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**TITLE** Restoration of Chinese Hamster Cell Radiation Resistance by the Human Repair Gene ERCC-5 and Progress in Molecular Cloning of this Gene

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RESTORATION OF CHINESE HAMSTER CELL RADIATION RESISTANCE  
BY THE HUMAN REPAIR GENE ERCC-5 AND PROGRESS  
IN MOLECULAR CLONING OF THIS GENE<sup>1</sup>

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**ABSTRACT** The UV-sensitive Chinese hamster cell UV-135 is being used to identify and isolate the human gene, ERCC-5, which corrects nucleotide excision repair in this incision-defective mutant. A cosmid library, constructed from a 3° transformant of UV-135, has been screened for transfected gpt and human Alu family sequences. An ordered physical map of overlapping positive cosmids has been determined. Molecular evidence suggests a region of this map of <40 Kbp contains the ERCC-5 gene.

#### INTRODUCTION

A fundamental issue of human genetics and molecular biology concerns the mechanisms by which cells prevent the accumulation of deleterious DNA alterations induced by various environmental chemicals and radiation. Recently, transformation of repair-deficient rodent cell lines with human DNA has provided a useful technique for the isolation of two genes, ERCC-1 and ERCC-2, which are involved in ultraviolet light (UV) excision repair (1,2). This report will update progress in our laboratory on the isolation and characterization of a third human excision repair gene, ERCC-5.

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## RESULTS AND DISCUSSION

Thompson and colleagues have identified six complementation groups or UV repair-deficient mutants of Chinese hamster ovary (CHO) cells (3,4). UV-135, a CHO UV-sensitive mutant of complementation group 5, has been reported to be corrected in cell-cell hybrid studies by human chromosome 13 (4). We have transfected UV-135 with calcium phosphate-precipitated, high molecular weight human fibroblast DNA that had been ligated with an excess of restriction enzyme-linearized pSV2gpt vector DNA. Selection of transformants was based on their resistance to MAX (mycophenolic acid/adenine/xanthine) indicating the presence of functional gpt DNA, and resistance to sequential UV treatment (5) indicating the presence of human DNA complementing the repair defect in UV-135. Our selection strategy also included a sibling enrichment procedure in which resulting MAX<sup>r</sup> colonies were dispersed before exposure to UV in order to suppress revertant colony formation induced by the UV-selection protocol (5).

Table 1 lists the serial co-transformation frequencies in the construction of 1°, 2°, and 3° MAX<sup>r</sup>, UV<sup>r</sup> co-transformants of UV-135. In all transfection experiments, MAX<sup>r</sup> colonies were first selected and when approximately 500 cells in size, dispersed into new dishes and subjected to a multi-dose UV selection protocol in addition to MAX. Formation of positive colony pooled samples (>20 colonies/dish) indicated the putative transformant was produced prior to UV selection, but presumably coincident with MAX<sup>r</sup> transformant selection. Mock genomic transfection controls with UV-135 DNA did not yield any MAX<sup>r</sup>, UV<sup>r</sup> co-transformants in over 20,000 MAX<sup>r</sup> transformants tested.

TABLE 1  
SERIAL CO-TRANSFORMATION FREQUENCIES

Transformant	MAX <sup>r</sup> , UV <sup>r</sup> : MAX <sup>r</sup>
1°	3 : 13,500
2°	1 : 120
3°	3 : 80

DNAs from 1°, 2°, and 3° MAX<sup>r</sup>, UV<sup>r</sup> co-transformants were digested with various restriction enzymes, electrophoresed in 0.8% agarose gels, transferred to nitrocellulose according to the methods of Southern (6) and hybridized with nick translated, <sup>32</sup>P-labeled gpt sequence isolated from the pSV2gpt vector (for specifics on experimental protocols see references 5,7). Analysis of these blots indicated a dramatic dilution of the number of copies of pSV2gpt incorporated in the genomic DNAs from an estimated >100 copies in the 1° transformant DNA to only 3 copies in the 3° transformant DNA.

A Southern blot of DNAs from UV-135 cells and 2° and 3° MAX<sup>r</sup>, UV<sup>r</sup> co-transformants, which had been digested with HindIII restriction enzyme, was hybridized with a nick translated, <sup>32</sup>P-labeled pBLUR 8 human Alu family sequence (8). The autoradiograph of this blot is shown in Figure 1. Several distinct human DNA fragments ranging in size from 1.7 to 15 Kbp are inherited concordantly with the UV<sup>r</sup> phenotype. One or more of these distinct fragments is lost in all MAX<sup>r</sup>, UV<sup>s</sup> segregants tested to date (data not shown).

