DNA RESEARCH 5, 229–233 (1998)

High-Resolution Mapping of Ribosomal Protein Genes to Human Chromosome 19

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(Received 16 July 1998; revised 23 July 1998)

Abstract

In a systematic effort for mapping of all the human ribosomal protein (rp) genes, we have found that an unusually large number (12) of rp genes are present on chromosome 19 and subsequently determined their locations on the chromosome by a radiation-hybrid procedure. For this, we isolated cosmid clones corresponding to each gene and placed nine of them on a metric physical map of chromosome 19. Although most genes are scattered over the chromosome, we found three genes are clustered in a 0.6-Mb region at 19q13.3 and two of them, *RPL13A* and *RPS11*, within a single cosmid only 4.3 kb apart. To explore a possible relationship between rp gene defects and human disease, we compared map positions of the rp genes and disease loci on chromosome 19, which led us to find *RPS9* gene in the same interval as the gene for retinitis pigmentosa 11. The disease locus has previously been mapped to the 6-cM interval at 19q13.4 between markers D19S572 and D19S926, which corresponds to less than 2-Mb region on the metric physical map. We mapped *RPS9* about 800 kb distal to D19S572.

Key words: ribosomal protein genes; human chromosome 19; gene cluster; human disease

1. Introduction

The mammalian ribosome is a massive structure composed of four RNA species and about 80 different proteins.[1] The nucleotide sequences of the four ribosomal RNAs and the deduced primary structure of all the ribosomal proteins (rp) have been determined.[2,3] Thus, the ribosome is the largest, most complex mammalian structure to have been completely described at the level of the nucleotide and amino acid sequences. Although the ribosome is known to be essential for cell growth and development, its possible role in human disease has been largely ignored. One might predict that genetic defects in ribosomal components would cause serious problems with the translational machinery and hence result in early embryonic death. However, there is strong evidence in *Drosophila* that quantitative deficiencies of ribosomal proteins may yield viable but abnormal phenotypes.[4,5] Moreover, 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.[6,7] We are intrigued by the possibility that deficiencies of other rp genes might underlie abnormal phenotypes associated with certain chromosomal disorders in humans.

To explore this possibility, we have been systematically mapping human rp genes using sequence tagged sites (STSs) specific to individual rp genes. Seventy-five rp genes have been localized and shown to be widely dispersed throughout the human genome.[8] However, an unusually large number of rp genes (12) were mapped to chromosome 19, even though it constitutes only 2% of the haploid genome. Here we describe a cosmid-based high-resolution mapping of rp genes to human chromosome 19, which enabled us to find the first example of rp gene cluster in mammals and to locate one of the rp genes in a critical region for retinitis pigmentosa 11 at 19q13.4.

2. Materials and Methods

2.1. Isolation and mapping of cosmid clones

Probes were generated by polymerase chain reaction (PCR) using partial genomic sequences of human ribosomal protein (rp) genes which had been mapped to human chromosome 19. Sequences of the PCR primers are listed in Table 1. To locate the genes on the established physical map of human chromosome 19,[9] these probes were

Communicated by Michio Oishi

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Table 1. Primers used for library screening.

Gene	Forward primer	Reverse primer	Size (bp)
RPS5	CATCTGGCTGCTGTGCAC	GTTGGACTTGGCCACACGCT	246
RPS9	AGTCCGTCCCCTAAATTTGG	CTTCGAACAGACGCCGTG	245
RPS11	TTCCAGATCGGACCAATTTA	TACTCGCCAATTCAACCTGT	1288
RPS15	AGACGAGTTTCAGTGTTGCC	AGACCACAGCCTCAGACAAG	319
RPS16	GGTGGCCTATTACCAGAAATGT	GGAAGCCTCATCCACATCT	130
RPS19	ACCTGACTAGGGCCCTCAGT	CTGTCCGGCGATTCTGT	191
RPS28	TTGATAGACCTTTGGTTGGTG	ACATGAGATGTTGACAGGCC	141
RPL13A	GCTAAACAGGTACTGCTGGGT	AGGAAAGCCAGGTACTTCACT	591
RPL18	CGCACAGCCACACCAAGT	GGAGCGGACGTAGGGTCT	400
RPL18A	CTCCCCTGGAGGGAAGTG	AGGCATGTTGAGGGGGTT	113
RPL28	ACCTCCTATGTGCGGACCAC	ATCCCTCTCCAGCCCATATT	198
RPL40	GTGTCAGTCTCAGACTCCCC	AGAAGGACGGCGACTGAG	220

