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Prokaryotic Nucleotide Excision Repair: The UvrABC System

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1. Introduction

DNA is the molecule chosen by nature to store the information required to build organisms. These organisms in turn serve to replicate that information. It was initially thought that DNA must be incredibly stable to maintain the integrity of the information, but it has been shown that DNA is in fact a dynamic molecule that is constantly damaged.[1,2] Damage can be caused by exogenous sources such as man-made mutagenic substances and naturally occurring agents, including sunlight and dietary mutagens, and endogenous sources, such as reactive oxygen species formed during cellular metabolism. The result can be base loss, base dimerization, base alkylation, base deamination, and base oxidation as well as single- or double-strand breakage leading to permanent changes in the information encoded by the DNA. Alterations in base sequence can also arise as a result of replication and recombination. Without maintenance, the information encoded by DNA would be altered so dramatically that the organism could not thrive. Nature has therefore devised a solution to this problem: DNA repair. A number of DNA repair systems have evolved including direct damage reversal, nucleotide excision repair (NER), mismatch repair, base excision repair, and recombinational repair. Each system has developed to specialize in the repair of certain types of damage. This review focuses on our current knowledge and understanding of NER in prokaryotes.

The first detection of NER was in the 1960s when investigators observed the excision of UV-induced DNA lesions from DNA in bacteria.[3,4] It was also discovered that, concomitant with dimer removal, insertion of short stretches of new DNA into "repair patches" took place.[5] Soon after these discoveries, complementation studies revealed the genes responsible for NER:[6] *uvrA*, *uvrB,* and *uvrC*. The expression levels of two of these genes, *uvrA* and *uvrB*, were found to be controlled by the SOS response,[7] a system involved in up regulating the expression level of a number of genes in response to DNA damaging agents. This is significant since constitutively in *E. coli* there are only ~25 molecules of UvrA and ~250 molecules of UvrB present. Upon SOS induction, the level of UvrA rises 10-fold to ~250 while UvrB levels rise 4-fold to ~1000 molecules per cell.[8] UvrC is estimated to be present at a mere <10 molecules per *E. coli* cell and is not regulated by the SOS response.[8]

The UvrABC proteins recognize and cleave damaged DNA in a multistep ATP dependent reaction[9] (Figure 1). In solution, ATP drives the formation of the UvrA dimer.[10] UvrA forms either a heterotrimeric ($UvrA_2B$)[11] or a heterotetrameric ($UvrA_2UvrB_2$)[12] complex with UvrB. The crystal structures of UvrB reveal a monomer,[13−15] which is also suggested by analytical ultracentrifugation (E. Karakas and Kisker, unpublished results) and gel filtration.[11] These data are in contrast to those of atomic force microscopy studies[12] (H. Wang and Van Houten, unpublished results) and cross-linking studies,[16] which suggest that UvrB is able to form a dimer in solution and on DNA. Discrimination of damaged versus nondamaged DNA by the UvrAB complex is much higher than that by the UvrA dimer alone, indicating a multistep approach to damage recognition.[17] UvrA initiates the DNA contacts and transfers the DNA to UvrB.[18] One UvrA dimer could load many UvrB proteins onto different damage sites, which would explain the higher concentration of UvrB compared to UvrA in the cell. UvrB's cryptic ATPase activity, which is activated in the presence of UvrA

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Caroline Kisker studied Biochemistry at the Freie Universtität Berlin and pursued her Ph.D. thesis in the group of W. Saenger with the structural characterization of the tetracycline repressor in complex with its inducer tetracycline. For this work she obtained the Karl Ramsauer Award. After completion of her Ph.D. thesis in 1994, she joined D. C. Rees' laboratory at the California Institute of Technology in Pasadena. She elucidated the structure of carbonic anhydrase and studied the sulfite oxidase deficiency at the atomic level. Since 1998 she has been a faculty member at the State University of New York at Stony Brook. She became a PEW Scholar in the Biomedical Sciences in 2000, and she pursues crystallographic and biochemical studies of DNA repair enzymes and enzymes containing the molybdenum cofactor and also pursues structure based drug design studies with an emphasis on new antituberculosis agents.

and damaged DNA, is necessary for damage recognition. Recognition and transfer of a DNA lesion to UvrB is hypothesized to trigger hydrolysis of UvrA's bound ATP, resulting in monomerization and dissociation of UvrA, leaving behind a salt stable UvrB:DNA preincision complex.[19] UvrC is responsible for both the 3′ and 5′ incision reactions.[20] Before 3′ incision by UvrC takes place, UvrB must be in its ATP bound conformation.[21] The first incision is made at the fourth or fifth phosphodiester bond 3′ to the damage, and the second incision, eight phosphodiester bonds 5′ to the damage.[9,22,23] After incision, UvrC dissociates and UvrD (DNA helicase II) is required to release the incised oligonucleotide. DNA polymerase I fills the resulting gap and removes UvrB from the DNA.[24,25] DNA ligase seals the newly synthesized end to the parental DNA, completing the NER pathway. Over the past 15 years several outstanding reviews have appeared, and the reader is encouraged to revisit them.[8,17,21,26−30] This review focuses on the important advances in the study of the structure and function of these remarkable proteins.

2. Understanding the Substrates

Nucleotide excision repair stands out from the other repair mechanisms in its ability to recognize a broad range of structurally unrelated DNA damages[8,26−28,31] (Table 1), which

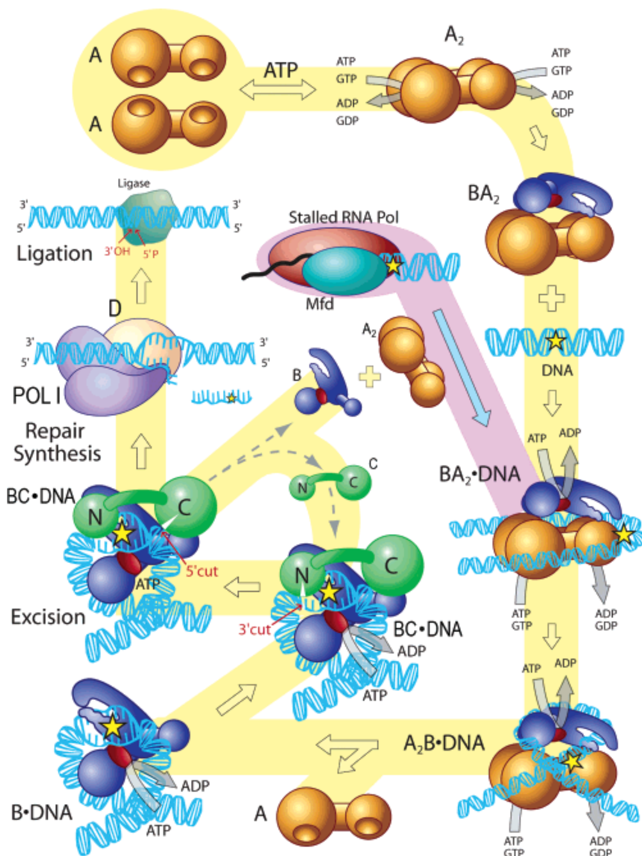

Figure 1. Recognition and repair of DNA damage by UvrABC. In the *global repair pathway*, UvrA and UvrB form an ATP dependent heterotrimer or heterotetramer (see text) that directly recognizes damaged DNA. In the *transcription coupled repair pathway*, Mfd (TRCF) recruits UvrA to the site of DNA damage marked by a stalled RNA polymerase. In both pathways, UvrA loads UvrB onto the damaged DNA and subsequently dissociates, leaving behind a stable UvrB:DNA preincision complex. UvrC binds to the preincision complex and mediates the incisions on both the 3′ and 5′ sides of the DNA. UvrD (DNA helicase II) removes the incised oligonucleotide and UvrC. DNA polymerase I fills the gap and triggers the release of UvrB. The newly synthesized ends are sealed to the parental strands by DNA ligase.

includes carcinogenic cyclobutane pyrimidine dimers and 6−4 photoproducts induced by UV radiation, benzo[*a*]-pyrene−guanine adducts caused by smoking, and guanine−*cis*-platinum adducts formed during cancer chemotherapy.[32] The strategy employed by NER is the same in all three kingdoms of life, and the same range of damages is recognized. Nevertheless, the UvrABC proteins are only utilized in prokaryotes and archae bacteria, whereas eukaryotes utilize a much larger number of proteins. No sequence homology to the UvrABC proteins was detected so far, with the exception of a small region at the C-terminus of UvrC and the ERCC1 protein.[33]

Several studies analyzed the structure and conformation of DNA adducts with respect to the rate of incision by the UvrABC system.[8] It has long been known that 6−4 photoproducts distort the DNA backbone more than cyclobutane pyrimidine dimers (TT pyrimidine dimers) and are incised at a higher rate than TT pyrimidine dimers both *in vitro* and *in vivo*, suggesting that DNA distortion is a major component of the recognition process.[34] Although there is no absolute consensus for damage recognition, the general rule seems to be the following: the larger the chemical substituents, the higher the rate and extent of incision by the UvrABC system becomes. For instance, Snowden and Van Houten found that UvrABC incised a ring closed abasic site (AP) < ring-opened AP < methoxylamine-AP < benzooxyamine-AP.[17] Likewise, Hoare et al. found that a nitropyrene−C^8-dG adduct is incised < (−)-*cis*-*anti*-BPDE−N^2-dG ≤ a (+)-*trans*-*anti*-methylcrysene−N^2-dG adduct.[185] There does, however, appear to be a size limitation. It has recently been shown that protein−DNA cross-links are substrates for UvrABC.[35,36] In this case, larger oligopeptide cross-links are repaired less efficiently than shorter cross-links. This suggests that larger cross-links have to be reduced in size by endogenous proteases prior to removal by UvrABC.[37] Interestingly, it was found that the type of damage is not the only entity that dictates the rate of incision. For example, Kow et al. found that thymine glycol is incised by the UvrABC system with different efficiencies depending on the sequence context.[38] Using defined substrates containing identical lesions, Verhoeven et al. found that the incision rate varied depending on the DNA sequence surrounding the lesion.[39] This finding indicated that the thermostability of DNA is affected not only by the type of lesion but also by the sequence of the DNA.[40]

3. UvrA

The *uvrA* gene has been sequenced from a large number of bacterial species and encodes a 103−105 kD protein. Structurally, UvrA is thought to be composed of two halves separated by a flexible protease sensitive linker region (Figure 2). Sequence homology and mutational analysis have revealed the presence of two C_4-type zinc fingers and two ATP Binding Cassette ATPase (ABC ATPase) domains.[41] The ABC ATPase domains each contain a Walker A and Walker B motif separated by intervening sequences. Preceding each Walker B motif is the signature sequence, Leu-Ser-Gly-Gly, characteristic of ABC ATPase family members. Located within the intervening sequences of the Walker A and Walker B motifs are the two zinc fingers (Figure 2).

