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Genetics

Recombination-dependent deletion formation in mammalian cells deficient in the nucleotide excision repair gene *ERCC1*

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ABSTRACT **Nucleotide excision repair proteins have been implicated in genetic recombination by experiments in *Saccharomyces cerevisiae* and *Drosophila melanogaster*, but their role, if any, in mammalian cells is undefined. To investigate the role of the nucleotide excision repair gene *ERCC1*, the hamster homologue to the *S. cerevisiae RAD10* gene, we disabled the gene by targeted knockout. Partial tandem duplications of the adenine phosphoribosyltransferase (*APRT*) gene then were constructed at the endogenous *APRT* locus in *ERCC1*$^-$ and *ERCC1*$^+$ cells. To detect the full spectrum of gene-altering events, we used a loss-of-function assay in which the parental *APRT*$^+$ tandem duplication could give rise to *APRT*$^-$ cells by homologous recombination, gene rearrangement, or point mutation. Measurement of rates and analysis of individual *APRT*$^-$ products indicated that gene rearrangements (principally deletions) were increased at least 50-fold, whereas homologous recombination was affected little. The formation of deletions is not caused by a general effect of the *ERCC1* deficiency on gene stability, because *ERCC1*$^-$ cell lines with a single wild-type copy of the *APRT* gene yielded no increase in deletions. Thus, deletion formation is dependent on the tandem duplication, and presumably the process of homologous recombination. Recombination-dependent deletion formation in *ERCC1*$^-$ cells is supported by a significant decrease in a particular class of crossover products that are thought to arise by repair of a heteroduplex intermediate in recombination. We suggest that the *ERCC1* gene product in mammalian cells is involved in the processing of heteroduplex intermediates in recombination and that the misprocessed intermediates in *ERCC1*$^-$ cells are repaired by illegitimate recombination.**

Eukaryotic cells defective in nucleotide excision repair (NER) are characterized by their inability to repair lesions such as pyrimidine dimers, bulky DNA adducts that are believed to distort the DNA helix (1, 2), and interstrand crosslinks (3). The role of NER in the repair of interstrand crosslinks, in particular, is believed to proceed by a pathway involving recombination between damaged and undamaged copies of DNA duplexes (1). In humans, defects in NER cause the genetic disease xeroderma pigmentosum (XP), which is associated with a high incidence of skin cancer and, in many cases, developmental and neurological abnormalities (1, 2). Some of the gene products involved in NER have been identified as components of the cellular transcription machinery (1, 2), but it is not known what other biological roles NER gene products might play in mammalian cells. In this study, we investigate the role of the NER gene *ERCC1* in recombination and gene stability.

The Ercc1 protein, in a multiprotein complex with the *XPF* gene product (4–8), forms an endonuclease that cleaves on the 5′ side of damaged DNA during NER (4, 6, 7). Another protein, the *XPG* gene product, is responsible for cleavage on the 3′ side of the damaged DNA (7–11). *In vitro*, these complexes are also capable of recognizing structures such as DNA bubbles and loops that might be found in heteroduplex intermediates in homologous recombination (4, 5, 8–11). In *Saccharomyces cerevisiae*, mutations in the *RAD1* and *RAD10* genes, which encode the 5′ structure-specific excision repair endonuclease, have variable effects on mitotic recombination (12–17) and severely reduce recombination between sequences containing terminal nonhomologies (18, 19). Mice with engineered disruptions of the *ERCC1* gene are defective for DNA repair as expected, but also exhibit a more complex set of phenotypes (20, 21). In mammalian cells, though, a role for NER proteins in recombination has not yet been determined.

