Journal of Cellular Biochemistry 18:271-283 (1982)
Mechanisms of Chemical Carcinogenesis 275-287

Repair Responses to DNA Damage: Enzymatic Pathways in E coli and Human Cells

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Bacteria and eukaryotic cells employ a variety of enzymatic pathways to remove damage from DNA or to lessen its impact upon cellular functions. Most of these processes were discovered in Escherichia coli and have been most extensively analyzed in this organism because suitable mutants have been isolated and characterized. Analogous pathways have been inferred to exist in mammalian cells from the presence of enzyme activities similar to those known to be involved in repair in bacteria, from the analysis of events in cells treated with DNA damaging agents, and from the analysis of the few naturally occurring mutant cell types.

Excision repair of pyrimidine dimers produced by UV in E coli is initiated by an incision event catalyzed by a complex composed of *uvrA, uvrB,* and *uvrC* gene products. Multiple exonuclease and polymerase activities are available for the subsequent excision and resynthesis steps. In addition to the constitutive pathway, which produces short patches of 20-30 nucleotides, an inducible excision repair process exists that produces much longer patches. This long patch pathway is controlled by the *recA-lexA* regulatory circuit and also requires the *recF* gene. It is apparently not responsible for UV-induced mutagenesis. However, the ability to perform inducible long patch repair correlates with enhanced bacterial survival and with a major component of the Weigle reactivation of bacteriophage with double-strand DNA genomes.

Mammalian cells possess an excision repair pathway similar to the constitutive pathway in E coli. Although not as well understood, the incision event is at least as complex, and repair resynthesis produces patches of about the same size as the constitutive short patches. In mammalian cells, no patches comparable in size to those produced by the inducible pathway of E coli are observed.

Repair in mammalian cells may be more complicated than in bacteria because of the structure of chromatin, which can affect both the distribution of DNA damage and its accessibility to repair enzymes. A coordinated alteration and reassembly of chromatin at sites of repair may be required. We have observed that the sensitivity of digestion by staphylococcal nuclease (SN) of

Received June 15, 1981; accepted June 17, 1981.

0730-2312/82/1803-0271$04.00

newly synthesized repair patches resulting from excision of furocoumarin adducts changes with time in the same way as that of patches resulting from excision of pyrimidine dimers. Since furocoumarin adducts are formed only in the SN-sensitive linker DNA between nucleosome cores, this suggests that after repair resynthesis is completed, the nucleosome cores in the region of the repair event do not return exactly to their original positions.

We have also studied excision repair of UV and chemical damage in the highly repeated 172 base pair α DNA sequence in African green monkey cells. In UV irradiated cells, the rate and extent of repair resynthesis in this sequence is similar to that in bulk DNA. However, in cells containing furocoumarin adducts, repair resynthesis in α DNA is only about 30% of that in bulk DNA. Since the frequency of adducts does not seem to be reduced in α DNA, it appears that certain adducts in this unique DNA may be less accessible to repair.

Endonuclease V of bacteriophage T4 incises DNA at pyrimidine dimers by cleaving first the glycosylic bond between deoxyribose and the 5′ pyrimidine of the dimer and then the phosphodiester bond between the two pyrimidines. We have cloned the gene (*denV*) that codes for this enzyme and have demonstrated its expression in *uvrA recA* and *uvrB recA* cells of E coli. Because T4 endonuclease V can alleviate the excision repair deficiency of xeroderma pigmentosum when added to permeabilized cells or to isolated nuclei after UV irradiation, the cloned *denV* gene may ultimately be of value for analyzing DNA repair pathways in cultured human cells.

Key words: E coli, DNA damage, excision repair

Although it was once believed that the intrinsic stability of DNA was responsible for genetic continuity, it is now known that an elaborate array of enzymes is required to maintain the integrity of the genetic material in living cells. DNA is exposed to a variety of environmental insults that would render life impossible were it not for repair mechanisms that remove lesions and tolerance responses that permit cells to survive in spite of persistent lesions. In addition to the damage inflicted by external agents, the cellular DNA is also subject to deleterious modification by endogenous events such as deamination of cytosine and depurination, as well as to replication errors resulting from the intrinsic infidelities of DNA polymerases and their associated proteins.