hybridized to high-density filter arrays of chromosome 19-specific cosmid libraries.[10] Cosmid clones in the libraries have been analyzed using a restriction digest fingerprinting scheme and assembled into contigs, as reported previously.[9] Distances between cosmid clones within the contigs were estimated using high-resolution fluorescence *in situ* hybridization (FISH) mapping methods.[11] Positive clones were isolated, then verified by PCR with the same primers used in the probe preparation, and assigned to specific positions on the metric physical map of chromosome 19.

2.2. *Analysis of* RPL13A *and* PS11 *cluster by PCR*

PCR assays were performed to analyze the genomic organization of *RPL13A* and *RPS11* genes. Primers were initially derived from their cDNA sequences (accession no. X56932 and X06617) and a partial genomic sequence of *RPS11* (accession no. AB007152). After determining the direction of the two genes by long PCR on DNA from a cosmid which carries both genes, we developed new primer pairs to estimate the genomic size of each gene or the distance between them. The primer sequences are 5′-AAGATGGCGGAGGTGCAG-3′ and 5′-GCAACGCATGAGGAATTAAC-3′ for *RPL13A*, 5′-GGGAAGATGGCGGACATTC-3′ and 5′-CTTCTGGAACTGCTTCTTGG-3′ for *RPS11* and 5′-CACAGAG GTCCTCAAGACCC-3′ and 5′-TCGAGAGCTGCTCTGATTGA-3′ for their distance. Thermal cycling conditions for the long PCR were as follows: (1) an initial denaturation at 94 °C for 3 min; (2) 30 cycles of denaturation at 94 °C for 0.5 min, primer annealing at 58 °C for 1 min, and polymerization at 72 °C for 10 min; and (3) a final extension at 72 °C for 5 min.

3. Results

3.1. *Isolation and assignment of the cosmid clones*

The 12 rp gene probes identified a total of 35 cosmids from the cosmid libraries used by Livermore's Human Genome Center to construct a metric physical map of chromosome 19.[9] These clones enabled us to place seven rp genes (*RPS15*, *RPL40*, *RPS16*, *RPS19*, *RPL18*, *RPS9* and *RPS5*) unambiguously on the physical map. Two genes (*RPL13A* and *RPS11*) were also placed with confidence by inferences drawn by mapping information on other clones in that location. The remaining three genes, however, have not been unambiguously mapped: *RPS28* identified clones which mapped to multiple positions; *RPL28* identified clones not yet placed in the metric physical map; and *RPL18A*, where only a single cosmid was identified whose location was inconsistent with data from radiation hybrid (RH) mapping. Although we failed to map *RPS28* due to multiple positions, we note that one of the sites was consistent with the result obtained by RH mapping. Moreover, we placed *RPL28* on 19q13.4 by FISH analysis using one of the two cosmids harboring this gene (data not shown). The mapped positions are listed in Table 2 along with estimated distances from the top of the short arm. They show strong agreement with the previous results of RH mapping except in two cases, *RPS9* and *RPS5* genes, where the RH mapping was unable to order these genes with high confidence since they were separated by only 10 centiRays (cR).[8] The current metric physical map, including detailed information on the locations of the rp genes, is available at an Internet site maintained at Lawrence Livermore National Laboratory (http://www-bio.llnl.gov/bbrp/genome/html/chrom_map.html).

3.2. *An rp gene cluster at 19q13.3*

Although most rp genes are dispersed over the human genome, we placed three genes within a small region of 19q13.3 about 0.6 Mb in length (Table 2). Furthermore,

Table 2. Mapped positions of rp genes.

