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Drosophila Acidic Ribosomal Protein rpA2: Sequence and Characterization

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Abstract A cDNA encoding the *Drosophila melanogaster* acidic ribosomal protein rpA2 was cloned and sequenced. rpA2 is homologous to the *Artemia salina* acidic ribosomal protein eL12′. In situ hybridization to salivary gland polytene chromosomes localizes the rpA2 gene to band 21C. It is a single copy gene, with an mRNA of 0.8 kb. Two-dimensional gel electrophoresis of *Drosophila* ribosomal proteins followed by immuno-blotting showed that the rpA2 protein has an apparent relative mobility in SDS of 17 kD and an isoelectric point less than pH 5.0. Although the *Drosophila* gene rp21C may be the same as rpA2, the reported sequences differ. Comparisons of the aligned nucleotide sequences coding for the acidic ribosomal proteins rpA1 and rpA2 of *Drosophila* with those of other eukaryotes support the view of two separate, though closely related, groups of acidic proteins. Comparison with the *Artemia* homologues suggests that nucleotide identity may have been conserved by some constraint that acts in addition to the requirement for substantial similarity of amino acid sequences. © 1993 Wiley-Liss, Inc.

Key words: *Drosophila,* ribosome, acidic ribosomal protein, molecular cloning, sequence comparison

While most proteins in a ribosome are present as a single copy, the stalk of the large ribosomal subunit contains pairs of acidic proteins. The *E. coli* equivalent of these proteins are the four copies of the acidic protein L7/L12. L7 is the acetylated form of L12. This pair of (L7/L12)-heterodimers has been implicated in GTP-dependent processes and in initiation, elongation, and release factor function [Liljas et al., 1985]. The eukaryotic structural and functional analogues of L7/L12 are paired, multicopy, acidic proteins, consisting of 106–115 amino acids. The ribosomes of *Drosophila melanogaster, Artemia salina,* rat, rabbit, and wheat germ each contain two distinct acidic proteins which are partially phosphorylated [Vidales et al., 1981]. The acidic ribosomal proteins of each of these different eukaryotic species are functionally equivalent and can reconstitute the activity of *S. cerevisiae* core ribosomal particles, from which the acidic proteins have been removed.

The two acidic ribosomal proteins from *Artemia,* eL12 and eL12′, have been sequenced at both the peptide and cDNA levels. They share 22 identical amino acid residues at their C-termini [Amons et al., 1982]. eL12 and eL12′ are partially phosphorylated on serine, have isoelectric points of less than 4.5, and have relative electrophoretic mobilities by SDS-PAGE of 13.5 kD and 15 kD, respectively [van Agthoven et al., 1978]. Cross-linking studies indicate that each of these proteins exists as a homodimer [Uchiumi et al., 1987]. One of each type of homodimer is anchored to a third protein, PO, forming a pentameric complex which is equivalent to the $(L7/L12)_4$ L10 pentameric complex found in the large ribosomal subunit of *E. coli.*

The two acidic, monophosphorylated ribosomal proteins from *Drosophila* are less well characterized. Their relative mobilities by SDS-PAGE are similar to the two *Artemia* proteins and their isoelectric points are below 4.5 [Vidales et al., 1981]. The *Drosophila* ribosomal protein rpA1 is the homolog of *Artemia* protein eL12 [Qian et al., 1987]. It is encoded by a single-copy, intronless gene which localizes to polytene chromosome band 53CD. The expression of the 0.6 kb rpA1 mRNA is translationally regulated during early *Drosophila* embryogenesis [Kay and Jacobs-Lorena, 1985].

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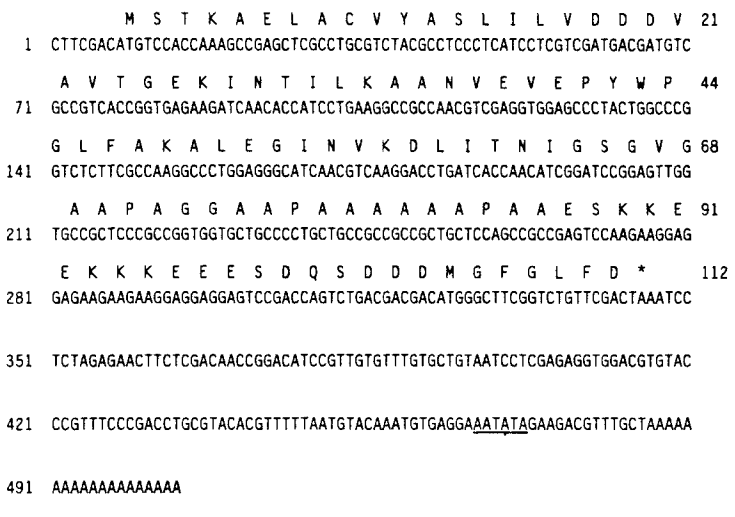

