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DNA EXCISION REPAIR

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ABSTRACT

In nucleotide excision repair DNA damage is removed through incision of the damaged strand on both sides of the lesion, followed by repair synthesis, which fills the gap using the intact strand as a template, and finally ligation. In prokaryotes the damaged base is removed in a 12–13 nucleotide (nt)–long oligomer; in eukaryotes including humans the damage is excised in a 24–32 nt–long fragment. Excision in *Escherichia coli* is accomplished by three proteins designated UvrA, UvrB, and UvrC. In humans, by contrast, 16 polypeptides including seven xeroderma pigmentosum (XP) proteins, the trimeric replication protein A [RPA, human single-stranded DNA binding protein (HSSB)], and the multisubunit (7–10) general transcription factor TFIIH are required for the dual incisions. Transcribed strands are specifically targeted for excision repair by a transcription-repair coupling factor both in *E. coli* and in humans. In humans, excision repair is an important defense mechanism against the two major carcinogens, sunlight and cigarette smoke. Individuals defective in excision repair exhibit a high incidence of cancer while individuals with a defect in coupling transcription to repair suffer from neurological and skeletal abnormalities.

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INTRODUCTION

A concept central to cancer biology is that mutations arising in oncogenes and tumor supressor genes as a result of replication errors or DNA damage lead to neoplastic transformation of cells (1). Base mismatches that result from replication errors or that occur during recombination are corrected by mismatch repair systems (2, 3). DNA lesions, which are noncoding or miscoding and include all types of base, deoxyribose, and phosphodiester bond modifications, are eliminated from the duplex by DNA damage–repair enzyme systems (3–5).

In general, mismatch and damage repair systems use the same overall strategy to maintain the integrity of genetic information: A mismatched or damaged nucleotide is removed and replaced by the correct and unmodified nucleotide using the intact strand of the duplex as a template. In addition to this basic mechanism, in direct damage repair the chemical bond(s) constituting the damage can be broken to restore the normal nucleotide. This review presents a brief survey of DNA damage repair mechanisms followed by a detailed analysis of nucleotide excision repair. The subject has been reviewed from different perspectives by several authors (5–11a).

DNA REPAIR MECHANISMS

There are three molecular mechanisms for repairing damaged DNA: direct repair, base excision repair, and nucleotide excision repair.

Direct Repair

In the direct repair mode, the abnormal chemical bonds between bases or between a nucleotide and an abnormal substituent are broken. The following are the currently known enzymes that catalyze direct repair.

1. DNA Photolyase (photoreactivating enzyme). This enzyme repairs cyclobutane pyrimidine dimers induced by ultraviolet light by splitting the cyclobutane ring using a light-initiated electron transfer reaction (12, 13). The crystal structure of the *E. coli* enzyme suggests that upon binding to DNA the enzyme flips the pyrimidine dimer out of the duplex into a hole that contains the catalytic flavin cofactor in the center of the protein (14). In

the absence of activating light, photolyase cannot catalyze the splitting reaction and remains stably bound to the damage. By an unknown mechanism this complex increases the rate of dimer removal by nucleotide excision repair both in *E. coli* (4) and in yeast (15, 16). Photolyase also binds to cisplatin-damaged DNA with relatively high affinity (16). However, the effects of these enzyme-substrate complexes on excision repair of cisplatin adducts in yeast and in *E. coli* are different. In yeast, photolyase inhibits excision repair and sensitizes cells to killing by cisplatin (16). In *E. coli,* photolyase stimulates excision repair and enhances resistance to killing by cisplatin (17). The distribution of photolyase in the biological world is erratic. Although photolyase is generally widespread in nature, many microorganisms, including *Bacillus subtilis* and placental mammals, lack photolyase. In contrast, photolyase is found in *E. coli* and other bacteria, and it is abundant in all tissues of some marsupial mammals such as *Monodelphus domesticus* (18–20). Why photolyase is present in internal animal organs has long been a mystery because the chances of dimer formation by exogenous ultraviolet radiation (UV) at these sites are essentially nil. The finding that *E. coli* photolyase stimulates excision of a nondimer lesion indicates that animal photolyases may play a similar accessory role in excision repair.

2. 6-4 Photoproduct Photolyase. This enzyme repairs the second major UV photoproduct by a light-initiated reaction (21). The enzyme has been found in Drosophila, silkworm, rattlesnake, and frog (21–23) but not in *E. coli*, yeast, or humans (21). During the formation of the 6-4 photoproduct, in addition to the C4-C6 bond formation between adjacent pyrmidines, the substitutent at C4 of one pyrimidine migrates to the C6 of the other pyrimidine. Interestingly, enzymatic photolysis reverses both reactions and hence restores the normal bases (21–23). The photochemistry by which this unusual reaction occurs remains to be elucidated.
3. Spore Photoproduct Lyase. UV irradiation of *B. subtilis* spores, which contain highly dehydrated DNA associated with small acid-soluble spore proteins, generates almost exclusively spore photoproduct (5-thyminyl-5,6-dihydrothymine) rather than cyclobutane dimers (24). This lesion is repaired by spore photoproduct lyase which breaks the C-C bond between the two thymines in a light-independent reaction. The enzyme is a 40 kDa protein with no apparent cofactor (25).
4. O^6-Methylguanine DNA Methyl Transferase. This enzyme is present in all species tested. It transfers the methyl group of O^6-methylguanine and (less efficiently) other alkyl groups at this position to a cysteine residue on the enzyme (26–28). The enzyme appears to play an important role in cellular defense against cancers induced by intrinsic and environmental alkylating agents (29, 30).

Base Excision Repair

In this mode of repair, usually, nonbulky DNA lesions such as uracil, thymine glycols and hydrates, N3-MeAde, and 8-oxo-guanine are removed from DNA in two steps. First, a DNA glycosylase releases the base by cleaving the glycosylic bond connecting the base to the deoxyribose. Second, the abasic sugar [apurinic/apyrimidinic (AP) site] is released by the combined actions of AP lyase and AP endonucleases (3, 5, 31, 32). In humans, there are uracil-, thymine glycol-, methylpurine-, and 8-oxoguanine DNA glycosylases with rather narrow substrate ranges and one major AP endonuclease (5, 31). Following removal of the AP sugar, the one-nucleotide gap is filled in to generate a 1–4-nt repair patch (3, 5, 31).

Nucleotide Excision Repair

The damaged base is removed by hydrolyzing phosphodiester bonds on both sides of the lesion. Two excision mechanisms could accomplish this removal. In removal by the endonuclease-exonuclease mechanism, an endonuclease makes an incision at a phosphodiester bond either 5′ or 3′ to the lesion, and then an exonuclease digests the damaged strand past the lesion. In removal by the excision nuclease (excinuclease) mechanism, an enzyme system incises phosphodiester bonds on either side of and at some distance from the lesion. The enzymes work in a concerted manner to excise the lesion in a fragment of relatively precise length.

REPAIR BY ENDONUCLEASE-EXONUCLEASE Two such repair activities are known. In the first, 8-oxoguanine endonuclease (8-oxoG), which was partially purified from human cell free extracts, incises immediately 5′ to 8-oxoG and then, presumably, the modified nucleotide is released by an exonuclease (33). The second involves *Schizosaccharomyces pombe* (*S. pombe*) DNA endonuclease (SPDE) or "UV-induced dimer endonculease," which was first detected in *S. pombe* (34, 35) and then in *Neurospora crassa* (36). The *S. pombe* enzyme is encoded by the *rad12* gene (35), and the partially purified protein can complement mutant cell-free extract in a repair synthesis assay. The *N. crassa* gene (*muts-18*) encoding the enzyme has been cloned and sequenced, and the en- zyme has been purified and characterized. The enzyme is a polypeptide of 74 kDa which cleaves the phosphodiester bond immediately 5′ to both cyclobutane pyrimidine dimers and 6-4 photoproducts. Mutants lacking the enzyme are sensitive to UV but not to UV-mimetic chemicals, suggesting that the activity is specific to these two UV photoproducts (36).

REPAIR BY EXCISION NUCLEASE Damage removal via concerted dual incisions on both sides of the lesion by an ATP-dependent enzyme system with an

essentially infinite substrate range is the most universal form of nucleotide excision repair. This system has been found in all free-living species tested, from the smallest free-living life form *Mycoplasma genitalium* to humans (11). This enzyme system is called excision nuclease (excinuclease), a term that describes its mode of action and acknowledges its uniqueness to repair (37, 38). For historical as well as practical reasons, the nucleotide excision repair process initiated by excision nucleases is referred to as excision repair (31). Therefore, in this review the terms excision repair and nucleotide excision repair are used interchangeably.

Currently, two types of excision nucleases are known. The prokaryotic type removes damage by incising the 8th phosphodiester bond 5′ and the 4th - 5th phosphodiester bond 3′ to the lesion, and hence it excises lesions in 12–13 nt–long oligomers (37, 39). The eukaryotic type incises the 20-25th phosphodiester bond 5′ and the 3rd - 8th phosphodiester bond 3′ to the lesion and thus excises 24–32 nt–long oligomers (38, 40–45). Genetic and biochemical data show the prokaryotic pattern in *E. coli, B. subtilis, M. genitalium, Micrococcus luteus, Streptococcus pneumoniae*, and *Deinococcus radiodurans* [see (10)]. The eukaryotic pattern has been found in humans (38), *X. laevis* (43), *S. pombe* (11a), and *S. cerevisiae* (45). Thus, *S. pombe* has both the endonuclease-exonuclease and the excinuclease modes of excision repair, and mutants defective in general excision repair are still capable of removing both 6-4 and cyclobutane pyrimidine dimers by SPDE (45a,45b).

Following the dual incision by excision nuclease, a protein-free gap does not form. Rather, one or more of the repair proteins remain bound to DNA and are dissociated by replication proteins concomitant with repair synthesis to fill the gap. These processes are followed by ligation. Although the overall strategy of excision repair is quite similar in prokaryotes and eukaryotes, the prokaryotic excinuclease consists of 3 subunits whereas the human excinuclease results from the concerted action of 16 polypeptides. Furthermore, the subunits of prokaryotic excinuclease do not share significant homology with any of the eukaryotic excinuclease subunits. These two systems are discussed in more detail below.

