DNA repair in the metallothionein gene increases with transcriptional activation

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ABSTRACT

We have studied DNA repair in the Chinese Hamster Ovary (CHO) metallothionein (MT) gene after UV-light induced damage. The repair was examined comparatively with or without transcriptional activation of the gene by incubation in the presence of the heavy metal $ZnCl_2$. Whereas the repair efficiency was very low in the uninduced state, it increased significantly after induction of the gene. The presence of $ZnCl_2$ did not appear to change other repair parameters in the cells. The overall genome DNA repair efficiency after UV irradiation was similar whether or not the gene was induced and the preferential DNA repair pattern in the essential dihydrofolate reductase (DHFR) gene which we have previously described was unaffected by the presence of $ZnCl_2$. Based upon repair analysis in two different restriction fragments containing the MT I gene, we conclude that the region of efficient repair after induction is considerably larger than the 1 kb size of the gene. The results suggest that the accessibility of a genomic region to DNA repair enzymes may be regulated by the local chromatin structure in a dynamic manner.

INTRODUCTION

We have previously examined the DNA repair characteristics in genomic sequences in rodent and human cell lines after UV irradiation, and presented evidence that active genes are preferentially repaired (1,2). Rodent cell lines are known to be deficient in the overall genome repair of UV-induced pyrimidine dimers, but active genes are repaired as efficiently as DNA in normal, repair proficient cells. The discovery of preferential repair has provided an explanation for why rodent cells are as UV-resistant as human cells (1). In normal human cells preferential DNA repair of active genes is manifested by more rapid repair in the genes than in the overall genome (2). Preferential repair of active genes thus appears to be a normal feature of mammalian DNA repair. Furthermore, there is evidence that UV-survival may be more closely associated with DNA repair in active genes than with the average repair in the overall genome (3).

In Chinese hamster ovary (CHO) cells, sequences located within the trans-

cribing region of the essential dihydrofolate reductase (DHFR) gene are repaired much more efficiently after UV-damage than are non-transcribing sequences located just 10 kb downstream from the gene (1). These changes in the DNA repair pattern within a small region may be ascribed to chromatin structural alterations in the genome (4). In mouse cells, DNA repair efficiency has been examined in different proto-oncogenes: whereas 80% of the pyrimidine dimers were removed from the c-abl proto-oncogene in a 24 hour period, only 20% were removed from the c-mos proto-oncogene in that period (5). These differences in DNA repair efficiency are likely to relate to gene activity since the c-abl gene is actively transcribed in these cells whereas the c-mos gene is not.

The results so far have indicated that transcribing regions are repaired more efficiently than are non-transcribed regions. We have searched for an experimental system which permits a more direct comparative study of DNA repair efficiency and transcriptional activity, such as a gene which can be modulated with respect to transcription. This would demonstrate whether DNA repair capacity is linked with a specific "permanent" chromatin structure surrounding a sequence, or whether it is dynamic and changes with the rate of transcription in specific regions at any given time.

A number of genes are inducible after activation by external inducers and one of the most studied is metallothionein (MT). Mammalian genomes encode two closely related metallothioneins, MT I and MT II, which are rapidly and transiently induced by exposure to heavy metals such as cadmium, zinc and copper, and by various circulating factors including hormones and interferon (6-8). This has been thoroughly described in mouse, hamster and human cells, and many features have been reviewed by Hamer (9). The optimal metal concentration for induction varies in different systems but is generally just below the level causing cell toxicity.

Crawford et al. (10) established a number of cadmium resistant CHO cell lines including the variant Cd^r200T1 which was found to carry an amplification of the MT genes and to express increased amounts of metallothionein. Griffith (11) determined the copy number of MT genes in the Cd^r200T1 cells to be about 12, and demonstrated a coordinate induction of all copies of the two MT genes with ZnCl₂ or CdCl₂. After induction with 100 μ M ZnCl₂, the level of MT I increases dramatically --more than 1000 fold, whereas the level of MT II increases about 100-fold (11). The induction of MT genes in the Cd^r200T1 cells has been demonstrated both at the mRNA and protein levels (10,11).

