10 DNA Repair in Specific Sequences and Genomic Regions

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10.1 INTRODUCTION

Analysis of repair processes in mammalian cells has been based largely on results obtained with prokaryotes, most notably *Escherichia coli*. In mammalian cells, the main enzymatic pathways for removing various damaged bases and bulky adducts appear to be similar to those in prokaryotes. However, some features that distinguish eukaryotes from prokaryotes are likely to play a role in determining the efficiency and specificity of the mammalian repair systems. The complexity of the substrate for repair enzymes in mammalian cells (i.e., the condensed structure of DNA in chromatin) could influence both the induction and processing of DNA damage within different parts of the genome. For most somatic cells, the expression of a large part of the genome is not needed for specialized function. Thus, DNA repair can operate with a strong preference for processing damage in functionally important genomic domains.

A large body of evidence suggests that regions of chromatin engaged in replication and transcription are in a more open conformation than the inactive bulk chromatin. From the observed hypersensitivity of transcriptionally active chromatin to certain nucleases, differences in chromatin conformation may play a role in determining the accessibility of genomic sequences to repair enzymes. Early studies aimed at elucidating the role of chromatin structure in DNA repair compared properties of bulk chromatin to those of chromatin that had been enriched for transcriptionally active sequences by selective digestion with enzymes that degrade DNA. Although such studies provided evidence that the initial distribution and removal of several bulky chemical adducts were influenced by chromatin structure (Bohr *et al.*, 1987), this approach has been limited by the digestion technique. Analysis of repair in the special case of non-transcribed α-DNA of

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monkey cells (Smith, 1987) revealed a complex pattern of repair heterogeneity, probably mediated by chromatin structure.

Recently, analysis of DNA damage and repair has become possible in specific sequences of the genome, allowing study of well-characterized loci in different cells and under different regulatory conditions. Although, most such studies have focused on damage induced by UV-light in cultured mammalian cells, limited data are now also available for *E. coli, Saccharomyces cerevisiae*, and *Drosophila melanogaster*.

In this chapter, evidence is summarized on the heterogeneity of repair of different types of damage in various cellular systems. Not an exhaustive description of various aspects and agents, this chapter is more of a discussion of the demonstration of "preferential repair" as the most notable result obtained from studying specific sequences. A more detailed discussion may be found in Smith and Mellon (1990).

10.2 REPAIR OF UV-INDUCED LESIONS

10.2.1 CYCLOBUTANE PYRIMIDINE DIMERS

The method to measure repair of cyclobutane pyrimidine dimers (CPD) in defined DNA sequences was described initially for CHO cells containing amplified dihydrofolate reductase (DHFR) gene sequences (Bohr *et al.*, 1985). The CPD content of DNA fragments obtained by restriction of genomic DNA can be determined by using the enzyme T4 endonuclease V, which makes single strand incisions specifically at CPD sites. DNA is denatured and fractionated by size in alkaline agarose gels, transferred to a supporting membrane and hybridized with an appropriate probe. Comparison of intensities of bands in DNA samples treated and untreated with T4 endonuclease V allows calculation of the actual frequency of CPD in a DNA fragment and as a measure of its repair.

10.2.1.1 Rodent cells

Removal of pyrimidine dimers from the *DHFR* gene in CHO cells has been studied most intensively. The demonstration that the removal of CPD was much faster and more efficient from the *DHFR* gene itself than from inactive flanking sequences or the genome overall (Bohr *et al.*, 1985; Table 10.1) suggests that the high UV-survival of cultured rodent cells—despite their levels of overall repair being lower than those of human cells—results from proficient repair of essential genes. However, this consequence may occur only in concert with the efficient removal of other photolesions such as (6-4) photoproducts (Smith and Mellon, 1990). The results also demonstrated that overall repair capacity is not necessarily a valid parameter to predict biological endpoints such as cytotoxicity and mutagenesis.

Table 10.1 Preferential removal of cyclobutane pyrimidine dimers from the DHFR gene in cultured mammalian cells

Cell	Time (h)	% T4 endonuclease-sensitive sites removed			Genome overall
		A	B	C	
Human 6A3	4	52	69	32	
	24	85	100	69	70
CHO B-11	4	51	82	12	
	24	62	89	10	15

Cells irradiated with 10 J/m^2
Data from Mellon *et al.* (1987)
A = both strands analyzed simultaneously; B = transcribed strand; C = non-transcribed strand.

Fine-structure analysis of repair in the *DHFR* region has been facilitated by its extensive characterization, and has made available probes for study of different regions of the *DHFR* gene and flanking sequences. The 3′ half of the *DHFR* gene appears to be less well repaired than the 5′ half (Bohr *et al.*, 1986). This feature is not generic to active genes in rodent or human cells, since efficient repair at 3′ ends of other genes has been described (Mayne *et al.*, 1988). A fragment located several kb 3′ to the gene and proximal to a putative replication origin region and nuclear matrix attachment region (MAR) exhibited poor repair. Whether this situation is common to this type of MAR and to what extent this might relate to association of DNA with nuclear matrix proteins has yet to be determined.

