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The structure of a gene containing introns and encoding rat ribosomal protein P2

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ABSTRACT

The single rat ribosomal protein P2 gene containing introns has been characterized. It has 2275 nucleotides distributed in 5 exons and 4 introns. The sequence of amino acids encoded in the exons corresponds exactly to that derived before from a cDNA. Only this one P2 gene in a family of approximately 9 members has introns and is expressed. There are two transcriptional start sites (adjacent cytidine residues) located in a tract of 10 pyrimidines flanked by GC-rich regions. The P2 gene, like other mammalian ribosomal protein genes, lacks a TATA box; however, it has at positions −30 to −27 the sequence TTTA which may be a degenerate TATA box and may serve the same function. The architecture of the P2 gene, and especially the structure of the promoter region, resembles that of other mammalian ribosomal protein genes. This suggests that the common features contribute to the coordinate regulation of their transcription and that the stoichiometry of P2 (it is present in 2 copies in the ribosome) is achieved by regulation of the translation of its mRNA.

INTRODUCTION

The large ribosomal subunits from all the species that have been analyzed, prokaryotic, archaebacterial, and eukaryotic, have a set of proteins that are distinguished by their chemical and physical properties. The prototypes are *Escherichia coli* L12 and L10 (1, 2). Protein L12 is acidic which in itself sets it apart from most other ribosomal proteins which are very basic; in addition, *E. coli* L12 has an unusually large number of alanyl residues, is deficient in aromatic residues, and has about 70%-helical content (3–5). Unlike the other proteins there are four copies (two dimers) of L12 in ribosomes (6, 7); the stoichiometry of the others is one (7). The NH_2-terminal serine of about half the population of L12 molecules is acetylated (5); this form has been designated L7 from its coordinates on two-dimensional gels and the two together L7/L12, although, they are the products of a single gene. No phenotype has been attached to this posttranslational modification (8). Four copies of L12 form an assembly with a single copy of L10; the complex occurs in solution and is a discrete ribosomal substructure (9, 10) that is located in the stalk and in the base of the stalk in the 50S ribosomal subunit (11, 12). The acidic proteins are involved in the binding of the elongation factors EF-Tu and EF-G, the initiation factor IF-2, the release factor RF, and in the hydrolysis of GTP (13–18).

Eukaryotic ribosomes contain proteins that are related to *E. coli* L12 and L10. The first indications of a structural relationship came from immunochemical studies: Antisera against *E. coli* L7/L12 cross-reacted with yeast and with rat acidic proteins, and with rat L40/L41 (19, 20); the latter were later designated P1 and P2 (21). In reciprocal experiments antisera against yeast acidic proteins and against rat L40/L41, reacted with *E. coli* L7/L12 (19, 20). The initial suggestion of a functional relationship derives from the observation that antisera against the eukaryotic acidic proteins were as effective as anti-*E. coli* L7/L12 in inhibiting the EF-G dependent binding of GDP to *E. coli* 50S ribosomal subunits in the presence of fusidic acid. Moreover, yeast acidic proteins could substitute for *E. coli* L12 in reconstituting the activity of PI cores (19, 20); the latter are L12 deficient 50S ribosomal subunits.

The three eukaryotic acidic proteins analogous to prokaryotic L10 and L12 have been designated P0, P1, and P2 (21); P because they are phosphorylated; phosphorylation appears to be necessary for assembly of the proteins into ribosomal subunits (22). The amino acid sequences of the P proteins from human (23), rat (24, 25), *Artemia salina* (26–28), and yeast (29–32) ribosomes have been determined. They have chemical and physical properties similar to the *E. coli* homologues but in addition the three P proteins have a highly conserved, indeed, almost identical, sequence of 17 amino acids near the carboxyl terminus (23–25). This conserved sequence is an epitope recognized by autoantibodies in an appreciable number of patients with systemic lupus erythematosus (33, 34); a rise in the titer of these autoantibodies sometimes correlates with the onset of episodes of psychosis (35).

