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DNA REPAIR IN HUMANS

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ABSTRACT

DNA repair is an important molecular defense system against agents that cause cancer, degenerative diseases, and aging. Several repair systems in humans protect the genome by repairing modified bases, DNA adducts, crosslinks, and double strand breaks. These repair systems, base excision, nucleotide excision, and recombination, are intimately connected to transcription and to cell cycle checkpoints. In addition, genotoxic stress induces a set of cellular reactions mediated by the p53 tumor suppressor and the Ras oncogene. These genotoxic response reactions may help the cell survive or enter apoptosis. Damage-response reactions may be utilized as targets of anticancer chemotherapy.

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INTRODUCTION

DNA damage from intrinsic and extrinsic agents causes cell death and tissue degeneration, aging, and cancer. Lesions are elminated from DNA by three basic mechanisms: direct reversal, base excision, and nucleotide excision. In direct repair, the chemical bonds that constitute the lesion or link a substituent to DNA are broken. In base excision repair, the abnormal or modified base in DNA is removed by a glycosylase that hydrolyzes the glycosylic bond, the resulting abasic site (AP site) is removed by AP lyase/endonucleases, and the nucleotide is replaced. In nucleotide excision repair, dual incisions are made on either side of the lesion, the damaged nucleotide is released in an oligomer (12–13 nt in prokaryotes and 27–29 nt in eukaryotes), and the resulting gap is filled in and sealed. As a general rule, direct repair has a narrow substrate range, base excision repair eliminates lesions that do not distort the helix, and nucleotide excision repair repairs DNA containing bulky adducts. However, there is a substantial overlap in the substrates of all repair systems; in particular, the nucleotide excision repair system repairs virtually everything.

The three repair systems have been found in both prokaryotes and eukaryotes. The enzymes performing direct repair and base excision repair are structurally and functionally homologous in the two systems. However, the subunits of the nucleotide excision repair nuclease (excinuclease) in eukaryotes do not share any homology with the prokaryotic system. Within the eukaryote

kingdom excinuclease composition and function are conserved in organisms ranging from *Saccharomyces cerevisiae* to humans. The yeast repair systems, in all aspects quite similar to those in humans, have been reviewed in detail recently (51, 150) and are not addressed here unless the human counterpart remains ill-defined and there is a need to refer to the yeast system to clarify a particular point. Similarly, the mismatch repair system, which corrects mismatches and not damaged DNA (127), is not reviewed here.

DIRECT REPAIR

The three known examples of direct repair are spore photoproduct repair, photoreactivation, and alkyl transfer. The first has been found in *B. subtilis* only and is not discussed. The other two repair systems are reviewed briefly.

Photoreactivation

Photoreactivation is the enzymatic reversal to monomers of pyrimidine cyclobutane dimers (Pyr< >Pyr). The dimers are formed by the ultraviolet component of sunlight and are the major cause of skin cancer (12, 169, 200). Dimers are reversed by photolyase that binds to Pyr< >Pyr in DNA, absorbs a blue-light photon, and splits the cyclobutane ring by electron transfer from the photoexcited $FADH_2$ at the active site (167). The enzyme is present in many species including nonplacental mammals but not in humans (84, 97, 101), and hence is not discussed further.

Alkyl Transfer

O^6-Methylguanine-DNA Methyltransferase (MGMT) is a suicide enzyme that repairs DNA by transferring the methyl group from O^6-meGua in DNA to a cysteine residue in the enzyme by an irreversible reaction (104, 125, 163). It also repairs other alkylguanines such as O^6-ethylguanine and O^6-butylguanine, as well as O^4-methylthymine at a considerably lower efficiency (218). In the laboratory, these lesions are induced by treatment of cells with synthetic alkylating agents such as MNNG. Under physiological conditions, natural methylating agents such as S-adenosylmethionine are thought to alkylate DNA as a side reaction.

MGMT has been found in all species tested. The human enzyme is a monomeric protein of $M_r = 21{,}700$ (125). In contrast, *E. coli* has two MGMTs: Ada and Ogt. The latter is about the same size as the human MGMT and like the human enzyme has a single function. In contrast, the Ada protein ($M_r = 39{,}000$), which was the first MGMT to be discovered (163), has three functions, methyltransfer from phosphotriesters, methyltransfer from O^6-meGua, and positive regulation of several genes involved in defense against alkylation

damage. The human enzyme has no regulatory function. It is a simple enzyme with no cofactor, with an active site cysteine in the sequence context of PCHRV that is conserved in all MGMTs. The reaction involves transfer of the alkyl group from DNA to the Cys residue at the active site. However, the active site is buried (128) and only becomes accessible upon conformational change induced by contact with DNA (56).

O^6-meGua is cytotoxic, mutagenic, and tumorigenic, effects that are ameliorated by MGMT. There are no known human cell lines mutated in the MGMT gene. However, about 20% of human tumor cell strains are sensitive to MNNG and do not have detectable MGMT activity and are said to have Mer^- (methylation repair minus) phenotype (125). Similarly, transformation of human cells by tumor viruses causes the MGMT-deficient phenotype, referred to as Mex^- (methylation excision minus) (125). Although these cell lines are not mutated in the MGMT gene, introduction of either the bacterial or the mammalian gene into Mer^- or Mex^- cells restores resistance to MNNG, which indicates a cause-and-effect relationship between lack of MGMT activity and sensitivity to alkylating agents (125).

Mer^-/Mex^- cells can acquire resistance to MNNG by other mechanisms. In one instance, a human cDNA clone expressing a protein related to the lysosomal *lamp2* glyco-protein conferred MNNG resistance to CHO cells without affecting their Mex^- phenotype (52). In addition, other ill-defined revertants have been described that confer resistance to MNNG without restoring the MGMT activity. One particular class of revertants is particularly interesting. These Mer^- (or Mer^+) and MNNG-resistant clones are defective in mismatch repair (80, 82). Apparently, in wild-type cells mismatch repair contributes to cytotoxicity by executing a futile mismatch repair cycle: The mismatch repair system recognizes the O^6-MeG:T mismatch and excises the "T strand"; however, upon repair synthesis the same mismatch occurs with 50% frequency, leading to repeated and futile attempts to repair every mismatch with the eventual consequence of chromosome degradation and death. This phenomenon poses a particular problem in cancer therapy with alkylating agents, because the treatment selects for mismatch-defective clones that are likely to undergo multiple mutations with serious consequences for the host.

MGMT likely plays a significant role in cancer prevention. In a number of cancers the oncogenic mutation resulted from G:C to A:T transition, which may have resulted from alkylation of the G residue (125). Indeed, overexpression of MGMT protects transgenic mice from cancers induced by alkylating agents. In one case, expression of human MGMT in transgenic mice protected them from thymic lymphomas induced by MNU (46). In another case, expression of the *E. coli* ada gene in transgenic mice protected the animals from liver cancers induced by dimethyl- or diethylnitrosamine (133). However, there is

no known disease associated with MGMT mutation. The Mer^- phenotype appears to be the effect and not the cause of the malignant transformation.

BASE EXCISION REPAIR

Base excision repair works mainly on nonbulky base adducts such as those produced by methylating agents and on oxidized, reduced, or fragmented bases resulting from ionizing radiation and oxidative damage. In base excision repair, the modified, damaged base or base remnant is removed by a DNA glycosylase. The resulting AP-deoxyribose is released by a pair of AP endonucleases that incise 3′ (AP lyase) and 5′ (AP hydrolase) to the AP site. The missing nucleotide is then replaced by a DNA Pol and ligated. There are five well-characterized glycosylases and one major AP endonuclease in humans.

DNA Glycosylases

Many DNA glycosylases are also AP lyases that cleave the base-sugar glycosylic bond and the phosphodiester bond 3′ to the damaged base in a generally concerted reaction involving a Schiff base intermediate (39).

