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DNA REPAIR ENZYMES

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Perspectives and Summary

Preventing an unacceptably high mutation rate is a challenge to the cell. Enzymatic mechanisms of great intricacy have evolved to ensure that DNA is replicated with high fidelity, and mismatch repair activities exist to remove the rare misincorporated residues that have escaped proofreading during replication. Potentially mutagenic alterations also arise in nonreplicating DNA by spontaneous hydrolysis under physiological conditions and by exposure to radiation and chemical mutagens. The spontaneous decay of DNA is greater than generally recognized; the loss of bases due to depurination and depyrimidination of DNA amounts to several thousand

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residues per genome per day for a mammalian cell. Moreover, premutagenic deamination of about one hundred cytosine residues and a few adenine residues also occurs under the same conditions. The recent observation that S-adenosylmethionine acts as a weak DNA alkylating agent indicates that nonenzymatic DNA methylation takes place in vivo. The sites methylated in this fashion are different from those modified by DNA methylases, and the amount of DNA damage could be similar to that caused by the total spontaneous hydrolytic decay (1).

Repair activities that recognize these various alterations in DNA, and also other important forms of damage, such as the most common radiation-induced lesions, presumably appeared very early during evolution as they seem to be universally distributed in living cells. The major pathways of DNA repair are, in fact, surprisingly similar in *Escherichia coli* and in mammalian cells. It would appear that the same kinds of repair enzymes, which presumably evolved to avoid mutations to lethality or auxotrophy in primitive organisms, may also be employed to prevent certain deleterious events unique to more complex structures, such as the persistence of lesions that might cause transformation to malignancy; no novel DNA repair pathways have been detected in higher cells for these purposes. (Cells from long-lived organisms such as man have more effective mechanisms of DNA repair than those from mice and other short-lived animals.) The universally occurring repair activities seem to serve efficiently to counteract transformation, as exemplified by the relatively harmless effects of sunlight on normal humans when compared to its strongly carcinogenic effects in xeroderma pigmentosum patients. Further, repair enzymes that function to correct radiation damage and unavoidable DNA decay in the intracellular milieu also provide protection against many potential, chemical mutagens recently introduced into the human diet and environment. For instance, the occurrence of sulfur dioxide as an air pollutant, and the use of its neutral aqueous form, bisulfite, as a component of many common beverages, is a recent phenomenon. The latter agent causes one of the same mutagenic DNA alterations produced by spontaneous hydrolysis, that is, deamination of cytosine to uracil (2), and is only a very weak mutagen, since the DNA damage it causes is efficiently repaired.

The most important DNA repair pathways, in *E. coli* as well as in human cells, depend on the excision of an altered residue or group, and many different enzymes initiating such reactions have been discovered recently. The two main types of activities reside within DNA glycosylases, which cleave the base-sugar bond of a nonconventional nucleotide residue, and nucleases, which incise DNA by the specific cleavage of a phosphodiester bond adjacent to a damaged residue. Repair activities of these kinds possibly comprise the largest group of enzymes acting on DNA in the cell. So far,

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about 15 different enzymes have been discovered that apparently serve exclusively in the early stages of DNA repair, and this no doubt represents only a fraction, perhaps one half, of those needed to fulfill the functions required for initiating the removal of commonly occurring lesions from DNA. In this review, the properties of the enzymes hitherto known to be involved in these processes are summarized.

In addition to the various types of excision mechanisms, post-replication repair occurs that, at least in bacteria, depends on recombination between daughter strands, and error-prone inducible repair functions have been demonstrated. As these latter processes have not yet been reproduced in cell-free systems, a discussion of the enzymes possibly involved in them is deferred. Further, factors postulated to make the DNA in mammalian cells accessible (or nonaccessible) to repair enzymes are not discussed. It has often been proposed that the DNA in nucleosomes could be refractory to repair, but this seems unlikely from a physiological point of view, and the experimental evidence is not convincing.

The present review covers enzymes that specifically recognize different kinds of altered DNA, and special emphasis is laid on progress made over the last three years (see 3). The more general activities that catalyze exonucleolytic excision, gap-filling, and ligation are not described. A summary of the latter has been included in a recent review by Grossman (4), and a general and comprehensive review of different repair pathways has already appeared in this series (5). A number of other reviews covering various aspects of DNA repair have also appeared (6–9), and the informative article by Friedberg et al (8) on inherited human diseases associated with defective DNA repair is especially noteworthy.

DNA Glycosylases

Several enzymes termed DNA glycosylases, which catalyze the cleavage of base-sugar bonds in DNA, have been found recently, and most of them are widely if not universally distributed. They have the common property of acting only on altered or damaged nucleotide residues. So far, attempts to find DNA glycosylases that might correct mismatched bases or remove 5-methylcytosine from DNA have failed. The physical and general biochemical properties of the different DNA glycosylases are similar; the enzymes are of relatively low molecular weight, with reported values between 18,000 and 31,000. Further, they do not require cofactors such as divalent metal cations, and apparently act by simple hydrolytic cleavage of the glycosyl bond. Double-stranded DNA is the preferred substrate; with the notable exception of uracil-DNA glycosylase, there is little or no activity against damaged single-stranded DNA, and no DNA glycosylase cleaves mononucleotides. Since these activities resemble each other also from the

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point of view that they have similar purification properties and appear to be present only in small amounts in cells (again with the exception of uracil-DNA glycosylase), it becomes an increasingly arduous task to show that each newly discovered member of this class is not identical to a previously known DNA glycosylase. Nevertheless, there appears to be little overlap between the various activities, although all of them have not yet been characterized in detail. Each enzyme has a narrow substrate specificity, and may have evolved as a defence against a single type of DNA lesion. Thus, two different DNA glycosylases are required to remove deaminated cytosine and deaminated adenine from DNA, and these enzymes cannot replace each other. Further, two distinct DNA glycosylases serve to remove 7-methylguanine and its imidazole ring–opened derivative from alkylated DNA, and neither of these two activities can release guanine that contains a bulky substituent group, such as an aflatoxin B_1 residue, at the 7 position. The properties and the substrate specificities of the DNA glycosylases strongly indicate that they are all involved in repair processes. However, direct evidence for this notion is only available in the cases in which enzyme-deficient mutant cells have been isolated and shown to be anomalously sensitive to DNA-damaging agents. It is clear, though, that the apurinic or apyrimidinic site generated by the release of a nonconventional base can be rapidly corrected by an excision-repair process that involves the replacement of a single nucleotide residue or at most a small number of such residues.

