COMMENTARY

DNA repair and transcriptional activity in genes

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Recent findings on the genomic heterogeneity of mammalian DNA repair have disclosed new features about molecular aspects of the repair processes and have also raised new interesting questions to help direct our research. The studies on preferential DNA repair in active genes demonstrate that there are variations in the repair processes corresponding to different parts of the genome and we are now attempting to study how these processes are regulated or controlled. In this commentary, it seems pertinent to discuss an important aspect of the preferential DNA repair mechanisms, the potential relationship between the DNA repair efficiency, the local chromatin structure and the transcriptional activity for a specific mammalian gene.

Most of the available results on DNA repair in specific genomic sequences are based on a technique to measure damage and repair after ultraviolet (u.v.) irradiation using quantitative hybridization (Bohr *et al.* 1985; Bohr & Okumoto, 1988) and have been reviewed recently (Bohr *et al.* 1987). The initial findings were that the essential, active gene dihydrofolate reductase (DHFR) was preferentially repaired in hamster and human cells (Bohr *et al.* 1985; Mellon *et al.* 1986), and some results have been outlined in Table 1. The fine structure of DNA repair in and around the DHFR gene in CHO cells has been examined, and it was found that the preferential DNA repair was confined to a genomic region of about 60–80 kilobases. This region has been called a DNA repair domain and its size corresponds well with described higher order structures or loops in chromatin (Bohr *et al.* 1986). However, further studies are needed to clarify whether DNA repair is regulated within such loops in chromatin.

Different genes are repaired with different efficiency. In a study on the repair of proto-oncogenes in mouse cells, it was reported (Madhani *et al.* 1987) that the c-*abl* proto-oncogene is repaired five times more efficiently than the c-*mos* proto-oncogene in the same cells (Table 1). Since the c-*abl* gene is actively transcribed and the c-*mos* gene is not, these findings indicate that a correlation exists between DNA repair efficiency and transcriptional activity. However, the c-*abl* gene is directly involved in the translocation leading to chronic myelogenous leukaemia whereas the c-*mos* gene is only indirectly associated with the translocation leading to acute myeloid leukaemia. This raises the alternative possibility that some relationship exists between DNA repair efficiency and another characteristic about a gene such as the potential 'importance' or 'danger' that a gene represents to a cell.

Which parameters regulate the DNA repair efficiency in individual genes? One attempt to address this question was to study the DNA repair efficiency after u.v. damage in a gene where transcriptional activity could be regulated (Okumoto & Bohr, 1987). The gene chosen for this study was the well-characterized inducible gene metallothionein I (MTI), which can be activated by incubation of mammalian cells with heavy metals. The repair study was carried out in CHO cells (amplified 12-fold for the MTI gene) where the MTI gene is activated 1000-fold by incubation of the cells with $ZnCl_2$. In a separate set of experiments, and as a control, the DNA repair in the overall genome was studied in the cells by use of repair replication. The overall genome repair was found to be unaffected by the incubation in $ZnCl_2$ (Table 2).

Table 2 shows the repair efficiencies in the MTI gene. As a comparison, it is seen that incubation of the cells in $ZnCl_2$ had no effect on the repair of another gene, the DHFR. The MTI gene is contained in a 6 kb and in a 18 kb fragment, which were each examined for repair efficiency before and after incubation of the cells in $ZnCl_2$. In both fragments, repair was enhanced by about 50% after induction of the MTI gene. This demonstrated that the repair in the MT gene increases with transcriptional activation. Since the MTI gene is only 1 kb long, repair must increase in a genomic region considerably larger than the gene in order for us to demonstrate such a substantial increase. Further studies are needed to determine the exact size of the repair domain at the MT gene, but it is possible that this domain is of a similar size to that found at the DHFR gene and that all such repair domains for genes have a similar size. It is thought that genes are individually located within a higher-order structure or loop formation of chromatin, and DNA repair may thus be directed towards such loops, which then might constitute a minimal region of repair.

