SHORT COMMUNICATION

Mapping of Ribosomal Protein S3 and Internally Nested snoRNA U15A Gene to Human Chromosome 11q13.3–q13.5

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The mammalian ribosome is a massive structure composed of 4 RNA species and about 80 different proteins. One of these ribosomal proteins, S3, appears to function not only in translation but also as an endonuclease in repair of UV-induced DNA damage. Moreover, the first intron of human RPS3 transcripts is processed to generate U15A, a small nucleolar RNA. We localized the nested RPS3/U15A genes to the immediate vicinity of D11S356 and D11S533 on human chromosome 11q13.3–q13.5 using a combination of somatic cell hybrid analysis, fluorescence *in situ* hybridization, and YAC/STS content mapping. These findings add to the evidence that genes encoding ribosomal proteins are scattered about the human genome.

In mammals, each ribosomal protein is typically encoded by a single gene, from which a number of silent, processed pseudogenes have been generated (4). These pseudogenes complicate the mapping of ribosomal protein genes to chromosomes, and this explains, at least in part, why only 16 of the 80 or more ribosomal protein genes have been chromosomally assigned (Refs. 1, 5–7, 9, 10, 13, 16, 21). The 16 genes that have been assigned map to 12 different chromosomes, suggesting that ribosomal protein genes, unlike ribosomal RNA genes, are dispersed throughout the genome.

The RPS3 gene, not previously mapped, is of particular interest. First, the protein that it encodes has two apparently distinct functions: (i) as a ribosomal protein, RPS3 contributes to the domain where translation is initiated (2), and (ii) as an endonuclease, RPS3 apparently participates in repair of UV damage (22; S. Linn, unpublished observations). Second, U15A, a small nucleolar RNA ("snoRNA"), is processed from the first intron of the RPS3 transcript (20). The function of snoRNA U15A is not well understood, but it may act in ribosomal RNA processing (19). Third, the nested RPS3 and U15A genes are overexpressed in colorectal carcinomas (14). We have recently sequenced part of the RPS3/U15A transcription unit (20). This facilitated chromosomal mapping by allowing us to design an intron-specific PCR assay that would not recognize pseudogenes derived from processed RPS3 transcripts.

To assign RPS3/U15A to a human chromosome, we used the polymerase chain reaction (PCR) to amplify human–rodent somatic cell hybrid DNAs with primers corresponding to the second intron of RPS3. This PCR assay is specific to human genomic DNA; the expected 263-bp product is observed with human but not mouse or hamster DNA as template (Fig. 1A). Results of screening a panel of multichromosome hybrids indicated that the nested RPS3/U15A genes reside on human chromosome 11 (Figs. 1A and 1B). We screened a second panel of monochromosomal hybrid DNAs to verify this result. As expected, the only hybrid positive for RPS3/U15A was that which retained human chromosome 11 (Fig. 1C).

To confirm and refine this localization, we assayed cell hybrids retaining portions of human chromosome 11 and hybridized an RPS3/U15A-containing YAC clone to human metaphase chromosomes *in situ*. PCR analysis of subchromosomal hybrids (8, 17) (Fig. 2) allowed us to localize RPS3/U15A to 11q13–q23 (Fig. 2). PCR screening of a chromosome 11-specific YAC library (15) enabled us to identify a single RPS3/U15A-containing clone, YAC yRP9A2. Fluorescence *in situ* hybridization analysis localized yRP9A2 to 11q13 with negligible background elsewhere in the genome (Fig. 3A). Testing of yRP9A2 for the presence of other 11q13 loci revealed that it contains D11S533 and D11S356 (Fig. 3B), previously mapped to 11q13.3–q13.4 and 11q13.4–q13.5, respectively (18). These results are entirely consistent with the somatic cell hybrid and *in situ* hybridization studies (Fig. 3C). We conclude that