Fig. 1. The cDNA and amino acid sequences of *Drosophila* ribosomal protein rpA2 The cDNA sequence of rpA2 is shown with the amino acid translation above The polyadenylation signal is underlined Nucleotide positions are indicated along the left margin and amino acid residues along the right margin

A cDNA sequence for the presumed *Drosophila* homolog of the *Artemia* ribosomal protein eL12′ was reported without further characterization of the putative protein. The gene was named rp21C according to its chromosome location at band 21C [Wigboldus, 1987]. During the screening of a *Drosophila* lambda gt11 expression library we fortuitously isolated a cDNA clone for a similar but not identical sequence. We report correlations to the corresponding mRNA and protein, and to the homologous ribosomal proteins of other species. As the *Drosophila* homolog of the *Artemia* acidic ribosomal protein eL12 was called rpA1 [Qian et al., 1987], we refer to this *Drosophila* homolog of *Artemia* protein eL12′ as rpA2.

MATERIALS AND METHODS

The materials and methods used were as described in Olson et al. [1990]. Antiserum was raised against a velocity sedimentation fraction, enriched in protein X, of a 45% saturation $(NH_4)_2SO_4$ fraction of *Drosophila* Kc 7E10(0) cell culture medium. Further details of protein X will be described elsewhere. The clone X4 was isolated with the ProtoBlot Immunoscreening System (Promega), subcloned into pBluescribe (Stratagene), restriction mapped, and sequenced by the dideoxy-chain termination method at least twice on each strand and across all restriction sites used in subcloning. Gel compression artifacts were resolved by substituting inosine for dGTP. Sequences were aligned and maintained using the DB system [Staden, 1982]. A crude ribosome fraction was prepared from Kc 7E10(0) cells by a modified version of the method of van Agthoven et al. [1978]. The two-dimensional electrophoretic analysis was carried out in the first dimension in 9.5 M urea with a 3/10 ampholyte (Biorad) gradient. The mobility of rpA2 in SDS gel electrophoresis was estimated relative to Prestained Protein Molecular Weight Standards (low range, BRL). Blots of the resolved proteins were stained with the above antiserum, using alkaline phosphatase-conjugated second antibodies. Amino acid sequences were aligned pairwise with the local alignment program SEQHP [Goad and Kanehisa, 1982] and with the Multiple Sequence Alignment program of Feng and Doolittle [1987].

RESULTS AND DISCUSSION

A lambda gt11 cDNA library made from total *Drosophila* Kc 7E10(0) cell RNA [Blumberg et al., 1987] was screened with rabbit antiserum against a partially purified 170 kD *Drosophila* extracellular matrix component provisionally named protein X. The 0.5 kb clone X4 was isolated and sequenced (Fig. 1). Codon usage analysis [Staden and McLachlan, 1982] using a *Drosophila* codon bias table was used to identify the correct reading frame.

Nucleotide and Amino Acid Sequences of rpA2

The predicted protein sequence of clone X4 was initially compared to the NBRF protein

database of January 1988, revealing a strong similarity with *Artemia salina* ribosomal protein eL12′ and a weaker similarity with *Artemia salina* ribosomal protein eL12. Subsequently, the amino acid sequence was also used to search the NBRF protein database of March 1991, and additional proteins were identified: *Drosophila* ribosomal proteins rpA1 and rp21C, and acidic ribosomal proteins from several eukaryotic and archaebacterial species.