URACIL-DNA GLYCOSYLASE The uracil-DNA glycosylase from human cells has recently been extensively purified and characterized (10, 11). It has a molecular weight of about 30,000, similar to the 25,000 found for the *E. coli* enzyme (12). The human enzyme, however, has a 20 times higher K_m (about 1 μM) for dUMP residues in DNA than the bacterial enzyme. Human uracil-DNA glycosylase is found mainly in the cell nuclei (13). Mitochondria also contain uracil-DNA glycosylase, but whether this minor activity is distinct from the nuclear enzyme is not known (14). The nuclear enzyme appears to be induced during cell proliferation in contrast to the mitochondrial activity (14a). Both the *E. coli* and human uracil-DNA glycosylase are product-inhibited by free uracil, with a K_i of about 2.10^{-4} M, and somewhat surprisingly, initial velocity measurements at different uracil concentrations indicate a noncompetitive form of inhibition (10, 12). One possible explanation of these data would be that the enzyme acts in a processive fashion, and that the free base interferes with the progression of the enzyme along the DNA. A similar model (15) to explain this kind of unusual product inhibition of a DNA glycosylase postulates separate enzyme domains for binding, with release of the base residue preceding dis-

sociation of the enzyme from DNA. Many different uracil analogues, including derivatives having modifications at positions 1–4, have been tested and show no detectable inhibition of the enzyme. Most derivatives modified at positions 5 and 6 are also ineffective, but 6-aminouracil and 5-azauracil inhibit almost as well as unsubstituted uracil, and 5-fluorouracil is a weak inhibitor (11). These data suggest that the enzyme is able to remove the latter derivaties if incorporated into DNA, and this has already been shown in the case of 5-fluorouracil (16–18), which is excised by either the *E. coli* or human uracil-DNA glycosylase, albeit 20 times more slowly than unsubstituted uracil. In addition, 5-fluoro-dUTP is a substrate for the cellular dUTPase, so the mechanisms for avoiding incorporation of 5-fluorouracil into DNA are almost the same as those employed to prevent uracil incorporation (16–18). These findings may be related to the cytotoxic and chemotherapeutic effect of 5-fluorouracil on mammalian cells. Another cytotoxic compound, methotrexate, causes a great increase in the intracellular dUTP level and a corresponding decrease in dTTP by inhibition of dihydrofolate reductase. This leads to an increased frequency of misincorporation of uracil into DNA, accompanied by increased fragmentation of the newly synthesized DNA because of the concerted action of uracil-DNA glycosylase and an endonuclease for apurinic and apyrimidinic sites (AP endonuclease) (19). It is possible that this process is responsible for the observed cytotoxicity.

An entirely different kind of inhibitor of uracil-DNA glycosylase is a small protein (mol wt 18,000) induced in *Bacillus subtilis* by the uracil-containing DNA phage PBS2. This protein apparently binds stoichiometrically to the host enzyme. It has been extensively purified (20) and shown to inhibit uracil-DNA glycosylases from several other sources, including *E. coli,* yeast, and human cells. On the other hand, DNA glycosylases acting on hypoxanthine or 3-methyladenine in DNA are resistant to inhibition by the PBS2-induced protein (21). A similar uracil-DNA glycosylase inhibitor is induced as an immediate early function by phage T5 in *E. coli* (22). Since T5 DNA does not normally contain uracil, the reason for the occurrence of this inhibitor is obscure.

The physiological role of uracil-DNA glycosylase is to correct for deaminated cytosine residues in DNA. This has been clarified by the isolation and characterization of enzyme-deficient *E. coli* mutants, *ung* (23), and also by the discovery that 5-methylcytosine residues are hot spots of spontaneous base substitutions (24). In the latter case, 5-methylcytosine would be deaminated to thymine, which cannot be removed by the uracil-DNA glycosylase. Direct evidence that the enzyme serves to remove spontaneously deaminated cytosine residues from DNA in vivo has been obtained recently by the demonstration that in an *E. coli ung* strain, the rate of spontaneous

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transitions at cytosine residues was raised to that found at 5-methylcytosine residues in wild-type cells (25). Thus, in an *ung* mutant, unmodified as well as modified cytosine residues are hot spots of mutation. On the other hand, incorporation of uracil instead of thymine in DNA does not appear to be markedly mutagenic, since *E. coli ung* mutants also deficient in dUTPase (*dut*) have as much as 15–20% of their thymine residues replaced by uracil, but retain their viability (26).

HYPOXANTHINE-DNA GLYCOSYLASE This enzyme presumably acts in an analogous fashion to uracil-DNA glycosylase; it removes spontaneously deaminated adenine residues (27). However, no bacterial mutant deficient in hypoxanthine-DNA glycosylase has been isolated, so there is only circumstantial evidence to suggest a role for the enzyme in vivo. The latter includes the observation that just as cytosine is deaminated to uracil, adenine in DNA is slowly converted to hypoxanthine by hydrolysis at neutral pH (28). Further, the enzyme does not release derivatives similar to deaminated adenine such as xanthine or alkylated purine bases from DNA. This narrow substrate specificity indicates that it specifically recognizes dIMP residues. A mammalian hypoxanthine-DNA glycosylase has recently been purified from calf thymus (28). It is similar to the enzyme from *E. coli,* but, in contrast, shows markedly higher activity in the presence of 0.1 M KCl. The calf thymus enzyme has a molecular weight of 31,000 and is not product inhibited by free hypoxanthine. As with uracil-DNA glycosylase, the hypoxanthine-DNA glycosylase acts equally well on matched or mismatched purine-pyrimidine base pairs; that is, hypoxanthine is removed from a double-stranded polydeoxynucleotide having either C or T residues in the complementary chain.

3-METHYLADENINE-DNA GLYCOSYLASE I One of the major alkylation products in DNA that has been treated with simple methylating agents is 3-methyladenine. The alkyl group of this derivative is located in the minor groove of the DNA helix, and in this regard 3-methyladenine differs from more innocuous methylated bases such as 7-methylguanine, 5-methylcytosine, and N^6-methyladenine. 3-Methyladenine in DNA is not well tolerated by cells, and it is released both in bacteria and in mammalian cells very rapidly after formation by a DNA glycosylase. Since this alkylation lesion is formed in similar amounts by weakly mutagenic and carcinogenic agents such as methyl methanesulfonate and dimethyl sulfate, and more strongly mutagenic compounds such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and N-methyl-N-nitrosourea, it seems likely that 3-methyladenine is a potentially lethal or inactivating lesion rather than a strongly mutagenic one. Traces of 3-methyladenine are continuously formed by the

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nonenzymatic alkylation of DNA by S-adenosylmethionine, under physiological conditions (1).

A distinct DNA glycosylase, which removes 3-methyladenine from DNA, has been found in *E. coli* (29, 30). It is now termed 3-methyladenine-DNA glycosylase I, because a glycosylase of overlapping specificity (3-methyladenine-DNA glycosylase II) has been recently discovered. A 3-methyladenine-DNA glycosylase has also been found in *Micrococcus luteus* (31, 32). The *E. coli* enzyme (molecular weight 20,000) shows a narrowly defined substrate specificity in that it does not catalyze the release of other alkylated purines such as 7-methylguanine, 7-methyladenine, 3-methylguanine, N^6-methyladenine, or 1-methyladenine from DNA. On the other hand, the ethylated base derivative analogous to 3-methyladenine, 3-ethyladenine, is released efficiently by the enzyme. 3-Methyladenine-DNA glycosylase I is similar to the uracil-DNA glycosylase from the point of view that no endonucleolytic activity has been detected in the highly purified enzyme. Thus, the end products of the reaction of the enzyme with alkylated DNA are free 3-alkyladenine and DNA that contains apurinic sites; no chain cleavage occurs at the latter sites even in the presence of high concentrations of enzyme. Another similarity is that the enzyme is product inhibited in a noncompetitive fashion, in this case by free 3-methyladenine.