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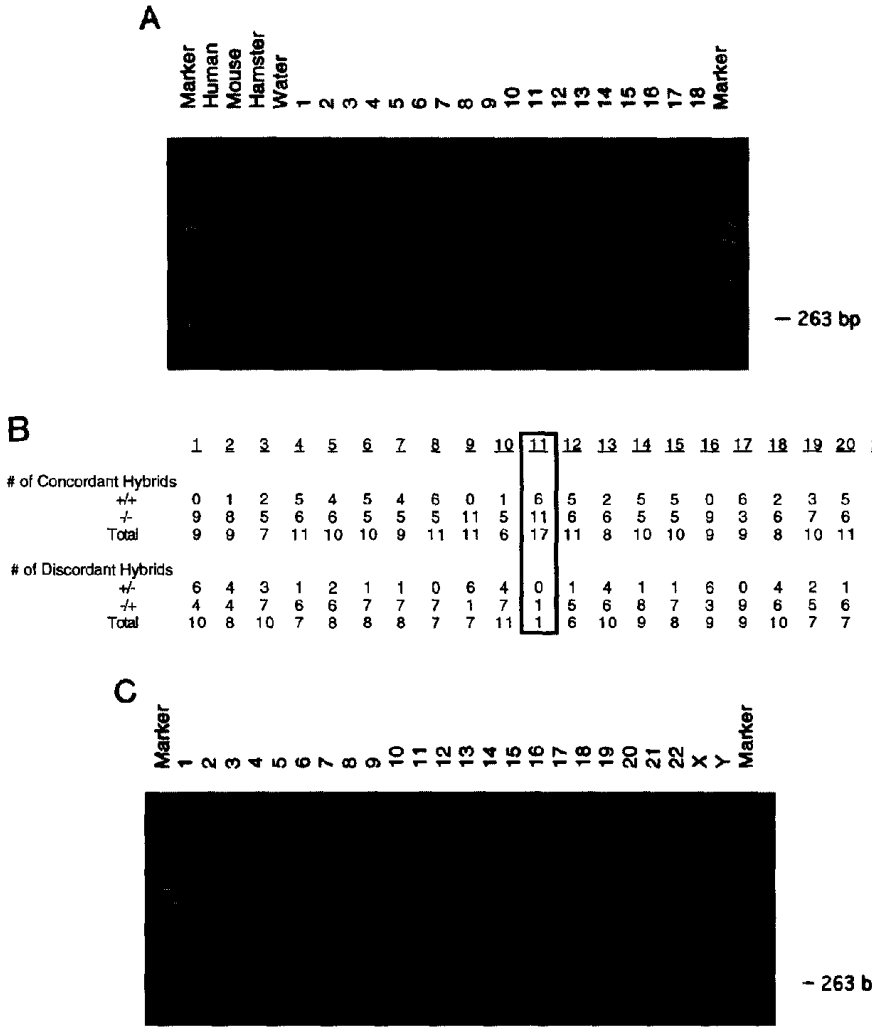

FIG. 1. Mapping of RPS3/U15A to human chromosome 11 by PCR analysis of human–rodent somatic cell hybrid DNAs. (**A**) Eighteen human–rodent hybrids containing multiple human chromosomes (NIGMS panel 1). "Marker" lane contains ϕX174RF DNA digested with *Hae*III. Thirty PCR cycles (1 min at 94°C, 1 min at 61°C, 1 min at 72°C) were carried out on 50 ng of genomic DNAs with primers (5′-CATGGTCCCACCTATTCC-3′ and 5′-GGGGGAAAAGTGACAATTCA-3′) specific to the second intron of RPS3 (20). Reaction products were analyzed by agarose gel electrophoresis in the presence of ethidium bromide. (**B**) Tabulation, by human chromosome, of number of concordant and discordant hybrids from **A**. Symbols before slash denote presence (+) or absence (−) of 263-bp PCR product, and symbols after slash denote presence (+) or absence (−) of chromosome. (**C**) Twenty-four hybrids containing single human chromosomes (NIGMS panel 2).

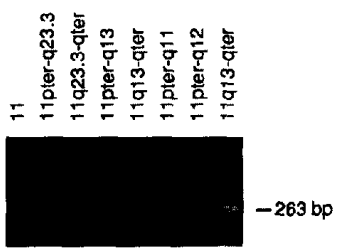

FIG. 2. Mapping of RPS3/U15A to 11q13–q23 by PCR analysis of human–rodent somatic cell hybrid DNAs containing portions of human chromosome 11. PCR was carried out as in Fig. 1 with genomic DNA from the following hybrids (8, 17) as template: J1 (retaining an intact human chromosome 11), PBR-3 (retaining a human 11;12 translocation product carrying 11pter–q23.3), PBR-6 (retaining 11q23.3–qter; the reciprocal of PBR-3), TKR-2 (retaining an 11;2 translocation product carrying 11pter–q13), TKR-33 (retaining 11q13–qter; the reciprocal of TKR-2), 15R1A (retaining 11pter–q11), J1-11 (retaining 11pter–q12), and EXR-5CSAZ (retaining an X;11 translocation product carrying 11q13–qter).

the RPS3/U15A transcription unit is located in 11q13.3–q13.5, near D11S533 and D11S356.