Gene	Cosmid map		RH map
		Mb[a)]	cR[b)]
RPS15	19p13.3	1.6	6
RPS28	19p13.2	—	39
RPL18A	—	—	90
RPL40	19p12	18.2	102
RPS16	19q13.1	43.0	314
RPS19	19q13.2	46.2	319
RPL18	19q13.3	53.3	337
RPL13A	19q13.3	53.9	350
RPS11	19q13.3	53.9	351
RPS9	19q13.4	59.3	377
RPL28	19q13.4	—	372
RPS5	19q13.4	63.6	367

a) Distance from the top of the short arm. b) Distance in centiRays from the most distal short-arm marker.[8]

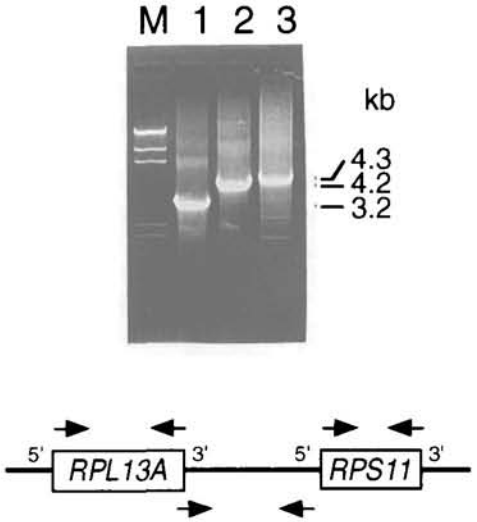

Figure 1. PCR analyses of *RPL13A* and *RPS11* gene cluster. PCR assays were performed on a cosmid DNA which carries both rp genes using a primer pair derived from *RPS11* (lane 1), *RPL13A* (lane 2) or the region between the two genes (lane 3) as diagrammed under the panel. Lane M: Lambda DNA digested with *Hin*dIII.

two of these genes (*RPL13A* and *RPS11*) were found within a single cosmid whose insert size was estimated at about 40 kb. PCR assays on the cosmid enabled us to determine an approximate distance between the two genes, their orientation, and approximate sizes. As shown in Fig. 1, *RPL13A* and *RPS11* genes are tandemly located with an interval of only 4.3 kb, and are about 4.2 kb and 3.2 kb in length, respectively. We sequenced this entire region and have confirmed these results (data not shown).

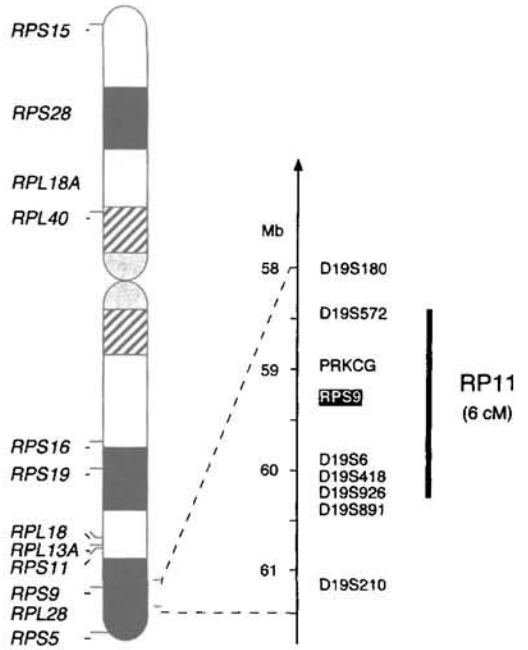

Figure 2. Localization of rp genes and markers at the RP11 locus to the metric physical map of human chromosome 19. Positions of *RPS28*, *RPL18A* and *RPL28* genes were determined by RH mapping (see Results). The marker D19S926 is placed between markers D19S418 and D19S891 based on the Généthon human linkage map.[16] The latest more detailed map is available at http://www-bio.llnl.gov/bbrp/genome/html/chrom_map.html.

3.3. *Localization of* RPS9 *gene to a critical region for RP11*

To investigate the possible relationship between rp gene deficiencies and chromosomal disorders in humans, the positions of the rp genes were compared with disease loci which had been mapped to chromosome 19. This search identified one gene (*RPS9*) in a critical region for retinitis pigmentosa 11 (RP11), an autosomal dominant form of inherited retinal degeneration (MIM 600138). The RP11 locus had been mapped to chromosome 19q13.4,[12,13] and its interval has recently been refined to the 6-cM region between markers D19S572 and D19S926.[14,15] Although D19S926 has not been placed on the physical map, it is known to be between D19S418 and D19S891 by genetic mapping.[16] Both of these markers are on the physical map. By inference, the largest possible extent between D19S572 and D19S926 would be less than 2 Mb apart on the metric physical map of chromosome 19.[9] Within that region, we have placed *RPS9* about 800 kb distal to D19S572 (Fig. 2).

