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Nucleotide Excision Repair in *Escherichia coli*

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BACKGROUND AND PERSPECTIVE

The genetic success of an organism is dependent upon the faithful replication and maintenance of its genetic material. Many physical and chemical agents in our environment pose a threat to this genetic continuity. Organisms have evolved a diverse array of enzymatic pathways for the removal of DNA damage. The bacterium *Escherichia coli* has served as a model organism for the investigation of many of these DNA repair pathways. One of the best-characterized and most widely studied DNA repair pathways in *E. coli*, as in most organisms, is nucleotide excision repair. This pathway consists of five basic steps: damage recognition, incision, excision, repair synthesis, and ligation (for a comprehensive review of this topic, see reference 60). These steps work together as follows. During damage recognition, one or several proteins bind to the damage-induced distortion. This preincision complex serves as a binding site for an endonuclease, which incises the DNA near the site of the altered nucleotide(s). The damaged nucleotide and several surrounding nucleotides are removed, and the incision complex is released. The gap created during excision is filled by the action of DNA polymerase. In the final step, ligation, DNA ligase seals the newly completed repair patch.

In the past few years, modern molecular techniques have allowed significant progress in our understanding of the proteins that mediate the individual steps in *E. coli* nucleotide excision repair. The first three steps of this process are carried out by three proteins encoded by the *uvrA*, *uvrB*, and *uvrC* genes. The UvrA, UvrB, and UvrC proteins act in a

series of steps to first recognize and bind to the damaged site and then hydrolyze two phosphodiester bonds, one 7 nucleotides 5′ and the other 3 or 4 nucleotides 3′ of the modified nucleotide (177, 278).

The enzymatic activity of these three proteins has been designated in several ways in the literature. Howard-Flanders et al. first called the proposed activity of the three *uvrA*, *uvrB*, and the *uvrC* genes the UvrABC excision endonuclease (85). During the initial characterization of these proteins, this name was shortened to UvrABC endonuclease (22, 189–193). Later, this activity was called ABC excision nuclease (ABC excinuclease), to underscore the novel property of producing two incisions which help mediate the excision of a short oligonucleotide containing the damaged nucleotide (177). In addition, the "Uvr" was dropped from the name to emphasize the property of acting on a plethora of DNA lesions in addition to UV-induced photoproducts (177). For the purposes of this review, the individual protein subunits will be designated as the UvrA, UvrB, and UvrC proteins and the concerted endonuclease activity of all three proteins will be referred to as the UvrABC nuclease complex. Each of these subunits performs partial reactions leading to the dual-incision event, but the actual excision of the damaged nucleotide and the capacity of the enzyme to undergo several catalytic cycles occurs only with the addition of other protein factors. Therefore, nucleotide excision repair should be viewed as a complex series of reactions, mediated by several proteins, each subsequent phase is dependent upon the previous step.

The enzyme activity of the UvrABC complex displays a remarkable substrate diversity and is capable of acting on a wide variety of DNA damage. It has been proposed that this broad specificity of the UvrABC complex is due to its ability to recognize damage-induced conformational changes in the DNA and not the chemically modified bases per se (73, 174, 177, 251, 270).

The goal of this review is to evaluate *E. coli* nucleotide excision repair by surveying the published literature through October 1989. The review first examines the individual protein components of the repair pathway and then details each of the five steps outlined above. The last section addresses some of the more important questions that remain to be solved. The review will focus on (i) the regulation and structure of the *uvrA*, *uvrB*, and *uvrC* genes; (ii) the properties of the Uvr subunits; (iii) the common structural elements of DNA damage that are repaired via the nucleotide excision repair pathway; (iv) the nature of the protein-DNA interactions involved in damage recognition; (v) the molecular interactions of the Uvr proteins and DNA polymerase I and helicase II, during incision and excision of the damaged strand; and (vi) the nature of nucleotide excision repair as it proceeds within the cell. This review will mention the following topics only briefly as they apply to nucleotide excision repair: mismatch repair, the SOS response, the repair of simple alkylation damage, and photoreactivation. Over the years, nucleotide excision repair has been examined to varying degrees in several reviews (61, 72, 73, 76, 120, 135, 136, 147, 163, 171, 179, 264, 265, 270). DNA repair was the topic of a recent UCLA Symposium on Cellular and Molecular Biology, the proceedings of which have been published (62).

GENETICS OF *E. COLI* NUCLEOTIDE EXCISION REPAIR

In the late 1950s, during experiments aimed at isolating strains of *E. coli* that would be more resistant to the killing effects of UV irradiation, Hill discovered mutants of *E. coli* B strains that instead were more sensitive (82). Later, in 1962, Howard-Flanders and Theriot were able to isolate mutants of *E. coli* K-12 with mutations at three genetically distinct loci, *uvrA*, *uvrB*, and *uvrC*, which displayed the phenotype of being more sensitive to the killing effects of ultraviolet light irradiation (86). A few years later, nucleotide excision repair was discovered in bacteria, and it was found that both of the radiosensitive mutants B_{s-1} and K-12 *uvrA* were defective in dimer excision (16, 201, 202). Howard-Flanders et al. went on to isolate 23 different strains with mutations that mapped to one of these three loci (85). No evidence was seen for any sequential action of the three gene products, and double mutants were not appreciably more sensitive than the single mutants. This led the authors to suggest that the *uvr* genes encoded a multiprotein "excision endonuclease" (85). Interestingly, certain *uvr* mutants displayed intermediate phenotypes in survival, dimer excision, or host cell activation of UV-irradiated bacteriophages. Van de Putte et al. isolated several more repair-deficient mutants of *E. coli* B, K-12_s, and CR_{34} and mapped the mutations to the *uvrA*, *uvrB*, and *uvrC* (247) genes. Unfortunately, little in the way of molecular characterization of these mutants has been done, and to date, only the *uvrA6* (19) and the *uvrB5* (4) mutants have been characterized at the molecular level. The sequences of these two mutant alleles revealed nonsense mutations which lead to the synthesis of a truncated protein.

More extensive mutant hunts identified several other genes that appeared to be involved either directly in nucleotide excision repair, *uvrD* (*uvrE*), *polA*, and *recA*, or that affected subsequent responses to UV light (17, 110, 141, 162, 213, 220, 226, 257; see reference 76 for a review). Furthermore, several other genetic loci have been shown to cause an altered sensitivity to the killing effects of UV light. This topic will be dealt with again in the final section of this review.

Promoter Structure of the *uvrA*, *uvrB*, and *uvrC* Genes

The *uvr* genes were molecularly cloned and sequenced by several groups, and this information led to a greater understanding of the roles of the three proteins in the process of damage recognition and incision (2, 4, 92, 165, 166, 176–178, 183, 234, 278, 279). A discussion of the protein motifs which have been found in each of the Uvr subunits follows a description of the organization of each of the genes.

SOS response. During the early 1970s, it was found that certain types of DNA damage could induce a cascade of events in *E. coli* termed the SOS response. These cellular responses included increased mutability, enhanced resistance to the killing effects of genotoxic agents, and cellular filamentation (for an extensive review, see reference 264). Damage-inducible genes were found to be under the control of the LexA repressor, which is regulated by the RecA protein. The SOS response appears to be induced by an alteration in DNA synthesis, either directly by DNA damage blocking to the replication fork or indirectly by antibiotics, such as novobiocin, that inhibit DNA synthesis. Under these conditions the RecA protein becomes activated (in a way that is not clearly understood) and helps facilitate autocleavage of the LexA protein. The cleaved LexA protein can no longer act as a repressor, allowing expression of specific genes.

***uvrA* gene structure.** The *uvrA* gene maps to position 92 on the 100-min *E. coli* map, near the gene for single-strand binding protein. Analysis of the 5′ region indicates that the gene has one promoter, with a characteristic TATA box.

TABLE 1. LexA-binding sites in selected repair genes[a]

Gene	Sequence	ln K_d
uvrA	ACTGTATATTCATTGAG	17.2
uvrB P2	ACTGTTTTTTTATCCAG	17.7
uvrD	TCTGTATATATACCCAG	ND[b]
recA	ACTGTATGAGCATACAG	20.0
LexA1	GCTGTATATACTCACAC	17.7
LexA2	ACTGTATATACACCCAG	17.7
uvrC	TCTGAACGTGAATTGCAG	NB[c]
RecQ	CCTGTTTTTATTT-CAG	ND
Consensus	ACTGTAYAYAYAYACAG T T T T T	

[a] Adapted from references 10 and 182.
[b] ND, Not determined.
[c] NB, no binding has been observed in vitro.

Kacinski et al. (99) and Kenyon and Walker (106) established that the *uvrA* gene is damage inducible. The *uvrA* gene is expressed at low constitutive levels, and the amount of UvrA protein has been shown to increase from about 20 to about 200 copies per cell following SOS induction. Analysis of the sequence revealed an SOS box between positions −35 and −65 (Table 1). DNase I footprinting studies have revealed that this site is bound by LexA protein in vitro (13, 180).

***uvrB* gene structure.** The *uvrB* gene maps to position 17, near the *gal* operon. Cloning and sequencing of the *uvrB* gene revealed a complex promoter structure. Transcription of the *uvrB* gene appears to be controlled by both SOS-dependent and SOS-independent promotors, leading to the relatively high constitutive expression of UvrB (approximately 200 copies per cell) which is 7- to 10-fold higher than UvrA and 15- to 20-fold higher than UvrC (55, 178, 182).

In 1981, Van den Berg et al. established by cloning and deletion studies that the *uvrB* gene contains tandem promoters P1 and P2 (244). Tandem promoters have been noted in several genes, including the *gal* and *lac* operons (128). One specific construct carried in plasmid pNP19, lacking the −35 region of P2 (244), caused a significant decrease in UvrB protein synthesis as monitored by the maxicell method (99). Inactivation of UvrC in the host was found to increase the expression of UvrB from plasmid pNP19. This observation led the authors to suggest that the UvrC protein might be involved in the expression of the *uvrB* gene (244).

Later in 1982, Sancar et al. reported the sequence of a 540-base-pair (bp) fragment from the 5′ region of the *uvrB* gene and established the existence of an additional promoter, P3, which is 320 bp upstream from P2 (182). Analysis of transcripts produced in vitro from this cloned fragment demonstrated that transcription from P2 is inhibited by binding of LexA, whereas P1 transcription is not inhibited in vitro, but may be inhibited by LexA binding in vivo (see below). Transcription from P3 terminates within P2 in vitro; it is not known whether this promotor functions in vivo.

In vivo analysis of the expression of the UvrB gene was performed by Van den Berg et al. (246) by using S1 nuclease mapping (208) of RNA derived from pNP12 plasmid carrying the *uvrB* gene. They found two transcripts consistent with expression from P1 and P2. Expression from P3 was not observed. The P1 transcript was expressed at 10- to 20-fold higher levels than the P2 transcript. This ratio was also seen when transcripts from the genome were analyzed. UV irradiation induced the expression from both promoters, with P1 remaining the stronger promoter. Using gene fusion studies, these researchers also examined β-galactosidase expression. UV induction of β-galactosidase activity was found to parallel the levels of RNAs.

Van den Berg et al. also found that deletion of P2 increased the expression from P1 two- to threefold (in the absence of UV induction). This is in contrast to their previous study of UvrB protein synthesis (244). These observations led them to suggest that expression from both promoters is regulated by LexA binding. Since this was in disagreement with the in vitro data (244), the authors suggested that the topological state of the DNA might affect regulation by altering the binding affinity of LexA. Alterations in supercoiling have been shown to affect the expression of several genes, presumably by altering the binding of regulatory proteins or RNA polymerase itself (128).

During the course of these studies, it was found that vectors carrying the region containing P3 could be propagated only in rich media or in strains with a *uvrB* deletion (172, 182, 245). Growth in minimal media led to two types of mutations that stabilized growth propagation of the plasmid: plasmid mutations or host mutations (245). Host mutants were not characterized. Plasmid mutations included specific alterations in a stem-loop structure at −35 or two insertions −10 and −20 disrupting P3. It was suggested that transcription from P3 leads to plasmid loss and that the stem-loop structure acts as a regulatory site for this promoter. Transcription from this site was never observed in vivo, probably because it leads to loss of plasmid. It is unfortunate that transcription of P3 in the host mutants was not examined.

The P3 (GTATCCACAG) stem-loop structure has strong homology to DnaA-binding sites in *oriC*, *dnaA*, *polA*, and pBR322 origin (245). Plasmids containing P3 could be maintained in a *dnaA*(Ts) mutant. The authors speculate that the DnaA protein might couple the expression of UvrB protein to replication. The *dnaA* protein functions as an initiator protein for *E. coli* DNA replication of the replication origin (*oriC*) by melting an A+T-rich region and facilitates the binding of *dnaB* and *dnaC* (18). It is important to point out that *polA uvrB* double mutants are inviable (133).

What function might the UvrB protein serve? As discussed below, the UvrB protein does not bind directly to DNA; rather, it forms a complex with the UvrA subunit that facilitates UvrB binding (96, 149). Other proteins that are involved in DNA replication may also facilitate UvrB binding to other DNA structures in an analogous manner.

Analysis of the 3′ region of the *uvrB* gene indicates the presence of two imperfect palindromic repetitive elements (REP sequences) (2, 4). REP sequences represent about 1% of the entire *E. coli* genome and have been found both 5′ and 3′ to many genes (5, 223). Various functions have been proposed for these elements, although no clear biological role has been established. It has been suggested that genes containing these elements 5′ to their transcription start site are down regulated, whereas genes which have these elements 3′ to the transcription start site may show higher mRNA stability (5). Other evidence indicates that these elements may be important for genome organization (223). It is interesting that both *uvrD* (which encodes helicase II) and *polA* (which encodes polymerase I) have REP sequences (4). It has recently been shown that REP sequences are binding sites for DNA gyrase, which suggests that these sequences may be involved in higher-order structure of the *E. coli* chromosome (274).

***uvrC* gene structure.** The *uvrC* gene (map position 41.5) is expressed at very low levels, approximately 10 copies per cell (282, 283). Using various assays, several groups have identified four potential promoters in the 5′ region of the

uvrC gene (132, 183, 205–207), at approximately 2.3, 1.0, 0.4 and 0.1 kilobases (kb) from the putative translation start site, P2.3, P1.0, P0.4, and P0.1, respectively. (This somewhat awkward terminology is an attempt to avoid confusion, as each of these promoters has been identified differently in the literature.) Van Sluis et al., using S1 nuclease mapping, suggested that transcription was initiated at P0.4 (258). However, Sancar et al., using BAL 31 deletion studies, showed that complete complementation of a *uvrC* mutation could be achieved by a plasmid carrying the most proximal promoter, P0.1 (183). RNA polymerase-binding studies identified an additional promoter, P1.0, which lies about 1 kb upstream from the 5′ end of the structural gene. Interestingly, complementation was lost when both P0.4 and P0.1 promoters were carried on a plasmid. Fusion of P1.0 to the *uvrC* structural sequences resulted in normal complementation (206).

Northern (RNA) analysis of the *uvrC* gene revealed that two primary transcriptional products of 2.8 and 1.6 kb are produced in vivo (258). It appears that transcription of the *uvrC* gene occurs predominately from P1.0. Analysis of promoter strength by fusing various combinations of all three promoters to the chloramphenicol acetyltransferase reporter gene, CAT (69), indicated that a complex regulatory circuit exists for the expression of the *uvrC* gene (258). Sharma et al. reported that a plasmid carrying P0.4 and P0.1 failed to express or complement the *uvrC* gene, whereas direct fusion of P1.0 with the *uvrC* structural region resulted in full complementation, as did a plasmid pUV7 carrying all three promoters (206). RNA polymerase binds tightly to P1.0 and weakly to P0.4 and P0.1. No synthesis of UvrC was detected by maxicells with the plasmid pUV7, although insertion of IS*1* downstream from the P1.0 promoter resulted in detectable levels of the UvrC protein. The authors suggested that a sequence downstream from P1.0 which is disrupted by IS*1* is a negative control element and that insertion of the IS elements leads to activation of either P2 and/or P3 (206).

The 5′ region flanking the *uvrC* gene contains two open reading frames, and maxicell analysis has shown the production of 28- and 24-kilodalton (kDa) proteins in addition to the UvrC protein (206). Further sequence analysis and survival data revealed a fourth potential promoter site, which is located 2.3 kb from the translational start point of the *uvrC* structural gene. The role of this promoter in the expression of the *uvrC* gene is not known. Sequences 5′ to P1 have been shown to increase the expression of the *uvrC* gene. Since transcripts from P2.3 were not found, it was suggested that the 28-kdal protein might play some role in expression of the *uvrC* gene, although this has not been shown conclusively (207).

Conflicting data have been presented about whether the *uvrC* gene is under LexA-RecA control (56, 258). Van Sluis et al. (258) reported that the *uvrC* gene is induced in vivo, and they also identified a putative SOS box. It was subsequently shown by DNase I footprinting (70) that this site did not bind LexA in vitro. If this sequence is a LexA-binding site, it is the only site which separates the palindrome CTG...CAG by 11 bases instead of the normal 10. It should be pointed out, however, that the *recQ* gene contains an SOS box which has a 9-bp interval between the CTG...CAG palindrome (10). Using fusions of the cloned UvrC promoter region to the CAT reporter gene, Foster and Strike failed to show any induction following UV irradiation or mitomycin C treatment (56). More complete analyses of the sequence of the *uvrC* gene indicates three putative LexA-binding sites (SOS boxes), but none of these bound LexA in vitro (56; D. Owen, unpublished data). It appears that the *uvrC* gene is regulated so as to maintain expression at very low levels.

PROTEIN PRODUCTS OF THE *uvr* GENES

Prior to molecular cloning and overexpression of the gene products, Seeberg et al. undertook the herculean effort of partially purifying the individual subunits by complementation assays with cell extracts from various *uvr* mutants (189–196). To summarize some of their findings, (i) the Uvr subunits worked together to incise chemically modified or UV-damaged DNA in an ATP-dependent manner; (ii) the UvrA protein, although being a DNA-independent ATPase, bound to DNA in an ATP-stimulated manner; and (iii) the binding affinity for damaged DNA was greater than that for nondamaged DNA, and this specificity could be completely abolished by the nonhydrolyzable substrate adenosine 5′-(γ-thio)triphosphate (ATP-γ-S) (194). Subsequently, several groups cloned each of the *uvr* genes into vectors designed for overexpression, which was greatly facilitated the purification of large amounts of the Uvr subunits to apparent homogeneity (4, 165, 166, 177, 178, 181, 234, 278, 279). The individual properties of each of the subunits are discussed in the following section.

