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NOTES

DNA-Mediated Transfer of a Human DNA Repair Gene That Controls Sister Chromatid Exchange

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The Chinese hamster cell line mutant EM9, which has a reduced ability to repair DNA strand breaks, is noted for its highly elevated frequency of sister chromatid exchange, a property shared with cells from individuals with Bloom's syndrome. The defect in EM9 cells was corrected by fusion hybridization with normal human fibroblasts and by transfection with DNA from hybrid cells. The transformants showed normalization of sister chromatid exchange frequency but incomplete correction of the repair defect in terms of chromosomal aberrations produced by 5-bromo-2'-deoxyuridine.

Sister chromatid exchange (SCE), a process first recognized in plant cells over 25 years ago (26), has become widely used as an indicator of genetic damage in both experimental systems and humans (20). SCEs are induced by a great variety of chemical mutagens and carcinogens as well as by UV and ionizing radiations (20), and they have been correlated with specific locus mutations for various agents (2, 3). Moreover, certain classes of chemicals that do not show mutagenic activity, viz., inhibitors of poly(adenosine diphosphoribose) polymerase, also can enhance SCE frequency (17). SCEs occur during the S phase of the cell cycle (28), probably at DNA replication forks or sites where replication is incomplete (13, 18). However, little is known about the molecular basis of SCE in terms of the chemical lesions that are responsible, the enzymes that are involved, and whether mutations occur at the sites of exchange.

A genetic approach involving the study of mutations that modify SCE frequency should provide insight concerning the origin of SCEs. One such mutation has been identified in the Chinese hamster ovary (CHO) cell line in a mutant strain referred to as EM9. This mutant was isolated on the basis of its hypersensitivity to killing by ethyl methanesulfonate and was found to have a 12-fold-elevated SCE frequency (27; see Table 1). The enhanced SCE apparently reflects an altered response to incorporated 5-bromo-2'-deoxyuridine (BrdUrd), which is used to visualize SCE; the SCEs occur predominantly when DNA replication proceeds on a BrdUrd-containing template (8). At the biochemical level, EM9 cells show a defect in rejoining DNA strand breaks after exposure to alkylating agents or ionizing radiation (27). At present, the function that is defective in mutant EM9 remains unknown, but DNA ligases (5), the poly(adenosine diphosphoribose) polymerase system (12), and two classes of apurinic/apyrimidinic endonucleases (14) appear to be intact.

By several criteria, EM9 cells are phenotypically like cells derived from individuals having the cancer-prone genetic disorder Bloom's syndrome (10). Cells from individuals with this disorder also show extraordinarily high SCE levels (4), sensitivity to BrdUrd (24), and enhanced production of SCEs when DNA replicates on a BrdUrd-containing template (24). The molecular defect in DNA metabolism in these cells has not been defined.

To learn whether normal human cells possess a gene that can correct the biochemical defect in EM9 cells, we developed a selective system to test for genetic complementation after fusing EM9 cells with human cells. 5-Chloro-2'-deoxyuridine (CldUrd) proved to be efficient in killing EM9 cells (7), thereby allowing recovery of complementing hybrid cells. Briefly, monolayer cultures consisting of an equal number of EM9 cells and human fibroblast strain 812 cells were treated with 47% polyethylene glycol 1000 plus 10% dimethyl sulfoxide for 1 min and then rinsed three times. After a 24-h incubation in fresh medium for hybrid formation, the cells were replated (5×10^5/100-mm dish) in selective medium consisting of CldUrd (8 μM), thymidine (32 μM), deoxycytidine (200 μM), and fluorodeoxyuridine (10 μM) to kill the EM9 cells and ouabain (10 μM) to kill the human cells. The CldUrd selective medium, which was renewed once during the incubation, killed all of the EM9 cells. One hybrid clone obtained after a fusion with the diploid fibroblasts was subcloned and examined. Subclone H350 was shown to contain a single rearranged human chromosome and to have about 1% human DNA as determined by dot-blot hybridization for human-specific repetitive sequences (data not shown). The SCE frequency of almost all (49/50) H350 cells scored was low, like that of the normal CHO cells, which showed that normalization of SCE had occurred through genetic complementation (Table 1).

