Human *RSU1* Is Highly Homologous to Mouse *Rsu-1* and Localizes to Human Chromosome 10

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The mouse *Rsu-1* cDNA, formerly referred to as *Rsp-1*, was isolated in our laboratory using an expression cloning assay designed to identify sequences capable of suppressing transformation by v-Ki-*ras* (2). When introduced into v-Ki-*ras*-transformed mouse fibroblasts, *Rsu-1/Rsp-1* suppressed anchorage independent growth. NIH3T3 cell lines constitutively expressing *Rsu-1/Rsp-1* under the control of a heterologous promoter are highly resistant to transformation by v-Ki-*ras* and v-Ha-*ras*, but not v-*src*, v-*mos*, or v-*raf* (2). We also reported that the *Rsu-1/Rsp-1* open reading frame encodes a 277-amino-acid protein that shares homology with *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* adenylyl cyclase in the region through which Ras activates adenylyl cyclase in *S. cerevisiae* (1, 3, 4). This region of homology consists of a series of 23 amino acid repeats defined by the positions of proline, leucine, and asparagine. The homology between these molecules suggests that Rsu-1 may physically associate with Ras p21 or other Ras regulatory molecules and may exert its effect in the transformation suppression assay by interfering with v-Ras signal transduction. Results obtained with murine *Rsu-1/Rsp-1* cDNA clones indicated that it is phylogenetically well conserved and is ubiqitously expressed (2). This suggests that Rsu-1/Rsp-1 may interact with other highly conserved proteins and that it may function in a Ras signal transduction pathway in higher eukaryotes. In this report, we confirm and expand our analysis of *Rsu-1* phylogenetic conservation by hybridization. We have demonstrated by isolation and DNA sequence analysis of human *RSU1* cDNA that human Rsu-1 exhibits more than 95% conservation with the murine Rsu-1 at the amino acid level. In addition, we localize *RSU1* to human chromosome 10.

The human *RSU1* cDNA was isolated from a λgt10 human primary skin fibroblast cDNA library. Using a probe encompassing the 5′ 200 bp of the mouse *Rsu-1* open reading frame, 50,000 plaques were screened by hybridization at high stringency and 4 strongly hybridizing plaques were isolated. The phage containing the largest insert, a 2.2-kb insert, was chosen for DNA sequence determination. The sequence of this cDNA has been submitted to GenBank (Accession No. L12535). The translation of the longest open reading frame encoded by the 2.2-kb human *RSU1* cDNA indicates that it encodes a protein identical in size and with a high degree of homology to mouse

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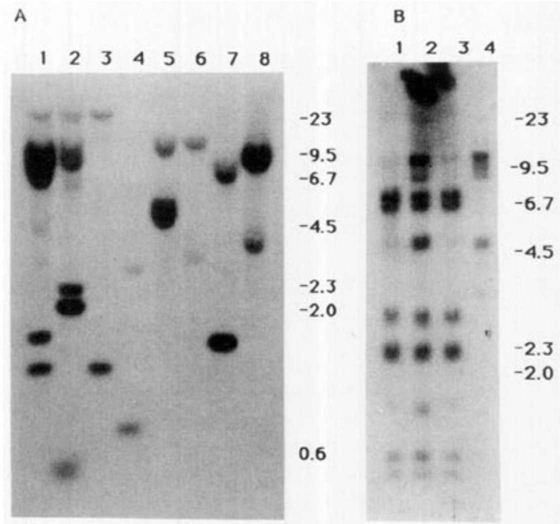

FIG. 1. (A) Hybridization to genomic DNA from several eukaryotic species. A probe corresponding to the first 304 bp of the human *RSU1* open reading frame was prepared by PCR and labeled by random priming. Hybridization of this probe to a blot containing 10 μg of *Eco*RI-digested genomic DNA from eight different species was performed at 42°C in 50% formamide. The blot was washed at 55°C in 0.1 × SSC and 1% SDS for 1 h and exposed to Kodak X-Omat AR film. Lane **1**, human; lane **2**, monkey; lane **3**, rat; lane **4**, mouse; lane **5**, dog; lane **6**, cow; lane **7**, rabbit; lane **8**, chicken. (**B**) Hybridization to genomic DNA from monochromosomal human–hamster somatic cell hybrids. A probe corresponding to nucleotides 1 through 1918 of the human *RSU1* cDNA was hybridized as described above to a blot containing 10 μg of *Eco*RI-digested genomic DNA. The DNA from monochromosomal hybrids was obtained from the Coriell Cell Repositories (Camden, NJ). Lane **1** hybrid NA10826B (containing human chromosome 2); lane **2**, hybrid NA10926B (containing human chromosome 10); lane **3**, hybrid NA11418 (containing human chromosome 15); lane **4**, primary human skin fibroblast.

