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Conserved Pattern of Antisense Overlapping Transcription in the Homologous Human *ERCC-1* and Yeast *RAD10* DNA Repair Gene Regions

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We report that the genes for the homologous *Saccharomyces cerevisiae RAD10* and human *ERCC-1* DNA excision repair proteins harbor overlapping antisense transcription units in their 3′ regions. Since naturally occurring antisense transcription is rare in *S. cerevisiae* and humans (this is the first example in human cells), our findings indicate that antisense transcription in the *ERCC-1–RAD10* gene regions represents an evolutionarily conserved feature.

The DNA excision repair pathway is one of the major repair systems in cells. It counteracts the mutagenic and carcinogenic effects of DNA lesions. The isolation and characterization of DNA repair genes is an important step toward the understanding of the mechanism and biochemistry of DNA repair. The recently isolated human DNA excision repair gene *ERCC-1* was found to encode a protein which is significantly homologous to the *Saccharomyces cerevisiae RAD10* repair protein, and similarity to parts of the *Escherichia coli uvrA* and *uvrC* proteins was also noted (8, 31, 33). These findings strongly suggest that DNA excision repair functions have been conserved throughout evolution. We report here that antisense transcription units in the *ERCC-1* and *RAD10* loci may be part of the evolutionarily conserved features of DNA repair.

The architecture of the 3′ region of the *ERCC-1* gene is schematically depicted in Fig. 1A. Alternative polyadenylation of *ERCC-1* transcripts yields mRNAs of 1.1 and 3.4 kilobases (kb) (32). These RNA species were visualized on Northern (RNA) blots of total poly(A)$^+$ HeLa RNA when it was hybridized with a randomly primed (9), ^{32}P-labeled 5′ *ERCC-1* cDNA probe (Fig. 2, lane 1). However, a genomic probe from the middle part of the extended version of exon X (the hatched *Bam*HI-*Pst*I fragment in Fig. 1A), expected to hybridize only with the 3.4-kb alternatively polyadenylated *ERCC-1* transcript, in addition detected a 2.6-kb RNA (Fig. 2, lane 2). This transcript was also detected in two other *ERCC-1*-expressing human cell lines examined (data not shown).

The 2.6-kb transcript was not recognized by 5′ *ERCC-1* probes. Southern blots of human genomic DNA probed with the unique *Bam*HI-*Pst*I fragment yielded hybridization patterns that were fully consistent with the physical map of this cloned region (data not shown), excluding the possibility that cross-hybridization with other sequences accounts for detection of the 2.6-kb RNA. To investigate the possibility that the 2.6-kb RNA was transcribed from the opposite strand, poly(A)$^+$ HeLa RNA was hybridized to ^{32}P-labeled strand-specific RNA probes of the *Bam*HI-*Pst*I fragment. The 0.9-kb *Bam*HI-*Pst*I fragment shown in Fig. 1A was subcloned in both orientations downstream of the T7 promoter in pTZ18/19R (Pharmacia). With the aid of T7 RNA polymerase, ^{32}P-labeled single-strand probes were synthesized under previously described conditions (21) and hybridized to Northern blots containing size-fractionated total poly(A)$^+$ HeLa RNA. The autoradiograms of this experiment (Fig. 2A, lanes 3 and 4) showed that the 3.4-kb *ERCC-1* RNA and the 2.6-kb RNA were recognized by different probes, indicating that these RNAs are transcribed from opposite DNA strands and that the 2.6-kb RNA represents an antisense transcript.

Screening of an Okayama-and-Berg (24) cDNA library with the 1.0-kb *Pvu*II fragment, which includes *ERCC-1* exon X (Fig. 1A), yielded two partial cDNAs (designated pcD2.1 and pcD3B) of the antisense RNA (Fig. 1A). The largest clone (pcD3B) had an insert size of 1 kb. Sequence analysis of both clones and the genomic DNA 3′ of exon X revealed that pcD3B was completely colinear with the genomic DNA. A compilation of the genomic sequences around exon X and the sequence of the 3′ half of pcD3B is presented in Fig. 1B. Both cDNA clones completely overlap with *ERCC-1* exon X and terminate in intron 9 at about 60 base pairs downstream of the splice acceptor of *ERCC-1* exon X. A polyadenylation signal, ATTAAA, is located at about 23 base pairs upstream of the start of the poly(A) tail, indicating that both cDNAs were derived from an authentic poly(A)$^+$ mRNA with a 5′-to-3′ orientation opposite that of *ERCC-1*. It can be deduced from these data that the 2.6-kb transcript has an overlap of 170 base pairs with the *ERCC-1* transcription unit, yielding the major 1.1-kb mRNA, whereas it is completely complementary to the 3.4-kb *ERCC-1* transcript for at least 1 kb. We have provisionally designated this antisense gene *ASE-1* (antisense *ERCC-1*). The *ASE-1* cDNA clone pcD3B harbors a 5′ truncated potential open reading frame (ORF) encoding 183 amino acids (data not shown), which is suggestive of a coding function of the antisense transcript. However, at this stage, we cannot rule out the possibility that the cloned portions of the cDNA represent part of the 3′ untranslated region of the 2.6-kb *ASE-1* RNA. To substantiate a coding function of the antisense transcript, poly(A)$^+$ RNA was prepared from HeLa nuclei and polysomes by previously described proto-