The repair levels of different regions of the *DHFR* gene locus can be influenced by altering DNA methylation levels (Ho *et al.*, 1989). In CHO cells containing only about 40% of the normal level of DNA methylation, repair in the 3′ end of the gene increased to resemble repair in the 5′ end. Although the overall repair increased two-fold, repair of the fragment containing the MAR was not increased.

Additional evidence for proficient repair of transcriptionally active sequences in rodent cells has come from studies of several other systems. The HMG CoA reductase gene in CHO cells and the HPRT gene in V79 cells have exhibited efficient repair in backgrounds of low overall repair. In the HPRT gene, the domain of preferential repair appears to extend out in the 3′ direction for several kb. Mouse 3T3 cells have been shown to remove CPD from the active c-*abl* protooncogene, but not from the inactive c-*mos* gene (Madhani *et al.*, 1986). Repair in genes of the major histocompatibility locus of the mouse has been shown to be inefficient in cells not expressing them (e.g., fibroblasts), but efficient in expressing them (e.g., B cells). In B cells, poor repair was observed in a fragment containing an unexpressed pseudogene that lies between two expressed (and repaired) genes (Haqq and Smith, 1987).

Further insight into the role of transcription in preferential repair was provided

by measurements of repair in two complementary strands (Table 10.1). Mellon *et al.* (1987) discovered that the preferential repair of the *DHFR* gene in CHO cells is confined to the transcribed strand, both in cell lines containing amplified *DHFR* loci and in those unamplified for *DHFR* loci. The removal of CPD from the non-transcribed strand was as poor as from the genome overall. This effect suggests that chromatin accessibility alone cannot account for preferential repair in these cases, and implies some role for the transcription process itself in the removal of CPD in these rodent cells in culture.

10.2.1.2 Human cells

Human cultured cells differ from rodent cells by an efficient and nearly complete removal of CPD from their genomes at biologically relevant UV doses. Therefore, what has been called "preferential repair" in human cells reflects primarily differences in rates of repair. A rapid repair of transcriptionally active DNA was postulated some years ago from the kinetics of recovery of UV-inhibited RNA synthesis in normal and UV-sensitive human cells. Resumption of RNA synthesis following UV-irradiation was much faster than the overall removal of CPD. Mellon *et al.* (1986) demonstrated that the *DHFR* gene in a human cell line is repaired two- to three-fold faster than the genome overall; further studies revealed that this preferential repair could be attributed solely to more rapid repair of the transcribed strand. The non-transcribed strand was repaired in a manner resembling the genome overall: more slowly and less completely than the transcribed strand (Table 10.1; Mellon *et al.*, 1987). In both human (Mellon *et al.*, 1987) and CHO cells, preferential repair has also been shown in the flanking region 5′ to the gene that occurs on the DNA strand opposite to the one in *DHFR*, a feature consistent with the presence of divergent transcription units in these regions.

In primary fibroblasts, the adenosine deaminase (ADA), β-actin, and *DHFR* genes have also been shown to exhibit preferential repair (Mayne *et al.*, 1988; Kantor *et al.*, 1990). For the ADA gene, the repaired domain includes some sequences directly 3′ to the transcription unit. However, DNA fragments at the 3′ end of the ADA gene exhibited no preference of repair of the transcribed strand, due perhaps to the presence of a convergent transcription unit overlapping the gene. An ADA antisense transcript has been reported recently, suggesting that both strands of this part of the gene are transcribed. Few data on transcriptionally inactive sequences are available for human cells. The transcriptionally inactive 754 locus has been found to be repaired much more slowly than active regions, perhaps even slower than the genome overall (Venema *et al.*, 1990; Kantor *et al.*, 1990), suggesting the possibility of different levels of repair among inactive sequences in human cells.

Heterogeneity in removal of CPD has been reported in UV-sensitive human cells. Analysis of CPD removal in active genes in xeroderma pigmentosum group C (XP-C) cells revealed a complex picture. Repair of the 3′ end of the ADA gene in XP-C was indistinguishable from that in normal cells, whereas the 5′ end of the ADA

gene (the *DHFR* and β-actin genes) were repaired less than in normal cells, but still much more efficiently than the genome overall (Venema *et al.*, 1990; Kantor *et al.*, 1990). Strand-specific analysis indicates that removal of CPD from active genes in XP-C cells is restricted to the transcribed strand only; the high repair in the 3′ end of the ADA gene may thus be the result of a convergent transcription unit. The inactive 754 locus exhibits the deficient repair characteristic of the genome overall in these cells. Together, these results suggest that the residual repair (15% of normal) in XP-C is confined largely to domains containing transcriptionally active DNA, perhaps explaining the relatively high UV-resistance of non-dividing XP-C cells compared to cells of other XP groups (Kantor and Elking, 1988).