Much of our current understanding of the biochemical reactions upon which repair and tolerance processes depend has been derived from studies of the bacterium Escherichia coli because of the ease with which this organism can be manipulated genetically. Resulting models for DNA repair and lesion tolerance have been used as guides for experimentation with the more complex eukaryotic systems. The subject of DNA repair has been treated comprehensively in the proceedings of a recent ICN-UCLA symposium [1] and critically in a number of reviews [2–5].

Some of the pathways for processing damaged DNA are constitutive, while others are activated or induced, either by the damage itself or by certain treatments that interfere with normal DNA replication without causing damage. In bacteria the *recA* system constitutes a large number of coordinately controlled processes induced by the presence of repairable damage in DNA and/or by the inhibition of DNA replication [2,5]. In mammalian cells some apparently similar effects have been described, but we do not yet know whether these reflect a cascade of inducible responses analogous to those in bacteria. Nevertheless, models for chemical carcinogenesis should take into account the possibility of generalized and inducible cellular responses to lesions that interfere with normal DNA metabolism as well as enzymatic actions at the sites of lesions.

Most chemical carcinogens or their activated intermediates have been found to interact with DNA and to cause mutagenesis in cellular test systems. The strong correlation of DNA damage with carcinogenesis is also seen when repairability of certain types of lesions is considered. Thus, a deficiency in the repair of UV-induced pyrimidine dimers in cellular DNA in the hereditary disease xeroderma pigmentosum (XP) has been shown to correlate with a high incidence of sunlight-induced skin cancers in the affected individuals. A number of other hereditary diseases conferring predisposition to cancer may also involve deficiencies in DNA repair processes [2,4]. Although accumulating evidence from a variety of studies implicates repairable DNA lesions in carcinogenesis, we do not know whether the initiating events are specific responses to lesions, aberrant repair of specific lesions, or some more generalized cellular response. In this paper, we will review known responses to damaged DNA in bacteria and mammalian systems and describe several of our recent approaches to the study of DNA repair.

CONSTITUTIVE AND INDUCIBLE REPAIR

Repair of damage in DNA involves either the direct reversal of defects in situ or their removal and replacement by normal DNA constituents (Fig. 1). For example, pyrimidine dimers may be directly reversed by enzymatic photoreactivation with no consequent interruption of the phosphodiester backbone [6]. The methylation or ethylation at the O^6 position of guanine in DNA has likewise been shown to be repairable by direct reversal in E coli, in this case by a transferase that removes the added alkyl group from the guanine [7,8]. Constitutive levels of both photolyase and the alkyltransferase are present in E coli but increased levels of either activity may be obtained under certain conditions. Thus, for example, conditioning of bacterial cultures by growth in the presence of low levels of alkylating agents, such as N-methyl-N′-nitrosoguanidine, results in a greatly enhanced capacity to reverse O^6 guanine alkylation [9]. Evidence is accumulating in support of an analogous process in mammalian cells [10,11]. In fact, the reduced efficiency of O^6-methylguanine repair in *Mer*$^-$ lines of human cells may in fact reflect a deficiency in a methyltransferase [12]. These lesion-reversal processes are highly specific; photoreactivation operates only on pyrimidine dimers and the alkyltransferase has been shown to deal only with O^6 guanine alkylation.

Also highly specific are the known DNA glycosylases that initiate pathways of excision-repair through release of damaged or inappropriate bases from deoxyribose in DNA (Fig. 1). Nine glycosylases have been identified, each specific for one of the following: uracil, 3-methyladenine, hypoxanthine, 7-methylguanine, 5,6-dihydroxy-dihydrothymine, urea, the adduct of activated aflatoxin B_1 to the N^7 position of guanine, 7-methylguanine with an opened imidazole ring, and pyrimidine dimers [3,8,13]. The apyrimidinic or apurinic site (AP site) resulting from glycosylase action (or from spontaneous base loss) is then a substrate for the action of one of several AP endonucleases, which produce backbone incisions from which excision and resynthesis can proceed. Alternatively, the missing base might be replaced directly, through the action of an insertase. Enzyme activities that insert purines into AP sites have been reported both in mammalian cells [14,15] and in E coli [16]. However, thus far, no mutants have been isolated to permit an evaluation of the role of insertase activities in vivo.

