The Acidic Phosphoproteins from *Saccharomyces cerevisiae* Ribosomes. NH_2-Terminal Acetylation Is a Conserved Difference between P1 and P2 Proteins†

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ABSTRACT: Isoelectrofocusing gels of acidic ribosomal proteins from most yeast strains reveal the presence of up to 10 bands which are the product of only 4 genes. The proteins have been characterized by NH_2-terminal amino acid sequencing, specific antibodies, HPLC, and by taking advantage of acidic protein-defective yeast strains obtained by gene disruption methods. The four most basic proteins coincide with the phosphorylated and dephosphorylated forms of the YP2 proteins, YP2α and YP2β, formerly named L44 and L45. Amino-terminal sequencing has shown that these two polypeptides have free amino-terminal ends starting at the first methionine residue. The bands defined earlier as L44′ correspond to the phosphorylated and dephosphorylated processed forms of protein YB1β lacking the first eight amino acids. The formation of this truncated YP1β form seems to be stimulated by salt during protein extraction and is also favored by some modifications at the amino termini of the protein. On the other hand, the previously uncharacterized band, called Ax, corresponds to an NH_2-terminal acetylated form of YP1β which starts at the serine in the second position of the nucleotide-derived sequence. Finally, the most acidic band is the phosphorylated product of the fourth acidic protein gene. This protein, called YP1α, which is very poorly stained by silver and Coomassie blue, has not been characterized in detail previously. It is also monophosphorylated in the ribosome and, like YP1β, is present as an NH_2-terminal acetylated form starting at the second serine residue. YP1 proteins, but not YP2 proteins, show a p*I* change when obtained from *Saccharomyces cerevisiae* NAT1, an NH_2-terminal acetyltransferase-defective mutant, confirming the N^{α}-acetylated condition of the first polypeptides. The difference in the amino end of the two acidic protein groups parallels the situation found in bacterial acidic proteins L7 and L12 and suggests that this conserved structural feature must have a significant role in the acidic proteins' function.

The ribosomes contain, among many strongly basic proteins, a set of very acidic and highly conserved polypeptides which are, in addition, the only multicopy components of the particle. The bacterial acidic proteins, called L7/L12, have been studied in some detail, and they seem to play an important role in the interaction of the ribosome with the supernatant factors during protein synthesis [see for a review Möller and Maassen (1986)]. In eukaryotic organisms, the acidic proteins, although playing a similar functional role, have some peculiar characteristics which suggest their implication in other cellular mechanisms. Thus, contrary to their bacterial counterparts, the eukaryotic acidic proteins are found phosphorylated in the ribosome (Juan-Vidales et al., 1981, 1984; McConnell & Kaplan, 1982; Sanchez-Madrid et al., 1981) and dephosphorylated in an unusually large pool of them present in the cell cytoplasm (Mitsui et al., 1988; Sanchez-Madrid et al., 1981; van Agthoven et al., 1978; Zinker, 1980). Moreover, the eukaryotic polypeptides seem to be involved in an exchange process between the ribosome and the cytoplasmic pool (Tsurugi & Ogata, 1985; Zinker & Warner, 1976). This process might be mediated by a phosphorylation–dephosphorylation mechanism since this modification has been shown to affect drastically the affinity of the proteins for the ribosome "in vitro" (Juan-Vidales et al., 1984) as well as "in vivo" (Naranda & Ballesta, 1991). These data are compatible with the existence in higher cells of a mechanism that can control the ribosome activity by regulating the amount of acidic protein bound to the particle, and, in fact, the amount of these ribosomal components present in the ribosome seems to be related to their activity situation (Saenz-Robles et al., 1990).

The analysis of *Saccharomyces cerevisiae* ribosomal proteins showed initially the presence of two acidic polypeptides, called either L44 and L45 (Kruiswijk & Planta, 1975) or L35 and L36 (Zinker & Warner, 1976). Afterward, the presence of an additional polypeptide, which was called L44′ (Juan-Vidales et al., 1984), was found in the etanol–ammonium chloride extracts of the yeast ribosomes, which specifically removes the acidic proteins from the particles (Sanchez-Madrid et al., 1979). The three polypeptides were purified and characterized (Juan-Vidales et al., 1984), and specific polyclonal (Juan-Vidales et al., 1984) and monoclonal (Vilella et al., 1991) antibodies to the proteins have been obtained. The genes encoding these three proteins have also been cloned by screening λgt11 expression libraries with the specific antisera (Remacha et al., 1988).