Analysis of repair in Cockayne's syndrome (CS) cells has provided valuable insight into the nature and importance of preferential repair. In these UV-sensitive cells exhibiting normal overall repair but poor recovery of RNA synthesis following UV, the active ADA and *DHFR* genes (Mayne *et al.*, 1988) as well as the c-*abl* gene were repaired at the same rate and to the same extent as the inactive 754 gene or genome overall, respectively. Thus they appear to lack the rapid repair observed in normal cells. The repair of active genes in CS may be even less efficient than the overall repair.

10.2.1.3 *D. melanogaster*

Preliminary studies on removal of CPD in the *Drosophila* cell line K^c has revealed no consistent differences between active and inactive genes. The rate of repair of the active gene coding for RNA pol II was exceptionally fast compared to the overall repair of the genome. However, repair of the active Gart and the inactive Notch and White loci resembled the removal of CPD from the genome overall. Further analysis of preferential repair in specific sequences in these cells may provide important clues to the determinants of preferential repair in those cell types exhibiting it.

10.2.1.4 Yeast

Repair of CPD in functionally different genomic regions in *S. cerevisiae* was reported by Terleth *et al.* (1989). These investigators took advantage of the mating type determinant system of yeast, comparing removal of CPD in two identical α-mating type loci differing only in their expression. The active MAT α-locus was found to be repaired significantly faster than the inactive HML α-locus in a wild-type strain. In a mutant, expressing both loci repair of the HML locus was similar to the active MAT locus in wild-type cells.

Recent results obtained from screening of RAD mutants for effects on preferential repair have revealed an interesting similarity to mammalian cell mutants. RAD 16 mutants, which exhibit moderate UV-sensitivity, are able to remove CPD from the

active MAT locus, but not from the inactive HML locus (Terleth *et al.*, 1989). In this respect RAD 16 cells resemble most cultured rodent cells and XP-C cells.

Smerdon and Thoma (1990) have examined repair of UV damage in exquisite detail in the yeast TRURAP mini-chromosome, actually a 2.6 kb plasmid whose chromatin structure has been mapped. Plasmid DNA prepared from irradiated cells was examined in a modification of the technique described above, in which the probe hybridizes to one end of the restriction fragment. This process allows mapping of the CPD sites to within about 40 bases, and formation and removal of dimers at individual sites can be monitored. The transcribed strand of the active *URA3* gene was repaired much more rapidly than the other strand, while slow repair occurred on both strands of a region containing the *ARS1* sequence and "stable nucleosomes." Still another region of the plasmid exhibited rapid repair of both strands; both strands of this region may be transcription templates. Any individual plasmid molecule contained only about one CPD.

10.2.1.5 *E. coli*

Recently, strand-specific preferential repair has been demonstrated for the genes of the *lac* operon in *E. coli* (Mellon and Hanawalt, 1989). In the uninduced state (after 40 J/m^2), repair in each of the two strands of a restriction fragment in the locus displayed roughly linear kinetics, with about 50% of the CPD removed in 20 minutes. Under inducing conditions, repair of the transcribed strand was very rapid, being more than 60% complete in only 5 minutes, whereas repair of the other strand was similar to that found in the uninduced cells. This clearly demonstrates that strand-specific preferential repair of active genes is not a unique feature of eukaryotes, and that chromatin structure *per se* is not a required factor for selective removal of CPD from active genes.

10.2.2 (6-4) PHOTOPRODUCTS

Although the rate of induction of (6-4) photoproducts in DNA by UV was initially considered to be about 10% of CPD (Franklin and Haseltine, 1986), recent data indicated that their induction may be significantly higher, and that the biological relevance of this lesion is greater than previously understood. Only limited information is available concerning the induction and repair of (6-4) photoproducts in different parts of the genome, because no convenient and quantitative assay is currently available. Very recent work suggests that these lesions, unlike CPD, may be highly favored to form in the inter-nucleosome linker regions of chromatin, like many chemical adducts (Gale and Smerdon, 1990; Mitchell *et al.*, 1990). This effect may be relevant to the more rapid repair of (6-4) photoproducts compared to CPD, and to the existence of several mutants that are altered in their repair of only one of these two major products.

Nairn *et al.* (1985) investigated the removal of (6-4) photoproducts in CHO cells using a radioimmunoassay and observed no differences in either their induction or removal between chromatin enriched in transcriptionally active sequences and bulk chromatin. About 40% was removed from both chromatin fractions in 2 hours following 20 J/m^2. In these experiments, the fractionation procedure relied on the selective precipitation of transcriptionally active chromatin after limited digestion with micrococcal nuclease. Whether the enrichment was high enough to detect preferential repair is unclear.

