

ISOLATING HUMAN DNA REPAIR GENES USING RODENT-CELL MUTANTS

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SUMMARY

The DNA repair systems of rodent and human cells appear to be at least as complex genetically as those in lower eukaryotes and bacteria. The use of mutant lines of rodent cells as a means of identifying human repair genes by functional complementation offers a new approach toward studying the role of repair in mutagenesis and carcinogenesis. In each of six cases examined using hybrid cells, specific human chromosomes have been identified that correct CHO cell mutations affecting repair of damage from UV or ionizing radiations. This finding suggests that both the repair genes and proteins may be virtually interchangeable between rodent and human cells. Using cosmid vectors, three human repair genes that map to chromosome 19 have cloned as functional sequences: *ERCC1* by D. Bootsma's group in Rotterdam and *ERCC2* and *XRCC1* in our laboratory. *ERCC1* was found to have homology with the yeast excision repair gene *RAD10*. Transformants of repair-deficient cell lines carrying the corresponding human gene show efficient correction of repair capacity by all criteria examined. Future experiments will test whether these genes correct the defects in the cells from human repair syndromes such as xeroderma pigmentosum and Bloom's syndrome.

INTRODUCTION

Mutations induced by environmental agents arise when unrepaired DNA damage is followed by DNA replication or when damage is repaired inaccurately. The nucleoside excision repair (NER) system, which acts on bulky covalent adducts and pyrimidine dimers induced by ultraviolet (UV) radiation is a major line of defense against mutagenesis and carcinogenesis in human cells. Cell cultures from individuals having the

genetic disorder xeroderma pigmentosum (XP) are defective in NER and show hypersensitivity to induced mutation and transformation (Maher et al. 1979; Maher and McCormick 1984). XP patients have an elevated risk for neoplasms in sunlight exposed tissues as well as some internal sites (Kraemer et al. 1984). The XP syndrome appears to be extremely complex genetically. Nine complementation groups have been reported using unscheduled DNA synthesis as the repair assay (Fischer et al. 1985), indicating at least nine genes may underlie the repair process. Cells from each of these complementation groups show varying degrees of both hypersensitivity to killing by UV and reduced activity at the incision step of the repair process (Cleaver 1983). To date, the efforts of several laboratories to transfect and isolate genes that will correct the XP defects have been unsuccessful (Lehmann 1985; Schultz et al. 1985), perhaps because of the poor integration of foreign DNA in most human cell lines (Hoeijmakers et al. 1987).

An alternative approach to isolating human repair genes has been developed using DNA-repair-deficient mutants isolated in rodent cell lines (for a review of mutants see Collins and Johnson 1987). The molecular cloning of the first human DNA repair gene was accomplished by Westerveld and coworkers (1984), who used a CHO (Chinese hamster ovary) cell mutant as the recipient for DNA-mediated gene transfer. This gene, designated *ERCC1* (*Excision Repair Complementing defective repair in Chinese hamster*), has been analyzed in detail. The amino acid sequence deduced from the cDNA has regions of homology with the protein encoded by the yeast excision repair gene *RAD10* (van Duin et al. 1986). This observation suggests that there may be significant evolutionary conservation of repair proteins even between lower and higher eukaryotes. If so, the study of mammalian repair proteins could be facilitated by what is known about

the NER system in the yeast *Saccharomyces cerevisiae*, in which at least five proteins are essential for the incision step (Friedberg 1987).

Our laboratory has recently isolated a second human gene that corrects a different complementation group of UV-sensitive CHO mutants. We have also isolated a human gene involved in sensitivity to ionizing radiation and DNA strand break repair. The efficient correction of mutant hamster cells with both these human genes suggests conservation of repair gene expression as well as repair protein structure and function. Therefore, the use of rodent cell mutants may be highly effective for dissecting human repair processes.

