adenine	++	[75–77]
	Cisplatin	N^7-Guanine, GG, AG/GxG	++/+++	[78–84]
	Cyclohexylcarbodiimide	Unpaired T and G residues	++/+++	[85]
	DNA-protein/DNA-peptide cross links	Chemically induced	+/++	[26,68,86–88]
	Mitomycin C, *N*-methylmitomycin A	N^7-Guanine; O^6-methyl guanine, N^2-Guanine	++	[6,89–93]
	N,N′-Bis(2-chloroethyl)-*N-nitrosourea*	Bifunctional alkylation	++	[89,94,95]
	Nitrogen mustard	Bifunctional alkylation	++	[89,93,95]
	N′-Methyl-*N*-nitronitrosoguanidine (MNNG)	O^6-Methyl guanine	++	[50,52,96]
	Psoralen	C^5, C^6-Thymine; bisadduct	+++	[20,55,70,74,97–102]
	UV irradiation	Pyrimidine dimer (C^5, C^6-pyrimidine), 6-4-photoproduct	++/+++	[20,28,68,103–106]

Table 1 (*Continued*)

Category	Damaging agent	Lesion or adduct description	Repair by UvrABC	References
III. Natural Bases	A-tracts	AAAA	Not repaired	[8,46]
	dsDNA		Not repaired	[8]
	Extrahelical bases or loops in DNA		Not repaired	[85]
	Mismatches	A-G; G-G	Not repaired/++	[73,85]
	Sequence-specific bends		Not repaired	[8,107]
IV. Backbone modifications	2-Aminobutyl-1,3-propanediol (ABPD)	Synthetically modified	+	[73]
	Azidophenacyl bromide	Synthetically modified, phosphorothioate linkage	+	[17]
	Cholesterol, Chol-S, Chol-P	Synthetically modified, tethered to backbone	+++	[15,47,83,108,109]
	Fluorescein	Synthetically modified, tethered to backbone	+++	[68,110–112]
	Phosphorothioate, methyl phosphorothioate	Synthetically modified	+	[17,113]
	Phosphotriesters		Not repaired	[8,46]
	Single nucleotide gap	Synthetically modified	+++	[17,108]
	Single strand nick (3′ or 5′) in dsDNA with modified bases	Synthetically modified	+++	[17,31,109,114]
	Single strand nick in dsDNA	Synthetically modified	+++	[17,108]
V. Intercalators	Actinomycin D		Inhibits repair	[115]
	Caffeine		Inhibits repair	[115–117]
	Chloroquine		Inhibits repair	[116]
	Ditercalanium	Noncovalent bisintercalator	++	[118,119]
	Doxorubicin/AD32		+	[120,121]
	Ethidium Bromide		Inhibits repair	[115]
	Hoechst 33258		Inhibits repair	[115]

Repair key: +, 0–25%; ++: 25–50%; +++: >50%; see individual references for greater detail.

opened AP < methoxyamine-AP < benzoxyamine-AP. The general rule seems to be that the larger the chemical substituents on the DNA, the higher the rate and extent of incision by the complete UvrABC nuclease system.

Studies by Hoare, using aromatic hydrocarbons further confirmed that the size of the chemical moiety greatly affected the extent and rate of incision [66]. She found that nitropyrene-C^8-dG adduct is incised at a rate < (−)*cis*-anti-BPDE-N^2-dG ≤ (+)-*trans*-anti-methylcrysene-N^2-dG adduct. Even the stereochemistry of identical chemical adducts can affect incision as we found with (±)-*cis* or -*trans*-BPDE-N^2-dG adducts [64]. (±)-*cis*-BPDE adducts form a base-displaced structure in which the BPDE is stacked into the helix and either one or both of the adjacent guanine and cytidine residues are displaced outside the helix. In contrast, the (±)-*trans*-BPDE adducts bind in the minor groove with little disruption of base pairs, but lead to a dynamic opening and bending of the DNA. The (+)-*trans* displays a stronger perturbation than the (−)-*trans*, suggesting an explanation for the differential recognition by the prokaryotic NER machinery [123]. The UvrABC system incised the *cis*-BPDE adducts better than the *trans*-BPDE and also the stereochemistry affected cleavage with the plus enantiomers being removed better than the minus. It is interesting to note that the (−)-*trans*-BDPE lies in the 3′ direction with respect to the modified dG and apparently interfered with the normal 3′ incision site [64].

Geacintov et al. [124] have suggested that the thermostability of the DNA helix is affected by both

sequence context and the overall extent of adduct disruption can greatly affect recognition by the NER proteins. Verhoeven tested this hypothesis by measuring incision of the same DNA adduct in several sequence contexts. They clearly showed that the incision of the same DNA adduct varied depending on sequence contexts [83]. Thus, surrounding sequence can alter the conformation of a DNA adduct.

DNA repair proteins must be finely tuned by evolution to be able to discriminate damaged DNA from non-damaged DNA. Otherwise, it has been suggested that the repair machinery might promiscuously incise non-damaged DNA, leading to potential mutagenesis during gratuitous gap filling [113]. To this end, Sancar and coworkers [113] have suggested that both bacterial and human NER systems can occasionally incise non-damaged DNA. It is interesting to note that single nucleotide gaps and even nicks are bonafide substrates for the UvrABC system [17,108]. The possibility exists that these proteins play a potential role in other DNA processes besides repair. Curiously, the double *polA*/*uvrB* mutant is not viable, thus suggesting a wider role for UvrB in DNA maintenance beyond just repair (reviewed in [8]).

Most recently, it has been shown that protein–DNA cross-links are substrates for UvrABC [87], and that larger protein–DNA cross-links are repaired less efficiently than oligopeptide cross-links. These data suggest that protein–DNA cross-links might be processed to smaller oligopeptides by endogenous proteases [88] and that there is an upper limit to the size of the lesion that can be acted on by the NER machinery. The current challenge for the field is to develop a recognition model that can account for the entire substrate repertoire using rigorous structural and thermodynamic principles that combine information regarding the structure and conformation of the DNA lesion, with the interaction of residues on UvrA and UvrB. Before we can discuss recognition in any greater detail, it is important to become familiar with the individual proteins.

5. UvrA

Bacterial *uvrA* genes encode proteins (103–105 kDa) whose primary sequence reveals the presence of two zinc finger motifs and two ATP binding cassette ATPase (ABC ATPase) domains [125,126]. Structurally, UvrA is thought to contain two domains separated by a flexible protease sensitive linker region (see Fig. 2). The N- and C-terminal domains each contain one ABC-type ATPase domain interrupted by a Cys_4-type zinc finger. While the N- and C-terminal domains contain a high degree of homology within the ATPase cassettes, they vary considerably outside these regions. The ABC ATPase domain is usually a contiguous stretch of about 200 amino acids. However, in UvrA, this domain is interrupted by the insertion of about 390 amino acids in the N-terminal domain, while the C-terminal domain contains an additional 130 amino acids. Besides the zinc fingers within these inserted domains, there are no other remarkable features. The function of the amino acids within these inserted domains is currently unknown.