3.1. The Zinc Finger Domains

The two zinc finger motifs of UvrA have a CX_2CX_{18-20}-CX_2C consensus sequence in which the four cysteine residues coordinate one zinc molecule.[42] The N-terminal zinc finger is less well conserved between UvrA molecules than the C-terminal zinc finger. Consistent with this observation, mutations in the N-terminal zinc finger that lead to a loss in zinc binding have no effect on NER. It was therefore concluded that the N-terminal zinc finger is not essential for NER *in vitro*.[43] However, bacteria containing mutations in the C-terminal zinc finger render the cells profoundly sensitive to cell killing by UV light.[43] In an independent study, Wang et al. created the C-terminal zinc finger mutation C763F, which retained no *in vivo* repair activity, failed to bind DNA, but retained vigorous ATPase activity.[44] They concluded that the C-terminal zinc finger is primarily responsible for UvrA's DNA binding capacity. While it has been shown that mutations in the cysteines of the C-terminal zinc finger give rise to dysfunctional proteins, it cannot directly be concluded from these studies that the zinc finger is the major motif responsible for DNA binding by UvrA. A prime example is the damage recognition protein in mammalian NER, XPA, which is also a C_4-type zinc finger protein. In XPA, the zinc finger subdomain does not directly interact with DNA.[45,46] Although the zinc finger is not

Table 1. DNA Damage Recognition by the UvrABC Nuclease

damaging agent	lesion or adduct description	repair by UvrABC[a]	refs
	I. Single Base Modifications		
4-nitroquinoline-1-oxide	4NQO−purine adducts	+	155−157
apurinic/apyrimidinic sites	abasic sites, reduced apurinic sites (ring opened)	+	17, 158−160
aflatoxin-B1	purine adducts, N^7-guanine, formamidopyrimidine	++	109, 161−164
alkoxyamine modified AP sites	AP analogue	++	165, 166
anthramycin	N^2-guanine	+++	167−169
CC-1065	N^3-adenine	++	147, 191, 192
cholesterol	synthetically prepared cholesterol adducted base	+++	170
fluorescein	synthetically prepared fluorescein adducted thymine	+++	18
ionizing radiation	dihydrothymine, *N*-glycoside-β-ureido iodobutyric acid urea residues/thymine glycol HO-C^5,C^6-thymine	not repaired/++	27, 38, 158
menthol	synthetically prepared menthol adducted base	+	149
multifunctional alkylating agents	O^4-alkyl thymine, O^6-methyl guanine, N^6-methyl adenine	not repaired/+	131, 171−174
N-acetoxy-2-acetylamino-fluorene (AAF), *N*-hydroxy-aminofluorene (AF)	C^8-guanine	++	82, 175−181
N′-methyl-*N*-nitronitrosoguanidine (MNNG)	O^6-methyl guanine	++	131, 173, 207
polycyclic aromatic hydrocarbons (PAHs)	N^2-guanine, benzo(*a*)pyrene diol epoxide, methylchrysene/C^8-guanine, 1-nitropyrene	+++/++	36, 103, 116, 126, 163, 181−186
psoralen	monoadduct (e.g. 8-methoxypsoralen (8-MOP) and 4,5′,8-trimethylpsoralen (TMP))	+++	9, 70, 177, 187−190
	II. Intra- and Interstrand Cross-links		
cisplatin	N^7-guanine, GG, AG/GxG	++/+++	39, 193−198
cyclohexylcarbodiimide	unpaired T and G residues	++/+++	199
DNA−protein/DNA−peptide cross-links	chemically induced	+/++	35−37
mitomycin C, *N*-methylmitomycin A	N^7-guanine; O^6-methyl guanine, N^2-guanine	++	6, 200−204
N,*N*′-bis(2-chloroethyl)-*N*-nitrosourea	bifunctional alkylation	++	200, 205, 206
nitrogen mustard	bifunctional alkylation	++	200, 204, 206
psoralen	C^5,C^6-thymine; bisadduct	+++	9, 99, 177, 187, 190, 208−212
UV irradiation	pyrimidine dimer (C^5,C^6-pyrimidine), 6−4-photoproduct	++/+++	9, 36, 108, 156, 213−215
	III. Natural Bases		
A-tracts	AAAA	not repaired	8, 27
dsDNA		not repaired	8
extrahelical bases or loops in DNA		not repaired	199
mismatches	A-G; G-G	not repaired/++	189, 199
sequence specific bends		not repaired	8, 216
	IV. Backbone Modifications		
2-aminobutyl-1,3-propanediol (ABPD)	synthetically modified	+	189
azidophenacyl bromide	synthetically modified, phosphorothioate linkage	+	18
cholesterol, Chol-S, Chol-P	synthetically modified, tethered to backbone	+++	39, 95, 98, 122, 149
fluorescein	synthetically modified, tethered to backbone	+++	36, 84, 88, 217
phosphorothioate, methyl phosphorothioate	synthetically modified	+	18, 105
phosphotriesters		not repaired	8, 27
single nucleotide gap	synthetically modified	+++	18, 95
single strand nick (3′ or 5′) in dsDNA with modified bases	synthetically modified	+++	18, 98, 159, 218
single strand nick in dsDNA	synthetically modified	+++	18, 95
	V. Intercalators		
actinomycin D		inhibits repair	219
caffeine		inhibits repair	219−221
chloroquine		inhibits repair	220
ditercalanium	noncovalent bisintercalator	++	222, 223
doxorubicin/AD32		+	224, 225
ethidium bromide		inhibits repair	219
Hoechst 33258		inhibits repair	219

[a] Repair key: +, 0−25%; ++, 25−50%; +++, >50%.

responsible for DNA binding, deletion of part of the finger or site-directed mutagenesis of the zinc-coordinating Cys-108 results in a loss of DNA binding activity.[47,48] The suggestion has been made that the zinc finger is required to maintain the proper conformation of this protein region and that in its absence conformational distortions arise that abolish DNA binding. Therefore, while it is *likely* that the C-terminal zinc finger of UvrA interacts with DNA to facilitate the NER reaction, it cannot be ruled out that this motif is also required for structural integrity.

3.2. The ABC ATPase Domain

The majority of proteins within the ABC ATPase superfamily with known function are involved in molecular

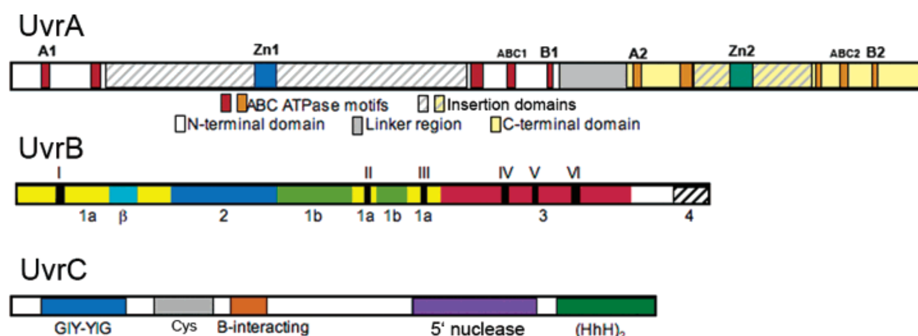

Figure 2. Domains of the UvrABC proteins. UvrA consists of two halves (white and yellow, respectively) separated by a protease sensitive linker (gray). Each domain contains one ATP binding site composed of a Walker A motif (A1 and A2), a Walker B motif (B1 and B2), and an ABC signature sequence (ABC1 and ABC2). Each domain also contains an insertion domain (crosshatched areas) and one zinc finger (Zn1 and Zn2) located between the Walker A and ABC signature. Conserved ATPase motifs are indicated in red and orange in the N-terminal and C-terminal domains, respectively. UvrB is color coded according to its domain architecture (see Figure 3) with domain 1a in yellow, 1b in green, 2 in blue, 3 in red, 4 in white (the crosshatch is the UvrC interacting region), and the β-hairpin in cyan. The six helicase motifs (I to VI) found in domains 1a and 3 are indicated. UvrC contains two distinct endonuclease centers, an N-terminal GIY−YIG family nuclease domain (blue) responsible for 3′ incision and a C-terminal endonuclease domain (purple) that performs the 5′ incision. The potential UvrB interacting domain (B-interacting) is colored in orange, the Cys rich region is gray, and the tandem helix−hairpin−helix domains implicated in DNA binding are shown in green.

transport. However, a few other DNA metabolizing enzymes, whose crystal structures have been solved, also possess this fold. These include Rad50, MutS, and the SMC proteins.[49−51] All of the ABC ATPase superfamily members function as dimers or higher order oligomers. On the basis of the available structures of ABC ATPases, a unifying model of how this domain folds has been adopted.[52] The ABC ATPase elements—the Walker A motif, Walker B motif, Q-loop, signature sequence, and His loop—are all integrally involved in forming an interface between two subunits such that ATP is sandwiched between them.[53] The composite ATP binding is created by the Walker A and Q-loop motif of one subunit and the signature sequence, Walker B motif, and His loop of the other subunit (for a review, see ref 52). Due to this architecture, each active site is capable of binding two molecules of ATP. Thus, ATP binding is thought to stabilize the dimer architecture, while ATP hydrolysis drives dissociation of the subunits.[54] On the basis of the observations that UvrA contains all the conserved ABC ATPase elements, it can be postulated that the ATPase domains of UvrA function in a way similar to the case for other ABC ATPases, although UvrA is slightly more complex because it possesses two ABC ATPases.

The original observation that UvrA must be a dimer to be functional came from UvrA dilution studies. In these studies, low concentrations of UvrA promoted monomer formation and caused a drastic decrease in the specific activity of UvrA's ATPase.[55,56] Additional evidence came from Myles et al., who cleaved UvrA into two domains and showed that only the N-terminal domain possesses the ability to dimerize independently, suggesting that UvrA dimerizes in a head-to-head fashion.[57] The ATPase motifs in the N-terminal domain alone are likely insufficient to stabilize the dimer, and an additional interaction, which could be provided by the N-terminal C_4 zinc finger motif, may be required.

In the crystal structure of ABC ATPases in complex with ATP, the Walker A motif (Gly-Lys-Ser) interacts with the phosphates of the ATP molecule.[49−53,58] Walker A motif mutants in the SMC protein have shown that substitution of Lys to Ala disrupts ATP binding.[54] Likewise, mutation of Lys to Met within *E. coli* MutS severely impaired its ATP binding.[59] Similar Walker A mutants have been created within both of *E. coli* UvrA's ABC ATPase domains: K37A and K37M. While binding of radiolabeled ATPγS in the presence of plasmid DNA suggested no difference between these mutants and the wild-type protein,[60] an independent study using equilibrium gel filtration to measure ^{3}H-ATP binding revealed defective ATP binding of the Walker A K37A mutant.[56]

Mutagenesis of the N-terminal or C-terminal Walker A motifs revealed that both domains of UvrA possessed active ATPases.[56,60] In both studies, mutagenesis of the N-terminal Walker A motif resulted in a protein that had a lower K_m value than the corresponding C-terminal Walker A mutant, suggesting that UvrA has a high and a low affinity binding site. These mutants also showed that the two ATPase domains are not equivalent, because the wild-type and C-terminal mutant proteins demonstrate cooperative ADP binding while the K37A mutant does not; therefore, cooperative nucleotide binding can be attributed to the N-terminal domain of UvrA.[56] As is the case with other ABC ATPases, the ATP binding sites in UvrA are not equivalent and appear to be allosterically regulated.[56,60,61]

Even though by sequence analysis and site-directed mutagenesis UvrA possesses two ABC ATPase domains with the potential for four nucleotide-binding sites per dimer, only one ATP per UvrA dimer has been observed experimentally.[56] In addition, only the N-terminal domain of UvrA could be cross-linked with ^{32}P-ATP, suggesting that the ATP is bound at the N-terminal ATPase site.[57] Considering that each Walker A site has the potential to be in one of three states (empty, bound to ATP, or bound to ADP), there are 4^3 or 64 potential configurations for UvrA's ATPase sites, and it remains to be determined why UvrA possesses multiple potential ATP binding sites and yet only binds one ATP per UvrA dimer.

The role of UvrA's ATPase activity has been the subject of numerous studies.[26,56,57,60,62−65] Interpretation of the data is complicated since UvrA contains four potential ATP binding sites per dimer and interacts with UvrB, which also possesses an ATP site. UvrA's ATPase is modulated by several factors such as UvrA protein concentration, UvrB, and DNA. UvrA is an ATP/GTPase while UvrB is strictly an ATPase. In the presence of UvrA, GTP, and damaged DNA, the addition of UvrB causes a decrease in GTP hydrolysis.[63,65] The opposite is observed if ATP is included

in this reaction.[63,65] The observed increase in ATP hydrolysis is most likely due to activation of UvrB's cryptic ATPase site and not associated with UvrA,[63] suggesting that the interaction of UvrB with UvrA and DNA leads to a suppression of UvrA's ATPase/GTPase activity.[63]

UvrA was originally described as a DNA-independent ATPase;[62] however, it was later shown that the apparent K_m for ATP or GTP decreases with increasing dsDNA concentration or upon addition of ssDNA.[63,64] In addition, nondamaged DNA causes the apparent K_m for ATP to decrease more than damaged DNA.[61] Since UvrA binds to DNA as a dimer, and ATP is believed to bind at the interface of the dimer, the presence of DNA may simply cause a lowering of the K_m for ATP by promoting dimerization. The relative changes in the K_m may be directly correlated with the relative binding affinities of UvrA for the various DNA substrates.