Mutants of *E. coli* deficient in 3-methyladenine-DNA glycosylase I, *tag*, have been isolated (33). One mutant shows a temperature-sensitive phenotype and contains an anomalously heat-labile enzyme, which indicates that the mutation is in the structural gene. In general, the *tag* mutants show two characteristic properties. First, after exposure to a high dose of a methylating agent, they are unable to eliminate 3-methyladenine rapidly from their DNA; they differ in this regard from wild-type cells. Second, the mutants are more sensitive to killing by alkylating agents such as methyl methanesulfonate in short-term experiments, and they also show impaired host cell reactivation of alkylated phage lambda and T7 (33, 33a). On the other hand, the *tag* mutants show normal resistance to agents such as ultraviolet (UV) light, X-rays, and nitrous acid. Initial attempts to map the *tag* mutants were complicated by the inducibility of a second 3-methyladenine-DNA glycosylase in *E. coli* (see below) and by the inadvertent isolation of a double mutant also deficient in the adaptive response to alkylating agents. It has recently been determined that the *tag* gene is located close to the gene for streptomycin resistance, at 70–74 min on the standard *E. coli* K-12 map (E. Seeberg, personal communication).

3-METHYLADENINE-DNA GLYCOSYLASE II The *E. coli tag* mutants are not totally deficient in 3-methyladenine-DNA glycosylase activity; the cell extracts retain 5–10% of the wild-type enzyme level. This residual

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activity is due to another enzyme, 3-methyladenine-DNA glycosylase II, which is present at similar levels in *tag* mutants and in wild-type cells. It differs from the *tag*$^+$ gene product in that it is a considerably more heat-stable enzyme and shows no product inhibition by free 3-methyladenine (33). A more detailed study of the general properties and substrate specificity of 3-methyladenine-DNA glycosylase II (which has a molecular weight of 27,000, significantly larger than the *tag*$^+$ gene product) has been performed recently (15). The highly purified but nonhomogeneous enzyme catalyzed the liberation of 3-methyladenine and 3-methylguanine equally efficiently from alkylated DNA, and in addition released 7-methyladenine and 7-methylguanine, albeit at about a twentyfold slower rate. The analogous ethylated derivatives were similarly liberated from DNA that had been treated with an ethylating agent. However, O^6-methylguanine residues or the imidazole ring–opened derivative of 7-methylguanine could not be released. The activities found to release 3-methyladenine and 7-methylguanine from DNA chromatographed together on several different columns and showed identical rates of heat inactivation, so they were most likely due to the same enzyme. A DNA glycosylase, present in small amounts in *E. coli* cell extracts, which can release 7-methylguanine from alkylated DNA, has been reported by Laval et al (34). This latter activity is presumably due to 3-methyladenine-DNA glycosylase II.

When *E. coli* are exposed to small amounts of alkylating agents such as MNNG, they gain increased resistance to the mutagenic and killing effects of a subsequent challenge with the reagent. This inducible repair pathway, termed the adaptive response, is not dependent on the *recA*$^+$ gene product (35–37). While the adaptation that allows mutation resistance is largely due to the induction of a repair function for O^6-methylguanine (38), the adapted resistance to killing of the cell shows somewhat different features. In particular, the latter is dependent on a functional *polA*$^+$ gene product (37), which implies that excision-repair of some alkylated residue that is a potentially lethal lesion occurs more efficiently in adapted than in normal cells. This lesion may well be identical with 3-methyladenine (or 3-methylguanine). It has been found recently that 3-methyladenine-DNA glycosylase II can be induced and occurs at a 20-fold increased level in adapted *E. coli,* whereas 3-methyladenine-DNA glycosylase I appears to be constitutively expressed and is present at an unchanged level of activity after adaptation (1). The expression of 3-methyladenine-DNA glycosylase II is dependent on a functional *alk*$^+$ gene, which maps 44 min, but the mechanism of the induction process remains unclear (G. B. Evensen and E. Seeberg, personal communication).

The apparently broad substrate specificity of 3-methyladenine-DNA glycosylase II is at first sight puzzling, since most other DNA glycosylases

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show such a restricted specificity. It seems possible that the unique feature recognized by this enzyme is a positively charged purine residue, rather than methylation or ethylation at some specific site. If this notion is correct, the enzyme should also be able to release unsubstituted adenine and guanine from DNA at low pH values. Unfortunately, the low activity and rapid inactivation of the enzyme under such conditions, as well as the acid-catalyzed depurination of the DNA substrate, make attempts at a practical demonstration difficult. The recognition of a common denominator such as an alteration in charge would make it easy to understand how a single enzyme can remove several different types of alkylated bases without attacking unsubstituted DNA.

A 3-methyladenine-DNA glycosylase has been partly purified from several mammalian tissues (39–41a). The enzyme is present in the cell nuclei and appears to be very labile. Consequently, it has not been highly purified, but partly purified preparations also release 7-methylguanine at a slow rate. In this regard, it therefore seems to resemble the *E. coli* 3-methyladenine-DNA glycosylase II rather than I. The mammalian activities against 3-methyladenine and 7-methylguanine co-chromatographed and showed the same heat lability (41). It is not known at present if mammalian cells resemble *E. coli* in having two different DNA glycosylases that release 3-methyladenine, or if only a single enzyme exists that preferentially releases 3-methyladenine over 7-methylguanine. Another possibility, not yet ruled out, is that in addition to a 3-methyladenine-DNA glycosylase, a distinct 7-methylguanine-DNA glycosylase occurs; two groups have reported on the presence of an activity of this kind in crude cell extracts (42, 43). After treatment of mammalian cells with methylating agents in vivo, 3-methyladenine is liberated much more rapidly from DNA than 7-methylguanine (44).

FORMAMIDOPYRIMIDINE-DNA GLYCOSYLASE 7-Methylguanine residues, the most abundant lesions in alkylated DNA, are susceptible to alkali-catalyzed cleavage of the imidazole ring. Thus, in methylated DNA incubated at high pH, the 7-methylguanine residues are converted to 2,6-diamino-4-oxy-5-N-methylformamido-pyrimidine. This ring-opening reaction occurs only at a very slow rate at neutral pH, so substituted formamidopyrimidine is a minor secondary alkylation lesion derived from 7-methylguanine. Nonenzymatic cleavage of the glycosyl bonds of 7-methyldeoxyguanosine residues in DNA to yield apurinic sites occurs about 300 times more rapidly at pH 7.4 than the purine ring-opening reaction (L. Breimer, unpublished information). Nevertheless, the latter reaction may be of significance because of the large amounts of 7-methylguanine generated by the action of methylating agents on DNA.

E. coli cell extracts contain a unique DNA glycosylase that effectively catalyzes the release of the substituted formamidopyrimidine from alkylated, alkali-treated DNA. The partly purified enzyme has a molecular weight of about 30,000, and does not release intact 7-methylguanine from DNA (45). Further, the enzyme does not catalyze the release of a similarly ring-opened guanine residue carrying an aflatoxin B_1 adduct rather than a methyl group, nor can it be induced in cells by MNNG treatment (T. Lindahl, unpublished data). It is not presently known if this enzyme has as its sole function the removal of the secondary alkylation product, which presumably is a dangerous lesion with miscoding or noncoding properties, or if it can also serve to remove unsubstituted guanine and adenine residues with opened imidazole rings. The latter derivatives are the major purine lesions in DNA exposed to ionizing radiation (46), and in addition, ring-opened adenine residues may be generated as spontaneous hydrolytic lesions (47). A formamidopyrimidine-DNA glycosylase with properties similar to the bacterial enzyme has been detected in rodent liver cell extracts (43).