5.1. UvrA zinc fingers: DNA binding motifs

The zinc fingers contained in UvrA have the consensus sequence of $CysX_2CysX_{18\text{-}20}CysX_2Cys$ in which the four cysteine residues coordinate one zinc molecule [127]. While the two zinc fingers share the same spacing, the primary amino acids are quite different. Furthermore, the N-terminal zinc finger is less well conserved than the C-terminal zinc finger. Consistent with this observation is that mutations in the N-terminal zinc finger have little effect on NER and therefore, it was concluded this zinc finger is not essential for NER in vitro [128]. However, amino acid substitutions that disrupt the C-terminal zinc finger lead to insoluble proteins and rendered the bacterium profoundly sensitive to cell killing by UV [128]. In an additional study, it was shown that the C-terminal zinc finger mutation C763F creates a UvrA mutant protein that retained no in vivo repair activity and failed to bind to DNA, but retained vigorous ATPase activity [129]. Based on these results, it was concluded that the C-terminal zinc finger is primarily responsible for UvrA's DNA binding capacity. While it has been shown that mutations of the cysteines in the C-terminal zinc finger give rise to dysfunctional proteins, we cannot conclude from these studies that the zinc finger is responsible for DNA binding. It is likely that global changes occurred in UvrA as a result of these cysteine mutations. Therefore, while it is *likely* that the C-terminal zinc fingers are involved in DNA binding, site-directed mutagenesis is required to address the role of the C-terminal

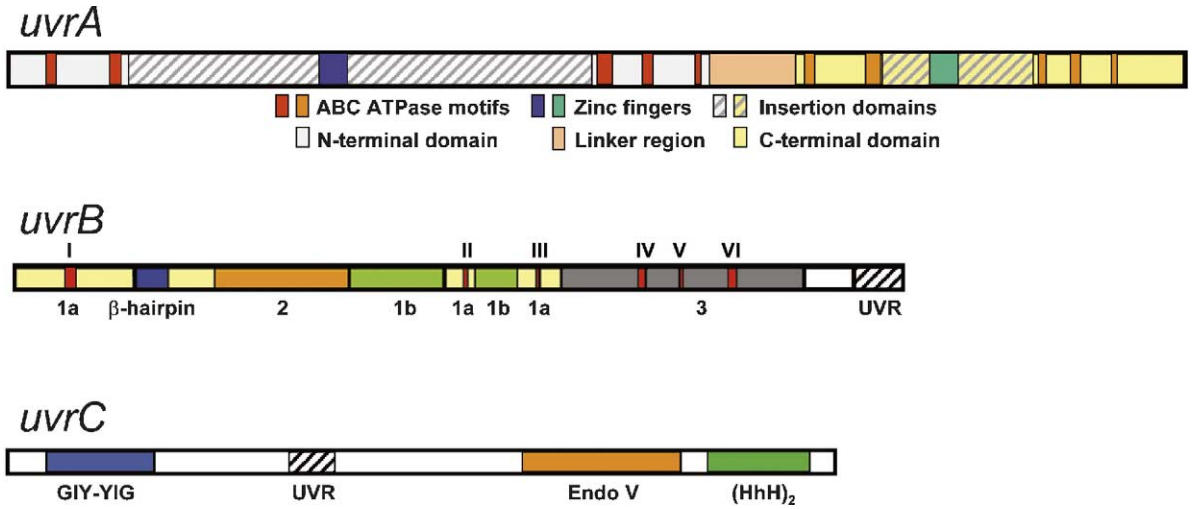

Fig. 2. Linear representation of the genes. UvrA: two domains are separated by a flexible protease-sensitive linker region. Within each domain, there is one ABC ATPase motif, a zinc finger and an insertion domain. The two domains are shown in gray and yellow, while the linker region is shown in beige. The conserved ABC ATPase motifs are shown in red and orange and the zinc fingers are shown in blue and green. The gray crosshatched area marks the inserted domains. UvrB: the six-helicase motifs (red) of uvrB are shown above the graphic, while the domain organization and β-hairpin element are listed below. UvrC: the position of the 3′ nucleolytic center (blue) is denoted by the GIY-YIG below the graphic. The UvrB interacting domain is known as the UVR domain (gray hatched). The 5′ nuclease center (orange) has homology to EndoV and the final element is the tandem helix–hairpin–helix (green).

zinc finger in DNA binding and possibly damage recognition.

5.2. Structure and function of ATP binding domains

The ABC-type ATPase superfamily is well established and the majority of proteins in this category, whose functions are known, are involved in transport events. There are a few DNA metabolizing enzymes, notably Rad50 [130], MutS [131,132] and the structural maintenance of chromosome (SMC) proteins [133,134], whose crystal structures are known that also possess an ABC-type ATP binding fold. All of the proteins in this superfamily function as dimers or higher order oligomers. The ABC-type ATP binding fold is responsible for creating a dimerization interface between two subunits. This interface is created between two molecules in such a way that ATP is bound between the Walker A motif of one subunit and the signature sequence and Walker B elements of the other subunit (for a review see [135]). In such an arrangement, each ABC dimer can bind two molecules of ATP along its dimerization interface. Generally, it is believed that ATP binding serves to stabilize the dimer architecture, while ATP hydrolysis is thought to drive dissociation of the subunits. We believe UvrA functions in a similar way as other ABC ATPases, although UvrA is slightly more complicated because of the fact that each monomer possesses two ABC-type ATPases domains.

UvrA forms a dimer with an association constant on the order of $K_A \sim 10^8\ M^{-1}$ [136]. Myles and Sancar [137] experimentally separated UvrA into two domains and showed that the N-terminal domain possessed both the ability to dimerize and hydrolyze ATP, while the independent C-terminal domain failed to do either. The C-terminal domain is reminiscent of other isolated ABC ATPases that are monomeric [138–140] when separated from their transmembrane domains. While the N-terminal and C-terminal ABC ATPase domains could potentially interact, Myles and Sancar [137] concluded that UvrA dimerizes in a head-to-head fashion. Several factors influence the dimerization status of UvrA: high protein concentrations, ATP and the poorly hydrolyzable ATP analog, ATPγS, each promote

dimerization [10,141,142]. Thus, UvrA dimerization could be a key point of regulation in the NER pathway.

UvrA is a DNA-independent ATPase that can hydrolyze both ATP and GTP [9,143,144]. Site-directed mutagenesis of the two Walker A motifs, GKS to GAS, a mutation which in other ABC ATPases abolishes ATP hydrolysis, demonstrated that the C-terminal Walker A site has a higher affinity for ATP than the N-terminal site [136,145]. It was also demonstrated that there is cooperativity in ATP hydrolysis between the two sites and mutagenesis of the N-terminal Walker A site destroys this cooperativity [136,145]. As is the case with other ABC ATPases, the ATP binding sites in UvrA are not equivalent, but are allosterically regulated [136,146]. The precise nature of this allosteric regulation is currently unknown.

In addition to the above-mentioned regulators of UvrA, both DNA and UvrB possess the ability to modulate the dimerization status and ATPase activity of UvrA. The consequences of UvrA's interactions with DNA and UvrB will be discussed within the context of damage recognition below.

6. UvrB

UvrB is considered the central recognition protein in bacterial NER as it interacts with all the components of the repair system: UvrA, UvrC, UvrD, polymerase I and damaged DNA [147,148] (see Fig. 2 for a linear graphic of the *uvrB* gene and Fig. 3 for the domain structure of the protein). In lieu of a historical review of the literature on UvrB, we have compiled a table of all the mutants created in UvrB thus far (see Table 2 and references cited therein) and have included a brief review below.

6.1. UvrB crystal structure reveals a helicase fold

Sequence analysis has shown that UvrB contains six helicase motifs [159] with similarity to XPD and XPB, two helicases involved in eukaryotic NER. Successful crystallization of the UvrB protein from different organisms [11–13] and most recently the Y96A variant from *Bacillus caldotenax* [144], have allowed a more in-depth view into this key component of bacterial NER. The original structure of UvrB determined by our groups revealed that it contained all the elements of an intact helicase, including all residues implicated in coupling ATP hydrolysis to strand translocation [13]. In addition, high structural similarity to two other helicases, NS3 and PcrA, was observed, thus suggesting that UvrB functions as a helicase adapted for NER. Furthermore, based on these similarities, it was proposed that the UvrB DNA binding sites would be located in or near domains 1a and 3 (Fig. 3) leading to a padlock model, which utilizes a β-hairpin emerging from the first helicase domain to clasp one strand of the DNA between the β-hairpin and domain 1b as the DNA is scanned for damage [13,160]. This model has been supported by DNA photoaffinity labeling in conjunction with site-directed mutagenesis of the UvrB protein (discussed below) together they have provided additional evidence that the non-damaged strand is being held between the β-hairpin and domain 1b during the pre-incision complex phase of NER [17].