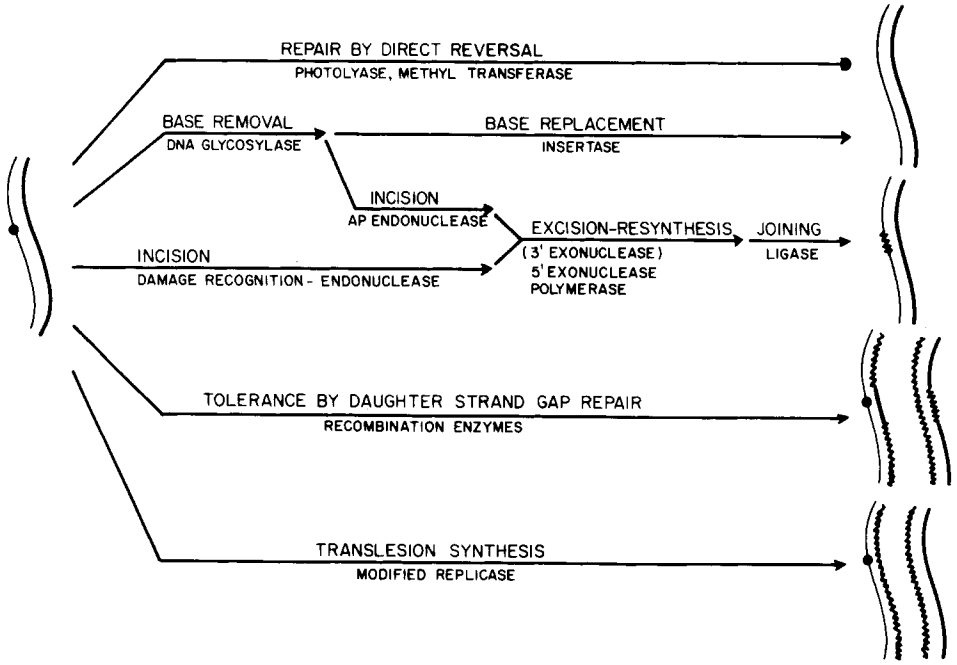

Fig. 1. Enzymatic processing of damaged DNA to restore function. Wavy lines designate newly synthesized DNA strands or repair pathces. The illustrated tolerance schemes, including daughter strand gap repair ("postreplication repair") and translesion synthesis are not discussed in the text. (See [2] for review)

The most general scheme for dealing with a variety of structural defects in DNA involves an enzymatic mechanism that incises the damaged strand in preparation for subsequent excision of a stretch of nucleotides containing the damage. Excision is usually coordinated with repair resynthesis, which replaces the excised nucleotides by utilizing the undamaged complementary DNA strand as template. The repair patch is ultimately joined to the contiguous parental strand by polynucleotide ligase. The initial incision may be effected by the direct action of an endonuclease activities from Micrococcus luteus and from bacteriophage T4 (T4 endo V) incise DNA strands containing pyrimidine dimers by a two step mechanism; first, the pyrimidine on the 5′ side is cleaved from its sugar by a DNA glycosylase, then the phosphodiester backbone is cut between the dimerized pyrimidines by an AP endonuclease that leaves an AP site on the 3′ end at the incision [17–22]. The 3′ terminal deoxyribose does not effectively serve as a primer for synthesis by DNA polymerase I, but it can be removed by a 5′ AP endonuclease normally present in E coli to provide a 3′-OH terminus, which would be a substrate for repair resynthesis [23]. In the case of T4 endo V, the glycosylase and AP endonuclease activities may be associated with a single protein coded by the *denV* gene of T4 [22].

In contrast, incision at pyrimidine dimer sites in E coli DNA occurs in an ATP-dependent reaction that requires the products of the *uvrA, uvrB,* and *uvrC* genes acting in a complex [24]. The incision evidently proceeds by a one-step

mechanism rather than sequential glycosylase-AP endonuclease action [19], and unlike the known glycosylase-mediated processes, the E coli incision complex acts on a variety of bulky lesions (reviewed in [2]). Each of the three *uvr* genes has been cloned and some of the properties of the individual protein products have been elucidated. The *uvrA* protein is a single polypeptide of 114,000 daltons normally present in about 20 copies per cell [25]. It binds to both single and double stranded DNA and has a DNA-independent ATPase activity [26]. The *uvrC* protein, with a molecular weight of 7000 daltons, also binds to DNA [27]. The *uvrB* protein, a single polypeptide of 84,000 daltons, lacks DNA binding activity and is normally present in about 140 copies per cell [28]. Contrary to an early report [29], none of these proteins by themselves exhibit incision activity [24].