RESULTS

The UV-sensitive mutants of CHO cells that show markedly reduced incision after UV exposure have been assigned to UV complementation groups 1-5 as presented in Table 1. The mutants in each of these five groups all have a similar degree of hypersensitivity to killing by UV (~7-fold compared to the parental line, AA8). However, with respect to one particular property, namely sensitivity to killing by DNA interstrand cross-linking agents, the mutants show phenotypic diversity. Mutants in complementation groups 2 and 4 are highly sensitive to this class of DNA-damaging agent while mutants in groups 1, 3, and 5 show little or no hypersensitivity (Hoy et al. 1985). Thus, mutants in these latter three groups appear very similar to XP cells in being hypersensitive to bulky monoadducts. (XP cells are not generally noted for being hypersensitive to cross-linking agents although this issue has not been systematically addressed). The sensitivity to cross-linking agents seen with the other two UV complementation groups indicates these

cells are defective in components that are essential for efficiently repairing both UV damage and cross-links. Sensitivity to cross-linking agents is a characteristic of cells from patients with Fanconi's anemia (Ishida and Buchwald 1982). The mutant UV61, which constitutes the sixth group of UV sensitivity in Table 1, differs from the others. Not only is this mutant less UV sensitive than the others (~3-fold), but it also appears to have normal incision as measured by alkaline elution (L.H. Thompson, unpublished results). Thus, these properties resemble those of cells from both the XP variant and from Cockayne's syndrome (Friedberg et al. 1979). The phenotype of line EM9 is totally different. This mutation confers a much reduced ability to rejoin radiation or chemical induced strand breaks, a 10-fold elevated baseline frequency of SCE (sister chromatid exchange), and hypersensitivity to killing by ionizing radiations and certain simple alkylating agents (Thompson et al. 1982a). High SCE is a well known feature of Bloom's syndrome cells.

As shown in Table 1, in each case we have examined there has proved to be a gene (or chromosome) in normal human cells that will correct the repair defect in the CHO mutant. This result could not have been fully anticipated since various studies have pointed to significant differences between rodent and human cells in the NER system (see Discussion). As a simple working hypothesis, we assume hamsters and humans share common genetic loci involved in repair and that these loci encode gene products that are functionally interchangeable between species.

We see that the first five human NER genes identified by functional complementation are distributed among four different chromosomes, suggesting that the CHO complementation groups correspond to distinct genes. The two NER genes assigned to

chromosome 19 must also differ from each other. The first NER gene to be cloned, *ERCC1*, was isolated using another CHO mutant (line 43-3B) belonging to complementation group 2 (Westerveld et al. 1984). We recently isolated a second NER gene, *ERCC2*, based on its ability to functionally correct mutant UV5 (C.A. Weber et al. in preparation). Both genes have been shown to correct only the mutants of the complementation group that was used in their isolation (A. Westerveld et al. in preparation; C. Weber et al. in preparation). This finding of specificity provides strong evidence that these two complementation groups involve different genes.

We have addressed in some detail the question of how well the human genes function in the CHO mutants. Transformants made with genomic DNA probably contain single copies of the human genes (based on band intensity in Southern blots) while cosmid transformants may contain multiple copies. Both genomic and cosmid transformants containing *ERCC2* show a full restoration of resistance to both killing and mutation induction at the *aprt* locus by UV radiation (C. Weber et al. in preparation). The rate of incision measured immediately after UV irradiation (Thompson et al. 1982b) is also returned to the normal level by the presence of the human gene in UV5 cells (C. Weber et al. in preparation). By using radioimmunoassays specific for Pyr(6-4)Pyo photoproducts and for cyclobutane (5-6) dimers, the kinetics of repair of UV damage can be examined in greater detail (Mitchell et al. 1985a). As shown in Figure 1, during the first 6 hr after irradiation UV5 cells appear grossly deficient compared with parental AA8 cells in removing (6-4) products. For (5-6) products, normal CHO cells are known to excise only low percentages (Meyn et al. 1974), and the kinetics of the antibody sites are similar for UV5 and AA8 cells. At times beyond 6 hr it is apparent that both classes of

lesions are somehow modified (Mitchell et al. 1987), even in UV5 cells since most sites are no longer recognized by the antibodies.