It is believed that both bacterial and eukaryotic NER systems employ helicase activity to unwind DNA for damage verification. It has been stated that the UvrAB protein complex can simultaneously "scan and sense" the DNA duplex for damaged sites [161], although it is not known how long the UvrAB complex will scan after each binding event. While it has been suggested that the UvrAB complex has helicase activity, it can only destabilize short oligonucleotides of less than 30 bases upon binding. Therefore, UvrB is not acting as a true helicase, but due to limited strand opening destabilizes short stretches of DNA [110]. Our padlock model [160] predicts the following: when the UvrAB complex locates a lesion, UvrB harnesses the energy of a bound ATP molecule in conjunction with the β-hairpin region of UvrB in order to impose an unfavorable conformation on the DNA, thus facilitating recognition and incision by UvrC.

6.2. Role of domain 2 and the β-hairpin for UvrA interaction and its effect on UvrB's ATPase activity

UvrB has been extensively mutagenized in order to decipher its biological functions (see Table 2 and Fig. 3). In Fig. 3, the black circles represent mutations that give rise to altered function, while the gray circles are mutants that have no significant defects. The structure of the Y96A variant allowed, for the first time, a detailed atomic analysis of domain 2 of UvrB [144], one

Table 2
UvrB mutations prepared in *E. coli* or *B. caldotenax*

Mutation[a]		Domain	ATPase	DNA protein complexes	Repair ABC incision	UV survival	References
E. coli	*B. caldotenax*						
WT	WT		+UvrA	AB, B, BC	3′ then 5′	WT	See references below
D15A (D16)		1a	NR	NR	NR	∼WT	[22]
G39D		1a	NR	NR	NR	Reduced	[149]
G39S		1a	NR	NR	NR	Reduced	[149]
G44R		1a	NR	NR	NR	Reduced	[149]
K45A		1a	Defective	AB, no B	Defective	Defective	[150]
K45D		1a	NR	NR	NR	Defective	[150]
K45R		1a	NR	NR	NR	Defective	[150]
N51A		1a	∼WT	∼WT	∼WT	∼WT	[150]
N51K		1a	NR	NR	NR	∼WT	[150]
V52D		1a	NR	NR	NR	∼WT	[150]
I53R		1a	NR	NR	NR	∼WT	[150]
D55A (Q55)		1a	NR	NR	NR	∼WT	[150]
F88W (F89)		1a	∼WT	∼WT	∼WT	∼WT	[151]
β-Hairpin mutations							
	Y92A	βh	Enhanced	AB, B ∼ WT	Reduced	NR	[112]
Y92W		βh	NR	∼WT	Reduced	NR	[68]
Y92A/Y93A		βh	Enhanced	Reduced B	Reduced/BC incision	NR	[152]
D93A (D94)		βh	NR	NR	∼WT	Reduced	[22]
	Y93A	βh	Reduced	AB, reduced B	Reduced	NR	[112]
Y95F		βh	NR	∼WT	NR	NR	[68]
Y95W		βh	NR	B enhanced	∼WT	NR	[68]
Y95A/Y96A		βh	Enhanced	AB, no B	Defective/BC incision	Defective	[152]
Y95W/Y96W		βh	NR	Defective	NR	NR	[68]
	Y96A	βh	∼WT	AB, no B	Defective	NR	[112,144]
Y96W		βh	NR	Defective	NR	NR	[68]
E98A (E99)		βh	NR	ND	3′ only	NR	[151]
	E99A	βh	Reduced	AB, no B	Defective	NR	[112]
Y101A/F108A		βh	Enhanced	AB, no B	Defective	Defective	[152]
Y101W		βh	NR	Defective	Reduced	NR	[68]
D105A (D106)		βh	NR	NR	∼WT	∼WT	[22]
F107W (Y108)		βh	∼WT	∼WT	∼WT	∼WT	[151]
	E110A	βh	NR	∼WT	∼WT	NR	[111]
	E110R	βh	Enhanced	∼WT	∼WT	NR	[111]
	K111A	βh	Reduced	AB, B ∼ WT	Reduced	NR	[112]
D111A (D112)		βh	NR	NR	∼WT	∼WT	[22]
	R123A	1a	Reduced	AB, no B	Defective	NR	[112]
	H124A	1a	∼WT	B enhanced	∼WT	NR	[112]
D134A (D135)		1a	NR	NR	∼WT	∼WT	[22]
Domain 2							
D167A (E168)		2	NR	NR	∼WT	∼WT	[22]
	R183E	2	↓/↑GTPase	Reduced B	Reduced	NR	[144]
F187W (F188)		2	∼WT	∼WT	∼WT	∼WT	[151]
	R194A/R196A	2	↓/↑GTPase	∼WT	∼WT	NR	[144]
	R194E/R196E	2	↓/↑GTPase	Reduced B	Reduced	NR	[144]
	R213A/E215A	2	↓/↑GTPase	∼WT	∼WT	NR	[144]

Table 2 (*Continued*)

Mutation[a]		Domain	ATPase	DNA protein complexes	Repair ABC incision	UV survival	References
E. coli	*B. caldotenax*						
F216W (F217)		2	~ WT	~WT	~WT	~WT	[151]
H247A (H248)		1b	NR	NR	~WT	~WT	[22]
	F249A	1b	Reduced	AB, B ~ WT	~WT	NR	[112]
E265A (E266)		1b	NR	ND	3′ only	NR	[151]
	R289A	1b	Reduced	AB, B ~ WT	~WT	NR	[112]
	R289A/R367A	1b	Reduced	AB, B ~ WT	Reduced	NR	[112]
	E307A	1b	Reduced	AB, reduced B	Reduced	NR	[112]
D326A (D327)		1a	NR	NR	~WT	~WT	[22]
D331A (D332)		1a	NR	NR	~WT	~WT	[22]
D337A (D338)		1a	Defective	AB, ~WT	<5% WT	Defective	[22]
E338A (E339)		1a	NR	ND	3′ only	Reduced	[151]
	D338N	1a	Reduced	AB, no B	Defective	NR	[111]
H340F (H341)		1a	NR	NR	~WT	Reduced	[22]
	H341A	1a	~WT	~WT	Reduced	NR	[111]
D353A (D354)		1b	NR	NR	~WT	Reduced	[22]
F365W (F366)		1b	~WT	~WT	~WT	~WT	[151]
	R367A	1b	Reduced	AB, reduced B	Reduced	NR	[112]
D372A (D373)		1b	NR	NR	~WT	~WT	[22]
D419A		3	NR	NR	~WT	~WT	[22]
D433A		3	NR	NR	~WT	~WT	[22]
D478A (E478)		3	Enhanced	AB, ~WT	< 5% WT	Defective	[22,151]
F496W (D496)		3	~WT	~WT	~WT	~WT	[151]
G502R (G501)		3	NR	NR	NR	NR	[149]
G502R/G509D (G501/G508)		3	NR	NR	NR	NR	[149]
	R506A	3	Reduced	Reduced	Reduced	NR	[111]
G509S (G508)		3	Reduced	AB, no B	ND	~WT	[149,153]
D510A		3	Reduced	Low/AB, ~WT	Defective	Defective	[21,22,151]
	D510A	3	Reduced	AB, no B	Defective	NR	[111]
	D510N	3	Enhanced	~WT	Reduced	NR	[111]
E514K (E513)		3	~WT	AB, ↓B, low C	Reduced	~WT	[149,153]
E514K/R541H (E513/R540)		3	Defective	AB, no B	ND	~WT	[149]
D521A		3	NR	NR	~WT	Reduced	[22]
D523A		3	NR	NR	~WT	Reduced	[22]
F527W		3	~WT	~WT	~WT	~WT	[151]
	F527A	3	~WT	Enhanced B	Reduced	NR	[112]
R544H (R543)		3	Defective	AB, no B	ND	~WT	[149,153]
H581F		3	NR	NR	~WT	~WT	[22]
D594A		3	NR	NR	~WT	Reduced	[22]
C-Terminal “coiled-coil”							
K634A		4	NR	NR	~WT	~WT	[22]
H636F		4	NR	NR	~WT	~WT	[22]
E637A		4	NR	NR	~WT	~WT	[22]
E639A (E640)		4	~WT	~WT	Defective	Defective	[22]
E640A		4	NR	Normal C	~WT	~WT	[153]
H645F		4	NR	NR	~WT	~WT	[22]
E650A		4	NR	NR	~WT	~WT	[22]
E652A		4	NR	NR	~WT	~WT	[22]
F652L		4	Hyper + UvrA	~WT	3′: ↓, 5′:~WT	NR	[154,155]