Although constitutive levels of the products of all three genes (*uvrA, uvrB,* and *uvrC*) are evidently adequate for incision at most of the UV induced dimer sites in E coli DNA, we now know that the cellular levels of the *uvrA* and *uvrB* proteins are controlled by the *recA-lexA* regulatory circuit (Fig. 2) and are therefore inducible by UV [30,31]. Although the biological consequences of induction of the *uvr* gene products are not yet understood, this induction is correlated with *uvr*$^+$-dependent survival enhancement, with the production of long excision repair patches (described below) and with the major component of Weigle reactivation (the enhanced survival of UV-irradiated bacteriophage on UV-irradiated, compared to unirradiated, host cells) [32–34].

Although all three of the known DNA polymerases in E coli are potentially able to perform repair resynthesis, two of these, polymerase I and polymerase III, have intrinsic 5′ exonuclease activities and are therefore attractive candidates for coupled excision and resynthesis. Mutants deficient in polymerase I are UV

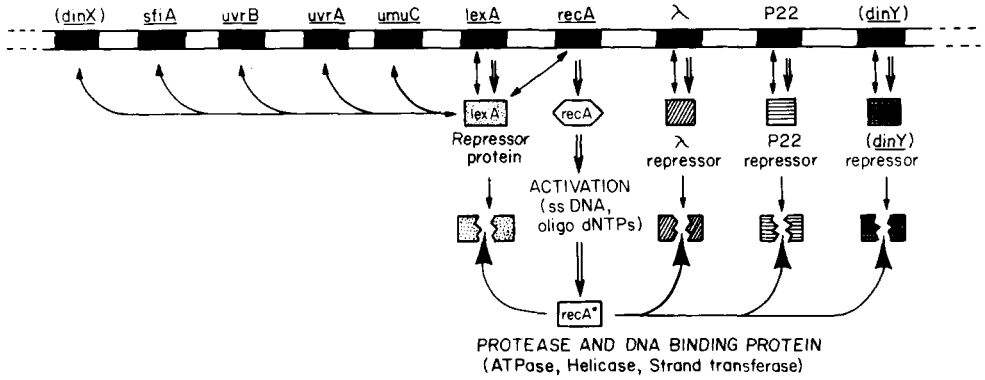

Fig. 2. The *recA-lexA* regulatory circuit in E coli. The *recA* gene product is activated in the presence of single-stranded DNA. In its activated form the recA protein is a special protease that cleaves various repressors including that for the λ prophage and the repressor for the Salmonella typhimurium prophage P22 as well as the lexA protein. The lexA protein is a repressor for the *recA* gene as well as for its own gene and for a number of other genes. (The sequence of genes shown is not meant to reflect their order in the genetic map.) The designation, *dinX*, is meant to cover other DNA damage inducible genes under *lexA* control but for which the functions and/or products are not yet known [30]. The designation *dinY* in like manner is meant to cover other genes for which the repressors may be substrates for the recA protein.

sensitive, although not as sensitive as are *uvrA, uvrB,* or *uvrC* mutants. Mutants deficient in polymerase III have only been obtained as conditional lethals, so it has not been possible to assess their UV sensitivity under conditions in which the enzyme is not functional. Mutants deficient in polymerase II are not UV sensitive. In studies of cells made permeable to deoxyriboside triphosphates by treatment with toluene, UV-stimulated repair synthesis is detected in mutants deficient in both DNA polymerases I and III, but not in mutants deficient in all three polymerases [35]. Although these results support the idea that all three polymerases may participate in repair synthesis, it has been difficult to evaluate their relative importance in vivo.