Homodimers of P1 and of P2 are integrated with a single copy of P0 into a coherent structure in eukaryotic ribosomes (36); the complex has the composition $P1_2 \cdot P2_2 \cdot P0$ where the molecular weights (in rat) are: P1, 11,490; P2, 11,684; and P0, 34,178 (25). P1 and P2 can be stripped selectively from 60S ribosomal

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subunits with ethanol and high concentrations of monovalent salts (19, 20, 22, 37). The particles deficient in P1 and P2 cannot catalyze protein synthesis: They do not associate with EF-1α nor EF-2 and, hence, do not bind aminoacyl-tRNA (38) nor do they hydrolyze GTP (22, 37). Polyclonal antibodies against P1 and/or P2 also inhibit EF-2-dependent GTPase activity (22, 39). Monoclonal antibodies against an epitope in the conserved carboxyl-terminal amino acid sequence inhibited the binding of EF-1α and EF-2 to ribosomes and the hydrolysis of GTP dependent on the elongation factors (40).

The genome in mammals has multiple copies of most (if not all) ribosomal protein genes (cf. 41 for a discussion and for references). However, where the analysis has been extensive only one of the genes has proven to contain introns and to be functional (42–48); the other members of the family are presumed to be retroposon pseudogenes. A possible exception is ribosomal protein S4 from humans; there appear to be separate transcribed genes for S4 on the X and Y chromosomes (49). Until recently the large number of pseudogenes (about 7–20) made identification of the single functional gene difficult and discouraged analysis of their structure. Indeed, the structure of only seven of perhaps eighty functional, intron-containing mammalian ribosomal protein genes had been determined (42–48). The preliminary indications are that the promoter regions share many common features and that they also have some unique aspects. Nonetheless, the number that have been analyzed is so small, especially as a fraction of the total number of ribosomal protein gene families, that one cannot be confident in generalizing the results. This is a crucial deficiency because of the bearing that the architecture of the promoters will have in formulating paradigms of how the regulation of the transcription of individual proteins is coupled to the coordination of the transcription of all seventy to eighty ribosomal proteins and then fine-tuned to the synthesis of rRNA.

The polymerase chain reaction has made the problem more amenable to analysis; it makes it far easier to identify an intron containing gene and helps in establishing its structure (50). We chose to determine the structure of a P2 gene because this ribosomal protein is one of the few to which one can ascribe a function, because it is related to P0 and P1 by a conserved amino acid sequence, and because its stoichiometry (two copies per ribosome) suggests the possibility that its gene might have distinct features.

MATERIALS AND METHODS

Materials

Restriction endonucleases and T4 DNA ligase were purchased from New England Biolabs; T4 polynucleotide kinase, DNA polymerase I (Klenow fragment), and calf alkaline phosphatase were from Boehringer Mannheim; reverse transcriptase was from Life Sciences, Inc; [γ-^{32}P]ATP (3000 Ci/mole), [^{35}S]dATP (1000 Ci/mole), and [α-^{32}P]dCTP (3000 Ci/mole) were from Amersham Corp; Taq polymerase (*Thermus aquaticus* DNA polymerase) was from Perkin Elmer Cetus; Sequenase was from United States Biochemical Corp. Oligodeoxynucleotides were synthesized on a solid support by the methoxyphosphoramidite method using an Applied Biosystems, Model 380B, DNA synthesizer; they were purified by polyacrylamide gel electrophoresis. The methods used for the preparation of rat ribosomal P protein cDNAs (25) and of nuclear DNA (51) have been described before.

Preparation of clones

A bacteriophage Charon 4A recombinant DNA library made from partially digested rat genomic DNA with *Alu*I and *Hae*III was a gift from T. Sargent (52). A random selection of 400,000 plaques were screened initially with a synthetic oligodeoxynucleotide probe (a 21-mer) that had the sequence that encodes the carboxyl-terminal peptide, MGFGLFD, of rat ribosomal protein P1 (25). Secondary screening was with radioactive cDNAs (53) specific for individual P proteins. A single plaque, designated RP2-12, hybridized strongly to a P2 cDNA probe.Two restriction fragments from RP2-12 were subcloned into the plasmid vector pUC9. The subcloned recombinant plasmids were isolated by the alkaline-sodium dodecyl sulfate method (54).