At a recent UCLA meeting (cf. Downes, 1988) some results were reported on the repair in specific sequences in the mammalian genome. Interestingly, there may now be a human disorder that is deficient in the preferential DNA repair of active genes. This is Cockayne's syn-

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Table 1. *Preferential DNA repair in mammalian cells after u.v. damage*

	% repair	
	After 8 h	After 24 h
CHO cells		
Bulk DNA		15
Non-coding regions		15
An active gene	50	75
Human cells		
Bulk DNA	35	80
An active gene	75	80
Mouse cells		
c-*abl* proto-oncogene		80
c-*mos* proto-oncogene		20

In rodent cells, the active genes are efficiently repaired whereas the non-coding regions or inactive genes have minimal repair. In human cells, the active genes are repaired much *faster* than the bulk of the genome or inactive genes.

There can be considerable variations in the repair efficiency of individual genes within a cell.

drome, and different laboratories find a lack of preferential repair for different active genes in cells from individuals with this disorder after exposure to u.v. damage.

Using an antibody to bromodeoxyuridine, Leadon & Snowdon (1988) separated repaired DNA and probed it for the frequency of specific sequences. They reported that the repair of u.v. damage in the human MT gene was enhanced after induction of the gene. This supports the above results on repair in the MT gene in CHO cells and confirms by a different approach that the DNA repair efficiency can be correlated with transcriptional activity. Thus, there is accordance between the studies on proto-oncogenes and MT genes demonstrating that repair activity can be related to transcriptional activity. However, Kessler & Ben Ishai (1988) reported preliminary results that DNA repair of u.v. damage did not increase with the induction of the creatine kinase gene in rat muscle cells. We await the completion of these studies, but the correlation between DNA repair and transcriptional activity in individual genes may not be universal. It is possible that the important parameter with regard to DNA repair is the stage of differentiation of the gene and/or cell rather than the level of transcription. Further studies in developing or differentiating systems are needed to address these questions. It is also important to correlate the repair of a specific gene with direct measurement of transcriptional activity at the DNA level using nuclear run-off experiments as a measure of expression rather than studies of the mRNA level by Northern assays.

Whereas the distribution of pyrimidine dimers induced after u.v. damage appears to be homogeneous over the genome (Williams & Friedberg, 1979), this is not the case for a large number of bulky agents. Studies have shown that many agents are preferentially located in DNase I-sensitive regions of chromatin which are considered to be the transcriptionally active regions (reviewed by Bohr *et al.* 1987). Several studies over the last decade have suggested that DNA damage and repair in the mammalian genome is governed by the 'openness' or accessibility of chromatin (Wilkins & Hart, 1974; van Zeeland *et al.* 1981; Bohr & Hanawalt, 1984; Bohr *et al.* 1987) and the notion of accessibility has become widely accepted in the field. In support of this, we have recently completed a study on the preferential repair of the DHFR gene in

Table 2. *Repair in the genes MTI and DHFR and in the overall genome*

Region	$ZnCl_2$	J m^{-2}	Repair (h)	% repair	Increase $\pm ZnCl_2$
MTI gene 6 kb fragment	−	10	8	22	
	+	10	8	30	1·4
	−	20	8	14	
	+	20	8	22	1·6
	−	20	24	31	
	+	20	24	52	1·7
MTI gene 18 kb fragment	−	10	8	28	
	+	10	8	39	1·4
	−	20	8	17	
	+	20	8	35	2·1
	−	20	24	38	
	+	20	24	47	1·2
DHFR gene 14 kb fragment	−	10	8	72	
	+	10	8	54	0·8
	−	20	8	60	
	+	20	8	56	0·9
	−	20	24	66	
	+	20	24	62	0·9
Total genome repair replication	−	10	8		
	+	10	8		1·04
	−	20	8		
	+	20	8		0·90
	−	20	24		
	+	20	24		0·85