In UV irradiated E coli, repair replication results in patches that are heterogenous in size. Most of the patches are short (20–30 nucleotides), while a minority are at least ten times longer, with a few being several thousand nucleotides in length ([36]; P. Cooper, unpublished). The short patches are produced by a constitutive system and may reflect a close coupling of excision and resynthesis by DNA polymerase I [37]. Although somewhat longer patches may be produced by DNA polymerases II or III constitutively [33], the very long patches are produced by an inducible system under control of the *recA-lexA* regulatory circuit [32–34]. Like constitutive short patch repair, long patch repair is dependent on the uvr^+ genotype; however, it requires protein synthesis for expression, and like other functions in the *recA-lexA* circuit it can be induced by irradiation [32] or by incubation at 42°C (in the presence of adenine) of the tif-1 mutant [34]. The substrate for long patch repair seems to be a specific class of lesions, the nature of which is presently unknown; we have speculated that the lesions may be pyrimidine dimers at certain configurations of the genome, such as near replicating forks or in regions of active transcription where the two DNA strands may have separated [33]. Long patch repair resynthesis does not correlate with mutagenesis. The *umuC* mutation, which eliminates UV induced mutagenesis, has little effect on long patch resynthesis, while the *recF* mutation, which does not affect UV induced mutation, eliminates long patch resynthesis (Cooper PK, manuscript in preparation). Although long patch resynthesis is correlated with enhanced survival of irradiated bacteria, further investigations are needed to determine whether this reflects a causal relationship.

In UV-irradiated human cells, repair replication produces only patches in the same size range as the constitutive short patches in E coli. No patches corresponding to the very long ones produced by the inducible excision repair system in E coli have ever been observed over the wide range of UV doses and postirradiation incubation times that have been studied. When determined by analysis of the density distribution of small DNA fragments containing repair patches synthesized in vivo in the presence of bromodeoxyuridine, the average size of these patches is found to be 20–30 nucleotides [33,38]. Some confusion in the literature has arisen because these short patches produced in UV-irradiated human cells have often been termed "long" or "wide," to distinguish them from a class of extremely short patches (perhaps only one to four nucleotides) reported for repair of certain other lesions. Before discussing this point, we should emphasize that the terms "long" and "short" have completely different meanings when applied to DNA repair in E coli than when applied to mammalian cells.

Two aspects of the patch size distributions in mammalian cells need clarification. The first is the actual size distribution, the second is the degree to which the patch size depends upon the lesion being repaired. For contact inhibited human fibroblasts irradiated with UV_{254}, we have repeatedly obtained a value of about 30 nucleotides using the density shift method and sonication to reduce the DNA fragment size [39–42]. We have recently refined our technique by fractionating the sonicated DNA on neutral sucrose gradients to obtain fragments more homogenous in size. The size distributions of these fragments have been analyzed both on alkaline sucrose gradients calibrated with restriction fragment markers of known lengths and by labeling the fragments with ^{32}P at their 5′ ends and analyzing them under denaturing conditions on polyacrylamide gels of the type used for DNA sequencing. The 20–30 nucleotide estimate is consistently obtained. In addition, we have shown that sonication does not selectively break DNA at sites of repair patches by comparing release of the ^{3}H (repair label) with that of ^{32}P (bulk label) from the fragments by BAL 31 nuclease, which degrades duplex DNA molecules from their ends [43]. The α DNA in African green monkey cells [44, see below] allowed us to analyze DNA fragments of a single length (172 bp) generated by restriction endonuclease cleavage rather than by sonication. For UV, the patch size in these fragments was 20 nucleotides, compared to a size of 20–30 nucleotides in DNA fragments from sonicated bulk DNA.

These values are in conflict with the value of about 100 nucleotides often reported by investigators using the bromouracil photolysis method for analyzing repair [42,45,47]. The quantitative aspects of that complex technique have recently been discussed [48]. That method gives weighted value to larger patches in a distribution, and it has usually been employed to compare the repair induced by different damaging agents rather than to make accurate estimates of the average patch size induced by a given agent. The values reported have varied considerably. For example, Ahmed and Setlow [49] reported patch sizes of 130 ± 60 nucleotides (11 measurements) for UV-irradiated normal human cells, 80 ± 20 (two measurements) for XP variant cells, and 320 ± 40 (three measurements) for XP-D cells, but were unwilling to ascribe significance to these apparent differences. More recently Francis et al [50] have reported 90 ± 14 nucleotide patches (ten measurements) for repair of UV damage in normal cells using the same technique.