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UREA-DNA GLYCOSYLASE Ionizing radiation causes ring opening and fragmentation of pyrimidine residues in DNA (48). Thus, urea and N-substituted urea derivatives are generated as remnants of pyrimidines. These residues, which may remain attached to deoxyribose in DNA, are unlikely to retain any coding information and presumably have to be repaired. In a search for DNA glycosylases acting on such species, a DNA glycosylase that cleaves a urea-deoxyribose bond was identified (49). The substrate used was a $KMnO_4$-treated polydeoxynucleotide containing a few fragmented thymine residues. This enzyme seems unable to release N-substituted urea derivatives such as formylpyruvylurea and formylurea, and it is presently not known if other DNA glycosylases exist for this purpose. The urea-DNA glycosylase appears to be a distinct glycosylase; it is present at similar levels in *E. coli* wild-type cells and in *ung* and *tag* mutants, and the partly purified enzyme has chromatographic properties different from several other DNA glycosylases. A similar urea-DNA glycosylase has also been observed in crude extracts of several types of mammalian cells (L. Breimer, unpublished).

THYMINE GLYCOL-DNA GLYCOSYLASE Ring-saturated pyrimidine residues are common base lesions in DNA exposed to ionizing radiation or UV light. Such residues may also be generated, without introducing other forms of damage, by treatment of single-stranded DNA with osmium tetroxide. A DNA glycosylase activity is present in *E. coli* that catalyzes the release of thymine glycol (5',6'-dihydroxydihydrothymine) and a com-

pound tentatively identified as 5,6-dihydrothymine from OsO_4 treated DNA (50). It is not known if the enzyme also would liberate analogous cytosine residues. This glycosylase activity appears to be associated with *E. coli* endonuclease III, an AP endonuclease discussed below, as judged from the fractionation properties, reaction optima, and heat sensitivities of the two activities. Thus, a single enzyme may in this case account both for the release of the damaged base and the subsequent cleavage of the DNA molecule at the apyrimidinic site.

PYRIMIDINE DIMER-DNA GLYCOSYLASE In organisms particularly resistant to UV light, such as *M. luteus* and phage T4–infected *E. coli,* a small "UV endonuclease," which specifically cleaves DNA at pyrimidine dimers, has been detected. The enzyme seems to have no counterpart in uninfected *E. coli,* or in mammalian cells. Recent studies on the mechanism of action of this enzyme have yielded the surprising result that it cleaves initially one of the two glycosyl bonds within a pyrimidine dimer in DNA (50–58). The initial partial unhooking of the pyrimidine dimer at its 5' side was first recognized from the anomalous properties and alkali lability of the resulting oligonucleotide observed by sequencing UV-irradiated, enzyme-treated DNA (51). Several laboratories have verified this mode of action by demonstrating the release of free thymine from the DNA product by photoreversal of thymine dimers at enzyme-cleaved sites, either by irradiation or by treatment with a photoreactivating enzyme. In bacteriophage T4, the pyrimidine dimer-DNA glycosylase (molecular weight 18,000) is the product of the *denV* gene. The enzyme also has a low intrinsic AP endonuclease activity, and is consequently able to cleave the DNA at the 3' side of the apyrimidinic site generated at a pyrimidine dimer by the glycosylase activity. It is now evident that the two activities reside within the same protein. In addition to cofractionation of the activities until a single homogeneous protein was obtained, an amber mutant in the *denV* gene was isolated, and both enzyme activities were partly recovered on infection of an amber suppressor host strain (54). Further, infection of *E. coli xth* mutants, which are deficient in the major host AP endonuclease, with T4 $denV^+$ phage yielded significantly higher AP endonuclease activity in cell extracts than that found in extracts from cells infected with T4 *denV* mutants (57). The DNA glycosylase function of the enzyme appears much more active than the AP endonuclease. In vitro, the two activities do not appear to act in a concerted fashion, so that many uncleaved apyrimidinic sites are generated in UV-irradiated DNA treated with a small amount of the $denV^+$ gene product (53, 54). The AP endonuclease activity of the enzyme may therefore only be an accessory activity whose role can be easily replaced by one of the AP endonucleases of the host cell. This notion is supported by the

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properties of the enzyme synthesized by a UV-resistant *denV* mutant. In this case, the DNA glycosylase activity was retained, but the AP endonuclease activity had been inactivated (57). The T4 *denV*$^+$ gene has recently been cloned in the plasmid pBR322, and the expression of such cloned DNA in *E. coli uvrA* and *uvrB* mutants renders them less sensitive to UV light (58). The T4 enzyme has also been introduced into human cells from xeroderma pigmentosum patients by concomitant treatment of cells with the purified enzyme and UV-inactivated Sendai virus. In this remarkable experiment the enzyme was shown to be able to correct the incision defect of the cells and permit repair to take place at pyrimidine dimers (59).

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AP Endonucleases

Apurinic and apyrimidinic (AP) sites in DNA may be generated by several routes, and two of the most important ones involve spontaneous hydrolysis and the action of DNA glycosylases (3). Such defects are repaired very efficiently, but in view of their frequent occurrence in DNA they may nevertheless contribute to occasional inactivation or mutagenesis of cells (60). It seems unlikely, however, that the generation of AP sites in DNA plays a major role in spontaneous mutagenesis, since *E. coli xth* mutants do not show increased spontaneous mutation frequency, although they are deficient in the major endonuclease for AP sites and presumably have an impaired ability to remove these sites from DNA (61, 62).

The correction of AP sites in DNA is initiated by endonucleases that cleave DNA specifically at these sites. Several enzymes of this type have been detected in *E. coli,* and recent extensive studies by Linn and coworkers (63–66) show that two classes of enzymes are present, those that cleave DNA at the 3' side of the AP site (class I enzymes) and those that cleave at the 5' side (class II enzymes). In either case, a 5'-phosphate and a 3'-OH group are generated at the cleavage site. Class II enzymes yield a terminal phosphate bound to a deoxyribose residue that does not carry a base and account for most of the AP endonuclease activity present in cell extracts from either *E. coli* or mammalian cells.