wild-type CHO cells and u.v.-sensitive CHO cells, some of which were transfected with repair genes. The transfectants contained either the bacterial gene *denV* coding for the pyrimidine dimer-specific repair enzyme bacteriophage T4 endonuclease V or the well-characterized human repair gene, ERCC-1 (Hoeijmakers, 1987). The u.v.-sensitive mutants did not repair u.v. damage, but the repair was restored in the transfectants. Cells containing the *denV* gene repaired all genomic sequences equally efficiently (Bohr & Hanawalt, 1987), whereas cells containing the human ERCC-1 gene repaired the active DHFR gene much more efficiently than the non-coding sequences (Bohr *et al.* 1988). Apparently the ERCC-1 gene product reacts more like the expected enzyme involved in normal repair in mammalian cells. Since the T4 endonuclease V is a small $16\times10^3 M_r$ enzyme, half the estimated size of the ERCC-1 gene product, these results support that the preferential repair of active genes is related to chromatin accessibility.

The recent report of strand specificity of DNA repair in mammalian cells (Mellon *et al.* 1987) raises some interesting questions and (in a sense) opposes the notion of accessibility. Although the exact structural or spatial arrangement of the coding and non-coding strands in the transcriptional complex is not known, it is unlikely that the two strands are located very far apart. Unless the non-coding strand is tightly shielded (e.g. by proteins), those results suggest that repair processes can be regulated by other features than chromatin accessibility. The DNA repair mechanism may be associated with the transcriptional complex or in some other manner directed towards the active gene. It is further possible that there is a separate part of the repair process that is responsible for the preferential repair component.

Further studies are needed to examine the relative importance of local chromatin structure, primary DNA sequence and function of the DNA for the determination of efficiency and organization of DNA repair. Certain molecules such as polyADP ribose and ubiquitin may play important roles in locating vital sequences for the DNA repair machinery. Also the importance of local levels of methylation may be of importance. No published studies have yet addressed this question. However, Ho *et al.* (1988) recently reported that demethylation of CHO cells by growth of the cells for many generations in azacytidine enhanced the overall genome DNA repair and changed the fine structure organization of the DNA repair domain in the DHFR gene. It had previously been reported that the 3′ end of the DHFR gene in CHO cells is less efficiently repaired than its 5′ end although both are better repaired than non-coding sequences (Bohr *et al.* 1986). In the demethylated cells, the non-coding regions surrounding the DHFR gene are still not repaired, but the 3′ end is repaired as efficiently as the 5′ end. This could possibly be ascribed to changes in the chromatin structure at the DHFR locus upon local demethylation.

Another set of molecules that may play important roles in the regulation of preferential DNA repair are the topoisomerases. The topoisomerase I and II enzymes regulate the tension or stress in DNA. Using inhibitors of topoisomerase II, it was originally shown by Collins & Johnson (1979) that this enzyme was involved in DNA repair in CHO and human cells. However, since then there have been many studies on the role of topoisomerase II in DNA repair at the level of the overall genome, some of them using new and more specific inhibitors, but the role of the enzyme has not been clarified. At the level of the individual gene, one study showed that novobiocin did not have any effect on the repair of the DHFR gene in CHO cells after u.v. damage whereas it inhibited the repair of the overall genome (Bohr & Hanawalt, 1987). This further supports the possibility that different repair mechanisms exist for the repair in genes and for the repair in the remainder or bulk of the genome. The repair of the active genes could involve topoisomerase I. This possibility is further attractive in the light of recent data on the binding of topoisomerases to specific sequences. In particular, topoisomerase I appears to bind to actively transcribed regions (Gilmour *et al.* 1986; Culotta & Sollner-Webb, 1988). It may thus be a current model for future experimentation that topoisomerase I is involved with the preferential repair whereas topoisomerase II is involved in the average genome repair.

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