To investigate the influence of the *ERCC1* gene product on homologous recombination in mammalian cells, we constructed tandemly duplicated adenine phosphoribosyltransferase (*APRT*) genes in *ERCC1*$^+$ and *ERCC1*$^-$ hamster cell lines (Fig. 1*A*). Because the tandem duplications in both cell lines were constructed at the endogenous *APRT* locus, we can directly compare measurements of recombination made in the presence and absence of the *ERCC1* gene product (22). To detect the full spectrum of events associated with recombination, we designed these experiments as a loss-of-function assay in which the parental *APRT*$^+$ tandem duplication could give rise to *APRT*$^-$ cells by homologous recombination (both crossovers and gene conversions), illegitimate recombination (all forms of gene rearrangements: deletions, duplications, inversions, and translocations), and small mutations (base changes and frameshifts) (22, 23).

MATERIALS AND METHODS

Vectors and Gene Targeting. The targeted disruption of the hamster *ERCC1* gene in RMP41 cells, which carry a single copy of the native *APRT* gene with a yeast FLP recombination target (FRT) recognition site in intron 2 (24), was carried out by using a replacement vector, pSL1, which contains a *NEO* expression cassette interrupting exon 5 of *ERCC1* (25). Recombination substrates (Fig. 1*A*) were constructed at the

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Abbreviations: APRT, adenine phosphoribosyltransferase; NER, nucleotide excision repair; XP, xeroderma pigmentosum; FRT, FLP recombination target.
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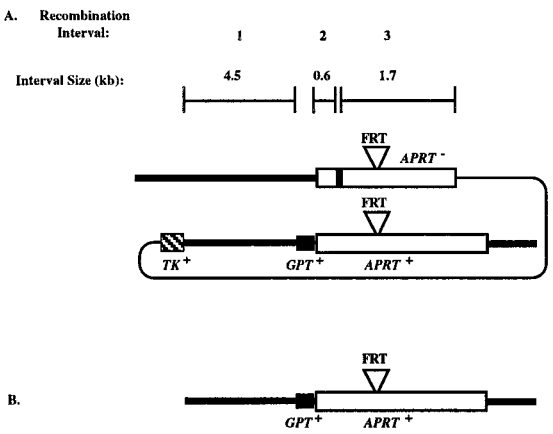

FIG. 1. Gene structures of the *APRT* locus and a possible recombination intermediate recognized by NER. (*A*) Tandemly duplicated *APRT* recombination substrate. In this and other figures, the heavy lines represent chromosomal sequences and the thin line represents plasmid backbone. The *APRT* gene is represented by an open box, the *GPT* gene by a solid box, and the *TK* gene by a crosshatched box. The FRT sequences located in intron 2 are represented by the inverted triangle above both *APRT* gene copies. The exon 2 mutation that destroys the *Eco*RV site is denoted by a heavy vertical line at the 5′ end of the upstream *APRT* copy; at other positions (see Table 2) the heavy line represents undefined *APRT* point mutations. The sizes of the three recombination intervals defined by the *TK* gene, *GPT* gene, and exon-2 mutation are shown above the *APRT* map. (*B*) Single copy *APRT* gene in cell lines used for measuring spontaneous rates of point mutations, deletions, and gene rearrangements.

native *APRT* gene in *ERCC1*$^-$ and *ERCC1*$^+$ RMP41 cells by using the yeast FRT/FLP recombinase site-specific recombination system and the FRT-containing targeting vector pGS73 (24).

Fluctuation Tests and Drug Selections. Fluctuation tests were done by seeding independent parallel cultures of 50–100 cells and expanding to 2–4 $\times$ 10^7 cells before replating for drug selections (22). The cultures were plated in 0.3 μM 1-(2-deoxy-2-fluoro-β-D]-arabinofuranosyl-5-iodouracil (FIAU) for *TK*$^-$ selections, 0.4 mM 8-azaadenine for *APRT*$^-$ selections, and 0.3 μM FIAU + 0.4 mM 8-azaadenine for *TK*$^-$*APRT*$^-$ selections, as described (22). Recombination rates (per cell per generation) in each experiment were calculated by the method of the median (26).