Determination of the sequence of nucleotides in DNA

The determination was by the enzymatic chain-termination method using modified T7 DNA polymerase (Sequenase) and synthetic oligodeoxynucleotides as primers (55). The sequence of nucleotides was determined in both strands of the P2 gene and in overlapping sequences for each fragment.

Determination of the site of the initiation of transcription of the P2 gene

An oligodeoxynucleotide primer (an 18-mer) complementary to a region near the 5′ end of the P2 mRNA was made radioactive at the 5′ end with T4 polynucleotide kinase and [γ-^{32}P]ATP (56). The primer (5 ng) and 6 μg of rat liver poly(A)$^+$ mRNA were heated at 80°C for 10 min in 50% deionized formamide, 40 mM PIPES (piperazine-N, N′-bis (2)-ethanesulfonic acid (pH 6.4)), 400 mM NaCl, and 1 mM EDTA. The solution was slowly cooled to room temperature over 4–6 hrs to ensure the hybridization of the primer to the mRNA. The hybrid was precipitated with ethanol in 0.3 M sodium acetate. The annealed

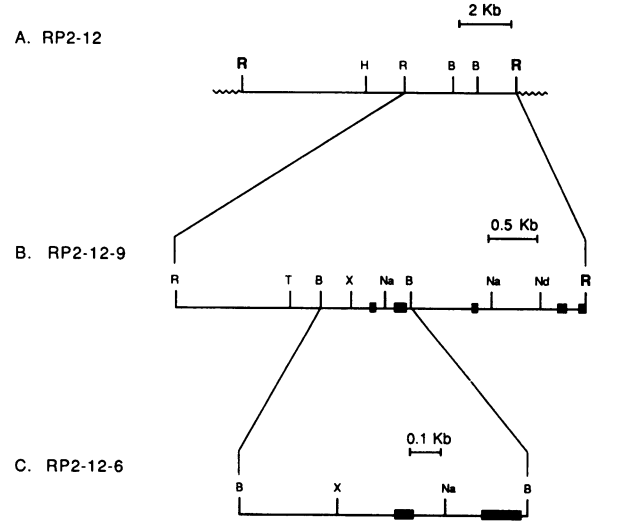

Figure 1. A restriction endonuclease map of a recombinant rat ribosomal P2 gene in bacteriophage Charon 4A. A, RP2-12 contains a rat ribosomal protein P2 gene (straight line) inserted into Charon 4A (wavy line) at *Eco*RI sites (large, bold Rs); B, RP2-12-9 is an *Eco*RI-*Eco*RI restriction fragment of RP2-12; C, RP2-12-6 is a *Bam*HI-*Bam*HI restriction fragment of RP2-12. The exons are designated by rectangles. Restriction sites are abbreviated: B, *Bam*HI; H, *Hind*III; Na, *Nal*I; Nd, *Nde*I; R, *Eco*RI; T, *Taq*I; and X, *Xmn*I.

primer was extended using avian myeloblastosis virus reverse transcriptase (57); the reaction was at 46°C for 40 min. DNA synthesis was terminated by the addition of EDTA (to a final concentration of 20 mM), the reaction mixture was extracted with phenol-chloroform-isoamyl alcohol (25:24:1), and the DNA was precipitated with ethanol. The primer extension product was resolved by electrophoresis in polyacrylamide gels containing urea; detection was by autoradiography.