A method devised by Clements et al (67) has been employed in several laboratories (64, 68, 69) to define the site of cleavage by different AP endonucleases. Alternating poly(dA-dT) was synthesized in the presence of small amounts of [α-^{32}P]dUTP, which led to the replacement of a few dTMP residues with radioactive dUMP residues. Treatment with uracil-DNA glycosylase was then used to convert the latter to apyrimidinic sites. Cleavage of the resulting polymer with class II AP endonucleases yielded the radioactive phosphate residues in a phosphatase-sensitive form, while this was not the case for class I enzymes. In a different approach to define the terminal structures at the site of cleavage, it was observed that the 3'-OH

termini generated by class II enzymes acted as good primers for *E. coli* DNA polymerase I, while this was not the case for the termini generated by class I enzymes (63). Thus the 3'→5' exonuclease function of DNA polymerase I removes a 3'-terminal deoxyribose residue without an attached base only very slowly. It is presently unclear if the two different kinds of AP endonucleases may act in concert, that is, if cleavage by a class I enzyme is followed by cleavage with a class II enzyme, or vice versa, to release the deoxyribose-5'-phosphate residue from an AP site (69a). This seems feasible since cells contain both kinds of activities. Alternatively, a sugar-phosphate residue may be excised by one of the cellular exonucleases after initial cleavage of the damaged DNA by an AP endonuclease. There may be no stringent borderline between class I and II AP endonucleases in all cases, because a homogeneous AP endonuclease from human placenta (68) has recently been observed to be able to incise DNA at either side of an AP site, although one of the modes of cleavage is preferred to the other one. A note of caution is expressed concerning the classification as an AP endonuclease of any protein that increases the rate of chain cleavage at apurinic sites, since basic proteins such as cytochrome *c* and pancreatic ribonuclease show some activity in this regard, as do polyamines. Further, it has been shown recently that at high concentrations the tripeptides Lys-Trp-Lys and Lys-Tyr-Lys catalyze chain cleavage at AP sites relatively effectively (69b, 69c). The general properties of AP endonucleases from different sources have been reviewed previously (3), and recent progress is summarized below.

E. COLI EXONUCLEASE III This is the major AP endonuclease of *E. coli* (62). It has also been called endonuclease VI (70). Although the enzyme was first discovered and characterized as an exonuclease, its AP endonuclease function may be of greater physiological relevance (61). The AP endonuclease activity of exonuclease III is of the class II type, and in addition the enzyme has associated RNase H, 3'→5' exonuclease, and phosphatase activities. This useful reagent enzyme has recently been cloned, and overproducing *E. coli* strains have been obtained (71). *E. coli* mutants deficient in exonuclease III, *xth,* show two known phenotypic differences from wild-type cells; they are slightly sensitive to methyl methanesulfonate (71a) and unusually sensitive to hydrogen peroxide (B. Demple, personal communication).

E. COLI ENDONUCLEASE III The apparent broad specificity of this enzyme for a variety of different types of damaged DNAs (72, 73) first appeared puzzling, but has now been largely resolved by the demonstration that it has an associated DNA glycosylase activity that releases ring-

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saturated thymine (50). Endonuclease III, a class I AP endonuclease, is a small enzyme (mol wt ~ 23,000) that does not require Mg^{2+} for activity (63). The AP endonuclease activities associated with certain DNA glycosylases have been consistently of the class I type, while the major AP endonucleases without any demonstrable glycosylase activity have been of the class II type (66).

E. COLI ENDONUCLEASE IV This is an enzyme activity first discovered in extracts of *E. coli xth* mutants that catalyzes the cleavage of DNA at AP sites in the same fashion as exonuclease III; that is, it appears to be a class II AP endonuclease (63, 74). *E. coli* endonuclease IV differs from the AP endonuclease function of exonuclease III in that it has no associated exonuclease activity, and it is present in similar amounts in wild-type cells and in *xth* mutants (74). The presence of this enzyme in *E. coli* may explain why *xth* mutants are resistant to most DNA-damaging agents, if it is assumed that endonuclease IV may substitute for the AP endonuclease activity of exonuclease III. *E. coli* mutants deficient in endonuclease IV have not been isolated, so a direct test of this hypothesis is not presently possible.

E. COLI ENDONUCLEASE V This endonuclease, of unclear biological function, has been characterized in some detail (75, 76). It is much more active on certain forms of damaged DNA than on native DNA, which suggests that it may be a DNA repair enzyme; but in contrast to the enzymes described above it will also attack intact DNA in an endonucleolytic fashion. In vitro, endonuclease V efficiently degrades DNA in which a large proportion of thymines have been replaced by uracils. It also attacks several other kinds of damaged DNA effectively in vitro and catalyzes the formation of both single-strand and double-strand breaks in undamaged DNA at a slow rate (76a). It seems unable to catalyze some of these reactions in vivo to any significant extent, however, since DNA containing large amounts of uracil is not degraded in *E. coli ung* mutants (26). Further, endonuclease V does not cleave circular PM2 DNA molecules that contain small amounts of uracil residues any faster than circles lacking such residues, in marked contrast to the action of uracil-DNA glycosylase. Endonuclease V cleaves DNA preferentially at the 3' side of AP sites; no associated DNA glycosylase activity has been detected. Possibly the enzyme recognizes some distorted form of secondary structure in DNA, perhaps associated with a reduction in base stacking interactions. A better understanding of the physiological role and specificity of this intriguing enzyme will almost certainly have to depend on the isolation of endonuclease V–deficient mutants. For comparison, the different exonucleolytic, endonu-

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cleolytic, and DNA-unwinding activities of the *recBC*$^+$ gene product would appear bewildering in the absence of any genetic information on its function.

Another strange enzyme activity recently discovered in *E. coli* extacts resides in an endonuclease (mol wt 55,000) that cleaves single-stranded DNA at apyrimidinic sites (E. Friedberg, personal communication). The enzyme does not act on double-stranded DNA containing either apyrimidinic or apurinic sites. Its physiological role has not been defined.

MAMMALIAN AP ENDONUCLEASES The dominant AP endonuclease in several different types of mammalian cells appears to be a class II enzyme (65, 68, 69), and there is no convincing evidence for the presence of more than one such enzyme (3, 68). This mammalian enzyme resembles *E. coli* endonuclease IV in its specificity. Thus, no mammalian AP endonuclease with an associated exonuclease activity has been found. The human AP endonuclease has been purified to homogeneity from HeLa cells (65) and from placenta (68). It is a monomeric protein (mol wt 32,000–41,000, depending on the method of determination) and requires Mg^{2+} for activity. In addition to this class II enzyme, an AP endonuclease of the class I type is present in human fibroblast extracts. This activity has so far only been partly purified and characterized; attempts to inhibit it with antibodies against the class II enzyme have yielded equivocal results (64). Confirmation is needed that this class I type AP endonuclease activity is absent in fibroblasts from xeroderma pigmentosum group D (64), which is an unexpected observation, since the gene product missing in this complementation group of xeroderma pigmentosum is required for incision at pyrimidine dimers (59, 77) and presumably is analogous to one of the *E. coli uvr*$^+$ gene products. Two AP endonucleases from mouse cells have been described (77a); one acts only at AP sites while the other has a broader substrate specificity and may be similar to the *E. coli* endonuclease III or V.