EXCISION REPAIR IN PROKARYOTES

The *E. coli* excision nuclease has been extensively characterized (9, 10, 46). The activity results from the combined actions of three subunits, UvrA, UvrB, and UvrC, and the enzyme is referred to as (A)BC excinuclease. However, as discussed below, a multimeric complex containing all three subunits does not exist. Some of the properties of the three subunits are summarized in Table 1. The three formal steps of excision repair are damage recognition, dual incisions, and repair synthesis and ligation. A model for the entire excision repair reaction is shown in Figure 1.

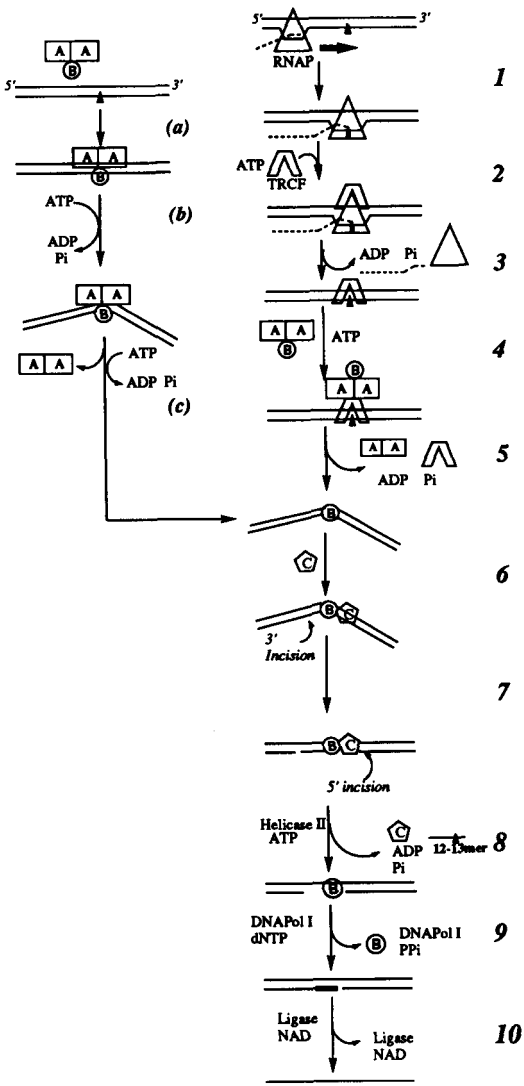
Table 1 The six proteins required for excision repair in *E. coli*

Protein	Mr	Sequence motifs	Activity	Role in repair
a. Excision nuclease subunits				
I. UvrA	$(104)_2$	a) Walker ATPase (2) b) Zinc finger (2) c) Leucine zipper d) UvrA superfamily	a) ATPase b) Damage-specific DNA binding c) UvrB binding d) TRCF binding	a) Damage recognition (Proximal) b) Molecular matchmaker c) TRC
II. UvrB	78	a) Helicase motif b) Homology to TRCF	a) Latent ATPase b) Latent "helicase" c) Damage-specific ssDNA binding d) Binds UvrA e) Binds UvrC	a) Damage recognition (ultimate) b) Unwinding duplex c) 3′-incision
III. UvrC	69	a) Limited homology to UvrB b) Limited (40 amino acid) homology to ERCC1	a) Nonspecific DNA binding b) UvrB binding	a) Induces 3′-incision b) Makes 5′-incision
b. Repair synthesis				
IV. Helicase II (uvrD)	70	Helicase motif	True helicase	Releases UvrC and excised oligo
V. DNA Pol I	103		DNA synthesis	Repair synthesis Displaces UvrB
VI. Ligase	75		Ligase	Ligation

Figure 1 Model for excision repair in *E. coli*. Transcription-independent (left) and transcription-coupled (right) forms are shown: A, UvrA; B, UvrB; C, UvrC; RNAP, RNA polymerase; DNA Pol I, DNA polymerase I; helicase II, UvrD protein; TRCF, transcription-repair coupling factor. In the transcription-independent mode the A_2B_1 complex locates the lesion by tracking along DNA. Locating the lesion is a slow process and is the rate-limiting step of the overall reaction. Once A_2B_1 arrives at a lesion site the reaction proceeds as follows: (*a*) a transient A_2B_1-DNA complex is formed guided by UvrA in an ATP-independent reaction, (*b*) the DNA is kinked and unwound in an ATP-dependent reaction leading to the formation of intimate contacts between UvrB and the damaged strand, and (*c*) the molecular matchmaker (UvrA) dissociates, leaving a stable UvrB-DNA complex. In the transcription-coupled repair, RNA Pol is used as a surrogate damage recognition protein. Upon encountering a lesion RNA Pol stalls (Step 1) and makes a stable complex. Steps 2–5: The stalled complex is recognized by the TRCF, which releases RNA Pol and the truncated transcript while simultaneously recruiting the A_2B_1 repair proteins, helps UvrA load UvrB onto the lesion, and pulls UvrA off UvrB to accelerate the rate of formation of the preincision UvrB-DNA complex. These steps are highly concerted. The subsequent steps of excision repair are identical in the transcription-independent and transcription-coupled modes. Step 6: UvrC binds to the UvrB-DNA complex, and UvrB makes the 3′ incision. Step 7: The DNA straightens enabling UvrC to make the

Damage Recognition by a Molecular Matchmaker

Excision nucleases in general and (A)BC excinuclease in particular excise bulky adducts such as cisplatin-1,2-d(GpG) diadduct, psoralen-thymine monoadduct, and benzo[a]pyrene-guanine adduct. However, with varying efficiencies they also excise lesions with minor helical distortions, ranging from AP sites to O^6-MeGua (41, 47, 48). Clearly, complementary surfaces between the enzyme and substrate cannot be the basis for recognition of these lesions



because the lesions have very few or no structural similarities. *E. coli* (A)BC excinuclease employs a molecular matchmaker, UvrA, to aid in recognition.

A molecular matchmaker (49, 50) is a protein that, in an ATP-dependent reaction, brings two compatible yet solitary macromolecules together, promotes their association, and then leaves the complex so it can engage in productive transactions. A molecular matchmaker must fulfill five criteria (50). First, in the absence of the matchmaker, the affinity of the matched protein for its binding site must be so low as to be physiologically insignificant. Second, the molecular matchmaker must promote stable complex formation between the matched components. Third, the matchmaker (or the matched protein) must be an ATPase, and ATP hydrolysis must be needed for association of the target molecules. Fourth, the matchmaker must make a complex with the matched components, causing a conformational change, but no covalent modification. Fifth, after stable complex formation the matchmaker must dissociate to allow the matched protein to carry out its effector function. UvrA meets all five criteria: It brings UvrB and damaged DNA together, promotes their association, and then leaves the complex in an ATP hydrolysis–dependent reaction.

Damage recognition proceeds as follows. UvrA dimerizes through a noncanonical leucine zipper (51) and makes an A_2B_1 complex with UvrB. This complex binds DNA nonspecifically with relatively high affinity ($K_{NS} \sim 10^{-7}$ M), and this binding activates the UvrB ATPase/helicase function (52) enabling the complex to probe the DNA for its propensity for local unwinding and bending. UvrA is a damage specific–DNA binding protein with specificity for damage in double-stranded DNA (53); UvrB is a damage specific–DNA binding protein with a specificity for single-stranded damaged DNA (54). The matchmaking step in which UvrA "loads" UvrB onto the damage exploits both the enhanced capacity of damaged regions to undergo deformation (bending and unwinding) and the intrinsic binding properties of UvrA and UvrB. Initial formation of the A_2B_1-DNA complex utilizes the damage-recognition specificity of UvrA. This process is followed by UvrB-dependent unwinding of 5 bp around the lesion (55, 56) and kinking of DNA by 130° into the major groove at phosphodiester bond 11 5′ to the lesion (57).

This unique conformation promotes extensive contacts between UvrB and the damaged strand. The initial contacts of UvrB with DNA are mainly ionic in nature and hence complex formation is sensitive to ionic stength. However, these interactions coupled to ATP hydrolysis by UvrB, which causes the local unwinding, expose the bases in the DNA and the hydrophobic core in UvrB, and they lead to a tight "hydrophobic bonding" between UvrB and the damaged strand, resulting in a salt-insensitive complex (49, 58). This model for damage recognition is in contradistinction to previous models (4, 9) which proposed that backbone deformity is the main determinant of recognition and that none

of the subunits are in direct contact with the lesion. UvrB appears to have a hydrophobic binding pocket, and because of the lack of a requirement for specific H-bond donors and acceptors or for formation of ionic bonds of unique orientations, a vast number of chemical groups can be accomodated within this pocket (54).

Recent findings show that three repair enzymes with rather narrow substrate specificities, namely DNA photolyase (pyrimidine dimers), uracil glycosylase (uracil in DNA), and exonuclease III (AP site) "flip out" the lesion from the duplex into a "hole" within the enzyme to bring the active site cofactor or residues in close contacts with the target bonds (14, 59, 60). Thus, it is possible that UvrB employs a flip-out mechanism of substrate binding. Whether the excinuclease system flips out only the damaged nucleotides or the entire excised fragment remains to be seen.

To recapitulate, formation of the A_2B_1-DNA complex involves an ATP-independent step of "recognition" of any anomaly in DNA structure by UvrA, followed by "creation" of the ultimate recognition structure through UvrB-mediated helix unwinding, and consequent conformational change of UvrB and formation of intimate contacts between DNA and UvrB. Thus, the substrate structure cannot be considered independently of the binding reaction because the structure evolves in the process of recognition. In any event, formation of intimate contacts between UvrB and DNA weakens the contacts at the UvrA-UvrB interface, leading UvrA to dissociate and leaving behind a stable UvrB-DNA complex (49, 58, 61). Dissociation of UvrA is essential for binding of UvrC to the UvrB-DNA complex, a process that initiates the dual incisions (54, 62).