Isolating a gene that complements the defect in EM9 cells would provide a useful tool for characterizing the molecular basis of this mutant phenotype. For the purposes of gene transfer and eventual gene isolation, the H350 cells were an advantageous source of the complementing human gene since the gene was effectively enriched 100-fold with respect to human DNA sequences. Various studies have shown that

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TABLE 1. Properties of parental strains and transformants

Culture[a]	Differential cytotoxicity[b]		SCEs per cell ± SE[c]	Total aberrations per cell ± SE[c]
	CldUrd	MMS		
AA8 (wild type)	1.0	1.0	9.9 ± 0.5	0.02 ± 0.01
EM9	6.0	9.0	117.6 ± 3.0	0.63 ± 0.08
H350 (low)	1.2	1.0	8.3 ± 0.5 (49)[d]	0.14 ± 0.04
H350 (high)			96.0 (1)	
9T1 (low)	1.0	1.0	8.8 ± 0.6 (36)	0.09 ± 0.04 (75)
9Tl (high)			92.9 ± 5.4 (14)	0.48 ± 0.14 (25)
9T9	1.0	1.0	9.1 ± 0.5	0.16 ± 0.04
9T12 (low)	1.0	1.0	9.2 ± 0.4 (49)	0.05 ± 0.02
9T12 (high)			111.0 (1)	
9T14	1.0	1.0	8.0 ± 0.5	0.13 ± 0.04

[a] Three cultures (H350, 9T1, and 9T12) had two distinct cell populations (high and low) with regard to SCE frequency.

[b] Differential cytotoxicity is a measure of relative sensitivity to killing by an agent determined in a 24-well-tray assay, which we recently developed and validated (11). Differential cytotoxicity is defined as the lowest concentration of agent that produces a detectable reduction in growth of wild-type cells divided by the lowest concentration that produces a similar reduction in growth of the cell strain under test in the same culture tray. Cells were exposed to CldUrd continuously for 4 days at concentrations corresponding to 5 to 100% substitution. MMS (methyl methanesulfonate) doses ranged from 1.5 to 30 μg/ml under similar incubation conditions.

[c] Fifty cells from each culture were scored for SCEs as described previously (27); 100 cells were scored for chromatid aberrations.

[d] The number of cells scored is given in parentheses.

human genes transfected into rodent cells can be detected and isolated on the basis of differential hybridization of human and rodent repetitive interspersed sequences (1, 16, 22), which are closely associated with virtually all genes.

For DNA transfection, EM9 cells were plated at 10^6 cells per 10-cm petri dish in growth medium and incubated for 20 h. Calcium phosphate precipitates (6) containing 20 μg of H350 DNA and 20 μg of pSV2gpt (15) DNA were vigorously pipetted and then added to each dish. Plasmid pSV2gpt contains a bacterial guanine phosphoribosyltransferase gene, which provides an efficient selectable marker for DNA transfer. Amphotericin B was added at 2.5 μg/ml. After a 24-h incubation, the DNA was removed by replacing the medium. For expression of the transferred genes, the dishes were incubated for 24 h; the cells were then trypsinized and divided into two dishes (2×10^6 cells per dish) containing medium that is selective for *gpt* transformation-competent cells (15). This medium, which we refer to as MAXTA, consisted of α minimal essential medium containing the following: mycophenolic acid, 10 μg/ml; adenine, 25 μg/ml; xanthine, 250 μg/ml; thymidine, 10 μg/ml; and amethopterin (0.25 μg/ml) plus 10% dialyzed fetal bovine serum. After 3 days, growth medium supplemented with CldUrd was added to the cultures to select for repair-competent cells, and after 6 additional days, medium containing both CldUrd and MAXTA was added. Vigorously growing 14-day-old colonies were isolated, transferred into CldUrd-MAXTA medium, grown to mass culture, and frozen.

From 1.2×10^8 cells treated with DNA, 13 independent clones were isolated. Any clone transformed by a repair gene is expected to also express the *gpt* gene through cointegration of DNA molecules (21). Thus, the transformed cells of interest are resistant to both CldUrd and MAXTA and can be distinguished from revertants of the EM9 mutation. The frequency of colonies arising in dishes containing only MAXTA was 1.6×10^{-3} per viable cell. The frequency of revertants among EM9 cells treated with EM9 DNA was 10^{-6} (determined in a separate experiment). Therefore, the expected frequency of MAXTA-resistant revertants would be 1.6×10^{-9}, which is 60-fold lower than the frequency of colonies actually obtained.

Four of the putative transformant clones were grown in nonselective medium for verification and further study. DNA samples from each of the four resistant clones and from control cultures were digested with restriction endonuclease *Eco*RI and analyzed by blot hybridization by using total human DNA as the probe (Fig. 1). The DNAs from human cells and hybrid H350 produced intense autoradiographic signals with no discrete bands, whereas DNA from EM9 cells gave only a faint background. In contrast, one or more distinct restriction fragments containing human DNA were present in the four transformants. In addition, inspection of the hybridization pattern revealed the presence of a common 3.8-kilobase human DNA fragment. Therefore, we conclude that this common fragment represents a portion of the complementing repair gene or a closely linked sequence. Since transformants 9T12 and 9T14 contain extremely small amounts of human DNA, these clones represent suitable material for construction of DNA libraries for isolating the gene.