The kinetics of removal for (6-4) photoproducts in the secondary transformant of UV5 (line 5T4-1), in which UV resistance has been restored by *ERCC2*, resembles that of AA8 cells (Fig. 1). In both human and rodent cells (6-4) products appear to be repaired much more rapidly than (5-6) products (Mitchell et al. 1985b). Moreover, in CHO cells the removal of (6-4) products appears to be more closely correlated with cell survival than does the repair of (5-6) products. The mutants in complementation groups 1-5 are all deficient at early times in repairing (6-4) products (Mitchell et al. 1987).

The CHO mutant EM9 is also corrected by a gene on human chromosome 19 (Table 1). This gene was cloned in our laboratory from a tertiary transformant using CldUrd, which is highly toxic to EM9 cells, as the selecting agent (Brookman et al. 1987). The gene is designated *XRCCI* (*X-ray Repair Complementing defective repair in Chinese hamster*). Cell survival and the kinetics of strand-break repair are restored to normal levels in *XRCCI* transformants, and the baseline frequency of sister-chromatid exchange is also returned to the normal range (Brookman et al. 1987). Thus, *XRCCI*, which is the first isolated mammalian repair gene affecting cellular response to ionizing radiations, efficiently corrects the defect in EM9 cells.

DISCUSSION

From the results presented here it is clear that using the repair mutants of CHO cells, and hopefully other rodent lines, provides a powerful way to identify and isolate human genes that are likely to be important in the repair processes of human cells. Both human

genes we have studied function efficiently in correcting the CHO defects. Similar results were obtained by Zdzienicka et al. (1987), who found that the *ERCC1* gene fully reversed the UV-induced hypermutability to ouabain resistance and restored cell killing to a near-normal level (for UV radiation) or normal levels (for several bulky mutagens). Several laboratories are currently using the other mutants listed in Table 1 in attempts to clone the complementing human genes using methods similar to ours (Thompson et al. 1987) and that of Westerveld et al. (1984). Within several years, a sizeable collection of human repair genes should be in hand, and efforts to characterize some of the encoded gene products should be underway.

A leading question is whether the human genes being isolated using rodent cell mutations will be capable of correcting the mutations responsible for any of the human repair syndromes such as XP, ataxia telangiectasia, or Bloom's syndrome. So far the *ERCC1* gene does not correct any of the complementation groups of XP cells in which it has been tested (J. Hoeijmakers and A. Westerveld, personal communication). We have tested the *ERCC2* gene for correction only in the highly UV-sensitive XP-A cells (line XP12ROSV) and have seen no evidence for complementation (L. Thompson and C. Weber, unpublished results).

Further isolation and characterization of human (and rodent) repair genes should help resolve the dilemma of why rodent cells tend to have lower overall levels of UV-induced NER than human cells (Thompson et al. 1980; Yagi et al. 1984; Vijg et al. 1984). This difference has been difficult to understand since rodent and human cells tend to have very similar resistance to killing by UV radiation (Takebe et al. 1974; Yagi et al. 1984). Evidence now points to there being preferential repair of active genes in rodent cells

(Bohr et al. 1985; Madhani et al. 1986). Human cells also show this property with respect to the rate of repair (Mellon et al. 1986). This preferential repair of essential genes appears to correlate with UV resistance (Bohr et al. 1986).

Our results also show that the *ERCC2* secondary transformant of UV5 has an efficiency of cyclobutane dimer removal (Fig. 1) more like that of normal CHO cells than normal human cells (Mitchell et al. 1985b; Mitchell et al. 1987). This finding suggests that *ERCC2* does not control a hypothetical, rate-limiting component of repair that might be responsible for the overall difference in repair capacity between CHO and human cells.

ACKNOWLEDGMENTS

This work was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48.

