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The human ribosomal protein L6 gene in a critical region for Noonan syndrome

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Abstract We have determined the genomic structure of the human ribosomal protein L6 gene (*RPL6*) and assigned it to the interval containing the Noonan syndrome locus. *RPL6* spans 4415 bp and consists of seven exons and six introns. The first exon is only 19 bp in length, containing a 5′ non-coding region and a polypyrimidine tract. The second exon starts with the initiator ATG. Although the overall structure of the protein is highly conserved among mammalian species, there is significant variation in the N-terminal portion. We have refined the position of *RPL6*, using two different radiation hybrid panels. *RPL6* was mapped to chromosome 12q24.1 between the markers D12S84 and D12S861, which is in the critical region for Noonan syndrome.

Key words Ribosomal protein · *RPL6* · Noonan syndrome · RH mapping · Genomic sequence · 12q24.1

Introduction

The ribosome is a cellular organelle responsible for protein synthesis in all cells. Its biogenesis in mammalian cells requires the equimolar accumulation of four RNA species and about 80 different proteins (Wool 1979). Although the ribosome is essential for cell growth and development, the effects of ribosomal mutations and their role in human disease have scarcely been explored. It is possible that genetic defects in ribosomal components would invariably result in early embryonic death. However, there is strong evidence in *Drosophila* that a quantitative deficiency of any one of the ribosomal proteins could yield viable but abnormal phenotypes (Lambertsson 1998). Moreover, a recent study has shown that the ribosomal protein S19 gene (*RPS19*) is mutated in patients with Diamond Blackfan anemia (Draptchinskaia et al. 1999; Willig et al. 1999).

To explore the possibility that ribosomal protein deficiencies or mutations cause certain human disorders, we have been systematically mapping the human ribosomal protein genes (Kenmochi et al. 1998a, 1998b). In this study, we determined the nucleotide sequence of the human ribosomal protein L6 gene (*RPL6*) and mapped the gene to the same interval as the Noonan syndrome locus.

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Materials and methods

Genomic sequencing

Genomic DNA was extracted from peripheral blood leukocytes of a Japanese male. The nucleotide sequence of the *RPL6* gene was determined by the polymerase chain reaction (PCR) method, using primers designed based on the cDNA sequence (accession no. D17554), which was originally identified as a cDNA encoding DNA binding protein TAXREB107 (Morita et al. 1993). After amplification of genomic fragments of *RPL6* through a series of steps using these exon primers, intron-containing PCR products were obtained and directly sequenced. Inverse PCR was employed to determine the sequence of the 5′-flanking region.

Radiation hybrid (RH) mapping

Two different RH mapping panels, Genebridge 4 and G3 panels (Research Genetics, Huntsville, AL, USA), were used for localizing *RPL6* within the human chromosome. The specific primers were 5′-GCGGGTGAAAAAGTTG AGAA-3′ from the second exon and 5′-GAAAATCCAA TTTACAGTCCCC-3′ from the third intron of the gene, which give a PCR product 445 bp in length. The data vectors were submitted to the RH servers at the Whitehead Institute/MIT Center for Genome Research (Genebridge 4;

http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl) or the Stanford Human Genome Center (G3; http://shgc-www.stanford.edu).

Results

Gene organization and sequence of *RPL6*

The human *RPL6* gene is 4415 bp long and consists of seven exons and six introns (Fig. 1). Based on the published 5′-untranslated region (UTR) sequence of a full length cDNA for *RPL6* (accession no. D28388), we identified the transcription start site, at a C residue within a polypyrimidine tract. This polypyrimidine tract is a unique feature of vertebrate ribosomal protein (rp) genes, and transcription always starts at a C residue within the tract (Meyuhas et al. 1996). Although no typical TATA box was found in the promoter region, there is a TATA-like sequence (TATAGA), 27 bp upstream of the start site. Human *Alu* sequences were found in the 5′-flanking region and the third intron. All intron-exon junctions conform to the consensus sequences established for intron donor and acceptor splice signals (Table 1). Analysis of the 5′-flanking region of *RPL6* revealed the presence of possible c-Ets-1 oncoprotein binding sites (5′-CCGGAAG) at positions −64 bp and −21 bp from the transcription start site. This is one of the elements frequently found in the 5′-flanking region of vertebrate rp genes (Maeda et al. 1993; Toku and Tanaka 1996).