Properties of the UvrA Protein

Translation of the nucleotide sequence of the UvrA protein predicts a protein of 940 amino acids with a molecular weight of 103,874 (92). The predicted protein sequence revealed that the UvrA protein may be a composite protein containing several interesting structural motifs, including nucleotide-binding sites and zinc DNA-binding fingers (49). It has been suggested that this protein could have arisen by gene fusion and subsequent gene duplication events (49).

Nucleotide-binding sites. One property of the UvrA protein which was elaborated early in the study of the UvrA protein is a DNA-independent ATPase activity (194). Analysis of the UvrA amino acid sequence revealed a Walker type A consensus sequence for three potential nucleotide-binding sites, with the second site being partially defective (Table 2) (see reference 92 for additional references). Comparison of the amino acid sequences around these sites with sequences of other ATPases revealed an extensive homology with bacterial ATP-dependent transport proteins, which are located in the cellular membrane (49). The significance of this homology, as well as the intracellular location of the UvrA protein, is not known.

ATPase activity. The ATPase activity of the UvrA protein has been examined in detail by several groups (33, 142, 194, 233). In the absence of DNA, the apparent K_m for ATP has been reported to be 150 to 200 μM and the reported turnover number varied from 16 to as many as 125 molecules of ATP per UvrA (as monomer) per min. Both ADP and ATP-γ-S are competitive inhibitors, with K_is of 21 and 53 μM, respectively (194).

The UvrA protein is a DNA-independent ATPase, although its kinetic parameters are influenced by the addition of DNA and the UvrB protein. UvrA also hydrolyzes GTP, with a K_i in the presence of ATP of 200 μM; the UvrA ATPase is also inhibited by GTP-γ-S (33, 142).

Addition of the UvrB subunit to the UvrA subunit resulted in significant changes in the ATPase activity of the complex (142) (see the section below on UvrA and UvrB subunit interactions). Oh et al. have performed kinetic analyses on

TABLE 2. Comparison of Walker A-type domains in several DNA-metabolizing proteins from *E. coli* and *S. cerevisiae*[a]

Protein	Residues	Amino acid sequence	Function
E. coli			
UvrA			
Site I	24–45	DKLIV V T G LS G S GKS SLAFDT L	Repair
Site II	633–654	GLFTC I T G VS G S GKS TLINDT L	Repair
UvrB	32–53	LAHQT L L G VT G S GKT FTIANV I	Repair
UvrD	22–43	RSNLL V L A GA G S GKT RVLVHR I	Repair, transcription(?)
RecA	59–80	GRIVR I Y G PE S S GKT TLTLQV I	Recombination
RecB	16–37	QGERL I E A SA G T GKT FTIAAL Y	Recombination
RecD	164–185	RRISV I S G GP G T GKT TTVAKL L	Recombination
RecN	22–43	SGMTV I T G ET G A GKS IAISAL G	Recombination
DnaA	165–186	YNPLF L Y G GT G L GKT HLLHAV	Replication
DnaB	223–244	SDLII V A A RP S M GKT TFAMNL V	Replication
S. cerevisiae			
RAD3	35–56	GGNSI L E M PS G T GKT VSLLSL T	Repair, replication
RAD18	353–373	GGISK L M I MK S N GKS SSYRKL L	Repair
PIF	251–272	GHNIF Y T G SA G T GKS ILLREM I	Mitochondrial recombination

[a] Adapted from reference 7. Additional references: RAD3, Sung et al. (225); RAD18, Jones et al. (95); PIF, Foury and Lahaye (57).

the UvrA ATPase activity and found that addition of DNA increases the binding affinity for ATP, but decreases the turnover number (142). This decrease in K_m is dependent on the state of the DNA. Double-stranded DNA (67 μM) had the least effect, single-stranded DNA (5 μM) had the greatest, and UV-irradiated DNA was intermediate (31 μM). These modulatory effects of double-stranded DNA on nucleotide binding were also observed by changes in the K_i for ADP, which increased 8-fold, whereas the K_i for ATP-γ-S decreased some 70-fold (0.7 μM) from the previously reported 50 μM. DNA seems to increase the binding affinity for ATP and decrease the binding affinity for the hydrolyzed substrate, ADP. The binding affinity for ATP is influenced by the number of single-strand regions (due to heat denaturation or by DNA damage). It is also important to note that Lineweaver-Burke plots of the ATPase activity in the presence of ADP are not linear, which suggest cooperativity between the two ATP-binding sites in the UvrA protein (142).

In an attempt to define the role of the nucleotide-binding sites in the action mechanism of the UvrABC complex, Brandsma et al. were able to alter the structure of the UvrA protein near both the presumed nucleotide-binding sites by linker insertion mutagenesis (20). They concluded that both ATP-binding sites needed to be intact for complete biological complementation. Site-directed mutagenesis studies of the Walker consensus sequences are currently in progress and may help to elucidate the role of these motifs in the action mechanism of the UvrABC complex.

DNA-binding activity of the UvrA protein. Seeberg and Steinum, using filter-binding studies, showed that purified UvrA protein binds to UV-irradiated DNA more efficiently than to nondamaged DNA and that this interaction is stimulated by the addition of ATP. ADP was found to decrease the binding affinity for either DNA substrate, whereas the addition of the nonhydrolyzable substrate analog ATP-γ-S stimulated binding to DNA but completely abolished all specificity (194, 195). A more thorough description of the DNA-binding activity of the UvrA protein is given in the section describing the formation of the preincision complex.

Zinc finger DNA-binding motif. Zinc fingers were first discovered in the transcription factor, TFIIIA, which stimulates RNA polymerase III transcription from the 5S ribosomal RNA gene in oocytes of *Xenopus laevis* (130). This protein contains nine tandem repeats consisting of X-X-C-$(X)_4$-C-$(X)_{12-18}$-H-$(X)_4$-H-X-X. Subsequent studies have shown that each finger of TFIIIA probably makes contact with the major groove of the recognition sequence (54). Each of the nine fingers in TFIIIA have conserved amino acids at specific sites including basic and hydrophobic residues. It is believed that these amino acid side chains help confer the sequence specificity. Many transcriptional-regulatory proteins that bind to specific sequences contain the zinc finger DNA-binding motif (8, 9). Analysis of the predicted amino acid sequence of the UvrA protein indicated two sets of four cysteines with spacing similar to the cysteines and histidines in TFIIIA (49) (Table 3; Fig. 1). Sancar and co-workers, using a colorimetric assay and inductively coupled plasma analysis, have shown that highly purified preparations of UvrA contain 1.6 zinc atoms per molecule (136, 139). Extended X-ray absorption fine structure analysis was used to show that these zinc atoms were chelated to four cysteine residues (139). Interestingly, substitution of one cysteine residue by either histidine, alanine, or serine by site-directed mutagenesis did not reduce the UV survival of strains carrying the mutations by more than 80%. This observation suggests that the zinc finger motif of UvrA can be stabilized by coordination with just three amino acid residues. It is

TABLE 3. Selected proteins containing putative DNA-binding zinc fingers[a]

Protein class	Organism
Regulatory proteins	
TFIIIA	*Xenopus laevis*
ADR1	*S. cerevisiae*
GAL4	*S. cerevisiae*
Seredipity	*Drosophilia melanogaster*
Kruppel	*Drosophilia melanogaster*
c-*erb*-A	Humans
Glucocorticoid receptors	Humans
Viral proteins	
Large T antigen	Simian virus 40
Gene 32 protein	T4 phage
DNA-metabolizing proteins	
UvrA	*E. coli*
Topoisomerase I	*E. coli*
poly(ADP) ribose polymerase	Cattle
RAD18	*S. cerevisiae*

[a] Adapted from reference 8; see text for additional references. The DNA-binding domain is C/H-$(X)_{2-4}$C/H-$(X)_{5-20}$-C/H-$(X)_{2-4}$-C.

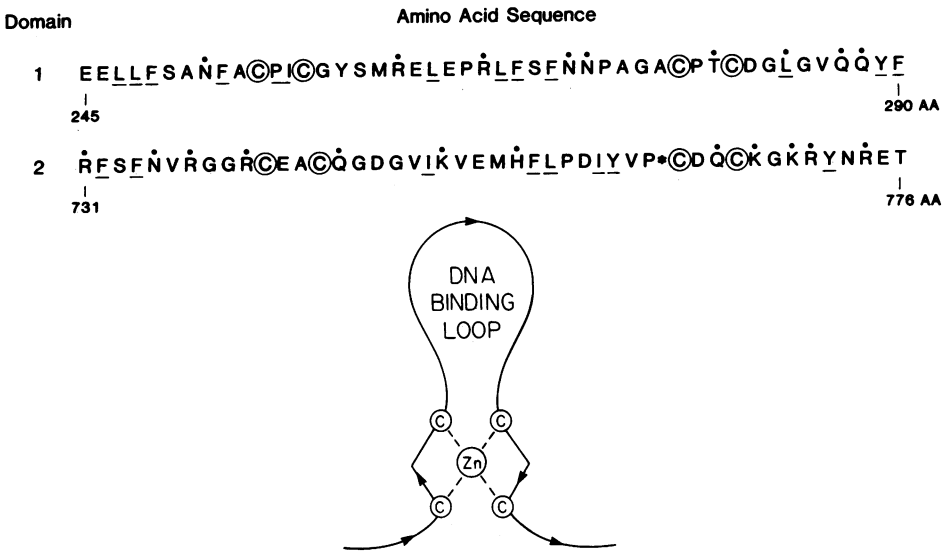

FIG. 1. DNA-binding zinc fingers of UvrA. (Top) The two protein domains which are believed to form zinc finger motifs are depicted. The four cysteines which have been shown to form a coordination complex with zinc are circled (139). Basic amino acids are dotted, and hydrophobic amino acids (AA) are underlined. (Bottom) The proposed structure for the UvrA zinc finger motif. However, the precise structure of the UvrA fingers is not known.

interesting that topoisomerase I (encoded by the *topA* gene) contains zinc and that the amino acid sequence reveals three potential zinc fingers (241). Topoisomerase does not display any sequence-specific DNA-binding activity. Quite recently, poly(ADP) ribose polymerase has been found to contain two large zinc fingers (126). This protein does not appear to have any sequence specificity, but is activated by DNA strand breaks. Analysis of the amino acid sequence of the yeast RAD18 protein has shown the presence of three putative zinc fingers. RAD18 belongs to the yeast *rad6* epistasis group involved in postreplication repair and mutagenesis. This protein also has been shown to contain a nucleotide-binding site (95).

Properties of the UvrB protein

Translation of the *uvrB* gene yields a protein of 672 amino acids with a molecular weight of 76,118 (2, 4). UvrB is hydrophobic, a property which is exploited for its purification. During isolation of the UvrB protein it was discovered that this protein is cleaved near the C terminus, yielding a 70-kDa protein, UvrB* (32, 181, 234). Comparison of the UvrB sequence with the Ada protein suggested conserved regions near the C-terminal end of the molecule, which is a potential cleavage site for the Ada protease:

UvrB: MSPKA|LQQ (626 to 633)
Ada 1: MTPKA|WQQ (156 to 163)
Ada 2: MTAKQ|FRH (122 to 129).

Data are from references 2 and 4.

Since cleavage of the UvrB protein results in complete loss of UvrABC nuclease activity, it has been suggested that proteolysis of the amounts of UvrB product is one way to down regulate the increase in the nucleotide repair proteins that results from the SOS response (32, 33). It has been shown that the UvrB* protein does form a complex with UvrA on DNA and that the formation of this complex is ATP dependent, although addition of the UvrC protein does not result in incision of the DNA. The protease appears to be under the control of the heat shock *htpR* gene, which encodes a sigma factor that guides RNA polymerase to specific promoters during the heat shock response.

Nucleotide-binding site. Analysis of the UvrB sequence revealed the presence of a Walker nucleotide-binding site (2, 4). Free in solution, the UvrB protein does not appear to have any ATPase activity. However, it appears that this cryptic ATP-binding site is unmasked, activating the ATPase activity during the proteolytic cleavage of the UvrB protein to the UvrB* or during its interaction with the UvrA protein (32, 33). It was found that the ATPase activity of UvrB* was dependent on single-stranded DNA but not on UvrA protein. Since UvrB* does not bind directly to DNA and the single-stranded DNA stimulatory effect could not be saturated even by very high DNA concentrations, Caron and Grossman suggested that the observed stimulation in the ATPase activity might be due to changes in ionic strength or water interactions (32, 33). In support of this idea, it was found that several chaotropic agents were stimulatory for the UvrB* ATPase activity (32, 33). The apparent role of the cryptic UvrB ATPase is discussed in more detail below.

The function of the ATP-binding site in the UvrB protein was investigated by using site-directed mutagenesis in the highly conserved GKT sequence (198) (Table 2). Seeley and Grossman found that changing lysine 45 to alanine (K45A) eliminated the increase in ATPase activity associated with the formation of the UvrAB complex and that this mutant UvrB protein was unable to support incision. Furthermore, this mutant gene could not confer UV resistance in a strain carrying a *uvrB* deletion (N364) (198).

Homologies with UvrC. Sequence analysis of the UvrB protein revealed two domains that share homology with the UvrC protein (Table 4) (4). The same study revealed that antibodies raised against the UvrB protein cross-reacted with the UvrC protein and, similarly, that antibodies raised against the UvrC protein reacted with the UvrB protein.

Homology with the AlkA protein. Backendorf et al. (4) reported that the UvrB protein contained a 19-residue

TABLE 4. UvrB domains which share homology with the UvrC protein[a]

Domain	Residues	Sequence
I		
UvrB	352–357	MYRGDRARLE
UvrC	326–331	LPRGDRARYL
II		
UvrB	648–661	HAQNLGFEEAAQINDQLH
UvrC	197–210	ASQNLGFEEAANINDQIQ

[a] Data from reference 4.

stretch which displayed a high degree of homology with a part of the AlkA protein (Table 5). Chou-Fasman predictions of secondary structure of this sequence in both proteins revealed a helix-turn-helix motif. This type of motif is often seen in proteins that bind to DNA (4). The UvrABC complex has a broad substrate specificity, and it is interesting that the AlkA protein (3-methyladenine glycosylase II) also acts on multiple substrates including 3-methyladenine. AlkA is inducible and is under the control of the Ada protein.

Properties of the UvrC protein

The translational start site of the UvrC protein has not been confirmed by N-terminal sequencing because it is apparently blocked (183). Sancar et al. have suggested that translation of UvrC begins at an ATG triplet, yielding a protein of molecular weight 66,038 (183). More recently, Moolenaar et al., using deletion analysis, have found that translation of the UvrC protein appears to be initiated at a GTG triplet 66 bp 5′ to the ATG mentioned above (132). Initiation of this site would lead to a protein 22 amino acids longer than that predicted by Sancar et al. (183), which is in closer agreement with the estimates of molecular weight by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. During the course of subcloning this gene, one particular construction, pDR3024, which truncates the protein by the loss of seven C-terminal amino acids, was unable to confer UV resistance in a host strain with a *uvrC* mutation (DR1984) (183). Inspection of the predicted amino acid sequence did not reveal any specific protein domains or motifs (132, 183), although, as noted above, two specific domains of the UvrC protein have homology with the UvrB protein. It has been estimated that there are approximately 10 copies of the UvrC protein in a RecA$^-$ cell.

The C-terminal domain of UvrC has also been shown to have a high degree of homology with the C-terminal domain of ERCC-1, the first human repair gene which has been cloned and sequenced (248, 249). The significance of this homology is not known (Table 6). It would be interesting to determine whether antibodies to the UvrC protein cross-react with the ERCC-1 protein. ERCC-1 has two other homologies with the known repair proteins RAD10 and UvrA. The first 200 amino acids of ERCC-1 share homology with RAD10, whereas the C-terminal domain shares homology with UvrC. These domains are separated by a short stretch of 42 amino acids which share some homology with the UvrA protein (residues 48 to 90) domain such that the entire ERCC-1 protein has significant homology with domains in each of these three proteins.

TABLE 5. Homologous domains of the UvrB and AlkA proteins[a]

Protein	Residues	Amino acid sequence[b]
UvrB	66–84	NLTLAAN-LYGQ-MKQFFPQN
AlkA	133–152	LTARVAN-LYGQ-RLDDFPQY

[a] Data from reference 4.
[b] The sequence is presented as helix-turn-helix.

Single-strand-binding activity. UvrC is a DNA-binding protein and binds to single-stranded DNA with relatively high affinity (175). This property has not been studied in any detail.

Interaction of the Uvr Subunits in Solution

Formation of UvrA dimer. Hydrodynamic studies indicated that the UvrA protein probably exists as a dimer in solution and that the distribution of monomers and dimers is affected by ATP, which shifts the equilibrium in favor of dimer formation. By velocity sedimentation techniques, it appears that UvrA exists in equilibrium between a monomer and a dimer population; the distribution between the two is greatly affected by the presence of ATP, ADP, or ATP-γ-S and the initial concentration of the UvrA protein (142, 148, 149). Addition of ATP, ATP-γ-S, or ADP greatly increased the protein recovery in these experiments. Gel filtration experiments indicated that the UvrA protein exists as a dimer with a Stokes radius of 5.92 nm and an apparent molecular weight of 210,000 in the absence and presence of ATP (149). Since neither of these two techniques is performed under conditions of equilibrium, it was important to examine the monomer-dimer distribution by equilibrium sedimentation analysis, a technique which should be affected only by the molecular weight and not by the hydrodynamic properties of the protein. When UvrA was sedimented in the absence of a nucleotide cofactor it appeared to act as a monomer, and in the presence of ATP it appeared to sediment with the molecular weight of a dimer (142). It should be pointed out that under both experimental conditions a plot of ln c/c_0 versus r^2 (an estimation of the molecular weight) was curvilinear, suggesting some heterogeneity in the sample.

Interactions of UvrA and UvrB in solution. The stoichiometry of the UvrAB complex both free in solution and on DNA was recently addressed (149). Gel filtration experiments indicated a Stokes radius of 6.01 ± 0.48 nm, which corresponds to a molecular weight of 201,400, a value inconsistent with any integral combination of the UvrA or UvrB proteins. Velocity sedimentation experiments indicated that the UvrA and UvrB subunits interact to form a complex in solution which is consistent with a UvrA-to-UvrB ratio of 2:1 ($UvrA_2B$).

Seeberg et al. found that during initial purification of the UvrB protein some UvrC was found to cochromatograph with UvrB on a DEAE column. UvrC did not bind to a DEAE column when purified from a *uvrB* mutant (189, 190, 195, 196). On the basis of this evidence, it had been suggested that these two proteins interact in solution. However, no evidence for the interaction of the UvrC protein with UvrB or the $UvrA_2B$ complex was detected by gel filtration or glycerol gradient velocity sedimentation (149).