Determination of the number of intron-containing P2 genes

Synthetic oligodeoxynucleotides (17−21 bases) having the same sequences as regions of a P2 cDNA were used as primers to amplify parts of the gene in the polymerase chain reaction (PCR). Pairs of primers were designed to bridge adjacent exons so that when extended the amplified DNA would contain the intervening intron. The PCR was with rat liver nuclear DNA (0.5−1 μg), or with plasmid RP2-12-9 DNA (2−10 ng), or with cDNA (2−10 ng) in 50 μl of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 μM each of the 4 dNTPs, 1 μM each of the two primer oligodeoxynucleotides, and 1.3 units of Taq polymerase. The reactions were in a Perkin Elmer Cetus DNA Thermal Cycler for 25 cycles: Each cycle was for 2 min at 94°C (denaturation); 2 min at 55°C (annealing); and 2 min at 72°C (elongation). The amplified DNA was resolved by electrophoresis in 1.2% agarose gels and located with UV light after staining with ethidium bromide.

Nucleotide sequence accession number

The nucleotide sequence reported here has been submitted to GenBank and assigned accession number X55153.

RESULTS AND DISCUSSION

Isolation and characterization of an intron-containing ribosomal protein P2 gene

The three rat acidic ribosomal P proteins, P0, P1, and P2, share a sequence of seven amino acids (MGFGLFD) near their carboxyl terminus; however, the sequences of nucleotides in the cDNAs encoding these common residues differ (25). A synthetic oligodeoxynucleotide (a 21-mer) that reproduces the relevant sequence in the P1 cDNA was used as a probe to screen for rat P protein genomic clones in a bacteriophage Charon 4A recombinant DNA library. The stringency of the hybridization was purposely low to permit annealing of the probe to all of the P protein genes. In the initial screening 149 plaques gave a positive signal. Forty-five of the plaques were characterized further using uniformly labeled cDNAs for P0, P1, and P2. Of these 45, 3 hybridized specifically, i.e. at high stringency, to P0; 12 to P1; and only 1 to P2. The latter was designated RP2-12 and it was characterized further. The phage DNA was isolated and digested with restriction endonucleases (Figure 1). Two of the restriction fragments that hybridized to a P2 cDNA had *Bam*HI sites not present in the cDNA suggesting that RP2-12 contained an intron. These two fragments, *Eco*RI-*Eco*RI (4.3 kb) and *Bam*HI-*Bam*HI (0.9 kb), were subcloned into pUC9; they are designated RP2-12-9 and RP2-12-6 respectively (Figure 1).

Determination of the sequence of nucleotides and analysis of the structure of an intron-containing ribosomal protein P2 gene

The sequence of nucleotides in RP2-12-9 was determined for both strands and in overlapping sequences for each restriction fragment (Figure 2). The nucleotide sequence of RP2-12-6 is contained in RP2-12-9 (cf. Figure 1). Comparison of the nucleotide sequences of RP2-12-9 and the P2 cDNA established that the former had introns. However, RP2-12-9 lacks 40 bp that are in the P2 cDNA including the codons for the two carboxyl-terminal amino acids. The genomic library was generated by partial digestion with the restriction enzyme *Hae*III and *Alu*I (52) and there is a *Hae*III site where the deletion occurs. To establish the nucleotide sequence at the 3′ end of the gene, a set of primers were synthesized for amplification of rat genomic DNA in the PCR. The first primer corresponded to the sequence at positions 2155−2180 which spans the junction of intron 4 and exon 5;