DO PURINE INSERTASES EXIST? There are several reports of an enzyme that can directly reinsert purines at apurinic sites in DNA, both in *E. coli* (78) and in human fibroblasts (79, 80). Such a proposed activity would be reminiscent of certain tRNA modifying enzymes such as the one that removes an unsubstituted guanine residue within the anticodon region by cleavage of a glycosyl bond and inserts a hypermodified guanine at the same site (81). However, the report that a certain *E. coli* DNA insertase activity requires purine deoxynucleoside triphosphates as donors has not been reproducible. M. Sekiguchi and H. Kataoka (submitted for publication) have established that while an *E. coli* activity reminiscent of an "insertase" could be observed using partly depurinated DNA as a substrate, the residue added

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at an apurinic site was a single purine mononucleotide and not a free purine. Further, the apparent insertase activity was absent in *E. coli polA* mutants, although homogeneous DNA polymerase I lacked ability to insert free purines into depurinated DNA. In conclusion, the results indicated that short-patch excision repair with replacement of a single nucleotide residue could account for the observations initially reported as a DNA insertase activity in *E. coli.*

The activity from human cells has been studied in greater detail and therefore requires more serious consideration. It appears to be due to a DNA-binding protein (molecular weight 120,000) that does not require Mg^{2+} for activity and employs free purines rather than deoxynucleoside triphosphates as donors. There are two puzzling aspects of this activity that need to be resolved before the notion of a DNA insertase can be accepted. First, there is no apparent energy source for the insertion reaction. We can postulate that the increase in base stacking interactions allowed in the repaired DNA can provide this energy, or alternatively that the enzyme itself occurs in an activated form such as an enzyme-adenylate complex. Second, the activity seems to prefer guanine over adenine. Because of its virtual insolubility in neutral aqueous solution, free guanine can bind very tightly to DNA in a noncovalent fashion, and this may have occurred during measurements of possible insertase activity.

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UVR^+ Endonuclease

DNA lesions such as pyrimidine dimers and polycyclic hydrocarbon adducts, which cause major helix distortions, are removed by an excision-repair process initiated by a complex endonuclease activity. In *E. coli,* this multisubunit enzyme is the product of the *$uvrA^+$*, *$uvrB^+$*, and *$uvrC^+$* genes. A similar endonuclease is presumably present in eukaryotes, and the gene products missing in human cells of various complementation groups of xeroderma pigmentosum and in certain UV-sensitive yeast mutants are believed to be analogous to the *E. coli uvr^+* gene products, although there is as yet no direct evidence for this. The *E. coli uvr^+* endonuclease function can be obtained in active form from gently lysed cells, and cell-free extracts from different *uvr* mutants complement each other. This endonuclease requires Mg^{2+} and ATP for activity (82). It does not seem to have any associated DNA glycosylase activity, and in the case of pyrimidine dimers, it acts by cleavage of a phosphodiester bond adjacent to a dimer (50). However, it is not known whether the enzyme incises DNA at the 5' or 3' side of the dimer, or if a 5'- or 3'-terminal phosphate is generated. The substrate for the enzyme in UV-irradiated DNA can be removed by treatment of the DNA with photoreactivating enzyme prior to exposure to the *uvr^+* endonuclease, which indicates that the enzyme is recognizing pyrimi-

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dine dimers and not some other type of UV-induced damage (83). It is known from in vivo data that the *uvr*$^+$ endonuclease attacks DNA at a variety of bulky lesions, and it has recently been shown that the partly purified enzyme incises DNA at photochemically bound psoralen residues and at N-acetoxy-2-acetamidofluorene adducts in vitro (83, 84). Thus, there is satisfactory agreement between the in vivo and in vitro properties of this complex enzyme. While the endonuclease attacked DNA at psoralen monoadducts as well as at psoralen-induced cross-links, no psoralen-base adducts were released in free form from DNA that contained radioactive psoralen residues, which again proves indirectly the absence of an intrinsic DNA glycosylase activity in the enzyme (84).

The different subunits of the *uvr*$^+$ endonuclease do not remain attached to each other in cell extracts, but they can be separately purified as three proteins of high molecular weight and identified by a complementation assay (82). Because of the low amounts and instability of the active proteins in cell extracts and the relatively complicated assay procedure, progress with purification has been slow. Recently, the different *E. coli uvr*$^+$ genes have been separately cloned on small multicopy plasmids, and techniques have been devised for their selective expression, which promises more rapid progress in this area. The development of a procedure to identify the proteins encoded by recombinant plasmids, the maxi-cell method (85, 86), has been of considerable importance in this regard. In this technique, *E. coli recA uvrA* double mutants carrying a multicopy plasmid such as pBR322 are irradiated with UV light. While many of the plasmids remain intact, this leads to degradation of chromosomal DNA and cell death. Following addition of cycloserine to prevent the outgrowth of any bacterial survivors, the cells are incubated and then labeled with [^{35}S] methionine. Only plasmid-coded proteins are radioactively labeled under these conditions, and such proteins can then be easily isolated in a radiochemically pure and biologically active form. A plasmid carrying the *uvrA*$^+$ gene coded for a protein of mol wt 114,000 as estimated by SDS gel electrophoresis. This was identified as the *uvrA*$^+$ gene product by its absence in cells carrying plasmids in which the cloned *uvrA* gene had been inactivated by the integration of an insertion sequence (86). The molecular weight of the biologically active *uvrA*$^+$ gene product has been found to be 100,000–130,000 (84, 86), so this protein is a monomer. By similar techniques, the *uvrB*$^+$ (87) and *uvrC*$^+$ (88) genes have been cloned and the proteins identified. The molecular weight of the *uvrB*$^+$ protein is 84,000, while that of the *uvrC*$^+$ protein is 68,000.

The different subunits of the *uvr*$^+$ endonuclease show no detectable endonucleolytic activity by themselves. The *uvrA*$^+$ protein, however, is a DNA-binding protein that also shows ATPase activity (84, 88a). It may be that the *uvrA*$^+$ protein is the subunit that initially recognizes the lesions in

DNA and binds at damaged sites, and that the *uvrB*$^+$ and *uvrC*$^+$ proteins then interact with the *uvrA*$^+$ subunit and catalyze the chain cleavage. The helical region destabilized by the *uvrA*$^+$ protein binding may determine the patch size of the region subsequently excised. The role of the cofactor, ATP, in the process is obscure. The *uvrB*$^+$ protein may have an additional repair function in the cell, since it seems to be present in larger amounts than either the *uvrA*$^+$ or *uvrC*$^+$ protein (86–88). Moreover, *polA uvrB* mutants are nonviable, whereas *polA uvrA* mutants have been constructed (89). The products of the *uvr*$^+$ genes have previously been regarded as being constitutively expressed, but the synthesis of *uvrA*$^+$ protein can in fact be induced severalfold by exposure of *E. coli* to UV light (90). This induction process is dependent on functional *recA*$^+$ and *lexA*$^+$ genes (91).

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Photolyase

The photoreactivating enzyme, which is the product of the *phr* gene in *E. coli* (92, 93), catalyzes the direct monomerization of pyrimidine dimers in DNA without any associated excision. The *E. coli* enzyme (molecular weight 37,000) seems to contain small amounts of carbohydrate and RNA (94). Visible light (340–400 nm) is required for the monomerization process. The nature of the light-absorbing cofactor has not been identified, in spite of many attempts.

Photoreactivation may also occur in the absence of enzymes, and can be promoted by tryptophan-containing peptides (95). Thus, as discussed above for AP endonucleases, it is important to distinguish between distinct enzymes, which catalyze the process efficiently, and proteins that promote the reaction in a relatively unspecific manner (96). Photolyase is found in bacteria and lower eukaryotes. Despite extensive debate, it is not yet clear whether enzymatic photoreactivation occurs in mammalian cells.