The ATPase activity of the UvrAB complex has altered properties from those of UvrA alone (32, 33, 142). Formation of the UvrAB complex results in a DNA-stimulated ATPase (142). The nature of the DNA has a great effect on the kinetic constants, with UV-irradiated DNA leading to a sixfold decrease in K_m (142). It has been suggested that the altered ATPase activity of the UvrAB complex is due to activation of the cryptic ATPase of UvrB (33).

TABLE 6. Homology of UvrC with ERCC-1[a]

Protein	Residues	Amino acid sequence
UvrC	533–588	TSSLETIEGVGPKRRQMLLKYMGGLQGLRNASVEEIAKVPGISQGLAEKIFWSLKH
ERCC-1	236–291	TECLTTVKSVNKTDSQTLLTTFGSLEQLIAASREDLALCPGLGPQKARRLFDVLHE

[a] Adapted from references 248 and 249.

Properties of the UvrA, UvrB, and UvrC proteins are compared in Table 7.

FORMATION OF THE PREINCISION COMPLEX

Several methods have been used to examine the formation of the preincision complex. These include filter-binding assays, DNase I footprinting, DNA unwinding, and hydrodynamic assays. Taken together, these studies have helped elucidate the stoichiometry and formation mechanism of the preincision complex.

Binding of the Uvr Proteins to a Damaged Site

The binding of the UvrA protein to DNA has been examined by filter-binding assays in several laboratories. To summarize some of these findings, it was found that (i) binding of the UvrA protein to DNA was not strictly UV or ATP dependent (194, 277, 278); (ii) UvrA bound to DNA with an increased affinity which increased with UV fluence, and this specificity could be completely abolished by the nonhydrolyzable substrate ATP-γ-S (194, 277); (iii) addition of ADP seemed to inhibit, but not totally eliminate, both specific and nonspecific DNA binding (194, 278); (iv) The $t_{1/2}$ for the UvrA-DNA complex was less than 5 s (278); and (v) the addition of both the UvrA and UvrB proteins to DNA containing UV-induced photoproducts or psoralen adducts led to a stable protein-DNA complex which was resistant to salt and had a half-life of >55 min (276–278).

These results were later extended by Van Houten et al., using DNase I footprinting techniques (252–254). Using a 137-bp fragment carrying a psoralen-thymine monoadduct at a defined position, they showed that the UvrA protein bound to this substrate with an apparent binding affinity (K_a) of approximately 0.7×10^8 to 1.5×10^8 covering a 33-bp region surrounding the modified thymine (254). It was also found that ATP stimulated UvrA binding fivefold, but in agreement with filter-binding studies, ATP was not required for specific binding of the UvrA to a psoralen monoadduct (252, 253). In addition, ADP decreased the UvrA-binding affinity two- to threefold and ATP-γ-S completely inhibited specific binding while enhancing nonspecific binding (252).

TABLE 7. Properties of UvrA, UvrB, and UvrC

Property	Value in:		
	UvrA	UvrB	UvrC
Mol wt	103,874	76,118[a]	66,038[b]
No. of amino acids	940	672	588[b] (610)
No. of Trp residues per molecule	3	0	2
Molar extinction coefficient	46,680	27,699	36,200
pI	6.5	5.0	7.3
Intrinsic metal	2 Zn	None	None
DNA binding	Yes	No[c]	Yes
Nucleotide-binding site	2	1	
ATPase[d]	125	25	
SOS regulation	Yes	Yes	No (?)
No. of molecules/cell[e]	20 (200)	200 (1,000)	10

[a] Molecular weight as predicted by Arikan et al. (2). The N-terminal Met is removed in the mature protein.

[b] Molecular weight as predicted by Sancar et al. (183). The UvrC protein may actually be 22 amino acids longer (132).

[c] UvrB does bind to DNA in the presence of UvrA, but shows no affinity for DNA in the absence of UvrA (96).

[d] Turnover number for UvrA and UvrB* (the 70-kDa form), respectively, in the absence of other protein subunits or DNA (33, 142).

[e] The number in parentheses are those obtained after SOS induction.

The addition of UvrB to a reaction containing UvrA and DNA leads to several important changes in the nature of the protein-DNA complex that is formed (252, 254). DNAse I footprinting indicated the appearance of a DNase I-hypersensitive site at the 11th phosphodiester bond 5′ to a psoralen monoadduct, an increase in the binding affinity of about three- to fivefold, and, surprisingly, a decrease in the footprint to 19 bp. This increase in binding affinity is consistent with the increase in the stability of the Uvr-DNA complex as assayed by filter-binding techniques described above (194, 276, 278).

Stoichiometry of the preincision complex. Measurements of the rate and extent of UvrABC nuclease incision for randomly damaged substrates indicated that a 1:1:1 ratio of freshly purified Uvr subunits resulted in maximum incision efficiency (91, 278). Oh and Grossman reported that maximum unwinding activity was seen with a UvrA-to-UvrB ratio of 1:2 to 1:3, although lower ratios of UvrA concentrations were not assayed (143–145). Filter-binding studies seemed to indicate maximum binding activity at a 1:1 ratio of UvrA and UvrB (278). DNase I footprinting experiments which monitored the appearance of the UvrB-induced hypersensitive site indicated that a DNA-to-UvrB ratio of 1:1 results in maximum enhancement (254). Since the stoichiometry of the Uvr proteins in solution is $UvrA_2B$, it might be expected that this same stoichiometry would be found for Uvr complexes which are bound to DNA.

Orren and Sancar have recently reported on the stoichiometry of the Uvr complex that forms on the DNA when both the UvrA and UvrB subunits are added to UV-irradiated pBR322 (148, 149). Using gel filtration, they found that as the ratio of UvrA to UvrB was decreased in solution, the composition of the bound-Uvr complex changed dramatically. As expected, the amount of UvrA bound to UV-irradiated DNA decreased as the amount of UvrA that was in the reaction was decreased, but, unexpectedly, the amount of UvrB bound to the DNA increased as the amount of UvrA in the reaction was decreased. Eventually, at a UvrA-to-UvrB ratio of 1:10, maximal UvrB binding was observed, while little, if any, DNA-bound UvrA could be detected. They also showed that addition of UvrC to the preformed UvrB-DNA complex resulted in DNA incision in the complete absence of UvrA. These results seem to indicate that UvrA acts to promote the formation of the UvrB-DNA preincision complex at the site of the damage, but does not itself stay bound. No data were presented which examined the fate of the UvrA subunit following its release. This complex (which is presumably a UvrB-DNA complex) is quite stable. Challenge with UvrA or additional DNA could not cause dissociation (149).

Thus, it appears that the formation of the UvrB-DNA preincision complex occurs through a nonreversible process in which the UvrA protein acts to direct UvrB onto the DNA at the site of lesion, and the UvrA protein may cycle through several rounds of UvrB loading. This hypothesis must be examined in more detail. The lack of reversibility suggests that a significant alteration in the protein conformation and/or DNA conformation must occur during the formation of the UvrB-DNA complex. Evidence for a stable alteration in DNA conformation was indicated by the appearance of DNase I-hypersensitive site as listed above and by the opening of the DNA helix as described below.

Unwinding of the DNA Helix: Evidence for the Formation of a Stable Open Complex

It is known that protein binding to its cognate recognition sequence can induce significant alterations in the DNA conformation. Three well-characterized proteins are *Eco*RI endonuclease (127), CAP-binding protein (121), and RNA polymerase (128). RNA polymerase has been found to unwind the DNA helix by 540° or about 17 bp in the formation of the stable RNA polymerase promoter complex (65). *Eco*RI induced a series of changes including three site-specific neokinks and unwinding of 25° ± 2° (50, 127). The CAP-binding protein induces a site-specific bend of 90 to 180° (121) and unwinding of 29° (50). Several studies have examined whether the binding of UvrA or the UvrAB complex induces any changes in the DNA helix.

One common method for examining protein-induced unwinding is to mix the protein of interest with supercoiled DNA, which is then relaxed to completion with DNA topoisomerase. After all the proteins are removed by phenol extraction, the distribution of topoisomers is analyzed on agarose gels in the presence of cholorquine (65). If the protein of interest induces DNA unwinding, a new distribution of topoisomers is observed. Knowing the linking number of the topoisomers and the amount of protein binding, it is possible to determine the average unwinding per protein molecule. Strike and Rupp used this approach to examine the interaction of UvrA and UvrB with DNA (224). Unlike the positive control RNA polymerase, the UvrA or UvrAB complex was not found to induce any unwinding of either nondamaged or UV-irradiated pBR322 in the presence or absence of ATP or ATP-γ-S (224). It was not clear from this report whether the DNA had been electrophoresed in the presence of cholorquine or ethidium bromide, which cause relaxed DNA to migrate differently from nicked open-circular DNA. Therefore, under the conditions of electrophoresis, any nicking of the DNA by the Uvr proteins would lead to what would appear as complete relaxation and, therefore, no unwinding of the DNA helix. These results are in contrast to a similar series of experiments reported by Oh and Grossman (143). They found that the addition of UvrA to nonirradiated pBR322 in the presence of ATP-γ-S induced significant unwinding of the DNA, about 100° or about 3 bp per $UvrA_2$ complex. A small but detectable amount of UvrA unwinding was observed with ATP. It was also found that addition of UvrA (in the presence of ATP) to UV-irradiated DNA induced unwinding of the DNA helix which are proportional to the UV fluence. In a reaction mixture containing ATP-γ-S and nonirradiated pBR322, addition of UvrB to the UvrA complex induced significantly more unwinding, about 180 to 220°. Addition of increasing amounts of UvrB to the reaction mixture which contained UvrA, ATP, and UV-irradiated pBR322 resulted in a gradual increase in the helical unwinding. These data strongly suggest that the interaction of the UvrAB complex with damaged DNA (in the presence of ATP) induced a significant unwinding at the site of the damaged nucleotide.

Protein-induced unwinding of a DNA helix can occur by simple protein binding as discussed above or through an active process in which the protein actively unwinds the two strands and is designated as a helicase activity (125). Oh and Grossman have investigated the helicase activity of the UvrAB complex in two separate experiments (144, 145). The stoichiometry of the complex was not determined in these experiments, but on the basis of the work cited previously, the ratio of UvrA molecules to UvrB molecules is probably 1:2. The basis of these experiments is an investigation of the ability of the UvrAB complex to displace a labeled DNA strand from a duplex in a helicase assay. The unwinding activity of the UvrAB complex was found to displace a 22-mer relatively efficiently, a 55-mer rather poorly, and a 346-mer not at all (145). This is in contrast to the activity of several helicases, which can displace long stretches of duplex DNA (125). Two explanations can be given for this limited helicase activity. Binding of the UvrAB complex may induce localized unwinding of the DNA helix, which is capable of destabilizing a short duplex sufficiently to release a 22-mer. An alternative explanation is that the UvrAB complex is incapable of displacing longer fragments because the duplex reanneals behind the complex as it migrates through DNA. This limited helicase activity was found to proceed in the 5′-to-3′ direction (with respect to the displaced strand).

These data, taken together with the studies of Orren and Sancar, suggest a model for the formation of the preincision complex (149). The $UvrA_2B$ complex first melts into the DNA helix and travels a short distance (20 to 50 bp) in the 5′-to-3′ direction. If the $UvrA_2B$ complex encounters a damaged nucleotide, the limited helicase activity is inhibited and the UvrB complex is inserted into the helix at the site of the lesion, with the concomitant loss of the $UvrA_2$ dimer (Fig. 2). This limited helicase activity is analogous to the isomerization reaction observed during the interaction of RNA polymerase with a promoter (128). Unwinding of the DNA helix at the site of the modified base must be confirmed by biophysical studies such as hypochromicity (260) and chemical methods for single-stranded DNA such as alkylation of cytosine with dimethyl sulfate (35).

Role of nucleotide cofactor in the action mechanism of the UvrABC complex. ATP is absolutely required for incision, although the exact step at which it acts is not known. ATP causes an increase in the binding affinity of the UvrA protein to damaged DNA, presumably by promoting the formation of a dimer. Both GTP and ATP are hydrolyzed by the UvrA ATPase (33, 142). ATP is required for the formation of the $UvrA_2B$ complex. It is not known whether GTP can substitute for the formation of this complex, but the data of Grossman would suggest that it cannot (142). Formation of the $UvrA_2B$ trimer results in a decrease in UvrA ATPase activity and an activation of the UvrB ATPase which hydrolyzes only ATP. ATP is required for the unwinding activity of the UvrAB complex on DNA (33, 142).

ATP hydrolysis does not seem to be needed for the maintenance of this structure, because experiments reported by Caron and Grossman indicated that ATP is not required for incision once the preincision Uvr-DNA complex had been formed (31). (The makeup of the Uvr complexes was not reported in that study, but from the work of Orren and Sancar (149), the preincision complex which Caron and Grossman studied most probably contained only UvrB.) They showed that incision occurred following the addition of

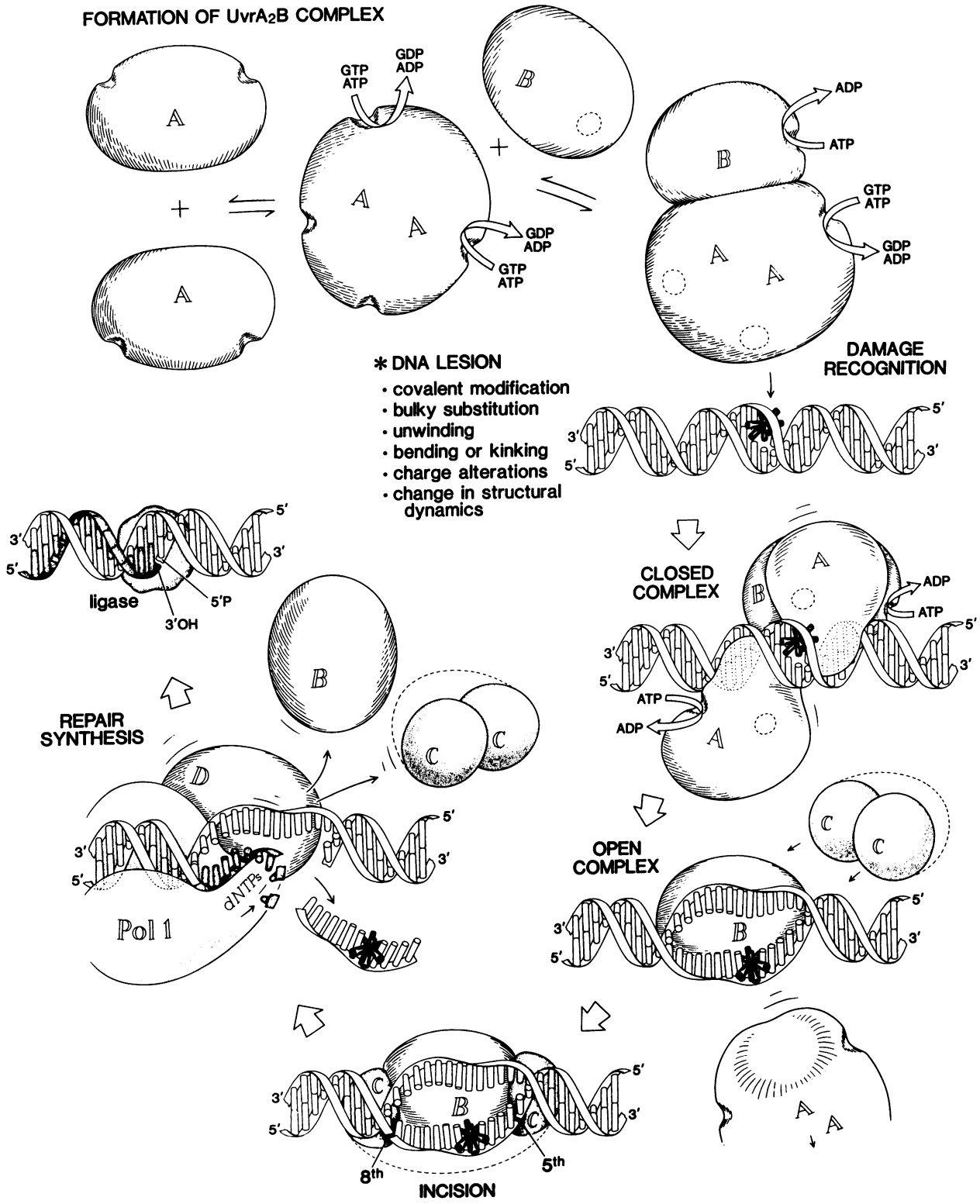

FIG. 2. *E. coli* nucleotide excision repair. A hypothetical scheme for the key steps in nucleotide excision repair is shown. Nucleotide excision repair proceeds in multiple steps (see the text for references and a more complete description). Solution studies suggest that two monomers of UvrA (A) form a dimer in an ATP-dependent manner. The UvrA dimer has four potential ATP-binding sites (shown as clefts), some or all of which may hydrolyze ATP and GTP (broad arrows). UvrB (B) interacts with this UvrA dimer, forming a protein complex with altered ATPase activity. It is believed that a cryptic ATP-binding site on UvrB (dotted elipse) becomes activated during the formation of the $UvrA_2B$ complex. During the damage recognition step, the UvrA dimer acts to target the UvrB subunit to the site of the damage (shown as a distorted base). Depicted in this model is the interaction of the UvrA dimer in the major groove both 5′ and 3′ to the modified nucleotide.

the UvrC subunit to the Uvr(A)B-DNA complex which had first formed in the presence of ATP and then purified away from ATP by gel filtration. The addition of UvrC in the presence of ATP-γ-S to the preformed Uvr(A)B-DNA complex actually resulted in higher frequency of incisions. This result suggests that ATP-γ-S actually stabilizes the Uvr(A)B-DNA complex once it had been formed in the presence of ATP.

Locating the Damage Site: Is There a Tracking Mechanism?

Nonspecific binding of a protein to DNA can either act to impede the binding to a specific site or can enhance binding to a specific site by facilitating the transfer of the protein from a nonspecific site to a specific site. This topic has been reviewed extensively (11, 12, 123, 260–262), the following serves as a short introduction.

Microscopic hopping. One property of DNA binding proteins is microscopic hopping. Once a protein binds nonspecifically, it will undergo a large number of microscopic dissociations and reassociations before macroscopic dissociation (to a position away from the DNA molecule) occurs. This microscopic hopping is strictly dependent on the linear nature of the DNA chain and occurs for all proteins that bind to DNA. Hopping is not a facilitating mechanism (123).

Intersystem transfer. Intersystem transfer occurs when a protein is nonspecifically bound to a site on DNA and is then transferred to another site on the DNA molecule by direct contact, and at no time is the protein free in solution (261, 262).