In this report, we have used a previously described assay (1,3,12) to deter-

mine the in vivo DNA repair efficiency of pyrimidine dimers in genomic restriction fragments after irradiation with UV light at 260 nm. Determination of DNA repair efficiency was based upon a comparison between the initial and final pyrimidine dimer frequencies. The repair efficiency was determined in restriction fragments containing the MT I and II genes after UV irradiation of the cells with 10 or 20 J/m². The level of DNA repair was compared with or without addition of ZnCl₂ to activate the genes.

METHODS

Cd¹⁰⁹Tc cells and c-DNA probes for MT I and MT II genes were generously provided by Dr. C.E. Hildebrand, Los Alamos National Laboratory, University of California, New Mexico. The probes have been described by Griffith et al., (13). Cells were grown in Minimal Essential Medium supplemented with 10% fetal calf serum, non-essential amino acids, penicillin-streptomycin, and 10 uM ZnCl₂ (10). For experiments, cells were prelabeled for 3 days with 0.3 uCi/ml ³H-thymidine and 10⁻⁵ M thymidine to uniformly label the DNA. They were then trypsinized, seeded at 2.5x10⁶ cells/dish and used for experimentation after 1 day during exponential growth.

The steps involved in the measurements of DNA repair in specific genomic sequences have been described in previous publications (1,3,4), and in a detailed protocol (12). The methodology will only be outlined here, and a flow-chart for the procedure is shown in Fig 1. Cells are irradiated, and incubated for repair over a 8 or 24 hour period in the presence of bromodeoxyuridine and fluorodeoxyuridine to density label DNA replicated during the repair incubation. The cells are then lysed, and the DNA is extracted, purified and restricted with the appropriate restriction enzyme. The parental DNA is then separated from that which has been replicated after the UV-damage by neutral CsCl density gradient centrifugation. This step is necessary since newly formed daughter DNA would be expected to be dimer-free and thus be assayed as repaired DNA. Samples of the purified parental DNA is treated with the pyrimidine dimer specific enzyme, T4 endonuclease V, from bacteriophage T4 (14) which incises the DNA at pyrimidine dimer sites (endonuclease sensitive sites). Alkaline agarose gels (0.4%) are prepared, and samples of DNA treated or not with T4 endonuclease V are run in parallel. The gels are Southern transferred and hybridized to specific probes. The density of the bands are determined by densitometry on the resulting autoradiograms.

The density of the full length size fragment is used for the calculation of the frequency of pyrimidine dimers using the Poisson distribution. The repair

Fig 1. Flow chart describing the experimental procedure.

efficiency is determined on the basis of initial pyrimidine dimer frequency and the dimer frequency after repair. We have previously discussed the reliability and precision of our measurements (1,5,12). At the UV doses used there is less error in the measurement when repair is efficient (60-85%) than when it is not (0-25%). Based on experience over the last 4 years, we estimate that the measured repair levels vary experimentally by approximately 5-10%.

Repair replication experiments including alkaline rebanding were done as

Fig 2. Autoradiogram showing the repair measurements in: a) the 6 kb BamHI MT I fragment, b) the 18 kb HindIII MT I fragment and c) the 14 kb KpnI DHFR fragment. Irradiation dose, repair period, addition of ZnCl₂, and treatment or not with T4 endonuclease V are indicated. A lighter exposure of these autoradiograms were used for the measurements shown in Table 1.

described by Smith et al. (15). Cells were preincubated for 2 hours before irradiation and for 8 or 24 hours repair with or without ZnCl₂ in the medium, similar to the conditions for the experiment in Table 1, Fig 2. The results are shown in Table 3.