Transmethylase for O^6-Methylguanine

The major mutagenic DNA lesion in cells exposed to simple alkylating agents such as MNNG or N-methyl-N-nitrosourea is O^6-methylguanine (38, 97, 98). On replication, frequent incorporation of thymine instead of cytosine residues occurs, leading to the accumulation of transition mutations. The persistence and repair of this form of DNA damage has been intensively studied because carcinogenesis induced by methylating and ethylating agents apparently is correlated with defective or insufficient repair of O^6-alkylguanine in the target cells and organs (99, 100). With regard to bacteria, *E. coli* cells usually have limited capacity for removing O^6-methylguanine from DNA, but an inducible repair function is expressed after the exposure of cells to low concentrations of alkylating agents. This repair pathway, termed the adaptive response, allows rapid and error-free

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repair of O^6-methylguanine residues (38, 101, 102). The adaptive response can be induced in *recA* mutants and is consequently different in nature from inducible error-prone repair processes. The signal for adaptation is not known, but a comparison of the relative efficiencies of various alkylating agents as inducers indicates that it is due to an O-alkylated rather than an N-alkylated residue, and possibly resides within O^6-methylguanine itself (P. Karran, personal communication). An important and unexpected finding has been that the repair activity is expended in its reaction with alkylated DNA and thus acts only once (103). *E. coli* mutants defective in the adaptive response have been isolated, and have been essential for the detailed analysis of the pathway. These mutants are either unable to express an adaptive response, *ada,* or express the response in a constitutive fashion, Adc (104, 105). Both types of mutations have been mapped at 47 min on the chromosomal map by P1 transduction (B. Sedgwick, *J. Bacteriol.* In press).

The development of an in vitro assay for the adaptive response (106) has allowed a biochemical analysis of the process. Cell extracts from adapted *E. coli* cause the specific disappearance of O^6-methylguanine from alkylated DNA in a reaction independent of divalent metal ions. A striking feature of this reaction is that the methyl groups are not released as low-molecular-weight material, but remain bound to an acid-precipitable macromolecule. The receptor has been identified as the methyltransferase[1] itself, which removes an alkyl group from the O^6 position of guanine onto one of its own cysteine residues. Thus, the reaction products are an S-methylcysteine in the protein and unsubstituted guanine in the DNA (107, 108). It would consequently appear that no excision of base or nucleotide residues from DNA is associated with the repair of O^6-methylguanine. The transferase is specific for methyl residues only at the O^6-position of guanine in DNA, and no mobilization of methyl groups from 7-methylguanine or 3-methyladenine occurs. However, the same protein repairs O^6-ethylguanine in ethylated DNA with the concomitant formation of an S-ethylcysteine residue, although this analogous reaction proceeds more slowly than with a methylated substrate (109). The reaction between the methyltransferase and O^6-methylguanine in DNA exhibits features that are in good agreement with the properties of the adaptive response in vivo. Thus, the transferase is consumed in the reaction (111). This suicide inactivation is not associated

[1]While an enzyme, strictly defined, should not be consumed in the reaction with its substrate, the present reaction has many features of the interaction between a suicide enzyme inactivator and its target enzyme (110). Further, it has not been ruled out that the transferase activity might not be slowly regenerated under some conditions. For these reasons, and to distinguish this reaction from the direct methylation of proteins by treatment with alkylating agents, we designate the protein induced during the adaptive response a methyltransferase.

with any detectable alteration in the size of the protein, since both the inactive methylated protein (in 6 M guanidium hydrochloride) and the active unmethylated species show a molecular weight of about 17,000. It would thus appear that the inactivation is due to blocking of a reactive cysteine residue in the protein, without any accompanying dissociation or degradation (107). While S-methylcysteine has been found previously in proteins directly exposed to alkylating agents (112, 113), it has not been detected in enzymatically methylated proteins; in the latter case, methyl groups are usually bound to either lysine, arginine, histidine, or glutamic acid residues (114). Certain chemical mutagens, however, in a detoxifying reaction catalyzed by a glutathione S-transferase, have been found to be bound to the cysteine residue of glutathione. Glutathione S-transferase can convert the *cis* isomer of dimethyl 1-carbomethoxy-1-propen-2-yl phosphate (Phosdrin) to its O-demethylated derivative, with the simultaneous formation of S-methylglutathione (115). It is not known at present if the active site of the methyltransferase has any structural similarity to glutathione. The removal of the methyl group from O^6-methylguanine in DNA is unusual in that it represents an example of enzymatic methyl group transfer to a protein in a situation where S-adenosylmethionine was not employed as the methyl donor. In this context, it should also be mentioned that it is not without precedent to find a protein-modifying enzyme that uses itself as the main target of modification. For example, a number of protein kinases that catalyze autophosphorylation are known, and the major acceptor for poly(ADP-ribose) in mammalian cell nuclei is the poly(ADP-ribose) synthetase itself (116, 117).

Uninduced *E. coli* B or *E. coli* K-12 cells growing in conventional media contain low but detectable amounts of the methyltransferase that acts on O^6-methylguanine in DNA, corresponding to about 20 protein molecules per cell (1). In contrast, adapted cells, or Adc mutants, contain 3000–10,000 molecules per cell. The Adc mutants show a high frequency of spontaneous reversion, which indicates that persistent, high levels of the protein are unfavorable to the cell (105). An adaptive response to alkylating agents, associated with the induction of a methyltransferase for O^6-methylguanine residues in DNA, has also been demonstrated in *M. luteus* (S. Riazuddin, personal communication). Mammalian cells have recently been found to contain a very similar methyltransferase, and a partly purified activity from mouse liver that employs a cysteine residue as its own methyl group receptor has been shown to be consumed in the reaction with alkylated DNA (118). O^6-Ethylguanine in ethylated DNA is apparently also repaired in this fashion in mammalian cells (119, 120). No obvious or striking induction of the mammalian activity has been achieved by treatment of tissue culture cells with alkylating agents, however, and it is presently unclear if this

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methyltransferase can be induced in mammalian cells (121–123; P. Karran, unpublished information). The most intriguing property of the mammalian O^6-methylguanine methyltransferase is that it no longer seems active in some human tumors and tumor cell lines, in particular in lines from cells transformed with DNA tumor viruses, whereas normal diploid fibroblasts and other control cells express this repair function (124–126). The tumor cells lacking the repair activity, termed Mer^- or mex^- cells, are markedly sensitive to simple alkylating agents, and it is an exciting possibility that a further clarification of the mechanisms involved may lead to a better rationale for the treatment of certain tumors with alkylating agents.

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Mismatch Repair

A number of mismatch repair systems occur universally in cells, and such functions are important in view of the vastly increased mutation frequency observed in certain bacterial strains defective in this form of DNA correction. Nevertheless, little is presently known about the biochemistry of mismatch repair, and this process may be regarded as a major remaining mystery of DNA metabolism. While it is easy to see that bacterial strains lacking uracil-DNA glycosylase, or having a defective DNA polymerase (127), might exhibit a moderately increased frequency of spontaneous mutation, the very high mutation rate (up to 10^4 times that of wild-type cells) found in *E. coli* strains such as *mutH, mutL, mutS, mutU* (*uvrDE*), *mutD* (*dnaQ*), and *mutT* (128) is not understood. The multitude of gene products involved implies that a correction system of considerable complexity exists.