Facilitated linear diffusion. Some proteins, most notably the Lac repressor, have been found to bind to the recognition sequence at rates that are higher than those for three-dimensional diffusion. Although it is apparent that RNA polymerase can slide along DNA, it is not necessary to evoke sliding as a rate enhancement mechanism (128).

Does the UvrAB complex scan for DNA damage by tracking along the DNA helix? At present, no experimental evidence has been offered to support or refute a facilitated-diffusion process. Since the binding activities of Uvr proteins are not extremely high, it is not necessary to invoke such a process. No systematic study has been performed on the effects of additional nondamaged DNA on the specific DNA-binding or incision activity of the UvrABC complex. However, when the 137-bp psoralen monoadducted DNA fragment was used, it was observed that addition of relatively large amounts of nondamaged DNA (up to 2 μg of pBR322 in a 50-μl reaction mixture) to this labeled DNA fragment (1 to 5 ng) caused relatively little inhibition of specific binding as monitored by DNase I footprinting or incision (B. Van Houten and A. Sancar, unpublished observation). These experiments suggest that the UvrAB complex can find a damaged site even when there is a relatively large number of nonspecific binding sites in the reaction. It is tempting to speculate that the UvrA protein might be shuttled from one section of DNA to another via an intersystem transfer mechanism involving its two DNA-binding zinc fingers.

Model for the Formation of the Stable UvrB-DNA Preincision Complex

On the basis of all the current information regarding the interaction of the UvrA and UvrB proteins with DNA, the following is a model for the formation of the preincision complex (see also Fig. 2).

1. Solution interaction:

$$A + A \overset{ATP}{\rightleftharpoons} A2$$

$$A + B \overset{ATP}{\rightleftharpoons} A_2B$$

2. Formation of the preincision complex:
   a. Nonspecific binding

$$A_2B + DNA \underset{K_{ns}}{\rightleftharpoons} [A_2BDNA^c] \overset{ATP}{\underset{ADP}{\rightleftharpoons}} [A_2BDNA^o]$$

limited linear diffusion to a damaged site

   b. Specific binding

$$A_2B + S \underset{K_s}{\rightleftharpoons} [A_2BS^c] \overset{ATP}{\underset{kf}{\rightarrow}} [A_2BS^o] \rightarrow BS^o$$

DNA binding (c, closed; o, open) isomerization

The model may be explained as follows. (i) ATP is required for the formation of the $UvrA_2B$ complex. (ii) The $UvrA_2B$ complex can transiently bind to DNA nonspecifically (K_{ns}). In the presence of ATP hydrolysis, this interaction is short lived and, probably owing to the rapid association and dissociation, could not be detected by gel filtration. (iii) This nonspecific binding leads to a transient melting of DNA strands. This $UvrA_2B$ open complex has a limited helicase activity which moves 5′ to 3′, traveling approximately 20 to 50 bases before dissociating. (The number of bases is a rough estimate based on the ability of the $UvrA_2B$ complex to dissociate duplexes of various lengths.) (iv) If this UvrAB complex encounters a damage site, the helicase activity is inhibited and there is a concomitant protein-DNA induced allostery in the binding mode such that $UvrA_2$ is lost and the UvrB-DNA complex becomes quite stable, with a $t_{1/2}$ of 100 min. (v) A reinterpretation of the filter-binding experiments suggest that the long-lived salt-stable complex that forms on

This type of interaction, although consistent with all known data, has not been shown directly, and this model should be viewed as one of many possible types of interactions. The model does predict that the DNA lesion, although surrounded by the UvrAB complex, is open to solution and is free to interact with other repair proteins. From filter-binding and DNase I footprinting studies, it is known that the UvrA dimer can interact with DNA in a nonspecific manner and also form a complex with the damaged site in the absence of UvrB (not shown). The ATPase activity of the UvrAB complex is also known to be affected by the binding of DNA. Formation of the stable UvrB-DNA open preincision complex in which several base pairs are unwound may occur concomitantly with the release of the UvrA dimer. This open UvrB-DNA complex creates a suitable binding site for the UvrC subunit, forming an incision complex. The stoichiometry of the incision complex is not known, although it is believed that one UvrB protein interacts with each DNA adduct. Dual DNA incision usually occurs 7 nucleotides 5′ and four nucleotides 3′ to the modified nucleotide. This postincision complex does not appear to dissociate without the dual actions of DNA polymerase I (Pol 1) which, through repair synthesis, fills in the patch, and the UvrD protein, helicase II (D), which helps to dissociate the postincision complex and the damage-containing oligonucleotide from the parental DNA. Finally, in the ligation step, DNA ligase seals the nick to complete the repair patch. dNTPs, Deoxynucleoside triphosphates.

TABLE 8. UvrABC nuclease substrates[a]

Damaging agent	Adduct(s)	Reference(s)
N-Acetoxy-2-acetylaminofluorene	*C*-8-Guanine	14, 64, 174, 229
N-Hydroxy-aminofluorene	*C*-8-Guanine	154, 229
Doxorubicin and AD32	Intercalator	97
Alkoxyamine modified AP sites	AP analog	Kow et al., in press
Anthramycin	*N*-2-Guanine	267
Apurinic/apyrimidinic sites	Abasic sites	118; A. S. Snowden, Y. W. Kow, and B. Van Houten, submitted for publication
Benzo[*a*]pyrene diol epoxide	*N*-2-Guanine	197, 243, 255
N,*N*'-Bis(2-chloroethyl)-*N*-nitrosourea	Bifunctional alkylation	58, 98
Cyclohexylcarbodiimide	Unpaired T and G residues	233
CC-1065	*N*-3-Adenine	200, 228
Cisplatin and *trans*-platinum	*N*-7-Guanine	6, 88, 156
Ditercalanium	Noncovalent bisintercalator	113, 115
Mitomycin C	*N*-7-Guanine	58, 85, 156a
N'-Methyl-*N*-nitronitrosoguanidine	*O*-6-Methylguanine	170, 256, 259
Nitrogen mustard	Bifunctional alkylator	58
4-Nitroquinoline-1-oxide	*C*-8, *N*-2-Guanine *N*-6-Adenosine	93, 235
Reduced apurinic sites	Ring-opened AP	Kow et al., in press
Psoralen	*C*-5, *C*-6-Thymine	94, 174, 177, 250, 251, 276
Pyrimidine dimer	*C*-5, *C*-6-Pyrimidine	137, 177, 192, 278
6,4-Photoproduct	*C*-6, *C*-4-PyC	59, 137, 236
Thymine glycol	*C*-5, *C*-6-Thymine	118; Kow et al., in press

[a] DNA lesions which are not substrates for UvrABC nuclease: base mismatches (233), dihydrothymine, extrahelical bases, sequence-specific bends, urea *N*-glycoside-ureidoisobutyric acid (Kow et al., Mutant. Res., in press).

damaged DNA is, in fact, an open UvrB-DNA complex. (vi) It would appear that the role of the UvrA subunit is to act as a "damage antenna" targeting the UvrB protein to the site of the damaged nucleotide. By analogy with RNA polymerase promoter formation, UvrA is acting like a sigma factor to give the specificity to the complex. Once a stable open complex is formed, UvrA may participate in another loading of UvrB to a damaged site.

Finally, we can ask how the UvrABC complex recognizes DNA damage and what the structural determinants for damage-specific binding are. These questions are addressed in the next section.

DAMAGE RECOGNITION BY THE UvrABC COMPLEX

One of the remarkable features of the UvrABC complex is its ability to work on a broad spectrum of DNA damage. Lists of all known DNA lesions that are substrates for the UvrABC complex are given in Tables 8 and 9. Examination of these lists reveals that the UvrABC complex can recognize DNA containing large chemical modifications such as

TABLE 9. DNA distortions induced by selected types of DNA damage

Adduct	Strand unwinding (degrees)	Helical kink (degrees)	Helical displacement (nm)	T_m (°C)	References
AF-guanine	22				83, 188
		Hinge			187
Anthramycin-*N*-2-guanine				+20 to +30	71
Apurinic site		Hinge			100, 187
BPDE-guanine	13				188
	26				188
	30–330				66
		25–35			84
Cisplatin-GG diadduct		40–50		−27 to −15	160, 209, 210
	12				188
	11–60				188
O-6-Methylguanine			Into the major groove		101, 102
Psoralen cross-link					
8-MOP	87.7	46.5	0.35		152
4-AMT	56.0	53.0	0.15		239
HMT		None			79, 219
Psoralen monoadduct					
4-TMP	28				188
Pyrimidine dimer	6				188
	14				188
	19.7	27	0.27		152
		30			89

N-2-benzo[*a*]pyrene diol epoxide (BPDE)- and *N*-acetoxyacetylaminofluorene-guanine monoadducts, linked bases such as pyrimidine dimers and cisplatinum guanine-guanine adducts, and relatively minor modifications such as thymine glycols, apurinic sites, and *O*-6-methylguanine. Even chemicals such as doxorubicin and the bisintercalator ditercalanium, which do not apparently form covalent linkages with the bases, are substrates (see references in Table 8).

Recognition Problem

How can one protein complex act on such a diverse set of DNA lesions? This is not a new question. As early as 1965, when it was discovered that the protein complex encoded by the *uvrA*, *uvrB*, and *uvrC* genes acted on several structurally distinct lesions, Hanawalt and Hayes suggested that this complex detects DNA damage by "gauge(ing) the closeness-of-fit to the Watson-Crick structure" (77). On the basis of this model, they speculated that DNA mismatches may even be substrates for this enzyme. Although it has recently been shown that the UvrABC complex does not, in fact, act on base mismatches (233), little progress has been made in the last 25 years at refining this wonderful description. How might the UvrABC complex act as a molecular calipers and systematically measure the dimensions of the DNA helix, binding to DNA with great affinity only if it encounters a structure that does not resemble normal B-form DNA? Stating this question another way, how do DNA repair enzymes, such as the UvrABC complex, exploit damage-induced alterations in the DNA helix to obtain their specificity?

These two questions are addressed in the following section. The first part discusses some of the unique conformational features that specific DNA damage induces in the DNA helix. The last part speculates on how the UvrAB complex may use specific protein motifs to bind to these structural alterations. Before the structural requirements for DNA damage recognition by the UvrABC complex are addressed, it is worthwhile to review the major structural aspects of DNA that are exploited by several DNA-binding proteins. Several outstanding reviews have been written on this subject, and the following is meant only to serve as an introductory review (48, 105).

Structure of DNA. DNA is a helical molecule consisting of two strands running in opposite polarity. The coding potential of this macromolecule resides in the sequence of four planar bases, adenine (A), guanine (G) cytosine (C), and thymine (T) (the first two are purines, and the second two are pyrimidines). These bases are attached to 2′-deoxyribose sugar residues, forming a glycosidic bond between the N-1 (pyrimidines) or N-9 (purines) position and the 1′-position of the sugar, forming nucleosides. The basic unit of DNA is the nucleotide, which consists of the nucleoside attached to one phosphate group. This phosphodiester linkage which connects the 3′ and 5′ positions of adjacent nucleosides is a strong acid and carries a negative charge. The four major forces that stabilize the structure of the DNA molecule are hydrogen bond formation between G · C and A · T base pairs, neutralization of the negative charges by cations, hydration by water, and the aromatic stacking interactions of the planar bases. DNA exists in many conformations; the three general families are A form, B form, and Z form. Most studies indicate that DNA in solution adopts a B form. For B-form DNA the average helical parameters are 10.5 bases per turn with a rise of 0.34 nm per base and a diameter of 2.2 nm. The DNA helix forms two grooves: the major groove has an average width of 1.02 to 1.22 nm and a depth of 0.8 to 1.0 nm, and the minor groove is approximately 0.46 to 0.64 nm wide and is slightly shallower. The floor of these grooves is paved with oxygen and nitrogen atoms which serve as hydrogen acceptors and donors. The specific dimensions of the grooves are determined by the sequence, with G · C runs producing a wide minor groove (resembling A-form DNA) and A · T runs producing a narrow minor groove (51, 52).

One of the factors that affects the structure of the DNA helix is the conformation of the sugar-phosphate backbone, which can be given by seven torsional angles. The deoxyribose sugar is found in two primary conformations, C-2′-endo, which separates adjacent phosphates by 0.70 nm, and C-3′-endo, which separates the phosphates by 0.59 nm. Another important consideration in the structure of the DNA helix is the orientation of the bases to each other and to the deoxyribose moiety. The N-glycosyl torsion angle determines the orientation of the base with regard to the sugar. In B-form DNA the bases are in the anti conformation, which is a more extended form than the syn conformation, in which most of the base lies over the sugar. Guanines adopt the syn form in the left-handed helix, Z-DNA, but certain DNA adducts, such as *N*-acetoxyacetylaminofluorene, cause guanine to adopt a syn rather than anti conformation (83). In B-DNA, the helical twist (the amount of twist which each successive base pair is offset from the base pair above or below it) is about 36°. This 36° offset is the result of optimization of stacking interactions which is the predominant force in forming a right-handed helix (186). Four other parameters, helix tilt, base pair tilt, propeller twist, and base pair roll, define the orientation of the bases in the DNA helix and give localized variation in the conformation of specific DNA sequences (48, 51). It is important to realize that the sugar-phosphate backbone and the orientation of the bases are intimately linked. Take, for example, differences between A-form and B-form DNA. In A-form DNA the distance between consecutive bases (the rise) is 0.23 nm, whereas in B-form DNA it is 0.34 nm. This smaller rise for A-form DNA is accompanied by a sugar conformation change from 2′-endo to 3′-endo, which, in turn, changes the base tilt from −5 to +12 and the distance between the phosphates of each consecutive base from 0.70 to 0.58 nm. These conformational changes, in turn, affect the dimensions of the major and minor grooves. Unlike B-form DNA, A-form DNA has a very shallow and wide minor groove, with a concomitant decrease in the width of the major groove.

It is easy, therefore, to see how modification of the base, as a result of a physical or chemical agent, could change the orientation of the bases, the base pairing, and the conformation of the sugar-phosphate backbone. It is these damage-induced conformational changes that create a new surface on which DNA repair proteins bind.

Potential binding sites for proteins. Viewing the DNA helix as a potential binding site for proteins, one discovers four major features of this molecule that could promote protein binding: (i) the negatively charged phosphates can form salt bridges with the positively charged amino acid side chains such as arginine and lysine; (ii) the floor of the major groove can act as hydrogen acceptors or donors for the formation of hydrogen bonds between the side chains of several amino acids; (iii) the aromatic bases can stack between aromatic side chains such as those of tyrosine and phenylalanine; and (iv) hydrophobic interactions, or Van der Waals contacts, can occur between moieties on the bases (such as the methyl group of thymine), and nonpolar amino acid side chains.

Damage-Induced Distortions: Important Determinants in Damage Recognition

With the above discussion as background, we can now ask whether DNA lesions alter any of these four structural features in such a way as to impart specificity for the binding of a DNA repair protein.

Dynamic structure of DNA. DNA is an extremely flexible and dynamic molecule undergoing rapid changes in its conformation and therefore existing as a family of conformers. DNA undergoes spontaneous bending and changes in base stacking over a very rapid time scale (117, 140, 157). The time scale of helical bending and twisting is on the order of 10^{-9} s, involving 5-nm movements (117, 140). The distribution of molecules among these various conformers is dependent upon many factors, including temperature, ion concentration, torsional stress, and sequence context.

Over the years, several approaches have been used to examine the types of helical alterations induced by specific DNA damage. These include X-ray diffraction studies of uniquely modified bases, nucleosides, or nucleotides; one- and two-dimensional proton nuclear magnetic resonance spectroscopy (NMR), as well as ^{31}P and ^{13}C NMR; molecular modeling studies using dynamic and energy minimization calculations; electrophoretic mobility shift assays; and DNA-unwinding assays. Three of the best-characterized DNA lesions that are substrates for the UvrABC complex are the UV-induced pyrimidine dimer, the cisplatin N^7-guanine-guanine diadduct, and the psoralen interstrand cross-link.

Development of kinked model for DNA damage. Using X-ray diffraction data of a thymine-thymine cyclobutane ring, combined with energy minimization techniques, Pearlman et al. developed a model of two photoproducts, pyrimidine dimers and psoralen cross-links (152). They suggested that three major structural distortions induced by these lesions are site-specific kink, localized unwinding of the two strands, and displacement of the bases away from the helical axis. These modeling studies, for the most part, have been supported by physical techniques including 2D-NMR and gel mobility assays (89, 104, 239). However, gel retardation assays and hydrodynamic studies have suggested that psoralen monoadduct and cross-links may not cause a site-specific bend (79, 219).

(i) Recognition of pyrimidine dimers by DNA photolyase. One DNA repair protein which has been extensively studied with regard to DNA binding and interaction with its substrate is DNA photolyase. It is worthwhile to discuss this protein not only because it represents one strategy for DNA damage recognition, but also because of its potential interaction with the UvrABC complex at pyrimidine dimers. DNA photolyase, therefore, also helps to give insight into what the UvrABC complex may be recognizing. DNA photolyase is a flavin protein which repairs UV-induced pyrimidine dimers in a two-step process in which the protein first binds to the dimer and then uses the energy of a photon of light in the range of 300 to 330 nm to photoreverse the cyclobutane pyrimidine dimer by electron donation (21, 179). Using the approach of Lohman (123), which consists of measurements of the apparent binding affinity (K_a) versus the salt concentration, Sancar et al. showed that DNA photolyase makes salt bridges with only two phosphates and that more than 50% of the free energy of binding is due to hydrophobic interaction (184). The nature of the protein interaction of DNA photolyase has been further studied by chemical and DNase I footprinting techniques, which have revealed that DNA photolyase probably binds directly to the face of DNA with the cyclobutane ring making strong contacts with the phosphates both 5′ and 3′ to the thymine dimer (90). The enzyme also makes contacts with one base and the second and third phosphates 3′ to the pyrimidine dimer, and it is believed that although these contacts stabilize the photolyase-DNA complex, they are not required for its formation.

(ii) DNA photolyase stimulates UvrABC-mediated incisions. Once both purified DNA photolyase and the subunits of the UvrABC complex became available, a very important question about the action mechanism of the UvrABC complex could be addressed. Is a pyrimidine dimer that is bound by DNA photolyase a substrate for the UvrABC complex? This interesting question was addressed by Sancar et al. (173), who showed that rather than inhibiting the UvrABC complex, DNA photolyase actually increased the initial rate and overall extent (by nearly 100%) of UvrABC-mediated incisions. It would be of interest to determine whether DNA photolyase stimulated UvrABC complex formation by protein-protein interactions or caused the pyrimidine dimer to adopt a significantly different conformation that was detected more efficiently by the UvrABC complex. It is not known whether the DNA photolyase stimulated UvrABC incisions by causing an increase in the binding affinity (K_s) or by altering the rate of hydrolysis of the two phosphate bonds (k_{cat}).