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FIGURE LEGENDS

Fig. 1: Kinetics of repair of UV-induced (6-4) photoproducts and cyclobutane dimers ((5-6) photoproducts). Photoproducts were measured using radioimmunoassays specific for each class of dimer (Mitchell et al. 1985a). Closed symbols represent (5-6) products and open symbols (6-4) products. Wild-type CHO line AA8 (■,□); UV5 cells (◆,◇); *ERCC2* secondary transformant 5T4-1 (●,○,▲,△). Lines were fit to the data for 5T4-1. Data for UV5 and AA8 cells were taken from Mitchell et al. (1987).

Table 1.

Status of Mapping and Cloning Human DNA Repair Genes

Mutant ^a	UV Group ^b	Chromosome ^c	Gene name	Cloned?
EM9	--	19	<i>XRCC1</i>	yes
UV5	1	19	<i>ERCC2</i>	yes
UV20	2	19	<i>ERCC1</i>	yes
UV24	3	2	<i>ERCC3</i>	no
UV41	4	16	<i>ERCC4</i>	no
UV135	5	13	<i>ERCC5</i>	no
UV61	6	ND ^d	--	no

^aMutants were isolated as described (Thompson et al. 1980; Busch et al. 1980; Busch 1980; Thompson et al. 1982).

^bComplementation group assignments were reported as follows: (UV5, UV20, UV24, and UV41) Thompson et al. 1981; (UV135) Thompson and Carrano 1983; (UV61) Thompson et al. 1987.

^cChromosomal assignments were made as follows: (EM9) Siciliano et al. 1986; (UV5 and UV41) M.J. Siciliano and L.H. Thompson, in preparation; (UV20) Thompson et al. 1985; (UV24 and UV135) L.H. Thompson et al. in preparation.

^dNot determined

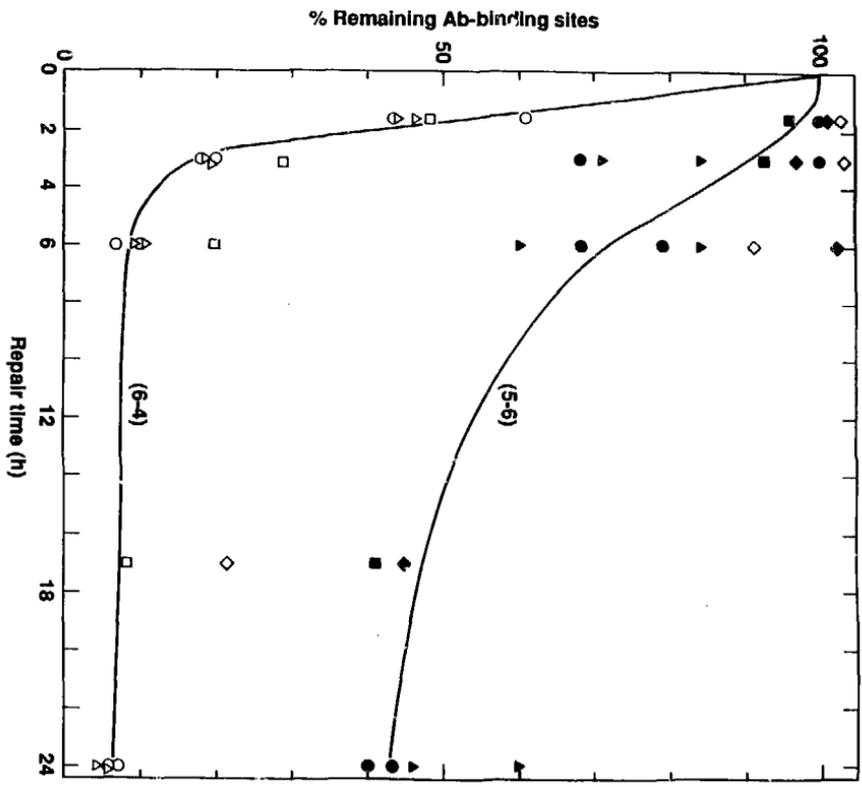


Fig. 1