Mismatch repair activities should be able to discriminate between a newly synthesized DNA strand, which might contain replication errors, and the parental template strand. For this reason, the suggestion (129) that methylation of the parental strand might serve to instruct the mismatch correction system has kindled considerable interest. Several types of evidence have been obtained for some kind of involvement of DNA methylation in mismatch repair. First, Marinus's isolation of *E. coli* mutants deficient in DNA methylation and his demonstration that strains defective in the adenine DNA methylase, *dam,* had a moderately increased spontaneous mutation frequency (130) implied that a connection exists between mismatch repair and methylation. The major weakness in this argument is that it is not known whether the dam^+ gene product is the methylase itself, or if it is a control function that regulates the expression of several different gene products, including the methylase. The DNA adenine methylase employs S-adenosylmethionine as methyl donor and has been found to methylate adenine at the N^6 position within the DNA tetranucleotide sequence, GATC (131). Apparent revertants of *E. coli dam* mutants, isolated as 2-aminopurine resistant clones, are second-site mutations in the *mutH,*

mutL, and *mutS* genes, which indicates a close connection between the *dam*$^+$ product and several mutator genes (132, 133).

In a different experimental approach to investigate the role of methylation in mismatch repair, transfection experiments with phage λ heteroduplex DNA molecules containing one methylated and one unmethylated strand have been performed. The data showed that for the correction of some mismatches, the unmethylated strand was preferentially repaired in wild-type cells, while in various mutator strains no strand bias was observed (132, 134). However, similar transfection experiments with phage T7 argue against a general role of methylation in mismatch correction (135). In the latter system, repair of phage heteroduplex DNA containing mismatched bases, though markedly reduced, was not totally eliminated in *E. coli mutH, mutL, mutS,* and *mutU* mutants. The observed repair did not seem to involve a T7 phage-coded function but rather depended upon the host cell machinery. In wild-type host cells, the H strand of the viral DNA was preferentially corrected, although T7 DNA contains very few methyl groups in either strand, and the GATC sequence is not methylated at all. Thus, the *dam*$^+$-controlled methylase does not seem to act on T7 DNA, and the mismatch repair system presumably recognized some structural feature of the viral H strand other than methylation. It is still possible that these differences are more apparent than real and may be accounted for by the different modes of replication of λ and T7 DNA. Mismatch repair activities have been observed in several other microorganisms. Thus, pneumococcus mutants, *hex,* have been isolated that are defective in mismatch repair of heteroduplex DNA (136). It seems likely the *hex*$^+$ gene product is analogous to the product of one of the *E. coli* strong mutator genes.

Two *E. coli* mutator genes with properties different from those described above have been found. The *mutD* strains show a strong mutator phenotype only when the growth medium has been supplemented with thymidine, and transversions as well as transitions and frameshifts have been observed (137). Only the latter two types of mutations are seen in *mutH, mutL, mutS,* and *mutU* strains. It has been proposed that the *mutD*$^+$ gene product (25,000 mol wt) may be a subunit of DNA polymerase III, since this enzyme purified from a *mutD* strain had altered chromatographic properties, anomalously low exonuclease activity, and reduced ability to discriminate between dATP and its 2-aminopurine analogue (138, 138a). The other unusual mutator gene, *mutT,* differs from other strains in that mutants exhibit a greatly increased frequency only of the specific transversion A·T→C·G (139). Transversion mutations usually result from purine·purine, rather than from pyrimidine·pyrimidine mismatches in DNA, with one of the purine residues being accommodated within the double helix in

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its *syn* conformation (140, 141). It would consequently appear that *mutT* mutants may be defective in a repair enzyme that specifically recognizes A·G mismatches and removes the G residue in DNA. Alternatively, the *mutT*$^+$ gene may code for a factor that serves to prevent the introduction of a purine deoxynucleoside triphosphate opposite to a DNA purine residue during replication (128).

Conclusions

The present wide-ranging studies on DNA repair activities will most probably reveal many previously unknown enzymes over the next few years, some of which may act on DNA in novel and unexpected ways. Even with regard to a relatively well-characterized group of chemical mutagens such as the simple methylating agents, the mechanisms of repair of several alkylated pyrimidines have not yet been biochemically characterized, although it is known that lesions of this kind are actively removed from DNA in vivo (142). Moreover, since both bacteria and growing mammalian cells contain long stretches of single-stranded DNA (143, 144), and since precursor deoxynucleoside triphosphates have been found to react readily with alkylating agents (145), significant alkylation probably occurs at sites normally protected by hydrogen bonding in double-stranded DNA. Modification at such sites would be expected to result in the formation of miscoding lesions. For example, 1-alkyladenine is readily produced by the action of alkylating agents on adenine residues in monomeric form or in single-stranded DNA (146). It seems likely that repair functions exist to deal with damage of this kind, although none has yet been reported. Many other types of potentially mutagenic DNA lesions, such as purine N oxides, have not been investigated so far with regard to possible repair. Further, many DNA repair enzymes no doubt exist that act on the major base lesions introduced by exposure of cells to ionizing radiation. A start has been made to investigate such enzymes (49, 50), but nothing is known about the removal of important X-ray lesions (48) such as 5-hydroxy-5-methylhydantoin and formylpyruvylurea. A clarification of the latter, presently hypothetical, repair mechanisms may well be a prerequisite for the definition of the specific repair activities apparently lacking in anomalously radiation-sensitive cells such as those obtained from ataxia-telangiectasia patients (147, 147a). (With regard to ionizing radiation, the induction of poly(ADP-ribose) synthesis in mammalian cell nuclei in response to chain breaks in DNA (148) suggests that this polymer may play a role in DNA repair, but the mechanism is not understood.)

In the past, it has often been technically difficult to observe the removal of a specific altered base from DNA in the presence of several other forms of damage. Liquid chromatography techniques (149) have in many cases

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now removed this experimental hurdle. Another practical problem in the elucidation of repair activities has been that DNA repair enzymes, with the exception of the ones acting at important spontaneous lesions such as apurinic sites and deaminated cytosine residues, seem to be present in low amounts in cells. Twenty enzyme molecules per cell represents a typical value in *E. coli.* This is probably a reflection of the fact that, under most conditions, cells are exposed to relatively low levels of DNA-damaging agents. Consequently, they are usually well equipped to deal with a small number of a certain kind of DNA lesion, whereas sudden exposure to a high dose of a mutagen might cause many mutations due to transient saturation of the repair capacity. In some cases, cells have the ability to adapt to new environmental conditions by the induction of repair enzymes. In *E. coli,* the *uvrA*$^+$ gene product (90), the methyltransferase acting on O^6-methylguanine (102), the 3-methyladenine-DNA glycosylase II (1), a repair activity apparently recognizing an unknown lesion introduced by long-wave UV light (150), and an elusive factor that seems to allow error-prone replication over potentially lethal lesions such as pyrimidine dimers (151) are all inducible. It seems likely that the expression of several other repair functions may also be dependent on exposure to the damaging agent, and if the conditions of induction can be experimentally established it will clearly be easier to define biochemically the activities involved. In addition, cloning of the genes for various DNA repair enzymes into multicopy plasmids is presently being carried out in many laboratories, and this should permit easier access to these enzymes in purified form.

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