Dual Incisions

UvrB and UvrC carry out the excision reaction. The UvrB-DNA complex is recognized with high affinity and specificity by UvrC, and binding of UvrC to the complex leads to the dual incisions. Although contributions of amino acid residues from both subunits to both incision active sites cannot be eliminated, current evidence indicates that UvrB makes the 3′ incision and UvrC makes the 5′ incision (63). The two incisions are concerted but nonsynchronous. The 3′ incision is made first, followed within a few seconds by the 5′ incision (55). The 3′ incision step requires ATP binding (but not hydrolysis) by UvrB. Because of its wide substrate range, a major challenge for (A)BC excinuclease is to discriminate between substrate and nonsubstrate DNA structures. The stepwise recognition and incision reactions help accomplish this goal. Thus, discrimination occurs at the following steps (46, 56): (*a*) binding of A_2B_1 to DNA, (*b*) dissociation of A_2 from A_2B_1-DNA complex, (*c*) dissociation of $UvrB_1$-DNA complex, (*d*) binding of UvrC to the $UvrB_1$-DNA

complex, and (*e*) dissociation of the UvrB-3′-incised DNA complex. Such a stepwise mechanism amplifies the modest specificity of each step and safeguards against futile excision and resynthesis reactions on undamaged DNA.

Repair Synthesis

Following the dual incisions, UvrB, UvrC, and the "excised" oligomer remain in the postincision complex although the excised oligomer is no longer H-bonded (49). UvrC is not very stably bound in this complex; it dissociates slowly and this process is facilitated by helicase II (UvrD) which accomplishes its function by simply binding to the nicks, not by protein-protein interactions (64, 65). The remaining UvrB-gapped DNA complex is stable; however, following dissociation of UvrC the 3′-OH at the 5′ incision site becomes accessible to DNA polymerase I, which fills the gap and displaces UvrB. Under conditions approximating the physiological concentrations of DNA Pol I and ligase, virtually no nick translation occurs, and as a consequence more than 90% of the repair patches are 12–13 nt in length (66).

Since Pol I$^-$ mutants are not as UV sensitive as Uvr$^-$ mutants it has long been assumed that, in the absence of Pol I, either Pol II or Pol III can carry out repair synthesis, albeit less efficiently than Pol I (3). In a defined in vitro system all three polymerases are capable of repair synthesis (J Bouyer & A Sancar, submitted). However, Pol II and Pol III need accessory factors. In the absence of the polymerase β-clamp and the γ complex molecular matchmaker (68), Pol III is capable of limited repair synthesis, but Pol II is not, and the residual repair synthesis is inhibited by single-stranded DNA binding protein (SSB). In contrast, the β-clamp plus γ complex enables both polymerases to perform repair synthesis, and this repair synthesis is stimulated by SSB (J Bouyer & A Sancar, submitted). Thus, these polymerases require the same accessory proteins to fill a 12-nt gap as they do for semiconservative replication. Each cell has only about 20 Pol III holoenzyme molecules (68) and since, upon DNA damage, Pol II is induced to a level comparable to that of Pol I by the SOS response (69), Pol II likely does most of the repair synthesis in the absence of Pol I (J Bouyer & A Sancar, submitted).

EXCISION REPAIR IN HUMANS

Excision repair in humans is the prototype for excision repair in eukaryotes. The basic mechanism is similar to that of prokaryotes in that a multisubunit, ATP-dependent nuclease makes dual incisions on the damaged strand, excises an oligomer, and the resulting gap is filled and ligated. However, 13–16 polypeptides are needed to accomplish the task that is achieved by three polypeptides in *E. coli*. The two repair systems appear to represent convergent

evolution. None of the excision repair proteins of humans shares significant sequence homolgy with *E. coli* excision repair proteins. In contrast, excision repair genes and proteins are conserved in eukaryotes ranging from *S. cerevisiae* to humans (3, 6, 70). As a consequence, the genetics and biochemistry of human excision repair is directly applicable to *S. cerevisiae* and other eukaryotes and vice versa. However, since the excision reaction entails coordinated action of 16 polypeptides and thus involves multiple protein-protein interactions, interspecies genetic or biochemical complementation is rare or absent.

Genetics of Human Excision Repair

Defective excision repair in humans is associated with three diseases: xeroderma pigmentosum, Cockayne's syndrome (CS), and trichothiodystrophy (TTD) (71).

XERODERMA PIGMENTOSUM Xeroderma Pigmentosum (XP) is caused by an absence or greatly reduced level of excision repair (72). The disease is hereditary with autosomal recessive inheritance. The frequency of the disease is 10^{-6} in the United States and Europe and 10^{-5} in Japan. Symptoms fall into two groups: photodermatoses and neurological abnormalities. Photodermatoses include increased sensitivity to sunlight with manifestations ranging from erythema to xerosis and skin atrophy. Nearly 90% of these individuals develop basal cell and squamous cell carcinomas in their teens. Malignant melanomas also occur at high frequency. The overall rate of these three types of skin cancers is 2000-fold higher in XP individuals under the age of 20 than in the general population (71). Cancers of internal organs also occur at a 10–20-fold higher rate than in the general population. Most XP individuals suffer from neurological symptoms which include mental retardation, progressive ataxia, and deafness (71).

Somatic cell genetics revealed heterogeneity in XP individuals (73) and led to the identification of seven classic XP complementation groups named XP-A through XP-G (71). In addition, a group of individuals with near-normal UV resistance at the cellular level and normal levels of excision repair at the biochemical level exhibit the dermatological symptoms of XP, including skin cancer, but not the neurological symptoms (74, 75). These individuals are called XP variants (XP-V). The biochemical defect in XP-V is not known; however, XP-V cells have a reduced capacity to resume DNA replication after UV damage compared to normal cells. XP-V cells are said to be defective in postreplication repair (3). In eukaryotes, postreplication repair is an ill-defined phenomenon encompassing all molecular mechanisms enabling the cell to generate, through replication, two intact duplexes without actually removing

the DNA damage (76–78). Clearly, the XP-V gene is not involved in excision repair.

XP complementation groups do not define all of the genes required for excision repair. Many repair defective–rodent cell lines have been isolated by screening mutagenized cell cultures for sensitivity to UV or chemotherapeutic agents such as mitomycin C (79, 80). These studies have resulted in the isolation of mutants falling into 11 complementation groups. Those in groups 2, 3, 4, 5, 6, and 8 are counterparts of human XP or CS mutants. The gene defined by complementation group 1 is required for excision repair but the genes in groups 6 to 11 are not. They either participate in transcription-repair coupling (6 and 8) or play accessory and as yet unknown roles in repair. Since all available data indicate a one-to-one correlation between the *S. cerevisiae* and human excision repair genes (6), yeast genetics has also aided in identifying human excision repair genes. The essential functions in excision repair of the transcription factor IIH (TFIIH) subunits p62 (hTFB1) and p44 (hSSL1) were revealed by the discovery that yeast *ssl1* (81) and *tfb1* (82) mutants are defective in excision repair. Human excision repair genes are called *XPA, XPB,* and so on or *ERCC1* (excision repair cross complementary group 1) and so on, depending on whether they were cloned by complementing human XP mutants or rodent UV-sensitive cell lines.

COCKAYNE'S SYNDROME CS patients suffer from cachetic dwarfism, mental retardation, and progressive neurological symptoms caused by demyelination, and they are moderately sensitive to UV (83). Mutations in five genes cause CS. Two genes, *CSA* and *CSB,* are associated with "pure" CS. The corresponding rodent complementation groups are 8 and 6, respectively. Hence the genes are referred to by the names of *CSA(ERCC8)* and *CSB(ERCC6)*, respectively (84, 85). In addition, some of the XPB, XPD, and XPG mutations give rise to XP/CS overlap syndrome (86). It has been suggested that CS is more of a transcription defect disease than a repair deficiency disease (87). CSA and CSB proteins are involved in coupling transcription to repair (88, 89), and XPB and XPD proteins are subunits of the transcription factor TFIIH (90–92); XPG is sometimes found to be associated with TFIIH (93).

TRICHOTHIODYSTROPHY TTD individuals have sulfur-deficient brittle hair and suffer from dental caries, ichthyosis, skeletal abnormalities, and progressive mental retardation caused by demyelination. Mutations in three genes cause the disease: TTD-A, XPB, and XPD (94). Like CS, TTD caused by mutations in XPB and XPD exhibits the symptoms of both diseases. Reportedly, TTD is also mainly a transcription disease, because the repair defect in cell lines from all three complementation groups can be restored by microin-

jection of the transcription factor TFIIH, although none of the known TFIIH subunits is mutated in TTD-A (87).

Structure and Function of Human Excinuclease

The structure-function relationship of human excision nuclease is now understood in considerable detail. Sixteen polypeptides in six fractions are sufficient to reconstitute excision nuclease (93). The corresponding fractions of *S. cerevisiae* were also found to be necessary and sufficient for damage removal by dual incision in a defined system (45), further evidence of the striking similarities of the two systems. With the exception of XPA and XPG, all the reconstitution fractions contain 2–7 polypeptides in tight assemblies. Since the individual polypeptides are not present in free form in significant amounts in the cell, these six fractions may be justifiably considered the subunits of human excinuclease. The properties of each fraction are reviewed here to help explain the reaction mechanism of human excinuclease. Table 2 summarizes some of the properties of the six fractions. A more detailed account is given below.

XPA The first of the six fractions is a zinc finger protein (95) with affinity for DNA and a marginally higher affinity for UV- or cisplatin-damaged DNA (96). In contrast, its yeast counterpart binds with high affinity and specificity to DNA containing 6-4 photoproducts (97). In fact, the real damage recognition entity of human excinuclease may be the XPA-RPA complex. XPA and RPA (HSSB) make a tight complex in vitro (98–100), and although each protein binds damaged DNA in isolation, when both are present increased amounts of both proteins are bound. However, whether enhanced binding results from complex formation is not clear. Even the increased affinity observed with the XPA-RPA complex is not sufficiently specific or avid enough to account for the ability of human excinuclease to locate rare lesions in the genome and excise them. Additional specificity may be conferred by interaction with other excision repair proteins. Indeed XPA binds to XPF-ERCC1 rather tightly (101, 102) and to TFIIH with modest affinity (103). However, standard gel retardation assays failed to reveal increased specificity upon association of these proteins with XPA (104).