An indication of preferential repair of (6-4) photoproducts in active genes was derived from a study of the *DHFR* region in UV-irradiated CHO cells. Thomas *et al.* (1989) measured (6-4) photoproducts in specific sequences by using two enzymes from *E. coli*: DNA photolyase to remove specifically the CPD, and UVR-ABC nuclease to incise the DNA at sites of remaining damage thought to be almost exclusively (6-4) photoproducts. No differences were found in the frequencies of (6-4) photoproducts in the *DHFR* gene or the transcriptionally inactive 3′ flanking sequence over doses up to 60 J/m^2. The (6-4) photoproducts appeared to be removed more efficiently from the *DHFR* gene than from the non-coding sequence, i.e., 55% and 35% removed, respectively, during 8 hours repair following 40 J/m^2 UV. However, the total extent and rate of removal were much less than previously reported for (6-4) photoproducts in the genome overall. The authors suggested that this might arise from the greater UV doses they used. In addition, whether repair of the 3′ non-coding sequence truly reflects repair of the bulk chromatin is questionable. More information on repair of genes and inactive sequences, including data about strand specificity, is needed to draw conclusions about preferential repair of these lesions. However, this study does document heterogeneity in repair of (6-4) photoproducts, at least for rodent cells.

10.2.3 REPAIR HETEROGENEITY DETERMINED FROM REPAIR SYNTHESIS

Preferential repair of DNA damage has also been analyzed by labelling the newly synthesized DNA resulting from excision-repair synthesis, either with radioactive thymidine or bromodeoxyuridine, and then fractionating the DNA according to some property and examining the distribution of repair label. For example, Cohn and Lieberman (1984) labelled repair patches with BrdUrd, isolated the DNA fragments containing BrUra with a specific antibody, and analyzed their molecular weight distributions. They concluded that for certain doses and times after irradiation, the distribution of repaired sites in human fibroblasts was not random.

This type of analysis may often be less specific with regard to the lesions under study than direct adduct measurements. For example, in the case of UV-induced damage, repair incorporation reflects the removal not only of CPD, but also of (6-4) photoproducts and other lesions. The rates of removal and associated patch sizes need not be the same for all lesions. Indeed, recent experiments suggest that the

repair synthesis observed immediately after UV irradiation originates predominantly from removal of (6-4) photoproducts. A class of human trichothiodystrophy cells and a mutant V-79 cell (VH-1) both exhibit alterations in repair synthesis at early times after UV that correlate well with deficiencies in the removal of (6-4) photoproducts (Zdzienicka *et al.*, 1988; Broughton *et al.*, 1990). Photoreactivation (mediated by microinjected photolyase) in human fibroblasts has little effect on repair synthesis (measured autoradiographically) early after UV, but produced significant effects later (Roza *et al.*, 1990). Cohn and Lieberman (1984) found an apparent random distribution of repaired sites in the first 2 hours after irradiation with moderate doses of UV, but a non-random distribution for many hours thereafter. This effect might argue against preferential repair of (6-4) photoproducts; but at the irradiation doses used, the interlesion distance of the (6-4) photoproducts may have been too large compared with the DNA fragment size to allow detection of a non-random distribution in this case.

In Leiden, much attention has been paid to the analysis of the distribution of UV-induced repair sites with respect to their association with the nuclear matrix, a structure known to be associated with transcriptionally active DNA. Under certain conditions, repair occurred preferentially at the nuclear matrix in confluent human fibroblasts (Mullenders *et al.*, 1988). This preferential repair was only apparent during a short (15 minute) period directly following irradiation and only at low UV-doses (5 to 10 J/m^2), and was not observed in primary fibroblasts after high doses (30 J/m^2; Mullenders *et al.*, 1988) or in transformed human cells (Harless and Hewitt, 1987). The association of those sites repaired immediately after UV with the matrix was stable during continued incubation. This matrix-associated repair may reflect primarily the removal of (6-4) photoproducts from transcriptionally active DNA, since it was observed only very early after irradiation. Removal of CPD seems to be too slow to account for this observation, even from the transcribed strands of active genes.

The preferential localization of repaired sites in DNA associated with the nuclear matrix had been observed previously in XP-C cells, even under conditions (high UV doses and longer times) where it is not observed in normal cells (Mullenders *et al.*, 1984, 1988). From these results and the hypersensitivity of repaired DNA to endogenous nucleases (Player and Kantor, 1987) XP-C cells can be inferred to be proficient in repair of at least some fraction of transcriptionally active DNA but deficient in repair of inactive DNA. A heterogeneity in the location of repair sites in XP-C cells was also demonstrated when XP-C DNA was treated with the CPD specific enzyme T4 endonuclease, and analyzed in alkaline sucrose gradients (Mansbridge and Hanawalt, 1983). On average, the molecules containing repair sites were considerably longer than the bulk of the DNA molecules suggesting that some domains in the chromatin of XP-C cells were repaired much more efficiently than the bulk chromatin. This phenomenon, also referred to as "domain-limited repair," was not observed in normal human fibroblasts, even in the first 2 hours following irradiation (Mullenders *et al.*, 1986). Direct analysis of the removal of CPD from active and inactive genes has confirmed that XP-C cells have the

capacity to repair domains containing active genes, but are unable to repair inactive DNA (Mayne *et al.*, 1988; Kantor *et al.*, 1990).