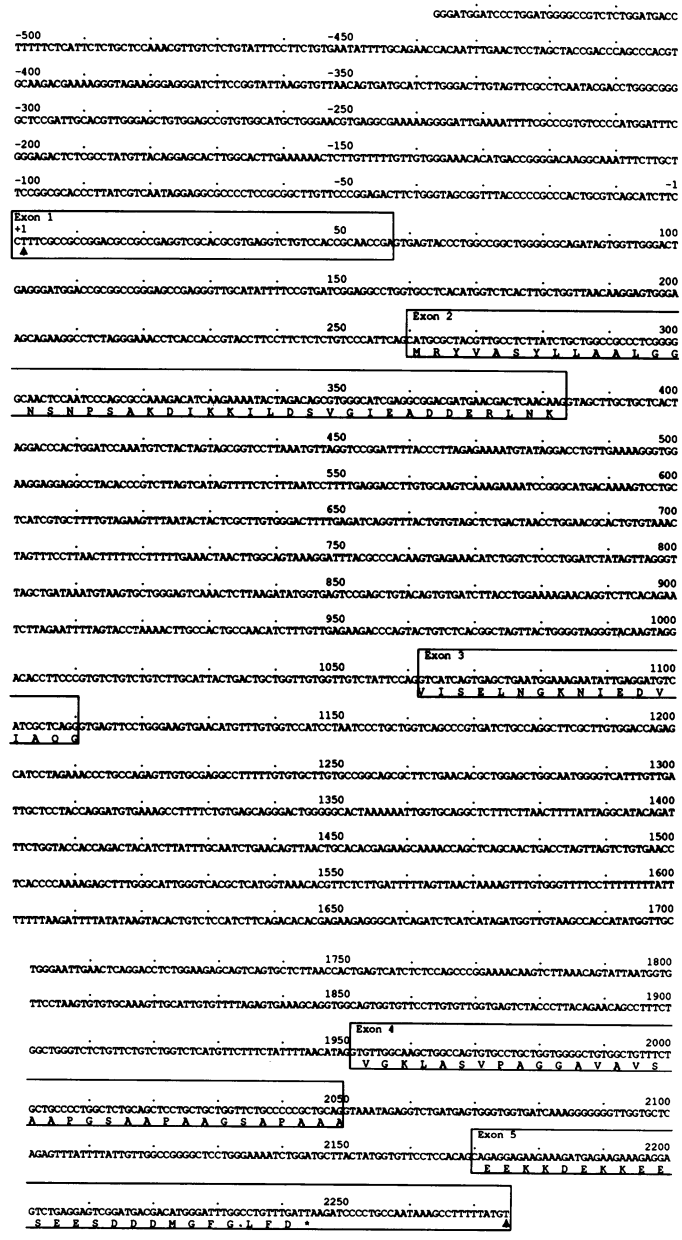

Figure 2. The sequence of nucleotides in a ribosomal P2 gene. The exon sequences in the gene are boxed and the amino acid sequence of protein P2 is given in the single letter code below the codons. Positive numbering starts at the major transcription initiation site; negative numbers designate the 5′ flanking promoter region. The 5′ and 3′ ends of the P2 cDNA are demarked by arrow heads.

the reverse primer reproduced the 3′ nontranslated region of the P2 cDNA (positions 2251−2275 in Figure 2). A single fragment of 121 bp was amplified and the nucleotide sequence determined; it did not have an additional intron. Thus, the nucleotide sequence of RP2-12-9 combined with that of the amplified fragment contains the complete ribosomal protein P2 gene. Comparison of the sequence of nucleotides in the genomic DNA with that of the P2 cDNA establishes that the gene has 2275 nucleotides distributed in 5 exons and 4 introns. The introns have 202, 678, 840, and 120 bp and together comprise 80% of the nucleotides in the gene. The intron-exon junctions have canonical GT-AG donor-acceptor splice site sequences and there are pyrimidine-rich sequences immediately upstream of the 3′ splice sites which is common in mammalian introns (58). The rat P2 gene has a short (57 bp) noncoding first exon as do the genes for mouse L30 (38 bp; reference 42) and L32 (46 bp; reference 43), and human S14 (54 bp; reference 45); the first exons in the human S17 (46) and mouse L7a (47) genes are even shorter, i.e. 28 and 29 bp respectively, but encode a single amino acid; the only substantial exception is mouse S16 (44) which has a first exon of 101 bp that encodes 16 amino acids. Intron 3 has (at positions 1591−1769) a sequence of 179 bp that has 85% identity with the mouse *Alu*-like B2 repetitive element (59); similar *Alu*-like nucleotide sequences have been found in the introns of other mammalian ribosomal protein genes (43−46). The open reading frame begins at the second nucleotide of exon 2 and ends in exon 5 (Figure 3). The sequence of 115 amino acids encoded in the exons is identical to that deduced from the cDNA (25).