RPA (HSSB) The second fraction is a trimeric protein (p70, p34, p11) with high affinity for single-stranded DNA, and it performs an essential function in DNA replication analogous to that of *E. coli* single-stranded DNA binding protein (105–107). The large subunit binds DNA (108). The p34 subunit is involved in protein-protein interactions (109), undergoes phosphorylation/dephosphorylation reactions during the cell cycle (110, 111), and becomes hyperphosphorylated upon DNA damage by UV or ionizing radiation (112,

Table 2 The 25 polypeptides required for excision repair in humans

Fraction	Number	Proteins (yeast homolog)	Sequence motif	Activity	Role in repair
a. Excision nuclease subunits					
I. XPA	1	XPA/p31 (RAD14)	Zinc finger	DNA binding	Damage recognition
II. RPA	2	p70		DNA binding	Damage recognition
	3	p34			
	4	p11			
III. TFIIH	5	XPB/ERCC3/p89 (RAD25)	Helicase	a) DNA-dependent ATPase	a) Formation of preincision complex
	6	XPD/ERCC2/p80 (RAD3)	Helicase	b) "Helicase"	b) Transcription-repair coupling
	7	p62 (TFB1)		c) GTF	
	8	p44 (SSL1)	Zinc finger	d) CAK	
	9	Cdk7/p41 (KIN28)	S/T kinase		
	10	CycH/p38 (CCL1)	Cyclin		
	11	p34	Zinc finger		
IV. XPC	12	XPC/p125 (RAD4)		DNA binding	a) Stabilization of preincision complex
	13	HHR23B/p58 (RAD23)	Ubiquitin		b) Protection of preincision complex from degradation
V. XPF	14	XPF/ERCC4/p112 (RAD1)		Nuclease	5′-Incision
	15	ERCC1/p33 (RAD10)			
VI. XPG	16	XPG/ERCC5/p135 (RAD2)		Nuclease	3′-Incision
b. Repair synthesis and ligation					
I. RFC	17–21	$(p140)_1(p40)_4$	ATPase	ATPase	Molecular matchmaker
II. PCNA	22	$(p32)_3$			Polymerase clamp
III. RPA					
IV. Polε(δ)	23	p258	Polymerase	Replicase	Repair synthesis
	24	p55			
V. Ligase	25	p102			Ligation

113). Phosphorylation of HSSB occurs in two steps: first by cdk-cyclin A and then by Ku antigen–stimulated DNA-dependent protein kinase (114). Up to five serine and threonine residues become phosphorylated by the combined actions of these kinases. The role of the small (p11) subunit is unknown.

Depletion of cell-free extracts of RPA by antibodies or chromatography (115, 116) was found to severely inhibit repair synthesis *in vitro* and moderately reduce the damage-specific nicking activity. This finding led to the proposal that HSSB was essential for repair synthesis and in addition played a role in earlier steps of excision repair such as stabilizing the postincision complex (117). However, when the excision nuclease was reconstituted with purified proteins the incision step was found to be absolutely dependent on RPA (93). This conclusion was confirmed in the yeast reconstituted excision nuclease system (45). Hence, RPA is an essential subunit of human excinuclease. In addition to its interaction with XPA through both the p70 and p34 subunits (99, 100) it binds the XPG and XPF subunits of the excinuclease (98). Thus, the XPA-RPA complex with its multiple interactions with TFIIH, XPF-ERCC1, and XPG might constitute the nucleation component for the remaining subunits of the excinuclease.

TFIIH The third fraction is one of the six general transcription factors (GTFs) (TFIID, TFIIA, TFIIB, TFIIF, TFIIE, and TFIIH) required for optimal transcription by RNA polymerase II (118–120). TFIIH has two enzymatic activities: helicase and CTD kinase, which phosphorylates the C-terminal domain (CTD) of the largest subunit of RNA Pol II. TFIIH is the last GTF to enter the initiation complex and is recruited to the complex by TFIIE. TFIIH is not required for transcription initiation but is required for promoter clearance which is the reaction encompassing the phosphorylation of CTD, the disruption of the initiation complex, and the synthesis of a transcript 30–50 nt in length (121, 122). After that reaction, RNA Pol II enters the elongation mode as TFIIH dissociates from the polymerase (122). The helicase activity of TFIIH is thought to be important for promoter clearance because in the absence of TFIIH aborted transcripts of less than 50 nt accumulate (121, 122a). Some genes such as the IgH gene can be transcribed without TFIIH. Furthermore, in in vitro systems with RNA Pol II lacking the CTD, a normal level of transcription occurs from several promoters. Even in TFIIH-dependent promoters this dependence can be abrogated by using a superhelical template (123). Nevertheless, TFIIH is an essential factor because mutations in its XPB (3, 6) and XPD (124, 125) homologs in yeast are lethal, indicating that TFIIH plays an essential role in transcription of genes important for normal cellular metabolism.

Depending on the purification scheme, TFIIH contains 5–10 subunits. Sequence analysis of its largest subunit (p89) revealed that it is identical to the

XPB/ERCC3 gene (91, 126). Concurrently, it was found that XP-B and XP-D human and rodent mutant cell-free extracts failed to complement in an excision assay (44), even though these cell lines complement upon cell fusion. The conclusion was that XPB and XPD make a tight complex and that the mutant subunits in these tight complexes exchange too inefficiently in vitro to complement each other. These observations, combined with the identification of the XPD homolog in yeast TFIIH (127), led to the eventual realization that TFIIH in its entirety is a repair factor (91, 92, 128). The highly purified TFIIH contains seven polypeptides (93, 129): XPB, XPD, p62, p44, p41 (cdk7), p38 (Cyclin H), and p34. Yeast genetics reveals that the first four are required for excision repair (119). Whether or not the other subunits are essential for excision repair is not known. However, the presence of the remaining three subunits in stoichiometric amounts in TFIIH does not interfere with excision (92, 129). Thus, the seven-subunit form of TFIIH is likely equally active in transcription and in repair.

However, the action mechanism of TFIIH in transcription differs in four important ways from that in repair. First, TFIIH is absolutely required for excising any type of lesion in any sequence context in both linear and supercoiled DNA. In contrast, TFIIH is not required for transcription from a subclass of RNA Pol II promoters or for transcription from any promoter in superhelical DNA. In this regard TFIIH is more of a repair factor than a transcription factor (93). Second, the XPA protein recruits TFIIH to the preexcision complex (103); in contrast TFIIE recruits TFIIH to the preinitiation complex (118, 120, 122a) and plays no role in general excision repair (103). Third, the ATPase ("helicase") activity of XPD is essential for excision repair (3, 6) but not for transcription. Finally, the CTD phosphorylating activity of TFIIH plays an important role in transcription initation but no protein is phosphorylated during excision repair (129). Anti-cyclin H antibodies which inhibit the cdk-activating kinase (CAK) activity of TFIIH (130–132) inhibit both transcription and excision repair. However, the inhibition of excision repair could simply result from steric hindrance by the antibody bound to a building block of TFIIH rather than from interference with the phosphorylating activity of CAK.

XPC-HHR23B The fourth fraction contains two proteins; a 125 kDa and 58 kDa protein. The XPC gene, as defined by the XP-C complementation group, encodes a protein of 125 kDa with a modest degree of homology to the *S. cerevisiae* Rad4 protein (133, 134). Purification of XPC protein using an in vitro assay for repair yielded a fraction with stoichiometric amounts of two polypeptides. Sequence analysis revealed that p125 was the *XPC* gene product and p58 had a high degree of sequence homology to the *S. cerevisiae* RAD23 gene. The gene for the p58 was cloned. Humans have two RAD23 homologs called *HHR23A* and *B*. Of these homologs, only the protein encoded by

HHR23B is found in complex with XPC (13). There is no known human syndrome associated with mutations in HHR23A or B. In yeast, the *rad23Δ* mutant is not as UV sensitive as other yeast strains with mutations in the basal subunits of excinuclease (6). Neither p125 nor p58 (HHR23B) has any sequence signature revealing what function they may perform in excision repair. However, the p58 subunit has an interesting feature: the N-terminal 70 amino acids of both yeast RAD23 (135) and human HHR23A and B (134) show 25–31% sequence identity to ubiquitin and thus belong in the family of ubiquitin-fusion proteins. In some ubiquitin fusion proteins, the ubiquitin moiety is thought to function as a chaperone enabling proper folding and assembly in multiprotein complexes. In yeast, Rad23 has been found to help in stabilize the Rad14(XPA)-TFIIH complex (136).

The role of XPC protein in excision repair is rather interesting. The protein binds DNA with high affinity ($K_D \sim 10^{-9}$ M) and no specificity. XPC null mutants carry out normal strand-specific repair of transcribed genes but are defective in overall repair (137, 138). In contrast, yeast *rad4*(XPC) mutants are totally defective in excision, and *Rad7* and *Rad16* mutants behave like the human XPC mutant in that they repair the transcribed strand of a gene but not the lesions elsewhere (139). Whether these differences are real or apparent remains to be seen. However, in vitro experiments reveal that human excinuclease can be reconstituted in the absence of XPC and of RNA polymerase, and the partial nuclease reconstituted in this manner excises 27 nt–long fragments by incising at the appropriate 3′ and 5′ sites relative to the lesion (129). However, under these conditions both the excised oligomer and the damaged strand in the preincision complex were extensively degraded. XPC appears to bind to the damaged strand in the preincision complex and help target the nuclease subunits of the excision nuclease to the proper site while protecting the rest of the DNA in the precincision complex (which appears to be extensively single-stranded) from attacks by the two nuclease subunits, XPG and XPF-ERCC1. XPC, because of its high affinity to DNA, may help stabilize the preincision subassemblies on nucleosomal DNA and thus ensure proper assembly of the preincision complex and recruitment of the nuclease subunits. In transcribed DNA, an elongation complex stalled at a lesion apparently obviates the need for XPC.