Table 2 (*Continued*)

Mutation[a]		Domain	ATPase	DNA protein complexes	Repair ABC incision	UV survival	References
E. coli	*B. caldotenax*						
R658A		4	NR	NR	∼WT	∼WT	[22]
D659A		4	NR	NR	∼WT	∼WT	[22]
H662F		4	Hyper - UvrA	NR	∼WT	∼WT	[22]
E666A		4	NR	NR	∼WT	∼WT	[22]
S672A		4	NR	NR	∼WT	∼WT	[22]
Domain deletions							
574Δ			NR	AB	1–2% WT	NR	[153]
609Δ (UvrB*)			↑ - UvrA	AB	<0.1–1% WT	Reduced	[22,143,153, 156–158]
630Δ			NR	AB, BC	1% WT	NR	[153]
649Δ			NR	AB, BC	2% WT	NR	[153]
	Δ2 (Δ154–247)		↓/↑GTPase	A enhanced	Defective	NR	[17,144]
	Δβ-hairpin (Δ97–112)		Hyper + UvrA	AB enhanced	Defective	NR	[17,110,144]
MBP/UvrB (115–250)			NR	Binds to A	NR	NR	[151]
MBP/UvrB(251–546)			NR	DNA binding, no A or C	NR	NR	[151]
MBP/UvrB(547–673)			NR	Binds to A and C	NR	NR	[151]

NR = not reported; ND = not detected; ∼WT = wild-type like activity, see individual reference for more detail; enhanced or ↑ = greater than WT; reduced or ↓ = less than WT; ATPase activity is in the presence of UvrA and UV-irradiated DNA unless otherwise noted; MBP = maltose binding protein. UvrB*, deletion mutant of UvrB.

[a] Mutations are listed under the species in which they were prepared as reported in the original literature cited in the far right column. The reader may observe slight discrepancies in numbering and/or residue due to the inclusion or omission of Met1 when numbering the protein sequences and the fact that the two proteins are not 100% conserved. When necessary, in the *E. coli* column, residues in parenthesis are included to indicate the analogous residue in the sequence of UvrB from *B. caldotenax* and are labeled as such on the UvrB structure shown in Fig. 3.

of the two regions responsible for the interaction with UvrA. Due to more favorable crystal packing, domain 2 was visualized and a new protein fold was determined for this domain, which shares sequence homology to Mfd [162]. Mfd is a bacterial transcription-coupling repair factor that recruits UvrA to sites of DNA damage marked by a stalled RNA polymerase [162]. Examination of several point mutations in highly conserved residues in UvrB and Mfd, as well as deletion of the entire domain 2, demonstrated that domain 2 [144] is essential for binding to UvrA, and thus important for subsequent: (1) destabilization of short duplex regions in DNA; (2) forming a UvrB–DNA pre-incision complex; (3) incision; and (4) coupled hydrolysis of ATP and domain 3 closure.

It is well documented that the UvrABC system requires ATP binding and hydrolysis to function properly. Using an enzyme-coupled assay, we have reported that in the presence of damaged DNA, UvrA exhibits similar levels of ATPase and GTPase activity, whereas wild type UvrB alone has very low ATPase activity that is barely above background and no GTPase activity [144]. An interesting observation reported in this study is a nearly seven-fold hyper-ATPase activity, and a decrease in GTPase activity when UvrA is combined with the Δβ-hairpin mutant (ΔβH, lacks the β-hairpin) in the presence of damaged DNA. These activities are attributed to the fact that the UvrA dimer can recruit the UvrB ΔβH protein to the site of the DNA damage, but the defective ΔβH cannot verify the damage. Thus, a successful hand off of the DNA from UvrA to UvrB is prevented. Therefore, the UvrB mutant hydrolyzes ATP continuously, unsuccessfully trying to engage the damage. This data correlates well with our padlock model.

6.3. Assessing the DNA hand-off from UvrA to UvrB using photoaffinity DNA substrates

It is important to reiterate that a key step of NER that is still poorly understood is the transfer of damaged

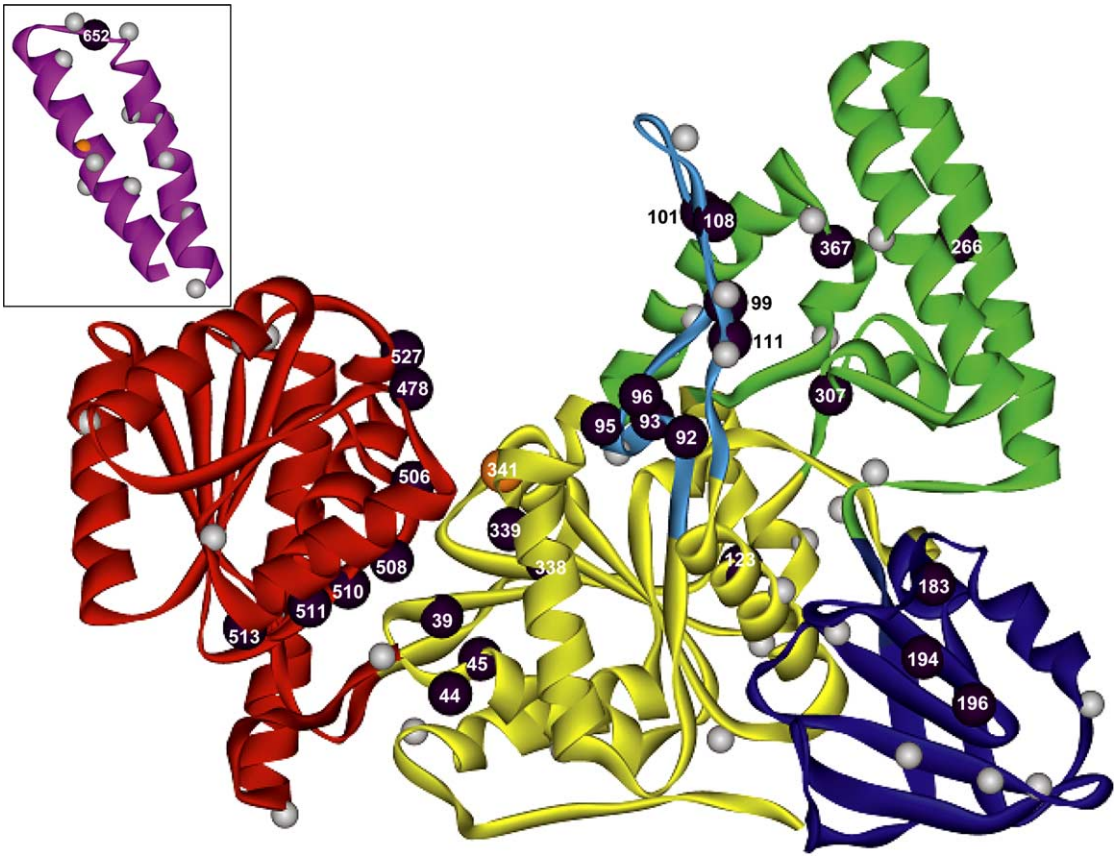

Fig. 3. Graphic representation of mutations created within UvrB. All of the mutations that have been made in the UvrB protein were overlaid onto the crystal structure of *B. caldotenax* (PDB 1T5L [144]) and the solution structure (PDB 1E52 [183]). The structural features of the UvrB protein are: domain 1a, yellow; β-hairpin, light blue; domain 1b, green; domain 2, blue; domain 3, red; domain 4, magenta (inset). Two mutations are depicted with orange balls to denote the fact that there are conflicting results reported in the literature regarding the activities of these mutants, E640 and H341. Mutations with wild type-like phenotypes and/or less than 20% reduction in incision activity are depicted as small gray spheres. Substitutions that produced a protein whose incision activity was reduced by more than 20% of wild type are shown as large purple spheres with the corresponding amino acid numbered within.