We designate the *Drosophila* protein that clone X4 encodes as ribosomal protein rpA2 for the following reasons. The predicted initiation Met residue aligns with the first Met residue of the homologous *Artemia* ribosomal protein eL12′, and the nucleotide sequence surrounding the initiation codon fits the consensus for a ribosomal binding site [Kozak, 1986]. The predicted rpA2 amino acid sequence is similar over its entire length to *Artemia* ribosomal protein eL12′. The predicted amino acid sequence of *Drosophila* rpA2 is 112 amino acids long, has a net negative charge of 10, and has a molecular weight of 11,513. For comparison, the amino acid sequence of *Artemia* eL12′ is 110 amino acids long, has a net negative charge of 14, and has a molecular weight of 11,538. The 3′-untranslated region contains a polyadenylation signal followed 14 bp downstream by a poly(A) tail (Fig. 1). The polyadenylation signal, AATATA, deviates from the consensus AATAAA [Proudfoot and Brownlee, 1976], but there is precedence for usage of this sequence in *Drosophila* ribosomal protein rp49 [O'Connell and Rosbash, 1984].

mRNA for rpA2

On blots of either total RNA from *Drosophila* Kc 7E10(0) cells, or of poly(A)+ RNA from 4–6 hour *Drosophila* embryos, rpA2 cDNA probes hybridized only to a 0.8 kb band (Fig. 2). With single-stranded RNA probes only the antisense probe hybridized. The mRNA for protein X, against which the antiserum had been raised, is at least 5 kb [personal communication, Dr. R. Nelson, UCLA] and was not detected with these probes, even though subsequent hybridization with a different probe showed that sufficiently large mRNA was present on the blots.

rpA2 Gene

In situ hybridization of an rpA2 cDNA probe to *Drosophila* polytene chromosomes gave only a single band at locus 21C at the tip of the second chromosome. Therefore, the two *Drosophila* acidic ribosomal proteins rpA1 and rpA2 are encoded by separate genes on different chromosomes. Hybridization of cDNA clone X4 to a Southern blot of digested *Drosophila melanogaster* OreR genomic DNA indicated that rpA2 is a single copy gene (not shown).

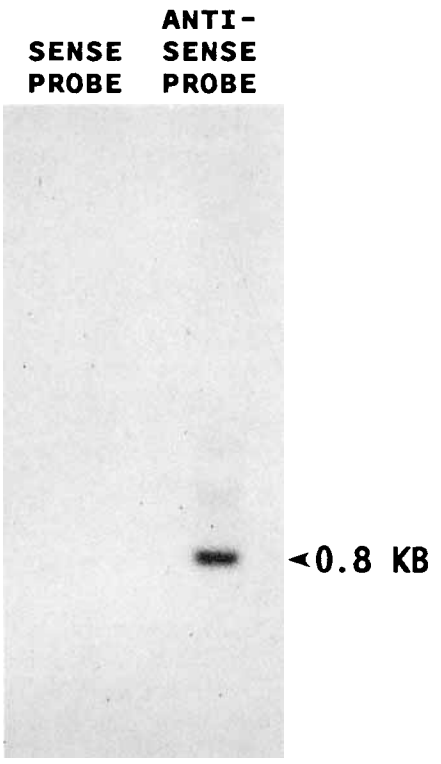

Fig. 2. Northern blot analysis of rpA2. Aliquots (2 μg) of 4–6 h *Drosophila* embryo poly(A)+ RNA were denatured in glyoxal and DMSO, electrophoresed on a 0.8% agarose gel, and capillary blotted onto nitrocellulose. The filters were probed with single-stranded [^{32}P]-labelled RNA probes which correspond to the sense (coding) or the antisense (non-coding) strand of the rpA2 cDNA. Not shown are the RNA size ladder (BRL, 0.24–9.5 kB) and rRNA bands apparent by UV shadowing.

The *Minute (M)* loci of *Drosophila* are a class of unlinked mutations whose common phenotype is ascribed to impaired protein synthesis caused by defective ribosomal protein genes [Kay and Jacobs-Lorena, 1987]. This hypothesis has been verified for the rp49 gene [Kongsuwan et al., 1985]. The *Drosophila* protein rpA2 maps at the well-established *Minute* locus *M(2)21C* and near the potential locus *M(2)21AB* [Lindsley and Zimm, 1990].

rpA2 Protein

Protein X and a ribosomal fraction were prepared from *Drosophila* KC cells and electrophoresed on, respectively, 5% and 17.5% polyacryl-