XPG The fifth fraction contains a solitary protein of 135 kDa (140–142). Its yeast homolog is RAD2 and both XPG and RAD2 show significant sequence homology to the human flap endonuclease (FEN1) which cleaves a DNA flap with a 5′-single stranded–end at the single-strand to double-strand DNA junction (143). XPG and Rad 2 proteins also have FEN activity (144) and thus XPG was predicted to be the 3′ nuclease of human excinuclease (143, 144). In fact XPG has three types of nuclease activities: (*a*) single-strand specific

endonuclease (144, 145), (*b*) exonuclease activity of 5′ to 3′ directionality (145), and (*c*) FEN activity (144) which is stimulated by RPA (T Matsunaya & A Sancar, unpublished observation).

Direct evidence that XPG makes the 3′ incision comes from studies with XPG antibodies and from reconstitution experiments (146). Anti-XPG antibodies specifically changed the site and level of the 3′ incision in human cell-free extracts without affecting the 5′ incision strongly, suggesting that XPG makes the 3′ incision. Omission of the XPF-ERCC1 complex resulted in normal 3′ incision without any 5′ incision (129). Since XPG and XPF-ERCC1 are the only excinuclease subunits that have nuclease activity, these results established XPG as the 3′ nuclease (146). This fact may explain why XP-G mutants have some of the lowest residual repair activity of all of the XP mutants: XPB and XPD are essential genes and hence the mutants are always leaky; XPC protein is not required for gene-specific repair, and excision of nontranscribed sequences can occur without XPC when naked DNA is used as substrate. XPE protein is not required for excision; it may have a stimulatory effect (93). Mutants lacking XPF-ERCC1 do make the 3′ incision, which probably leads to some abnormal excision by a 3′ to 5′ exonuclease. As a consequence, XP-A and XP-G mutants have the lowest unscheduled DNA synthesis (UDS) of all the XP cell lines (71).

XPF-ERCC1 These two proteins (the sixth fraction) make a complex (44) of 1:1 stoichiometry (93, 147). The complex is a single strand–specific endonuclease which at the penultimate step of purification (or pure protein in the presence of RPA) also has junction endonucleolytic activity on a "bubble structure" on the strand which makes the transition from duplex to single-stranded DNA in the 5′ to 3′ direction (147). This activity is similar to that observed with the yeast Rad1-Rad10 complex which is the counterpart of XPF-ERCC1(148). These data, which are consistent with XPF-ERCC1 making the 5′ incision, were confirmed with antibody inhibition experiments. Anti-ERCC1 antibodies specifically inhibited the 5′ incision in a defined system giving rise to uncoupled 3′ incision (146). The same results were obtained by omission of XPF-ERCC1 in a reconstitution experiment (129). Moreover, these results showed that in the assembly of human excinuclease, XPF-ERCC1 is perhaps the last subunit to arrive and that in a normal excision reaction the 3′ incision may precede the 5′ incision even though the reaction is concerted.

Finally, XPF-ERCC1, like their yeast counterparts (149), are involved in recombinational repair as evidenced by the unusual sensitivity of these mutants to crosslinking agents such as mitomycin C (150).

XPE AND DDB XP-E patients show mild symptoms of XP, and the XP-E cell lines are only moderately UV sensitive and have 50% of normal UDS activity

(71). XP-E cell-free extracts have reduced excision (44) and repair synthesis (134). Gel retardation assays using UV-irradiated DNA revealed that 2 out of 13 XP-E cell lines lacked a protein that specifically bound to damaged DNA. This activity was named damaged DNA binding protein (DDB) (151–153). The protein has been purified to homogeneity (154–156), and it is a heterodimer of $(p127)_1$ $(p48)_1$ composition (157). The genes for both subunits have been cloned and sequenced (158–160). The purified protein binds to 6-4 photoproducts with high affinity ($K_D \sim 10^{-10}$ M; 155) but not to pyrimidine cyclobutane dimers or to psoralen monoadducts (155). Microinjection of DDB into XPE-DDB$^-$ cells has been reported to restore the UDS to normal level but has no effect on the UDS of XPE-DDB$^+$ cells (161). However, DDB does not complement the excision activity of cell-free extracts from either DDB$^-$ or DDB cells. In fact, DDB inhibits excision in vitro (162). Of the six fractions that are necessary and sufficient to reconstitute human excinuclease in vitro only RPA restores the excision activity of XP-E cell-free extract; yet no mutation was found in any of the three subunits of RPA in an XPE-DDB$^-$ cell line (162). Clearly the relationship between the XP-E phenotype, DDB, and RPA remains to be elucidated.

Another class of proteins that binds to damaged DNA are high mobility group (HMG) domain proteins. Some members of this family bind to cisplatin-1,2-d(GpG) diadduct with high affinity (163–165). However this binding inhibits human excinuclease (42) and hence is not of direct relevance to excinuclease function. The binding may be relevant, however, to the tissue specifity of certain anticancer drugs.

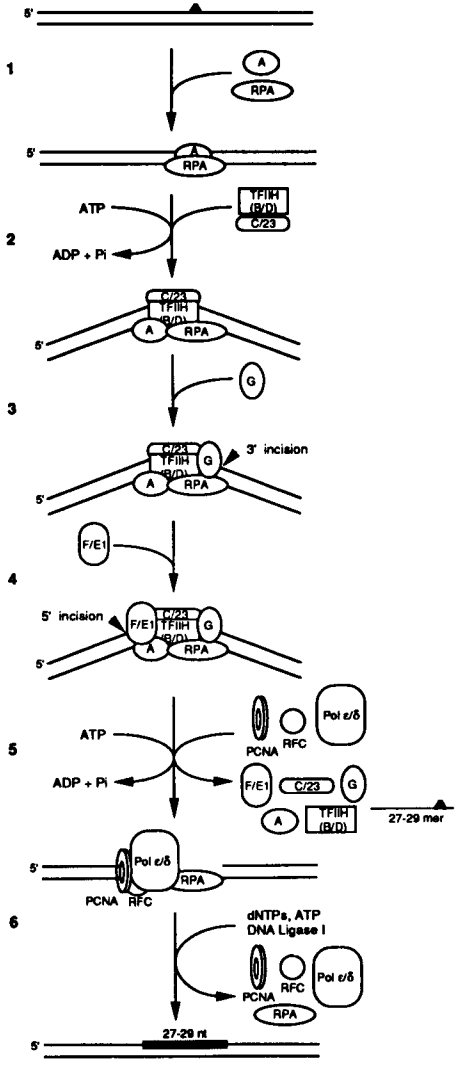
In addition, human ribosomal protein S3 cleaves the phosphodiester bond of heavily UV-irradiated DNA at unknown lesions as well as at the intradimer phosphodiester bond. This activity, which has been referred to as AP endonuclease I or UV endonuclease III, is missing in some XP-D cell lines (166). The significance of these findings to XP pathogenesis is unknown (166).

Mechanism of Excision Repair

The three formal steps of excision repair are damage recognition, dual incisions, and repair synthesis and ligation. Figure 2 summarizes our current understanding of human excision repair which is based on (*a*) properties of individual components, (*b*) reactions with subsets of proteins, and (c) experiments with immobilized substrates. The three main steps are discussed below.

DAMAGE RECOGNITION Although it has not been demonstrated experimentally, damage recognition is almost certainly the rate-limiting step of excision repair for two reasons. First, rare DNA lesions must be located among the 10^{10} bp present in the human genome. Second, lesions of infinite variety (Table 3)

must be recognized by a small set of proteins (XPA, RPA, TFIIH) without recourse to the combinatorial recognition mechanism of transcription regulation which employs hundreds of proteins (transcription factors) in different combinations to activate specific genes or a small set of genes (167). Instead, damage recognition occurs in at least two stages: an ATP-independent step of low discrimination followed by an ATP-dependent step which leads to the



formation of a long-lived preincision complex. Although molecular matchmaking may be employed by human excinuclease to achieve high specificity, in contrast to *E. coli*, this possibility has not yet been experimentally demonstrated.

Binding of XPA-RPA Both XPA (96) and RPA (168) have slightly higher affinity for damaged DNA than for undamaged DNA. The functional form of the recognition entity appears to be the XPA-RPA complex, because these two proteins associate tightly (98–100). Since RPA is quite abundant, in vivo all the XPA may be in XPA-RPA complex. This complex has higher affinity for damaged DNA than does either component alone (98–100). However, the binding data were qualitative and hence the level of improvement in damage-binding specificity by the complex compared to the individual components is not known at present. Theoretically, the occupancy of the target site in any DNA-protein interaction can be increased by two means. Either a protein of low abundance but high specificity finds its target because of its intrinsically high affinity for its site, or a protein of high abundance and low specificity occupies its target by the law of mass action while simultaneously occupying many nontarget sites as well. The second mechanism is utilized by RPA-XPA to find damage. RPA is one of the most abundant cellular proteins (106–108, 169) so it may occupy the damage sites and help recruit XPA to the lesion. In excinuclease reconstitution from purified components, 200–300 nM of RPA were needed for optimal activity as compared to picomolar amounts of XPF-ERCC1 (129, 170). In addition to XPA-RPA, XPC likely also contributes to damage recognition by stabilizing the XPA-RPA complex. This contribution may explain why XPC is not needed for transcription-coupled repair because in that case the stalled RNA polymerase is used as a surrogate damage-recognition protein.