DNA from UvrA to UvrB. Most recently, our laboratory has analyzed this transfer utilizing photoaffinity probes incorporated into DNA [17]. We have utilized two types of arylazido-modified photoaffinity reagents that, by design, probe protein contact sites on both the damaged and non-damaged sides of the DNA. DNA-protein photoaffinity cross-linking in conjunction with several site-directed mutations and two domain deletions, Δ2 and the Δβ-hairpin, of UvrB has allowed a visualization of the architecture of the DNA when it is in complex with UvrA and UvrB. We can now dissect the molecular handoff of DNA from UvrA to UvrB into discrete steps. The most important step being Uvr isomerization in which UvrB is in close proximity to the adduct within the $UvrA_2B$ complex, prior to UvrA dissociation. We believe that the β-hairpin deletion and Y96A mutants are arrested during this normally transient step that proceeds by insertion of the non-damaged strand into the pocket between the β-hairpin and domain 1b. This is followed by engagement of the damaged strand at the base of the β-hairpin through aromatic residues, primarily Tyr96.

6.4. UvrB as a model for the human repair protein, XPD

UvrB shares 15% sequence identity and 62% similarity with XPD [111]. By modeling the XPD protein based on the crystal structure of UvrB [13], several xeroderma pigmentosum disease-like mutations were successfully mimicked in vitro in UvrB. The success of this study demonstrates the ability to gain a molecular understanding of the NER process in humans by studying their bacterial protein counterparts [111]. These data strongly suggest that while UvrB and XPD show little sequence conservation, they play homologous roles of strand opening and damage verification in the bacterial and human NER repair pathways, respectively.

While we have learned a great deal from the site-directed mutagenesis studies, much is still to be discovered, such as where does the DNA lie in the protein–DNA complex? Also, since the coiled-coiled domain (Fig. 3, inset) has remained elusive in the crystal structures that have been solved thus far, it is not known where this element exists structurally and what other functions it contributes.

7. UvrC

Similar to UvrA, UvrC is weakly expressed constitutively, resulting in about 10–20 copies per cell [115]. UvrC contains two constrained endonuclease active sites, which mediate incision of damaged DNA only after recognition of the UvrB–DNA pre-incision complex [20]. Site-directed mutagenesis revealed that the N-terminal nuclease center is responsible for 3′ incision, while the C-terminal center carries out the 5′ cutting [21,23]. The 3′ incision is believed to occur prior to the 5′ incision [20–23].

Aside from the two distinct catalytic sites, UvrC possesses a coiled–coiled region and a tandem Helix–hairpin–Helix (HhH) domain (see Fig. 2). The coiled-coil domain of UvrC is proposed to interact with the C-terminal domain of UvrB [153, Moolenaar, 1998 #18]. This interaction is critical for 3′ incision, whereas the 5′ incision is apparently independent of this interaction [153,154]. The two HhH motifs are implicated in DNA binding [163,164].

7.1. Coordination between damage recognition and UvrC incision

Although UvrC does not interact with UvrA, earlier studies suggested that it might interact with the UvrB protein in solution because both proteins co-migrate during protein purification steps [165]. Once UvrA has been displaced from the UvrAB–DNA complex, UvrC is believed to interact with the C-terminal coiled–coiled domain of UvrB forming the UvrBC–DNA complex, which catalyzes the dual incisions.

Not surprisingly, the UvrC binding and incision reactions are highly dependent upon the previous steps. It has been shown that incision efficiency can vary six-fold on DNA substrates containing the same acetylaminofluorene-deoxyguanine (AAF-dG) adduct, but in different sequence contexts [166]. However, there was no difference in the formation of the UvrAB complex [166,167]. The incision reaction was also shown to be inversely related to the stability of the UvrB–DNA pre-incision complex [167]. These data suggest that after the release of UvrA from the UvrAB–DNA complex, an isomerization step converts UvrB from an inactive pre-incision complex to an active form that is required for the formation of a productive incision complex. More recently, Moolenaar reinvestigated the UvrBC transition and discovered that ATP hydrolysis and subsequent ATP binding by UvrB is required to create an active UvrBC–DNA incision complex [19]. Thus, the isomerization step could be the ATP exchange reaction that couples the UvrB and UvrC protein activities.

Examination of the incision efficiency on DNA substrates containing a flap or bubble of varying size revealed that the UvrAB complex may induce local DNA structure alterations including unpaired bases [65]. These unpaired regions on DNA might serve as the key feature of the damage verification process allowing the UvrB–DNA pre-incision complex to be recognized by UvrC further setting the stage for incision.

It was suggested that UvrBC might have two DNA binding modes: one that supports 3′ and another that supports 5′ incision [65]. In the mode leading to 3′ incision, the binding of UvrBC to DNA includes a dsDNA region three bases 3′ to the damaged site. The 3′ incision can relieve the DNA stress and lead to further opening of the DNA. Meanwhile, the 5′ incision mode

may require a large number of unpaired bases 3′ to the damaged site. This is consistent with the fact that the HhH DNA binding domain is required for stabilization of UvrBC–DNA complex after the 3′ incision [163]. In addition, a truncated UvrC protein lacking this HhH domain no longer binds to ssDNA [163]. Furthermore, it has been reported that 3′ incision is dependent on ATP binding by UvrB, whereas the 5′ is not [19]. This observation also supports the notion that the two catalytic sites on UvrC require the DNA be presented in structurally different ways for each incision event to occur.

Moolenaar et al. [153] concluded that the UvrAB complex processes the DNA into an incision competent structure and that the C-terminal coiled–coiled domain of UvrB interacts with a similar internal domain of UvrC in order to recruit UvrC to the sites of incision. However, several intriguing questions arise: does the same UvrC molecule produce both cuts? What are the other structural features of the UvrB–DNA pre-incision complex that UvrC recognizes and interacts with? What conformational changes take place within UvrC and the DNA substrate after recognition of the UvrB–DNA pre-incision complex?

7.2. Catalytic mechanism of UvrC

A common feature of the catalytic mechanism observed with many nucleases is the requirement for a bound metal ion as a cofactor. Metal ions can act as Lewis acids by lowering the pK_a of their directly coordinated water molecules. Most importantly, positive charges of the divalent metal ions help to neutralize the negative charges in the transition state [168]. However, the requirements regarding the types and numbers of metals (one, two or even three) involved in catalysis are complicated.

As mentioned before, UvrC has two catalytic sites, one on the N- and C-terminal halves of UvrC, respectively. The region of UvrC that is located between the UVR domain and the HhH motifs in the C-terminal half showed subtle but statistically significant similarity to endonuclease V [169]. Multiple sequence alignments of EndoV family members with the UvrC sequence revealed the conservation of two aspartic acids and a lysine [169]. Previously, it was shown that mutation of the conserved Asp399 and Asp466 in *E. coli* UvrC abolished the 5′ incision [21]. UvrC with mutation of Asp438 or His538 were also defective in 5′ incision [21]. However, so far, there is no available structure of the C-terminal catalytic domain of UvrC. Therefore, the exact catalytic mechanism of the 5′ incision still remains to be deciphered.