3.3. Damage Recognition and DNA-Binding by UvrA

UvrA plays a vitally important role in the NER mechanism due to the fact that it is the first component of the system to recognize DNA damage. How UvrA facilitates DNA damage recognition remains to be elucidated. UvrA displays no sequence-specific DNA binding, and it binds and recognizes a wide variety of DNA perturbations. Zou et al. has conducted a thermodynamic analysis of UvrA's DNA binding. In this study, the authors took advantage of the intrinsic fluorescence of benzo[*a*]pyrene diol epoxide (BPDE) DNA adducts to evaluate the effects of temperature and salt concentrations on UvrA's DNA binding ability.[66] It was concluded that although some electrostatic interactions are involved, only a small number of counterions were released from the DNA upon UvrA binding. This suggests that hydrophobic forces are largely responsible for complex formation. Dimerization of the ABC ATPase domains is also a hydrophobically driven process and therefore would account for some of the observed hydrophobic forces.[10]

It has been proposed that UvrA possesses two DNA binding sites, one within the N-terminal domain of UvrA and the other within the C-terminal domain.[57] In an attempt to localize the DNA binding domain of UvrA, Wang and Grossman identified a putative helix−turn−helix motif within the N-terminal domain of UvrA.[67] They randomly mutagenized this region and obtained eight single substitutions, of which they characterized two: G502D and V508D. These mutant proteins are located between the signature sequence and the Walker B motif, and they lost their ability to distinguish damaged from nondamaged DNA. However, on the basis of the available crystal structures of ABC ATPases, these amino acids would not be solvent exposed. It therefore seems unlikely that these amino acids are directly responsible for DNA binding, but they could instead perturb ATP hydrolysis in the N-terminal ATPase domain and thereby disrupt UvrA's functions.

UvrA has been proposed to bend and unwind DNA upon binding.[68] When UvrA is incubated with plasmid DNA in the presence of topoisomerase, the DNA undergoes a change in the linking number, suggesting that UvrA unwinds the DNA.[68] How UvrA induces this change on the DNA structure is currently unknown. DNA footprint studies have shown that UvrA specifically binds to damaged DNA in the presence and absence of nucleotide cofactor and produces a DNA footprint that is 33-bp in length.[69] Because the number of protected base pairs does not change in the presence or absence of cofactor, the suggestion was made that the UvrA:DNA complex does not undergo a significant conformational change upon nucleotide hydrolysis.

Nonspecific and specific DNA binding constants have been derived for UvrA on the basis of several different binding assays. From DNase I footprinting and titrations of UvrA on a 137-bp DNA fragment, equilibrium DNA binding constants of $K_s = (0.7-1.5) \times 10^8\ M^{-1}$ and $K_{ns} = (0.7-2.9) \times 10^5\ M^{-1}$ were determined (monomer concentration).[70] Therefore, UvrA binds to damaged DNA with a specificity ratio of 10^3 compared to nondamaged DNA. While the UvrA:DNA complex shows specificity, it is also salt sensitive, is short-lived, and is weaker than the UvrB:DNA complex.[10,71]

Functional ABC ATPase domains are required for efficient DNA damage recognition by UvrA. Two laboratories independently created Walker A ATPase deficient point mutants and assessed DNA binding. One study reported that the specific binding by the N-terminal mutant was indistinguishable from that by wild-type UvrA.[56] The other reported that the N-terminal mutant had lost its damage specific binding.[60] In both studies the C-terminal mutant was more defective in DNA binding, as judged by DNase I footprinting[56] and filter binding assays,[60] and displayed an increase in nonspecific DNA binding relative to that of wild-type UvrA. It was therefore concluded that ATP hydrolysis in the C-terminal domain of UvrA is essential to allow dissociation from nondamaged DNA sites. Additional support comes from the substitution of ATPγS in DNA binding experiments; ATPγS promotes tight nonspecific dsDNA binding.[69] These studies underscore the importance of UvrA's dynamic ATPase as an integral part of UvrA's DNA damage detection mechanism.

Early nitrocellulose filter binding studies in the presence of ATP showed that UvrA possessed a higher affinity for ssDNA than dsDNA, but bound nonspecifically to ssDNA.[62] Subsequently it was reported that retention of UvrA:ssDNA complexes was "abysmally low", and therefore Mazur and Grossman extended the study of UvrA and ssDNA via competition experiments.[10] They investigated the association and dissociation rate constants for UvrA binding to ssDNA. The equilibrium association constants for UvrA binding to ssDNA were $7 \times 10^6\ M^{-1}$ and $2 \times 10^7\ M^{-1}$, while the dissociation rate constants were $1.4 \times 10^{-4}\ s^{-1}$ and $7.5 \times 10^{-5}\ s^{-1}$, in the absence and presence of ATP, respectively. Thus, ATP significantly decreased the rate of dissociation from ssDNA,[10] leading to higher overall binding affinity. In addition, they noted that dissociation of UvrA from ssDNA was 40-times slower than dissociation from UV-irradiated dsDNA in the presence of ATP.[10] These intriguing observations suggest that UvrA binds to ssDNA and damaged DNA through different types of interactions and that UvrA might have some other uncharacterized function with respect to ssDNA *in vivo*. In the presence of ATPγS, UvrA exhibits a reduced binding to ssDNA. Strike and Rupp showed in cross-linking experiments an increase in the percentage of UvrA cross-linked to nondamaged ssDNA in the presence of ATP, but a decrease in cross-linked protein when ATPγS was included.[72] They suggested that UvrA has two modes of association with ssDNA: a loose initial interaction that permits trapping by filter binding assays and a second close association that is achieved after ATP hydrolysis. Further studies to analyze the role of UvrA and ATP in the presence of ssDNA led to dissociation constants for *Thermus thermophilus* HB8 UvrA (*Tth* UvrA) and damaged ssDNA of

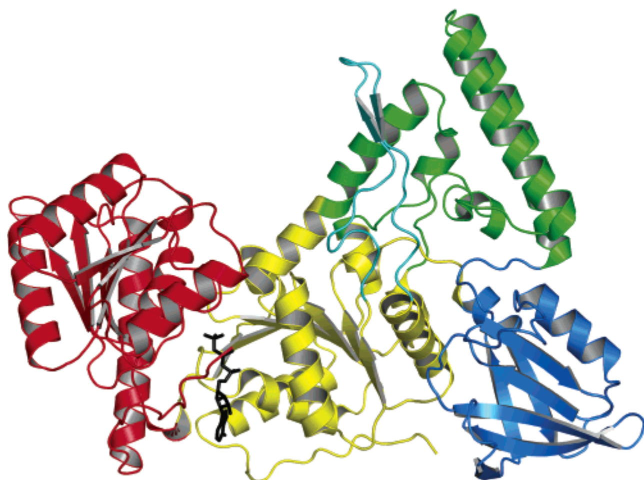

Figure 3. Structure of UvrB in complex with ATP. Helicase domains 1a and 3 are shown in yellow and red, respectively. Domain 2, the UvrA interacting domain, is shown in blue. The β-hairpin is shown in cyan and observed to interact with domain 1b in green. The ATP molecule, located between domains 1a and 3, is shown in all-bonds representation and color-coded by element.

$K_d = 0.43\ \mu$M and $0.1\ \mu$M in the absence and presence of ATP, respectively.[73] Binding to nondamaged ssDNA was dramatically influenced by the presence or absence of nucleotide cofactor. The K_d for UvrA binding to nondamaged ssDNA was greater than $30\ \mu$M while in the presence of ATP the K_d was $0.12\ \mu$M. These results suggest that ATP drastically affects the affinity of *Tth* UvrA for nondamaged ssDNA and *Tth* UvrA binds with at least 100-times higher affinity to damaged ssDNA than nondamaged ssDNA.[73]

4. UvrB

UvrB plays a central role in NER since it interacts with all the components of the system: UvrA, UvrC, UvrD (helicase II), DNA polymerase I, and DNA. As part of the UvrAB complex, UvrB is critical for the second phase of damage recognition. After initial identification of a potentially damaged site by UvrA, UvrB is involved in verifying the nature of the damage and remains bound to the DNA forming the preincision complex, which is recognized by UvrC (Figure 1). The crystal structures of UvrB from *Bacillus caldotenax*[15] (PDB code 1D9Z) and *Thermus thermophilus*[13,14] (PDB codes 1C4O and 1D2M) have been solved. The structure of *B. caldotenax* UvrB has also been solved in complex with ATP[15] (PDB code 1D9X). UvrB is composed of five domains referred to as 1a, 1b, 2, 3, and 4. The structure of the UvrB:ATP complex is shown in Figure 3 and illustrates the protein's overall architectural fold. Domain 4 is completely disordered in both the *B. caldotenax* and *T. thermophilus* structures. A UvrB fragment encompassing domain 4 has been isolated, and it was found to form a coiled−coiled structure[74,75] (Figure 4). Domain 2 is mostly disordered in the original UvrB structures. The structure of domain 2 was elucidated when a point mutant of UvrB from *B. caldotenax* (Y96A) crystallized in a different space group as compared to the case of wild type.[65] Figure 3 shows a hybrid structure containing the wild-type UvrB:ATP complex from *B. caldotenax* and domain 2 from the Y96A mutant. Domains 1a, 1b, and 3 are well ordered in both the *B. caldotenax* and *T. thermophilus* structures.

UvrB is classified as a member of the helicase superfamily on the basis of the presence of six conserved helicase motifs

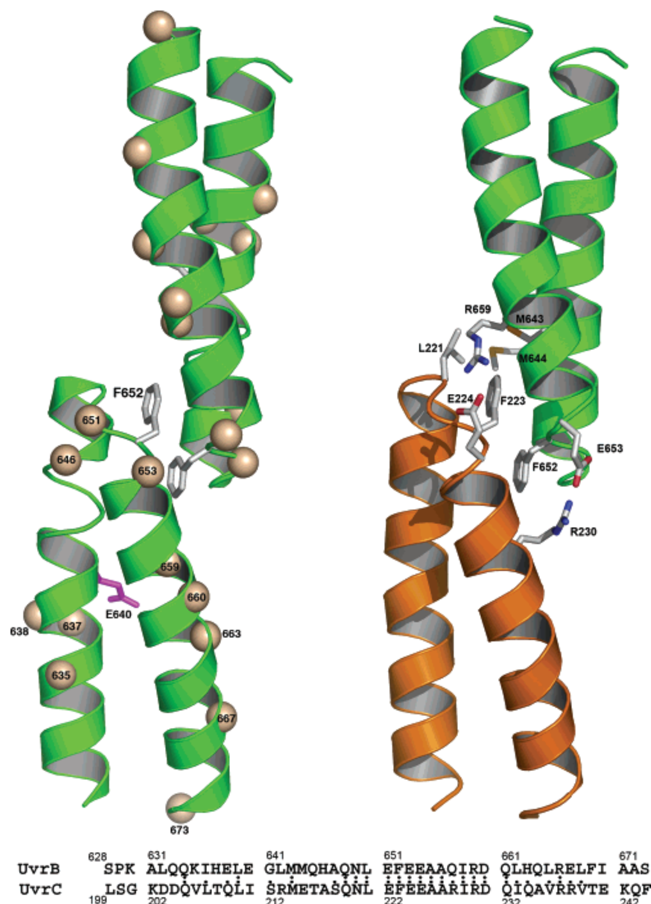

Figure 4. Structure of *E. coli* UvrB's domain 4. Left, interaction between two domain 4 molecules as observed in the crystal structure. Residues that have been mutated are indicated by spheres and labeled. The side chains of Phe 652 and Glu 640 are depicted in all-bonds representation. Glu 640 is colored magenta because conflicting results for this mutant have been reported. Right, the hypothetical model for the interaction between UvrB (green) and the similar region of UvrC (orange) based on the interaction seen in the domain 4 crystal structure. Side chains of residues located at the interface are drawn. An alignment of the two regions that are similar in *E. coli* UvrB and UvrC is shown.