Analysis of Recombination Products. One colony from each parallel culture and each drug selection was picked for further analysis as described (22). Cell DNAs from each colony were used to determine the gene structures of the *APRT* recombinants by PCR and Southern analysis. Locations of PCR primers used in these experiments are indicated in Fig. 2; their sequences are available on request. The significance level for the difference in crossover with gene conversion products between *ERCC1*$^-$ and *ERCC1*$^+$ cells was calculated by a Fisher's exact test (27).

RESULTS

Generation of *ERCC1*$^-$ Cells and Experimental Rational. The *ERCC1*$^-$ cell line used here was generated by targeted insertion of a neomycin (*NEO*) expression cassette into exon 5 of the hamster *ERCC1* gene as described for a closely related Chinese hamster ovary cell line (25). In these Chinese hamster ovary cells, the *ERCC1* gene is present as a single copy (25). The *ERCC1*-targeted insertion initially was produced in RMP41 cells, which carry a single copy of the native *APRT* gene that contains the yeast FRT recognition site in intron 2 (24). Tandemly duplicated *APRT* genes (Fig. 1*A*) then were constructed in *ERCC1*$^-$ and *ERCC1*$^+$ RMP41 cell lines by site-specific recombination with an *APRT* targeting vector by using the yeast FRT/FLP recombination system (24). The targeted *NEO* insertion was judged to eliminate the Ercc1 protein activity by three criteria: (*i*) no *ERCC1* transcript was detected by Northern analysis, (*ii*) no Ercc1 protein was detected by Western analysis, and (*iii*) the cells were mitomycin c sensitive, as expected for the loss of Ercc1 activity (ref. 25, data not shown).

The tandem duplications in the *ERCC1*$^+$ cell line (GS21–15) and in the *ERCC1*$^-$ cell line (GS21–13) share 6.8 kb of homology that is divided into three intervals defined by two sequence differences (the *GPT* gene and the *APRT*-inactivating exon-2 mutation in the *Eco*RV site) between the upstream and downstream copies (Fig. 1*A*). Homologous recombination between the duplicated segments yields crossovers and gene conversions. Crossovers, which eliminate one copy of the repeat and the intervening plasmid sequences, were selected in two ways: crossovers in any of the three intervals were selected as *TK*$^-$ cells by plating in medium containing 1-(2-deoxy-2-fluoro-β-D]-arabinofuranosyl-5-iodouracil (FIAU) (22); crossovers in interval 3 were selected as *TK*$^-$*APRT*$^-$ cells by plating in medium containing FIAU and 8-azaadenine (22). Gene conversions, which involve the unidirectional transfer of the exon-2 *Eco*RV mutation from the upstream *APRT* copy to the downstream copy, were selected as *APRT*$^-$ cells by plating in 8-azaadenine (22). In wild-type cells, gene conversions typically outnumber crossovers by 5–10 to 1, accounting for about 80% of all events that lead to an *APRT*$^-$ phenotype (22, 23).

Recombination in *ERCC1*$^-$ Cells. Rates at which *TK*$^-$, *TK*$^-$*APRT*$^-$, and *APRT*$^-$ cells were generated in the *ERCC1*$^+$ and *ERCC1*$^-$ cell lines were measured by fluctuation analysis (26) and are shown in Table 1. Rates in the *ERCC1*$^-$ cell line were 2- to 4-fold higher than in the *ERCC1*$^+$ cell line. When adjusted to reflect only homologous recombination events (by subtracting out the proportion of events that were caused by point mutations and deletions; see below), the rates in the *ERCC1*$^-$ cell line were minimally increased, about 1.5- to 2.5-fold, over those in the *ERCC1*$^+$ cell line (Table 1). These changes in rate are comparable to results in *Drosophila*, where mutations in the mei-9 gene, the fly homologue of *XPF* (*RAD1*) (28), have been shown to cause a small elevation of mitotic recombination (29). In *S. cerevisiae*, however, mutations in *RAD1* or in *RAD10* have been shown to reduce mitotic recombination 2- to 10-fold (13, 15–17), have no effect on recombination (14), or moderately stimulate recombination 3- to 4-fold (12).