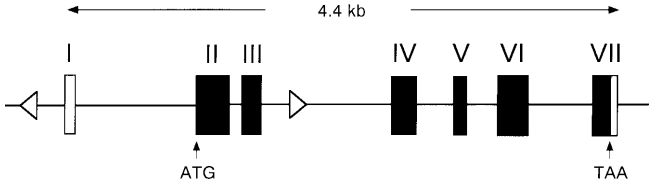

Fig. 1. Structure of the *RPL6* gene. *Filled* and *open boxes* represent coding and untranslated regions, respectively, and *open triangles* show *Alu* repeats. The position of the initiator ATG and the terminator TAA are indicated with *vertical arrows*. The *RPL6* nucleotide sequence data will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB042820

Comparison of the amino acid sequences of mammalian rp L6

We compared the amino acid sequences of rp L6 of several mammalian species, and found significant differences in the N-terminal portion of the protein (Fig. 2). The overall identity of the amino acid sequences between human and rat L6 (Chan and Wool 1996) and between human and mouse L6 (Nacken et al. 1995) is 87% and 85%, respectively. In general, ribosomal proteins are well conserved in the course of evolution. For instance, the amino acid sequences of related human and rat proteins are nearly identical; the average for 72 comparisons of complete sequences is 99%, and for 32 comparisons, it is 100% (Wool et al. 1996). Therefore, the extent of variation observed in the mammalian L6 proteins is extremely unusual. Although the role of the NH_2 terminus of rp L6 is unclear, there may be some particular function associated with this portion of the protein.

Mapping of *RPL6*

RPL6 was localized within the human chromosome by typing two kinds of radiation hybrid panels, the Genebridge 4 and G3 panels, using primers generated based on the second exon and the third intron of the gene. The data vector on Genebridge 4 was 0010100001 0000000001 1101111100 0010010010 0100110000 1000010000 0000001010 1111100010 0100011010 010 and the consequent report indicated that the gene is located at 7.8 cR distal to the marker WI-7485. The data vector on the G3 panel was 0100011100 0000001000 0000101110 0010000000 0000000000 1000000000 1101110000 1000001110 000, where the gene was placed at 17 cR distal to SHGC-9870. These mapping results agreed with each other, and the gene was thereby assigned to chromosome 12q24.1 between markers D12S84 and D12S861 (Fig. 3).

Discussion

We have mapped the human *RPL6* gene to chromosome 12q24.1 between the markers D12S84 and D12S861, which is the interval containing the Noonan syndrome (NS) locus. NS is an autosomal dominant disorder characterized by a

Table 1. Structure and splice site sequences of the *RPL6* gene

Exon no.	3′ Splice acceptor	Exon (bp)	5′ Splice donor	Intron (bp)
1	gaccttaatt/CTCTTTCCCA[a]	19	TCTTGCAAG/gtaagaatcg	930
2	atatttttag/ATGGCGGGTG	237	TAAATCCAAG/gtaagggaag	81
3	gtccttgtag/GTTGAAAAGA	99	TCGCAAAATG/gtaagatgtg	1349
4	ctctccacag/CCTAGATATT	144	CAGGGGCAAG/gtgagagtac	404
5	taacttacag/AGGGTGGTTT	49	CTTGTGACTG/gtaagaaaat	256
6	tccccttag/GACCTCTGGT	185	AGAAAAAGAG/gtaagtttct	476
7	ctttatttag/AAATATGAGA	186	AGCTGACTAC	