(I−VI) distributed throughout domains 1a and 3.[29] The two domains are connected to each other by a short linker and have an α/β/α sandwich-fold (Figure 3). The structures of domains 1a and 3 are similar to the core domain of other helicases including PcrA,[76] Rep,[77] and NS3.[78] In addition, all the residues necessary to couple ATP hydrolysis to strand translocation are present.[15] At the interface of domains 1a and 3 is UvrB's ATP binding site (Figure 3). The ATPase site of UvrB becomes activated in the presence of UvrA and damaged DNA, or when the coiled-coil domain of UvrB (domain 4) is cleaved off.[63,64,79] A detailed structural analysis of UvrB and its ATP binding site, including a detailed comparison of UvrB to related helicases, was reviewed by Theis et al.[29]

On the basis of the presence of the helicase domains in UvrB, it has been envisioned that the UvrAB complex could simultaneously "scan and sense" the DNA duplex for damage.[80] However, in contrast to "true" helicases, which couple ATP hydrolysis and subsequent domain motion to the unwinding of long stretches of DNA,[81] the ATPase activity of UvrB is associated with very limited DNA unwinding, only displacing up to 22 nucleotides depending on the DNA's melting temperature.[82,83] Thus, more recent

work describes this activity as "strand destabilization" activity rather than helicase activity.[84] The current model suggests that UvrB does not use its helicase domains to scan along DNA, but rather to distort the DNA at lesion sites, facilitating recognition and incision by UvrC (discussed below). UvrB mutants deficient in ATPase activity (Table 2) are also defective in repair and the associated supercoiling activity, as well as DNA strand destabilization.[83,85−87] Since the strand destabilization assay will be mentioned a number of times throughout the review, it should be briefly explained here. The assay involves adding UvrA, UvrB, and ATP to a DNA substrate composed of a short oligomer with a centrally located lesion annealed to a single-stranded M13 circle DNA. The UvrAB complex is able to destabilize the short damaged oligonucleotide to the extent that the addition of SDS and EDTA will lead to the dissociation of the oligomer from ssM13 DNA as analyzed by native PAGE.[88]

4.1. The UvrA–UvrB Interaction

UvrB interacts with both UvrA and UvrC in solution and in complex with DNA.[8] However, no complex containing all three proteins has ever been observed. Sequence comparisons have suggested that residues in domains 2 and 4 of UvrB are responsible for these protein−protein interactions.[89,90] Domain 2 is located between domains 1a and 1b (Figure 3) and is homologous to a domain present in the TRCF (transcription repair coupling factor) protein (also referred to as Mfd).[66,230] TRCF releases a stalled RNA polymerase from a lesion and interacts directly with UvrA to initiate damage repair[91] (Figure 1). UvrA is able to bind UvrB and TRCF, suggesting that the homologous portion of these two proteins (domain 2 in UvrB) is the UvrA binding domain.[91] Furthermore, a fusion protein of the maltose binding protein and residues 116 to 251 from *E. coli* UvrB, which encompasses domain 2, was shown to interact with UvrA.[90] A similar fusion protein using domain 4 of UvrB (*E. coli* residues 548 to 674) interacted with both UvrA and UvrC.[90] Despite the interaction with UvrA, deletion of domain 4 results in a UvrB variant that can still form the preincision complex and hence still interacts with UvrA,[92−94] but no longer recruits UvrC.[92,93,95] Since UvrA and UvrC both interact with domain 4 of UvrB, the UvrA interaction might serve to block UvrC binding to UvrB prior to the dissociation of UvrA. This would ensure that both phases of damage recognition have been achieved and completed prior to the incision reaction.

The structure of UvrB's domain 2 has a $\beta\alpha\beta\beta\beta\beta\beta\beta\beta$ topology (Figure 3). The core is formed by a six-stranded antiparallel β-sheet. One face of the β-sheet is exposed to the solvent while the other interacts with an α-helix and a two-stranded antiparallel β-sheet. Domain 2 is structurally similar to no other known protein and only contains sequence homology to the aforementioned TRCF. On the basis of the crystal structure as well as phylogenetic analysis of UvrB and Mfd, conserved surface residues were identified and subsequently mutated. The *B. caldotenax* UvrB mutants were assayed for their ability to interact with UvrA via pull-down assays. Four mutants displayed impaired UvrA:UvrB complex formation: R183E, R194A/R196A, R194E/R196E, and R213A/E215A. These results suggest that UvrB interacts with UvrA through arginine mediated electrostatic interactions.[65]

Although much is known about the regions of UvrB responsible for the UvrA interaction, the UvrB interacting region of UvrA is poorly understood. Only a few reports have probed for which domains of UvrA are required for the interaction with UvrB. Claassen et al. created a series of C-terminal deletion mutants of UvrA.[96,97] The mutants were insoluble and thus purified from inclusion bodies. For each UvrA mutant, a productive UvrA−UvrB interaction was inferred on the basis of the observation that addition of UvrB, or the K45A UvrB ATPase deficient mutant (Table 2), led to an increase in ATPase activity. All of the mutants, the largest with 710 deleted amino acids from the C-terminal end, interacted with UvrB in this study, and therefore, it was concluded that the N-terminal 260 amino acids of UvrA are sufficient for UvrB interaction. However, previously it was shown that the elevated ATPase activity measured in the UvrA:UvrB:DNA complex was due to the activation of UvrB's cryptic ATPase site and not to UvrA.[79] In addition, the UvrA mutant with 710 amino acids deleted would have removed the ATPase catalytic residues and therefore could not have produced the observed elevated ATPase activity. Thus, the conclusion that the first 260 amino acids are sufficient for interaction between UvrA and UvrB has to be re-evaluated.

The ABC ATPase motifs of UvrA are important for the interaction with UvrB. Mutation of the Walker A sequence, GKS to GAS, of UvrA either in the N-terminal or C-terminal domain of UvrA lead to greatly reduced complex formation between the proteins.[56] The authors noted that the two mutants behaved differently in DNase I footprinting experiments, designed to monitor the appearance of an UvrB-dependent DNase I hypersensitive site as an indication of productive complex formation. In this assay, the N-terminal Walker A mutant possessed 10% of the UvrB loading activity, compared to wild-type UvrA, while the C-terminal mutant exhibited about 1% activity.

Investigating the precise role of UvrB's ATPase activity for the formation of the UvrA:UvrB complex is complicated since both UvrA and UvrB possess ATPase activity. Mutants of UvrB were created to evaluate whether ATP binding or hydrolysis by UvrB is required for the interaction with UvrA (Table 2). A mutation in the Walker A motif of UvrB (K45A) results in a UvrB variant that is unable to hydrolyze ATP but is still capable of interacting with UvrA as measured by the recovery of salt-resistant nucleoprotein complexes.[79] While it is impossible to infer the true nature of these complexes, the fact that they were resistant to high salt suggests that they were UvrB:DNA complexes. Therefore, hydrolysis by UvrB is not a prerequisite for UvrA:UvrB complex formation in the presence of DNA.[79] Further support comes from studies using the helicase mutants of UvrB (Table 2).[83] The UvrB mutants in this study lacked ATPase activity, yet they were shown to interact with UvrA on the basis of the findings that UvrA's ATPase activity was reduced when these UvrB mutants were added to UvrA:DNA complexes, and by diagnostic DNase I DNA footprinting analysis. Moreover, in the presence of UvrA, UvrB, GTP, and UV-irradiated plasmid DNA, filter-binding assays were used to capture salt-resistant complexes.[63] Since UvrB cannot accommodate GTP into its nucleotide-binding pocket,[15] the results suggest that UvrB does not require cofactor binding at all to interact with UvrA.

4.2. Generation of the UvrB:DNA Preincision Complex

In the absence of UvrA, UvrB does not bind to dsDNA, and its affinity for ssDNA is in the micromolar range whether

Table 2. UvrB Mutations Prepared in *E. Coli* or *B. Caldotenax*[b]

Mutation[a]		Domain	ATPase	DNA Protein Complexes	Repair		References
E. coli	*B. caldotenax*				ABC Incision	UV survival	
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	83
G39S		1a	NR	NR	NR	Reduced	83
G44R		1a	NR	NR	NR	Reduced	83
K45A		1a	Defective	AB, no B	Defective	Defective	79
K45D		1a	NR	NR	NR	Defective	79
K45R		1a	NR	NR	NR	Defective	79
N51A		1a	~ WT	~ WT	~ WT	~ WT	79
N51K		1a	NR	NR	NR	~ WT	79
V52D		1a	NR	NR	NR	~ WT	79
I53R		1a	NR	NR	NR	~ WT	79
D55A (Q55)		1a	NR	NR	NR	~ WT	79
F88W (F89)		1a	~ WT	~ WT	~ WT	~ WT	90
β-hairpin mutations							
	Y92A	βh	Enhanced	AB, B ~ WT	Reduced	NR	84
Y92W		βh	NR	~ WT	Reduced	NR	36
Y92A / Y93A		βh	Enhanced	Reduced B	Reduced / BC incision	NR	106
D93A (D94)		βh	NR	NR	~ WT	Reduced	22
	Y93A	βh	Reduced	AB, Reduced B	Reduced	NR	84
Y95F		βh	NR	~ WT	NR	NR	36
Y95W		βh	NR	B enhanced	~ WT	NR	36
Y95A / Y96A		βh	Enhanced	AB, no B	Defective / BC incision	Defective	106
Y95W / Y96W		βh	NR	Defective	NR	NR	36
	Y96A	βh	~ WT	AB, no B	Defective	NR	65,84
Y96W		βh	NR	Defective	NR	NR	36
E98A (E99)		βh	NR	ND	3' only	NR	90
	E99A	βh	Reduced	AB, no B	Defective	NR	84
Y101A / F108A		βh	Enhanced	AB, no B	Defective	Defective	106
Y101W		βh	NR	Defective	Reduced	NR	36
D105A (D106)		βh	NR	NR	~ WT	~ WT	22
F107W (Y108)		βh	~ WT	~ WT	~ WT	~ WT	90
	E110A	βh	NR	~ WT	~ WT	NR	217
	E110R	βh	Enhanced	~ WT	~ WT	NR	217
	K111A	βh	Reduced	AB, B ~ WT	Reduced	NR	84
D111A (D112)		βh	NR	NR	~ WT	~ WT	22
	R123A	1a	Reduced	AB, no B	Defective	NR	84
	H124A	1a	~ WT	B enhanced	~ WT	NR	84
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	65
F187W (F188)		2	~ WT	~ WT	~ WT	~ WT	90
	R194A / R196A	2	↓ / ↑GTPase	~ WT	~ WT	NR	65
	R194E / R196E	2	↓ / ↑GTPase	Reduced B	Reduced	NR	65
	R213A / E215A	2	↓ / ↑GTPase	~ WT	~ WT	NR	65
F216W (F217)		2	~ WT	~ WT	~ WT	~ WT	90
H247A (H248)		1b	NR	NR	~ WT	~ WT	22
	F249A	1b	Reduced	AB, B ~ WT	~ WT	NR	84
E265A (E266)		1b	NR	ND	3' only	NR	90
	R289A	1b	Reduced	AB, B ~ WT	~ WT	NR	84
	R289A / R367A	1b	Reduced	AB, B ~ WT	Reduced	NR	84
	E307A	1b	Reduced	AB, reduced B	Reduced	NR	84
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	90
	D338N	1a	Reduced	AB, no B	Defective	NR	217
H340F (H341)		1a	NR	NR	~ WT	Reduced	22
	H341A	1a	~ WT	~ WT	Reduced	NR	217
D353A (D354)		1b	NR	NR	~ WT	Reduced	22
F365W (F366)		1b	~ WT	~ WT	~ WT	~ WT	90
	R367A	1b	Reduced	AB, reduced B	Reduced	NR	84
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	22,90
F496W (D496)		3	~ WT	~ WT	~ WT	~ WT	90
G502R (G501)		3	NR	NR	NR	NR	83
G502R / G509D (G501 / G508)		3	NR	NR	NR	NR	83
	R506A	3	Reduced	Reduced	Reduced	NR	217
G509S (G508)		3	Reduced	AB, no B	ND	~ WT	83,92
D510A		3	Reduced	Low/ AB, ~ WT	Defective	Defective	22,23,90
	D510A	3	Reduced	AB, no B	Defective	NR	217
	D510N	3	Enhanced	~ WT	Reduced	NR	217
E514K (E513)		3	~ WT	AB, ↓ B, low C	Reduced	~WT	83,92
E514K / R541H (E513 / R540)		3	Defective	AB, no B	ND	~ WT	83
D521A		3	NR	NR	~ WT	Reduced	22
D523A		3	NR	NR	~ WT	Reduced	22
F527W		3	~ WT	~ WT	~ WT	~ WT	90
	F527A	3	~ WT	Enhanced B	Reduced	NR	84
R544H (R543)		3	Defective	AB, no B	ND	~ WT	83,92
H581F		3	NR	NR	~ WT	~ WT	22
D594A		3	NR	NR	~ WT	Reduced	22

Table 2 (Continued)

Mutation[a]		Domain	ATPase	DNA Protein Complexes	Repair		References
E. coli	*B. caldotenax*				ABC Incision	UV survival	
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
E640A		4	NR	Normal C	~ WT	~ WT	92
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	74,93
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							
574 Δ			NR	AB	1-2% WT	NR	92
609 Δ (UvrB*)			↑ - UvrA	AB	< 0.1 – 1% WT	Reduced	16,22,63,92,226,227
630 Δ			NR	AB, BC	1% WT	NR	92
649 Δ			NR	AB, BC	2% WT	NR	92
	Δ2 (Δ154-247)		↓ / ↑GTPase	A enhanced	Defective	NR	18,65
	Δβ-hairpin (Δ 97-112)		Hyper + UvrA	AB enhanced	Defective	NR	18,65,88
MBP/UvrB(115-250)			NR	Binds to A	NR	NR	90
MBP/UvrB(251-546)			NR	DNA binding, No A or C	NR	NR	90
MBP/UvrB(547-673)			NR	Binds to A and C	NR	NR	90

[a] Mutations are listed under the species in which they were prepared *as reported in the original literature cited in the far right column*. There may be slight discrepancies in numbering due to the inclusion or omission of Met1 when numbering the protein sequences. When necessary, in the *E. coli* column, residues in parentheses 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 Figure 6. [b] 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.