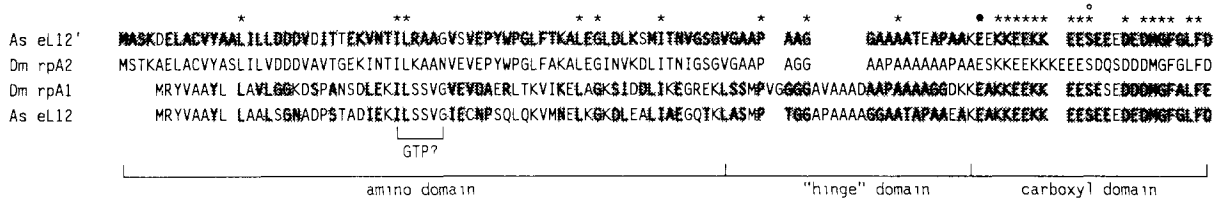

Fig. 3. Amino acid sequence alignment of *Drosophila* and *Artemia* acidic ribosomal proteins. The amino acid sequences of *Artemia* eL12' (As eL12'), *Drosophila* rpA2 (Dm rpA2), *Drosophila* rpA1 (Dm rpA1), and *Artemia* eL12 (As eL12) were aligned using the Multiple Sequence Alignment program of Feng and Doolittle [1987]. An asterisk above the sequences indicates amino acid identity in all four proteins. The Ser residue which is known to be phosphorylated in As eL12' and As eL12 is indicated by ° above the sequences. Amino acid residues which are either identical or conservatively substituted relative to the Dm rpA2 sequence are indicated by shading. The putative GTP binding site, as well as the three structural domains proposed for eukaryotic acidic ribosomal proteins (see text), are indicated.

amide-SDS gels. Western blots reacted with rabbit antiserum raised against partially purified protein X showed an apparent Mr-17 kD band in the ribosomal fraction and, separately, the 170 kD protein X. Two-dimensional electrophoretic separation indicated that the immunoreactive ribosomal protein has a pI less than 5.0 (not shown). These results are consistent with the expected properties of ribosomal protein rpA2. The acidic ribosomal proteins from *Drosophila* have isoelectric points not greater than pH 4.5, and their relative mobilities by SDS-PAGE are reported as 13.5 kD and 15.0 kD [Vidales et al., 1981]. The sequence of rpA2 predicts a molecular weight of 11.5 kD and an acidic protein with a net charge of 10 negative residues over its length of 112 residues. Anomalous migration of acidic proteins during SDS-PAGE has been well documented and can vary significantly with specific conditions [Eley et al., 1979; Olson et al., 1990]. The *Drosophila* rpA1 protein and the *Artemia* acidic ribosomal proteins also migrate in SDS-PAGE as if they had larger molecular weights than those calculated from their amino acid sequences.

Sequence Comparison of rpA2 and rp21C

The nucleotide sequence of Figure 1 differs from that of rp21C at positions 33 (G vs. C), 34 (C vs. G), 165 (G vs. C), 166 (C vs. G), and 357 (G vs. A). These differences cause two changes of amino acids and mostly occur in GC-rich regions where gel-compression artifacts of sequencing bands can lead to errors. We took special precautions to avoid such errors, and the resulting amino acid sequence of rpA2 is more consistent with homologous proteins. Specifically, residue 9 in several homologous proteins is an invariant Cys, as it is in rpA2, while this is a non-conserved Ser in rp21C. The residue at position 53 in several aligned homologs is a consensus Gly, as it is in rpA2, while rp21C has an Ala at this site. Presumably the two sequences, which map to the same chromosome site 21C, represent the same gene of *Drosophila melanogaster,* though strain-specific differences cannot be ruled out. (The rpA2 sequence was obtained from the Oregon R strain; the source of the rp21C sequence is not published.) Restriction site polymorphisms were observed outside of the rpA1 gene, but the coding regions of rpA1 from the Canton S and Oregon R strains of *Drosophila melanogaster* are identical with the exception of 1 conservative Gly to Ser substitution [Qian et al., 1987]. The term rpA2 seems better for this protein than rp21C, because a quite different *Drosophila* ribosomal protein, with its gene at chromosome locus 80C, is already established in the literature as protein rp21 [Kay et al., 1988].