Gene Rearrangements in *ERCC1*$^-$ Cells. To determine if the products of the various selections were similar in the *ERCC1*$^+$ and *ERCC1*$^-$ cell lines, we isolated independent colonies and characterized the structures of their *APRT* loci by PCR and Southern analysis. As shown in Table 2, the proportions of colonies that fell into various classes were very similar in *ERCC1*$^+$ and *ERCC1*$^-$ cell lines with one striking exception: gene rearrangements were prominent among the products from *ERCC1*$^-$ cells, but were absent among the products from *ERCC1*$^+$ cells. Because the *ERCC1*$^+$ cell line yielded no rearrangements in 275 products (less than 0.4%), whereas the *ERCC1*$^-$ cell line gave 38 rearrangements among 141 analyzed products (27%), rearrangements were increased more than 50-fold in the *ERCC1*$^-$ cell line.

To identify the specific types of rearrangements formed in these experiments, we analyzed 15 in detail by using both PCR and Southern analysis. Their molecular structures, shown in Fig. 2, indicate that deletions are the predominant rearrangement; only rearrangement 1 is more complex, because it had other bands in addition to the predicted Southern pattern. Deletions involving the tandem duplication are shown in Fig. 2*A*; deletions associated with crossovers, are shown in Fig. 2*B*. The combination of PCR and Southern analysis allowed us to