Table 3. Intermediates and Products Formed by UvABC in the Presence of Various DNA Constructs[a]

DNA construct	Intermediates and products formed in presence of UvrA	Intermediates and products formed without UvrA	References
dsDNA with lesion	A AB B BC 3' 5'	-	17,28,31,89,228
mismatches			
3-6 bp	A AB B BC 3' 5'	3' 5'	125,126
8 bp	A AB B BC 3' 5'	-	126
> 8 bp	A AB B BC 5'	5'	102,126
truncations			
opposite strand at 3' site	A AB B BC	-	98
both strands at 3' site	A AB B BC	-	98
damaged strand at 3' site	A AB B BC 5'	-	98
both strands at 5' site	A AB	B (BC)	98
damaged strand at 5' site	A AB	(B)	98
opposite strand at 5' site	A AB (B BC 3' 5')[1]	B BC 3' (5')	98
nicks			
3'-pre-nicked	A AB B BC 5'	-	92,93,98,102
5'-pre-nicked	A AB	-	98
opposite 3' incision site	A AB B BC	-	98
opposite 5' incision site	A AB B BC 3' 5'	(3') 5'	98
dsDNA, no lesion	(A)	-	10,70
5' overhanging end	(A)	BC 5'	95,180
3' overhanging end	(A)	-	95
10 bp mismatched	A AB	-	229
mismatched, pre-nicked	(A)	BC 5'	102
ssDNA with lesion	-	(B)	90
ssDNA, no lesion	-	((B))	90

[a] A, AB, B, and BC refer to the formation of different nucleoprotein complexes containing UvrA/UvrB/UvrC as demonstrated in footprinting or gel shift experiments. 3′ and 5′ refer to incision products detected after the reaction. Parentheses around the complex or incision products (i.e. (BC) or (3′)) indicates that the amount of complex or incision product is minimal. [1]UvrA likely does not participate in the formation of these complexes since they also form in the absence of UvrA (see following column).

or not the DNA is damaged.[90] ATP hydrolysis, presumably by UvrA, is required in order to generate UvrB:DNA complexes since ATPγS and ADP cannot be used as substitutes.[19,69] This is consistent with the notion that UvrA catalytically loads UvrB onto sites of DNA damage.[11] How the loading is achieved has been studied extensively by analyzing the nucleoprotein complexes formed by UvrA, UvrB and/or UvrC in the presence of various DNA substrates (Table 3). Moolenaar et al. studied the role of the DNA flanking the lesion.[98] DNA substrates with both strands truncated 5′ or 3′ to the lesion, with respect to the damaged strand, were examined (Table 3). Experiments using DNA truncated on the 3′ side of the damage, in the absence of UvrA, yielded no UvrB:DNA preincision complex. However, in the presence of UvrA, formation of the UvrB:DNA preincision complex and the UvrB:UvrC:DNA complex is observed. In contrast, experiments using 5′ truncated DNA in the presence of UvrA showed no preincision complex formation, suggesting that the UvrAB complex is approaching the DNA damage from the 5′ side. Interestingly, in the absence of UvrA, UvrB is able to bind and form a stable preincision complex in a damage-dependent fashion when the DNA is truncated at the 5′ end but not if it is truncated 3′ to the damage. These results also suggest that dsDNA 5′ to the lesion impedes formation of the preincision complex and one of the roles of UvrA must be to overcome this barrier. Truncating only the nondamaged strand on the 5′ side leads to formation of the preincision complex as well as 3′ and 5′ incision in the absence of UvrA. Further evidence for a 5′ approach by the UvrAB complex is observed when cross-linked DNA is used as a substrate. In this case, translocation of the UvrAB complex stops prematurely since it is not able to separate the strands at the lesion. Assuming that the complex approaches the damage from the 5′ side, the incisions should be 5′ shifted, which is exactly what is observed for certain cross-links.[99]

It has been proposed that DNA repair events including base excision repair and NER proceed in an orderly manner in which each successive enzymatic step in the reaction cascade recognizes a product−enzyme complex rather than binding to free DNA intermediates.[11,100,101] Various models have been proposed for damage recognition by the bacterial NER system. In 1996, Sancar and Hearst coined the phrase "molecular matchmaker" to describe a number of protein−DNA interactions that involve a handoff of DNA from one protein partner to the next in an arranged marriage between two partners that would not occur otherwise. They suggested five criteria that UvrA must follow to be a molecular matchmaker for UvrB and damaged dsDNA.[11,100] First, in

the absence of UvrA, the affinity of UvrB for dsDNA should be physiologically insignificant. Electrophoretic mobility shift assays have shown that this is true; UvrB can bind ssDNA in the micromolar range but does not display measurable affinity toward dsDNA.[90] Second, UvrA must promote a stable complex between UvrB and DNA. Using numerous independent techniques, several laboratories have demonstrated that UvrA is required to load UvrB onto damaged dsDNA.[19] Third, the UvrA or UvrB proteins should bind or hydrolyze ATP. As has been discussed, both proteins are endowed with ATPase activity. Fourth, UvrA should form a complex with UvrB and DNA, inducing a conformational change, but not covalently modify either UvrB or DNA. This is a key feature of damage recognition: the action of one protein promoting a conformational change to allow recognition by a second protein. In the context of the UvrA:UvrB:DNA complex, it was shown that UvrA must induce a conformational change in UvrB, endowing it with the ability to bind tightly to damaged DNA.[11] Simultaneously, UvrB is promoted to go from an inactive state to a conformation that is capable of hydrolyzing ATP.[70,88] UvrA is required for strand opening around the lesion since UvrB can interact with and promote incision on a lesion in the context of an unpaired flap or bubble in the absence of UvrA.[95,102,103] Fifth, after the UvrB:DNA complex is created, UvrA should dissociate from the complex. The departure of UvrA from the complex is supported by a number of observations including DNA footprinting and electrophoretic mobility shift assays (EMSA).[70,104] While UvrA fulfills the role of a molecular matchmaker in all five criteria, this analogy fails to fully appreciate the role of UvrB in damage verification.

The description "molecular matchmaker" suggests that the matched molecules are passively joined, like an arranged marriage; in this regard it has to be clearly stated that UvrB actively participates in the selection of its "spouse", the damaged DNA. UvrA binds both damaged and nondamaged DNA and furthermore attempts to handoff both of these DNAs to UvrB. However, neither gel shift assays nor DNA footprints led to the formation of UvrB:DNA complexes with nondamaged DNA even though there is a small but measurable amount of incision seen on nondamaged DNA.[105] Thus, UvrB and DNA are not simply being matched by UvrA, but rather UvrB is actively engaged in the "damage verification" step of the reaction.[84,88,106]

In the normally transient UvrA:UvrB:DNA complex, UvrB must determine if damage is present. If this is the case, UvrB will grasp the damaged DNA from UvrA to create a stable UvrB:DNA complex. Otherwise, UvrB rejects the DNA and helps to promote UvrA dissociation. Support for this notion comes from DNA footprinting experiments and cross-linking experiments. In the DNase I footprint experiments, ATP and UvrB increase the specific binding of UvrA by decreasing the amount of time UvrA resides at nondamaged DNA sites.[107] In cross-linking experiments, it was shown that the β-hairpin mutant of UvrB, which forms a stable UvrA:UvrB:DNA complex, does not engage the DNA and therefore the majority of the cross-linking captured UvrA.[18] These results suggest that, prior to transferring the DNA from UvrA to UvrB, the presence of damage has to be verified by UvrB. If no damage is present, UvrB dissociates. This could also weaken UvrA's affinity for DNA and thereby lead to a release of both proteins. In this way, UvrA initially searches for "irregularities" in the DNA and through successive steps of recognition UvrB takes a closer look at whether the DNA contains a damaged base. The joint actions of UvrA and UvrB imposes a high degree of damage specificity on the NER process.

4.3. The UvrB:DNA Preincision Complex

During characterization of the NER reaction, it was immediately apparent that the addition of UvrB to filter binding experiments increased the recovery of DNA–protein complexes.[108] The half-life of UvrB:DNA complexes varies depending on the lesion but ranges from 135 min using UV treated DNA to 400 min for the UvrB:aflatoxin-B1 DNA complex.[19,109] The stable UvrB:DNA preincision complex serves as a scaffold for the binding of UvrC. The unique stability of the preincision complex, before and after excision of the lesion[110] and at high ionic strength,[311] lies in what is perhaps the most prominent feature of UvrB, a flexible β-hairpin that extends out of domain 1a (Figure 3). The hairpin contains a large number of highly conserved hydrophobic and aromatic residues at its base and tip (Figure 5A). The aromatic and hydrophobic residues at the tip of the hairpin form van der Waals interactions with domain 1b. The aromatic residues at the base of the hairpin are predominantly solvent exposed and show no obvious structural constraints suggesting functional importance. The majority of conserved residues in UvrB, including those that have been mutated and led to an altered phenotype (Table 2), are located on the same face as the β-hairpin (Figure 6). A homology model of UvrB bound to DNA[15] was built on the basis of the structures of three helicases in complex with DNA: NS3, PcrA, and Rep.[76−78] The model predicts that the β-hairpin inserts itself between the two strands of the DNA, thereby clamping one of the strands between the β-hairpin and domain 1b (Figure 5B). This mode of DNA binding by UvrB was coined *The Padlock Model*.[29] The hydrophobic residues at the tip of the hairpin are predicted to be involved in strand opening and clamping of the DNA. There is no direct evidence indicating whether UvrB grasps the damaged or nondamaged DNA strand. On the basis of the available biochemical data, it has been suggested that the nondamaged strand is tightly clasped.[110] Additionally, DNase I footprinting experiments have shown that UvrB preferentially protects the nondamaged DNA strand from cleavage.[70]

The padlock model explains the remarkable stability of the UvrB:DNA preincision complex. It also agrees with the observation that the preincision complex does not spontaneously form, since a conformational change in UvrB would be required to move the β-hairpin away from domain 1b to allow insertion between the strands of duplex DNA. It is thought that UvrA triggers this conformational change in UvrB, leading to the release of the tip of the β-hairpin from domain 1b. Further support for this model comes from the studies using truncated DNA by Moolenaar et al. mentioned above (Table 3). Substrates with both strands truncated 5′ to the damage, relative to the damaged strand, were able to form preincision complexes in the absence of UvrA. Since the damage is close to the end of the DNA, the model predicts that UvrB could "slide" onto the end of the DNA without requiring UvrA to unwind the DNA and assist UvrB in inserting its hairpin between the strands. If the damage were farther from the end, then UvrB would not be expected to bind, which is exactly what is observed when using a substrate that is not truncated. If UvrB would slide on the nondamaged strand, then the amount of preincision complex

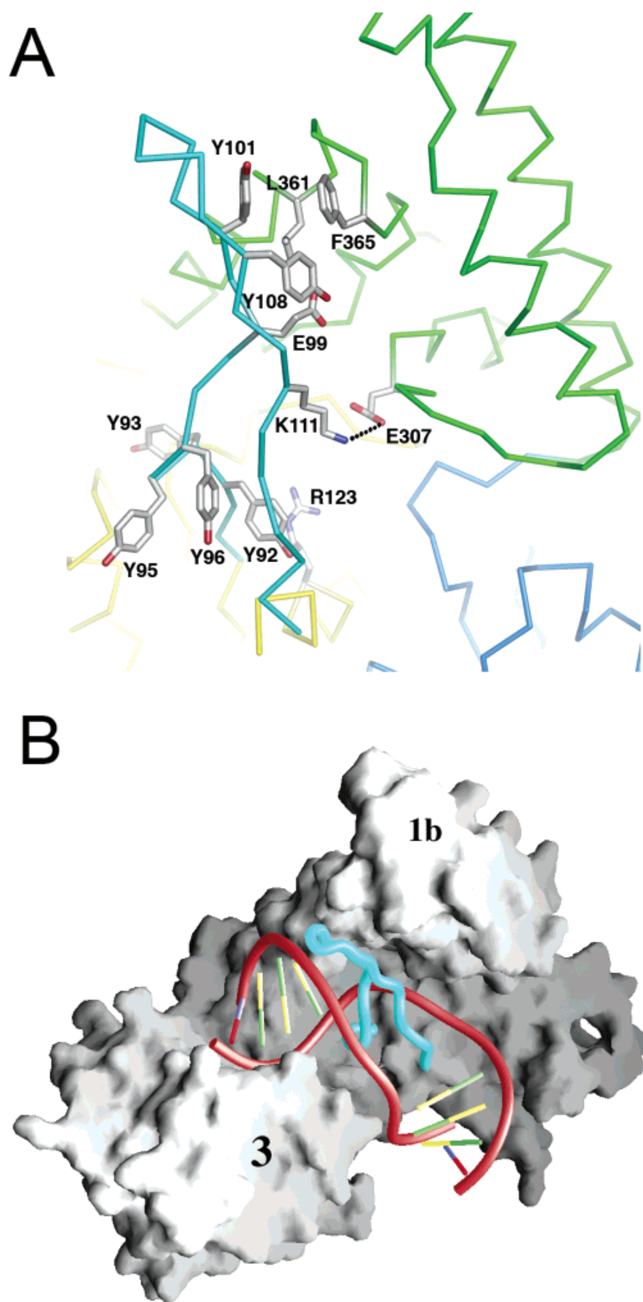

Figure 5. β-hairpin region of *B. caldotenax* UvrB and the UvrB:DNA preincision complex model. (A) The Cα-trace of UvrB is shown and color-coded as in Figures 1 and 3 with the β-hairpin in cyan, domain 1b in green, domain 1a in yellow, and domain 2 in blue. Selected residues are drawn in all-bonds representation. The salt bridge between Lys 111 and Glu 307 is indicated by a black dotted line. (B) Hypothetical model of the UvrB:DNA preincision complex. A surface representation of UvrB is shown with the domain orientation based on the superposition with the NS3–DNA complex. Domains 1b and 3 are indicated. The DNA is shown with its phosphate backbone in red and the undisrupted base pairs as spokes. The β-hairpin of UvrB is shown in cyan inserting between the two strands of the double helix.

