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Hypothesis

Structural basis for autogenous regulation of *Xenopus laevis* ribosomal protein L1 synthesis at the splicing level

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It is known that the injection of the *Xenopus laevis* ribosomal protein L1 gene into oocytes causes the accumulation of immature L1 transcripts due to a specific block of splicing of the second and third introns. In this paper the secondary structures of these introns in pre-mRNA have been constructed. It has been shown that they share homology with 28 S rRNA. The putative RNA-binding segment of L1 has also been predicted. These results are interpreted as the structural basis for autogenous regulation of *X. laevis* ribosomal protein L1 synthesis at the splicing level.

Ribosomal protein; RNA splicing; Nucleic acid conformation; ribosomal RNA; Protein conformation

1. INTRODUCTION

The co-regulated synthesis of ribosome components in *Escherichia coli* is determined by translational autoregulation of ribosomal protein biosynthesis [1]. This regulation is the result of protein binding to initiator regions of mRNAs structurally similar to the protein-binding ones of rRNA. The presence of analogous homologies of mRNA and rRNA secondary structures in yeast [2] suggests the possibility of translational regulation of ribosomal protein biosynthesis in eukaryotes. This possibility is confirmed by measurements of production of one of the yeast ribosomal proteins in a system with an increased dosage of the protein gene [3]. On the other hand, it was shown that the increase of gene copy number of L1 protein from *Xenopus laevis* [4] and of L32 from *Saccharomyces cerevisiae* [5] resulted in the accumulation of immature mRNAs still containing introns. These facts indicate that eukaryotes may have the bacterial-like autogenous mechanism of ribosomal protein biosynthesis regulation, which is characterized by the protein interaction with sequences in introns rather than with the mRNA initiator region and occurs at the level of RNA splicing [4,5]. Such autogenous regulation in eukaryotes, similar to that in bacteria, is based on the competition between rRNA and mRNA for protein binding.

In this paper, we have built the possible secondary structures of three introns of *X. laevis* L1-pre-mRNA. The comparison of these structures with fragments of rRNA allows one to reveal the pre-mRNA regions which may serve as L1-binding sites during autogenous regulation at the splicing level.

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2. METHODS

The mRNA secondary structures were constructed by a method of complementarity matrices described in [2]. The protein super-secondary structure α-helix-turn-α-helix was predicted by a method based on the stereochemical requirements of an amino acid sequence [6] obtained by modifica-

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tion of the stereochemical requirements of Ohlendorf et al. [7]. A segment having the best sequence agreement with the set of α-helix-turn-α-helix segments from [8] was chosen according to the algorithm described in [7].

3. RESULTS AND DISCUSSION

The gene coding for L1 protein from *X. laevis* contains 9 introns [9]. The increase of gene copy number results in the block of splicing of the second and the third introns [4]. Bearing this in mind we have constructed the possible secondary structures of these introns in pre-mRNA (fig.1). The figure also shows the possible structure of the fourth intron. The latter contains a sequence of 60 nucleotides, which is homologous to the fragment of the second intron [9]. Such sequences are present in the seventh and eighth introns as well, indicating that they may have some common function [9]. The construction of secondary structures (fig.1) shows that spatial arrangements of these sequences, at least in the second and the fourth introns, are not similar. However, the secondary structure formed by this fragment in intron 2 has an analog in *X. laevis* 28 S RNA, helices 86, 69 and 87 of the model of Clark et al. [10] (fig.2a,c). Moreover, intron 3 also includes a fragment whose secondary structure is homologous to this region of rRNA (fig.2b). These analogies suggest that L1 can block the splicing of introns 2 and 3 by interaction with their fragments shown in fig.2 and such interactions must be realized similarly to the interaction with 28 S RNA, the

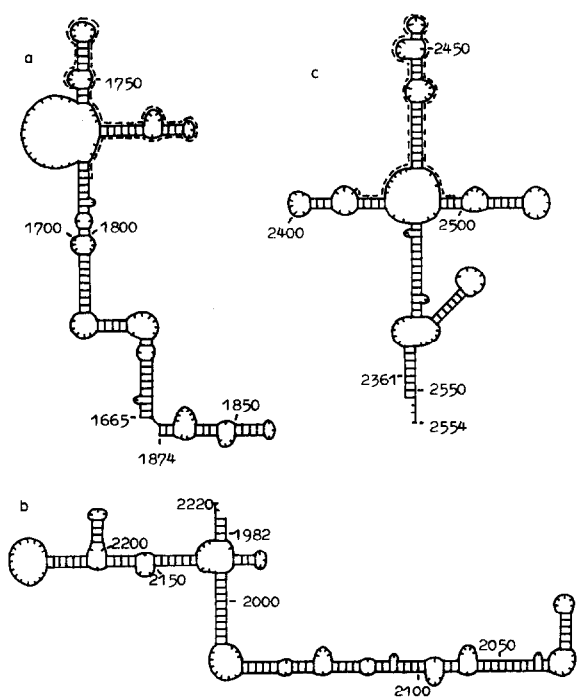

Fig.1. The possible secondary structures of L1-mRNA in the regions of the second (a), the third (b) and the fourth (c) introns. The homologous sequences [9] in introns 2 and 4 are shown by dashed lines.