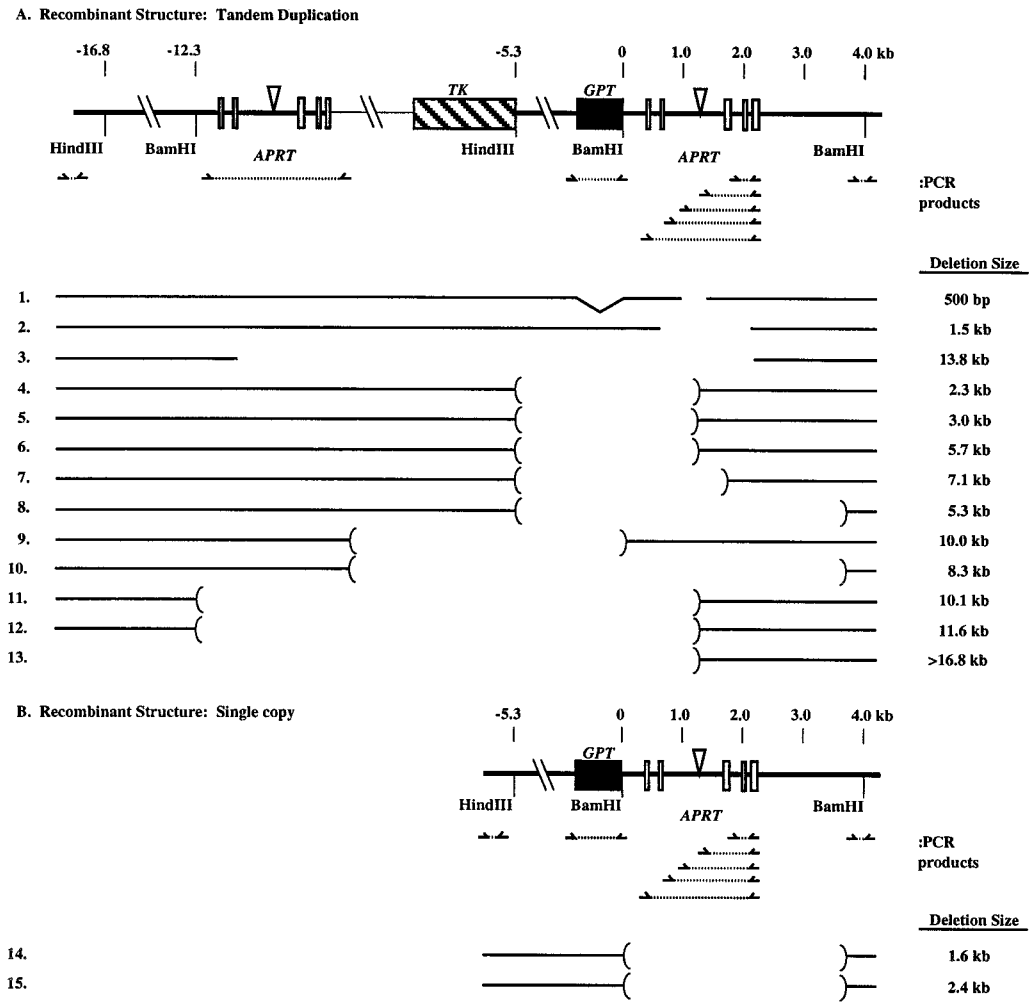

FIG. 2. Molecular structure of *APRT*$^-$ gene deletions and rearrangements recovered from *ERCC1*$^-$ cells. *APRT* exons are indicated as open boxes. Numbering in kilobase pairs (kb) for these maps is relative to the *Bam*HI site 5′ of the downstream *APRT* copy (map position 0). The PCR primers used to map the extent of deletions and rearrangements are shown below the *APRT* maps. The open areas between the brackets for each gene structure represent regions that did not yield PCR products; thus the bracketed regions indicate the interval in which deletion and rearrangement junctions map. The sizes of the deletions as estimated from Southern analysis are indicated at the right. Deletions whose endpoints were determined precisely by sequencing across PCR products are indicated without brackets. A straight line under *GPT* for both tandem duplication and crossover recombinant structures indicates *GPT* is present (by PCR); an indentation under the *GPT* gene indicates it is absent, which may indicate conversion to the wild-type *APRT* sequence. Rearrangement 1 is missing the *GPT* gene, perhaps because of gene conversion, but it also has other rearrangements that are apparent from Southern analysis.

map approximate locations for the deletions (Fig. 2, brackets) and to estimate their size (Fig. 2, *Right*). Rearrangement 1 and deletions 2 and 3 were amplified by PCR primers that spanned the deletion junctions and sequenced. The DNA sequences at the deletion junctions shared 1–4 bp of homology similar to other well characterized *APRT* and mammalian cell deletions and rearrangements (23, 30–33).

Gene Stability in *ERCC1*$^-$ Cells. The prevalent deletions in the *ERCC1*$^-$ cell line might reflect an abnormality in the recombination process associated with the absence of the *ERCC1* gene product, or alternatively they could have arisen independently of recombination as a general effect on gene stability caused by abnormal NER. To distinguish between these alternatives, we isolated from the *ERCC1*$^-$ cell line GS21–13 a derivative, GS96, that contained a single copy of the *APRT*$^+$ gene with an adjacent *GPT* gene (Fig. 1*B*). In the absence of the second copy of the *APRT* gene, the homologous recombination events detected in the above experiments could not occur; however, other events, including deletions and small mutations, would be detectable as *APRT*$^-$ cells. The rate at which *APRT*$^-$ cells were generated in the *ERCC1*$^-$ cell line GS96 was about 7-fold higher than in the corresponding *ERCC1*$^+$ cell line (GS7B2), which contains an identical *APRT* gene structure (Table 1). Consistent with this observation, the rates at which *TK*$^-$ and *APRT*$^-$ mutant cells arose in the *ERCC1*$^-$ cell line GS21–13 were increased 2- to 3-fold (Tables 1 and 2), not significantly different from GS96. (Because the overall rates of appearance of *TK*$^-$ and *APRT*$^-$ cells in GS21–13 were increased 2- to 3-fold and the proportion of *TK*$^-$ and *APRT*$^-$ mutant cells remained the same relative to the GS21–15, the overall rates of *TK*$^-$ and *APRT*$^-$ mutations increased 2- to 3-fold.)