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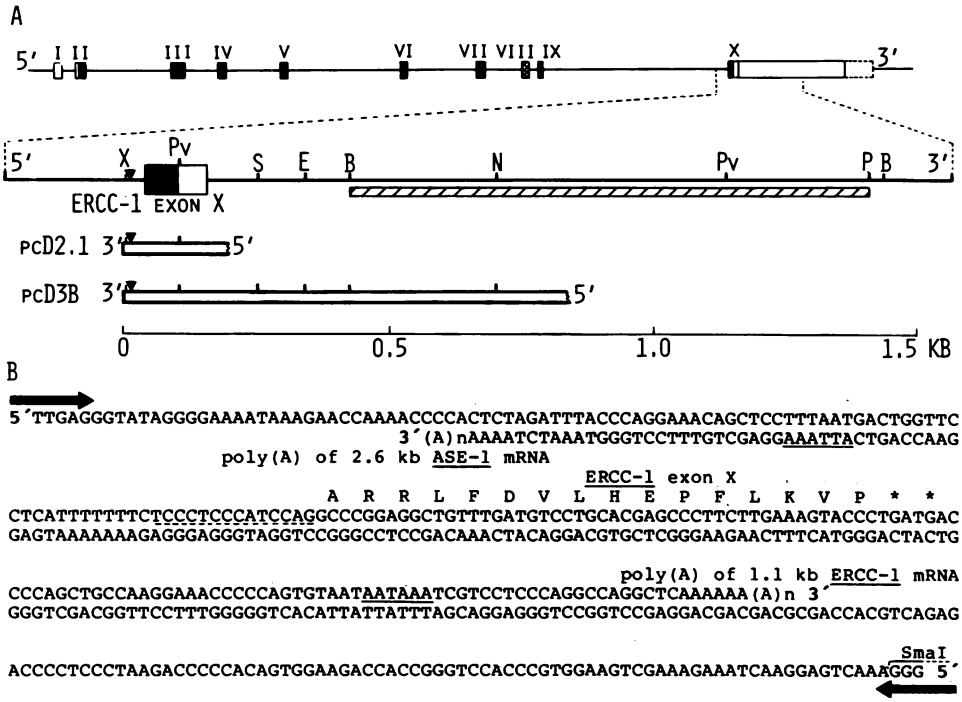

FIG. 1. (A) Schematic representation of the *ERCC-1* gene and its 3' flanking region. Boxes with roman numerals refer to *ERCC-1* exons. Filled boxes represent *ERCC-1* coding sequences. The alternatively spliced exon VIII is hatched. The variable size of exon X is due to alternative polyadenylation yielding longer transcripts of 3.4 and 3.8 kb in addition to the major mRNA of 1.1 kb (32). The pcD2.1 and pcD3B antisense cDNA clones were isolated from a cDNA library of simian virus 40-transformed fibroblasts (24). An inverted black triangle marks the polyadenylation signal (ATTAAA) of the antisense transcript. Restriction sites: B, *Bam*HI; E, *Eco*RI; N, *Nun*II; P, *Pst*I; Pv, *Pvu*II; S, *Sma*I; X, *Xba*I. The 0.9-kb *Bam*HI-*Pst*I fragment used as a probe for the experiment shown in Fig. 2 is shown by the hatched bar. (B) Nucleotide sequence of the *ERCC-1* exon X region and cDNAs of the *ASE-1* gene. The arrows indicate the orientations of *ERCC-1* (upper line) and the antisense transcription unit (lower line). The double-strand region indicates the overlap between the 1.1-kb *ERCC-1* mRNA and the 2.6-kb *ASE-1* mRNA. The overlap with the longer 3.4-kb *ERCC-1* RNA (not shown) continues over the entire *ASE-1* sequence shown. The sequence of *ERCC-1* exon X and part of the flanking DNA has been reported earlier (31, 32). The amino acids encoded by *ERCC-1* exon X are given in one-letter code. The *ERCC-1* exon X splice acceptor is shown by a broken line.