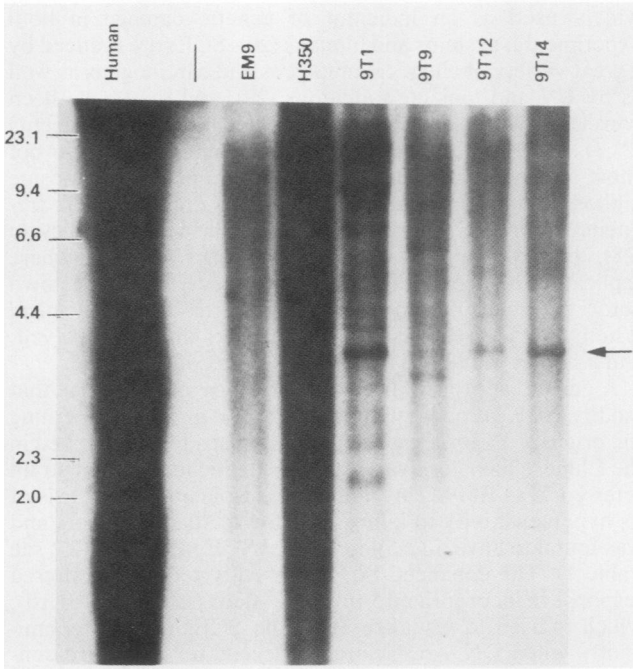

FIG. 1. The presence of human DNA sequences in transformants as detected by blot hybridization. High-molecular-weight DNA (10 μg) was cleaved with *Eco*RI, fractionated on a 0.8% agarose gel, and transferred to a nitrocellulose filter (25). Human DNA was radiolabeled with ^{32}P by nick translation (23) to a specific activity of 10^8 cpm/μg and used as a hybridization probe. Hybridization conditions were as described previously (9) except that the temperature was 42°C with 50% formamide. DNA was prepared from human foreskin fibroblasts, mutant EM9, hybrid H350, and MAXTA-CldUrd-resistant transformants 9T1, 9T9, 9T12, and 9T14. *Hin*dIII fragments of λ phage DNA were used as molecular-weight markers (in kilobase pairs). The arrow indicates a 3.8-kbp restriction fragment that is common to the four transformants.

The four transformants were characterized for several parameters that are pertinent to the phenotype of the EM9 mutant (Table 1): the resistance to killing by CldUrd or by methyl methanesulfonate and the frequencies of SCE and chromosomal aberrations. Cell killing was determined by a rapid assay developed for detecting DNA-damaging agents (11). Compared with the other strains tested, EM9 cells were ninefold more sensitive to methyl methanesulfonate, which agrees with earlier results (27), and sixfold more sensitive to CldUrd. All transformants had the same levels of resistance to killing by methyl methanesulfonate and CldUrd as did the wild-type AA8 cells and hybrid H350.

SCE measurements revealed that normalization of SCE had occurred in H350 and each transformant, although in strains H350, 9T1, and 9T12 a minority of the cells (2, 28, and 2%, respectively) had high SCE levels. In these three cultures, a subpopulation had apparently lost the complementing repair function. For the low-SCE cells, the SCE frequencies were not significantly different from those of the AA8 cells. Conversely, the values for the high-SCE cells were similar to those of the EM9 cells.

Chromatid-type aberrations were measured in second-division cells from the same samples used for SCE analysis. EM9 cells were extremely sensitive to aberrations arising from the exposure to BrdUrd. The transformants and the hybrid had greatly reduced levels of aberrations, but in no case were the values as low as for the AA8 cells. These results suggest that the repair defect, with respect to the production of aberrations, is not fully complemented by the human gene. Because SCEs were completely normalized in the same cells, the two cytogenetic endpoints show partial uncoupling. The reason for this divergence is unknown. Possibly the prevention of aberrations requires either a greater amount of repair gene product or a higher degree of specificity of that product. In the 9T1 strain, a fraction of the cells (25%) showed the high level of chromosome aberrations characteristic of EM9. This pattern is consistent with the pattern of heterogeneity in SCEs seen with 9T1.

Through interspecies complementation, this study shows that a human gene is capable of correcting the DNA repair defect in a CHO cell mutant that exhibits elevated SCE levels, hypersensitivity to mutagens, and retarded repair of DNA strand breaks. In summary, three points of evidence indicate that a human gene was taken up and expressed by EM9 mutant cells: (i) the frequency of resistant colonies in selective medium was much higher than that of revertants; (ii) a DNA restriction fragment containing human sequences was common to independent clonal isolates; and (iii) phenotypic instability, a common property of transformants (19), was evident in two of the four isolates examined. Isolation of the complementing gene from a transformant may assist in defining the biochemical defect in EM9 cells.

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