Significantly, the higher rate measured in GS96 was not accompanied by an increase in deletions or rearrangements: among 49 independent colonies from the *ERCC1*$^-$ cell line, 48 were point mutants and only one was a rearrangement. These numbers fit with the general expectation of a 10:1 ratio of point mutations to deletions at the *APRT* locus in wild-type Chinese hamster ovary cells (30, 31). They also agree with other studies that show that deletions in cell lines with a single copy of *APRT* were stimulated less than 2-fold in Chinese hamster ovary cell lines with two different *ERCC1*$^-$ mutant alleles (refs. 30, 34, and 35; data not shown). Collectively, these studies (refs. 30, 34, and 35; data not shown) and ours indicate that *ERCC1*

Table 1. Recombination and mutation rates

Recombination rates ($\times 10^{-7}$)*		
	Cell lines	
Cells	GS21-15, *ERCC1*$^+$	GS21-13, *ERCC1*$^-$
TK$^-$	15 ± 3 (11)	48 ± 23 (29)
TK$^-$ *APRT*$^-$	1.4 ± 0.5 (1.4)	6 ± 3 (3.4)
APRT$^-$	14 ± 3 (11)	31 ± 25 (19)
Mutation rates ($\times 10^{-7}$)†		
	Cell lines	
Cells	GS782, *ERCC1*$^+$	GS96, *ERCC1*$^-$
APRT$^-$	1.8 ± 0	13 ± 8

*These rates represent the average ± SD of three fluctuation tests. Rates adjusted to reflect only homologous recombination events (by subtracting out the proportion of events caused by point mutations and rearrangements) are indicated in parenthesis. Because the recombination rates from the *ERCC1*$^+$ cell line GS21-15, which contains the identical *APRT* gene structure as GS21-13, are the same as previously reported results (22), we have included the previously reported rates in these data.

†These rates represent the average ± SD of two fluctuation tests for GS7B2 and three fluctuation tests for GS96.

deficiency does not have a substantial effect on gene stability as assessed by deletions and other rearrangements.

DISCUSSION

As a component of a structure-specific endonuclease, the Ercc1 protein is expected to play two roles in DNA metabolism: (*i*) cleavage on the 5′ side of damaged DNA in NER (6, 9) and (*ii*) by analogy to the *S. cerevisiae* RAD1/RAD10 homologs, removal of nonhomologous tails from invading single strands during recombination (18, 19). The 50-fold increase in deletions among the products of intrachromosomal recombination in *ERCC1*$^-$-deficient cells suggests that the Ercc1 protein may have an additional role in recombination and gene stability. The possibility that the deletions were generated as a consequence of a genome-wide instability induced by the *ERCC1* deficiency was ruled out by showing that deletions were not prevalent in *ERCC1*$^-$ cells that carry a single copy of the *APRT* gene: an observation consistent with previous studies that used two different *ERCC1*$^-$ mutant alleles (30, 34, 35). Because deletions were increased only in *ERCC1*$^-$ cells that contained tandemly duplicated *APRT* genes, it is likely that excision repair proteins are involved in the recombination process itself.

The absence of an increase in deletions in *ERCC1*$^-$ cells with a single copy of *APRT* also suggests, indirectly, that nonidentical copies of the *APRT* locus may be necessary to trigger the formation of deletions. For mammalian cells it is estimated that about 80% of mitotic recombination events between tandemly duplicated copies occurs by recombination between sister chromatids (36). In the absence of a tandem duplication, recombination between the single *APRT* copies on sister chromatids presumably still occurs; such recombination events normally would not be detected because there would be no genetic consequence if the copies were identical. If the recombination process were rendered abnormal, however, by the absence of the Ercc1 protein, detectable deletions should be generated. The low incidence of deletions in the *ERCC1*$^-$, single-copy *APRT* cell line argues that the Ercc1 protein may interact with the recombination machinery only when the recombining genes are not identical.

Table 2. Recombination products

A.

Events	Gene Structure	*TK*$^-$ GS21-15 [a] *ERCC1*$^+$	*TK*$^-$ GS21-13 *ERCC1*$^-$	*TK*$^-$ *APRT*$^-$ GS21-15 [a] *ERCC1*$^+$	*TK*$^-$ *APRT*$^-$ GS21-13 *ERCC1*$^-$
CROSSOVER		66 (72%)	29 (62%)	72 (100%)	24 (51%)
TK MUTATION		26 (28%)	13 (28%)	0	0
REARRANGEMENT		0	5 (10%)	0	23 (49%)
		92	47	72	47

B.