(iii) Kinked-DNA model. By using three different pieces of information, i.e., the results of the UvrABC incision studies in the presence of DNA photolyase, molecular models of the types of helical deformities that are induced by a pyrimidine dimer, and the position where DNA photolyase actually contacts such a lesion, a model was developed to explain how the UvrABC complex might bind to DNA containing a pyrimidine dimer. Van Houten et al. suggested that the UvrABC complex binds to the damage-induced kink which is on the face of the DNA helix, opposite the pyrimidine dimer (251). In a sense, the enzyme binds to the elbow of the kink that is induced by the pyrimidine dimer, making significant contacts on the nondamaged strand while making incisions on the damage-containing strand a little more than one helix turn away. Finally, by analogy with TFIIIA (130), which binds in the major grooves via its zinc fingers, it is believed that UvrA contacts DNA in a similar manner (147, 171). Therefore, on the basis of all available evidence, it has been suggested that the UvrABC complex makes contacts in the major groove on either side of the damaged base. Chemical and enzymatic footprinting experiments that probe the major and minor grooves will be necessary to help refute or support this model. It is important to realize that the conformation of the DNA near the site of modification might be altered to such a degree, either by the presence of the damage or by the interaction with the UvrA and UvrB subunits, that the term "major or minor groove contacts" is a misrepresentation.

Other substrates appear to kink DNA. Two questions arise: (i) how accurate is this kinked-elbow model for UvrABC binding, and (ii) is a site-specific kink either necessary or sufficient for DNA damage recognition by the UvrABC complex? One other DNA lesion that has been widely studied and supports this kinked model for UvrABC recognition is the adduct that results from covalent attachment of cisplatin to the N-7 position of two adjacent guanines (160). This cisplatin adduct is a good substrate for the UvrABC complex (6, 156). Molecular modeling calculations with X-ray diffraction data, as well as other physical studies,

indicate that this lesion induces a site-specific bend or kink of approximately 40° in the helix and localized DNA unwinding similar to the psoralen cross-link and the pyrimidine dimer (Table 9) (209, 210). DNA sequences containing stretches of $(A)_5$ have been found to adopt a static bend into the minor groove. By ligating this sequence to DNA containing specific DNA damage, it has been possible to determine the extent and orientation of the lesion-induced bend. This experiment has been performed for the cisplatin GG adduct, and it has been found to induce a ca. 40° bend into the major groove (160, 210).

One other DNA lesion that is acted on by the UvrABC complex and supports the kinked model is the BPDE-guanine adduct (194, 255). BPDE has also been suggested to cause a wedge in the DNA helix which would lead to a site-specific bend (84).

Shortcomings of the kinked-DNA model. Does all DNA damage induce a site-specific kink? Examination of Table 8 shows that not all DNA lesions that are substrates for UvrABC cause site-specific bends or kinks, and a few of these lesions, such as CC-1065 and anthramycin, are minor groove binders and greatly stabilize, rather than destabilize, the DNA helix.

Kinked DNA structures that are not substrates. Because the UvrABC complex appears to recognize a broad series of DNA lesions, it is informative to ask what types of DNA lesions are not substrates for the complex (Table 8 footnote). Inspection of Table 8 and its footnote reveals that base mismatches or extrahelical bases are not substrates and that the naturally bent kinetoplast DNA does not appear to be incised by the complex. It seems remarkable that the UvrABC complex can recognize *O*-6 MeG · C or *O*-6 MeG · T (259) but not G · G, T · C, or C · A base mismatches (233). *E. coli* contains an efficient mismatch correction system encoded by the *mutS*, *mutH*, and *mutL* system which can recognize all but C · C mismatches (for a review, see reference 131). It would be interesting to determine whether C · C mismatches are acted on by the UvrABC nuclease system. With regard to bent DNA, it has recently been shown that extrahelical (or deleted) bases appear to cause a static bend in the DNA, the amount of which is proportional to the number of extra bases (87, 157, 159). The lack of incisions seen with these DNA structures, particularly structures that promote kinking, strongly suggests that a DNA kink is not sufficient for incision to occur. However, no experiments have been reported to determine whether the UvrAB complex actually binds specifically to any of these altered DNA conformations. The absence of incisions may be due to the absence of covalent modification or to lack of alteration in the electronic configuration of the base. It is possible that the complex recognizes this lesion as damage but that the nuclease centers cannot form properly in the absence of any clear chemical alteration. Footprinting experiments, band shift assays, or filter-binding assays may help to address this matter.

Structural determinants that are important for damage recognition. We can now attempt to describe the types of distortion that appear to be either necessary or sufficient to be recognized by the UvrABC complex. These distortions fall into six categories: covalent modifications, bulky substituents, localized unwinding of the two DNA strands, site-specific bend or kink, charge distribution around the site of the damage, and changes in the structural dynamics of the DNA helix.

(i) Covalent modification. Most types of DNA damage that are substrates for the UvrABC complex are caused by covalent modification of the bases of the DNA. The three important exceptions are the base intercalators ditercalanium (113, 115), doxorubicin, and AD32 (97). It is important to note, however, that these intercalators, notably ditercalanium, may cause DNA bending, DNA strand unwinding, disruption of base stacking, and changes in the phosphate conformations leading to changes in counter ion distribution. This idea is most certainly testable by using other intercalators such as anthracyclines, ethidium bromide, triositin A, or echniomycin. However, not all intercalating agents may be substrates, since *E. coli* strains carrying mutations in the *uvrA* gene do not appear to be hypersensitive to killing by ethidium bromide (114).

(ii) Bulky substituents. Until recently it had been widely believed that a bulky substitution was a prerequisite for recognition by the UvrABC complex. The N-substituted aryl compounds *N*-acetylaminofluorene (AF) and *N*-acetoxyacetylaminofluorene (AAF) both react with the C-8 position of guanine, forming AF-G and AAF-G adducts, respectively. Several physical and molecular studies of these two structurally similar compounds have revealed that the AF-G adduct probably lies on the outside of the helix and does not lead to a large distortion (83), whereas, the AAF-G can cause several changes in the DNA in which the fluorene ring is inserted into the helix, causing the base to be displaced out of the helix. AAF-G can also induce the guanine to flip the normal anti conformation to the syn conformation, which has been shown to exist in left-handed Z-DNA. The UvrABC complex recognizes both of these lesions in linear or supercoiled DNA, but Tang and co-workers have shown that the AAF-G adduct is recognized two- to threefold more efficiently (154, 227). Several genetic experiments suggested that the UvrABC complex might act on simple alkylation damage, including *O*-6-methylguanine (37, 170, 256).

Voigt et al. have recently shown that even the relatively small helical distortion induced by *O*-6-methylguanine is a substrate for the UvrABC complex (259). The UvrABC complex must have a very fine molecular caliper indeed. One important question is whether *O*-6-methylguanine leads to helix destabilization, a bend or kink in the DNA helix? Oligomers containing *O*-6-alkylguanine at a defined position have been used in proton two-dimensional NMR and ^{31}P-NMR studies which have revealed that the alkylation at the *O*-6 position of guanine leads to changes in the torsional angles of the phosphate across from the *O*-6-methylguanine (101, 102). In addition, the *O*-6 G · C base pair is greatly destabilized, with the formation of only one hydrogen bond. Furthermore, the entire base pair has been shown to be moved into the major groove of the DNA helix.

Recently, two groups have independently shown that the oxidative DNA damage, thymine glycol, is recognized by the UvrABC complex (119; Y. W. Kow, S. S. Wallace, and B. Van Houten, Mutant. Res., in press), although the structurally similar dihydrothymine, in which the 5 and 6 positions are substituted by hydrogens instead of hydroxyl groups, is not a substrate for the enzyme (Kow et al., in press). It was also found that apurinic (AP) sites are poor substrates for the enzyme, but reduced AP sites or alkoxyamine substrates (which contain an open ring structure) are in fact recognized more efficiently by the enzyme (Kow et al., in press). It would thus appear that bulky substitutions are not necessary for damage recognition.

(iii) Localized unwinding of the two DNA strands. Most of the DNA lesions listed in the Tables 8 and 9 have been shown to induce a localized unwinding of the DNA helix. In most cases, this unwinding of the base pairs leads to a

destabilization in the DNA helix. This can be monitored by examining the thermal stability of the helix and the rate of proton exchange on the imino groups on the bases (101, 102). Disruption of hydrogen-bonded base pairs will also lead to distortions of the phosphate backbone.

Ramstein and Lavery have calculated the energetics of DNA bending and strand unwinding and have found that once a base pair is disrupted, DNA bending becomes energetically favored, and, similarly, that DNA bending lowers the amount of energy that is needed to open the DNA helix (157). This relationship suggests that DNA damage that unwinds the two strands might not necessarily lead to static bends, but could act as a hinge joint and facilitate greater DNA bending at the site of the damage. This has been shown to occur for *N*-AAF–C-8-guanine adducts and reduced apurinic sites (100, 187). Both of these lesions are substrates for the UvrABC complex (154, 174; Kow et al., in press). Although hydrogen bond formation is important in maintaining the structure of DNA, the major driving force in helix stability appears to be stacking interactions (186). This is nicely illustrated by psoralen-DNA adducts. Psoralen adducts have been shown to unwind the DNA helix anywhere from 28 to 90° (Table 9), yet thermostability measurements indicate that a psoralen monoadduct actually stabilizes the helix by about 10 to 20°C (212) depending on the sequence. Therefore, DNA damage that causes DNA unwinding does not necessarily lead to helix destabilization. Apparently, the energy contribution of the stacking interaction of psoralen and the surrounding base pairs is great enough to overcome the destabilizing effects of DNA unwinding and base pair disruption.

(iv) DNA bending or kinking. The development of a kinked model for DNA damage that is a substrate for the UvrABC complex is discussed above. Since base pair disruption and DNA bending appear to be energetically linked, DNA damage might have a greater propensity for bending without necessarily displaying a static bend. As described previously, the interaction of the UvrA or UvrAB complex with DNA produces a local unwinding of the helix (143–145). It is conceivable that this unwinding at the site of damage could lead to bending as well. These ideas must be tested by using defined substrates and gel migration assays, such as those of Liu-Johnson et al., which have been used to measure the kinking induced by the CAP protein (121).

(v) Alterations in the charge distribution around the site of damage. Binding of chemicals to DNA could lead to disruption of the pi electron cloud around the bases, counter-ion distribution around the phosphates, and the shells of solvation surrounding the entire helix. The potential anticancer compound CC-1065 helps to illustrate these alterations (228). This drug interacts over several base pairs in the minor groove. It forms a covalent bond at the N-3 position of adenine, disrupting the normal electron configuration of this base. In addition, it appears to interact with a neighboring phosphate, which would affect the counter-ion distribution. The spine of hydration that runs down the minor groove would also be expected to be disrupted.

(vi) Alterations in the structural dynamics of the DNA helix. In a recent report on the recognition of anthramycin damage by the UvrABC complex, Walter et al. suggested that certain DNA lesions could "affect the fluidity of the DNA helix around the adduct and consequently may hinder nearby transitions of the dynamic structure" (267). Since DNA is a dynamic molecule undergoing many conformational changes, including bending and localized melting, on a very rapid time scale, both helix-stabilizing and helix-destablizing lesions could greatly affect this dynamic structure. Just as frays or knots affect the propagation of waves in a rope, damage could affect the passage of waves of energy through the DNA helix. These lesions may act to impede the passage of these waves, or the wave action may help to destabilize the helix. The interaction of many intercalating agents, such as dactinomycin, has been envisioned to interact with DNA in this way (222).

To summarize, DNA is a dynamic molecule which undergoes transient alterations in its structure, including breathing (localized melting of the base pairs) and bending (changes in the sugar-phosphate backbone conformation) (117, 140, 199). DNA damage can lead to changes in the dynamic structure by stabilizing specific conformational alterations at the site of the modification. Six types of structural and conformational changes are outlined above. It is these alterations that are potential substrates for the UvrABC complex.

In future studies, it will be important to find the structural determinants of DNA damage that are required for recognition as substrate by the UvrABC complex. It may be possible to describe the interaction of the UvrABC complex by a matrix of the six factors mentioned above. For example, is it possible to show a correlation between the amount of helix distortion induced by a particular DNA adduct and the affinity of the UvrABC complex for this damage? To help resolve the conflicting data for the recognition process of the UvrABC complex, it is necessary to undertake a study of a series of DNA lesions which change the structure of the DNA helix systematically and in a manner that is amenable to physical study and which can be correlated to the action mechanism of the UvrABC complex. These properties include the relative binding affinity, the incision kinetics, and the position of the incision sites. Current research in several laboratories is directed toward this goal.

Function: the Process of Damage Recognition

Having classified the sorts of helical deformities that are induced by DNA lesions, we can now turn to the second part of damage recognition, namely, the types of protein interactions that the UvrAB complex might use to identify damaged nucleotides and the protein motif that could be used to achieve these interactions. These two questions will be considered below.

Types of protein-DNA interactions. There are four major types of protein-DNA interactions: hydrogen bonds, ionic interactions, Van der Waals contacts, and hydrophobic interactions. Since it appears that the UvrA protein is involved in damage recognition and the UvrB protein is involved in the formation of the active nuclease complex, the protein-DNA interactions involved in these two steps are probably distinct (it should be pointed out, however, that the interaction of the UvrB protein with the UvrA protein most certainly affects the binding properties of the UvrA protein).

(i) Hydrogen bond formation. The most extensively studied protein DNA interactions are those between proteins that bind to DNA at specific sequences, such as repressor or activator proteins, restriction enzymes, and RNA polymerases (10–12, 123, 127, 128, 146, 150, 260–262). The formation of these complexes is stabilized by hydrogen-bonding interactions between the unique sequence of hydrogen acceptors and donors on the floor of the major groove and specific amino acid side chains on the protein. Since the UvrABC complex acts on many types of DNA damage which affect different bases and is almost unaffected by the sequence

context, it seems unlikely that unique hydrogen bond formation between the floor of the major or minor groove and the amino acid side chains is a major factor in specificity. Although the hydrogen acceptors and donors in the major groove may be too specific for generalized recognition by the UvrABC complex, it is conceivable that a general pattern of hydrogen bond formation helps promote complex formation. Since the interaction of the UvrAB complex with DNA appears to unwind the DNA helix, a more general type of hydrogen bond formation could be achieved by disruption of the hydrogen bonds between bases and reformation of hydrogen bonds between bases and amino acid side chains. This type of melted structure is similar to the open complex formed between RNA polymerase and a promoter.

(ii) Ionic interactions. Von Hippel and colleagues have suggested that sequence-specific proteins first interact nonspecifically with DNA mainly by ionic interactions with the phosphates (11, 12, 262). Once the protein locates a sequence with complementary hydrogen acceptors and donors that is in the proper orientation, several of the phosphate contacts are lost. This phenomenon helps to explain why specific DNA interactions are inhibited by salt to a lesser degree than are nonspecific interactions (123).

Our knowledge of the interaction of the UvrAB complex with DNA is limited to filter-binding studies and DNase I footprinting experiments. Formation of UvrB-DNA complex (149) is favored by a shift from nonspecific to specific binding (open-complex formation) and may involve fewer ionic interactions. Therefore, the specific complex should be more resistant to salts or polyanions. Grossman, Yeung, and co-workers (33, 276, 278) have shown by filter-binding assays that when bound to UV-induced DNA damage, the UvrAB complex is quite resistant to high-salt buffers.

This situation is analogous to RNA polymerase formation of an open complex which becomes resistant to heparin, a polyanion. Spermidine and putrescine are two polyanions that are abundant in *E. coli*; it would be of interest to study the effects of such polyanions on the formation of the specific DNA complex.

Using DNase I footprinting techniques, it has been observed that concentrations of greater than 100 mM KCl inhibit specific binding, and it appeared that as many as 10 salt contacts were formed with the UvrAB complex and a psoralen-modified thymine (Van Houten and Sancar, unpublished). A significant amount of the free energy of binding was due to factors other than ionic interactions. The 19-bp footprint of the UvrB-DNA complex is certainly large enough to account for these 10 phosphate contacts. Assuming the UvrB is a spherical molecule, it would have a diameter of 5.6 nm, which could cover a maximum of 16 bp. By deletion analysis, it was shown that as few as 3 bp 5′ and 1 bp 3′ to the normal incision sites are required for the formation of the active UvrABC incision complex, although at least 15 bp 5′ or 3′ to the incision sites is needed to maximize the incision efficiency (250). This requirement may be because the UvrABC complex binds more tightly to the ends of DNA molecules and might lead to the inhibition of specific complex formation (Van Houten and Sancar, unpublished).

Specificity with ion pair formation could be achieved only if the sugar-phosphate backbone surrounding the damaged base adopts a common conformation that is complementary to positively charged amino acids on the protein surface.

(iii) Hydrophobic and van der Waals contacts. Single-strand binding proteins such as single-strand-binding protein of *E. coli*, the gene 5 product of M13 phage, and the gene 32 protein of T4 phage interact with single-stranded DNA in a sequence-independent manner by two major interactions: ionic interactions between arginine side chains and phosphates and stacking interactions between the bases and tyrosine side chains (146). As mentioned above, unwinding of the base pairs in the formation of the open UvrB-complex would lead to the loss of free energy, which must be compensated for by the formation of new protein-DNA contacts unless new protein-DNA hydrogen bonds are established. One way to compensate for the unwinding of the DNA helix would be to establish hydrophobic interactions by stacking interactions between the bases and the amino acids side chains.

Protein motifs. The UvrA and UvrB subunits contain at least three structurally distinct motifs representing six separate domains including: three nucleotide-binding sites, two zinc fingers, and a marked hydrophobic domain of the UvrB protein. It follows from the preceding section that any of these motifs must be sufficiently flexible to accommodate the various helical alterations that are imposed by the broad spectrum of DNA damage. How might these domains be used to confer specificity of binding to DNA damage?

(i) UvrA zinc fingers. It appears that the DNA-binding zinc finger motif is used for specificity in many DNA-binding proteins, and it is of interest to determine whether the fingers of the UvrA subunit might serve a similar function. One model structure that has been suggested for the finger motif is an N-terminal β-sheet, a β-turn, and another β-sheet followed by a turn and an α-helix (8, 9). Analysis of the secondary structure prediction for the UvrA protein in the regions of the putative zinc fingers is consistent with this type of structure. Using two-dimensional NMR, Lee et al. have determined the three-dimensional solution structure of a single zinc finger DNA-binding domain from the *Xenopus* protein Xfin (116). The structure that was determined was similar to Berg's model (9), except for a 3_{10} helix instead of an α-helix; this might be due to the coordination with zinc at the two histidines.