There are two open reading frames in the 5′ flanking region of the P2 gene (Figure 2) that could encode proteins of 124 amino acids (positions −534 to −160) and of 202 residues (positions 255 to −354, i.e. the protein is encoded in the opposite orientation). There are no proteins related to the sequences encoded in these two open reading frames in the data banks. There is a shorter open reading frame (56 amino acids) in intron 3 at positions 1096 to 1266.

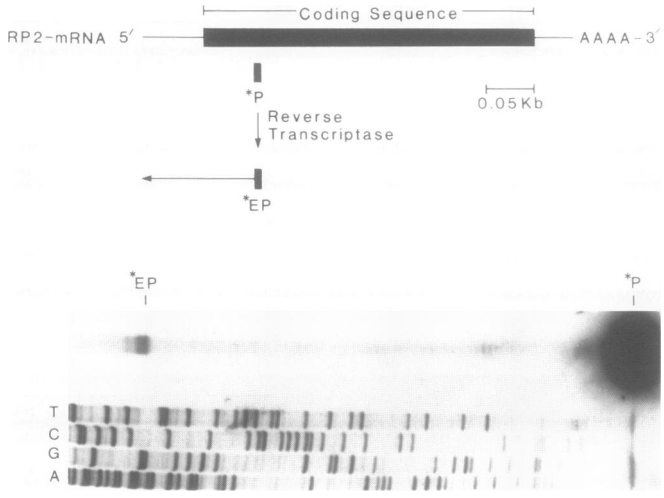

Figure 3. Determination of the site of the initiation of transcription of a rat ribosomal protein P2 gene by primer extension. A synthetic oligodeoxynucleotide (an 18-mer) corresponding to the sequence of nucleotides at positions (3′) 40 to 57 (5′) in the P2 cDNA (64) was made radioactive at the 5′ end and used as a primer with reverse transcriptase and poly(A)$^+$mRNA (cf. the schematic at the top). The product from the extension of the primer (*EP) and the unextended primer (*P) were resolved by electrophoresis in 6% polyacrylamide-urea gels. The positions of nucleotides in an unrelated DNA fragment (rat ribosomal protein S6) which was analyzed on a sequencing gel using [^{35}S]dATP and Sequenase served to establish the length of the extended primer (*EP).

Determination of the site of the initiation of transcription of a ribosomal protein P2 gene

A synthetic oligodeoxynucleotide primer (an 18-mer) that replicates positions (3′) 40 to 57 (5′) in the P2 cDNA was made radioactive at the 5′ end with polynucleotide kinase (56), annealed to poly(A)$^+$mRNA, and extended with reverse transcriptase (57). The extension products had 115 and 116 nucleotides (Figure 3). Approximately 75% of the primer extended DNA mapped at the second C in the pyrimidine sequence 5′-CCTTTC-3′ in the first exon; the remainder (approximately 25%) at the preceding C (Figure 3). The seven mammalian ribosomal protein genes that have been analyzed initiate transcription at a C residue in a polypyrimidine stretch (42−48); in the P2 gene this C is in a sequence of 10 pyrimidines (5′-TCTTCCTTTC-3′ at positions −5 to +5) that is flanked by GC-rich regions (Figure 2); these are hallmarks of mammalian ribosomal protein genes.

Characterization of the 5′ flanking region of a rat ribosomal P2 gene

Transient expression of a mouse ribosomal protein L32 gene required only 36 nucleotides upstream of the cap site suggesting that the promoter is close to the latter (60). The P2 gene has no canonical TATA box in the −20 to −30 region, a characteristic that it shares with the other mammalian ribosomal protein genes (42−48); however, all seven genes have an altered but recognizable form of the sequence. The rat P2 gene has, preceding the initiation site, a pyrimidine stretch followed by a GC-rich region with an embedded TTTA (at positions −30 to −27); the last could be a degenerate TATA box and hence the site of the binding of RNA polymerase II.