The catalytic domain in the N-terminal of UvrC shares limited homology with a small module found in members of the GIY-YIG homing endonuclease family [169]. Quite recently, the N-terminal catalytic domains of UvrC from *B. caldotenax* and *Thermotoga maritima* have been solved [170]. The structure of $UvrC^{N\text{-}Tma}$ revealed for the first time the exact coordination geometry of the metal ion that is required for catalysis of a member of the GIY-YIG superfamily. It was suggested that this domain employs a novel one-metal mechanism to cleave the phosphodiester bond [170]. This suggestion is based on the identification of a patch of highly conserved residues on the surface of the N-terminal domain of UvrC surrounding a divalent cation-water cluster. Mapping of the sequence conservation of UvrC from different organisms onto the surface revealed six strictly conserved residues that formed a patch on one side of the surface, namely Tyr19, Tyr29, Lys32, Arg39, Glu76 and Asn88 in *T. maritima* UvrC. Four highly conserved residues surround these residues: Tyr43, Glu69, Phe73 and Ile80. Together, these residues form a concave surface large enough to accommodate double stranded DNA. Glu76 is the only residue that makes direct contact to the bound metal ion (Mn^{2+} or Mg^{2+}), while Tyr29, Ile30, and Lys32 form hydrogen bonds with one of the five water molecules coordinating the metal ion in an octahedral arrangement. Mutational analyses of the residues on the conserved surface in full length UvrC have shown that mutation of the sole metal ligand, Glu76 (which is invariant in all known GIY-YIG family members), to alanine inactivates its incision activity. Mutation of Ile80, that forms part of the metal binding pocket, to glutamate, also renders the protein inactive. It was suggested that one of the water molecules coordinated to the metal could act as the general acid required to catalyze the cleavage of the phosphodiester bond [170]. Of the highly conserved tyrosines, Tyr29 most likely serves as the general base to activate a water molecule for nucleophilic attack on the phosphodiester bond. This suggestion is consistent with the observation that the side chain hydroxyl group of Tyr29 is in close proximity to the divalent cation, and Y29A and Y29F mutants are completely inactive.

Arg39 and Lys32 were suggested to be involved in stabilizing the negative charge of the free 5′-phosphate after DNA cleavage. Consistent with this idea, an Arg39 to alanine mutation inactivates the protein, while, in a previous study, an equivalent mutation of Arg42 to alanine in *E. coli* UvrC produced the same defect [23]. The Lys32 to alanine mutation reduced its activity by 25–30% compared to the wild type UvrC. It was speculated that Asn88 might play an important role in positioning the catalytic domain correctly with respect to the other domains of UvrC and mutation of Asn88 to alanine inactivated the protein. Finally, mutation of Phe73 to alanine or glutamate does not affect the incision activity of UvrC, suggesting that this residue is not directly involved in the catalytic reaction.

It is worth mentioning that magnesium appears to be coordinated with nucleases in a more transient manner compared to zinc and manganese [168]. Indeed, this is the case for another nuclease involved in DNA repair, namely Ape1 [168]. This relatively weak binding contributes to the fact that the number of bound magnesium ions in crystal structures can vary depending on different crystallization conditions. For RnaseHI, one magnesium or two manganese ions have been captured in different crystal structures [168]. In the case of UvrC, either soaking of $UvrC^{N\text{-}Tma}$ in $MnCl_2$ or $MgCl_2$ gave rise to only one cation in both structures, supporting the one-metal mechanism for UvrC 3′ endonuclease activity [170]. However, crystal structures of UvrC bound to DNA are needed to further elucidate the details of catalytic mechanism of UvrC.

7.3. UvrC homolog

It was surprising that the well annotated genome of *E. coli* did not reveal the existence of a second UvrC gene, until gene expression profiling experiments combined with careful homology searching revealed a C-homolog, Cho [47]. Cho is homologous to the N-terminal domain of UvrC and can elicit the 3′ incision, but not 5′ incision. Like UvrC, Cho is an UvrAB-dependent nuclease. Interestingly, Cho and UvrC interact with different domains of UvrB. Consequently, 3′ incision activity mediated by Cho is four nucleotides further away from the damaged site compared to incision by UvrC [47]. Curiously, the expression of Cho is inducible, while UvrC is not [171]. Thus, it was suggested that Cho functions as a backup nuclease for NER acting on very bulky substrates as well as protein–DNA crosslinks that block the 3′ incision site of UvrC. Cho and UvrC coexist in only a small number of bacterial species including *E. coli*. Many more species only contain UvrC. Surprisingly, mycoplasmas and *Borrelia burgdorferi* only contain Cho, in which case the 5′ incision activity might originate from these Cho's additional exonuclease domain or the exonuclease activity of another enzyme [172]. This speculation seems to be supported by the finding that Cho proteins of the Mycobacterium species are predicted to be larger than that of *E. coli*. The additional domain has strong homology to the epsilon 3′ exonuclease, which is the proof-reading subunit of DNA polymerase III holoenzyme [172]. These molecular speculations need to be validated through direct experimentation.

8. DNA damage recognition and processing by UvrA, UvrB and UvrC

A working model for damage recognition is shown in Fig. 4. We have dissected the NER mechanism into several discrete steps: UvrA dimerization, UvrAB complex formation, initial damage detection by UvrA and DNA bending, DNA opening and Uvr isomerization, damage engagement, pre-incision complex formation and DNA wrapping. In reality the trajectory of damage detection and processing is a continuous function, such that the steps shown in this figure represent snapshots of critical features along the entire reaction pathway.

Six characteristics of damaged DNA that UvrABC could use for damage recognition include: (i) covalently linked damage, (ii) bulky substituents, (iii) localized unwinding, (iv) bending or kinking, (v) change in charge distribution at the damage site, and (vi) changes in the dynamics of DNA [8]. Pioneering work by Naegeli and coworkers has suggested that both prokaryotic and eukaryotic NER proteins employ "bipartite substrate discrimination" in which both the chemistry of DNA and the base pairing must be altered (reviewed in [173]). This idea has been reinforced by recent studies from several laboratories [43,174,175]. For example, different stereoisomers of BPDE are recognized with different efficiency by UvrABC [64,67]. Zou et al. [60] further showed that by placing the BPDE adduct N^2-G in a six base unpaired region

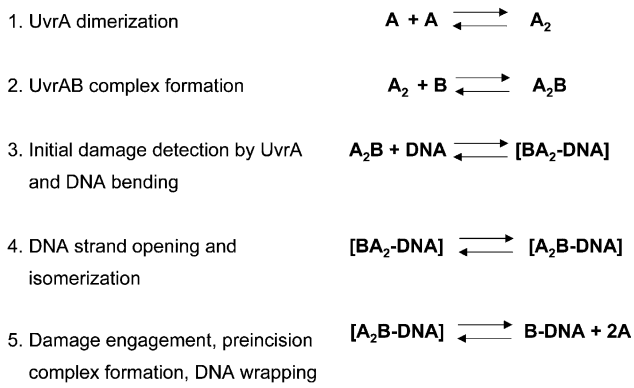

Fig. 4. DNA damage detection and processing by the bacterial nucleotide excision repair system. The figure depicts a more detailed glimpse of the initial steps of the reaction. The model proposes that UvrA dimerizes, followed by formation of the UvrAB complex. UvrA mediates the initial detection event then it prepares the DNA for UvrB that performs the damage verification step. The UvrB protein confirms that DNA damage is present by opening the DNA strands and inserting the β-hairpin; thus, the DNA lesion is now in closer proximity to UvrB. Therefore, we have depicted the isomerization reaction by changing the position of the protein in the complex relative to DNA: (B-A_2-DNA) verses (A_2-B-DNA). Finally, UvrB engages the DNA lesion, promotes DNA wrapping and signals UvrA to depart. A = UvrA, B = UvrB, C = UvrC.

this differential recognition could be eliminated. Interestingly the AAF-G adduct only required the opening of three base pairs to be maximally recognized which is consistent with earlier work by Gordienko and Rupp [58]. Gordienko and Rupp [58] had shown that the UvrAB interaction with AAF-dG adducts leads to an alteration in one to three base pairs including the adduct. Thus, base pair opening appears to be an important criterion for recognition and, as mentioned previously, supports the prediction that different sequence contexts, with different propensities for strand opening, affect recognition. This is precisely the case as Kow et al. [45] found that thymine glycol is recognized and incised in a sequence dependent manner. More recently, Zou et al. [60] found that BPDE, AF and AAF dG adducts in the sequence, TG*T, were incised more efficiently than in the sequence, CG*C. This difference appeared to be greater with AAF than with AF. Similar results were obtained with the BPDE adducts by Geacintov and coworkers (unpublished observation).