formed should be dependent on the length of the nondamaged strand, since the damage will effectively be further from the end. The length of the damaged strand should be irrelevant. This is precisely what Moolenaar et al. observed.[98] If the damaged strand is truncated and the nondamaged strand is not truncated 5′ to the damage, formation of the preincision complex is reduced. In contrast, if the nondamaged strand is truncated and the damaged strand is not, the preincision complex is formed, similar to the case for the substrate in which both strands are 5′ truncated. Similar UvrB loading experiments in the absence of UvrA were performed using a DNA substrate with both strands truncated on the 3′ side, and no preincision complex was observed. If UvrB is in fact sliding along the nondamaged strand, these results suggest that UvrB has a preference for 3′ to 5′ movement because the 3′ truncated substrate would require UvrB to move in the 5′ to 3′ direction along the nondamaged strand. Intriguingly, NS3 helicase is a 3′–5′ acting helicase,[111,112] as are the other two helicases that are structurally similar to UvrB, Rep[113,114] and PcrA.[115]

To test the validity of the *Padlock Model*, the tip of the β-hairpin (Gln 97 to Asp 112) from *B. caldotenax* UvrB was removed.[88] The $\Delta\beta$h UvrB variant was unable to form a stable preincision complex and was inactive in UvrABC-mediated incision, but it was still able to interact with UvrA and form the UvrAB:DNA complex in competition with wild-type UvrB. The $\Delta\beta$h UvrB variant does not destabilize a damaged duplex. Surprisingly, the ATPase activity of the $\Delta\beta$h mutant in the presence of UvrA and UV irradiated DNA was elevated 7-fold in comparison to wild-type UvrB while the GTPase activity of UvrA decreased. These results are attributed to the fact that the UvrA dimer can recruit the $\Delta\beta$h UvrB mutant to the damaged site, but the mutant is unable to verify the damage and continuously hydrolyzes ATP while unsuccessfully trying to engage the lesion. The model was further strengthened when residues Tyr 101 and Phe 108, located at the tip of the hairpin in *E. coli* UvrB (Figure 5A), were mutated to alanine to test if they were in fact involved in DNA strand separation.[106] The Y101A/F108A mutant was unable to bind or promote incision of a 50-bp DNA substrate with a cholesterol adduct located in the center of the oligonucleotide. It was concluded that the Y101A/F108A UvrB double mutant is a separation of function mutant that possesses ATPase activity but lacks the strand separating activity.

The padlock model predicts that the highly conserved aromatic residues (Tyr 92, Tyr 93, Tyr 95, and Tyr 96) at the base of the hairpin interact with the DNA (Figure 5). It has been suggested that hydrophobic and/or base stacking interactions with aromatic amino acid side chains play a role in damage recognition by the UvrAB complex,[17,90,116] making these residues prime candidates for the damage verification center of UvrB. Thus, residues Tyr 92 and Tyr 93 were mutated to alanine, which led to an UvrB variant that is lethal to the cell and can only be expressed in an *E. coli* strain that lacks the uvrA gene. *In vitro* experiments showed that this variant still forms preincision complexes on damaged DNA. However, it was observed that this mutant also formed preincision complexes with nondamaged DNA. On the basis of this observation, Moolenaar et al. suggested that these two tyrosines are important for damage recognition and prevent UvrB from binding to nondamaged DNA.[106] This mutant was further characterized using dsDNA with a single nucleotide gap and a similar substrate with a single-stranded nick. The nucleotide gap substrate is an optimal substrate for damage-independent incision by the wild-type UvrBC nuclease. The single-stranded nick is also incised, but to a much lesser extent. Incision of these substrates takes place seven nucleotides from the gap/nick, which is analogous to the 5′ incision event on damaged DNA. Therefore, it is assumed that the gap is positioned at the same location where the lesion would normally be. The nicked substrate mimics

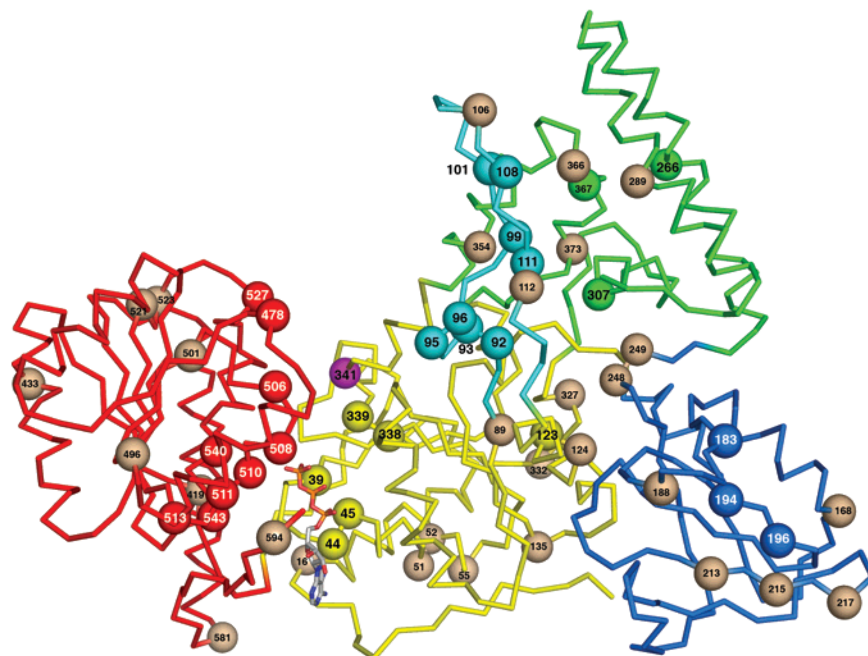

Figure 6. Structural representation of mutations created in UvrB. All of the mutations made in UvrB have been overlaid onto the crystal structure of *B. caldotenax* (PDB 1T5L[65]). The structure is color-coded by domains as in Figures 1 and 3. H341 is colored magenta because of conflicting results regarding the activity of this mutant. Mutations leading to wild-type phenotypes and/or less than 20% reduction in incision activity are shown as beige spheres. Mutations leading to more than 20% reduction in incision activity as compared to wild-type UvrB are colored according to their domain. All of the mutations are labeled with their corresponding residue number.

the lesion with the nucleotide at the nick. Apparently, this nucleotide at the lesion site inhibits UvrBC incision in the context of wild-type UvrB. However, the nucleotide appears to have no effect on incision when the Y92A/Y93A variant is used, suggesting that the nucleotide that occupies the lesion site sterically clashes with Tyr 92 and/or Tyr 93 and supports their role to prevent binding of UvrB to nondamaged DNA. Wild-type UvrB can still promote incision to a small extent on the nicked substrate because the nick in the substrate may allow the nucleotide at the lesion site to move out of the way of Tyr 92 and/or Tyr 93. However, with intact dsDNA, such flexibility would not exist and the presence of the nucleotide and the tyrosines at the lesion site would be mutually exclusive, resulting in no preincision complex formation. The question then arises: how is the preincision complex formed in the presence of a lesion? Moolenaar et al. propose that the damaged nucleotide is flipped out of the DNA helix in the preincision complex and Tyr 92 and/or Tyr 93 occupy the vacated space.[106] It has been suggested that the only common denominator among all types of damage recognized by NER is an alteration in stacking interactions.[17] The lesion-induced alterations in base stacking could facilitate base-flipping. Thus, damage recognition would be driven by the ease of extracting the damaged nucleotide.[106] In fact, extrusion of a nucleotide is a common feature in many DNA repair systems.[117−119] A number of structures have been solved including uracil−DNA glycosylase and 8-oxoguanine glycosylase, which reveal the damaged nucleotide to be turned out from the DNA helix and inserted into the active site pocket of the protein. Amino acid side chains insert into the DNA helix and take the place of the expelled nucleotide, stabilizing the nucleotide-flipped configuration. This type of mechanism, however, is not expected for UvrB since NER can recognize damages that vary greatly in composition and size: some of which can be extremely bulky (Table 1). An active site pocket that could accommodate all these different lesions is unlikely. The notion of flipping the nucleotide out of the double helix and away from UvrB instead of flipping it into an active site pocket is further supported by the observation that UvrB and photolyase can simultaneously bind to a pyrimidine dimer.[120] The structure of photolyase predicts that the cyclobutane pyrimidine dimer will flip out of the DNA helix and into a cavity formed by the protein that contains the cofactor. If UvrB expels the pyrimidine dimer outward, the photoproduct would be presented to photolyase and both could bind to the lesion.

To evaluate the relative contributions of Tyr 92 and Tyr 93 to the reaction mechanism, each residue has been mutated individually to alanine.[84] The results indicate that the Y93A variant is functionally more impaired than the Y92A mutant. In addition, Zou et al. reported that mutation of Tyr 92 to the more hydrophobic tryptophan resulted in an increase in incision efficiency of a protein−DNA cross-linked substrate (DPC), as compared to wild-type UvrB, but a decrease in efficiency when using a 50-bp DNA substrate with a centrally located *cis*-BPDE.[36] The relative importance of Tyr 92 and Tyr 93 may depend on the type of lesion being repaired.

To analyze Tyr 95 and Tyr 96, two additional aromatic amino acids in the β-hairpin motif (Figure 5A), the Y95A/Y96A double mutant was made. This mutant is unable to form the UvrB:DNA preincision complex with damaged DNA, and no incision is observed in the complete UvrABC reaction.[106] The mutant also lacks strand destabilization activity and is not able to form the preincision complex in the absence of UvrA when using a 31-bp dsDNA substrate containing a cholesterol adduct 8 bp from the 5′ end of the DNA. This substrate is similar to the 5′ truncated substrate mentioned above and does not require UvrA to form the wild-type UvrB:DNA preincision complex. However, the Y95A/Y96A mutant was still able to promote damage-independent incision of the gapped and nicked substrates. Moolenaar et al. suggest that these results indicate a direct role for Tyr 95 and/or Tyr 96 in damage-specific binding,

perhaps by stabilizing the extruded lesion, since the system only appears to function in the absence of damage. Tyr 96 has also been individually mutated to an alanine to determine the importance of this residue alone.[84] It was found that the mutant was unable to form the UvrB:DNA preincision complex, it had an extremely limited capacity to destabilize dsDNA, and there was almost no incision observed when this mutant was used in a complete UvrABC incision assay.[84] Likewise, Tyr 95 of UvrB was also further analyzed, but instead of mutating it to alanine, it was mutated to a tryptophan.[36] The Y95W mutant was shown to bind to DNA adducts in a bubble structure in the absence of UvrA with a dissociation constant of less than 100 nM, a much tighter binding than observed for wild-type UvrB. In contrast to the Y95A/Y96A mutant, Y95W is able to function in UvrABC incision reactions and forms a preincision complex that is more stable than the wild-type UvrB:DNA complex. Zou et al. attribute this effect to the increased hydrophobicity of a tryptophan compared to a tyrosine. Substitution of Tyr 95 by tryptophan could enhance the hydrophobic interactions to a lesion, resulting in the lowered dissociation constant compared to wild-type UvrB. The Y95W mutant is also able to bind to bubble substrates without damage, suggesting that it interacts with natural bases as well, and may intercalate between the adduct and a neighboring unmodified base in the preincision complex. In support of this conclusion, fluorescence quenching has suggested a direct involvement of Tyr 95 in DNA interactions and indicates binding to be driven by nonelectrostatic forces.[36] Thus, the current model predicts that Tyr 95 interacts with a damaged nucleotide that has been turned away from UvrB while Tyr 92 and Tyr 93 assume the place of the everted base, thereby stabilizing the conformation of the DNA. The position of Tyr 96 relative to Tyr 95 makes it unlikely that Tyr 96 also interacts with the damaged nucleotide. Tyr 96 may instead stack with the base adjacent to the damage, further stabilizing the conformation of the DNA.