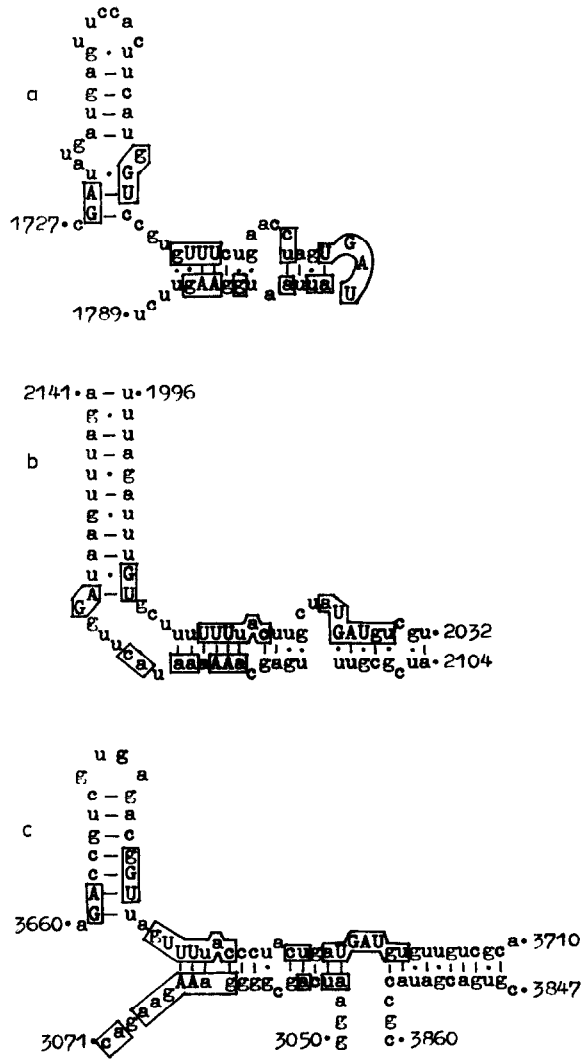

Fig.2. The fragments of secondary structures of L1-mRNA in introns 2 (a) and 3 (b) and of 28 S RNA (c) [10] from *X. laevis*. The sequences homologous in one of the mRNA fragments and in rRNA are boxed; the nucleotides conserved in all three structures are shown by capital letters.

fragment shown in fig.2c being the protein-binding site on rRNA.

In [2] it has been suggested that ribosomal repressor proteins can recognize RNA through the super-secondary structure α-helix-turn-α-helix, first found in DNA-binding proteins [8]. In *X. laevis* L1 the most probable localization of this structure is predicted (see section 2) to be in the segment 122–144 (other probable localizations are 79–101, 178–200, 123–145, 183–205, 247–269 in order of decreasing probability).

The proposed regulation mechanism is very similar to the regulation of ribosomal protein synthesis in *E. coli* [1]. Similarly to bacterial ribosomal repressor proteins, L1 protein from *X. laevis* binds to mRNA segments whose secondary and primary structures are homologous to those of protein-binding sites on rRNA. The only difference is that *X. laevis* L1 inhibits the maturation of mRNA in contrast to *E. coli* proteins which block the translation process. As noted in [5], such analogy is described well in terms of evolutionary development of eukaryotes from prokaryotes. The eukaryotic compartmentalization of protein synthesis in the cytoplasm and RNA synthesis in the nucleus does not interfere with autogenous regulation, because protein transport through the nuclear envelope is possible [1,3–5].

It is noteworthy that secondary structures homologous to the same rRNA fragment do exist in introns 2 and 3 in accordance with experimental data [4] on their participation in the regulation of mRNA processing and are not found in intron 4 in spite of the marked similarity of the primary structures of introns 2 and 4 [9]. This homology of primary structures extends to introns 7 and 8 [9] but these are much longer than introns 2–4, and the construction of their secondary structures without any additional data is ambiguous. On the other hand, we have built the possible secondary structure (not shown) of the initiator region of L1-mRNA (exon 1). We have failed to find a fragment homologous to this structure in 28 S mRNA.

The secondary structure homology of mRNA initiator regions and rRNA fragments in eukaryotes seems to be characteristic only for ribosomal proteins with translational regulation of biosynthesis; in yeast such a homology was found only for proteins coded by unsplit genes [2]. The further search for secondary structure homologies in mRNAs (or pre-mRNAs) and rRNAs along with the accumulation of sequenced genes may help in the elucidation of various aspects of both ribosomal protein biosynthesis regulation and protein-RNA interaction in the ribosome.

NOTE ADDED IN PROOF

This paper was being prepared when it was reported that introns 2 and 3 of the *Xenopus laevis* L1 pre-mRNA are involved in the regulation of the expression of the L1 gene [11]. It may be considered to support results.

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