URACIL-DNA GLYCOSYLASE Uracil in DNA originates either from misincorporation by DNA Pols or from deamination of cytosine. A potent (kcat ~ 600 min^{-1} with preference for ssDNA) UNG is present in human cells to cope with the problem. The gene (UNG) has been cloned; it encodes a 34-kDa protein that is processed by cleavage of N-terminal regions to a 26-kDa polypeptide (142). It has no AP lyase activity. This form is functional both in the nucleus and in the mitochondria (182). It has also been reported that the 37-kDa subunit of human glyceraldehyde-3-phosphate dehydrogenase (123) and a 36-kDa protein with sequence homology to human cyclins A and B (131) have uracil glycosylase activities. However, the specific activities of these proteins are extremely low, and it is doubtful if they function as such in vivo. Interestingly, a number of pox viruses, including HSV1, HSV2, and varicella zoster, encode their own uracil-DNA glycosylases (170), indicative of the significance of this enzyme for DNA maintenance. The structure of HSV-1 UNG indicates that uracil flips out of the duplex to fit into the uracil-binding pocket of the enzyme (170). This action mechanism explains the preference of UNG for ssDNA. There are no known human diseases associated with uracil glycosylase defect. Yeast mutants lacking the enzyme have a 20-fold increase in the rate of spontaneous mutation, which is explainable by failure to remove uracil in DNA generated by cytosine deamination (75).

HYDROXYMETHYLURACIL DNA GLYCOSYLASE Hydroxymethyluracil DNA glycosylase (HmUra) is formed by oxidation of the methyl group of thymine

or the deamination of 5-hydroxymethylcytosine. It is found in human and rat urine and is taken to be a measure of oxidative DNA damage in these organisms. The lesion is removed by a specific glycosylase that has been partially purified from mouse plasmacytoma cells. The enzyme has been found in all vertebrate cells but not in prokaryotes, presumably because prokaryotes do not have a high fraction of their cytosine residues in the 5-methylcytosine form (8). The enzyme is specific for HmUra and apparently does not have an AP lyase activity. Interestingly, this enzyme works with equal efficiency on ssDNA and dsDNA, in contrast to UNG, which prefers ssDNA, and other glycosylases, which prefer dsDNA.

THYMINE GLYCOL DNA GLYCOSYLASE The prototype of enzymes that remove saturated, oxidized, and fragmented thymines is the *E. coli* endonuclease III (31), although this enzyme and its mammalian functional homolog have been isolated under a variety of names that reflect either the damaging source (X-ray endonuclease, γ-ray endonuclease, UV endonuclease, redoxyendonuclease) or the damage (thymine glycol, urea, AP site). All of the damaging agents including UV (40) generate monomeric base damage of pyrimidines at relatively high frequency: 5,6-dihydrothymine (thymine glycol), methyltartronylurea, urea, 5,6-hydroxy-5-methylhydantoin, and 5,6-dihydrocytosine. The enzyme releases all of these lesions by first cleaving the glycosylic bond and then the 3′ phosphodiester bond by glycosylase/AP lyase mechanism, which is quite common to DNA glycosylases.

The crystal structure of the *E. coli* endonuclease III has been solved (91). The enzyme is a 27-kDa monomer with a [4Fe-4S] center that is apparently involved in substrate recognition. The human enzyme has not been purified to homogeneity. However, two enzymes purified extensively under the names of UV endonuclease I (43 kDa) and II (28 kDa) from murine plasmacytoma are functional homologs of endonuclease III (88). Both enzymes as well as their human counterparts, like endo III, also cleave AP sites by β-elimination (31). There is no known human disease or animal model associated with thymine glycol glycosylase deficiency.

N-METHYLPURINE DNA GLYCOSYLASE Alkylating agents attack at least 12 nucleophilic sites in DNA (181). The major adducts that form with agents acting by S_N2 mechanism such as dimethylsulphate are 7-alkylguanine and 3-methyladenine. The N7-alkyl group destabilizes the glycosylic bond and leads to rapid uncatalyzed cleavage of this bond, generating an AP site that is processed by AP endonuclease/lyases. As a consequence, this lesion is not highly mutagenic or toxic even in the absence of repair enzymes. In contrast, N3-MeAde is relatively stable and is toxic and mutagenic. Two enzymes that repair N3-MeAde were identified in *E. coli*; these removed 3-MeAde from

DNA and were named 3-MeAde Glycosylase I and II (Tag I and Tag II). The first is highly specific for 3-MeAde, whereas Tag II removes essentially all alkylated purines (165).

In humans there is only one N-methylpurine DNA glycosylase. The gene encodes a 33-kDa protein (125). The mammalian enzyme is functionally homologous to the *E. coli* Tag II with regard to substrate specificity; however, it has no sequence homology to either Tag I or Tag II. Thus, the name N-methylpurine DNA glycosylase is preferred for the human enzyme. However, even this name does not completely define its substrate spectrum. The enzyme removes 8-oxoG, hypoxanthine and 1,N^6-ethenoadenine at a rate ($k_{cat} \sim 1\ min^{-1}$) comparable to that of 3-MeAde (4, 42, 159, 168). It does not have an AP lyase activity. *E. coli tag* mutants are extremely sensitive to mutagenic and toxic effects of alkylating agents. There is no known human disease associated with MPG malfunctioning.

8-HYDROXYGUANINE DNA GLYCOSYLASE One of the most abundant lesions induced in DNA by ionizing radiation and oxidative stress is 7,8-dihydro-8-oxoguanine (8oxoG) (38). The 8oxoG glycosylase was first identified in *E. coli* as an activity that released formamido-pyrimidines (generated as secondary products of alkylated purines) from DNA. The enzyme was named FAPy glycosylase, and the *fpg* gene was cloned (31). Later, it was discovered that this is the major enzyme for eliminating 8oxoG from DNA both in *E. coli* (196) and in mammalian cells (5, 93).

This lesion, 8oxoG, is an important source of "spontaneous" mutation (58), and the expression of the *E. coli fpg* gene in mammalian cells protects them against γ-ray mutagenesis (93). This may explain why both in prokaryotes and eukaryotes there are five repair/cleansing systems for excluding it from DNA (58): (*a*) An 8oxodGPTPase (126), a homolog of *E. coli* MutT protein, sanitizes the nucleotide pool by specifically hydrolyzing 8oxodGTP and preventing its incorporation into DNA. (*b*) The 8oxoG glycosylase removes the base from DNA (5, 196). (*c*) MPG removes 8-oxoG by glycosylase action (4). (*d*) A glycosylase, MutY in *E. coli* (58) and MYH in humans (121), removes the adenine residue from the 8oxoG:A mismatch. The 8oxoG mispairs with A at high frequency, and although 8oxoG:C base pair is a good substrate for 8oxoG glycosylase, 8oxoG:A base pair is not (196). Hence, the MYH glycosylase helps repair 8oxoG lesion indirectly by initiating a mismatch repair reaction that converts 8oxoG:A mismatch to 8oxoG:C base pair. (*e*) In addition to these three pathways, human excision nuclease also is expected to remove 8oxoG (166) and hence contribute to the repair of this rather important lesion.

The *E. coli* Fpg is a 30-kDa monomer; it has a zinc finger at the DNA binding site (138, 196). The enzyme releases the base by a glycosylase action and then the AP site is released by β- and δ-elimination (139). The human enzyme also releases the base and the deoxyribose in a two-step reaction.

However, whether it functions in a manner similar to the *E. coli* enzyme is not known; it has been reported that 8oxoG in humans is removed by a glycosylase and by an endonuclease (5). Further work is needed to clarify the enzymology in humans.

AP Endonuclease

AP endonucleases cleave phosphodiester bonds adjacent to AP sites. Class I enzymes cleave the bond 3′, and class II enzymes cleave the bond 5′ to the abasic sugar (31). All known class I enzymes are glycosylase/AP lyases. The extreme susceptibility of the AP site to be cleaved by β-elimination makes it very difficult to prove that a class I activity associated with a particular protein is physiologically relevant unless there are supporting genetic data. Thus, class II AP endonucleases are the only bona fide AP endonucleases without associated glycosylase activity. In *E. coli* there are two well-characterized class II AP endonucleases (31): exonuclease III and endonuclease IV. As the name implies, exonuclease III, in addition to AP endonuclease, has a potent 3′→ 5′ exonuclease activity specific for double-stranded DNA. Endonuclease IV is purely a class II AP endonuclease with no other known activity. In eukaryotes, there is a curious dichotomy: *Saccharomyces cerevisiae* has a major AP endonuclease, Apn1, which is the structural and functional homolog of *E. coli* endo IV, whereas *Drosophila* and humans have only one major AP endonuclease, which is the structural and functional homolog of exonuclease III (31).