cols (6) and hybridized with *ASE-1* probes (Fig. 2B). By using the *Xba*I-*Pst*I fragment covering the region of *ERCC-1* exon X as a probe (Fig. 1A), the 3.4-kb *ERCC-1* and 2.6-kb antisense transcripts could be detected in both RNA samples, and the 1.1-kb *ERCC-1* transcript showed weak hybridization due to the presence of exon X sequence information in the probe. The relative amounts of the 2.6- and 3.4-kb transcripts differed in nuclei and polysomes. The alternatively polyadenylated 3.4-kb *ERCC-1* RNA appeared to be mainly of nuclear origin, whereas the antisense transcript was found predominantly in the polysome fraction, suggesting that *ASE-1* mRNA is translated into a protein.

The human *ASE-1* DNA specifically cross-hybridized to cloned DNA of the region downstream of mouse *ERCC-1* exon X, and strand-specific RNA probes identified an antisense RNA of 2.3 kb in this region (data not shown). Hence, it appears that the genomic organization of *ERCC-1* and *ASE-1* is conserved between humans and mice.

The *ERCC-1* homolog in *S. cerevisiae*, the *RAD10* gene, and its transcripts are shown schematically in Fig. 3A. The indicated restriction fragments were cloned in both orientations into M13 to investigate transcripts encoded by the *RAD10* gene and to determine the gene organization 3' of *RAD10*. Isolation of poly(A)$^+$ RNA from Rad$^+$ strain DBY747 (*MAT***a** *his3-1 leu2-3 leu2-112 trp-289 ura3-52*), RNA blotting, and hybridization were done as previously described (18). Poly(A)$^+$ RNA was dissolved in 1 M glyoxal, fractionated on a 0.8% agarose gel in 10 mM $NaPO_4$ (pH 6.5), transferred to GeneScreen, and hybridized to ^{32}P-labeled M13-derived single-strand probes which were synthesized by previously published procedures (14, 28). Northern blot analysis of yeast RNA with ^{32}P-labeled single-strand M13-derived DNA probes of the various fragments is shown in Fig. 4. A probe corresponding to the transcribed *RAD10* DNA strand of the *Pvu*II-*Xba*I fragment hybridized to three transcripts of 1.0, 1.5, and 1.8 kb. Strand-specific probes of the adjacent *Xba*I-*Eco*RV and *Eco*RV-*Eco*RV fragments hybridized to the larger two bands, whereas the more downstream *Eco*RV-*Bgl*II fragment recognized only the 1.8-kb *RAD10* transcript (Fig. 4A). The *RAD10* origin of these transcripts was confirmed by analysis of a *RAD10* disruption strain (data not shown). Since the 5' ends of the *RAD10* transcripts map at positions −17 and −32 (26), we conclude that the size heterogeneity of the *RAD10* transcripts arose mainly from differences at the 3' end and that, as with *ERCC-1*, *RAD10* displays alternative polyadenylation.

Surprisingly, hybridization of yeast poly(A)$^+$ RNA with radiolabeled probes corresponding to the noncoding *RAD10* DNA strand revealed a 1.9-kb transcript. As shown in Fig. 4B, this transcript was recognized by five different strand-specific probes of the entire region between the *Xba*I and *Nru*I sites indicated in Fig. 3A. Hence, the opposite DNA strand in the 3' *RAD10* region encodes a transcript that overlaps the 1.8-kb *RAD10* transcript by at least 600 nucle-