When repair synthesis was analyzed in confluent CS cells (Mullenders *et al.*, 1988), results opposite to those observed for XP-C cells were found. In these cells, DNA associated with the nuclear matrix appeared to be reduced in repair synthesis when compared with the remainder of the loop DNA. These results suggest that CS cells may not only be deficient in the preferential repair of active sequences, but that these sequences may actually be repaired less efficiently than the bulk of the DNA. Whether these observations in CS cells reflect only the repair of (6-4) photoproducts is unknown. Direct analysis of removal of these lesions from specific sequences in CS cells has not been reported. For CPD, thorough analysis of the repair of the separate complementary strands will be needed to determine whether the repair deficiency extends beyond a lack of preferential repair. Presently, factors involved in preferential repair may govern not only the rate but also the extent of repair of transcriptionally active genes.

Leadon and Snowden (1988) studied UV-induced repair synthesis in defined fragments of genes by labelling the repair sites with BrdUrd, separating repaired from non-repaired restriction fragments with an antibody specific for BrUrd-containing DNA, and determining the distribution of specific sequences between the two classes of DNA by hybridization. This method does not measure actual lesion frequencies, and does not distinguish the effects of repair patches produced during removal of different adducts. Transcribed members of the metallothionein (MT) family in human cells were initially repaired more rapidly than non-transcribed MT genes or the genome overall. This effect was observable at shortly (2 hours) following irradiation, and thus suggests that (6-4) photoproducts are preferentially repaired in the active MT genes compared with the genome overall.

For rodent cells, domain-limited repair and the association of repair with the nuclear matrix have been studied in confluent primary Syrian hamster cells (Mullenders, 1986), that exhibit the poor repair of CPD characteristic of established rodent cell lines. Repair patches labelled with ^{3}HdThd during a 2-hour period following UV-exposure were found to be uniformly distributed within the genome when the DNA was analyzed for domain-limited repair or for the preferential association with the nuclear matrix. Also when repair was measured over longer (24 hours) periods, no heterogeneity in distribution of repaired sites was observed. Given the very selective removal of CPD from active genes in rodent cells, this lack of domain-limited repair and matrix-associated repair is unexpected. The most likely explanation seems to be that in these rodent cells, unlike XP-C, that are deficient in repair of (6-4) photoproducts, the repair of these lesions accounts for a large fraction of the repair synthesis observed. The distribution pattern of repaired sites, therefore, most likely represents primarily the repair of (6-4) photoproducts, that are repaired proficiently in the entire genome, thus masking the observation of domain-limited and matrix-associated repair. These experiments were carried out before the effect of dose on the matrix association of repaired DNA was observed with normal human cells, and used relatively high doses; at lower doses these kinds

of measurements will likely reflect the repair heterogeneity in rodent cells.

10.3 ALTERABLE REPAIR OF UV DAMAGE IN MAMMALIAN GENES

With two exceptions, the studies discussed above compared repair in sequences of different transcriptional activity in the same cells. In a few cases, attempts have been made to study the relation between repair and transcription in mammalian cells by modulating gene activity in a given cell system by exogenous factors, and examine effects on repair. What is considered rapid repair in mammalian cells (i.e., a considerable fraction of lesions removed in several hours) could be facilitated by rates of transcription that would be considered low. Interpretation of such experiments can also be complicated by the fact that inducing treatments can also bring about changes in chromatin structure of the genes under study, and may influence overall repair efficiency. MT genes, whose transcription can be strongly enhanced by metals and steroids, have been studied in rodent and human cells. The possibility that UV itself may induce MT gene transcription complicates the interpretation of such experiments. Okumoto and Bohr (1987) measured removal of CPD in a CHO cell line containing amplified MT I and MT II genes. Repair measurements were made in DNA fragments which, in addition to the small MT genes, consisted largely of flanking sequences of unknown organization. The outcome of this study was that a 1000-fold increase in transcription of MT I gene by induction with zinc (assumed from results of others) led to variable increases of repair up to a maximum of two-fold. No enhancement of repair was observed in the *DHFR* gene. Although the results suggest that gene activity may determine the efficiency of repair of CPD in active genes in CHO cells; a precise description of its role in this system cannot be made mainly due to the lack of characterization of the DNA fragments used. The same problem was faced in the study of Leadon and Snowden (1988) who used an immunological technique to investigate repair in defined DNA fragments in human cells. This method allows simultaneous determination of repair in various size classes of DNA fragments. At early times after UV the MT genes that are constitutively expressed at a basal level exhibited about twice the amount of repair as the inactive MT pseudogenes or the average for the genome overall. Induction by either cadmium or steroids increased the repair observed by another factor of two, suggesting that the rate of repair in these genes was related to the rate of transcription. However, the nuclease hypersensitivity of the mouse MT genes increases after stimulation with cadmium, indicating changes in chromatin structure as well.

Another method to modulate gene activity is to study genes in cells at different stages of differentiation in culture. Studies with rat cells have revealed interesting, but unexpected, results. Kessler and Ben-Ishai (1988) studied repair of CPD in the creatine kinase (CK) gene in rat myogenic cells, that differentiate to form myotubes upon serum deprivation, and express the CK gene. They were unable to observe any significant removal of CPD in 24 hours in the CK gene in growing or

differentiated cells.