Bipartite recognition also seems to apply to the two damage recognition subunits of UvrA and UvrB, in which each subunit recognizes different aspects of the helix in order to develop high sensitivity and discrimination. UvrA appears to monitor the DNA helix for gross deformations, whereas UvrB seems to have adapted its helicase fold and β-hairpin to function as a ‘close fitting sleeve’ for damage recognition. Early work by Snowden and Van Houten showed that while UvrA readily binds to abasic sites, subsequent loading of UvrB is greatly inhibited, and the action of UvrB actually leads to dissociation of both UvrA and UvrB. Thus, abasic sites are poorly incised (reviewed in [32]). As suggested in this 1993 review, we believed that UvrB might be making strong stacking interactions between aromatic amino acid side changes and the damaged base. As discussed above, crystallographic results of UvrB combined with site-direct mutagenesis has confirmed the notion that UvrB is making strong contacts with the DNA using aromatic side chains and UvrB provides the damage discrimination activity to the recognition reaction.

DNA bending and kinking are common in protein–DNA interactions. Using the FREEHELIX algorithm, Dickerson [176], and Dickerson and Chiu [177] have analyzed the DNA trajectory in 86 separate co-crystal structures of proteins bound to DNA. They found that protein–DNA complexes fell into three types of DNA bending: a sharp kink, a general writhe or a smooth curve. Analysis of sequence-specific deformability lead to them to hypothesize that “the radical bending observed in many protein/DNA complexes, and the observed dependence of bending on base sequence, suggests that differential deformability of the helix may itself be a significant component of the recognition process” [177].

UvrA interacts with damaged DNA about 10–1000-fold more tightly than non-damaged DNA. Upon binding to DNA UvrA causes a site-specific bend and base pair opening is facilitated by UvrB binding [16,178]. UvrA can actually bind tightly to static bends such as those produced by the sequence 5′-AAAA-3′ [8,46]. DNA bending and base pair opening are energetically linked thus one aids the other. Thus, both UvrA and UvrB appear to use bending of the DNA helix to help facilitate damage recognition. Furthermore, the ability to bend and open the DNA helix could allow access to the damaged site by the insertion of the β-hairpin into the DNA helix. The padlock model of damage recognition hypothesizes that the non-damaged strand is locked between the β-hairpin and the wall of UvrB's domain 1b [13,160]. The damage-containing strand is believed to cross in front of the β-hairpin and allow access by UvrC. Goosen and coworkers [152] have suggested that the aromatic side chains that are strictly conserved Tyr92, Tyr93, Tyr95 and Tyr96, at the base of the β-hairpin, allow efficient nucleotide flipping of the damaged nucleotide out of the helix [152]. We have shown that mutating Tyr96 to an alanine completely destroys UvrB's engagement of the adduct prompting us to the hypothesis that UvrB might make strong stacking interactions with the flipped out adduct or the remaining bases in the DNA helix [112]. This hypothesis is supported by the findings by Zou and Geacintov who

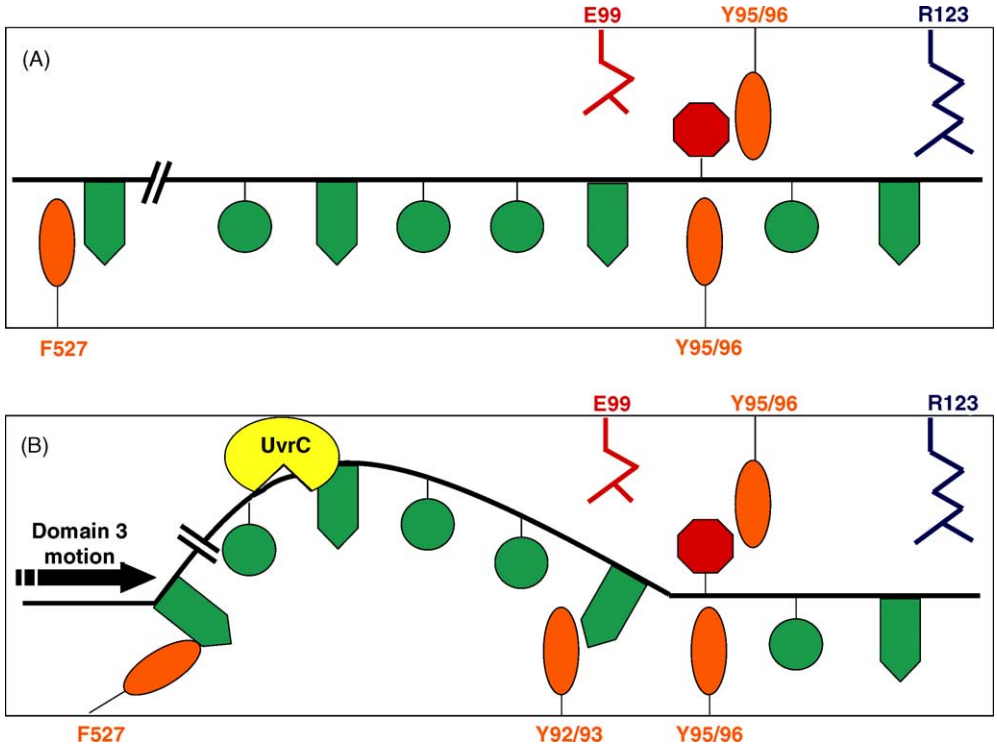

Fig. 5. Graphic representation of important amino acids within UvrB. Using the convention first developed for monomeric helicases by Wigley and coworkers [184], the bases along the damaged DNA strand are shown in green and the adducted base is shown as a red hexagon. The black bar across the DNA strand indicates that the point of interaction between F527 and the DNA is several bases downstream (more 3′) to the incision site. We have depicted, in various colors, the UvrB amino acid side-chains that are thought to be important for DNA damage recognition and processing [17,68,152]. Panel A: UvrB has engaged the DNA adduct (red) using Tyr95 and Tyr96. These residues either stack into the double helix, displacing the damaged base or stabilize the displaced damaged base [152]. Panel B: UvrB's domain 3 motion, possibly linked to ATP binding, pushes Phe527 and/or another, yet to be identified residue into the double helix causing the damaged strand to be pushed up into UvrC's 3′ nuclease center leading to incision. Arg123 and Glu99 are proposed to hold the DNA in place through attractive and repulsive forces, respectively. See text for additional details.