AP sites are transcriptional and replicational blocks and hence are cytotoxic; they are also highly mutagenic. The AP endonucleases perform two functions: first, they eliminate AP sites generated by glycosylases, or "spontaneous" base loss (especially depurination, which occurs at a relatively high frequency); second, they "trim" the 3′ ends of strand breaks generated by reactive oxygen species or ionizing radiation. Such breaks often have 3′-phosphoglycolate or 3′-phosphate. Class II AP endonucleases eliminate these species generating a 3′-OH primer terminus that can be used by DNA polymerase.

The human AP endonuclease (APE, HAP1, APEX, REF1) is a 36-kDa monomer. It has a high degree of sequence identity with *E. coli* exonuclease III. Functionally, it is also similar to exonuclease III in that in addition to its 5′ AP endonuclease activity it has a double-strand specific 3′ → 5′ exonuclease (31). However, the exonuclease activity is rather weak compared to that of the bacterial enzyme. The human enzyme has an additional function: It reduces and thus activates the transcription factors Fos and Jun through a cysteine residue in the N-terminal half of APE, hence the name reducing factor 1, Ref 1 (209, 217). The bacterial enzyme cannot substitute for this function.

Bacterial and yeast mutants defective in exonuclease III and Apn1, respectively, are very sensitive to ionizing radiation, H_2O_2, and alkylating agents.

There are no known human mutants. Leaky APE minus phenotypes of rat glioma and human HeLa cells have been generated by expressing antisense RNA (143, 210). The transfectants have apparently normal growth characteristics but are sensitive to killing by alkylating agents, H_2O_2, and to killing by either hypoxia or hyperoxia.

Repair Synthesis

Lesions repaired by base excision produce 3–4 nt patches in vivo (155). Work conducted in vitro with model substrates indicates that Polβ is better suited for filling in small gaps than the other two nuclear DNA Pols (151). Recent studies in yeast (213) and *Xenopus laevis* (117) in vitro systems have also implicated Polε and Polδ, respectively. It is possible that under physiological conditions both PCNA-independent Polβ and PCNA-dependent Polδ and Polε function in gap filling. However, in a partially purified system from bovine testis nuclear extract, only Polβ carried out repair synthesis in base excision initiated by a uracil residue (181), in agreement with in vitro studies with inhibitors (35, 215) and with the finding that Polβ is optimally suited for filling in short gaps. The repair patch, perhaps depending on the particular pol, is 1–4 nt in length.

Poly (ADP-Ribose) Polymerase

This enzyme synthesizes poly (ADP-ribose) from NAD. Its activity is greatly stimulated by DNA nicks and breaks generated by ionizing radiation and alkylating agents (30). The enzyme attaches poly (ADP-ribose) to many nuclear proteins including histones and itself. It has been suggested that the enzyme binds to nicks and prevents access to repair and recombination enzymes (30); upon auto-poly ADP-ribosylation the enzyme dissociates and allows access to the nick. How such an action aids in cell survival is unclear. However, the enzyme promotes survival because the inhibition of the enzyme activity by benzamidine, or its synthesis by antisense RNA, increases the cell's sensitivity to ionizing radiation and alkylating agents (30).

NUCLEOTIDE EXCISION REPAIR

Nucleotide excision repair (excision repair) is the sole repair system for bulky DNA adducts such as acetylaminofluorene-guanine, cisplatin-guanine, psoralen-thymine adducts, thymine dimers, and 6–4 photoproducts. In addition, all other lesions that are repaired primarily by direct repair or base excision repair are also excised by this repair system. There is no known covalent base modification that is not a substrate for the nucleotide excision repair system (72).

The basic strategy of excision repair is similar in prokaryotes and eukaryotes (116). In both systems, a multisubunit ATP-dependent nuclease (excision nuclease, excinuclease) makes dual incisions, one on either side of the lesion, and excises an oligonucleotide carrying the damage. Both *E. coli* and humans incise the 5th phosphodiester bond 3′ to the lesion. On the 5′ side, *E. coli* excinuclease incises the 8th and the human excinuclease incises the 24th phosphodiester bond (73, 116). There is some variability in the exact sites of incision depending on the lesion and the sequence context (70, 129); however, as a rule the *E. coli* enzyme excises lesions in 12–13 mers, and the human excinuclease removes the lesions in 24–29 mers (72). In *E. coli* three subunits are necessary and sufficient to carry out the dual incisions. In humans, 16 polypeptides, none of which has any homology to the *E. coli* excinuclease subunits, are required to perform the dual incisions (130). The excised oligomer is then replaced by repair synthesis by DNA Pols and accessory factors.

Excinuclease

Human excinuclease is the enzymatic activity resulting from the combined actions of 16 polypeptides that exist in the cell either in solitary form or in tightly held complexes. The complementation groups of the human disease xeroderma pigmentosum define seven polypeptides (XPA–XPG). The other subunits were defined by the rodent mutant (ERCC1), which does not correspond to any of the XP groups (51), and by biochemical assays for excision repair (43, 172). The following are the 16 polypeptides: XPA (p31), the general transcription factor, TFIIH [p89/XPB-ERCC3, p80/XPD-ERCC2, p62(TFB1), p44/hssL1, p41/cdk7, p38/cyclinH, and p34)], XPC (p125)-HHRA D23(p58) heterodimer (114), XPF(p112)-ERCC1(p33) heterodimer (152), XPG(p160), and HSSB (p70, p34, p11). In addition to the several rather stable complexes present in the system there are several weaker interactions: XPA binds to HSSB (115), to ERCC1/XPF complex with relatively high affinity (147) through the N-terminus of XPA (98), and to TFIIH with lower affinity (146) through the C-terminal 40 amino acids of XPA. TFIIH binds with moderate affinity to XPC (43) and to XPG (130).

Taking these interaction and the biochemical activities associated with individual subunits into account, the following model has been proposed for the excinuclease function (130, 166). The XPA binds to the damage site and facilitates the entry of HSSB; the XPA/HSSB complex recruits the TFIIH and ERCC1/XPF complexes. The helicase-like activity associated with TFIIH locally unwinds DNA and perhaps kinks and thus primes it for the dual incision. XPA/TFIIH recruits ERCC1/XPF complex, which makes the 5′ incision (51, 61, 118), and HSSB/TFIIH recruit XPG, which makes the 3′ incision (60, 64,

118, 141, 166). The dual incision is absolutely dependent on ATP hydrolysis (191). In contrast to the bacterial excinuclease where the 3′ incision precedes the 5′ incision, in human excinuclease the incisions may form in random order (118). There is no evidence that all of the excinuclease subunits exist as a complex (repairosome). In all likelihood some function as molecular matchmakers and the preincision complex contains only a few of the 16 polypeptides (70, 166). Following the dual incisions, a subset of the excinuclease subunits remain in the postincision complex (130) such that a single-stranded gap free of protein never exists as a repair intermediate.

Repair Synthesis and Ligation

The postincision complex is dissociated by replication/repair synthesis proteins. Repair synthesis requires PCNA and since of the five human DNA Pols (211) only Polδ and Polε require PCNA, it was proposed that repair synthesis is carried out by Polδ and or Polε (137, 179). Antibody inhibition studies with cell-free extract suggest that Polδ is the repair synthesis enzyme (220). In defined systems, Polδ, Polε, and even *E. coli* PolI can carry out repair synthesis but Polβ cannot (JT Reardon, personal communication). In DNA replication, the multisubunit RFC factor acts as a molecular matchmaker for PCNA (140, 164) to load it onto DNA as a polymerase clamp and hence confer processivity to DNA Polδ and Polε. In repair synthesis, RFC/PCNA appear to have two functions: the dissociation of the postincision complex (137) and the formation of the polymerase clamp for repair synthesis, likely in a manner analogous to the replication function (137, 179). The "gap" is filled in a precise manner without enlargement in either the 3′ or the 5′ direction (73) and hence the repair patch matches precisely the excision gap. The repair patch is then sealed by any of the four ligases (103), most likely ligase I.