Comparison of rpA2 With Other Acidic Ribosomal Proteins

The amino acid sequence of *Drosophila* rpA2 is consistent with the three structural domains proposed for other eukaryotic acidic ribosomal proteins [Liljas et al., 1985] in homology to the domains of the *E. coli* protein (indicated in Fig. 3). A putative GTP binding site is conserved in the amino acid sequence of the amino domain of approximately 65 residues. The hinge region of about 25 amino acids is rich in Ala, Pro, and Gly, and the carboxyl-terminal domain of approximately 25 residues has a high proportion of the charged amino acids Glu, Asp, and Lys, combined with a quite negative net charge. The carboxyl domain contains a conserved Ser (Fig. 3), which is phosphorylated in the *Artemia* homologues [Maassen et al., 1985]. Though the *Dro-*

sophila acidic ribosomal proteins are phosphorylated, the site of phosphorylation is not known [Vidales et al., 1981].

The *Drosophila* rpA2 and *Artemia* eL12′ proteins are 70% identical in amino acid sequence, or 82% similar after allowing for conservative replacements [Dayhoff, 1978] (Fig. 3). There is also a 68% nucleotide identity, so that even at the third base many codons have been retained, especially in the carboxyl coding region. While the amino acid similarities extend over the total proteins, they are particularly strong at the carboxyl ends, where 17 out of the last 24 amino acids are identical in all four sequences (Fig. 3). The carboxyl-most 8 amino acids are conserved in the homologous members of the paired acidic ribosomal proteins from a wide range of species, including human, rat, and yeast. Strikingly, the nucleotide identity between rpA1 and rpA2 is confined to the coding region and does not extend into the 3′ untranslated portions of the *Drosophila* mRNAs. The aligned sequences of rpA1 and rpA2 were examined for silent nucleotide changes, which would not cause changes of amino acids, by the method of Perler et al. [1980]. The actual number of silent nucleotide changes was calculated as a percentage of the total possible number of silent changes for the carboxyl portions (50%) and, separately, for the remaining, non-carboxyl regions of the proteins (80%). The ratio of these two percentages was 0.6 (0.64 if the statistical corrections of Perler et al. [1980] were applied and 0.59 without this correction). Thus, of the possible silent nucleotide changes only 0.6 as many occurred in the carboxyl portion of the rpA1/rpA2 pair as in the total remaining portions of these two proteins. The conservation of nucleotide sequence between portions of *Drosophila* rpA1 and rpA2, and between *Drosophila* rpA2 and *Artemia* eL12′ transcripts, might be functionally important for transcriptional or translational control.

The *Drosophila* rpA1 and rpA2 ribosomal proteins share 39% amino acid identity and 53% nucleotide identity over their aligned coding regions. Thus they are closely related, yet each also clearly belongs to a different set of homologous acidic ribosomal proteins. The *Drosophila* rpA1 protein is homologous to the *Artemia* eL12 protein, and the *Drosophila* rpA2 protein is homologous to the *Artemia* eL12′. There is a slightly lower degree of amino acid identity, 62%, between rpA1 and eL12 than the 70% identity between rpA2 and eL12′. The two *Artemia* proteins eL12 and eL12′ share 49% amino acid identity and 58% nucleotide identity, so that they have diverged less from each other than the *Drosophila* rpA1 and rpA2 proteins of 39% amino acid identity. Remarkably, the aligned nucleotide coding sequences of even the *non*-homologous cross-pairs [*Drosophila* rpA2: *Artemia* eL12] and [*Drosophila* rpA1: *Artemia* eL12′] have, respectively, 51% and 54% nucleotide identity. For comparison, the highest nucleotide identity, 68%, is between the aligned sequences of the homologues *Drosophila* rpA2 and *Artemia* eL12′. We conclude that some unexplained restraint may have conserved the nucleotide coding sequences of these acidic ribosomal proteins in addition to a constraint of amino acid conservation of the carboxyl end of the proteins. As the preferred codon usage is not the same in *Drosophila* and *Artemia* it is unlikely that a common distribution of tRNAs restrained silent nucleotide changes. Interestingly, the mRNA for rpA1 is specially sequestered as a translationally inactive nucleoprotein complex during early *Drosophila* development [Kay and Jacobs-Lorena, 1985, 1987; Hongo and Jacobs-Lorena, 1991]. The state of the rpA2 mRNA during this time is unknown.