In addition to the aromatic residues, there are two charged residues in the vicinity of the β-hairpin that are essential for binding of damaged DNA: Arg 123 and Glu 99 (Figure 5A). Each of these residues has been mutated to alanine.[84] Incision was severely compromised in the presence of both the R123A and E99A mutants, as was the ATPase activity. No preincision complex was observed, and the mutants were unable to destabilize dsDNA. Arg 123 is located just below the β-hairpin (Figure 5A) and is thought to provide ionic interactions with the phosphates of the nondamaged strand. Glu 99 is located in the center of the β-hairpin (Figure 5A) and is predicted to be involved in contacts that promote the interaction between the β-hairpin and domain 1b.

The role of UvrB's ATPase activity in the UvrB:DNA preincision complex has been analyzed in detail. Using special DNA substrates, it is possible to form the UvrB:DNA preincision complex in the absence of UvrA and ATP.[98] Addition of ATPγS is not sufficient to allow 3′ incision by UvrC, and ATP is necessary for the first incision reaction to proceed. These results demonstrate that one round of ATP hydrolysis is necessary prior to incision. If a typical UvrA/ATP-dependent preincision complex is formed, and the ATP is removed from the preincision complex prior to adding UvrC, incision still does not occur, but UvrC can bind to the preincision complex.[19] In this case, addition of ATP or ATPγS restores both 3′ and 5′ incision. However, the addition of ADP does not restore 3′ incision capability unless a 3′ nicked substrate is used, which mimics a completed 3′ incision reaction, in which case ADP restores 5′ incision.[21,102] These results demonstrate that, after ATP hydrolysis occurred, an additional ATP molecule must bind to UvrB, leading to a conformational change that allows 3′ incision by UvrC.[21] This conformational change is supported by DNA footprinting experiments where the binding of ATP or ATPγS to the preincision complex results in a DNase I-hypersensitive site not observed in the ADP bound form. This hypersensitive site indicates an alteration in the minor groove width, providing further evidence that the DNA has undergone a conformational change. These data suggest that two conformational changes within the UvrB:DNA preincision complex are required to form a preincision complex that presents the DNA to UvrC in such a way that 3′ incision can occur. The first conformational change is caused by the hydrolysis of ATP, thereby forming the pro-preincision complex. This complex has been shown to be unstable, and the DNA 3′ to the lesion is in a strained conformation.[98] The second conformational change, induced by the binding of ATP, leads to the stable preincision complex.

The UvrB:DNA preincision complex has been visualized using both electron microscopy and atomic force microscopy. The results indicate that the DNA in the preincision complex is bent by ~127° [121] and wrapped around UvrB.[122] The atomic force microscopy experiments suggest that approximately seven helical turns of DNA are wrapped around UvrB.[122] In a random site-directed mutagenesis study, Lin et al. identified a UvrB mutant, D487A, that produced a less pronounced DNase I hypersensitive site.[22] This mutant was subsequently evaluated by electron microscopy, and it was shown that it failed to support DNA bending.[123] The hypersensitive site could be due to alterations in the 5′ region of the DNA flanking the UvrB contact sites either through strand opening at the site of the adduct or by bending and wrapping of the DNA. This agrees with the fact that DNase I binds in the minor groove, and the dimensions of this groove dictate DNase I binding efficiency and the extent of incision.[124]

Several studies indicate that the DNA in the preincision complex is not extensively unwound.[102,125,126] In one study, DNA substrates were designed with a modified base as part of a bubble of mismatched bases. If the bubble contained three to five mismatches, 3′ and 5′ damage specific incision by UvrBC was observed independent of UvrA. When the bubble was increased to eight mismatches, a significant reduction in UvrA-independent incision was observed, probably due to inhibition of the 3′ incision, and further increases in the size of the bubble lead to uncoupled 5′ incision in the absence of 3′ incision.[126]

It was shown that UvrA loads UvrB onto damaged DNA catalytically and not stoichiometrically[11,127] and that the presence of excess amounts of UvrA inhibits the incision reaction.[11,128−131] This is due to UvrA's ability to reassociate with the UvrB:DNA complex and drive the reaction backward to the $UvrA_2B$:DNA complex.[109,129] Since UvrA dissociation is believed to be required for UvrC binding, an UvrAB complex would preclude UvrC binding to the UvrB:DNA complex and effectively inhibit the incision reaction. Under physiological conditions, the concentration of UvrB far exceeds that of UvrA. Therefore, although conditions where UvrA exceeds UvrB can be created *in vitro*, they are probably not relevant *in vivo*. Zou et al. also showed that *E.coli* UvrA is extremely sensitive to thermal inactivation

and the addition of chaperone proteins not only protected UvrA from heat lability but also allowed up to 10 cycles of UvrB loading per one dimer of UvrA.[127]

5. UvrC

UvrC has not been as extensively studied as UvrA and UvrB. This may be due in part to difficulties in protein purification, which can lead to a loss of the protein's native activity.[132] Until recently it was not clear whether UvrC carried out both incision reactions or if UvrB and UvrC were responsible for the 3′ incision reaction. However, mutagenesis studies and sequence alignments have shown that UvrC catalyzes both the 3′ and 5′ incisions and each of these incision reactions is performed by a distinct catalytic site, which can be inactivated independently.[9,20,22,23]

5.1. The UvrB–UvrC Interaction

The C-terminal domain of UvrB, domain 4, consists of approximately 60 moderately conserved residues separated from domain 3 by a highly variable stretch of 24 to 72 residues depending on the organism. This domain has been shown to interact with UvrC,[90] and deletion of this domain abolishes UvrABC-mediated incision. Site-directed mutagenesis has been used to characterize a number of residues in domain 4 (Table 2). The most severely affected mutant, F652L, resulted in a UvrB deficient in promoting incision (Table 2 and Figure 4).[22] The interaction between domain 4 and UvrC has been shown to be necessary for 3′ incision but is not required for 5′ incision.[92,93] No structural information on the linker region or on the position of domain 4 relative to the remainder of UvrB exists. The structure of the very C-terminal 55 residues of UvrB has been determined. The domain adopts a helix−loop−helix conformation stabilized by hydrophobic interactions and salt bridges between the helices (Figure 4). In the crystal, two molecules interact in a head-to-head arrangement with each other through hydrophobic and ionic interactions using residues in the loop region (Figure 4). There is a region in UvrC that shares sequence homology to domain 4 of UvrB and is predicted to have a similar fold (Figure 4). Because of this similarity, this region is speculated to be the UvrB interacting region of UvrC. A model for this interaction based on the head-to-head interaction seen between two UvrB domain 4 molecules in the crystal structure has been proposed where Phe 652 of UvrB interacts directly with Phe 223 of UvrC (Figure 4).[74] Mutation of either of these phenylalanines to leucine has been shown to disrupt the interaction between UvrB and UvrC.[93] In addition, other residues at the interface between the two molecules are also conserved. However, there is no direct evidence for such an interaction, and removal of this domain in *B. caldotenax* UvrC did not result in inhibition of incision (M. Skorvaga and Van Houten, unpublished results).

5.2. The N-Terminal Half of UvrC

The domain responsible for the 3′ incision resides at the N-terminus of the UvrC protein and constitutes approximately the first hundred residues (Figure 2). The crystal structures of the N-terminal endonuclease domain of UvrC from both *T. maritima* and *B. caldotenax* have been solved (Figure 7A).[133] This domain shares structural and sequence similarity to the catalytic domain found in I-TevI, a GIY−YIG homing endonuclease (PDB ID 1LN0 and 1MK0).[134−136] The domain constitutes an $\alpha\beta\beta\alpha\alpha\beta\alpha$ topology. The core is composed of a three-stranded β-sheet flanked by the first three α-helices on one side and helix 4 on the other (Figure 7A). There are at least 60 known members of the GIY−YIG superfamily including UvrC. These family members are present in bacteriophage T4, bacteria, archae, algal chloroplasts and mitochondria, and fungal mitochondria. Members of this family are characterized by a conserved GIY−(X_9−X_{11})−YIG motif within the catalytic module. This module is the only entity in common among all superfamily members. All known GIY−YIG endonuclease domains contain four invariant residues: Gly 31, Arg 39, Glu 76, and Asn 88. In addition to these four residues, this domain in UvrC contains three additional strictly conserved residues Tyr 19, Tyr 29, and Lys 32; Tyr 19 and 29 are highly conserved in all GIY−YIG family members. These seven residues and a few additional residues including Tyr 43 form a highly conserved patch on the surface of this domain (Figure 7A). The active site of the N-terminal endonuclease of UvrC resides within this patch.

A common feature of nucleases is the use of one, two, or even three divalent cations in the active site to lower the free energy of activation and stabilize the negative charge of the pentacovalent phosphoanion transition state formed during phosphodiester bond cleavage.[137] The metal can also be used to lower the pK_a of coordinating water molecules, resulting in either a hydroxide that can act as a nucleophile or as a general base, abstracting a proton from the nucleophilic water, or a metal-bound water that can act as a general acid and protonate the 3′ OH leaving group. The N-terminal endonuclease domain of UvrC contains a single divalent cation in its active site bound by Glu 76 and coordinated to five additional water molecules in an octahedral arrangement (Figure 7A and B). The position of the metal explains the strict conservation of Gly 31, which is positioned behind the metal. Any other residue with a side chain at this position would impede metal binding (Figure 7A).

Mutants of the highly conserved active site residues in the N-terminal domain of UvrC were generated, and their ability to perform 3′ incision was analyzed.[133] Mutants Y29A, Y29F, R39A, E76A, and N88A were unable to mediate 3′ incision. The activity of K32A was also reduced, but not to the same extent as seen for the other mutants. On the basis of the structural and mutational results, a reaction mechanism was proposed where the pK_a of Tyr 29 would be lowered due to its close proximity to the bound divalent cation. This would allow it to act as a general base, stripping a proton from a nucleophilic water while donating its own proton to a metal bound hydroxide (Figure 7C). The metal would act as a Lewis acid, stabilizing the transition state. A water coordinated to the metal would fulfill the role of the general acid and donate its proton to the 3′ OH leaving group of the phosphate. The invariant Arg 39 may be required to stabilize the negative charge of the product, much like the strictly conserved arginine in the active site of the homing endonuclease I−PpoI.[138] It is currently hypothesized that Lys 32 might have a similar, although not as critical, role as Arg 39 since the activity of K32A only decreased by ∼25−30%.

