Human repair gene restores normal pattern of preferential DNA repair in repair defective CHO cells

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ABSTRACT

The pattern of preferential DNA repair of UV-induced pyrimidine dimers was studied in repair-deficient Chinese hamster ovary (CHO) cells transfected with the human excision repair gene, ERCC-1. Repair efficiency was measured in the active dihydrofolate reductase (DHFR) gene and in its flanking, non-transcribed sequences in three cell lines: Wild type CHO cells, a UV-sensitive excision deficient CHO mutant, and the transfected line of the mutant carrying the expressed ERCC-1 gene.

The CHO cells transformed with the human ERCC-1 gene repaired the active DHFR gene much more efficiently than the non-transcribed sequences, a pattern similar to that seen in wild type CHO cells. This pattern differs from that previously reported in CHO cells transfected with the denV gene of bacteriophage T4, in which both active and non-transcribed DNA sequences were efficiently repaired (Bohr and Hanawalt, Carcinogenesis 8: 1333-1336, 1987). The ERCC-1 gene product may specifically substitute for the repair enzyme present in normal hamster cells while the denV product, T4 endonuclease V, does not appear to be constrained in its access to inactive chromatin.

INTRODUCTION

There has been increasing interest in the genetic control of DNA repair in mammalian cells. It has been shown that the defects in DNA repair deficient mutants isolated from established rodent cell lines can be genetically complemented by human genes using DNA-mediated gene transfer (1) or cell fusion (2,3). Using DNA-mediated gene transfer to the UV-sensitive Chinese hamster ovary (CHO) cell line 43-3B (originally isolated by Wood and Burki (4)), the first human repair gene (ERCC-1) was cloned by Westerveld et al. (5) and characterized by van Duin et al. (6). The ERCC-1 gene abolishes most of the DNA repair deficiency in the UV-sensitive CHO mutant cell line 43-3B and other mutants belonging to complementation group 1 (CHO complementation group numbering was recently changed to comply with the ERCC-gene numbering, i.e. the previous CHO complementation group 2 is now group 1) as determined by cell survival after treatment with UV (254 nm) or mitomycin C (MMC) (7). The level of UV-induced unscheduled DNA synthesis in these transfected cells indicated

at least partially restored repair proficiency (5). Characterization of the mutant 43-3B has shown that in addition to the already known sensitivity to UV and MMC this mutant shows increased sensitivity to alkylating agents Ethyl-nitrosourea (ENU), ethyl methanesulphonate (EMS) and methyl methanesulphonate (MMS) (7), and the increased sensitivity to UV (254 nm), 4 nitroquinoline-1-oxide (4NQO) and MMC is accompanied by increased mutability. The ERCC-1 gene restores the resistance of this mutant not only to UV but also to 4NQO, N-acetoxy aminofluorene (N-Ac-AAF) and alkylating agents. The frequency of mutation induced by UV at the Na⁺/K⁺ ATPase locus and the level of induced and spontaneous chromosomal aberrations are restored to the level of CHO wild type cells (8). Additionally, the rate of cyclobutane dimer removal approaches that in wild type CHO cells. These results indicate that the human ERCC-1 gene restores impaired functions in 43-3B, and that the gene may be functionally homologous to the defective or missing one in the 43-3B cell line. However, some evidence was found for a difference between the human gene product and its rodent counterpart, since the restoration of normal sensitivity to 4NQO, ENU and N-Ac-AAF was complete whereas it was not complete for UV and MMC.

We have previously studied the repair of pyrimidine dimers in genes and non-transcribing sequences in rodent and human cell lines (9-13). In all normal mammalian cells studied so far, evidence was found for preferential DNA repair of active genes (9). Most of these results documenting preferential repair are obtained after damage with UV light, but similar results have now been reported after damage with other agents (14,15). The findings suggest that preferential DNA repair of active genes is a general phenomenon in normal mammalian cells. The importance of measuring DNA repair in genes is emphasized by the demonstration that biological endpoints such as cellular survival correlate better with the repair of active genes than with overall, average repair of the genome (11). Since preferential DNA repair of active genes appear to be an essential feature in normal mammalian cells, it is important to examine whether this function has been restored in mutants transfected with repair genes. An optimal approach to measuring preferential DNA repair is to comparatively study the repair in transcribing and non-transcribing genomic sequences within the same cells. We have taken this approach in cells containing transfected DNA repair genes.