The relationships of the *Drosophila* proteins rpA1 and rpA2 to other eukaryotic acidic ribosomal proteins were explored by pairwise alignment using the program SEQHP, and the results are given in Table I. We conclude from this tabulation that one member of each homologous pair of acidic ribosomal proteins of a species can reasonably be assigned to be more similar to the *Drosophila* rpA1 protein and the other member to the *Drosophila* rpA2 protein, according to their different numbers of identical amino acids in the pairwise comparisons with the two *Drosophila* proteins. The relationship of the four acidic ribosomal proteins of *S. cerevisiae* to the two *Drosophila* proteins is in accord with the proposal for two groups of these proteins [Shimmin et al., 1989a]. Thus, although the paired proteins of any one species are closely related to each other, each protein can be assigned to a separate subgroup of the eukaryotic acidic ribosomal proteins [Ramirez et al., 1989; Shimmin et al., 1989b].

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TABLE I. Eukaryotic Ribosomal Proteins Homologous to *Drosophila* rpA1 and rpA2*

	Dm rpA1 (113)	Dm rpA2 (112)	As L12 (111)	Rn rpP2 (111)	Hu rpP2 (115)	Sc L45 (110)	Sc rpA2 (106)
Dm rpA2 (112)	42/104 15 SD	112/112 58 SD	40/104 17 SD	38/103 12 SD	42/103 12 SD	39/106 17 SD	42/106 18 SD
Dm rpA1 (113)	113/113 52 SD	42/104 15 SD	69/113 35 SD	65/113 29 SD	69/113 34 SD	56/113 29 SD	53/113 22 SD
As L12p (110)	43/109 14 SD	78/112 42 SD	50/102 17 SD	34/101 11 SD	34/60 20 SD	33/104 11 SD	111/101 14 SD
Rn rpP1 (115)	37/108 14 SD	69/112 36 SD	39/105 16 SD	33/105 12 SD	34/61 18 SD	27/105 14 SD	40/108 17 SD
Hu rpP1 (114)	37/108 13 SD	69/112 39 SD	39/105 14 SD	32/105 11 SD	35/61 18 SD	28/105 12 SD	41/108 16 SD
Sc L44p (106)	33/89 10 SD	43/103 27 SD	31/74 11 SD	28/82 11 SD	33/96 9 SD	50/96 16 SD	49/96 19 SD
Sc rpA1 (106)	29/89 9 SD	57/112 24 SD	38/98 11 SD	24/50 13 SD	34/95 11 SD	37/100 11 SD	43/100 10 SD

*The amino acid sequences of acidic ribosomal protein pairs from several eukaryotic species were aligned using SEQHP, and each sequence was shown to be homologous to either Dm rpA1 or Dm rpA2 (shaded). The total length of each amino acid sequence is given in parentheses below the abbreviated name of each protein. The number of identical residues/the length of the aligned sequences is reported in the top half of each box. The statistical significance of each local alignment was determined with the program SEQDP and is reported in the bottom half of each box in standard deviation units (SD) relative to the mean score obtained when one sequence was randomly scrambled and realigned to the other sequence 100 times. (All possible combinations of scrambled amino acid sequences will not occur in 100 scrambles; therefore, the SD values of a given sequence compared with itself varies within this table.) As L12, *Artemia* ribosomal protein eL12 (NBRF accession number A25208) [Maassen et al., 1985]; As L12p, *Artemia* eL12′ (B25208) [Maassen et al., 1985]; Dm rpA1, *Drosophila* rpA1 (A26401) [Qian et al., 1987]; Dm rpA2, *Drosophila* rpA2; Hu rpP1, human rpP1 (B27125) [Rich and Steitz, 1987]; Hu rpP2, human rpP2 (C27125) [Rich and Steitz, 1987)]; Rn rpP1, rat rpP1 (S08022); Rn rpP2, rat rpP2 [Lin et al., 1982)]; Sc L44p, *Saccharomyces* L44p (C28104) [Remacha et al., 1988]; Sc L45, *Saccharomyces* L45 (A28104) [Remacha et al., 1988]; Sc rpA1, *Saccharomyces* rpA1 (S00678) [Mitsui and Tsurugi, 1988a]; Sc rpA2, *Saccharomyces* rpA2 (S00679) [Mitsui and Tsurugi, 1988b].

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