At Stanford University, repair has also been studied using two rat cell cultured cell systems which can be induced to differentiate, L8 muscle cells and PC12 nerve cells. In both types of undifferentiated cells, inactive sequences were poorly repaired, as expected. However, CPD were removed from active genes much more slowly than anticipated from our observations with genes in CHO, mouse, and human cells. In the differentiating L8 cells, repair of both constitutively active (pyruvate kinase) and induced (creatine kinase) genes remained slow, requiring several days to reach appreciable repair levels. This situation might explain the results of Kessler and Ben-Ishai (1988). However, even unexpressed genes (e.g., serum albumin, GAP43) exhibited slow, but proficient, repair. In PC12 cells, growth associated protein (GAP-43) was induced from a basal level in undifferentiated cells to a very high level by supplementing the culture medium with nerve growth factor. However, this treatment increased only moderately (30 to 50%) the repair of the GAP-43 gene.

10.4 REPAIR OF OTHER TYPES OF DAMAGE

Although the excision-repair system in mammalian cells recognizes a large variety of lesions, the rate and extent of processing of various adducts can be different, and can depend on the cell type. Both rodent and human cells in culture remove (6-4) photoproducts rapidly and efficiently; whereas human cells exhibit extensive removal of CPD from the genome overall, whereas rodent cells do not. Information about repair of other types of adducts in defined regions of the genome is vital to basic mechanisms of preferential repair and its biological importance.

Studies of the influence of chromatin structure on the induction and repair of DNA damage other than UV-induced lesions have been mainly concerned with damage induced by reactive chemicals. The scarce data available for ionizing radiation are contradictory. Nose and Nikaido (1984) observed no differences in the induction of DNA strand breaks and alkaline sensitive lesions in defined DNA fragments of active and inactive genes, whereas Chiu *et al.* (1986) reported that DNA regions containing transcriptionally active DNA were more susceptible to damage by ionizing radiation. The reasons for this discrepancy are unclear. Several chemical agents have been characterized with respect to the distribution and repair of their adducts within chromatin. Heterogeneity in distribution of DNA adducts has been found at the levels of the nucleosome, in DNAase I hypersensitive regions of chromatin, in nuclear matrix associated DNA and loop DNA, and, in a few cases, in specific DNA sequences. With chemical agents, the possibility of heterogeneity in the initial distribution of adducts, which may in itself influence rates or extent of repair must be considered. The study of preferential repair of CPD in eukaryotic cells has been greatly facilitated by the uniform distribution of CPD within the genome. With the exception of ribosomal DNA, all genes investigated so far have exhibited CPD frequencies comparable with the average

frequency in the genome. The low frequency of CPD in ribosomal DNA can be attributed to its high guanine and cytosine content (Rajagopalan *et al.*, 1984).

10.4.1 AFLATOXIN B_1 ADDUCTS

Leadon and Snowden (1988) used their immunological technique to examine excision-repair in DNA fragments containing MT genes and pseudogenes in human cells treated with aflatoxin B_1. Shortly after treatment, results resembled those for UV irradiation. The fragments encompassing active genes contained more repaired sites than did fragments with inactive genes or the bulk of the DNA. However, at later times, unlike the case for UV, the repair in the fragments containing silent sequences remained low. Interpretation of results with aflatoxin are complicated by the fact that the rate of spontaneous release of the adducts is considerable, leaving apurinic sites in the DNA subject to repair by a different pathway.

10.4.2 ACETYLAMINOFLUORENE ADDUCTS

Differential repair of lesions induced by *N*-acetoxy-acetylaminofluorene (NA-AAF) has been reported for CV-1 monkey cells infected with simian SV40 virus (Brown *et al.*, 1987). The initial adduct frequency in cellular DNA was about twice that in viral DNA. Viral DNA repair was on average twofold faster than that of cellular DNA, but a viral DNA region containing the major nuclease hypersensitive sites was repaired at twice the rate of the rest of the viral genome. The more open structure of SV40 chromatin could result in greater accessibility to repair systems (Shelton *et al.*, 1980), but does not explain the lower adduct frequency in viral DNA. The formation of NA-AAF adducts in the non-transcribed highly condensed α-DNA of monkey cells was found to be slightly higher; their removal was slightly slower than in the bulk DNA. Removal of NA-AAF adducts from α-DNA was enhanced following UV-irradiation, suggesting that the heterochromatic α-chromatin has to be converted into a less condensed configuration to facilitate access to repair enzymes (Smith, 1987).