It has been shown that zinc stabilizes the formation of a non-B-form structure in the direct repeats of a β-globin gene promoter. This had led to the suggestion that the zinc chelated by the protein could act to stabilize unusual DNA structures (108). It could be hypothesized that the zinc of the UvrA fingers might serve a similar purpose. The addition of zinc (0.1 to 0.5 mM) greatly inhibits the incision reaction, although no DNA-binding experiments have been performed to see whether this concentration of zinc inhibited complex formation or the incision step (Van Houten and Sancar, unpublished). Site-specific mutagenesis studies of the zinc fingers are in progress (139) and should help define the role of these motifs in the damage recognition process.

(ii) Formation of the open UvrB-DNA complex and role of ATP hydrolysis. The role of ATP hydrolysis is dealt with in detail above in the section on formation of the preincision complex, but one function for which ATP is absolutely required is DNA duplex unwinding (144, 145). In addition, it has been proposed that ATP hydrolysis may be used to drive damaged DNA into a common conformation which can be incised (induced allostery) (4, 144). From studies with truncated UvrB* protein, it appears that this unwinding ability is absolutely required for the formation of the active incision complex (32, 33). This ATP-dependent unwinding activity of the UvrAB complex is analogous to promoter selection by RNA polymerase, in which an isomerization event occurs, resulting in the change from a closed to an open complex. As discussed above, this terminology can be extended to the

UvrB-DNA complex as well. How might the formation of this open UvrB-DNA complex be used in the process of damage recognition and allosteric interaction prior to the incision step?

Von Hippel et al. pointed out that local bending of the helix or changes in groove geometry could thermodynamically improve protein binding (260–262). Such distortions of the DNA helix require energy and would reduce the gain in binding affinity. For example, the cost of forming a DNA bubble by RNA polymerase is about 1.4 kcal (5.9 kJ) per A · T base pair and 3.3 kcal (13.8 kJ) per G · C base pair. If the UvrA or UvrB complex unwinds up to 10 bases, approximately 20 kcal (84 kJ) would have to be added by the formation of the open complex to overcome this loss in hydrogen bonding. One other source of energy to disrupt base pairs could be ATP hydrolysis, which might be used to lower the activation energy for the formation of the open complex, thus constraining the damaged DNA into a common conformation which favors UvrAB binding.

(iii) DNA unwinding new protein-DNA contacts. UvrB does not appear to bind to DNA in the absence of UvrA, but it forms a very tight complex with DNA once the UvrA protein has prepared the site (96, 149). How might this binding of the UvrB protein to DNA be achieved? If the DNA is truly melted (which must be confirmed by other methods), we can envision interaction of UvrB with the bases in the melted DNA. The formation of the UvrB-DNA complex could help to potentiate new interactions of UvrB with the bases or phosphates of the melted DNA helix.

FORMATION OF THE INCISION COMPLEX

During the initial characterization of the UvrABC complex, Seeberg found that incision of DNA only occurred in the presence of all three subunits, Mg^{2+}, and ATP (189, 190). Subsequent studies indicated that incision did not occur until the addition of the UvrC subunit (191, 192). Yeung et al. were able to show that the amount of incision was directly proportional to the amount of UvrC in the reaction (278). By using DNA fragments that were randomly modified by UV irradiation, cisplatin, *N*-acetoxyaminofluorene or psoralens, several groups were able to show that the UvrABC complex incised DNA in a highly conserved manner. The general incision pattern that emerged from these experiments is that the UvrABC complex usually hydrolyzes the eighth phosphodiester bond 5′ and the fourth or fifth phosphodiester bond 3′ to a damaged nucleotide (reviewed in reference 179 and discussed below). It should be pointed out, however, that not all DNA damage in all sequences is incised in this manner, a point that is examined in the last section (Effects of Alternative DNA Structure).

Damage-Processing: the Interactive Recognition Step

The invariance of the position of the incision sites has been cited as evidence for a common processing step by the UvrABC complex (73). It has been suggested that the formation of the active UvrB preincision complex occurs by an induced allostery between the surface of the DNA damage and the Uvr proteins. The interaction of the UvrB and UvrC proteins at the damaged site which results in the dual-incision event could occur in a process that is analogous to DNA binding and incision of the restriction endonuclease *Eco*RI. The binding of *Eco*RI is known to induce changes in the structure of the DNA backbone, a process that Kim et al. have called interactive recognition (107). Because of the potential similarities of the two systems, a brief description of *Eco*RI is informative.

The crystal structure of *Eco*RI bound to the recognition sequence GGAATTCC has lead to a significant understanding of the formation of this protein-DNA complex (127). *Eco*RI binds to its recognition sequence as a dimer and forms three neokinks in the DNA helix. The center kink is a result of localized unwinding of the bases, which allows the protein to make good contacts in the enlarged major groove. The two outside neokinks are at the site of phosphate cleavage. These neokinks represent strain introduced into the backbone. Hydrolysis occurs at these sites with the addition of Mg^{2+} ion. By using limited proteolysis, it was found that loss of the N-terminal arm leads to loss of incision but not binding of the *Eco*RI dimer (127). Since the nuclease center is located in another site in the molecule, it is believed that the N-terminal arm brings the correct phosphodiester bonds into the nuclease clefs.

At present, little is known about the interaction of UvrC with the preinicision complex, the location of the nuclease domains, or the mechanism of phosphate bond hydrolysis. No sequence homology between known endonucleases and the UvrC or UvrB has been reported.

Dual-Incision Mechanism

The observation that the UvrABC complex actually produced incisions on each side the damaged nucleotide was first reported by Sancar and Rupp (177) and confirmed by Yeung et al. (278) in the same year. Using DNA sequencing gels, together with *Micrococcus luteus* UV endonuclease or T4 endonuclease V, these groups were able to map exact sites of UvrABC incisions at various DNA lesions. Together, these reports demonstrated that the UvrABC complex catalyzed the hydrolysis of the eighth phosphodiester bond 5′ and the fourth or fifth phosphodiester bond 3′ to UV-induced photoproducts, psoralen monoadducts, or cisplatin adducts (177, 278). It has been suggested that this "incision at a distance" helps avoid both the possibility of nucleotide damage due to steric interference and the alteration of the DNA helix, resulting in the inhibition of the nuclease step (177). Incision resulted in the production of a 3′ hydroxyl group and a 5′ phosphate at each incision site (177). This dual incision facilitates the release of a 12- or 13-base oligonucleotide containing the lesion (31, 112, 137, 177). In two of these studies (31, 112), an oligomer of 8 or 9 bases was also produced. The nature of these shorter oligonucleotides was not examined, although it has been suggested that they could result from a 3′-to-5′ exonuclease activity (31, 112).

The UvrABC nuclease incision mechanism appears to be highly conserved. Several groups have mapped the incision sites produced in radiolabeled DNA fragments that have first been randomly damaged with various physical and chemical agents and then digested with the UvrABC complex. These agents include acid depurination (118), *N*-acetoxy-2-aminofluorene and *N*-hydroxyaminofluorene (154, 174), anthramycin (267), *cis*-platinum (6, 156, 175), CC-1065 (200, 228), osmium tetroxide (118), UV light-induced pyrimidine dimers and 6,4-pyrimidine-pyrimidone photoproducts (137, 177, 278), and psoralens plus UV (174, 276). Since chemical treatment produces adducts at various sites randomly, it was essential to prove the incision mechanism for a chemical-DNA adduct that was introduced at a defined position in a DNA fragment. To achieve this goal, Van Houten and Sancar, in collaboration with Gamper and Hearst, examined

the UvrABC incision sites for a DNA fragment in which a psoralen-thymine monoadduct had been engineered into a defined position (250, 251). They found that the UvrABC nuclease complex incised the eighth phosphodiester bond 5′ and the fifth phosphodiester bond 3′ to the monoadducted thymine. Using similar technologies, Jones and Yeung later confirmed and extended these results (94).

Yeung et al. were the first to suggest that the DNA incisions may be uncoupled at specific lesions, or under certain conditions (278). This observation raises the question of whether the 5′ and 3′ incisions are sequential or simultaneous. Grossman has mentioned in several of his papers that the dual incisions occur in a sequential manner proceeding in a 5′ to 3′ direction, although no data have been given (73). Analysis of the incisions which occur in the first 3 min on the psoralen-monoadducted substrate described above appeared to indicate that the 3′ incision preceded the 5′ incision (Van Houten and Sancar, unpublished). By using this substrate, it was also seen that incision under suboptimal conditions, such as elevated pH or aged subunits, led to a decrease in the incision efficiency and to a specific loss of the 5′ incision (200; Van Houten and Sancar, unpublished). Furthermore, it has been reported that particular types of DNA adducts, such as CC-1065, are more prone to this uncoupling phenomenon (200, 228).

Role of UvrC Subunit in the Incision Process

It is clear from experiments performed in vitro that UvrC is absolutely required for the dual incision event, although the role of UvrC in vivo has been equivocal. DNA incisions can be observed in the DNA of *E. coli* following UV irradiation (229–231). Characterization of several *uvr* mutants indicated that although no incisions occurred in the *uvrA6* mutant, DNA incisions could be detected in *uvrC34* or *uvrD56* mutants. Although the incisions seen in the *uvrC* mutants did not result in the removal of pyrimidine dimers (103, 193, 204, 206, 231). These incisions appeared to be due to true hydrolysis of phosphodiester bonds and not to alkaline cleavage of apurinic sites (231). These DNA incisions could occur because these *uvrC* mutants are leaky or other host endonucleases could perform the incision step on a preformed Uvr-DNA preincision complex. The appearance of a DNase I-hypersensitive site 5′ to the DNA adduct in the formation of the preincision complex seems to support this latter idea (254). Walters and colleagues went on to characterize the residual DNA incision activity in *uvrC* mutants and found that incisions in a *uvrC34* mutant seem to be under the control of the SOS response and that rifampin treatment completely eliminated SOS induction (268). By using insertional mutagenesis, they showed that DNA incision is completely defective in a *uvrC*::Tn*10* mutant. They mapped this insertion site to 370 ± 20 bp upstream from the 3′ end of the gene that encodes the C-terminal portion of the protein. They concluded that the residual incisions seen in the *uvrC34* and *uvrC56* mutants indicate a leaky phenotype due to partial activity. The fact that SOS induction increased this incision activity suggested the formation of more preincision complexes, which could act as sites for UvrC incision. It would be of interest to characterize the nature of these mutant proteins in an attempt to determine the role of UvrC in the incision mechanism.

UvrC may function in the incision reaction in at least three different ways: (i) it may interact with the preincision complex to activate a nuclease center in the UvrB protein; or (ii) it might, itself, contain a nuclease center that is only activated when bound to the UvrB-DNA complex; or, finally, (iii) the active nuclease center might be created by domains from both the UvrB and UvrC proteins. Since UvrB and UvrC share extended regions of homology, it is tempting to speculate that these proteins form a heterodimer at the site of the damage such that the phosphodiester bonds to by hydrolyzed are brought into the nuclease centers.

In an attempt to discriminate between these possibilities, Caron and Grossman investigated the incision activity of UvrC (31). They found that addition of UvrC to preformed Uvr(A)B-DNA complex resulted in incisions even in the absence of ATP. They also reported that the UvrABC complex can incise "nondamaged" DNA. This is a controversial finding because it is exceedingly difficult to demonstrate that any DNA that has gone through the rigors of DNA labeling and purification does not contain any damage. Incision of nondamaged DNA by the complete UvrABC system was reported to produce a nonamer instead of the typical 12- or 13-base oligonucleotide (31). Production of this nonamer was also seen by the addition of only UvrC to nondamaged DNA; addition of UvrB stimulated the production of the oligonucleotide. This latter observation suggests that the UvrB and UvrC proteins might interact in solution, and it is reminiscent of the earlier reports by Seeberg that the UvrC and UvrB activities coeluted from a DEAE column (191, 192, 195, 196). This UvrC-associated nuclease activity was also shown to coelute with UvrC from a UvrC monoclonal antibody column (31). It is obvious that direct proof of UvrC nuclease activity will require the isolation and characterization of specific mutants which are defective in this activity or the production of antibodies to specific oligopeptides from UvrC. With regard to the former point, it has been shown that a deletion in the UvrC gene, which leads to the production of a UvrC protein lacking the last seven C-terminal amino acids, is very poor at complementing a *uvrC* mutation. This mutant protein has not been characterized further (56, 132).

Another unusual feature of the UvrABC complex is that it will produce only stoichiometric incisions and the entire complex will not turnover. This topic is examined in the next section.

POSTINCISION EVENTS

During a discussion of A. Yeung's data at a 1983 Keystone Meeting on Mechanisms of DNA Repair, Friedberg pointed out that the UvrABC complex does not turn over and other protein products might be required for the turnover of the UvrABC complex. Later, using filter-binding assays, Yeung et al. (277) demonstrated the presence of a postincision Uvr protein-DNA complex which is more stable than the UvrAB-DNA complex.

Role of Helicase II and DNA Polymerase I in the Release of the Uvr Subunits from the Postincision Complex

One of the first demonstrations that the UvrD protein, helicase II, was involved in nucleotide excision repair was the observation of Kuemmerle and Masker that the UvrD protein stimulates UvrABC nuclease activity in cell extracts (111). Using purified Uvr proteins, Kumura et al. were able to show that addition of helicase II to a reaction containing the UvrABC nuclease and DNA polymerase I leads to an increase in the number of incisions, the amount of incorporation of radiolabeled nucleotides, and the amount of excised product (112). These studies were extended by the Gross-

man and Sancar groups (34, 91). To summarize their findings, (i) both helicase II and polymerase I are absolutely required for the UvrABC complex to incise DNA in a catalytic manner, presumably by allowing the postincision complex to turn over; (ii) although both T4 DNA polymerase and the Klenow fragment of polymerase I stimulate turnover of the UvrABC complex, only DNA polymerase will yield repair patches which could be sealed by DNA ligase; (iii) the Rep protein, which is a 5′-to-3′ helicase, would not substitute for helicase II; (iv) addition of helicase II appeared to allow the turnover of the UvrC subunit; (v) helicase II greatly stimulated the incorporation of nucleotides by polymerase I; and (vi) some of the UvrABC postincision sites were susceptible to resealing by DNA ligase in the absence of other factors.

Using defined DNA substrates that contained a psoralen-thymine monoadduct at a precise location, Van Houten et al. (252, 253) were able to investigate the nature of the postincision complex in greater detail. They found that the 5′ incision site of the postincision complex is more accessible to DNA ligase than the 3′ incision site. Using two different assays, they also found that DNA polymerase could insert nucleotides into the repair patch in the absence of helicase II. Addition of both helicase II and polymerase I leads to the loss of the postincision DNase I footprint. The addition of only helicase II to the postincision complex revealed a hypersensitive site which is consistent with helicase II making contact on the nondamaged strand near the 5′ incision site of the postincision complex. No change in the size of the footprint was observed. Contact of DNA helicase at this location is consistent with its aiding in the binding of DNA polymerase to the 5′ incision site to initiate repair synthesis. This model was first proposed by Matson, who showed that helicase II translocates unidirectionally in a 3′-to-5′ direction (125). It has been suggested that helicase II may participate in the turnover of the Uvr subunits by direct protein-protein interactions (125).

In contrast to the earlier study (34), it was found that DNA polymerase I can perform gap filling in the absence of helicase II, an event that would presumably result in the release of the excised oligomer (252, 253). One explanation for this discrepancy is that in the former study no DNA ligase was added. Under such conditions, extensive nick translation would lead to high levels of incorporation of radiolabeled nucleotides. Both helicase II and polymerase I are required for the production of catalytic rounds of incision by the Uvr proteins. What then is the role of helicase II? It is conceivable that unless helicase II is present during the polymerase step, UvrC, owing to its high affinity for single-stranded DNA, will remain bound to the excised oligomer and be unable to participate in the incision reaction. If this model is correct, the number of DNA incisions that occur in a reaction containing DNA polymerase I and limiting amounts of UvrA and UvrB should be directly proportional to the amount of UvrC that is added. This model also predicts that UvrC and helicase II might interact in solution.

It has been recently reported that helicase II will completely unwind circular pUC8 containing, on the average, one nick per molecule (164). These results, combined with the footprinting data described above, suggest that helicase II may participate in the turnover of the postincision complex by binding to the 5′ incision site and, through its unwinding activity, facilitate the release of the excised oligonucleotide and the Uvr subunits.

Patch-Size Measurements

Prior to the development of an in vitro repair system, several groups made estimates of the size of the repair patches that are produced during nucleotide excision repair in *E. coli* (reviewed in reference 252). Two types of repair patches were observed. The majority of the repair sites were relatively short, ranging from 13 to 25 nucleotides. A minor class of repair patches (representing 2 to 5% of the total repair sites) were observed that contained 1,600 to 2,000 nucleotides inserted per repair site (109). These long tracts of repair synthesis are SOS dependent and do not appear to be due to polymerase I incorporation (44, 76). It has been suggested that these long repair patches are caused by recombinational type repair.

Using a defined system containing purified proteins, Caron et al. found that the average number of nucleotides inserted in each repair site was 12.4 (34). Using a defined double-stranded substrate that contained a psoralen adduct in the polylinker region of M13mp19, Van Houten et al. (252, 253) were able to physically map the size of the repair patch following incision by the UvrABC complex. In 83% of the patches, DNA polymerase simply filled in the gap that was created by the excised oligomer. The other 17% of the repair patches had label distributed over an additional 33-bp region. No patch could be detected that was longer than 45 nucleotides.

MODEL OF NUCLEOTIDE EXCISION REPAIR

Having described the details of the five basic steps of *E. coli* nucleotide excision repair, it is worthwhile to give a brief overview of the entire process as it is believed to occur in vitro (Fig. 2). The last section of this review examines several important issues regarding the nature of *E. coli* nucleotide excision repair as it may occur in vivo.

Damage Recognition

Prior to the damage recognition step, the UvrA and UvrB subunits form an ATP-dependent $UvrA_2B$ complex. Although we do not presently know the exact requirements for this damage recognition, it is believed that the specificity is obtained by binding to an altered DNA conformation that is induced by physical or chemical DNA-damaging agents. This specific binding is believed to be mediated by the UvrA zinc fingers (Fig. 1).

Formation of the Preincision Complex

The $UvrA_2B$ complex binds to nondamaged DNA in a transient manner, causing localized unwinding. The $UvrA_2B$ complex interacts with the damaged site in a manner that is not clearly understood. This interaction leads to the dissociation of the UvrA dimer and the formation of a very stable UvrB-DNA preincision complex. It is believed that the DNA in this preincision complex is unwound and in a strained conformation. The released $UvrA_2$ complex is now free to bind to another molecule of UvrB, which can be loaded onto a new site of DNA damage. It appears that the hydrolysis of the nucleotide cofactor, ATP, is absolutely required for the formation of the preincision complex.