The protein factor Sp1 binds in a sequence specific manner to DNA and enhances by 10- to 50-fold the transcription by RNA

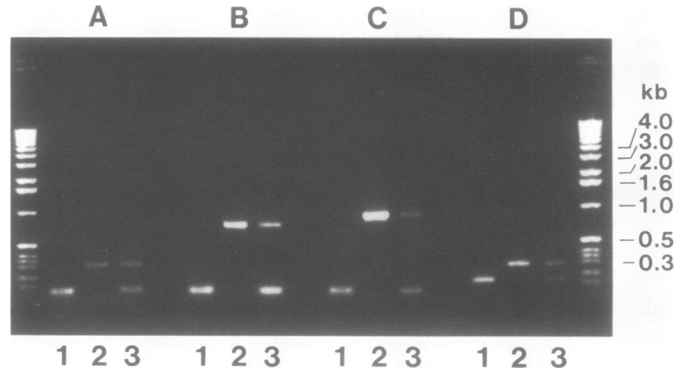

Figure 4. Analysis of a rat ribosomal protein P2 multigene family. The DNA obtained by amplification in the PCR using synthetic oligodeoxynucleotide primers that spanned introns in the P2 gene was analyzed by electrophoresis in agarose gels. Four sets of primers (designated P and RP for primer and reverse primer respectively) were selected: The primers in *A*, span intron 1 and include the 3′ end of exon 1 and the 5′ end of exon 2 (P11−31 and RP300−317); in *B* they span intron 2 (P335−351 and RP1093−1109); in *C* they span intron 3 (P1069−1085 and RP1986−2003); and in *D* they span intron 4 (P1956−1973 and RP2226−2243). The DNA for the PCR was: *lanes 1*, RP2-9 cDNA; *lanes 2*, RP2-12-9 DNA; *lanes 3*, rat genomic DNA. On the left and on the right there are 1 kb DNA ladders that serve as molecular size standards.

polymerase II of selected genes (61). The Sp1 consensus recognition sequence is GGGCGG and its inverted form CCGCCC. The rat ribosomal protein P2 gene has two potential Sp1 responsive elements in its 5′ flanking region: There is a high affinity binding site, TGGGCGGGGC, at positions −308 to −299; and a medium affinity binding sequence, CCCCCGCCCA (inverted form), at positions −26 to −17 just 3′ to the putative degenerate TATA-box, i.e. the TTTA sequence at −30 to −27. Whether Sp1 binds to these cis-acting sequences and enhances the transcription of the P2 gene has not been tested. The mouse ribosomal protein S16 gene has a Sp1 element 165 nucleotides upstream of the cap site and the factor enhances transcription two-fold (62).

There is at positions −81 to −78 in the P2 gene (Figure 2) the sequence CAAT (the larger context is GTCAAT). This motif occurs only in the mouse L7 gene (48) of the mammalian ribosomal protein genes that have been examined. However, a closely related sequence, CCAAT, has been observed at the same location in some cellular and viral genes (63) and is believed to participate in the regulation of the activation of transcription by binding a protein designated CTF for CAAT-binding transcription factor (61).

Finally, the P2 gene like the other mammalian ribosomal protein genes has in the upstream 250 nucleotides clusters of AT- and GC-rich sequences.