Figure 2 Model for excision repair in humans. A-G, XPA through XPG (except XPE) proteins; C/23, XPC-HHR23B complex; F/E1, XPF-ERCC1 complex; Pol ε/δ, DNA pol ε or δ. Step 1: XPA-RPA recognizes damage in an ATP-independent reaction. Step 2: XPA-RPA recruits TFIIH to the lesion, and TFIIH unwinds DNA which leads to intimate DNA-protein contacts and makes the damaged strand accessible to XPC-HHR23B, which is recruited to the damage site through interactions with TFIIH and XPA. Step 3: XPG is recruited by RPA and TFIIH to the damage site and makes the 3′ incision 3–5 nucleotides 3′ to the lesion. Step 4: XPF-ERCC1 is recruited to the site by XPA, XPF, and TFIIH and makes the 5′ incision 20–24 nucleotides 5′ to the lesion. In vivo steps 3 and 4 are likely to be tightly coupled. Whether all proteins shown in steps 3 and 4 are simultaneously present in the incision and postincision complexes is not known. Step 5: The postincision complex is dissociated by the RFC molecular matchmaker, which loads the PCNA trimeric circle onto DNA and facilitates its (166a) association with Pol ε or δ, thus replacing excision proteins with repair synthesis proteins. Step 6: The gap is filled and the repair patch, corresponding in size to the excised fragment, is ligated. This is the transcription-independent mode of repair. In transcription-repair coupling, RNA Pol II, through its interaction with CSA/CSB proteins, is presumed to recruit XPA-RPA and TFIIH to the lesion that stalls transcription.

Table 3 Substrate spectrum of human excinuclease[a]

Bulky adducts
Cholesterol
Acetylaminofluorene
Cisplatin-1,3-d(GpXpG)
6 - 4 Photoproduct
Cisplatin-1,2-d(GpG)
Thymine dimer
Texas Red
Biotin
Psoralen
Synthetic abasic analogs
Methylated nucleotides
O^6-Methylguanine
N^6-Methyladenine
Mismatches
A : G
G : G

[a] The lesions are listed in approximate order of catalytic efficiency. With the exception of N^6-MeAde all lesions are substrates for the *E. coli* excinuclease as well, although the order of preference is somewhat different.

Binding of TFIIH This factor participates both in excision repair (92, 128) and in transcription initiation (118, 120). In transcription initiation, TFIIH is the last factor to enter the initiation complex and is recruited to the complex by TFIIE (121, 122, 122a). Binding of TFIIH sets in motion helix unwinding and CTD phosphorylation which result in promoter clearance and entry into elongation mode by RNA polymerase. In repair, the DNA-protein complex of (XPA-RPA)-DNA may be assumed to be the "closed form" preincision complex. The precise sequence of events leading to the formation of preincision "open complex" is not known at present. XPA is known to bind specifically to TFIIH, and therefore it must be involved in recruiting TFIIH to the damage site. Equally relevant is the fact that TFIIE, which specifically binds to TFIIH through XPB/ERCC3 (92, 122a), has no role in general (as opposed to transcription-coupled) excision repair. In *S. cerevisiae*, Rad23 stabilizes the TFIIH-Rad14(XPA) complex (97). Since XPC also associates with TFIIH (92) a reasonable assumption is that XPC-HHR23B binds to XPA-TFIIH in humans as well and aids in formation of a more stable preincision complex. However, this function is not essential for a functional pre-excision complex formation because excision can be achieved without XPC. In the absence of XPC,

however, the reaction is not optimal and DNA is degraded extensively (129). XPG is also known to interact with RPA (98) and TFIIH specifically (93) and hence may play an important role in stabilizing the preincision complex.

No direct evidence is available on the nature of DNA conformational change caused by TFIIH in the preincision complex. However, based on its role in transcription (promoter clearance), and on the phenotype of yeast Rad3 (XPD) helicase active-site mutant (no repair; 6), TFIIH may reasonably be assumed to open the duplex at the lesion site, leading to evolution of new DNA-protein and protein-protein interactions that result in formation of a stable preincision complex. In fact, in reconstitution studies omission of XPC from the reaction makes the damaged strand uniquely susceptible to degradation by XPG in a TFIIH-dependent manner, suggesting that TFIIH creates a single-stranded region around the lesion which is attacked by this single-strand specific nuclease (129).

The composition of the preincision complex is not known at present, but XPA, RPA, TFIIH, and XPG are required for complex formation. The complex can form in the absence of XPC and XPF-ERCC1. However, since DNA degradation occurs in complexes without XPC, under physiological conditions XPC is likely also present. Analogous to the role of TFIIH in transcription—disengaging RNA Pol from other GTFs—TFIIH may, upon being recruited to the damage site, disengage some of the preincision (molecular matchmaker) proteins to prepare the preincision complex for the entry of the 5′ nuclease.

DUAL INCISIONS/EXCISION XPG makes the 3′ incision and XPF-ERCC1 makes the 5′ incision (143, 144, 146). The 3′ incision is made first, followed within seconds by the 5′ incision (129). A significant difference from the *E. coli* excinuclease is that the 3′ incision is not triggered by the binding of the subunit that makes the 5′ incision. In a reaction mixture lacking XPF-ERCC1, the 3′ incision is made at almost normal levels by XPG (129). As a consequence both in cell-free extracts and in defined systems a significant amount of uncoupled 3′ incision occurs even in the presence of XPF-ERCC1. In contrast to uncoupled 3′ incision, uncoupled 5′ incision is not observed in cell-free extracts or reconstituted systems, suggesting that XPG must be present in the preincision complex for XPF-ERCC1 to bind and make the 5′ incision. However, with anti-XPG antibodies, which apparently do not interfere with assembly of XPG in the preincision complex but inhibit its nuclease activity, extensive uncoupled 5′ incision was observed (146). This finding suggests that a conformational change caused by the 3′ incision is not necessary to prime the DNA for the 5′ incision by XPF-ERCC1.

The excised fragments range in size from 24 to 32 nt. The minimum size substrate for the excision reaction is about 100 bp (40). The incision sites are influenced by adduct type (41, 42, 93), and sequence context (40, 171) and

reaction conditions. The 5′ incision sites range from the 20 to 26 phosphodiester bond 5′ (146) and from the 2nd to 10th phosphodiester bond 3′ to the lesion (129, 146). However, in cell-free extracts the incisions occur over a more narrow range. Furthermore, both in cell-free extract and in defined systems the excised fragments are as a rule 24–32 nt with, in most cases, 27–29 mers being the dominant species (45, 93, 146). Thus, the nuclease appears to measure an "exact" distance. If the first 3′ incision is made too far from the lesion, then the 5′ incision is at the close end of the 5′ incision range, which results in a narrow range of excised fragments.

The composition of the dual incision complex is not known. Naturally, the complex contains XPG and XPF-ERCC1. Considering that extensive degradation of DNA occurs in the absence of XPC, the complex most likely contains XPC as well. Whether XPA-RPA, which recruits XPG and XPF-ERCC1, and TFIIH, which forms the open complex, are present following the recruitment of the nuclease subunits is not clear. The composition of the postincision complex is currently not known. However, even though the excised oligomer is released (129), a single-stranded gap, free of DNA does not form. At least some of the excinuclease subunits remain bound in the postincision complex with the gap (93), and they must be displaced by the polymerase accessory factors RFC and PCNA before the polymerase can fill the gap.

REPAIR SYNTHESIS Excision repair, in the strict sense, is the recognition and removal of damage from DNA. However, these two steps create a gap which must be filled to create a functional duplex. Of the five known human DNA polymerases (172–173) Pol δ and Pol ε carry out repair synthesis. The role of Pol ε in repair synthesis was discovered when a polymerase responsible for UV damage–induced DNA synthesis in permeabilized cells was purified (174) and later identified as Pol ε (175). A different set of in vivo experiments revealed that PCNA, which is the polymerase clamp of Pol δ and Pol ε, can be detected in association with chromatin of nonproliferating cells upon UV irradiation (176, 177), implicating the two PCNA-dependent polymerases, Pol δ and Pol ε, in repair synthesis. Indeed, depletion of cell-free extract of PCNA completely eliminates repair synthesis (116, 178), providing strong evidence that one or both of these polymerases carry out repair synthesis.

Studies using Pol δ antibodies indicate that nucleotide incorporation into UV-irradiated DNA in cell-free extract, which is commonly used as an assay for excision repair, prevents incorporation, and for this reason Pol δ was proposed as the repair polymerase (179). A different study, using gapped DNA as substrate and partially fractionated cell-free extracts, found that Pol ε was more efficient in generating ligatable products, suggesting that Pol ε was better suited to being a repair polymerase (180). As expected, in this latter system, gap filling was dependent on proliferating cell nuclear antigen

(PCNA) and was stimulated by the PCNA molecular matchmaker RFC (50) which loads the PCNA ring onto the primer-template. In fact a recent study with Pol δ and Pol ε yeast mutants showed that conditional mutations in either polymerase make cells UV sensitive (181). Thus the combination of available data is consistent with both polymerases being responsible for repair synthesis. Similarly, Drosophila (182) and yeast (183) conditional PCNA mutants are UV sensitive, providing in vivo support for PCNA in repair synthesis.

DNA Pol β, which is capable of filling in 25–30-nt gaps efficiently (172, 173), is the repair polymerase for certain types of base excision repair but apparently plays no role in nucleotide excision repair. In contrast, Pol δ and ε participate in filling gaps generated by either repair pathway (3, 31, 184). The inability of Pol β to carry out repair synthesis in nucleotide excision repair may stem from the fact that after excision of the oligomer the gap is occupied by RPA, which has a binding site of 30 nt (185), and other excision repair proteins. These proteins are apparently displaced by RFC-PCNA and, upon loading of PCNA at the 3′-OH of the gap, this primer terminus is no longer accessible to Pol β. Even though the main repair polymerases are quite different in prokaryotes (Pol I) and in eukaryotes (Pol δ and Pol ε), the *E. coli* replicase Pol III is also capable of repair synthesis, and when it carries out this function it is strongly dependent on the RFC-PCNA functional homologs δ and β proteins and on the RPA homolog SSB (J Bouyer & A Sancar, submitted).

The repair patch resulting from resynthesis was determined to be in the 30–50-nt range by a variety of in vivo methods (186–188). Considering the limitations of the in vivo methods, these studies gave remarkably accurate estimates. Determination of the repair patch size by the phosphorothioate method (66) revealed that within a resolution of 1–3 nt the 5′ and 3′ borders of the repair patch produced in cell-free extracts precisely matched the borders of the excision gap (38). Thus in humans, as in *E. coli,* the gap is filled without 5′ enlargement or 3′ nick translation.