[a] Upper- and lower-case letters denote exon and intron sequences, respectively

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Hm   MAGEKVEKPDTKEKKPEAKKVDAGGK---------VKKGNLKAKKPKKGKPHCSRNPVLV   51
     ***** **** ** ** ***                  ***   ****  * ***********
Rt   MAGEKAEKPDKKEQKPAAKKAGGDATAPRA-GAWCVKKSSSKAKKLRKSKPHCSRNPVLV   59
               * ******** ** * *   *   **   * **  * ***********
Ms             MEKKPAAKKAGSDAAASRPRAAKVAKKVHPKGKKPKKAKPHCSRNPVLV   49

Hm   RGIGRYSRSAMYSRKAMYKRKYSAAKSKVE-KKKKEKVLATVTKPVGGDKNGGTRVVKLR  110
     ***************** ********* *** ************ ***************
Rt   RGIGRYSRSAMYSRKALYKRKYSAAKTKVEKKKKKEKVLATVTKTVGGDKNGGTRVVKLR  119
     ************************************************************
Ms   RGIGRYSRSAMYSRKALYKRKYSAAKTKVEKKKKKEKVLATVTKTVGGDKNGGTRVVKLR  109

Hm   KMPRYYPTEDVPRKLLSHGKKPFSQHVRKLRASITPGTILIILTGRHRGKRVVFLKQLAS  170
     ***************************** ** ****** ****************** *
Rt   KMPRYYPTEDVPRKLLSHGKKPFSQHVRRLRSSITPGTVLIILTGRHRGKRVVFLKQLGS  179
     ************************************************************ *
Ms   KMPRYYPTEDVPRKLLSHGKKPFSQHVRRLRSSITPGTVLIILTGRHRGKRVVFLKQLDS  169

Hm   GLLLVTGPLVLNRVPLRRTHQKFVIATSTKIDISNVKIPKHLTDAYFKKKKLRKPRHQEG  230
     ********* ******************** *** *************** *********
Rt   GLLLVTGPLALNRVPLRRTHQKFVIATSTKVDISKVKIPKHLTDAYFKKKPLRKPRHQEG  239
     *********  ********************** ************** *********
Ms   GLLLVTGPLVINRVPLRRTHQKFVIATSTKVDISDVKIPKHLTDAYFKKKQLRKPRHQEG  229

Hm   EIFDTEKEKYEITEQRKIDQKAVDSQILPKIKAIPQLQGYLRSVFALTNGIYPHKLVF    288
     ***************** ************** ********* * **** *******
Rt   EIFDTEKEKYEITEQRKADQKAVDSQILPKIKAVPQLQGYLRSQFSLTNGMYPHKLVF    297
     ************************ ********************************
Ms   EIFDTEKEKYEITEQRKADQKAVDLQILPKIKAVPQLQGYLRSQFSLTNGMYPHKLVF    287
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Fig. 2. Comparison of the amino acid sequences of human (*Hm*), rat (*Rt*), and mouse (*Ms*) ribosomal protein L6. Gaps (*hyphens*) are introduced to maximize the alignment among the sequences. Identical residues between any two of the three species are indicated by *asterisks*