POSTREPLICATION REPAIR

Bulky lesions block the progression of the replication fork and generate short DNA fragments. Postreplication repair refers to the molecular processes that enable the cell to complete replication without removing the lesion. Three mechanisms have been proposed (85, 132): translesion synthesis (197), template switching, and recombination. Cells from xeroderma pigmentosum variant (XP-V) exhibit normal excision repair yet are sensitive to UV and defective in postreplication repair. No in vitro model exists for postreplication repair and it is not known which of the three proposed mechanisms accounts for postreplication repair in vivo.

CROSSLINK REPAIR

Interstrand crosslinks are caused by a variety of carcinogens and chemotherapeutic agents such as nitrous acid, nitrogen mustard, psoralen, cisplatin, and mitomycin C (180). In *E. coli*, the repair of interstrand crosslink occurs in three basic steps (165): dual incisions of one strand by (A)BC excinuclease, homologous pairing with a sister duplex mediated by RecA, and finally dual incisions in the second strand by (A)BC excinuclease. Interstrand crosslinks of all of these agents are removed in vivo by mammalian cells (162). Furthermore, it has been reported that human cell-free extracts remove crosslinks induced by psoralen (154) and cisplatin (96). However, no mechanistic details are available at present. Based on mutagenesis studies with psoralen-adducted DNA, it has been proposed that crosslinks are eliminated from human cells by two mechanisms (162). In the error-free mechanism, dual incisions on one strand are followed by strand transfer from the intact duplex and ligation and dual incisions of the second strand followed by release of the crosslink and repair synthesis, in a manner analogous to crosslink repair in *E. coli*. In error-prone repair, following the dual incisions a DNA Pol fills in the gap by passing the lesion to generate a three-strand intermediate that is then repaired like any other excinuclease substrate. Naturally, all excision repair deficient cell lines but ERCC1 and ERCC4(XPF) mutants in particular are extremely sensitive to crosslinking agents (199). In addition, cell lines from Fanconi's anemia are sensitive to crosslinking agents but are not particularly sensitive to other damage, which is repaired by excision repair.

DOUBLE-STRAND BREAK REPAIR

Double-strand breaks (dsb) are made under physiological conditions during somatic recombination and perhaps during transposition (102). In addition, they are one of the major products of ionizing radiation (38) and of oxidative stress (31). However, there is a difference between dsb involved in somatic recombination and those generated by ionizing radiation: In somatic recombination one of the recombination events involves signal sequences and hence signal joint formation may be considered a site-specific recombination event. The coding joint formation is more similar to the double-strand break repair both formally and enzymologically, and both are thought to involve enzymes of homologous recombination. Thus not all cells incapable of somatic recombination are defective in dsb repair and, conversely, not all dsb repair mutants are defective in somatic recombination. Thus the RAG1 and RAG2 genes are required for V(D)J recombination but not for dsb repair (53). Conversely, mutations in a number of dsb repair genes are without a detectable effect on somatic recombination.

There are two naturally occurring mammalian mutants deficient in repair of dsb: the scid mouse and human ataxia telangiectasia (AT) patients. Cell lines of scid and AT are extremely sensitive to ionizing radiation. Scid mice are also defective in somatic recombination, whereas AT patients are not. Of the many mammalian genes thought to be involved in dsb repair the following are well characterized.

DNA-PK Genes

DNA-dependent protein kinase (DNA-PK) is a heterotrimer consisting of a p450 catalytic subunit and Ku86 and Ku70 regulatory subunits (2). It is a serine/threonine kinase absolutely dependent on free DNA ends. It phosphorylates many cellular proteins including p53 and the p34 subunit of (RPA). However, its physiological target is not known. Ku70 and Ku86 make a heterodimer with DNA end-binding activity that is an important antigen in autoimmune diseases such as scleroderma. Recently, by analyses of rodent mutants sensitive to ionizing radiation (ten complementation groups; 25) the three genes encoding the subunits of DNA-PK have been identified and cloned.

The p86 Ku gene CHO mutants in complementation group 5 are defective in V(D)J recombination and dsb repair and are sensitive to ionizing radiation (9, 54, 183, 195). The Ku86 gene (XRCC5) restores both functions as well as DNA end-binding activity to mutant cell extracts. The predicted p86 Ku protein sequence has no remarkable features. It lacks helicase motifs but in combination with p70 Ku constitutes a $3' \rightarrow 5'$ helicase (202). Mutants of p86 are defective in both coding and signal joint formation.

THE p70 Ku GENE Recently it was found that the gene encoding p70 Ku complements the rodent sxi-1 mutant, which falls into the complementation group 10 (XRCC10). The gene restores both the somatic recombination and dsb repair defect in mutant cells (94a).

THE p450 DNA-PK GENE Somatic cell genetics revealed that CHO V-3 and mouse *scid* mutants fall into the same complementation group (194) and that all mutants within this complementation group (number 7) are corrected by the gene encoding the p450 subunit of DNA-PK (6, 89, 95). Mutants in DNA-PK p450 subunits are not affected in signal joint formation but are defective in coding joint formation and repair of double-strand breaks.

The XR-1 Gene

The CHO XR-1 mutants (XRCC4, complementation group 4) are sensitive to ionizing radiation, defective in dsb repair, and fail to form V(D)J coding joints

and signal joints, and thus they are similar to XRCC5 (p86 Ku) mutants (193). The XR-1 gene has not yet been cloned.

The XRCC1 Gene

This gene corrects the repair defect in CHO-EM9 cells, which are sensitive to alkylating agents and ionizing radiation and have an increased rate of sister chromatid exchange. The gene encodes a 70-kDa protein, which is tightly associated with ligase III, and XRCC1 mutants have a diminished level of ligase III polypeptide (16). The mutants are not defective in V(D)J recombination and the main function of this gene may involve base excision repair rather than double-strand break repair.

In Vitro Systems for Double-Strand Break Repair

Conceptually, repair of double-strand breaks is simple: the ends are brought together, trimmed, and filled in as necessary to generate 3′-OH and 5′-P termini, and then the two pieces are ligated. However, the presence of ten complementation groups indicates that the process is more complicated. Certainly, recombination proteins are involved in repairing dsb as evidenced by the fact that yeast recombination genes (RAD52 epistasis group) are involved in dsb repair (51, 150). A number of groups have reported in vitro systems capable of dsb repair by homologous recombination (79, 108), and a recombination complex of 550–600 kDa containing at least five polypeptides of M_r 230, 210, 160, 130, and 40 kDa has been extensively purified from calf thymus (79). This fraction, which contains Polε and ligase III, can repair double-strand gaps by a gene conversion-like phenomenon. The reaction is stimulated fourfold by bovine SSB. The relation of this complex to other proteins involved in dsb repair remains to be determined.

TRANSCRIPTION AND REPAIR

Transcribed sequences are generally believed to be more accessible to repair enzymes because of "loose" chromatin structure (62). However, in addition to this passive connection between the two processes, transcription and repair appear to cross paths at all stages of transcription, i.e. activation, initiation, and elongation.

Activation: AP-1 and APE/Ref-1

AP-1 and NF-kB are nuclear and cytoplasmic transcription factors, respectively, that are involved in activation of genes involved in cellular proliferation. Both factors are regulated by oxidation status of specific cysteine residues.

AP-1 is a heterodimer of Jun and Fos proto-oncoproteins. The heterodimer

is inactivated in vitro as a result of oxidation of cysteine residues at the DNA binding domains of Jun and Fos. This reaction is reversed by the AP endonuclease (APE) (216). A specific cys residue (C65) in the N-terminal half is responsible for this Ref-1 activity and the C-terminal half carries the APE function (210, 217). There are three possibilities regarding the physiological role of the Ref-1 activity of APE. First, the effect might be a fortuitous in vitro reaction with no in vivo relevance where Jun and Fos are always reduced in the highly reducing nuclear environment. Second, APE may perform the AP endonuclease and Ref-1 functions in vivo independently of one another in a manner similar to other multifunctional enzymes. The third possibility is that APE not only activates AP-1 but through its interaction with Jun and Fos is also recruited to genes regulated by AP-1 and hence is placed in proximity of lesions in these genes, enabling APE to carry out transcription-coupled repair. Indeed, it has been reported that AP sites are repaired preferentially in actively transcribing genes even in the absence of nucleotide excision repair (94). However, the particular genes investigated are not controlled by AP-1, and at this point, transcription-repair coupling via APE transcription activators, though conceptually attractive, is a remote possibility.