The induction and repair of NA-AAF adducts in specific sequences has been measured in the CHO cell line originally used by Bohr *et al.* (1985) to measure gene-specific repair of CPD (Tang *et al.*, 1989). In this case, the *E. coli* UVR-ABC nuclease was used to cleave DNA at the site of adducts. Similar frequencies of adducts were formed in 14 kb fragments of the *DHFR* gene and the 3′ non-transcribed sequence. Removal of adducts from the two specific fragments and from the genome overall followed the same kinetics, with 60 to 70% removal after 24 hours. This level of repair is surprisingly high, since less efficient repair has been reported in monkey cells (10% in 6 hours), CHO cells (30% in 24 hours, exclusively C^8-AAF), and human cells (50% in 24 hours, mainly C^8-AAF). Thus, removal of NA-AAF adducts appears to be clearly different from the selective

removal of CPD observed in the same CHO cell line, and resembles more closely the repair of CPD in human cells. However, the results of other experiments may indirectly suggest the existence of selective repair of NA-AAF adducts in active genes. Repair in the CHO line AT3-2 and in a UV-hypersensitive derivative have shown cross sensitivity to NA-AAF. Only C^8-AAF adducts were formed, and repair of the genome overall was slow but identical in both cell lines. Although repair of bulk DNA was similar, unobserved differences in extent of repair of active genes may account for different cytotoxic responses. In addition, assuming that NA-AAF adducts were formed exclusively with guanine, the observation that mutations in the *DHFR* gene were selectively recovered from the non-transcribed strand of the gene (Carothers *et al.*, 1989) may indicate preferential repair. However, this effect might also reflect a biased distribution of potential sites for the type of mutation selected in these experiments.

10.4.3 PSORALEN ADDUCTS

One of the first examples of intragenomic repair heterogeneity in mammalian cells was the extremely inefficient removal of psoralen photo-induced monoadducts and cross-links from the heterochromatic, non-transcribed α-DNA of quiescent cultured monkey cells. Since this effect was not observed in actively growing cells and since UV damage was efficiently repaired in α-DNA, the poor removal of psoralen adducts seemed likely to arise from the unusual chromatin structure of α-DNA, rather than from its inactivity (Smith, 1987). Interestingly, total adduct frequencies in α-DNA resembled those in bulk DNA; a lower cross-link to monoadduct ratio was observed, however, and might be the result of lower flexibility of DNA in α-chromatin.

Removal of psoralen DNA cross-linking and cross-linkable monoadducts from specific sequences has been studied by two methods. The first is an adaptation of that used for CPD, in which rapidly renaturing (cross-linked) restriction fragments are separated from fragments containing no cross-links by gel electrophoresis, and cross-link frequencies in specific sequences are determined by hybridization to specific probes. Cross-linking is measured directly by this method, and cross-linkable monoadducts are measured by determining the increase in cross-linking brought about by further irradiation of the purified DNA. These monoadducts comprise about 65% of the initial monoadducts formed by the irradiation of cells. Using this method, Vos and Hanawalt (1987) showed that the removal of DNA cross-linking from the *DHFR* genes of human and CHO cells proceeds more rapidly than removal of cross-linkable monoadducts. Comparison of repair in active genes with repair in silent sequences has not been reported using this method. Whether the observed repair is more rapid than that in the overall genome is difficult to assess due to the wide variation reported in the literature for rates of repair of these adducts. The rates of removal of monoadducts observed certainly appear slower than corresponding rates for CPD removal, but the possible effects

of the cross-links also present are difficult to assess.

A second method to measure repair of psoralen adducts involves shearing isolated genomic DNA to an appropriate size and separating cross-linked and noncross-linked molecules on special density gradients. Fractions from gradients can be assayed for DNA radioactivity to analyze the overall genome, and also slot-blotted to filters for hybridization to various specific probes. Using this method, cross-links were reported to be formed to a greater extent in the human *DHFR* gene than in the bulk of the genome, but to a much lesser extent in the inactive c-*fms* protooncogene. Removal of cross-links was efficient in *DHFR* (90% in 24 hours), barely detectable in c-*fms* and at an intermediate level in the overall genome (31%). More investigation, especially comparisons between neighboring active and inactive sequences and genes in different states of activity, is clearly needed.

10.4.4 ADDUCTS OF SIMPLE ALKYLATING AGENTS

Several investigators have utilized fractionation of chromatin to investigate selective alkylation or repair in different regions of chromatin. Such studies have revealed remarkable heterogeneity in the distribution of lesions within chromatin, perhaps most strikingly demonstrated by distinct hotspots of O^6-ethylguanine at the bases of DNA loops in rat brain cells exposed to *N*-ethyl-*N*-nitrosourea (Nehls *et al.*, 1984). Ryan *et al.* (1986) fractionated chromatin into "active" chromatin, nuclear matrix associated DNA, and bulk chromatin; they measured the methylated purine content of these fractions at various times after exposing rats to dimethyl-nitrosamine. Regions of active chromatin and nuclear matrix DNA tended to be methylated more readily than bulk chromatin. Removal of *N*-methylated purines was rather uniform in the various fractions, although repair in active chromatin was slightly faster than in the other fractions. In contrast to N-methylpurines, wide variations in repair of O^6-methyl guanine were observed in the various chromatin fractions, repair being most rapid in active chromatin. Nuclear matrix DNA, representing about 2.5% of the total DNA and being depleted of active DNA by the isolation method employed, was nearly unrepaired for up to 20 hours. The latter may be due to the presence of "bent" DNA at the bases of loops (Sykes *et al.*, 1988), since non-bent DNA has been shown to be refractory to repair of O^6-methyl-guanine *in vitro* (Boiteux *et al.*, 1985).