The three proteins have a highly conserved carboxyl terminal, and by using synthetic oligonucleotides derived from this amino acid sequence as probes, the identification of an additional gene encoding a fourth polypeptide which has the same carboxyl end, and shows all the characteristics of a typical acidic ribosomal protein, was also reported (Mitsui & Tsurugi, 1988; Newton et al., 1990). The product of this gene has not been detected in previous reports on yeast acidic ribosomal proteins, but "in vitro" coupled transcription–translation of the cloned gene showed the expression of a polypeptide with a low p*I* (Mitsui & Tsurugi, 1989) moving to a region of the isoelectrofocusing gel where some still not well-characterized

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acidic polypeptides, generically called Ax, are present (Juan-Vidales et al., 1984; Sanchez-Madrid et al., 1979).

Recently, a uniform nomenclature for the eukaryotic acidic proteins was proposed in which proteins L44′, L44, and L45 are called P1β, P2α, and P2β, respectively, and the product of the fourth gene, P1α (Wool et al., 1991).

An implication of the yeast acidic proteins in a possible regulatory mechanism of the ribosome has been proposed (Saenz-Robles et al., 1990; Naranda & Ballesta, 1991), but the individual role that each one of the components of this polypeptide family is playing in this process is still unsolved. A characterization of the still not completely well-defined set of proteins is a previous requirement for any further assessment of their respective functional roles.

MATERIALS AND METHODS

Organisms and Growth Conditions. Saccharomyces cerevisiae Y166 and W303 have been used as parental strains. Obtention of *S. cerevisiae* W303D6 and W303D7, lacking proteins L44′ (YP1β) and YP1α, by gene disruption methods has been previously described (Remacha et al., 1992). Cells were grown either in rich YEPD medium (1% yeast extract, 2% peptone, and 2% glucose) or in minimal SD medium (Sherman et al., 1983) supplemented with essential requirements. Cells were harvested at exponential growth phase, washed with 20 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 60 mM NH_4Cl, and 5 mM β-mercaptoethanol (buffer 1), and either used immediately or stored frozen at -70 °C.

Preparation of Ribosomes and Ribosomal Proteins. Cells were broken by grinding with sea sand in buffer 1 and the extracts centrifuged at 15K rpm for 15 min in a Sorvall SS-34 rotor to remove the cells' debris. The supernatant was centrifuged again at 100000*g* for 2 h, an the pelleted ribosomes were resuspended in 20 mM Tris-HCl, pH 7.4, 100 mM $MgCl_2$, 500 mM NH_4Cl, and 5 mM β-mercaptoethanol (buffer 2). The particles were then washed by centrifugation through 20–40% sucrose in buffer 2 and finally resuspended in buffer 1 and stored at -70 °C.

Acidic proteins were extracted from the ribosomes by washing with 50% ethanol and variable concentrations of ammonium chloride ranging from 0.3 to 1.0 M in the conditions previously described (Sanchez-Madrid et al., 1979). A split protein fraction (SP) containing mostly acidic proteins and a core particle deprived of acidic proteins were obtained. Proteins were precipitated with acetone and dissolved in appropriate buffer.

Electrophoretical Techniques. Electrophoresis was carried out in SDS–polyacrylamide gels according to Leammli and Favre (1973). Electrofocusing was performed in 5% polyacrylamide gel slabs (150 × 130 × 2 mm) using 2% Pharmacia ampholytes, pH 2.5–5.0, over a water-refrigerated plate, in the cold room, and applying a constant current of 6 mA until the voltage reached 600 V and then a constant voltage of 250 V for 14 h. Proteins were detected by staining of the gels with either silver or Coomassie blue.

Antibodies and Immunological Methods. Monoclonal antibodies (mAbs) were prepared from hybridomas obtained by fusing spleen cells from yeast acidic protein-immunized Balb/c mice to myeloma cells as described elsewhere (Vilella et al., 1991). Fused cells were grown in Eagle's medium containing 15% fetal calf serum, and the culture supernatants were tested for anti-acidic protein activity by ELISA. The cells giving a positive reaction were cloned to limit dilution. The specificity of the mAbs was determined by ELISA, using purified proteins, and by immunoblotting of ribosomal proteins resolved by SDS–polyacrylamide gel electrophoresis and isoelectrofocusing. Monoclonal specificity was confirmed using phages carrying cDNA inserts expressing the yeast acidic proteins [see Vilella et al. (1991) for details].

Indirect and inhibition ELISA, for estimation of acidic proteins in cellular fractions were performed as previously indicated (Vilella et al., 1991). Immunoblots were carried out according to Towbin et al. (1979).

HPLC of Acidic Proteins. Separations of acidic proteins were performed as reported (Saenz-Robles et al., 1988). Proteins extracted from ribosomes by ammonium chloride–ethanol washing were injected into a C3 alkyl-chain-bonded silica column (75 × 4.5 mm, 5-μm particle size and 30-nm pore size; Beckman RPSC ultrapore). Elution was carried out with a 30–50% gradient of acetonitrile in 0.1% trifluoroacetic acid at room temperature and at a constant rate of 0.5 mL/min. Proteins were detected by UV absorption at 220 nm. When required, fractions were collected and vaccum-dried.