The fact that the 3′ end of the repair patch matches the 3′ end of the gap means that DNA ligase is not a limiting factor in repair synthesis. Once the gap is filled, any of the four DNA ligases present in human cells (189–191) should be able to seal the patch.

TRANSCRIPTION-REPAIR COUPLING

Transcribed DNA is repaired faster than nontranscribed DNA both in humans (192) and in *E. coli* (193). Furthermore, the preferential repair is largely confined to the template strand (193, 194) and in humans only to genes transcribed by RNA Pol II (195). Several factors contribute to this phenomenon (195, 196): chromatin structure, topology of transcribed DNA, and the effects of lesions on the

progression of RNA polymerase. The chromatin structure and topology of transcribed DNA undoubtedly play a role in repair (197); however, the major repair modulating factor appears to be the interaction of RNA polymerase with a lesion. Bulky lesions such as pyrimidine dimers in the template strand block transcription both in *E. coli* (198) and in humans (199); in contrast lesions in the coding strand have no effect on progression of the transcription complex. Such a stalled polymerase inhibits excision in a defined system of repair and transcription factors alone (198). This finding appears to be in conflict with the in vivo data indicating that stalled RNA polymerase enhances the repair rate of the lesion blocking transcription (198). A single protein in *E. coli* and a complex of at least two proteins in humans displace stalled RNA Pol and recruit the excision nuclease to the damage site and thus provide a solution for this apparent paradox. The mechanism of transcription-repair coupling (TRC) is well understood in *E. coli*; however, no in vitro system for coupling exists for humans and thus the mechanistic details remain unclear.

Transcription-Repair Coupling in Escherichia coli

In *E. coli*, enhanced repair of the transcribed strand is mediated by the transcription-repair coupling factor (TRCF) which is encoded by the *mfd* gene (200). The TRCF is a protein of 130 kDa with helicase motifs but no helicase activity (201). TRC in *E. coli* occurs as follows (Figure 1): TRCF specifically recognizes RNA pol stalled at a lesion, and then it dissociates the ternary complex, releasing RNA pol and the truncated transcript while simultaneously recruiting the A_2B_1 damage binding component of (A)BC excinuclease to the damage site by specifically binding to UvrA (201). The reaction is highly concerted such that the release of RNA polymerase and the delivery of A_2B_1 to the damage site occur simultaneously, and capturing an intermediate involving all these components has not been possible. The UvrA-binding domains of TRCF and UvrB partially overlap; hence after recruiting the A_2B_1 complex to the damage site TRCF helps UvrA dissociate from the A_2B_1-DNA complex, facilitating formation of the preincision B_1-DNA complex (202, Figure 1). The TRCF also dissociates RNA Pol stalled by nucleotide starvation or by a protein road block and hence it may play additional roles in transcription and repair (203). However, *mfd*$^-$ mutants are only moderately UV sensitive (204) and have about a threefold increase in spontaneous mutation rate (205, 206). Null mutants have normal growth properties, indicating that *mfd* is not an essential cellular gene under physiological conditions.

Transcription-Repair Coupling in Humans

In humans, individuals defective in TRC suffer from CS (88). The two genes necessary for TRC in humans, *CSA/ERCC8* and *CSB/ERCC6*, have been cloned (85, 207). The CS mutant cell lines are slow to resume RNA synthesis

after DNA damage (208, 209) and show phenotypic properties similar to *E. coli mfd*⁻ mutants (85, 196): They are moderately sensitive to UV and have an increased rate of UV-induced mutations, and the majority of these mutations are caused by lesions in the template strand, in contrast to normal cells in which most of the UV-induced mutations are caused by lesions in the coding strand (204, 210–212).

The mechanism of TRC in man is not known. The CSA protein is 46 kDa in size, and it has the WD motif (207) found in many proteins including those involved in skeletal assembly, membrane trafficking, and RNA metabolism. The motif may be used for protein-protein interactions (213). In contrast, the sequence of CSB/ERCC6 is rather revealing. CSB/ERCC6 is a protein of 160 kDa with "helicase motifs," and it almost certainly performs a function analogous to that of TRCF in *E. coli* (85, 201). Indeed, CSB/ERCC6 does bind to the proximal (XPA) and ultimate (TFIIH) damage recognition subunits of human excinuclease (119, 196) and to CSA (207). Based on the properties of CSA and CSB proteins and the known facts of transcription by RNA Pol II in humans (118, 120, 214), the following model has been proposed for transcription-repair coupling in humans (195, 196, 215). RNA Pol II stalls at a lesion, and the stalled complex is recognized by the CSA/CSB heterodimer which perhaps with the aid of TFIIS backs off RNA Pol without dissociating the ternary complex. CSA/CSB also recruit XPA and TFIIH to the lesion site and thus increase the rate of assembly of excinuclease. Following excision and repair synthesis, RNA Pol II resumes its transcription on the repaired template by elongating the truncated transcript.

The main difference between this model and the prokaryotic one is that in humans RNA Pol II is believed to back up rather than dissociate from the lesion site during repair. Currently, there is no experimental evidence to support this view. However, the argument has been made (195) that some human genes are so large that transcribing them without encountering a lesion is practically impossible (transcription may take up to 24 hr for the dystrophin gene). Had the transcripts been discarded, making full length proteins of such genes would never have been possible. However, this model is based on certain assumptions about the in vivo rates of transcription, damage formation, and repair, and some of these assumptions may not be entirely justified. Hence in humans, as in *E. coli*, the truncated transcript may be discarded during transcription-repair coupling.

CELL CYCLE AND REPAIR

Intuitively, one would predict that excision repair is tightly coupled to the cell cycle. Thus, it would appear that lesions present during a prolonged G2 phase have more time to be repaired. The lesions in G2 would also pose less of a

threat to genomic integrity than lesions present during G1 or S phases, which would cause cellular death by blocking replication or by inducing mutation when bypassed by translesion synthesis. Studies on cell cycle and DNA repair have progressed along two lines of inquiry.

Excision Repair Potential as a Function of Cell Cycle

When the repair of pyrimidine dimer was measured in total genomic DNA in either CHO cells (216) or human fibroblasts (217) no significant differences were found except for an apparent decline during mitosis. These studies of low resolution were followed by high-resolution studies which analyzed gene-specific repair as a function of the cell cycle (218–221). Using flow cytometry to separate cells in various phases without using any synchronization procedure that might interfere with cell physiology, strand-specific repair of the actively transcribing CHO DHFR gene was found to be essentially constant during the entire cell cycle. This finding reveals that not only the overall repair but also the efficiency of TRC was constant during phases of the cell cycle (219). CHO cells were also obtained at high purity at various points of the cell cycle using a noninvasive synchronization method (treating cells with the plant amino acid mimosine) (220). Tests of these cells for gene-specific repair showed that the rate of excision of pyrimidine dimers was essentially constant throughout the cell cycle (221); however, DNA damage during S or G2 phases increased the length of G2 and hence allowed more time for repair, which explains the relatively high resistance of G2 cells to DNA damage compared to other phases. The consensus from these studies is that excision repair capacity of the cell does not change with cell cycle.

Cyclin-Dependent Kinase-Activating Enzyme, Transcription Factor IIH, and Excision Repair

Cyclin-dependent protein kinases such as cdk2 and cdc2 regulate the cell division cycle (222, 223). The activities of these kinases, in turn, are regulated by their associations with cyclins, which in general go through cyclic changes in concentration during the cell cycle, and by phosphorylation of serine or threonine residues of the kinases themselves. An activity that phosphorylates cyclin-complexed cdc2 and cdk is called cyclin-dependent kinase-activating enzyme (CAK). Recently CAK was purified from HeLa cells and found to be in two forms (222): a low molecular form containing cdk7 (41 kDa), cyclin H (37 kDa), and a third subunit (p36); and a large form of 300–400 kDa. Interestingly, the large form turned out to be TFIIH (130–132). Thus, two out of the seven subunits of TFIIH are cdk7 and cyclin H. The significance of this unexpected finding is unknown at present. However, Cdk7-Cyclin H needs to be phosphorylated by yet another kinase (CAKAK) to become CAK, and the

levels of cyclin H and CAK remain constant during the cell cycle (223). Hence, whether or not CAK regulates excision repair or transcription-repair coupling, its potential effect is independent of the cell cycle. However, the full implications of CAK-TFIIH connection remain to be explored.

Another issue that remains to be explored is the effect of the replication complex on excision repair. Although the effect of a replication complex on a transcription complex moving in the same or in the opposite direction has been investigated (224), no direct evidence shows that a stalled replication complex affects excision repair. A stalled complex may hinder excision repair in a manner analogous to a stalled RNA Pol, or it may stimulate repair by increasing the local concentration of RPA, thereby facilitating the assembly of excinuclease. In vitro replication/repair experiments are needed to directly determine the effects of the replication complex on repair.

SOS RESPONSE IN *ESCHERICHIA COLI* AND IN MAN

The SOS response was originally defined in *E. coli* as a coordinated cellular response to DNA damage by UV and other agents that cause bulky lesions in DNA; a response that aids cell survival (225, 226). The response results from induction of about 30 cellular genes that have a common regulatory element called the SOS box with the consensus sequence of CTG-N_{10}-CAG (225, 226). Attempts to find a similar response in human cells have revealed interesting damage response reactions which are often called SOS responses. The relation of these responses to excision repair will be discussed below after a brief review of the excision repair component of the SOS response in *E. coli.*

SOS Regulation of Excision Repair in Escherichia coli

The genes for rate-limiting subunits of (A)BC excinuclease, *UvrA* and *UvrB*, have SOS boxes (4) that are bound by the LexA repressor under physiological conditions. Upon DNA damage, the RecA protein binds to single-stranded DNA resulting from replication blocks and acts as a coprotease for autoproteolysis (and inactivation) of LexA. The levels of UvrA and UvrB increase, as does the cell's excision repair activity. In addition to *uvrA* and *uvrB,* other genes that play a role in excision repair, such as *uvrD* (helicase II) and *polB* (DNA Pol II), are induced by the SOS response and contribute to increased repair capacity. Upon completion of repair, the inducing signal disappears and the cell returns to preinduction conditions. A cardinal sign of the SOS response is increased repair capacity (4, 69), which led to Weigle's initial discovery of the response (226). Hence, increased repair capacity might be used as a reference point in describing a cellular response to damage as an SOS response.