The second aspect which needs clarification is the degree to which the patch size is specific for the lesion being repaired. Workers using the BrUra photolysis technique have classified damaging agents into two general categories. These have been termed "UV-like" or "long patch," and "ionizing radiation-like" or "short patch." [45,47,50]. These categories have been operationally defined by the characteristics of the BrUra photolysis analysis of DNA from cells treated with various agents. In general, when the value for the change in molecular weight of the DNA with increasing photolytic dose reaches a plateau or appears significantly curved, a patch size can be calculated from these data alone, and the value obtained is usually in the range of 35–150 nucleotides, termed "long." Agents initially classified in this category all share the property that they do not seem to produce single-strand breaks in the DNA directly, and their lesions are not efficiently repaired in XP cells. UV_{254}, NA-AAF, ICR-170, benzo (a)pyrenediolepoxide-1, and diolepoxide 2, and 7,12-dimethylbenz(a)anthracene-5,6-oxide have been placed in

this category. With a number of agents, however, the change in molecular weight with increasing photolytic dose is small and apparently linear up to the maximum practicable photolytic dose. In these cases, either the patches are too small to contain enough broumouracil to be broken, or the number of patches is very small, or both. For determination of the patch size in these cases an independent estimate of the number of patches is required. This behavior was reported for ionizing radiation, methylmethane sulfonate (MMS), ethylmethane sulfonate (EMS), and propane sulfone (45). For ionizing radiation, when the number of patches was assumed to be equal to the number of single strand breaks initially formed, the calculated patch size was three to four nucleotides. Initially, it appeared that agents in this category produced lesions that were repaired normally in XP cells. The situation is now less clear. Using BrUra photolysis, Francis et al [50] have recently classified a number of additional agents. EMS and MMS have apparently now been reclassified into the "long patch" category. N-hydroxy-1-naphthylamine, which produces lesions not repairable in XP cells, has now also been assigned to this category.

To date, the hypothesis that certain agents produce very "short" patches of three to four nucleotides has not been confirmed by the density shift analysis. Using this method, I.G. Walker (personal communication) was unable to distinguish the patch size distribution in HeLa cells irradiated with γ rays from that induced by UV_{254}, and D. Phillips obtained the same distribution for repair in cells irradiated with UV_{254}, treated with 1′-acetoxysafrole (these proceedings) or treated with 1′-acetoxyestragole (personal communication). The latter two agents have been classified by BrUra photolysis as of the "short patch" type [50].

ROLE OF CHROMATIN STRUCTURE IN DNA REPAIR

The complex chromatin structure in mammalian cells may necessitate important differences in their repair mechanisms compared with those in bacteria. The highly specific protein-DNA associations of chromatin may render eukaryotic cells subject to DNA-protein cross-linking in a fashion not found in bacteria. The proteins may also protect the DNA and render it less reactive to some agents, even at the level of sites of attack within a given base. Since chromatin structure obviously plays a large role in transcription and replication of eukaryotic DNA, it is likely that it is an important factor in DNA repair processes as well. The accessibility of sites of DNA damage to repair systems may be affected by their position in the chromatin structure at the most basic level. For example, in UV-irradiated cells made permeable to enzymes under conditions of low ionic strength, T4 endo V incises DNA at only about half the sites of pyrimidine dimers, but can make incisions at nearly all of the sites if the permeable cells are first subjected to a brief exposure to high ionic strength that presumably alters chromatin structure in a nonreversible way [51]. Similar results were also reported with preparations of pyrimidine dimer-specific nuclease from M luteus [52]. At a higher level of organization, certain large regions of DNA may be more or less accessible to repair systems depending upon their state of expression or function in the cell [2]. It seems likely that chromatin structure in a local region undergoing excision repair must be modified to allow the enzymatic events to occur; perhaps the organization of higher order domains, such as replicating units, must be altered as well. The sequence of

repair reactions cannot be considered complete until the original chromatin structure at the site of damage is restored.