NF-kB is a heterodimer (p50/p65) located in the cytoplasm in complex with IkB as an inactive trimer. Oxidative stress activates specific kinases that phosphorylate IkB; this makes it a target for proteolytic degradation and liberation of the NF-kB, which migrates into the nucleus to act on target genes (2, 173). It has been also reported that HIV Tat protein suppresses Mn-SOD production and creates a pro-oxidative condition that ultimately leads to NF-kB activation and tissue necrosis factor transcription and cytotoxicity (214).

Initiation: TFIIH

Transcription initiation by human RNA PolII is accomplished with the aid of seven general transcription factors (26, 219). Of all GTFs the TFIIH is the most elaborate factor. It is made up of seven subunits: p89(XPB), p80(XPD), p62 (yeast TFB1), p44 (yeast SSL1), p41 (cdk7), p38 (cyclin H), and p34. The helicase activities of XPB and XPD help in open complex formation and promoter clearance, and the cdk7/cyclin H (CAK) phosphorylates the CTD of RNA PolII (159, 176, 190).

The discovery of XPB and XPD in TFIIH led to the eventual realization that the entire TFIIH is a repair factor (43, 44, 171, 172). In addition, yeast strains with mutations in TFB1 (p62) and SSL1 (p44) are also defective in excision repair (51). Some ill-defined syndromes with mild UV sensitivity and non XP-like other symptoms are likely caused by mutations in other TFIIH complementation groups (77, 186).

That TFIIH is both a transcription and repair factor raises the question of

whether this dual function is relevant to coordinating cellular transcription and repair activities and whether TFIIH is directly involved in transcription-repair coupling. Several issues must be considered in any attempt to answer these questions. First, TFIIH is required for all nucleotide excision repair, both in transcribed and nontranscribed sequences. Second, TFIIH is recruited to promoters by TFIIE and to the lesion sites in DNA by XPA; TFIIE plays no role in repair (146). Third, transcription from a supercoiled substrate does not depend on TFIIH (219), but excision of damage is absolutely dependent on TFIIH with any type of DNA. Fourth, after promoter clearance TFIIH dissociates from the elongation complex (219), and hence delivery of TFIIH by RNA PolII to a lesion downstream of the promoter is unlikely. However, a local increase of TFIIH concentration as a result of promoter binding could conceivably lead to enhanced repair of both strands of transcribed genes.

Finally, because TFIIH is required for transcription initiation and for repair, it is conceivable that in a cell under genotoxic stress transcription and repair might compete for TFIIH, causing a reduced rate of transcription initiation. However, although DNA damage causes transcription inhibition there is no convincing evidence that transcription initiation is inhibited by damage. In fact, in UV-irradiated cells transcription recovers at a faster rate than replication because of transcription-repair coupling (116). The latter observation, at face value, would imply that damage in nontranscribed sequences may not compete efficiently with promoters in recruiting TFIIH. Clearly, this issue must be addressed in a well-defined in vitro experimental system before it can be decided whether competition for TFIIH is a factor in the turn-off of RNA synthesis upon DNA damage.

Elongation: Transcription-Repair Coupling

Transcribed sequences are repaired more rapidly than nontranscribed sequences (7) and lesions in the template (transcribed) strand are repaired at a faster rate than lesions in the coding strand (122). The mechanistic aspects of these phenomena (transcription-repair coupling, TRC) are reasonably well understood in *E. coli* (174). RNA Pol stalls at a lesion and makes it inaccessible to the excinuclease. A transcription-repair coupling factor (TRCF) encoded by the *mfd* gene recognizes the stalled complex and releases the stalled RNA Pol while simultaneously recruiting the UvrA damage–recognition subunit of (A)BC excinuclease. The net result is about tenfold enhancement in the repair rate of lesions in the template strand. Gene and strand-specific repair in humans appear to be similar to that in *E. coli* in outline; however, the mechanistic details are more complex.

Two genes, apparently, are required for coupling transcription to repair and

hence performing a function akin to that accomplished by a single gene (*mfd*) in *E. coli*. Cockayne's syndrome is characterized by lack of preferential repair (205, 206), and cell fusion studies reveal two complementation groups, CSA and CSB. The corresponding CHO genes are ERCC8 and ERCC6 (178, 201). Mutations in either gene eliminate preferential repair. The ERCC6/CSB gene has been cloned. It encodes a 160-kDa protein with helicase motifs but without extensive homology to the *E. coli* TRCF protein (201). Biochemical data on ERCC6/CSB are scant. Existing data are consistent with a model whereby a stalled RNA PolII is recognized by the CSA/CSB heterodimer, which displaces the stalled complex and recruits the excinuclease subunits XPA and TFIIH to the lesion site (175).

However, there is a theoretical problem with this model. It takes over 20 h to transcribe certain human genes (the dystrophin gene is 1.6×10^6 bp) and within this period it is more than likely that the gene would sustain a transcription terminating lesion. Were coupling to occur by release of truncated transcript the cell would never make dystrophin (62). This is an interesting argument but it critically depends on the accuracy of the estimated rate of transcription in vivo and hence would be irrelevant if the actual transcription rate is tenfold faster. Although some controversy exists regarding various points of TRC in humans, the following data are taken to be factual on the phenomenology of preferential repair (44, 62, 175).

(*a*) TRC occurs only in genes transcribed by RNA PolII. (*b*) TRC is observed with any lesion which blocks transcription; these are generally bulky lesions that can only be eliminated by excision repair. (*c*) Enhanced gene repair is mainly confined to the template strand although there are some reports of a moderate increase in the rate of repair of the coding strand. (*d*) Mutations in five genes cause Cockayne's syndrome: CSA/ERCC8, CSB/ERCC6, XPB, XPD, and XPG. In CSA and CSB mutants overall genomic repair is normal (152, 206), hence it is assumed that the lack of preferential repair causes the disease, which manifests neuroskeletal abnormalities without increased rate of skin cancer, a hallmark of XP syndrome. Only some of the XP-B, XP-D, and XP-G patients exhibit CS. However, in these cases the residual repair activity is so low as to make it impossible to measure whether the residual repair is strand specific.

Taking into account all of these observations, the following is a reasonable model for TRC in humans. RNA PolII stalls at a lesion, and the stalled complex is recognized by TFIIS (41) and CSA/CSB proteins; TFIIS enables RNA PolII to back up while CSA/CSB complex with the aid of TFIIE recruits XPA and TFIIH to the lesion site that is now accessible; the excinuclease then assembles at this site, and the dual incisions and repair synthesis restore a lesion-free duplex that can now serve as a template for RNA PolII to elongate the truncated transcript. The main difference between this model and the prokaryotic model

is that in *E. coli* the truncated transcript is discarded, whereas in humans it is reused.

Transcription-coupled repair is not essential for cell survival. *E. coli* (174), yeast (204), and human (206) mutants lacking TRC are only moderately sensitive to genotoxic agents and have about tenfold increase in induced mutation rate. An important function of TRCF may be to remove protein roadblocks (such as stalled RNA Pol, or specific DNA binding protein at their target sequences) from the path of the replication fork (175). RNA PolII stalled at these roadblocks is likely removed by a family of proteins of which TFIIS and ERCC6 are but two examples. Some of these elongation factors such as ERCC6 may, in addition to clearing the path for replication machinery, have acquired specialized functions such as coupling transcription to repair. The reconstitution of an in vitro transcription/replication/repair system may be necessary to answer these questions.

CELL CYCLE AND REPAIR

Two critical factors in cell cycle are the integrity of DNA before the cell enters the S phase and the presence of two sets of intact chromosomes before the cell enters mitosis. Hence it would be beneficial if the cell had regulatory mechanisms to stop the cell cycle at the G1/S and G2/M boundaries when the genome contained lesions. A number of studies have concluded that the cell cycle is tightly coupled to excision repair and dsb repair.

Change in Repair Capacity with Cell Cycle

A number of in vivo studies have failed to yield a consistent pattern of change in excision repair activity with cell cycle. However, the repair of genes expressed in a cell cycle–dependent manner varies in the predicted manner because of TRC (160). There is no evidence of cell cycle–dependent regulation of expression of excision repair genes or change in efficiency of preferential repair (106).