TABLE 1
PYRIMIDINE DIMER REMOVAL IN THE MT I AND DHFR GENES

Gene	Fragment Size	ZnCl ₂	J/m ²	Hrs Repair	Dimer frq /10 kb	Percent Repair	Increase +/-ZnCl ₂
MT I	6	-	10	0	1.03		
MT I	6	-	10	8	0.80	22	
MT I	6	+	10	8	0.72	30	1.4
MT I	6	-	20	0	2.07		
MT I	6	-	20	8	1.78	14	
MT I	6	+	20	8	1.61	22	1.6
MT I	6	-	20	24	1.42	31	
MT I	6	+	20	24	1.00	52	1.7
MT I	18	-	10	0	0.61		
MT I	18	-	10	8	0.44	28	
MT I	18	+	10	8	0.37	39	1.4
MT I	18	-	20	0	1.22		
MT I	18	-	20	8	1.02	17	
MT I	18	+	20	8	0.80	35	2.1
MT I	18	-	20	24	0.76	38	
MT I	18	+	20	24	0.64	47	1.2
DHFR	14	-	10	0	0.66		
DHFR	14	-	10	8	0.19	72	
DHFR	14	+	10	8	0.31	54	0.8
DHFR	14	-	20	0	1.32		
DHFR	14	-	20	8	0.54	60	
DHFR	14	+	20	8	0.59	56	0.9
DHFR	14	-	20	24	0.45	66	
DHFR	14	+	20	24	0.50	62	0.9

The 6 kb fragment is a BamHI digest, the 14 kb fragment a KpnI digest, and the 18 kb fragment a HindIII digest. Initial dimer values at 20 J/m² were calculated from the values measured at 10 J/m².

RESULTS AND DISCUSSION

The data in Table 1 show the repair analysis for two different restriction fragments containing the MT I gene, a 6 kb BamHI fragment and a 18 kb HindIII fragment, as well as a 14 kb KpnI fragment constituting the 5' half of the

TABLE 2
REPAIR OF MT I, NO PREINCUBATION WITH ZnCl_2

Gene	Fragment Size	ZnCl_2	J/m^2	Hrs Repair	Dimer frq /10 kb	Percent Repair	Increase +/- ZnCl_2
MT I	6	-	10	0	0.97		
MT I	6	-	10	8	0.92	6	
MT I	6	+	10	8	0.54	44	7.3
MT I	6	-	10	24	0.88	9	
MT I	6	+	10	24	0.55	44	4.9

DHFR gene (1). The autoradiograms are shown in Fig. 2a,b,c respectively. The initial pyrimidine dimer frequency was measured at 10 J/m^2 (Table 1) and was 0.66 per 10 kb in the 14 kb DHFR fragment, which correlates well with previous findings (1,3) and was very similar to that in the 18 kb HindIII fragment (0.61 per 10 kb). In the 6 kb fragment, a somewhat higher initial pyrimidine dimer frequency was found. We believe that this is a technical aberration consistently found due to the limiting sensitivity in such small fragments (12).

Table 1 shows the data from the repair measurements with and without the addition of 100 uM ZnCl_2 during the incubation period. Repair after 10 J/m^2 was measured at 8 hours, and the repair after 20 J/m^2 UV was measured at 8 and 24 hours. In both MT I containing fragments, we found an increase by factors of 1.2 to 2.1 in the repair efficiency after 8 and 24 hours of repair in the presence of ZnCl_2 . This increase was observed in cells preincubated for 2 hours in the presence of 100 uM ZnCl_2 before irradiation as well as during the repair period. In another, similar experiment, the ZnCl_2 was added only at the time of irradiation, Table 2. Although the level of repair in the uninduced gene is somewhat low in this experiment, a rather dramatic stimulation of DNA repair is apparent upon induction of the gene. This may indicate that the most dramatic increase in DNA repair is immediately after gene activation and it may correlate to studies on the level of pulse-labeled MT in the Cd^{2+} 200T1 cells. It was reported that maximal level of MT protein was reached 2 hours after addition of ZnCl_2 and declined by about 30% over the next 8 hours (11).