Protein Sequences. About 20 μg of the total acidic proteins was resolved by isoelectrofocusing. Proteins in the gel were electrophoretically transferred to an Immobilon-P membrane (Millipore) using LKB Novablot buffer (39 mM glycine, 48 mM Tris-HCl, 0.0375% SDS, and 20% methanol) as both anode and catode electrode solutions. Electrophoresis was performed at 0.8 mA/cm^2 for 1 h. Bands were located by Coomassie blue staining, cut, and directly used for Edman degradation microsequencing in an automatic Applied Biosystems 477 protein sequencer. Bands derived from two gels, in the case of protein YP1β(Ax), and from four gels, in the case of YP1α, were used side-by-side to obtain adequate results. Proteins YP2α and YP2β were also sequenced by directly loading about 50 pmol of HPLC-purified protein into the sequencer.

RESULTS

Identification of Protein YP1β Derivatives. Isoelectrofocusing of acidic ribosomal proteins from *S. cerevisiae* Y166 reproducibly shows the presence of up to eight protein bands. The four most basic proteins, which correspond to the phosphorylated and dephosphorylated forms of proteins YP2α and YP2β, formerly named L44 and L45 (Juan-Vidales et al., 1981, 1984), are usually found in constant amounts. However, the relative intensity of the more acidic bands, originally named L44′ and Ax, changed with the acidic protein preparations (Juan-Vidales et al., 1981, 1984).

It has been now found that the conditions used to extract the proteins from the ribosomes determine the proportion of the L44′ and Ax bands. Thus, treatment of the same ribosome preparation with increasing concentrations of ammonium chloride in 50% ethanol decreases the Ax bands and proportionally increases the L44′ forms (Figure 1), suggesting a close association between both types of proteins. This relationship was verified using monoclonal antibodies specific to L44′ (Vilella et al., 1991), which also cross-reacts with the Ax forms when tested by immunoblotting (Figure 2). Moreover, the absence of both bands Ax and L44′ from the YP1β-disrupted strain D6 (Figure 3B) confirms that they are encoded by the same gene. These two proteins must then be called YP1β and YP1β′, respectively, according to the uniforming nomenclature (Wool et al., 1991).

Identification of Protein YP1α. After the report on the existence of four acidic protein genes in yeast (Mitsui & Tsurugi, 1988; Newton et al., 1990), many unsuccessful attempts were made to detect by isoelectrofocusing one acidic

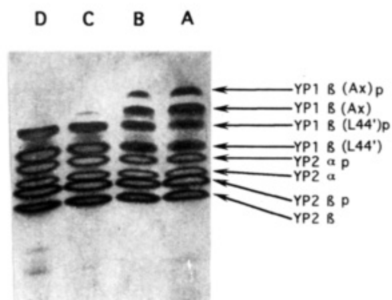

FIGURE 1: Acidic proteins extracted from *S. cerevisiae* Y166 ribosomes at increasing ammonium concentrations. Ribosomes from the same preparation were extracted with 50% ethanol in the presence of 0.3 (A), 0.5 (B), 0.7 (C), and 1.0 M (D) NH_4Cl and resolved by isoelectrofocusing in the 2.5 (top) to 5.0 (bottom) pH range. Gels were stained with silver.

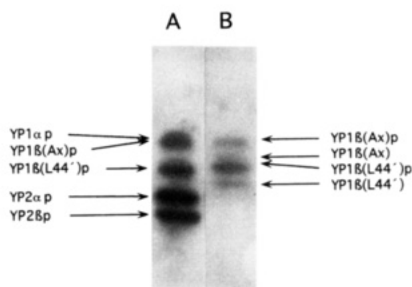

FIGURE 2: Immunoblot of acidic ribosomal proteins. Acidic proteins were separated by isoelectrofocusing [pH 2.5 (top) to pH 5.0 (bottom)], blotted to nitrocellulose paper, and treated with monoclonal antibodies ppA1, specific to the common carboxyl end (A), and 44'-1, specific to protein YP1β (B). ppA1 reacts preferentially with the phosphorylated forms of the acidic proteins when tested on immunoblots (Vilella et al., 1991).