Damage Response and Human Excision Repair

Ultraviolet and other DNA-damaging agents elicit a complex set of responses ranging from growth delay to apoptosis. Two response reactions are relatively well understood. One is mediated by the growth-stimulatory Ras signal transduction pathway, and the signal for this response is reactive oxygen species generated in the membrane (227). The other response reaction is mediated by the growth-inhibitory p53 pathway and the signal is DNA damage (228). Currently, no evidence shows convincingly that either of these response reactions has the *sine qua non* of SOS response (226): increased repair capacity. However, DNA damage does induce significant changes in cellular physiology, so these response reactions must be taken into account in any model of the role of excision repair in cellular survival of DNA damage.

EXCISION REPAIR IN INDUCED CELLS Attempts have been made to detect Weigle Reactivation–like phenomena in human cells. In one such study UV irradiated–herpes virus had higher survival and mutation rates when plated on UV-irradiated cells than on untreated host (229). This finding was taken as an indication of increased repair capacity of UV-induced cells. However, repair was not measured directly in such cells. Considering the profound effect of UV on cellular physiology (230) alternative explanations are more likely.

p53 AND EXCISION REPAIR *p53* is a tumor suppressor gene which plays an important role in the molecular pathogenesis of up to 50% of human cancers (231–233). The p53 protein is a transcriptional regulator and plays an important role in cell cycle regulation (234, 235). DNA damage by UV, ionizing radiation, and alkylating agents, which directly or indirectly cause single-strand breaks, results in increased levels of p53 by way of posttranslational modification and stabilization (236–238). p53 has several DNA binding properties that make it a candidate for a multifunctional DNA metabolism master regulator (239): (*a*) p53 binds to a specific sequence upstream of the target genes. (*b*) p53 binds to ends of DNA fragments and can promote strand exchange between single-stranded DNA and a homologous duplex. (*c*) p53 promotes the annealing of complementary single strands. (*d*) p53 binds single-stranded DNA 25–30 nt in length, and this binding causes a conformational change (allosteric regulation) that increases p53's affinity for its target sequences (240–242). (*e*) p53 binds to mismatches and bulges (243). In addition, p53 binds to certain proteins and protein assemblies: (*a*) p53 binds RPA and inhibits RPA-dependent replication initiation of SV40 in an in vitro system (244). (*b*) p53 binds XPB/ERCC3 (245). (*c*) p53 binds TFIIH and inhibits its helicase activity (246).

In light of these properties, suggestions have been made that, upon DNA damage, p53 induces several proteins that block the cell cycle at the G1/S

boundary and perhaps increases the level of some excision repair proteins. It has been suggested that by binding to RPA, p53 may convert RPA itself from a "replication form" to a "repair form," and by binding to TFIIH, p53 may aid in transcription-repair coupling. Furthermore, the affinity of p53 for TFIIH may enable it to interact with TFIIH associated with RNA Pol II stalled at a lesion, become activated by phosphorylation by the CAK activity associated with TFIIH, and somehow couple transcription to repair (247).

This model predicts that p53-deficient cells would be more sensitive to UV and at least partially defective in excision repair and transcription-repair coupling. Indeed it has been reported that human p53 (–/–) cells have diminished capacity to excise pyrimidine dimers, suggesting that they have a direct role in excision repair (248). However, a comprehensive study which measured UV survival and pyrimidine-dimer and 6-4-photoproduct excision in p53(+/+), p53(+/–), and p53(–/–) mouse fibroblasts showed no difference between the three cell types (249). The nuclear accumulation of p53 in XP-A and CS-B cells that do not repair transcribed DNA, with lower doses of UV compared to normal cells (250, 251), may be a consequence of more replication gaps or stalled transcription bubbles in mutant cells. An attractive model is that binding of p53 to bulges and other lesions (243), or to the 27–29 mer excised by human excinuclease (241), activates the protein as a transcription factor by an allosteric mechanism, initiating the chain of events leading to G1/S arrest (241). Finally, even though p53 reportedly inhibits SV40 replication by binding to RPA (244) and inhibits the helicase activity of TFIIH (246), micromolar concentrations of p53 have no detectable effect on an in vitro excision repair system absolutely dependent on RPA and TFIIH (A Kazantsev & A Sancar, unpublished observations). Thus, no direct evidence shows that p53 has any direct effect on transcription-independent or -dependent repair. The reports regarding the p53 effect on excision repair activity through its regulatory function are discussed below.

p21, Gadd45, AND EXCISION REPAIR These two genes are induced by p53 and contribute to G1/S arrest induced by p53 (234, 235, 252). The mechanism of cell cycle inhibition by p21 is well understood: It binds to Cdk/Cyclin complexes and inhibits kinase activity (234). p21 also binds PCNA with high affinity (253, 254). The PCNA-p21 complex reportedly inhibits replication but does not inhibit repair and thereby aids cell survival (255, 256). However, a comprehensive in vitro study with several different substrates did not confirm these preliminary results (257). The PCNA-p21 complex cannot participate either in replication or in repair, so increased p21 inhibits both cellular reactions and does not aid cell survival via differential effect on excision repair (257).

Similarly, it was reported that Gadd45 binds to PCNA and inhibits replica-

tion but stimulates excision repair either directly or in the form of a Gadd45-PCNA complex (252). However, this preliminary study has not been confirmed either. Up to micromolar concentrations of Gadd45 neither stimulated nor inhibited repair as measured by excision and repair synthesis assays (258). Thus, all available evidence is consistent with the following: (*a*) p53 does not directly participate in excision repair. (*b*) p53 does not induce the transcription of excision repair genes or modulate the activity of excision repair proteins by post-translational modification. (*c*) p53 does not upregulate proteins that stimulate excision repair. However, p53 undoubtedly plays a central role in cellular response to DNA damage, and signal transduction by p53 binding to nicks and abnormal DNA structures (243) or the excision product (241) remains an attractive possibility.

RPA (HSSB) PHOSPHORYLATION AND EXCISION REPAIR RPA is phosphorylated as a result of DNA damage by UV and ionizing radiation (112, 113), and extracts from UV-irradiated cells that contain hyperphosphorylated HSSB are reportedly unable to sustain SV40 replication, leading to the suggestion that phosphorylation converts RPA from a replication form (RPA) to a repair form (RPA-P). However, subsequent studies revealed that inhibition of RPA phosphorylation did not affect replication (169, 259) and that unphosphorylated and hyperphosphorylated RPA were equally active in replication and in excision repair (170). Phosphorylation requires replication intermediates or single-stranded DNA to act as coactivators for DNA-PK, so phosphorylation of RPA during replication or repair may initiate a signaling pathway that prevents cell cycle progression while replication or repair intermediates exist (259). The significance of RPA phosphorylation in cellular physiology remains to be determined. However, RPA clearly plays no direct role in coordinating replication and repair.

SUMMARY AND CONCLUSIONS

Nucleotide excision repair is an important cellular defense mechanism in prokaryotes and eukaryotes. The basic mechanism is the removal of damage by dual incisions by an ATP-dependent multisubunit enyzme system called excision nuclease (excinuclease) followed by the filling and ligating of the single-stranded gap. Several similarities and differences between these two systems are enumerated below.

Similarities Between Escherichia coli and Human Excision Repair Systems

The excision repair systems of *E. coli* and humans share the following features. 1. They both have a wide (and essentially identical) substrate spectrum. 2. They are the sole repair mechanism for bulky adducts. 3. In general, they

perform sequence-independent repair. 4. Damage recognition consists of an ATP-independent step followed by an ATP-dependent step. 5. DNA is unwound and kinked in the preincision complex. 6. The damage is removed by concerted but nonsynchronous dual incisions. 7. The 3′ and 5′ incisions are made by separate subunits. 8. The 3′ incision precedes the 5′ incision. 9. Following the dual incisions a subset of the subunits remains bound to DNA. 10. A helicase is required to dissociate the postincision complex. 11. The patch size equals the gap size. 12. Transcription is coupled to repair through a transcription-repair coupling factor.

Differences in Excision Repair Between Escherichia coli and Humans

The excision repair systems of *E. coli* and humans show the following differences. 1. Excision requires 3 polypeptides in *E. coli* and 16 polypeptides in humans. 2. The excinuclease subunits of the two systems show no sequence homology. 3. A replication protein, RPA(HSSB), is required for excision in humans but not in *E. coli*. 4. A transcription factor (TFIIH) is required for excision in humans but not in *E. coli*. 5. Although substrate spectra are similar the preferences are different, and sequence effect on excision affects the two systems differently. 6. Chromatin structure plays an important role in controlling excision in humans. 7. The nuclease subunits of *E. coli* do not show overt nuclease activity in isolation whereas the human nuclease subunits do. 8. In humans 3′ incision can occur with a subassembly of the repair proteins; in *E. coli* all subunits are needed to elicit the nuclease activity. 9. *E. coli* excises the damage in 12–13 mers; humans excise 27–29 mers. The "excised" oligomer is released by the human but not by the *E. Coli* excinuclease. 10. Replication polymerases (Pol δ and Pol ε) are responsible for repair synthesis in humans; in *E. coli* the repair polymerase Pol I carries out repair synthesis. 11. *E. coli* excision repair proteins do not participate in recombination; human XPF-ERCC1 complex is involved in recombination. 12. *E. coli* excision nuclease is regulated by SOS response; human excinuclease is not.

The concepts and methodology of prokaryotic excision repair greatly aided in studies on eukaryotic repair. These studies have led to a detailed understanding of human excision repair. However, as indicated above, the two systems differ in significant ways. Furthermore, excision repair in humans transcends the realm of scientific curiosity: It is the most important defense mechanism against DNA damage caused by tobacco smoke, which accounts for more than 30% of cancer deaths worldwide (260). A concerted effort to improve understanding of human excision repair in relation to other cellular phenomena may lead to new ways of thinking about cancer prevention and treatment.

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