Events	Gene Structure	*APRT*$^-$ GS21-15 [a] *ERCC1*$^+$	*APRT*$^-$ GS21-13 *ERCC1*$^-$
CROSSOVER		17 (15%)	4 (9%)
CONVERSION		88 (79%)	30 (64%)
APRT MUTATION		6 (5%)	3 (6%)
REARRANGEMENT		0	10 (21%)
		111	47

C.

Events	Gene Structure	*TK*$^-$ *APRT*$^-$ GS21-15 [a] *ERCC1*$^+$	*TK*$^-$ *APRT*$^-$ GS21-13 *ERCC1*$^-$
CROSSOVER		51 (75%)	23 (96%)
CROSSOVER + GENE CONVERSION [b]		17 (25%)	1 (4%)
		68	24

[a]Recombination products from *ERCC1*$^+$ cell lines are the combined results from experiments done here by using the cell line GS21-15 and previously reported results (22).

[b]This is a subclass of the crossover events obtained from the *TK*$^-$ *APRT*$^-$ selections summarized in *A*. Of the 72 *TK*$^-$*APRT*$^-$ recombinants derived from *ERCC1*$^+$ cells with a crossover structure, 68 were tested for the accompanying gene conversion of GPT. All 24 *TK*$^-$ *APRT*$^-$ recombinants derived from *ERCC1*$^-$ cells were analyzed for the accompanying gene conversion of *GPT*.

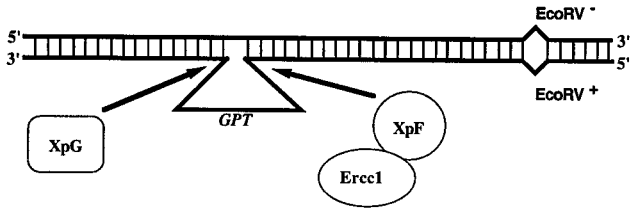

FIG. 3. A recombination heteroduplex intermediate that could be a substrate for the Ercc1/XpF endonuclease. The heteroduplex DNA was created by annealing the top DNA strand from the upstream *APRT* gene to the bottom strand from the downstream *APRT* gene so that the heteroduplex spans the *GPT* gene (see Fig. 1). This heteroduplex creates an ≈800-bp deletion loop similar to *in vitro* substrates processed by purified NER proteins, whose putative sites of action are indicated.

A candidate substrate for NER that might arise during recombination between nonidentical, tandemly duplicated genes is the heteroduplex indicated in Fig. 3. We have argued previously (22) that this heteroduplex is common in recombination at the *APRT* locus because of the prevalence of recombination products that are crossovers associated with a gene conversion; in *ERCC1*$^+$ cell lines this class accounts for 25% of the *TK*$^-$*APRT*$^-$ crossovers (see Table 2). This class is thought to arise by repair at one or both mismatches in the heteroduplex with a crossover downstream of the *Eco*RV mismatch (22). Significantly, this class of products appears to be substantially reduced ($P < 0.05$) in *ERCC1*$^-$ cells; only one of 24 crossovers (4%) had an associated gene conversion (Table 2). The reduction in crossovers associated with a gene conversion is similar to results observed in *RAD1*$^-$ *S. cerevisiae* cells, where this class of products was significantly reduced (13).