METHODS

The wild type CHO cells, CHO-9 and the UV-sensitive 43-3B cell line were generously provided by R.Wood. The 83-G5 transformed cell line has previously

been described by Westerveld (5). Cells were grown in Dulbecco's Minimal Essential Medium supplemented with non-essential amino acids and 10 % fetal bovine serum. Selection for the dominant marker was in modified mycophenolic acid medium (5).

The methodology for the repair measurements has been described in detail by Bohr et al., (10,11,16), and will only be briefly described here. Cells were uniformly prelabeled with ^3H -thymidine before UV irradiation. After irradiation, the cells were either lysed immediately or after incubation for 8 hours in the presence of 10 μM bromodeoxyuridine and 1 μM fluorodeoxyuridine. After proteinase treatment, the DNA was phenol extracted, treated with RNAase and resuspended in TE (10 mM Tris, 1mM EDTA) buffer. The genomic DNA was then incubated with endonuclease KpnI (6 U/ μg DNA for 3 hours, 37°) and the restriction reactions were checked for completion by electrophoresis on minigels. Samples were centrifuged to equilibrium in CsCl gradients, and the fractions containing parental density DNA were pooled, dialyzed and concentrated. Two 10 μg aliquots were removed from each sample, one was treated with T4 endo V and the other with endonuclease buffer only. Alkaline loading buffer (10X concentration = 500 mM NaOH, 10 mM EDTA, 25% Ficoll, 0.25% bromocresol purple) was then added and the samples were electrophoresed on 0.5% alkaline agarose gels for approximately 20 hours at about 30 V in a buffer consisting of 30 mM NaOH, 1mM EDTA. After standard gel washes, the DNA was transferred to Genatran nylon support membrane (Plasco Inc.). Prehybridization, hybridizations, and washes were according to the manufacturer's suggestions (with minor modifications (16)). When probing with the Cs-14 cosmid, a hybridization with 50-100 $\mu\text{g}/\text{mL}$ $\text{C}_{\text{ot}}100$ DNA (10) prepared from a wild type CHO cell line (K1) was included between the prehybridization and the hybridization with a radioactive probe to suppress hybridization to repetitive sequences present in the probe. Hybridization was carried out at 45°C in 5mL Hybrisol (containing 50% formamide, Oncor Inc.). Nick translated ^{32}P labeled probe (about 6×10^8 cpm/ μg) in the amount of 2×10^7 cpm was included in the hybridization solution. After standard washes of the support membrane, the final wash was at 0.1X SSPE, 0.1% NaDodSO₄, at 65°C . Support membranes were exposed to Kodak XAR 5 X-ray film for 1-14 days without intensifying screens. After development, the film was scanned with a Shimadzu densitometer.

The calculations of dimer frequencies are based on a comparison between the densitometric intensity of the bands in the autoradiograms of the two parallel samples, with or without T4 endo V treatment. This provides the fraction of dimer-free molecules (zero-class), and the Poisson distribution can then be

utilized to calculate the frequency of pyrimidine dimers in the fragment under study: $(= -\ln(\text{fraction of dimer free molecules}))$.

RESULTS AND DISCUSSION

We first studied repair in specific sequences in a CHO cell line carrying the denV gene coding for the pyrimidine dimer specific T4 endonuclease V (T4 endo V). This cell line, established by Valerie et al. (17), was shown to repair all genomic sequences examined whether or not they were being transcribed (18). We concluded from these experiments that the 16 kD T4 endo V enzyme was small enough to have access to DNA in all chromosomal regions regardless of the chromatin structure.

Fig 1. Map of the CHO DHFR gene indicating the regions analyzed. KpnI sites are indicated. The coding region is represented by fragment A, and the non-coding regions are represented by fragment B, which is flanking, downstream sequences to the gene. The probes used have been described previously (12). The autoradiogram shows the analysis of the dimer frequencies in the 3 cell lines tested: wild type CHO-9, UV sensitive 43-3B and transfectants 83-G5 containing the ERCC-1 gene. The filter has been probed for A) the coding sequences and B) the flanking, downstream sequences. Cells were irradiated at 20 J/m². The cell type, repair period and the presence or not of T4 endonuclease V is indicated.