We also measured the repair efficiency in a 7 kb fragment containing the MT II gene (data not shown) and consistently found an increase of the repair efficiency after stimulation, but the increase was smaller and less evident than that for the MT I gene. As mentioned above, the MT II gene is coordinately

TABLE 3
REPAIR REPLICATION, ALKALINE REBAND

Irradiation J/m ²	Hrs Repair	ZnCl ₂	Incorporation ³ H/ug DNA	Increase +/-ZnCl ₂
10	8	-	216	
10	8	+	224	1.04
20	8	-	331	
20	8	+	298	0.90
20	24	-	588	
20	24	+	502	0.85

activated in the presence of 100 μ M ZnCl₂ in these cells, although to a lesser degree than the MT I gene.

It was important to determine whether addition of ZnCl₂ had any independent effect on the general DNA repair characteristics of the cells. This control was carried out at two levels: Repair replication as an average measurement of repair over the entire genome, and repair measurements in a gene where expression should not be affected by ZnCl₂. Table 3 shows the results of a repair replication experiment. No appreciable difference in the overall level of genome repair in these cells is evident whether or not the MT genes were activated by ZnCl₂. We also studied the repair characteristics in the essential, constitutively active DHFR gene with or without addition of ZnCl₂. The autoradiogram from this experiment is shown in Fig 2c, and the data are recorded in Table 1. The repair efficiency in the DHFR gene was about 66% after 24 hours which is in agreement with our previous findings (1), but with no increase in repair in the presence of ZnCl₂. These results suggest that the activation of the MT gene is correlated with a significant increase in the DNA repair efficiency of that gene without an appreciable effect on the overall genome DNA repair level or on preferential DNA repair in essential genes. Thus a correlation exists between the level of transcription and DNA repair efficiency.

In both restriction fragments analyzed, we observe an average increase in DNA repair efficiency of about 50% after MT gene activation, Table 1. Repeated experiments have shown a significant and reproducible increase in repair efficiency with induction of the gene. The size of the MT I and MT II genes are each about 1 kb (D. Grady pers. comm.), but our measurements are done over 6

kb and 18 kb fragments containing the MT I gene. An increase in the repair efficiency confined to the 1 kb gene region alone would not be easily detectable in these much larger fragments, and particularly not in the 18 kb fragment. This suggests that the genomic region with increased repair efficiency (the repair domain) may be substantially larger than the size of the gene. In our studies on the fine structure of DNA repair in the DHFR gene, we found a repair domain with a size of about 60-80 kb which is between 2 and 3 times the size of the gene (4). It is possible that the repair domain surrounding the MT gene also is in the range of 60-80 kb, but our measurements at this point are not refined enough to firmly establish its size.

We have previously discussed aspects of the function of DNA repair enzymes in relation to higher order chromatin structure and organization (4,16,17). Hildebrandt and co-workers (pers. comm.) have recently studied nuclear matrix associated DNA in the Cd²⁺200T1 cells. They found an increase in the amount of nuclear matrix associated MT genes after activation of the genes with ZnCl₂. This may suggest that DNA repair of MT genes occur preferentially at the nuclear matrix. Preferential repair of nuclear matrix associated DNA has previously been reported (18).

Active genes have been proposed to have a more open chromatin structure than non-active genomic regions (19), and it is likely that the "openness" or accessibility of a genomic region determines the efficiency with which it is being repaired after damage. Our findings here, although confined to one example, suggest that the changes which govern the accessibility of repair enzymes can be dynamic in character and that the repair efficiency of a region may depend on the local chromatin structure at any given time. Since DNA repair presumably precedes transcription (20), regulation of DNA repair may be closely related with that of transcription.

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