4. Discussion

In this study, we have localized human rp genes to the cosmid and BAC-based high resolution physical map of chromosome 19 generated at the Lawrence Livermore National Laboratory. The map consists of cosmids as-

sembled into contigs by automated restriction fragment analysis. Selected cosmids from these contigs were ordered by high-resolution FISH mapping in which the distance between cosmid markers was determined.[11,17] Complete digest *Eco*RI restriction maps currently span approximately 90% of the euchromatin of the chromosome. Therefore, the resolution of this map is considered to be very high and hence the positions of the rp genes determined here should be more precise than ordinary FISH or radiation hybrid (RH) mapping. In the previous RH mapping, *RPS9* and *RPS5* were not ordered precisely because the distance between the two genes was only 10 centiRays (cR), which is smaller than the minimal value (15 cR) where markers could be ordered with high confidence.[8] By contrast, the high-resolution cosmid mapping described here enabled us to determine their precise positions and order.

In prokaryotes, rp genes are organized into a small number of operons where the gene expression is coordinately regulated under a single promoter.[18] On the contrary there has been little or no evidence of rp gene clustering in mammals, although most mammalian ribosomal proteins have recognized homologs in prokaryotes.[3] Here we found the first example of an rp gene cluster on human chromosome 19. Three genes were localized to a 0.6-Mb region at 19q13.3, and two of them, *RPL13A* and *RPS11*, are separated by only 4.3 kb. Although a recent study on 75 human rp genes strongly suggested that rp genes are dispersed throughout the human genome,[8] this cluster suggests that there is a similar control mechanism for rp gene expression in mammals as observed in bacteria. Detailed analysis of genomic sequences in this region has to be accomplished to examine such a possibility.

The current study was initially motivated by the possibility that mutations on ribosomal proteins contribute to human disease.[19] In this regard, a careful look at the *Drosophila Minutes* would be valuable. *Minutes* are a group of mutations (over 50) scattered in the *Drosophila* genome which are all associated with similar dominant phenotypes, including reduced body size, diminished fertility, and short, thin bristles.[20] Several *Minute* loci have been molecularly characterized, and all have been found to encode ribosomal proteins.[4,21–24] Thus, it is likely that quantitative deficiencies in human ribosomal proteins will result in reduced translational capacity and yield certain phenotypes.

In this study, we demonstrated that *RPS9* is located in a critical region for retinitis pigmentosa 11. Although it is unlikely that degeneration of the retina results from reduced translational capacity due to an *RPS9* defect, recent findings on extra-ribosomal functions of ribosomal proteins might explain the role of *RPS9* in retinal formation. There is cumulative evidence that a number of ribosomal proteins have a second function apart from both the ribosome and protein synthesis.[25] For example, in mammals and *Drosophila*, *RPS3* functions as both a ribosomal protein and an endonuclease.[26–28] In *Drosophila*, *RPS6* functions as a tumor suppressor in the hematopoietic system,[29] and *RPS2* functions in oogenesis.[30] In humans, *RPS4* is postulated as a cause of certain features of Turner syndrome,[6,7] *RPL5* forms a protein complex with *Mdm2* and p53,[31] and *RPL22* forms an RNP complex with the Epstein-Barr virus-encoded RNA.[32] All these facts probably reflect extra-ribosomal functions. Given these precedents, we need to carefully examine the possibility that ribosomal protein mutations could disturb various cellular functions without affecting translation. Therefore, it is worth pursuing to investigate in more detail about *RPS9* and its role in retinal degeneration.

Acknowledgments: We thank Tomoko Kawaguchi and David C. Page (Whitehead Institute) for their initial contribution to this work, Anne S. Olsen for suggestions in cosmid mapping, and Maki Yoshihama for help in cluster analysis. This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan. A portion of this work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under Contract No. W-7405-ENG-48.

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