Role of p53 in Coupling Cell Cycle to Repair

The p53 tumor suppressor is a tetrameric phosphoprotein that is mutated in a large fraction of human cancers (65). It performs its tumor suppressor function, in part, by acting as a transcriptional activator for genes that block the cell cycle at the G1/S boundary. Two of these genes in particular have been implicated in mediating the effect of p53 on excision repair: p21 (cip1, WAF1) and Gadd45.

THE ROLE OF p21 (CIP1/WAF1) This protein is induced by p53 (62), binds to cdk/cyc complexes (cyclin interacting protein), inhibits the activity of cyclin-dependent kinases (cdk) and thus blocks the cell cycle progression. Recently, it was found that p21 also binds PCNA (49, 100) and inhibits replication, but

apparently not excision repair (100). It was suggested that the PCNA-p21 complex was incapable of acting as a polymerase clamp for Polδ or Polε but was capable of carrying out its repair function. It was proposed that such a response would help the cell in avoiding the untoward effects of DNA damage by stopping replication until the integrity of DNA was restored by repair. However, more recent studies have shown that p21 inhibits both replication and repair synthesis (223). Whether there is a differential effect to confer a selective advantage in vivo remains to be determined.

Gadd45 AND EXCISION REPAIR Gadd45 is one of many proteins induced by growth arrest and DNA damage (50). The induction of Gadd45 is regulated by p53 and overproduction of this 16-kDa protein causes growth inhibition (17). It was also reported that addition of Gadd45 to cell-free extract (CFE) stimulated excision repair by a factor of 3 (184). However, a subsequent study failed to confirm any effect of Gadd45 either on the excision or resynthesis steps of nucleotide excision repair, and it was concluded that Gadd45 has no function in DNA repair (A Kazantsev & A Sancar, unpublished data).

Role of HSSB in Coupling of Cell Cycle to Repair

Human single-stranded DNA binding protein (HSSB, also known as replication protein A, RP-A) is required for DNA replication (74) and excision repair (28, 130) and it interacts with certain transcription factors (99). It is thought to play an important role in all DNA transactions. Indeed, a mutation in the p70 subunit of the yeast HSSB is conditionally lethal and the mutant is defective in replication, recombination, and repair (107). HSSB is a trimeric protein (p70, p34, and p11) and the p34 subunit becomes phosphorylated at the G1/S boundary of the cell cycle (37) and upon DNA damage by ionizing (105) or UV (19) radiation. In vitro studies indicate that cdks and DNA-PK are involved in phosphorylating p34 (144).

Of special significance, UV and ionizing radiation cause rapid phosphorylation of HSSB and cessation of DNA replication (19); furthermore, it was reported that CFE from UV-irradiated cells could not support the replication of SV40 and that addition of unphosphorylated HSSB restored the replication capacity of the extract (19). It was hypothesized that, if phosphorylated HSSB functioned normally in excision repair, damage-induced phosphorylation would enable the cell to repair its DNA before resuming replication. However, later studies revealed that phosphorylation had no effect on HSSB function in replication (15, 67, 144, 145) or repair (145). Thus it appears that HSSB plays no role in coordinating the cell cycle with repair.

CAK and DNA Repair

The phosphorylation of p34^{cdc2}-cyclin complex at thr161 of p34^{cdc2} is necessary for the cell to make the G2-M transition. This phosphorylation is carried out by the cdk-activating kinase, CAK (185). Recent research has revealed that the CAK activity is associated with the cdk7–cyclin H complex (185). This complex is found in two main forms in the cell: either as cdk7–cyclin H heterodimer or as part of TFIIH general transcription and repair factor (159, 176, 190). The significance of these finding vis-á-vis coordinating repair with the cell cycle is unknown. That both cyclin H and CAK activity are constant throughout the cell cycle (185) makes the prospect of cell cycle–dependent control of repair through CAK rather uncertain.

CELLULAR RESPONSE TO GENOTOXIC STRESS

In *E. coli* there are three well-defined DNA damage response systems: the SOS response (208), adaptive response to alkylating agents (104), and adaptive response to oxidative stress (32). To put the studies on human response reactions to DNA-damaging agents in perspective, it is useful to review the prokaryotic reactions briefly because the results with human cells are often interpreted within the context of the prokaryotic model.

In the SOS response (208) the initiating signal is ssDNA generated by a replication block by bulky DNA lesions such as pyrimidine dimers. The RecA protein binds to these regions, becomes activated as a co-protease, and promotes autocatalytic cleavage of the LexA repressor. Autocleavage of LexA turns on the transcription of genes involved in recombination (*recA, recO*), repair (*UvrA, UvrB, uvrD*), replication (*umuDC, dnaQ, polB*), and cell division (*sulA*). Cells stop dividing, repair lesions at a faster rate, or bypass them by recombination or translesion synthesis. These events generate two uninterrupted chromosomes that are transmitted to the daughter cells.

In the adaptive response to alkylating agents (104) the Ada protein, a positive regulator, becomes activated by alkylation of Cys69 by transfer of the alkyl (usually methyl) group from a DNA phosphorotriester. Upon activation, Ada turns on genes involved in inactivating the alkylating agent (*aidB*), and genes that eliminate the lesion by either alkyltransferase (*ada*) or glycosylase (*alkA*) action.

The adaptive response to oxidative stress (32) differs from the other two genotoxic response reactions in one important aspect. In the SOS response and adaptation to alkylating agents the signal for the response is DNA damage. In contrast, in the defense responses to oxidative stress the signal is the reactive oxygen species (ROS) itself. There are two distinct responses to oxidative stress: the OxyR regulon and the SoxRS regulon. The OxyR regulon is con-

trolled by the OxyR protein. OxyR acts as a positive regulator for genes encoding proteins that inactivate the ROS such as *katG* (catalase) and *ahpC* (alkylhydroperoxide reductase). OxyR binds upstream of the cognate promoters and upon oxidation of its cys199 residue undergoes a conformational change, resulting in contact with RNA Pol and transcriptional stimulation. The SoxRS regulon consists of a two-component (sensor protein/regulator protein) regulatory system and target genes. SoxR is the sensor of superoxides by virture of an Fe_4S_4 center. Upon oxidation, it binds to the SoxS promoter and turns the gene on. The SoxS protein, in turn, stimulates transcription of genes encoding proteins that detoxify ROS (MnSOD and Glucose-6-phosphate dehydrogenase) and enzymes that repair DNA (endoIV AP endonuclease).

Considerable research has been conducted on the cellular response to genotoxic agents in human cells; many responses are elicited by agents that damage DNA as by any other external stimulus. However, a critical analysis of the vast literature on the topic leads to the conclusion that human cells do not have an SOS response, an adaptive response to alkylating agents, or an adaptive response to oxidative stress in the sense of a set of coordinated molecular reactions set off by the offending agent and aimed at detoxifying of, or eliminating the damage caused by the genotoxicant. Nevertheless, DNA-damaging agents cause a series of changes in gene expression in human cells, including induction and repression. These changes may be incidental and of no consequence, important for cell survival, or lethal to the cell but beneficial for the survival of the organism. Without attempting to classify these responses in mechanistic or functional terms, the phenomonology is presented below.

The p53 and Growth Inhibition

p53 is an important tumor suppressor as evidenced by the fact that nearly 30% of human cancers carry p53 mutations (65). It is a tetrameric phosphoprotein and acts as a transcriptional activator for genes that negatively control cellular growth. p53 is unstable; however, upon DNA damage by UV (111) or ionizing radiation (81) it becomes hyperphosphorylated and stable and blocks the cell cycle at the G1/S border (90). The increased level of p53 has been proposed to aid cell survival by several mechanisms. First, p53 induces the synthesis of p21 (63), which inhibits cdk/cyclin activity by directly binding to these complexes and hence inducing cell cycle arrest at the G1/S border, and thus prevents attempts to replicate damaged DNA. The p53-induced Gadd45 also arrests cell cycle by some unknown mechanism (184) and hence may help genomic stability through Gadd45 induction. The growth inhibitory effect of p53 is well documented.