We have now examined DNA repair of coding and non-coding sequences in CHO cells carrying the human repair gene, ERCC-1. For comparison we have measured the repair in wild type CHO cells and in the parental UV-sensitive cell line 43-3B. We confirmed the presence of the ERCC-1 gene in the transfected cells by Southern blotting and hybridization with the cDNA (plasmid pcD3A (6)) (data not shown). DNA repair efficiency was examined in the DHFR gene and in its adjacent, downstream non-coding fragments. The fragments, produced by restriction with KpnI, both had lengths of 14 kb (Fig 1). The data are presented in Fig 1, and the calculations are shown in Table 1. For a comparison, the previously published data on repair in the denV⁺ cells (18) are included in the Table. It is seen that the initial pyrimidine dimer frequency is very similar in the gene and in non-coding sequences in all cell lines. The value of about 2.0 ($= 7 \times 10^{-3}$ per J/m² per kb) corresponds well with previous results (11). In the wild type CHO cells, about 50% repair within the gene is obtained after 8 hr whereas little or no repair has occurred in the non-coding sequence in that time period. This represents the normal pattern of preferential repair which we have previously described (10). In the UV-sensitive cell lines, repair was not detected in either the gene or in non-coding sequences, consistent with no or very little overall genome repair efficiency. In cells expressing the transfected repair genes, some interesting results

TABLE 1
DNA REPAIR EFFICIENCY IN CODING AND NON-CODING SEQUENCES IN CHO CELLS

Cell Type	Initial Dimer frequency		PERCENT DNA REPAIR			
			Gene		Downstream	
	Gene	Downstream	8 hr	24 hr	8 hr	24 hr
Wild type AAB*	2.0	1.9	49		0	
Wild type CHO-9	1.8	1.9	52	58	13	18
UV-sensitive IID3*	1.9	2.0	10		0	
UV-sensitive 43-3B	2.1	2.3	11	16	20	10
<u>denV</u> ⁺ (in IID3)*	2.0	2.0	70		66	
<u>ERCC-1</u> ⁺ (in 43-3B)	1.9	2.2	53	66	14	38

Cells were irradiated at 20 J/m²

* Data from Reference 18.

appear (Table 1). The denV⁺ cells repair the DHFR gene about 50% more efficiently than the wild type cells. However, they also repair the non-coding sequences very efficiently, as proficiently as the coding sequences. In contrast, the cells containing the ERCC-1 gene repair the DHFR gene and non-coding sequences at rates similar to those seen in the wild type CHO cells. The repair efficiency in the non-coding sequences is a bit higher than in the wild type cells, but considered to be inefficient repair. The situation might, however, have a resemblance to the preferential repair seen in normal human cells where all regions of the genome eventually are repaired proficiently, but the active genes are repaired about twice as fast as the bulk of the genome (14). Further studies in different genes and noncoding sequences are needed to fully characterize the pattern of preferential repair in the ERCC-1 containing transfectants.

Although the ERCC1 gene was transfected into CHO complementation group 1 and the denV gene into group 2, we believe that the transfectants can be readily compared. Our results suggest that whereas the denV⁺ cells repair all genomic sequences very efficiently, the ERCC-1⁺ cells repair the active gene efficiently and the non-coding sequences much less efficiently. The pattern of repair in the ERCC-1⁺ cells appears to be similar to the situation in the wild type cells. The ERCC-1 gene thus seems to restore a "normal" mode of repair in the UV-sensitive cell line. The ERCC-1 gene codes for an enzyme roughly twice the size of the T4 endo V which has an estimated MW of 32 kD. It has been previously suggested that a normal mammalian repair enzyme would be much larger than the T4 endo V (18,19), and may thus be able to access DNA only at sites of more open chromatin structure such as active gene regions. However, the ERCC-1 gene product may play a role in the early recognition of damaged sites in need of repair. It has recently been shown that the preferential DNA repair of the DHFR gene in CHO cells is largely due to the selective repair of the transcribed strand (20). Since the non-transcribed strand is poorly repaired, the organisation of preferential repair may be less dependant upon the accessibility or "openness" of chromatin structure than previously speculated. The function of the ERCC-1 gene product may thus be to locate the active genes in the genome for the repair complex based on a specific recognition rather than just chromatin accessibility.

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