How might the intermediate in Fig. 3 lead to deletions? One possibility is that the heteroduplex is misprocessed in cells lacking the Ercc1/XpF endonuclease activity. *In vitro* experiments that used purified proteins have shown that similar substrates can be bound and cleaved by the Ercc1/XpF complex and by the XpG protein (4–11). In *ERCC1*$^-$ cells, XpG-mediated cleavage of the loop in the heteroduplex intermediate shown in Fig. 3 would leave a nicked DNA duplex with an ≈800-bp single-stranded tail. In the absence of further processing by the Ercc1/XpF complex, this abnormal structure could serve as an entry point for proteins that resolve the structure by illegitimate recombination; alternatively, the single-strand tail could infiltrate elsewhere in the genome and form structures that lead to deletions or other gene rearrangements (23, 37, 38). If misprocessing of the heteroduplex intermediate by the NER machinery is the cause of the observed deletions, then deletion breakpoints might be expected to encompass *GPT*, as appears to be the case: 11 of 15 deletions have breakpoints that bracket the *GPT* gene (Fig. 2*A*, deletions 3–13). Elimination of the mismatched heteroduplex, by using tandemly duplicated *APRT* genes without the *GPT* gene, might be expected to decrease the frequency of deletions, as would a second mutation in the *XPG* gene, which might prevent misprocessing of the heteroduplex. We currently are pursuing these experimental approaches and others to clarify the nature of detected interaction between the NER and recombination machinery in mammalian cells.

The high incidence of deletions recovered in our recombination experiments was not observed in similar experiments that used *RAD1*$^-$ or *RAD10*$^-$ *S. cerevisiae* (12–17). In part, this difference is likely to be a consequence of experimental design. Our experiments used a loss-of-function recombination substrate, which allowed detection of homologous recombination, rearrangements, and mutations. By contrast, the gain-of-function substrates used in *S. cerevisiae* were designed to select specifically for homologous recombination events, and thus were blind to rearrangements and mutations (12–17). Even if loss-of-function substrates had been used in *S. cerevisiae*, however, rearrangements still might not have been observed because illegitimate recombination rarely is detected in *S. cerevisiae* except in *RAD52*-defective cells or in cells where no homolog is available for repair by homologous recombination (39–41). Because illegitimate recombination is very active in mammalian cells (32, 33), the consequences of *ERCC1* deficiency may be fundamentally different than in *S. cerevisiae*.

Our results, and those from *in vitro* studies that used purified proteins, suggest that NER is the mammalian cell pathway that processes bulky DNA adducts and DNA structures containing bubbles and loops greater than about 14 bp (11), whereas mismatch repair is responsible for single base mismatches and structures containing bubbles and loops fewer than 14 bp (42–44). Cells defective for mismatch repair often display an increased polymorphism in dinucleotide repeat lengths (45–51). It is believed that slippage of these sequences during DNA synthesis and the resulting out-of-register mispairing, which goes unrepaired in mismatch defective cells, lead to the observed sequence expansions and contractions. Other sequences with the potential to generate DNA structures such as H-DNA, stem-loop structures, and DNA structures associated with some triplet repeat sequences (52–54), also may generate substrates for processing by NER. In that context, it is interesting to note that triplet repeats do not show instability in human cell lines deficient in mismatch repair (55). We currently are testing the influence of NER on the stability of these candidate sequences by introducing them into the *APRT* gene in *ERCC1*$^+$ and *ERCC1*$^-$ cells.

Our results suggest that the *ERCC1* gene product helps to maintain genome integrity by ensuring the correct processing of intermediates in homologous recombination. The loss of Ercc1 activity, or loss of other excision repair activities, may contribute to carcinogenesis by promoting deletions and chromosomal rearrangements, similar to the deletions observed here. NER may be especially important in recombination events that take place between partially diverged members of gene families (56). If the Ercc1 protein played the same role in meiotic recombination, it would help to maintain genome stability across generations. Interestingly, mutations in the *Drosophila mei-9* gene, which encodes an *XPF* homologue, affect both mitotic and meiotic recombination (29, 57–59). *Mei-9* mutants exhibit an increased incidence of chromosomal instability (29, 60, 61), increased frequency of postmeiotic segregation (58), and lowered rate of meiotic recombination (58, 59), all of which could be explained by misprocessing of heteroduplex intermediates in homologous recombination. It will be interesting to see if NER proteins also play a similar role in meiotic recombination in mammals.

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