It has also been suggested that p53 promotes DNA repair by a variety of mechanisms. p53 binds to XPB, suggesting that this may aid repair (212). It

was also claimed that the increased amount of Gadd45 stimulates excision repair in vitro by a factor of 3 (184). However, the p53-XPB binding appears to have no effect on repair because neither CFE depleted of p53 nor a defined excision repair system repair better upon supplementing with p53. Similarly, the report on Gadd45 stimulation of excision repair has not been confirmed (A Kazantsev & A Sancar, unpublished data). Finally, p53-deficient mouse cells have UV survival indistinguishable from wild-type cells and excise Pyr-Pyr and (6–4) photoproducts at a rate identical to that of normal cells (76). Clearly, there is no convincing evidence at present that either p53 or p53-induced proteins play any direct role in any repair mechanism.

The Ras Pathway and Growth Stimulation

In addition to activating a cell cycle inhibitory response mediated by p53, UV also induces a cell growth stimulatory response mediated by the Ras signaling pathway. This genotoxic response reaction is referred to as "UV response" rather than DNA-damage response because existing data suggest that the initiating signal originates in the membrane and not in DNA (33, 34, 161). The proposed signal transduction pathway is as follows: UV generates ROS in the membrane, which activate growth factor receptors without inducing growth factor release. The phosphorylation of tyr residues of growth factors activates the signal transduction pathway, which goes through GFR → Src → Ras → Raf → MAPKK → MAPK → transcription factors, through a series of phosphorylation/dephosphorylation reactions. Of the many target transcription factors Jun, Fos, and NF-kB are perhaps the most important. Jun and Fos are activated by phosphorylation of their activating domains, whereas NF-kB is activated by proteolytic degradation of IkB in the cytoplasm, liberating NF-kB and thus enabling it to enter the nucleus (3). It has been suggested that this response reaction helps the cell survive because if cells are treated with N-acetylcysteine, which scavenges ROS, the Jun-Fos induction is inhibited; similarly, treatment of cells with the tyrosine kinase inhibitor, tyrphostin, makes them more sensitive to UV radiation (33). The UV activation of NF-kB has also been implicated in UV induction of HIV because NF-kB regulates HIV expression (68). In addition to the immediate UV response mediated by Ras and AP-1, there is a delayed response involving the genes of collagenase, metallothionein, and TPA (68). However, the protective effect of this response is not known and even the protective effect of immediate response is only moderate; further studies are needed to resolve the question of its usefulness to the cell. A UV response mediated by Ras2 and affecting the yeast AP-1 homolog GCN4 confers a moderate survival advantage (48). It is conceivable, however, that in humans the UV response represents a cellular signal transduction pathway subverted from its physiological role and is simply a symptom

of a genotoxic stress with no beneficial effects for the cell but with potential beneficial effects for the organism.

DNA Damage and DNA-Dependent Protein Kinase

Although both the p53-mediated and Ras-mediated response reactions involve protein phosphorylation, these phosphorylations are mainly part of the signal transduction reaction. In contrast, protein phosphorylation mediated by DNA-PK is hypothesized to directly affect the repair proteins (2, 57). The DNA-PK heterotrimer binds to DNA ends generated by ionizing radiation or by repair of UV damage, and the catalytic p450 subunit becomes phosphorylated and in turn phosphorylates HSSB and p53, which are known or thought to participate in repair directly. Phosphorylation of HSSB has no effect on its activity (145) and there is no evidence that p53 directly participates in repair. However, DNA-PK plays an essential role in dsb repair as evidenced by the hypersensitivity of mutants to ionizing radiation (6).

DUN Genes and Ribonucleotide Reductase

Ribonucleotide reductase (RNR) converts rNDPs to dNDPs and hence is an essential enzyme for DNA synthesis. RNR is damage inducible in all organisms tested ranging from *E. coli* to man (47). Although it has not been demonstrated that induction of RNR aids in cell survival, the inducibility of RNR genes has provided a useful tool in isolating genes involved in damage response reactions. Several such genes have been identified on the basis of damage uninducibility (DUN) of yeast mutants, and two have been characterized. DUN1 is a Ser/thr kinase and appears to play an important role in cell survival following DNA damage by MMS and UV because mutant cells have increased sensitivity to these agents (222). It does not affect the cell-cycle checkpoints nor does it regulate the RAD3 epistasis group (excision nuclease) genes. DUN1 becomes autophosphorylated upon DNA damage. The signal for phosphorylation is assumed to be single-stranded DNA, and DUN1 is assumed to exert its effect on RNR1 and RNR2 genes by phosphorylating some transcription factors. DUN2 is the large subunit of DNA Polε and appears to function upstream of DUN1 (136). In contrast to Dun1 mutants, Dun2 mutants are defective in both S phase checkpoint and in DUN1 autophosphorylation and hence RNR induction. Mutation analysis suggests that the N-terminal half of Polε has the polymerase active site whereas the C-terminal half contains the S-phase checkpoint domain. It has been proposed that Polε acts as a sensor of DNA damage/replication block and transmits the signal to DUN1. No human homolog of DUN1 has yet been identified and thus it is not known whether human Polε plays a role in damage response reactions.

DNA Damage–Inducible Genes and Proteins

In addition to the response reactions described above, a number of other genes and proteins are induced by DNA damage. There are about 20 *gadd* (growth arrest DNA damage) genes defined by relative enrichment of their transcripts following damage (50). Some, such as *gadd153*, are induced at the transcriptional level and some, such as *gadd45* and *gadd34*, are enriched as a result of increased mRNA stability (78). Similarly, several UV-inducible DNA binding proteins were detected by the "northwestern" hybridization technique (55) and 13 damage-inducible RNA-binding proteins were detected with the HIV-1 TAR element as a probe (17).

Of the repair proteins, the induction of Polβ by alkylating agents is well documented (135), and there is evidence that MGMT is induced by MNNG and other damaging agents including UV in certain cell types (92) by an unknown mechanism. A damaged DNA binding protein (DDB) that binds with high affinity to certain lesions such as (6–4) photoproducts but not to *cis-syn*-T<>T or cisplatin adducts (153) is absent in some XP-E patients (22) but present in others (83, 86). DDB is inducible by UV (69) and by cisplatin but it does not bind to cisplatin-damaged DNA nor does its level in the cell correlate with the cisplatin resistance level of the cisplatin-resistant cells (203). Finally, heme oxygenase is strongly induced by near UV and H_2O_2 and there is some evidence that it plays a role in cellular defense as antioxidant (87).

DNA REPAIR AND CANCER CHEMOTHERAPY

Most anticancer drugs damage DNA. Ionizing radiation, bleomycin, and neocarzinostatin induce double-strand breaks; cisplatin and mitomycin C make bulky adducts and interstrand crosslinks; and BCNU and nitrogen mustard cause nonbulky alkylation products as well as interstrand crosslinks. The susceptibility of cancer cells to chemotherapy is influenced by many factors including cell type, drug uptake and efflux, biotransformation of the drug, translesion replication, and repair capabilities of the cell. From the standpoint of DNA repair two topics are of interest. First, how DNA damage kills cells and second, whether the cell's ability to replicate past lesions or remove the lesion increases in drug-resistant cells.

Cell Death by DNA Damage

The conventional view of cell death by DNA damage holds that lesions at a level beyond the repair capability of the cell lead either to permanent inhibition of replication or incomplete replication, resulting in gapped duplexes and eventual degradation of DNA and other cellular constituents by nucleases and proteases and other lysosomal enzymes (necrosis). Thus, it was logical to

presume that rapidly dividing cancer cells would be more susceptible to killing by agents that damage DNA. However, recent research has revealed that genotoxic agents often kill cancer cells by activating the programmed cell death (apoptosis) response (109, 198). In most cell types, p53 is required to induce apoptosis by inducing many genes including the ICE (interleukin converting enzyme) protease. The Bcl-2 oncoprotein, which apparently generates ROS (187), functions as an antiapoptotic protein. Thus, p53-deficient lymphoid cells are more resistant to killing by a variety of DNA-damaging agents than wild-type cells (188), and Bcl-2 is involved in preventing both p53-dependent and p53-independent apoptosis (188) as well as death by necrosis.