Incision of the Phosphate Backbones

The UvrB-DNA complex represents a binding site for the UvrC protein. Hydrolysis of two phosphodiester bonds

occurs once the UvrC protein binds to the preincision complex. ATP does not appear to be required for this step. The catalytic centers or the mechanism of action for these cleavage reactions are not known.

Excision, Repair Synthesis, and Release of the Postincision Complex

It is believed that the postincision complex cannot turn over in the absence of other protein factors. In vitro, both helicase II and DNA polymerase I are required for the production of catalytic incisions by the UvrABC complex, presumably by releasing the excised oligomer and the UvrB and UvrC subunits. In the majority of the repair patches, DNA polymerase appears to fill the excised region with 12 nucleotides.

DNA Ligation

Although several polymerases have been shown to perform the gap-filling step and allow the turnover of the subunits, only DNA polymerase I will add sufficient nucleotides that the repair patch is sealable by DNA ligase. In the absence of DNA ligase, DNA polymerase will perform extensive nick translation, although the large repair patches observed in *E. coli* are probably due to recombinational events.

REPAIR OF PSORALEN CROSS-LINKS

Unlike the repair of DNA lesions that involve only one strand, lesions that covalently join the two strands of the DNA helix represent a special challenge to the *E. coli* repair system.

Uvr-Dependent Recombinational Repair

Prior to the isolation and characterization of the UvrABC subunits, several groups examined the repair of psoralen cross-links in *E. coli* by using a variety of biochemical and genetic approaches (36, 42, 43, 119, 134, 161, 217, 218, 280, 281). They found that the repair of psoralen-cross-linked DNA required ATP, functional UvrA, UvrB, UvrC, RecA, UvrD, and DNA polymerase I, as well as the presence of multiple genomes. On the basis of these results, Cole proposed a model for the repair of psoralen cross-links (42). This incision-recombination-incision model predicted that the UvrABC complex will incise the psoralen cross-link on one strand (Fig. 3). It was hypothesized that following the first incision, a new copy of the incised strand was brought in through a RecA-mediated recombinational event. The recombination event would result in a three-stranded intermediate in which the psoralen is cross-linked to one strand and an oligonucleotide of undetermined size. The next step of the model suggested that the UvrABC complex performs a second round of incision on the other side of the cross-link, leading to the release of two oligonucleotides which are cross-linked by the psoralen adduct. The gap created by this excision would then be filled by DNA polymerase I, and the newly made repair patch would be sealed by DNA ligase.

This model was remarkable in that all the data were generated from biochemical experiments performed on *E. coli* cells (42). This model of cross-link repair was later modified by Van Houten et al. to incorporate the precise locations of the UvrABC complex incisions on one strand of the psoralen cross-link, a detail that was not known at the time of the earlier experiments (251). The position of the UvrABC nuclease incisions for a psoralen DNA cross-link was determined by using a DNA substrate carrying a 4′-hydroxy-4,5′,8, trimethylpsoralen (HMT) cross-link at a defined position. It was shown that the UvrABC complex incised the psoralen cross-link on only one strand, with an atypical incision pattern in which the ninth phosphodiester bond 5′ and the third phosphodiester bond 3′ to the furan-modified thymine were hydrolyzed (251). Jones and Yeung have extended these studies and examined the UvrABC nuclease incision patterns of psoralen cross-links in several different sequences (94). While confirming the earlier experiments of Van Houten et al. (251), they also showed that furan-side incisions do not always occur and that the pyrone side of the cross-link is sometimes incised. No double-strand breaks were observed, indicating that the UvrABC complex does not incise both strands of the psoralen cross-link (94, 251).

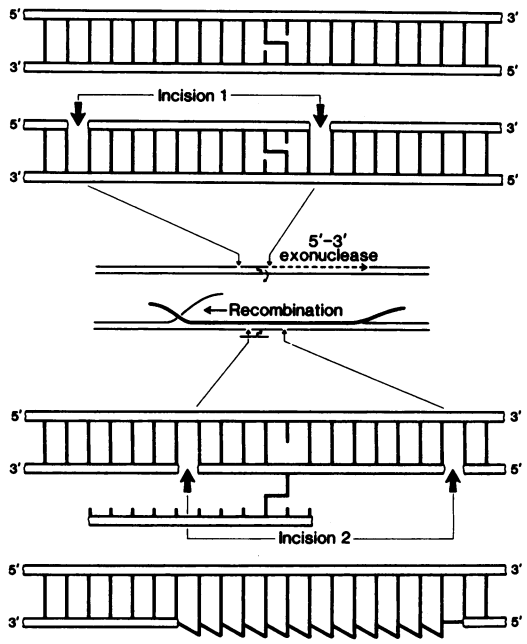

FIG. 3. Model of DNA interstrand cross-link repair. The incision-recombination-incision model of Cole (42) has been expanded to incorporate the exact sites of UvrABC nuclease incisions in DNA containing a psoralen cross-link (94, 251). It has been proposed that following the first set of incisions, the 5′-to-3′ exonuclease activity of DNA polymerase I will make a large gap 3′ to the cross-linked site. This gap is a site for RecA-mediated recombination (221). The invading strand (heavy line) displaces the cross-linked incision product. Following the recombination step, the UvrABC complex is free to incise the other strand of the cross-link (39, 40, 221). The dual action of DNA polymerase and helicase II leads to the release of an 11-mer or 12-mer cross-link concomitantly with repair synthesis and the turnover of the Uvr subunits (39, 40, 221). The repair patch is sealed by the action of DNA ligase.

It was important to determine whether the UvrABC nuclease complex will incise the triple-stranded intermediate that is formed after the recombination step. In a recent study, Cheng et al. (39, 40) found that (i) the RecA protein was able to catalyze the formation of the three-stranded complexes that contained psoralen-modified oligonucleotides which could be covalently attached by irradiation with UV light; (ii) the UvrABC complex incised three-stranded structures consisting of cross-linked oligomers of 30 to 107

nucleotides; (iii) the addition of helicase II, DNA polymerase I, and deoxynucleoside triphosphates to the UvrABC complex resulted in the release of a 12-mer cross-linked to the original oligomer; and (iv) this complete system was able to repair a triple-stranded structure containing psoralen cross-links, so that it could be incised by the *Kpn*I restriction enzyme.

Once it had been proved that two rounds of UvrABC incision could occur as described in the model, the remaining challenge was to show that a RecA-mediated recombination could fill the gap created by the first incision. This step was worked out by Sladek et al. (221), who used a double-stranded circular substrate that resembled the product of the first UvrABC nuclease incision. They found that this substrate did not lead directly to strand exchange, but that an additional step was needed. It was shown that DNA polymerase I could bind to the 3′ incision site and, through its 5′-exonuclease activity, create a long single-strand gap so that RecA could initiate strand exchange 3′ to the incised cross-link site. Strand exchange could then proceed past the incised cross-link, displacing the oligomer that is covalently attached to the other strand, thus forming a three-stranded intermediate. It remains to be seen whether RecA can mediate strand exchange through a UvrABC postincision complex.

Uvr-Independent Pathway

Over the years, several studies have appeared that suggest that psoralen adducts, including DNA cross-links, might be repaired in a manner different from that described above (1, 23–27, 47, 224, 284). Bridges and Von Wright have shown that the *uvrA rep* double mutant is more sensitive to the killing effects of psoralen cross-links than is the *uvrA* mutant alone (27). Since a *recA rep* double mutant was no more sensitive than either of the single mutants, it was suggested that the Rep protein, a DNA helicase, might work in conjunction with RecA to make the psoralen cross-link more accessible to other repair enzymes, such as a glycosylase. Using the toxicity of acriflavine as a measure of repair, Bridges et al. went on to show that the repair of cross-links also occurred in *uvrA*, *uvrA uvrB*, and *uvrA polA* mutants (23, 24). In an attempt to ascertain whether this repair of cross-links might be error prone, mutation frequencies of the *trp* gene were determined in a *uvrA* mutant that had been treated with psoralen plus UV light and then a second dose of UV light to induce cross-links. Under these conditions, a 12-fold increase in the mutation frequency for the psoralen cross-links was observed, suggesting that the residual cross-link repair in a *uvrA* mutant was indeed error prone.

The mutagenesis of psoralen-DNA adducts that have been targeted or site-specifically placed into DNA has been recently examined (155, 167, 168). Saffran and Cantor used mercurated nucleotides and sulfhydryl-containing psoralen to target monoadducts and cross-links in the *tet* gene of pBR322 (167, 168). In an initial study, they found that both repair and mutagenesis were dependent upon prior exposure to UV light (SOS induction). Using a negative *tet* selection system, they found that the mutations were primarily transitions, although some transversions and single-base deletions were also observed (167). They extended this initial study using colony hybridization techniques which can score mutations that are unconstrained by phenotypic changes (168). All the mutations, except one, scored in this manner could be attributable to a potential cross-link site. Transitions were more frequent than transitions at each site (occurring at a ratio of 26:7). Using the *lacI* system that had been randomly modified with 8-methoxypsoralen, Yatagi et al., while finding a similar pattern of mutagenesis, observed a significant number of single-base deletions (275). Piette et al. were able to introduce a HMT monoadduct or cross-link into the *Kpn*I site of the polylinker region of closed-circular double-stranded M13mp19 (155). Using these substrates, they could determine the types of mutation induced by the lesions. The most common mutation was a deletion of the modified thymine, and T · A to G · C transversions were the second most frequent. Unexpectedly, it was found that the HMT cross-link, while being highly toxic, had an infectivity of 1.6% compared with the control. Although part of this survival could be attributed to contaminating monoadduct, it was proposed that *E. coli* might possess an alternative error-prone repair pathway that does not involve homologous recombination Yatagi et al. have suggested a pathway in which gap filling by DNA polymerase I assisted by helicase II can substitute for the recombination step (275).

Bridges and Stannard have also presented data for the repair of psoralen monoadducts that is Uvr-independent and is decreased by a *polA* mutation (26). Ahmad and Holland (1) have isolated a strain of *E. coli* that shows enhanced resistance to treatment with psoralen plus UV light. Resistance in this strain is not due to altered psoralen uptake. This strain was found to overproduce a 55-kdal protein. On the basis of survival data, Zhen et al. (284) have proposed a novel glycosylase activity for the repair of cross-linked psoralen adducts. Sladek et al. have isolated a novel enzymatic activity from *uvr E. coli* that acts only on psoralen monoadducts, leads to incisions of supercoiled DNA, is Mg^{2+} independent, and leads to the release of labeled psoralen (F. M. Sladek, B. Dynlacht, and P. Howard-Flanders, J. Cell Biochem. Suppl. **12a:**276, 1988). It will be of interest to determine whether the activity characterized by Sladek et al. can be attributable to Ahamd's 55-kDa protein (1). A Uvr-independent activity for the repair of cisplatin adducts has also been proposed (68).

WHAT IS GOING ON IN VIVO?

Factors Influencing Nucleotide Excision Repair

The availability of purified UvrA, UvrB, and UvrC subunits, as well as DNA polymerase I and the UvrD protein helicase II, allowed the reconstitution of the entire process of nucleotide excision repair in the test tube, with rates of incision within a factor of 5 of the estimated in vivo rate (91, 230). An area of research that must be pursued is the careful analysis of factors within the intact cell that might alter the activity of the UvrABC complex. This line of inquiry falls into at least three categories: the effects of (i) local and global DNA structure, (ii) other protein factors, and (iii) transcription on the rate and extent of UvrABC nuclease incision. Furthermore, this type of research on *E. coli* will form a basis for our understanding of analogous processes in eucaryotic cells and will serve as a paradigm for formulating experimental designs in these organisms.

Recently, Gruskin and Lloyd have reported on a novel method to determine the molecular events of nucleotide excision repair in vivo (74, 75). With this approach they examined the fate of UV-induced pyrimidine dimers in the endogenous plasmid, pBR322. They found that UvrABC-initiated excision repair proceeds by a "limited processive" mechanism in which a majority of the dimers are repaired prior to the dissociation of the UvrABC complex. This

model is not entirely consistent with the report by Orren and Sancar which suggests that the $UvrA_2$ complex dissociates during the formation of the preincision complex (149). It remains to be determined whether the Uvr subunits can bind to a second damage site without undergoing macrodissociation following the postincision events. Interestingly, Gruskin and Lloyd were also able to show that repair occurred without a detectable accumulation of DNA strand breaks or the loss of superhelicity (75). The authors suggest that these data provide evidence that damage recognition is the rate-limiting step in nucleotide excision repair and that once incisions occur, resynthesis and ligation proceed quite rapidly (see also reference 3a).

Effects of Alternative DNA Structure

Two types of structural alterations that could affect the activity of the UvrABC complex are sequence-specific alterations and torsional stress induced by DNA supercoiling.

Sequence. It has been shown that the position and extent of incision by the UvrABC complex for UV-induced photoproducts are sequence specific (94, 137). The local sequence could affect damage recognition in at least two ways. The conformation of the damaged site will most certainly be affected by the local sequence surrounding the site (48, 52, 203). Part of the sequence context of DNA damage recognition could be in the localized variations in the DNA helix. Damage-induced alterations in the conformation could be amplified or suppressed depending on the sequence context. Second, if localized unwinding is necessary for the formation of the preincision complex, more energy would be necessary to melt into a run of G · C than of A · T base pairs. It takes more than twice the amount of energy to disrupt a G · C base pair (3.3 kcal/mol [13.8 kJ/mol]) than an A · T base pair (1.4 kcal/mol [5.9 kJ/mol]). The $UvrA_2B$ complex could melt into an A+T-rich region more easily than into a G+C-rich region. Therefore, DNA damage that is buried in a particular sequence could be recognized with different affinities. Resolution of this problem will require analysis of site-specific substrates in which the neighboring bases are systematically changed. Ideally, it would be of great benefit to perform these types of studies in conjunction with physical measurements such as two-dimensional NMR (38, 140, 151).

Stabilization of the helix in the vicinity of the damage could help explain the following observations.

(i) Recognition of psoralen adducts. The furan side of a psoralen-DNA interstrand cross-link is incised more frequently than the pyrone side (94, 221, 251). NMR studies have suggested that the DNA helix adjacent to the furan side has a thermostability of about 20°C less than the helix adjacent to the pyrone side (239). It might be expected that attack from the 5′ side of the cross-link (the furan side) would be enhanced by the lack of stability in this region, whereas 3′ attack of the complex (from the pyrone side) would be less likely, since this section is more stable. It has also been observed that incisions at a psoralen cross-link occur at the ninth phosphodiester bond 5′ and the third phosphodiester bond 3′ to the modified thymine (94, 251). Since the two strands of the cross-link are covalently linked and therefore cannot be completely melted, it might be expected that the preincision complex could not be accommodated in the same manner as an adduct involving only one strand. Jones and Yeung have also shown that the extent and position of the incision sites of psoralen monoadducts are quite sequence dependent (94). Their data strongly suggested that on rare occasions the UvrABC complex might actually incise the nondamaged strand which is adjacent to a psoralen monoadduct (94).

(ii) Recognition of anthramycin adducts. It has been noted that digestion of DNA containing anthramycin–*N*-2-guanine adducts results in an atypical incision pattern, in which cleavage occurs at 5 or 6 bases 5′ and 3 or 4 bases 3′ to the modified site (267). This altered incision pattern observed with the UvrABC complex could be due to the increase in helix stability induced by the anthramycin adducts (71). It would be interesting to determine whether the Uvr preincision complex that is formed at an anthramycin adduct has an altered DNase I footprint compared with the footprint observed with a psoralen monoadduct.

DNA topology. The DNA of *E. coli* is under negative superhelical stress (45): it is in an underwound state. Since it has been shown that binding of the UvrAB complex to DNA causes a localized unwinding, it might be expected that the UvrAB complex might bind more efficiently to supercoiled DNA. Although this hypothesis has not been addressed directly, several experiments have shown that the topological state of the DNA can affect the rate of damage recognition and incision.

(i) In vivo experiments with mutants. In *E. coli*, the topology of the DNA is regulated by the opposing activities of two major topoisomerases, DNA topoisomerase I and DNA topoisomerase II (DNA gyrase). Topoisomerase I can relax both negatively and positively supercoiled molecules and DNA gyrase and can also induce negative supercoils in an ATP-dependent manner (reviewed in reference 67). These two antagonistic activities maintain the *E. coli* DNA in a negative supercoiled state. It might be expected that inhibition of either of these activities by drugs or mutations could affect the nucleotide excision repair pathways. It has also been shown that UV-induced pyrimidine dimers inhibit the activity of *M. luteus* topoisomerase I (153). Several studies have been reported which examined the role of DNA topology in nucleotide excision repair. Hayes and Boehmer (80) showed that the DNA gyrase inhibitors coumermycin and oxolinic acid blocked repair and reduced recombination of UV-irradiated lambda phage. It has been shown that mutations in either the *gyrA* or *gyrB* genes led to increased sensitivity to the killing effects of UV light (46). These studies were extended by Von Wright and Bridges (263), who showed that a mutation in the *gyrB* gene resulted in a decrease in the negative superhelical density of *E. coli* DNA and that although the *gyrB* mutation led to an increase in UV survival in a *recA* host, the *gyrB* mutation had no effect on a *recA uvrA* double mutant. These authors concluded that following UV irradiation, the topology of the DNA was important for postreplication repair but not for UvrABC-mediated nucleotide excision repair.

(ii) In vitro experiments. Recent experiments suggest a role for torsional stress in the recognition of specific DNA adducts. For example, it was found that *trans*-platinum-guanine adduct could be recognized efficiently by the UvrABC nuclease complex only when present in supercoiled plasmid DNA, and not in linear DNA fragments (6), whereas the *cis* isomer was recognized equally well in both types of DNA. Walter et al. have shown that the anticancer drug anthramycin is recognized much more efficiently in relaxed DNA than in supercoiled DNA (267). Lambert et al. (113) have shown that the bis-intercalator ditercalanium is incised by UvrABC much more efficiently in negatively supercoiled than in relaxed plasmid. In the same study, it was also shown that recognition of UV-induced photoproducts was not significantly different in either supercoiled or

relaxed plasmids. Since DNA is under negative superhelical stress within the *E. coli* cell, it is important to understand the contribution of this factor to the interaction of the UvrABC complex with damaged DNA.