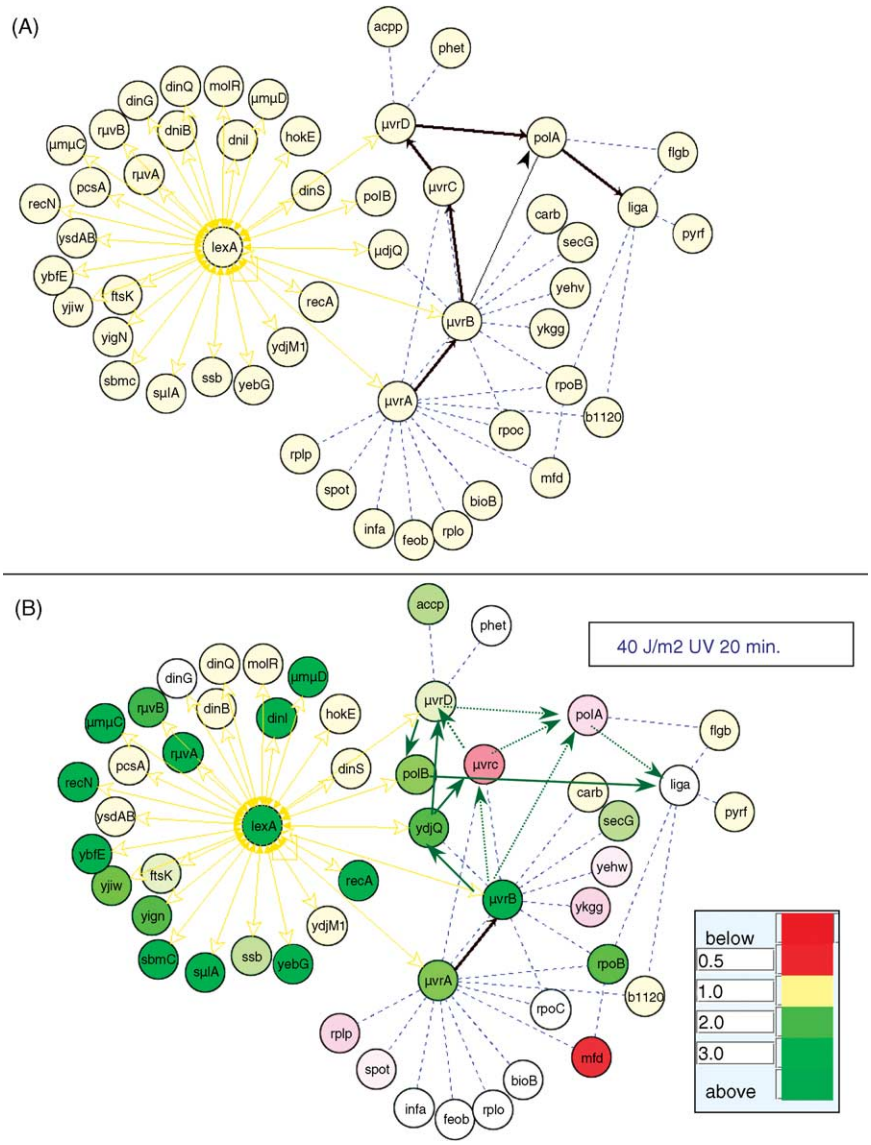

Fig. 6. Gene expression profiling of the bacterial nucleotide excision repair network. Panel A: protein–protein and protein–DNA interactions of the UvrABC system. Protein–protein interactions are shown in dashed blue lines; protein–DNA interactions are shown as orange arrows. Nodes are proteins, or in the case of LexA interaction promoter sequences in genes. Reaction pathway is shown as black arrow. NER interacting proteins: *Acpp* = Acyl carrier protein (ACP); B1120 = hypothetical protein; *BioB* = biotin synthase (EC 2.8.1.6) *Carb* = carbamoyl-phosphate synthase large chain; *YdjQ* Cho = UvrC homolog, b1741; *Feob* = IRON(II) transport protein; *Flgb* = flagellar basal-body ROD protein (FLGB) (proximal ROD protein); *Infa* = translation initiation factor IF-1; *Phet* = phenylalanyl-tRNA synthetase beta chain; *Rplo* = 50S ribosomal protein L15; Rplp = 50S ribosomal protein L16; *RpoB* = DNA-directed RNA polymerase, beta subunit (RPOB); *RpoC* = DNA-directed RNA polymerase, beta subunit (RPOB'); *SecG* = protein-export membrane protein; *Spot* = penta-phosphate guanosine-3′-pyrophosphohydrolase (Spot); *Yehv* = HspR; *Ykgg* = hypothetical protein HP0137. See text for references. Panel B: alterations in the NER network under UV-stress. Layered onto the network in panel A are gene expression changes that occur in *E. coli*, 20 min after 40 J/m^2 of UV light. Red, genes that are repressed; green, genes that are induced; yellow, genes that showed no change; white indicates no data. Green lines indicate possible remodeling of the NER system in response to UV damage (solid lines indicate new interactions predicted after UV light, dashed, pre-existing interactions). Note the induction of *ydjQ* CHO, the UvrC homolog, *polB* and the repression of *polA*, UvrC and Mfd.

placed an abasic site across a dG-BPDE adduct [67]. Rather than an increase in recognition and subsequent incision they found the opposite: an abasic site across from a (+)-*cis*- BPDE-N2-dG lesion decreased incision efficiency by a factor of five. These data suggest that the base opposite the adduct is important for stabilizing the UvrB–DNA complex probably through the aromatic tyrosine sides chains near the base of the β-hairpin.

Within UvrB, two charged residues in the vicinity of the β-hairpin are also essential for binding of damaged DNA Glu99 and Arg123. Arg123 is believed to provide ionic interactions with the phosphates of the non-damaged strand since it is located below the β-hairpin. The negative charge of Glu99 may be important to guide the negatively charged phosphate backbone to the base of the β-hairpin [112].

Site-directed mutagenesis of two other conserved aromatic side chains, Phe249 and Phe527, located in domains 1a and 3 of UvrB has provided insight into how UvrB might use it's helicase fold to process DNA damage. Mutating Phe527 to alanine, while not disrupting the formation of the UvrB–DNA complex, decreased incision activity to about 50% of wild-type UvrB [112]. These data combined with homology modeling and alignment of the helicase motifs in PcrA with UvrB suggest that Phe527 in UvrB intercalates into the helix and moves towards the β-hairpin as a consequence of ATP binding between domains 1a and 3. We believe this large domain motion is used to further distort the DNA helix forcing the correct nucleotides into the UvrC 3′ cleavage site, thus allowing catalysis by Arg42 (see Fig. 5). Surprisingly mutating Phe249 into alanine facilitates the transition from the UvrAB–DNA complex to the incision competent UvrB–DNA complex suggesting that this residue might impose some steric clash with the DNA bases [17].

9. So what remains hidden higher up the sleeve: what is the nature of repair in vivo?

While much has been learned about the reaction mechanism of the UvrABC system using purified proteins in biochemical assays, it is essential that nucleotide excision repair be placed in the biological context of the entire cell. In vivo, the UvrABC system does not work in isolation but is part of a complex network that responds to stressful conditions. Using UvrA fused to the green-fluorescence protein, Walker and coworkers [179] were able to visualize the location of UvrA in living cells of *Bacillus subtilis*. They discovered that UvrA underwent a dramatic re-localization after DNA damage whereas during normal growth, it was uniformly localized to chromatin. This redistribution was reversible. Grossman and coworkers [180] had previously found that following UV-irradiation, UvrA and UvrC join an ensemble of 15 other proteins, including three subunits of RNA polymerase, topoisomerase I, and DNA gyrase, to relocate near the inner membrane of *E. coli* at DNA-membrane junctions.

Using high density DNA microarrays, Hanawalt and coworkers [181] performed a global genome analysis of genes induced by UV light in *E. coli*. They found a number of new genes that were induced with putative LexA binding sites, and many more, which did not apparently have LexA SOS boxes. They also observed a number of repressed genes. Using these data, and interaction maps in bacteria [182], we have assembled a bacterial nucleotide excision repair interactome, Fig. 6. The network undergoes significant changes following UV irradiation and suggests that bacteria employ alternative repair proteins, and may follow a significantly different reaction pathway in response to DNA damage (Fig. 6B). These and other regulatory mechanism are yet to be explored in the bacterial NER system.

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