To date, most studies on the role of chromatin structure in DNA repair have focused upon the basic repeating unit, the nucleosome, consisting of a core (in which about 140 base pairs of DNA are wound about a complex of histones) and a linker region of variable extent but in the range of 50 base pairs. DNA associated with a core is much more resistant to attack by staphylococcal nuclease (SN) than is DNA in the linker region, or than DNA not organized into these units.

We have been studying the role of chromatin in repair in two ways. We have compared the repair response of human cells to agents that produce different amounts of damage in core than in linker regions, and we have compared repair in the highly repeated α DNA sequence in African green monkey cells to that in the bulk DNA. In these studies, we have taken advantage of the unique properties of the furocoumarins as DNA damaging agents. These are conjugated tricyclic compounds that intercalate into DNA and can form covalent adducts to pyrimidines when activated by long-wavelength UV light (UVA). The adduct forms a cyclobutane ring at the 5 and 6 carbons of the pyrimidines like that in the pyrimidine dimer. A number of different types of furocoumarins are available. Angelicin has rings in an angular configuration and forms only monoadducts to pyrimidines under the conditions we use. Psoralens such as 8-methoxypsoralen (8MOP) and the more soluable 4′ aminomethyl trioxalen (AMT) have their rings in a linear configuration. Certain of the monoadducts formed in DNA with these latter compounds can absorb a second photon and form an additional adduct to a properly positioned pyrimidine in the complementary strand, resulting in an interstrand cross-link. The cross-links in DNA from chromatin treated with psoralen and UVA have been shown by electron microscopy to be distributed as if they are formed only in the linker regions [53], and studies using isotopically labeled psoralen have shown that the adducts in chromatin are sensitive to SN digestion to the same degree as linker DNA [54]. It thus appears that cross-links and most, if not all, of the monoadducts in furocoumarin-treated cells are formed only in linker regions.

In human cells, repair resynthesis following treatment with furocoumarins and UVA resembles that following near UV irradiation (UV_{254}) [41,55]. Unlike furocoumarin adducts, the pyrimidine dimers produced by UV_{254} are located both in cores and linkers. At biologically relevant doses, the pyrimidine dimer frequency per unit DNA may even be higher in the nucleosome cores than in linker DNA [56].

The newly synthesized repair patches in cells irradiated with UV_{254} or treated with a number of chemical agents are initially much more sensitive to digestion by SN than is the bulk DNA [57–60]. Lieberman and co-workers initially reported that this enhanced SN sensitivity of repair patches nearly disappears within a few hours. Although this phenomenon has been the subject of some controversy [58,59], it has recently been confirmed and accepted [60], at least for repair following irradiation with UV_{254}. The interpretation of this phenomenon has evolved considerably since its description. At first it was supposed that repair could only be initiated at damage in linker regions and that with time nucleosome rearrangement randomized the positions of repair patches with respect to core structures and concomitantly moved remaining damage from core to linker DNA, thereby making it susceptible to repair. The favored current hypothesis suggests that the initial SN sensitivity is due to a

lack of native nucleosome structure at sites undergoing repair and that complete restoration of such structures requires a few hours. Because the damage sites are nearly random in the DNA for the case of UV_{254}, exact restoration of nucleosome cores to their former positions along the DNA would eventually cause repair patches to exhibit the SN sensitivity of bulk DNA. The study of repair of furocoumarin adducts has allowed up to probe further into this question. The time course for repair resynthesis is the same for UV_{254} damage and for a variety of furocoumarin adducts; this suggests that damage in linker DNA is not recognized and repaired more efficiently than is damage in core DNA. The nuclease sensitivity of newly synthesized repair patches in chromatin of cells either treated with angelicin and UVA or irradiated with UV_{254} changed in the same manner with time. This suggests that even if the initial enhancement of nuclease sensitivity reflects only the disruption of chromatin structure in the regions undergoing repair, the nucleosome cores do not return precisely to their original positions on the DNA during restoration of chromatin structure. If they did, the repair patches due to angelicin should remain more SN sensitive than those due to UV_{254} damage.