Three other ribosomal protein genes have been mapped to human chromosome 11, but no two genes appear to be in close proximity: RPS17 maps to 11p13–pter (6). RPS25 maps to 11q23.3 (9). RPS30, while mapping to 11q13 (10), the same band as RPS3, is not present on YAC yRP9A2 (not shown). Thus, the rule that ribosomal protein genes are scattered about the human genome (3) continues to hold.

Band 11q13 is a frequent site of structural abnormality, amplification, or loss of heterozygosity in certain human cancers, including multiple endocrine neoplasia type 1, breast and squamous cell carcinomas, and B-cell neoplasms (11). We do not know whether RPS3 or U15A plays any role in the development of such neoplasms, but we note that the genes are overexpressed in colorectal carcinomas (14) and that the 3;21 translocations observed

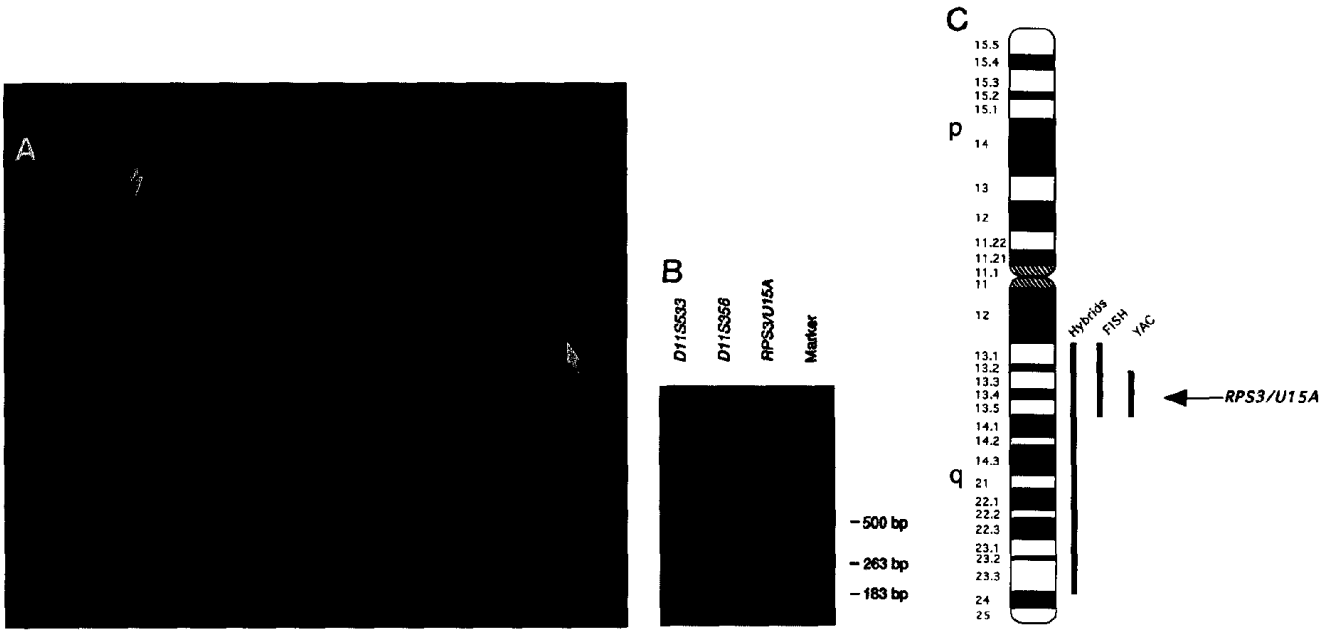

FIG. 3. (**A**) Mapping of RPS3/U15A to 11q13 by fluorescence *in situ* hybridization (FISH) of YAC yRP9A2 to human metaphase chromosomes. FISH was performed basically as described (8, 15), using the digoxygenin-labeled "IRE-bubble" PCR product (12) from YAC yRP9A2. Arrows indicate fluorescent hybridization signals on DAPI-banded chromosomes. (**B**) Presence of three STSs (D11S533, D11S356, and RPS3/U15A) in YAC yRP9A2 demonstrated by PCR. Primers for D11S533 and D11S356, as described by Smith *et al.* (18). (**C**) Idiogram of chromosome 11 summarizing localization of RPS3/U15A by subchromosomal hybrid analysis, FISH, and YAC/STS content mapping.

in myelodysplasia disrupt another ribosomal protein gene, RPL22 (13). Given RPS3's role in DNA repair (22; S. Linn, unpublished observations), it would be of great interest to know whether any heritable disorders of DNA repair map to 11q13.

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