Nose and Nikaido (1984) measured induction and repair of methylnitroso-guanidine-induced DNA strand breaks, alkaline labile sites in the active collagen and of the inactive β-globin gene of human fibroblasts. The rate and extent of repair in both loci was similar. This finding is in agreement with the study of Ryan *et al.* (1986), provided that lesions under study are assumed to be apurinic sites formed from *N*-methylpurines.

Similar conclusions can also be drawn from studies with CHO cells. Scicchitano and Hanawalt (1989) measured the repair of *N*-methylpurines in the *DHFR* gene and flanking sequences in CHO cells exposed to dimethylsulphate. To assay these

adducts, their heat lability was exploited to introduce apurinic sites in the DNA, which were then converted into single strand breaks. Formation of *N*-methylpurines was found to be the same in the *DHFR* gene and in the 3′ inactive sequence. Repair reached a level of 70% after 12 hours following treatment, and was virtually complete after 24 hours. No differences were observed between the gene and its flanking sequence, and analysis with strand specific probes indicated no differences in repair rates of the transcribed and non-transcribed strands of the *DHFR* gene. Overall repair in the genome measured by alkaline sucrose gradient centrifugation was somewhat slower and less complete (i.e., 70% repair in 24 hours).

Together, these results suggest that repair of O^6-methylguanine may occur preferentially in transcriptionally active DNA. Repair of *N*-methylpurines is uniform within chromatin, and in the case of active genes not dependent on transcription itself. The absence of preferential repair of *N*-methylpurine may be related to the repair system involved in processing these adducts. Preferential repair of 3-methyladenine would probably have gone unobserved in these experiments due to the preponderance of 7-methylguanine adducts. Chromatin structure may not restrict access of DNA to small glycosylases and AP endonucleases, and the absence of strand-specific repair may be due to lack of blockage of transcription, assuming that such blockage accounts for strand-specific repair of CPD. However the same arguments could apply to O^6-methylguanine, that is repaired by a small, specific methyltransferase. In this case, a preferential localization of the enzyme in transcriptionally active chromatin could underlie preferential repair, but further verification of this possibility awaits both data on gene-specific repair of O^6-methylguanine as well as availability of purified enzyme.

10.5 CONCLUSIONS

Current understanding of repair heterogeneity is blurred considerably. In areas such as removal of CPDs, enough data existed from different sources to begin to discern some detail. The strand-specific repair of active genes in *E. coli*, yeast, and cultured mammalian cells suggested that this process is probably not mediated by simple accessibility (diffusion controlled), but is somehow coupled to transcription itself. A model for the mechanism of this preferential repair is that transcription blockage at these lesions somehow targets the repair system. This model can be tested using *E. coli*, in which advanced knowledge of the repair process exists. Recently, findings have indicated that CPDs block transcription *in vitro* at the site of the lesion and that a relatively stable RNA polymerase–DNA complex is formed. In the system so far developed, purified UVR-ABC nuclease proteins have no increased affinity for this substrate.

How strand-specific repair fits into the overall picture of repair heterogeneity is unclear. Results with CS cells raise the possibility that a specific factor not only hastens repair of active genes, but is also required for their efficient repair, at least for CPD. Clearly, some intragenomic repair heterogeneity is not mediated directly

by the transcription process. In several cases, inactive DNA or regions flanking genes are repaired better than the genome overall. XP-C cells appear to restrict repair of both CPDs and (6-4) photoproducts—perhaps all bulky adducts—to certain chromatin domains; these domains may be larger than transcription units. These observations suggest that accessibility factors influence repair as well. The difference in the rate of removal of (6-4) photoproducts and CPD from human and rodent genomes may reflect different requirements for some accessibility factor due to their differing distributions between core and linker DNA. However, the simple idea that rodent cells carry out primarily repair of active genes (because they lack some factor necessary to make inactive chromatin accessible) seems inconsistent with their proficient removal of AF adducts from inactive sequences.

The complex systems developed by eukaryotic cells for managing their large amounts of DNA, containing sequences with differing functions, undoubtedly result in multiple hierarchies for DNA repair; that a simple picture is not emerging should not be surprising. An attempt to reconcile the available data with models of chromatin scanning and processing by repair systems may be found in the article by Smith and Mellon (1990), but the monograph serves primarily to emphasize our ignorance of such processes. The usual statement that more information is needed to understand the phenomenon under study certainly applies in this case. However, besides the need for more data about repair of various lesions in different genes, cell types, tissues, and organisms, knowledge of the details of chromatin architecture must advance considerably before understanding of repair heterogeneity increases in clarity.

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