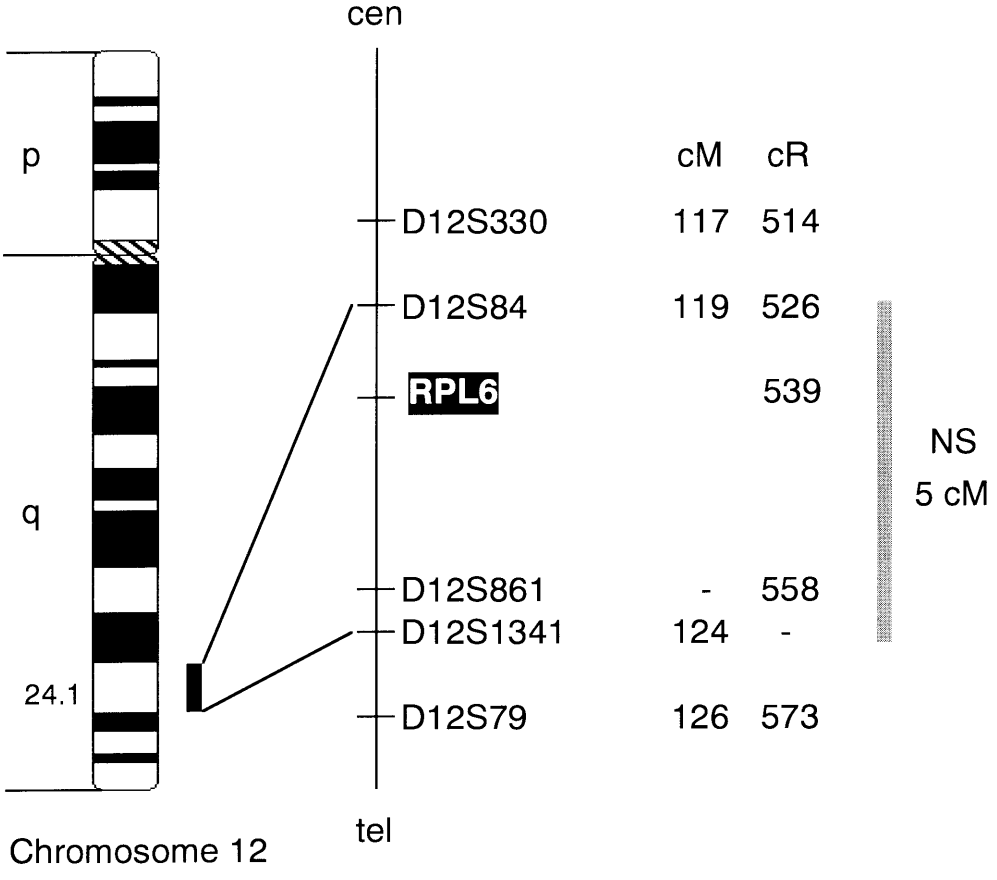

Fig. 3. Chromosomal placement of the *RPL6* gene at a distance relative to framework markers on the Whitehead Institute/MIT Center for Genome Research radiation hybrid map of the human genome (http://www-genome.wi.mit.edu). The approximate corresponding cytogenetic location of the gene on chromosome 12 and the critical region for Noonan syndrome (*NS*) are indicated. Distances of markers are in centirays (*cR*) and centimorgans (*cM*) from the top of the chromosome 12 linkage group

congenital heart defect, typical facial dysmorphy, and short stature, with an estimated incidence of between 1:1000 and 1:2500 (Allanson 1987; Noonan 1994). Linkage analysis in a large pedigree with NS localized the disease locus to 12q22-qter (Jamieson et al. 1994), and the location has recently been further refined to a 5-cM interval between the markers D12S84 and D12S1341 (Legius et al. 1998). It is of interest to investigate the possible involvement of the human rp L6 in the NS phenotype.

In *Drosophila*, the *Minute* phenotype (reduced body size, diminished viability and fertility, and short, thin bristles) results from heterozygous deficiencies (deletions) at any one of 50 loci scattered about the genome (Kay and Jacobs-Lorena 1987). Several of these *Minute* loci have been characterized at the molecular level, and all have been found to encode ribosomal proteins (Kongsuwan et al. 1985; Lambertsson 1998). Thus, the *Minute* phenotype appears to result from a reduced capacity for protein synthesis in flies with one allele rather than two of a given rp gene. Because ribosomal proteins are highly conserved among eukaryotes, it is likely that quantitative deficiencies in human ribosomal proteins, as in *Drosophila*, will result in reduced translation capacity and thereby yield abnormal phenotypes. Interestingly, it has been speculated for a long time that ribosomal protein S4, encoded by both X and Y chromosomes, is an important factor in Turner syndrome, a complex human disorder classically associated with a 45,X karyotype (Fisher et al. 1990; Watanabe et al. 1993). Because many investigators have noticed similarities between the Turner and Noonan phenotypes (Allanson 1987; Noonan 1994), our finding that *RPL6* is located in the critical region for NS is extremely intriguing. Screening for mutations of this gene in NS patients is now underway.

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