The potential role of the remaining two active site tyrosines (Tyr 19 and Tyr 43) remains to be explored. They are not positioned as close to the metal as Tyr 29 and would not be expected to have a reduced pK_a. The side chains of these two residues form a hydrogen bond to each other, leading to the speculation that these two tyrosines might only be

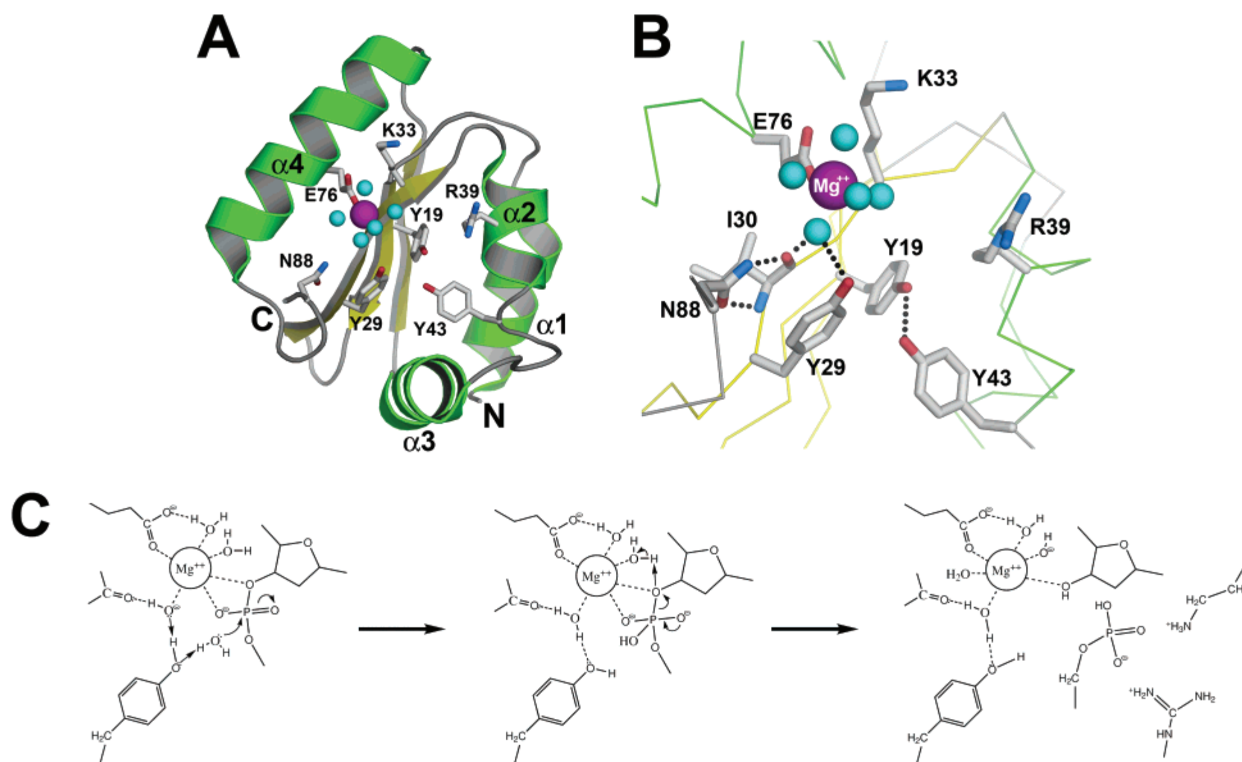

Figure 7. The N-terminal endonuclease domain of UvrC and its proposed reaction mechanism. (A) The overall structure of the GIY−YIG N-terminal endonuclease domain from *T. maritima* UvrC. The central β-sheet is shown in yellow, and the surrounding α-helices are colored green. Selected highly conserved residues are shown in all-bonds representation and labeled. The Mg^{2+} ion is shown in magenta, and the surrounding water molecules are colored cyan. The N- and C-termini are labeled along with the α-helices. (B) Close up view of the active site. The color coding is similar to that for part A. Hydrogen bonds are drawn as black dotted lines. Residues in close proximity to the magnesium are shown in all-bonds representation and labeled. (C) Proposed reaction mechanism for the 3′ phosphodiester bond cleavage by UvrC. The role of the Lewis acid is fulfilled by the divalent cation. One of the coordinating water molecules assumes the role of the general acid. Tyr 29 is the general base, accepting a proton from a nucleophilic water while concurrently transferring its proton to a metal bound hydroxide. Arg 32 and Lys 32 are responsible for stabilizing the negative charge of the product.

important for structural integrity of the domain (Figure 7B). However, the crystal structures of Y19F and Y43F have been solved for the isolated domain, and only very little structural variance was observed between the structures of the two mutants and the wild-type structure. The involvement of these two tyrosines in catalysis has not been ruled out. The role of Asn 88 appears to be structural, since its side chain forms two hydrogen bonds to the backbone of Ile 30, which in turn forms a hydrogen bond with Tyr 29, and Tyr 29 forms a hydrogen bond to one of the metal-bound waters (Figure 7B).

Adjacent to the N-terminal endonuclease domain is a cysteine rich region (Figure 2) containing four highly conserved cysteine residues with the consensus sequence $CX_{6-14}CX_7CX_3C$. The importance of these cysteines is not yet known.

5.3. The C-Terminal Half of UvrC

The C-terminal half of UvrC contains an endonuclease domain and a helix−hairpin−helix DNA binding domain (Figure 2). The endonuclease domain shares sequence homology with no other known protein. Mutagenesis studies have been carried out on the C-terminal endonuclease domain of *E. coli* UvrC to identify the residues involved in catalysis.[23] Lin et al. mutated a subset of Glu, Asp, and His residues and analyzed these mutants on the basis of UV resistance *in vivo* and incision activity *in vitro*. Four mutants were identified that conferred extreme UV sensitivity and showed defective 5′ incision activity: H538F, D399A, D438A, and D466A. It was initially thought that His 538 would act as the general base and strip a proton from a nucleophilic water molecule. However, when His 538 was mutated to either Asn or Asp, the protein remained active.[23] In fact, UvrC molecules have now been sequenced that have an Asp at the position of His 538 in *E. coli* UvrC. The three aspartates are more critical since attempts to conservatively mutate any of these to asparagines is as detrimental as mutating them to alanines, both of which result in no detectable 5′ incision.[23] It was thus concluded that the C-terminal endonuclease domain of UvrC is similar to nucleases such as RNase H and to Klenow 3′−5′ exonuclease, where Asp and Glu amino acids have prominent roles in catalysis, and is less akin to nucleases such as DNase I and RNase A, which rely on a histidine. It is likely that at least one of these aspartates is involved in coordinating one or more divalent metal ion(s).

The structure of the C-terminal helix−hairpin−helix (HhH) domain of *E. coli* UvrC has been solved by heteronuclear NMR (Figure 8).[139] The domain consists of two helix−hairpin−helix motifs that are homologous to the C-terminal domain of ERCC1, which in combination with XPF is accountable for 5′ incision in human NER. Several DNA repair proteins encode sequences that are predicted to form HhH motifs and mediate nonspecific DNA interactions.[134,140,141] The domain shows preferential binding to single-stranded−double-stranded DNA junctions with a strong preference toward DNA containing a loop or "bubble" of at least six unpaired bases.[139] It binds only weakly to

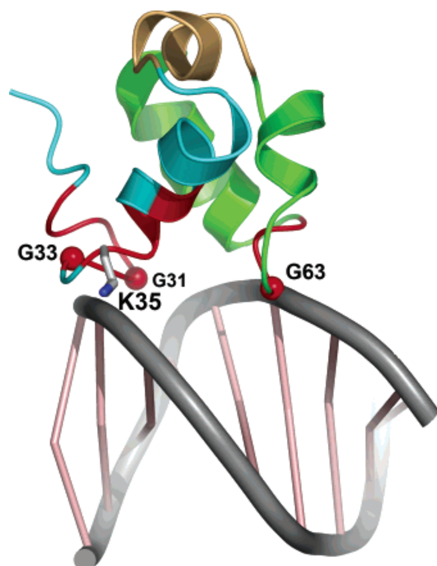

Figure 8. Hypothetical model of the helx−hairpin−helix (HhH) domain of UvrC bound to DNA. The image shows the two helix−hairpin−helix motifs (cyan and green) and the linker (gold) that joins them. The regions predicted by NMR to interact with DNA are shown in red. Selected glycines predicted that are important for the protein−DNA interactions are indicated with red spheres and labeled. The side chain of Lys 35, also predicted to take part in the protein−DNA interactions, is shown in all-bonds representation. The DNA's phosphate backbone is shown in gray with its bases as spokes in wheat.

ssDNA or dsDNA. Bubble substrates may be closer in structure to what the HhH domain of UvrC encounters *in vivo* since it was hypothesized that UvrB forms a preincision complex, which requires opening of about six base pairs. UvrC will interact with this complex to perform the incision reactions. It has been shown that the HhH domain is required for 3′ and/or 5′ incision depending on the sequence context of the lesion.[39] Verhoeven et al. identified three classes of DNA substrates: substrates where the HhH domain is required for both the 3′ and 5′ incision, substrates where it is predominantly required for 5′ incision with a minor role in 3′ incision, and substrates where it is required only for 3′ incision.[39] In addition, deletion of this domain results in a UvrC variant that is unable to bind ssDNA, suggesting that this domain is directly involved in DNA binding.[33] NMR chemical shift mapping of the C-terminal HhH domain of *E. coli* UvrC predicts a similar interaction to DNA as observed for the recombination protein RuvA and DNA ligase I,[139] both of which have structurally very similar helix−hairpin−helix domains.[142−146] The regions of proposed interaction are the hairpin loops from each of the two HhH motifs as well as positively charged residues from the second helix of the first helix−hairpin−helix motif. The residues with the largest chemical shifts were two conserved glycines, Gly 31 and Gly 33, in the first hairpin and Lys 35 at the N-terminus of helix 2 in the first HhH motif. A model for the interaction of the HhH domain with DNA is proposed on the basis of the RuvA:DNA complex (Figure 8).

5.4. The Oligomeric State of UvrC

The oligomeric state of UvrC is unclear. UvrC from *E. coli* has been reported to exist as both a monomer (UvrCI) and a tetramer (UvrCII) both *in vitro* and *in vivo*.[147,148] UvrC from *B. caldotenax* has also been observed as a monomer and tetramer in solution (J. J. Truglio and C. Kisker, unpublished result). The two forms differ in DNA binding properties and incision modes of certain types of DNA lesions. Nazimiec et al. used a DNA substrate containing a CC-1065-N3-adenine adduct within a very specific sequence (GATTA*CG) to show that monomeric UvrC can make both the 3′ and 5′ incisions in the presence of UvrA and UvrB, but the tetrameric form will only perform the 5′ incision.[147] However, tetrameric UvrC fails to incise the adduct formed at DNA sequences other than (GATTA*CG). It was also shown that the UvrC tetramer bound specifically to the drug−adduct site (GATTA*CG) in the absence of UvrA and UvrB while the monomer did not and that this binding was more favorable than binding by the UvrAB complex. The authors hypothesize that *in vivo* an alternative mechanism may exist where the tetrameric form of UvrC has a higher affinity than UvrA for damage in specific sequences or for certain types of damage resulting only in a 5′ incision. Interestingly, a UvrC homologue, Cho, is homologous to the N-terminal half of UvrC including the cysteine rich region and extends for approximately 100 more residues with little similarity to UvrC. Cho can only perform the 3′ incision reaction,[149,150] which would complement the activity of the tetrameric form of UvrC. Like UvrC, Cho interacts with UvrB, although sequence alignments do not identify the presence of UvrC's putative UvrB interacting domain. This suggests that the conserved cysteine region of UvrC and Cho might be the region responsible for interaction with UvrB. The incision made by Cho is four nucleotides further away from the lesion than the incision made by UvrC.[149] Unlike the case for UvrC, the expression of Cho is inducible by the SOS system.[151] It was thus suggested that Cho functions as a backup nuclease for NER acting on very bulky substrates including bulky DNA cross-links that block the 3′ incision site of UvrC. However, only a small number of bacterial species including *E. coli* encode genes for both UvrC and Cho.

6. UvrABC in the Context of the Cell

Much has been learned about the UvrABC reaction mechanism through biochemical and structural studies with the purified proteins. However, it is important that this information will be analyzed in the biological context of the entire cell.[30] After all, the UvrABC system does not work in isolation *in vivo* but as part of a complex network that responds to stress placed on the cell. The location of UvrA has been visualized in *Bacillus subtilis* using an UvrA−GFP fusion protein.[152] It was observed that UvrA is localized to the chromosome before and after DNA damage. *E. coli* UvrA is also localized to the chromosome before UV irradiation. However, upon radiation, 40% of UvrA shifts toward the inner membrane, joining a group of 15 other proteins including UvrC, three subunits of RNA polymerase, topoisomerase I, and DNA gyrase.[153] The reason for this discrepancy between *B. subtilis* and *E. coli* is not understood and implies that the two bacteria have evolved differently in their approach to repairing damaged DNA.

7. Beyond Repair

Finally, UvrA and UvrB might have other roles in DNA transactions, beyond DNA repair within the cell. The primary role of DNA polymerase I (polI) during replication is processing of the lagging strand by using its 5′ to 3′ exonuclease activity to remove the RNA primer and using

its polymerase activity to resynthesize the DNA. Nevertheless, *E. coli* cells without the *polA* gene (lacking DNA polymerase I) still survive when grown in synthetic media,[154] which implies that other enzymes are substituting for the exonuclease and polymerase activities of polI. It has been shown that UvrA and UvrB, but not UvrC, are essential for this alternative replication system and both ATP sites of UvrA and the ATP site of UvrB must be active.[154] It has been suggested that UvrA and UvrB would bind to the RNA−DNA hybrids of the Okazaki fragments similarly to the way these proteins bind to DNA damage. Notably, the N-terminal zinc finger of UvrA, which was shown not to be critical for DNA repair,[43] is vital in DNA replication.[154]

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