Rsu-1. The mouse and human Rsu-1 proteins are >95% identical and the majority of the amino acid changes that occur are conservative substitutions.

In mouse and human RNA from fibroblasts, as well as all other tissues and cell lines analyzed, the major *Rsu-1* transcript is 1.7 kb(2; M. L. Cutler, unpublished observation). There are two additional transcripts of 4 and 2.5 kb. The 2.5-kb transcript is abundant in epithelial cell lines and some tissues, but constitutes a minor transcript in both primary and established fibroblast cell lines (M. L. Cutler, unpublished observation). While the human *RSU1* cDNA ORF probe hybridized to 4- 2.5- and 1.7-kb transcripts on Northern blots of RNA from human cell lines, the 5′ untranslated region of the 2.2-kb human *RSU1* clone hybridizes to only the 2.5-kb RNA (data not shown), suggesting that this clone is a cDNA derived from the 2.5-kb transcript. The 3′ untranslated region of the 2.2-kb human cDNA clone is homlogous to the 3′ untranslated region of murine 1.7-kb transcript cDNAs. However, the 1.7- and the 2.2-kb transcripts terminate "downstream" of different polyadenylation signals. Because a 5′ 200-bp probe from the mouse *Rsu-1* ORF hybridizes to a single *Eco*RI fragment in DNA from most species (2), the RNA data mentioned above indicate that *Rsu-1* is a single-copy gene from which there are at least two primary transcripts or distinct splicing patterns.

Using the 5′ 304 bp of the human *RSU1* ORF as a probe, a Southern blot containing 10 μg of *Eco*RI-digested genomic DNA from a number of species was hybridized at high stringency (Fig. 1A). The human probe hybridized well to all the higher eukaryotic species represented on the blot, including the avian species. We had previously reported similar information, but this publication expands our original observation to bovine, canine, and avian species. In addition, there is a 700-bp *Eco*RI fragment in the murine sample homologous to the *RSU1* 5′ end probe that was not previously detected, suggesting that the earlier blot contained an incompletely digested sample (2).

A human *RSU1* cDNA probe representing 1–1918 bp of the ORF was hybridized to a set of hamster–human somatic cell hybrids that represented most human chromosomes. There were no hybrids in that panel that contained human *RSU1*-specific sequences (data not shown). However, it was determined that the human chromosomes that were underrepresented or not represented at all in this set of hybrids were chromosomes 2, 10, and 15. Therefore, DNA from monochromosomal human–Chinese hamster somatic cell hybrids containing the human chromosomes in question were digested with *Eco*RI and compared to *Eco*RI-digested primary human fibroblast cell line DNA for the presence of human *RSU1*-specific hybridizing fragments. Using a probe representing 1–1918 of the human *RSU1* ORF, hybridization was performed at high stringency as described above. The data shown in Fig. 1B indicate that the monochromosomal hybrids for human chromosomes 2 (NA 10826B) and 15 (NA 11418) did not contain human *RSU1*-specific fragments. However, the monochromosomal hybrid for chromosome 10, NA 10926B, contained human *RSU1*-specific *Eco*RI fragments (approximately 9.5, 7.5, 4.5, and 1.7 kb) in addition to Chinese hamster *Eco*RI fragments. One of the human *RSU1* fragments (approximately 8.5 kb) detected in human fibroblast DNA was not detected in hybrid NA 10926B. It is not known if the human parent of the hybrid NA 10926B contained the 8.5-kb *RSU1* *Eco*RI fragment. The detection of human *RSU1* fragments in the DNA from the NA 10926B hybrid cell line assigns *RSU1* to human chromosome 10.

Because *Rsu-1* can suppress transformation by activated Ras, it may be involved in regulation of Ras signal transduction. Therefore, loss or alteration of *RSU1* in specific tumors would be significant. The assignment of *RSU1* to human chromosome 10 raises some interesting possibilities in light of the neoplastic disease loci that map to regions of that chromosome. We are currently in the process of localizing *RSU1* to a subchromosomal region to aid in characterizing its potential involvement in neoplastic diseases resulting from chromosome 10 loss or alteration.

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