Analysis of the rat ribosomal protein P2 multigene family

Perry and colleagues (42−44) were the first to document that the mammalian ribosomal protein gene families have a single intron-containing transcribed member and 7 to 20 retroposon pseudogenes. The rat P2 family has approximately 9 genes (25) one of which has now been isolated, analyzed and shown to contain introns (Figure 2). We wished to determine if any of the others had introns. The strategy was to use PCR to amplify the genes employing primers that spanned adjacent exons. If there is a second intron-containing P2 gene the amplified DNA should be longer than the corresponding nucleotide sequence in the cDNA and should differ in length from that of the same region of the P2 gene that was sequenced. Four primer sets were chosen; they bridged exons 1 and 2, 2 and 3, 3 and 4, and 4 and 5 (Figure 4). In each case, amplification of the P2 cDNA (lanes 1, Figure 4) gave a single band of the expected length; amplification of P2 genomic DNA, i.e. RP2-12-9, with the same sets of primers gave in each experiment a single band that corresponded to the length of short regions of the adjacent exons and the included intron (lanes 2, Figure 4); finally, amplification of rat genomic DNA with the same primer sets gave two bands (lanes 3, Figure 4). The larger of these two amplified DNAs was the same length as the corresponding region of the P2 gene that had been sequenced whereas the smaller was the same length as the relevant region of the cDNA; the latter must derive from intron-lacking members of the P2 multigene family and are presumed to be retroposon pseudogenes. To the extent that introns define the functional, transcribed gene in a multigene family and to the extent that the lack of introns define genes that do not function there is one active rat ribosomal protein P2 gene. One reservation is required: It is possible, but most unlikely, that there is a second functional P2 gene that has four introns that are all exactly the same length as those in the gene that was sequenced. Thus, it is reasonable to assume that the P2 family has a single functional member and approximately 8 inactive pseudogenes.

There is one surprising aspect of the results in this experiment (Fig. 4) and it is the deviation from expectation in the intensity of the bands. In lane 3 of Fig. 4 part D the two bands are nearly the same size, 168 and 288 base pairs, and have almost the same intensity. The smaller band in our interpretation was amplified from the P2 pseudogenes and the larger one from the functional gene. There are approximately eight pseudogenes and only one functional P2 gene, therefore, the smaller band should be about eight times more intense. A similar discrepancy in a similar experiment was observed before (50), although, it was not commented on. Our tentative explanation is that the disparity derives from the hybridization conditions: The PCR primers (18-mers) used in the amplification reaction were annealed to genomic DNA at 55°C well above the calculated T_m of 45°C. These are very stringent conditions unlikely to tolerate even a single mismatch; pseudogenes are prone to mutations (58). Hence, we expect that not all of the pseudogenes are amplified with this set of primers and that this explains the lack of a significant difference in the intensity of the bands in lane 3 of Fig. 4 part D.

The structures of 8 functional mammalian ribosomal protein genes have been determined. They are: mouse L30 (42); mouse L32 (43); mouse S16 (44); human S14 (45); human S17 (46); mouse L7a (47); mouse L7 (48); and now rat P2 (Figure 2). Although, the number is relatively small, only 8 of a total of 70 to 80, the similarities in their constitution are striking; this is especially the case when one examines the promoter regions. Most have a small first exon which may be noncoding as with the P2 gene. The transcriptional start sites are in each gene at a cytidine residue embedded in a pyrimidine tract and flanked by islands rich in guanidine and cytidine. None of these genes has a TATA box; however, the P2 gene has at the usual site (i.e. positions −30 to −27) TTTA which because of its similarity to the canonical sequence may function in RNA polymerase II binding. These shared features of promoter region architecture may account for the coordination of the transcription of the 70 to 80 mammalian ribosomal protein genes. However, this presumption must of necessity be conditional until a systematic and detailed survey has been made of cis-acting sequences in the 5′ flanking regions of the genes and until the attendant trans-acting factors have been isolated and characterized. This latter has only just begun (62, 64). Moreover, the structure of the mammalian ribosomal protein genes has not yet provided clues as to how the synthesis of the proteins is adjusted to that of the rRNAs.

There are two copies of P2 in mammalian ribosomes, a stoichiometry it shares only with P1; all other ribosomal proteins occur in molar amounts. Thus, ribosome biogenesis requires twice as much P1 and P2 as of the other proteins. Since, no feature of the architecture of the promoter region of the P2 gene has been uncovered yet that sets it apart from the other ribosomal proteins, it may be that the increased amounts of P2 that are required for ribosome biogenesis are obtained by regulation during translation of the protein's mRNA.

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