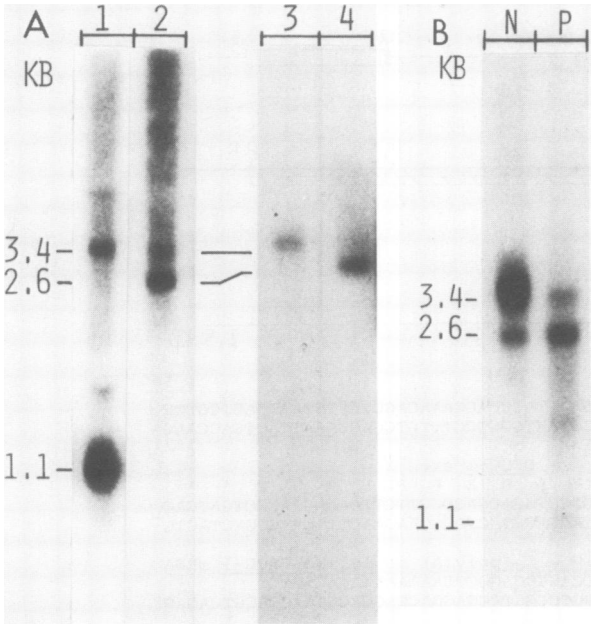

FIG. 2. Northern blot analysis of poly(A)$^+$ HeLa RNA. (A) Either 20 μg (lanes 1 and 2) or 10 μg (lanes 3 and 4) of poly(A)$^+$ RNA isolated by the lithium chloride-urea method (2) and passaged twice over oligo(dT)-cellulose by routine procedures (19) was size fractionated and hybridized to the following probes: lane 1, *ERCC-1* cDNA probe harboring exons I to V; lane 2, double-strand *Bam*HI-*Pst*I fragment shown in Fig. 1A; lanes 3 and 4, ^{32}P-labeled single-strand RNA probes of each strand of this *Bam*HI-*Pst*I fragment. After hybridization, the filters hybridized with the RNA probes were washed at 68°C in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and subsequently treated with RNase A (1 μg/ml in 2× SSC for 15 min) to reduce nonspecific background labeling. (B) Poly(A)$^+$ RNA (6 μg) of HeLa nuclei (N) and polysomes (P) hybridized with the ^{32}P-labeled *Xba*I-*Bam*HI fragment of the genomic *ERCC-1* 3′ region (Fig. 1A).

otides (Fig. 3A). We have provisionally designated the yeast antisense gene as *ASR10* (antisense *RAD10*).

To examine whether the *ASR10* gene encodes a protein, the nucleotide sequence of the 3′ *RAD10* DNA region was determined. The 1.9-kb antisense RNA contains an ORF encoding 525 amino acids with a calculated M_r of 59,505 (data not shown). The last two codons of the *ASR10* ORF overlap with those of the *RAD10* ORF (Fig. 3B).

Screening of the EMBL and NBRF data banks showed no significant similarity between *ASR10* and other genes or proteins. The predicted amino acid sequence of the yeast *ASR10* protein showed no homology with the partial sequence of the 183-amino-acid ORF present in *ASE-1* cDNA clone pcD3B. It will be of interest to determine whether the missing portion of the *ASE-1* cDNA encodes a protein which shows similarity to the *ASR10* amino acid sequence. On the basis of these findings, we conclude that the phenomenon of overlapping antisense gene organization is shared between the homologous mammalian *ERCC-1* and yeast *RAD10* genes.

Recently, several examples of antisense transcription in higher eucaryotes have been reported (1, 5, 13, 22, 30, 34). However, in all cases, the biological significance or conservation of this phenomenon is unknown. The *ERCC-1/ASE-1* locus represents the first example of naturally occurring antisense transcription in the human genome. Also, in *S. cerevisiae*, antisense transcription is unusual, although not without precedent. Recently, convergent overlapping transcription for two yeast *CDC* genes was described and divergent overlapping RNAs for the *HAP3* locus were reported (3, 12). Although the occurrence of overlapping gene transcription in the related mammalian *ERCC-1* and yeast *RAD10* regions can be purely coincidental, we consider this unlikely in view of the rarity of this phenomenon.

One can only speculate about the function of the antisense transcripts. The presumed coding capacity of both yeast and human antisense RNAs makes it unlikely that the antisense transcripts are solely regulatory RNAs with no other function than, e.g., regulating translation, as in procaryotes (11) and as applied in reverse-genetic experiments in eucaryotes (4, 7, 15–17, 20, 23, 27). Several advantages might accrue from partially complementary transcripts derived from over-