High molecular weight DNA was isolated from the tertiary MAX<sup>r</sup>, UV<sup>r</sup> co-transformant 40.2, partially digested with Sau3a enzyme and used in the construction of a complete genome library using BamHI linearized cosmid cloning vector

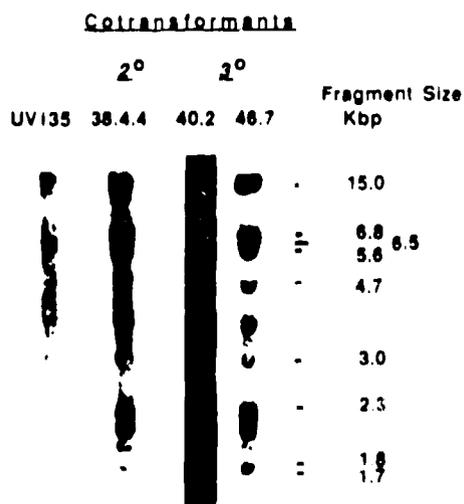


FIGURE 1. Autoradiograph of a blot of DNA from 2° and 3° MAX<sup>r</sup>, UV<sup>r</sup> co-transformants of UV-135 cells indicating the presence of human DNA hybridizing sequences.

pHC79. The resulting library of ~250,000 colonies was screened for the presence of both gpt and human repetitive sequences. An unusually low yield of only 15 hybridizing clones was isolated. Each clone was amplified in mini-preparations and its DNA was used in transfection experiments with UV-135 cells. No significant numbers of MAX<sup>r</sup>, UV<sup>r</sup> co-transformants were observed indicating that a functional copy of the putative ERCC-5 gene did not exist in any of the 15 tested cosmids.

Eight of the cosmid clones were ordered into a linear map using double restriction enzyme (HindIII and BglII) digests and Southern blot analysis using either gpt sequence or human repetitive DNA probes. A low resolution, physical map is shown in Figure 2 for four of these overlapping cosmids. The approximate positions of the three integrated pSV2gpt sequences and the location of DNA containing both human repetitive and other unique (presumably CHO in origin) sequences in this ~100 Kbp region of the 3<sup>o</sup> transformant's DNA are indicated. Additional ordering information has been obtained from similar analysis of other restriction enzyme digests and using other related cosmids (data not shown).

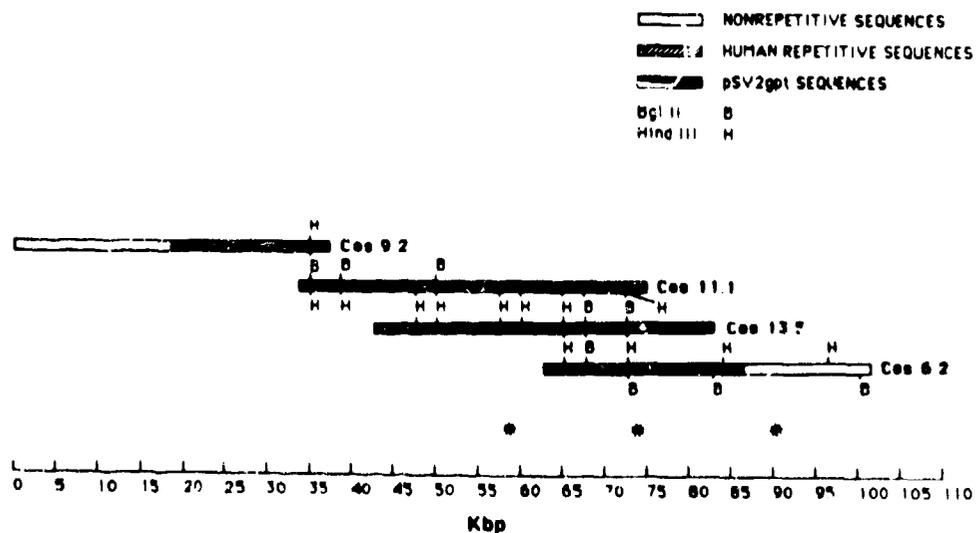


FIGURE 2. Physical map of four cosmids (Cos 6.2, 9.2, 11.1 and 13.5) overlapping the pSV2gpt region of DNA from the MAX<sup>r</sup>, UV<sup>r</sup> 3<sup>o</sup> co-transformant 40.2. The symbol (\*) denotes locations of C/G islands.

Recently, it has been shown that all housekeeping genes and many tissue-specific genes in vertebrates contain CpG sequence clusters (islands) at their 5' ends or 3' ends, or both (9). These islands contain a high density of non-methylated CpG and can be detected in chromosomal DNA by the use of rare-cutting (C-G) restriction enzymes (10). Using SacII, BssHII, and EagI restriction enzymes, we have located at least 3 putative CpG islands to the right of the pSV2gpt sequences on the physical map shown in Figure 2. One CpG-rich region is located in a region of CHO DNA whereas the other two are located in human-specific segments. Unique sequence probes from these latter two regions and other human sequence regions of the map are being subcloned using vector pUC19. We suspect that one or more of these probe fragments will show concordant inheritance both in MAX<sup>r</sup>, UV<sup>r</sup> co-transformant DNA as well as in the DNA of UV<sup>r</sup> human-CHO hybrids (used for the mapping of the ERCC-5 gene). Such probes will also be used to screen for unique mRNA transcripts by RNA slot blot and Northern analysis.

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