Drug Resistance and Increased Repair

Some cancers are intrinsically resistant to anticancer drugs and some are initially sensitive, but a subpopulation of cells that survives the original treatment no longer responds to the same drug. While the intrinsic resistance could be due to many factors, it has been reported that some subclones derived from a human melanoma cell line had increased rate of Pyr<>Pyr and 6–4 photoproduct excision (66). Most studies with the second type of resistance, i.e. resistance developed after the genotoxic treatment, have been conducted with cisplatin-resistant tumors and cell lines. The main findings may be summarized as follows.

First, in some resistant cell lines both intra- and interstrand crosslinks disappear faster than controls (113, 221). Whether this occurs by excision repair is not known. Experiments with CFE have failed to detect increased excision repair activity (207); however, it has been reported that CFE from a human epithelial cell line transformed with ras oncogene has more than twofold increase in excision repair activity (96). Second, some resistant cell lines perform replicative bypass more efficiently than sensitive cells (112). However, whether this represents a change in the repair or replication machinery or is a manifestation of the cancerous phenotype is still unclear. Third, a number of proteins that bind to damaged DNA have been implicated in resistance (23). Two of these have been well investigated. The DDB protein is increased in certain cisplatin-resistant cell lines (20, 21, 203); however, it does not bind to cisplatin lesions with high specificity and in the in vitro excision assay it has no effect on cisplatin repair. The HMG-domain proteins bind to cisplatin-GG diadduct with high affinity (14, 149) and they do not stimulate, but actually inhibit repair (13, 71). Hence, the significance of HMG domain proteins in resistance is unclear. Finally, an increase in mRNA levels of certain excision repair genes such as ERCC1 (29) in cells from cisplatin-resistant cancer tissue has been reported. However, it is not known if the corresponding proteins are limiting factors in excision repair or if this increase is a simple

manifestation of relative disregulation of most genes in cancer cells. Incidentally, increased expression of ERCC1 (11) and methylpurine DNA glycosylase (27) in CHO cells increases their sensitivity to DNA-damaging alkylating agents, indicating that increase in a repair enzyme level does not necessarily result in increased resistance to the lesion repaired by that enzyme.

MITOCHONDRIAL REPAIR

Oxidative phosphorylation in mitochondrial membrane accounts for about 90% of total cellular oxygen consumption. Furthermore, almost 30% of the electrons entering the electron-transfer system are apparently diverted from their normal path and into the mitochondrial membrane or matrix where they generate ROS. As a result, mitochondrial DNA is exposed to more oxidative damage than nuclear DNA. It has been found that 8oxoG is present in mitochondrial DNA at a frequency of ca 10^{-4}, compared to a frequency of 10^{-5} for nuclear DNA (177). This high frequency of oxidative damage in mitochondrial DNA is thought to play a critical role in aging (1, 177). Mitochondria have thymine glycol glycosylase and APE, which eliminate some of the oxidative base lesions from DNA (45). Similarly, alkylated bases such as O^6MeGua and methylpurines are repaired by the nuclear enzymes (125). However, there is no nucleotide excision repair in human mitochondria (177).

DNA REPAIR AND HUMAN DISEASES

Several human hereditary diseases are caused by mutations in DNA repair genes or in genes involved in eliminating ROS.

Xeroderma Pigmentosum

The disease is caused by a defect in excision repair. It is characterized by photodermatoses including skin cancer at early age, and in some cases neurological abnormalities (24). Of the seven complementation groups, A–G, the A, B, D, and G forms manifest neurological symptoms. In addition to these groups, there is a heterogeneous group called XP variant (XP-V) in which cells have normal excision repair but are defective in the so-called postreplication repair (10). The molecular basis of XP-V is not known.

Cockayne's Syndrome and Trichothiodystrophy

Cockayne's syndrome is characterized by photosensitivity but in its classic form not by increased cancer rate, and by dwarfism, and mental retardation (134). The classic form is caused by mutations in CSA and CSB genes, which are necessary for TRC. In overlapping forms the patients have mutations in

XPB, XPD, or XPG genes. Since XPB and XPD are subunits of TFIIH, and XPG tightly binds to TFIIH, it is conceivable that in the XP/CS form the residual repair activity is not coupled to transcription and that lack of TRC leads to Cockayne's syndrome, whether the basal excision repair machinery is impaired or not (62).

Trichothiodystrophy (TTD) patients are photosensitive but do not have increased rate of skin cancers, are physically and mentally retarded, and have brittle and sulfur-deficient hair and ichthyosis. The patients fall into three clinical forms corresponding to mutations in three genes. Those with XP-like symptoms have mutations in either the XPB or most commonly the XPD gene; the third complementation group does not manifest photosensitivity and is presumably due to a mutation in a TFIIH subunit other than XPB or XPD (77, 186). It is possible that some of the unclassified patients with TTD and related syndromes have mutations in any of the TFIIH subunits. Presumably, these mutations interfere more with the transcription function of TFIIH than the repair function.

Ataxia Telangiectasia

Ataxia Telangiectasia (AT) is an autosomal recessive disease characterized by cerebellar ataxia, high incidence of cancers, and hypersensitivity to ionizing radiation but not to UV (191). It has been reported that heterozygotes have a high incidence of cancer due to exposure to natural and iatrogenic ionizing radiation (192). The genetic basis of AT has recently been elucidated. It appears that a single gene (ATM) that encodes a PI-3-like kinase is mutated in all four complementation groups (224). AT cells manifest IR-resistant DNA synthesis indicating a defect in cell-cycle checkpoints. However, a report that AT gene(s) is necessary for IR-induced elevation of p53 protein (81) has not been confirmed (110).

Bloom's Syndrome

Patients with Bloom's syndrome (BS) have moderate photosensitivity and suffer from dwarfism and mental retardation. BS cells have an increased rate of sister chromatid exchange and slow rate of joining of single-strand nicks. Reports of a ligase I defect in BS syndrome have not been confirmed (124, 148). Similarly, claims of defects in uracil glycosylase or SOD have been discarded based on the fact that the chromosome 15, which complements BS, does not carry the genes for these enzymes (120).

Fanconi's Anemia

Patients with Fanconi's anemia (FA) manifest aplastic anemia, which is eventually followed by leukemia and death at a young age. FA cells exhibit chromosomal aberrations and are extremely sensitive to crosslinking agent

such as psoralen and mitomycin C. There are four complementation groups, FA-A through FA-D. The FAC gene has been cloned; it encodes for a 63-kDa protein; its sequence does not reveal any structural motif (189). Recently, it has been reported that a cyclin-related protein of 7.9 kDa reverses the crosslink-induced permanent S-phase block of group FA-A (36). The significance of this observation is unknown.

Amyotrophic Lateral Sclerosis (Lou Gehrig's Disease)

The disease is characterized by progressive motor neuron degeneration throughout the nervous system. The familial form is autosomal dominant. Some of the familial forms are associated with missense mutations in Cu/Zn-SOD (157). This finding led to the suggestion that decreased SOD leads to accumulation of superoxide radical and other ROS with catastrophic effects on motor neurons. However, later studies have revealed that the missense mutations found in some amyotrophic lateral sclerosis (ALS) families do not alter SOD activity, yet transgenic mice expressing human genes with these mutations develop an ALS-like syndrome, whereas control animal overexpressing wild-type human SOD do not (59, 156). It has been suggested that this gain of function may represent altered specificity of SOD so that it generates other free radicals particularly toxic to motor neurons (54, 158). Whatever the mechanism, clearly ROS metabolism plays an important role in ALS.

CONCLUSION

For historical reasons the study of DNA damage and repair was initiated and has been conducted for many years by scientists interested in the effects of radiation on biological systems. As a consequence, these topics have been only of peripheral interest to the majority of biological scientists interested in replication, transcription, gene regulation, and cell cycle. In fact DNA damage and repair occur under physiological conditions as part of normal cellular events. Hence, even a reductionist description of cellular processes must necessarily encompass DNA repair. Indeed, an important conceptual breakthrough in molecular biology in recent years has been the realization of the intimate interconnections between basic cellular reactions (cell cycle, replication, transcription, recombination, repair) previously thought to function more or less independently. It is expected that such an integrative approach would lead to a better understanding of the cell and the organism. Such an understanding would help in designing better approaches to cancer treatment and may even help with our long-standing quest for slowing the aging process.

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