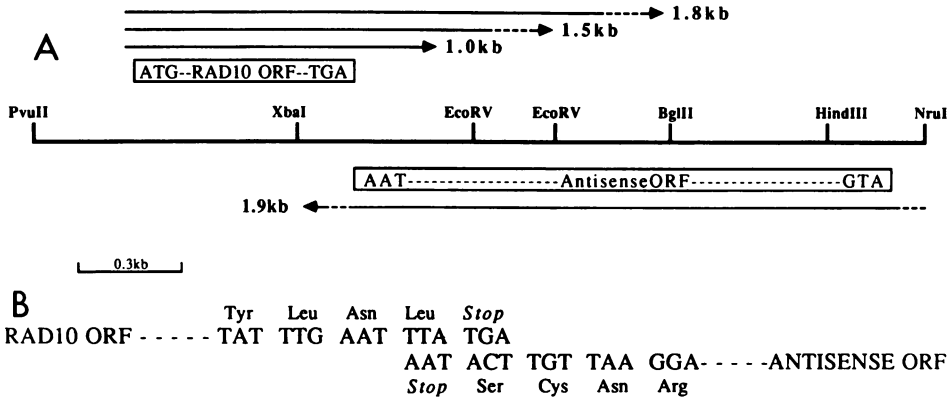

FIG. 3. (A) Transcript map of the *RAD10* gene and antisense gene region. The horizontal line in the middle is a restriction map of *RAD10* DNA (25). Only the relevant restriction enzymes sites with six-base recognition are shown. The positions of the *RAD10* and *ASR10* (antisense) ORFs are indicated by the open bars. The arrows above the *RAD10* ORF and below the antisense ORF represent the directions and approximate sizes of *RAD10* transcripts and the antisense transcript, respectively. The broken lines at the 5′ and 3′ ends of the transcripts indicate that the positions of these ends were not accurately determined by S1 nuclease mapping. The 5′ ends of *RAD10* transcripts were mapped to positions −17 and −32 by S1 mapping (26). The 3′ ends of the *RAD10* 1.0-kb transcript map at positions +869 and +938, as determined by S1 mapping (unpublished data). (B) Nucleotide and amino acid sequences of the overlapping coding regions of *ASR10* and *RAD10*.

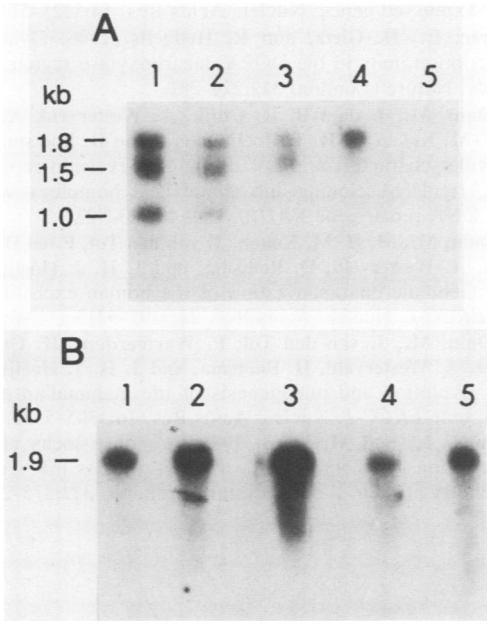

FIG. 4. Northern blot analysis of yeast poly(A)$^+$ RNA with strand-specific DNA probes of the *RAD10* region. (A) *RAD10* transcripts hybridizing to strand-specific probes from the *RAD10* gene. Poly(A)$^+$ RNA (10 μg) was loaded in each lane. The following probes were used after cloning in M13mp8, M13mp9, M13mp18, or M13mp19 (Fig. 3; given in parentheses): lane 1, *Pvu*II-*Xba*I (mp19); lane 2, *Xba*I-*Eco*RV (mp18); lane 3, *Eco*RV-*Eco*RV (mp19); lane 4, *Eco*RV-*Bgl*II (mp18); lane 5, *Bgl*II-*Hin*dIII (mp9). (B) *ASR10* transcript recognized by strand-specific probes from the *RAD10* antisense gene. Poly(A)$^+$ RNA (5.6 μg) was loaded in each lane. Probes: lane 1, *Xba*I-*Eco*RV (mp19); lane 2, *Eco*RV-*Eco*RV (mp19 in an orientation opposite to that of the clone used for Fig. 2A); lane 3, *Eco*RV-*Bgl*II (mp19); lane 4, *Bgl*II-*Hin*dIII (mp8); lane 5, *Hin*dIII-*Nru*I (mp9).

lapping genes. A tail-tail duplex configuration of the two mRNAs involved could mediate their transport to a common location in the cytoplasm. Furthermore, the stability of such a tandem transcript could be affected by the duplex state and serve to protect against degradation. At the translational level, several options are open. Translation could be inhibited or slowed down by the hybridized 3′ tail. Furthermore, unwinding of the 3′ termini could offer the possibility of induction of gene expression at the translational level. In these respects, it is worth noting that transcription levels, translation initiation, codon usage, and protein level (10, 29, 33) suggest that *RAD10* and *ERCC-1* are low-abundance proteins. Another intriguing possibility at the protein level, particularly for low-expression genes, is that tail-tail association of sense and antisense transcripts could provide an opportunity for complex formation between the two nascent proteins that are in close proximity to one another when the ribosomes on the opposite transcripts encounter one another. The possible regulatory effect of the conserved overlapping antisense transcription on the expression of *ERCC-1–RAD10* is being examined.

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