We have also examined African green monkey cells, comparing repair in α DNA to that in bulk DNA. The α DNA exists mostly in the form of tandem repeats of a 172 bp sequence, located at multiple positions and constituting about 20% of the genome. The monomeric unit of α DNA can be isolated by digestion of the purified DNA with the restriction nuclease Hind III followed by separation according to size on agarose gels [44]. In cells irradiated with UV_{254}, the amount of repair resynthesis per unit DNA in α is nearly the same as that in bulk DNA up to 48 h after irradiation. After exposure to N-acetoxy-2-acetyl aminofluorine (NA-AAF), for which lesions also appear to be random with respect to cores and linkers (Thea Tlsty, personal communication), repair resynthesis in α is only 60% of that in bulk DNA over a period of 48 h. With angelicin or AMT plus UVA, values of 30% were obtained in α compared to bulk DNA. Our results with UV_{254} show that α DNA is not inherently less accessible to repair enzymes than is bulk DNA. The sizes of repair patches in α DNA have been found to be the same as those in bulk DNA for all three agents studied. The results with the chemical agents suggest that either the efficiency of production of repairable adducts is less in α DNA or that repair of these adducts is less efficient in α than in bulk DNA. Preliminary results with isotopically labeled AMT indicate that initial total adduct frequencies are similar for α and bulk DNA. On the basis of these results, we suggest that repetitive DNA may differ significantly from bulk DNA with respect to repairability of some adducts. The difference in repair may reflect a difference in chromatin organization of these sequences within the genome.

ACTIVITIES OF T4 ENDO V AND PROPERTIES OF THE CLONED denV GENE

T4 endo V, the product of the *denV* gene of bacteriophage T4 [22], comprises a DNA glycosylase specific for pyrimidine dimers and an apyrimidinic-apurinic (AP) endonuclease activity. It incises DNA by cleaving the glycosylic bond between deoxyribose and the 5′ pyrimidine of a dimer and then the phosphodiester bond between the two pyrimidines [18]. It incises double-stranded DNA more actively than single-stranded DNA. In the presence of 10 mM NaCl, the enzyme acts processively

on UV-irradiated DNA. Under these conditions, incomplete digests contain two predominant classes of DNA molecules, those with an incision at every dimer and those with no incisions [62]. In contrast, in the presence of 100mM NaCl, the enzyme does not act processively and the incomplete digests contain more nearly homogeneous populations of DNA molecules that have incisions at some, but not all, of the dimers (P.C. Seawell, unpublished results).

T4 endo V is required for the first step in excision repair of UV-irradiated T4 DNA, and it contributes significantly to the survival of the irradiated phage. When introduced into transiently permeable cells of E coli, the enzyme enhances the survival of *uvrA, uvrB,* and some (but not all) *uvrC* mutants [63] even though the mechanism of incision of DNA by T4 endo V is different from that of the *uvrABC* complex of E coli as described above. Furthermore, when introduced into UV irradiated XP cells, T4 endo V increased the level of unscheduled DNA synthesis in cells from several different complementation groups [64]. In the case of complementation group A, it has also been shown to enhance the survival of the UV-irradiated cells [65] and to stimulate repair resynthesis when added to their isolated nuclei under appropriate conditions. [40]. Because of these observations, it appeared likely that the *denV* gene would confer UV resistance on *uvrA* or *uvrB* mutants of E coli or on XP cells if it could be maintained and expressed in them. We therefore cloned the *denV* gene by inserting restriction fragments of cytosine-containing T4 DNA into the SalI site of the plasmid pBR322, and transformed *uvrA recA* and *uvrB recA* derivatives of E coli with the chimeric plasmids [66]. Evidence for the expression of the *denV* gene in the transformed cells included not only their enhanced resistance to UV compared with the recipient cells, but also an increase in the survival of UV-irradiated λ and T4 *denV1* phages when plated on the transformants, the removal of pyrimidine dimers from the cellular DNA of UV irradiated transformants, and the presence in cell extracts of an activity that specifically incised UV-irradiated DNA. Of particular importance was the increased survival of the UV-irradiated *denV1* phage that does not produce T4 endo V and that are not reactivated by the *uvrABC* system normally present in E coli [66]. Because expression of the *den V* gene has been observed in transformants cured of detectable plasmids, it appears that the gene can be expressed after it has been integrated into the E coli chromosome.

ACKNOWLEDGMENTS

The work from our laboratory has been supported by a contract with the Department of Energy and by research grants from the National Institute of General Medical Sciences and the American Cancer Society.

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