Role of UvrB Proteolysis in Down Regulation of the SOS Response

As mentioned previously, UvrB has been found to undergo proteolytic cleavage to form UvrB*. Caron and Grossman have examined some of the in vitro properties of the UvrB* protein (32, 33). Using filter-binding assays, they have found that unlike the normal UvrB protein, which forms a tight complex with damaged DNA in the presence of the UvrA protein, UvrB* protein dissociation is considerably faster and the addition of UvrC to the UvrB*-DNA complex results in the rapid dissociation of the Uvr-DNA complex (32). The UvrAB complex has been shown to have altered ATPase activities, as well as DNA-unwinding activities. Both of these properties are lost in the UvrAB* complex. The role of UvrB proteolysis is unknown, but several *E. coli* proteins undergo proteolysis, including the Ada protein, which, like the UvrB protein, is damage inducible. It has been suggested that one role for proteolysis of the UvrB protein might be to reduce the excess levels of the UvrABC complex following the SOS response or to allow the turnover of the UvrABC complex (32, 33). Caron and Grossman also characterized a membrane-bound protease which cleaves the UvrB protein and found that it is under the control of the *htpR* gene, which codes for a sigma factor and directs transcription from unique promoters during the heat shock response (32).

Intracellular site of DNA Repair

Based on the total concentration (10 to 20 nM) of UvrA (as monomer) within the cell, its nonspecific binding affinity for DNA, and the total DNA concentration within the cell, it could be expected that very little if any UvrA would be free within the cell. The question arises of how the UvrA protein can direct the binding of the UvrB subunit to specific damaged sites in a vast excess of nonspecific binding sites. The DNA of *E. coli* appears to be highly organized with the cell. It is conceivable that the Uvr proteins are partitioned in such a way as to facilitate more rapid and efficient scanning of the DNA. It has been suggested that DNA replication might occur on the surface of the cellular membrane (81), and indirect evidence suggests that the Uvr proteins might also be membrane bound (237, 238). The UvrB subunit is a hydrophobic protein, which is cleaved by a membrane-bound protease (32, 33). Yonei and co-workers, in a series of papers, have shown that membrane-disrupting drugs such as lidocaine, procaine, and phenethyl alcohol inhibit nucleotide excision repair (237, 238, 240). Phenethyl alcohol, which clearly disrupts the DNA-membrane association, was shown to inhibit the incision step, whereas procaine, which does not inhibit the membrane-DNA associations, was shown to inhibit a later excision or resynthesis step (240). Both drugs greatly reduce the removal of pyrimidine dimers. Although disruption of the cell membrane fluidity would be expected to have pleiotropic effects on the cell, its direct effect on nucleotide excision repair certainly warrants more research on whether specific steps of nucleotide excision repair do indeed occur in conjunction with the cell membrane.

Ancillary Proteins Involved in Nucleotide Excision Repair

Photolyase. UV-induced pyrimidine dimers in *E. coli* can be repaired by two separate enzymatic pathways: nucleotide excision repair and photoreactivation. As mentioned in the previous section, the UvrABC nuclease complex will incise DNA-containing pyrimidine dimers at a higher rate if the dimers are first bound by DNA photolyase (173). Overexpression of DNA photolyase was found to lead to more rapid dark repair in vivo (267). This observation is important since it demonstrated that (i) two different enzymatic repair systems will cooperate to remove a particular DNA lesion and (ii) a specific DNA lesion, when bound by a protein, is still a substrate for the UvrABC complex. Recently, in the study of human repair proteins, Chu and Chang have shown that XP cells from complementation group E lack a protein factor which binds to pyrimidine dimers (41). These observations suggest that pyrimidine dimers in both bacterial and eucaryotic cells may be repaired by repair proteins which act as "damage antennae," marking the site for incision by the repair endonuclease.

Transcription and DNA repair. Bohr et al. have shown that in mammalian cells, the repair of UV-induced photoproducts is faster in genes that are actively transcribed than in sequences of DNA that are not expressed (15). Terleth et al. have found that the mating type gene that is actively transcribed in the yeast *Saccharomyces cerevisiae* is repaired faster than the same gene in a nontranscribed conformation (232). Recent repair studies of the *E. coli lacI* gene have also shown that induction of a gene leads to higher rates of repair (I. Mellon and P. Hanawalt, submitted for publication). These observations raise many important questions regarding the effects of transcription on DNA repair. At least three non-mutually exclusive explanations can be offered: (i) RNA polymerase antenna model, (ii) twin supercoiling domain model, and (iii) RNA-DNA heteroduplex model (221a) (Fig. 4).

(i) RNA polymerase antenna model. In the discussion of the interaction of the UvrAB complex with DNA, several parallels were drawn with the interaction of RNA polymerase with a promoter. These similarities may be more than just coincidental. Mellon et al. (129) have found that the enhanced repair in expressed genes could be attributed to efficient repair of only the transcribed strand. This strand specificity has also been observed in the gene-specific repair of *E. coli* (128a). One hypothesis which has been suggested to explain this observation is that DNA damage on the transcribed strand is an absolute block to RNA polymerase, whereas lesions on the other strand are not. It has been suggested that this lesion-stalled RNA polymerase acts as a damage antenna for the repair complex. The DNA in the transcription bubble is in an open state which might facilitate the formation of the preincision-UvrB complex. Alternatively, the preincision complex could be mediated by direct protein-protein interactions. Shi et al. have found that a psoralen-DNA adduct inhibited transcription by *E. coli* RNA polymerase (211).

(ii) Twin supercoiling domain model. Liu and co-workers have shown that passage of RNAP through a gene leads to two waves of superhelical tension in the DNA; a positive supercoil wave in front of the transcription complex and a negative supercoil wave following behind the RNA polymerase complex (242, 272). Since DNA topology has been shown to influence the UvrABC complex incision efficiency (see above), it is reasonable to suggest that these localized supercoiled waves could influence the repair of DNA lesions. To date, no systematic study of the effect of negative or positive supercoiling has been reported.

(iii) RNA-DNA heteroduplex model. The exact conformation of the DNA in the transcription bubble is not known, although at least 12 nucleotides are base paired with the

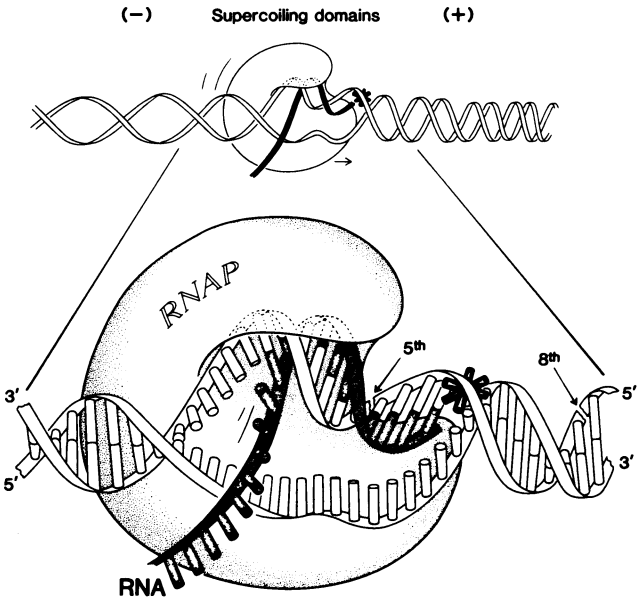

FIG. 4. Role of transcription in nucleotide excision repair. There are three possible modes of interaction between a transcription complex and DNA repair proteins: (i) twin supercoiling domains, (ii) RNA-DNA hybrid, and (iii) stalled RNA polymerase (RNAP). See text for a complete description. (Top) Motion of RNA polymerase through a transcribing region generating positive supercoil waves ahead of and negative supercoil waves behind the complex (241, 272). (Bottom) Enlarged view of the stalled RNA polymerase molecule (211) at the site of the DNA damage (shown as a distorted base). It is not known whether the UvrABC complex will incise the damaged strand while RNA polymerase is still bound. However, if the stalled transcription complex remains stably bound, the 3′ incision site of the UvrABC complex would occur in the RNA-DNA hybrid as shown.

growing RNA chain (260). Since the conformation of a DNA-RNA hybrid molecule is more A form than a DNA-DNA duplex, it might be expected that a DNA lesion in close proximity to this RNA-DNA hybrid junction would be in an altered conformation.

Each of these models can be tested experimentally in defined systems by using purified proteins and defined DNA substrates. It is important to point out that mammalian gene-specific repair might be limited to specific types of DNA damage. Gene-specific repair has been shown only for UV-induced pyrimidine dimers, and 6,4-photoproducts (15, 236). Recently it has been shown that chemical damage, such as aminofluorene–C-8-guanine adducts, is not repaired in a gene-specific manner (227).

Other protein factors. As mentioned above, studies have clearly shown that a genetic alteration in the *uvrA*, *uvrB*, and *uvrC* genes greatly diminishes or completely inhibits the repair of pyrimidine dimers, whereas mutations in the *polA* and *uvrD* genes greatly decreases both the rate and extent of repair. Genetic studies (3, 17, 110, 122, 141, 162, 213, 220, 226, 257) have implicated other gene products as playing a role in the repair of certain types of DNA damage (reviewed in reference 76). The RecA protein plays an important role in the repair of UV dimers by several pathways, including recombination repair and the SOS response. Lu and Echols have shown that activated RecA protein actually binds to pyrimidine dimers (124).

As mentioned above, genes that affect the superhelical state of the DNA within the cell also affect survival after UV irradiation. These include genes coding for the topoisomerase I and DNA gyrase (46, 263). The topology of *E. coli* DNA is also affected by how the DNA is packaged within the cell. The DNA of *E. coli* is organized into nucleosome-type structures composed primarily of DNA-binding protein II, the HU protein (146). Several other proteins that share homology with the HU protein have been identified (53). One such protein is the IHF (integration host factor) protein. Both the HU and IHF proteins have been shown to have important effects on DNA replication, recombination, and transcription (53, 63). The conformation of DNA is altered when bound by HU protein. It is highly likely that DNA damage would behave differently when the DNA was bound by the histonelike proteins of *E. coli* (29). No systematic UV survival studies on strains carrying mutations in the genes encoding these proteins have been reported. The effects these proteins might have on UvrABC complex in vitro are not known.

Role of the Uvr Proteins in Other Cellular Processes

Direct evidence. Genetic studies have indicated that both *polA uvrB* and *polA uvrD* double mutants are inviable (133). More recent studies demonstrated that the *uvrA polA12*(Ts) combination was not viable at the restrictive temperature, although a *uvrC polA12* was viable at the restrictive temperature (115). These findings suggest that the *uvrA*, *uvrB*, and *uvrD* gene products, either separately or in a concerted manner, perform some function that, in the absence of polymerase I activity, is essential for viability. Since the *uvrB* gene has been shown to contain a DNA-binding site for the DnaA protein, it has been suggested that the UvrB protein might be involved in DNA replication, as discussed in the section on the *uvrB* gene (61).

Indirect evidence. If the UvrB-DNA complex is fairly long lived in vivo, it might be expected that it would interfere with transcription or DNA replication through this region. Recently, Brouwer et al. have found that cisplatin-induced mutations in *E. coli* are dependent upon the presence of the UvrA and UvrB protein (28). The spectrum of these mutations was consistent with the major lesion being an intrastrand GG adduct (30). One possible interpretation of these data is that the replication fork can proceed past a cisplatin lesion that is bound by the UvrAB complex in what would be a mutagenic process. The effect of the Uvr proteins on DNA replication has also been suggested as a reason for the lethality of AF-G adducts. In *E. coli*, AAF-G adducts, but not AF-G adducts, are blocks to replication. It is also interesting that AF-G adducts do not trigger the SOS response, whereas AAF-G adducts do (169). It was first observed that AF damage was lethal to *uvrC* mutants, but not to *uvrB* or *uvrA* mutants (229). This observation was first interpreted as evidence that only the *uvrC* gene product was involved in the repair of AF-G. Later in vitro studies with purified proteins indicated that all three proteins were necessary for the incision of DNA containing AF-G adducts (174). It was then found that AF-G adducts were not lethal in the *urvA uvrC* double mutant, but were lethal in the *uvrB45 uvrC* double mutant, although they were not lethal in a strain carrying a *uvrB* deletion (14). One way to reconcile these data is by assuming that the AF-G lesion does not become lethal until it is bound by the UvrAB complex. If the *uvrA* gene is mutated or if the UvrB protein is absent (as in a deletion), the UvrABC complex cannot form and replication can proceed normally. This model would predict that the *uvrB45* mutation allows for the expression of a mutant protein that interacts with the UvrA protein and forms an inactive complex at the site of the damage.

Potential Interaction of Proteins from Different Repair Pathways

In *E. coli*, several enzymes are important for the repair of oxidative DNA damage. These enzymes include endonuclease III, endonuclease IV, and exonuclease III, encoded by the *nth*, *nfo*, and *xth* genes, respectively (reviewed in reference 266). It has been recently shown that the triple mutant containing the *uvrA nfo xth* mutation may be inviable (185). These data suggest that (i) normal cellular activities generate sufficient superoxide to kill the cell if there are no repair pathways that can remove oxidative DNA damage, and (ii) the UvrABC complex may be involved in the removal of specific oxidative DNA damage that would otherwise be lethal to the cell. This hypothesis is supported by recent studies that have shown that the UvrABC nuclease complex can incise DNA containing thymine glycols, a lethal form of oxidative DNA damage (118; Kow et al., in press). In addition, transfection experiments indicate that the survival of φX174 phage containing thymine glycol residues is much lower in the *uvrA nth* double mutant than in either of the two single mutants alone (Kow et al., in press).

The observations that the UvrABC complex (i) acts on relatively nondistorting DNA damage, such as *O*-6-methylguanine (259), (ii) may in fact inhibit the action of alkytransferases (37, 170), and (iii) acts on oxidative lesions, thymine glycol, or apurinic sites (118; Kow et al., in press) (which are removed by other repair pathways [266]) raise an interesting hypothesis. The UvrABC proteins may cooperate with or antagonize the action of other repair proteins in the removal of specific DNA lesions. This idea is testable, since many of these proteins and their DNA substrates have been purified to homogeneity and exist in large amounts for biochemical study. For example, it will be of interest to see whether O^6-methylguanine, when bound by the UvrAB complex, is indeed a substrate for O^6-alkyl transferase I. Thus, the study of *E. coli* can serve as an important model for the elucidation of multiprotein interactions involved in DNA damage recognition and repair. In this regard, it is interesting that pBR322 containing thymine glycols, when mixed with the UvrA and UvrB subunits, was still a substrate for endonuclease III and endonuclease IV (118). However, no evidence was given that the UvrA and UvrB subunits actually bound to thymine glycols under the conditions used in these experiments.

HOW WIDESPREAD ARE UvrABC-LIKE ENZYMES?

Bacteria

The UvrABC nuclease complex is a generalized repair system capable of recognizing a broad spectrum of DNA damage. The question arises of whether a homologous repair system exists in other organisms. *M. luteus* contains a very active endonuclease which acts on pyrimidine dimers. Somewhat unexpectedly, it was found that UV-sensitive mutants still retained this activity and mutants lacking this activity were UV sensitive (138). These UV-sensitive mutants were also sensitive to the killing effects of mitomycin C and 4-nitroqunioline oxide, which is reminiscent of the *E. coli uvr* phenotype. *M. luteus* apparently has two different pathways for the repair of UV-induced DNA damage, one mediated by the UV endonuclease and one mediated by a nucleotide excision repair pathway. Using the two excision repair-deficient strains DB7 and UV^sN1, Nakayama and Shiota cloned and sequenced two genes that complemented these defects (138, 214, 215). The genes that corrected the phenotypes in the DB7 and UV^sN1 mutant strains were found to be homologous to the *E. coli urvA* and *uvrB* genes, respectively. When optimally aligned, the predicted amino acid sequence of each protein had more than 50% identity with the UvrA and UvrB proteins. At present, no *uvrC* homology has been identified. It will be interesting to determine whether these *M. luteus* proteins could replace the UvrA or UvrB proteins in an in vitro reaction.

E. coli DNA Repair as a Paradigm for Eucaryotic DNA Repair

Genetic studies suggest that protein complexes which have broad substrate specificity, such as the UvrABC complex, exist in eucaryotic cells such as yeast and human cells (reviewed in reference 60). Remarkably, recent cloning and sequencing of both yeast and human DNA repair genes indicate that these gene products have significant homology with the Uvr proteins, and biochemical data suggest that they have similar functions (78, 216, 248, 249, 269, 271). For example, the yeast RAD3 protein has strong homology with both the UvrA and UvrD proteins in that it has an ATP-binding site (158). The RAD3 protein has been shown to have ATPase activity and function as a helicase (225). More recently, the human repair gene *ERCC-2* has been cloned and sequenced, and its product displays a 50% identity to the RAD3 protein (269; E. Freidberg, C. Weber, and L. Thompson, personal communication). Therefore, studies of the repair processes in *E. coli* help to formulate working models which serve as a basis for understanding these events in higher organisms. However, as stated by Walker et al. (265), we must be careful not to draw too close a parallel between procaryotes and eucaryotes. The inability of the UvrA, UvrB, UvrC, and UvrD proteins to restore UV-induced unscheduled DNA synthesis in xeroderma pigmentosum cells seems to indicate that procaryotic proteins cannot complement a defect in human cells (285).

CONCLUSIONS

Research into *E. coli* nucleotide excision repair has progressed rapidly in the last few years. The purpose of this review was to examine the major achievements in *E. coli* nucleotide repair through October 1989. Although I have tried to be as thorough as possible, there are sure to be some omissions, for which I apologize in advance. It was my intent not only to summarize the current state of knowledge, but also to point out some of the important differences, unresolved problems, and sometimes controversial findings that have arisen in the field.

Studies of the molecular nature of *E. coli* nucleotide excision repair have allowed the development of new approaches and techniques that will certainly be applicable to the study of similar processes in eucaryotes. Although recent in vitro experiments, with highly purified proteins and defined DNA substrates, have been successful in reconstituting the entire process of nucleotide excision repair, many important questions remain regarding the action mechanism of the UvrABC nuclease complex. In particular, it is important to remind ourselves that events which occur inside the cell are most certainly more complex than the conditions which we can duplicate within the sterile environment of the test tube. An understanding of *E. coli* repair processes serves as a conceptual framework for understanding similar processes in eucaryotes, including humans. However, DNA repair processes in eucaryotic organisms, although similar to

their procaryotic counterparts, are sure to have their own peculiarities. Although *E. coli* nucleotide excision repair has been fully reconstituted in vitro with proteins purified, much less is known about how these processes occur in vivo or about the enzymology of the similar events in mammalian cells. Recent reports suggest exciting advances in both these directions (41, 78, 128a, 129, 216, 271; Mellon and Hanawalt, submitted). Furthermore, the recent findings that gene-specific repair occurs in both *E. coli* and eucaryotes gives strong impetus to the idea that *E. coli* may serve as a paradigm for the study of nucleotide excision repair in eucaryotes.

Finally, by taking stock of our current knowledge and speculating on where the science may lead, it was my hope to stimulate research and generate more discussion in this most fascinating field.

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