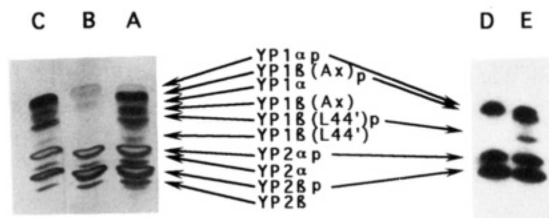

FIGURE 3: Isoelectrofocusing separation of yeast acidic ribosomal proteins in a polyacrylamide gel. Unlabeled (A, B, C) and ^{32}P-labeled (D, E) acidic ribosomal proteins from *S. cerevisiae* W303 (A, E), *S. cerevisiae* W303D6 (B, D), and *S. cerevisiae* W303D7 (C) were resolved by isoelectrofocusing in a 2.5 (top) to 5.0 (bottom) pH range. Gels were either silver-stained (A, B, C) or autoradiographed (D, E). The position of the different proteins is marked by the arrows.

polypeptide in addition to the three proteins, YP1β(L44'), YP2α(L44), and YP2β(L45), previously identified in *S. cerevisiae* Y166 ribosomes (Juan-Vidales et al., 1984). However, when *S. cerevisiae* W303 was tested, the presence of two additional poorly staining bands with a p*I* slightly lower than the p*I* of these previously characterized could be detected in the electrofocused gels (Figure 3A). These new bands were particularly evident in samples in which protein YP1β was missing (Figure 3B), obtained from *S. cerevisiae* W303D6, a yeast strain carrying a disrupted YP1β gene (Remacha et al., 1992).

Identification of these bands as the product of the YP1α gene was carried out in two ways. First, isoelectrofocusing of acidic proteins extracted from ribosomes of *S. cerevisiae* W303D7, which carries a disrupted YP1α gene (Remacha et al., 1992), showed the absence of these new bands (Figure 3C). Second, an in vitro transcription–translation assay using the YP1α gene, cloned in pGEM under the control of the T7

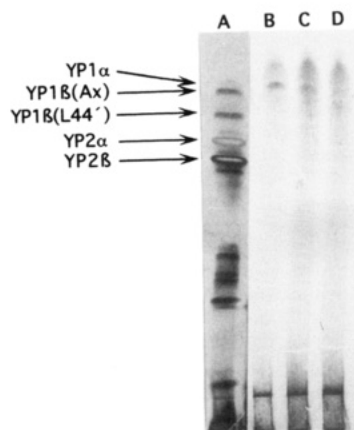

FIGURE 4: Isoelectrofocusing of the in vitro coupled transcription–translation of gene-encoding protein YP1α. The proteins from transcription–translation samples, including 0.3 μg (B), 1.5 μg (C), and no DNA (D), were mixed with carrier acidic proteins and resolved by isoelectrofocusing in a pH range between 5.0 (bottom) to 2.5 (top). (A) Stained gel; (B–D) autoradiogram.

Table I: Determination of the Amino-Terminal Amino Acid Sequence of Yeast Acidic Ribosomal Proteins[a]

protein	amino-terminal sequence	
	nucleotide-derived	experimental
YP1α[b]	MSTESA	STES
YP1β(Ax)[b]	MSDSII	SDSI
YP1β(L44')	MSDSIISFAAFILADA	AAFILADA
YP2α(L44)	MKYLAAYLLL	MKYLAA
YP2β(L45)	MKYLAAYLLL	MKYLAA

[a] Sequence from protein blotted in nitrocellulose paper obtained by automatic Edman degradation. [b] Protein from *S. cerevisiae* mutant NAT1 defective in an NH_2-terminal acetyltransferase (Takakura, 1992).

promotor, expressed one protein. When analyzed by isoelectrofocusing, the protein is found in the most acidic position of the gels where protein YP1α is expected to move (Figure 4).

Direct Amino-Terminal Sequencing of the Proteins from Isoelectrofocusing Gels. To characterize more precisely the different bands present in the acidic protein isoelectrofocusing gels, they were blotted to Immobilon-P transfer membranes and directly sequenced by Edman degradation to obtain the amino-terminal sequence. The bands corresponding to proteins YP2α(L44) and YP2β(L45) yielded the expected amino acid sequence derived from the nucleotide sequence of the genes (Table I).

Surprisingly, the sequence derived from the L44' band started at the alanine at position nine in the nucleotide-derived sequence, lacking, therefore, the first eight amino acids. No amino acid sequence could be derived from bands Ax or YP1α, indicating that they probably have a blocked amino-terminal end. This fact was confirmed when similar proteins, obtained from *S. cerevisiae* NAT1, a yeast strain defective in an NH_2-terminal acetyltransferase (Takakura et al., 1992), were sequenced; in this case, the expected sequences of proteins YP1α and YP1β were obtained, but starting in the second serine residue (Table I).

As expected, the position of the bands corresponding to these proteins, YP1β(Ax) and YP1α, is displaced to a more basic protein in the isoelectrofocusing gels of extracts from the NAT1 mutant due to the presence of an additional positive charge at the amino end (Figure 5).

Phosphorylation of the Acidic Proteins. It has been shown previously that proteins YP2α(L44), YP2β(L45), and YP1β(L44') can be monophosphorylated and, therefore, they usually

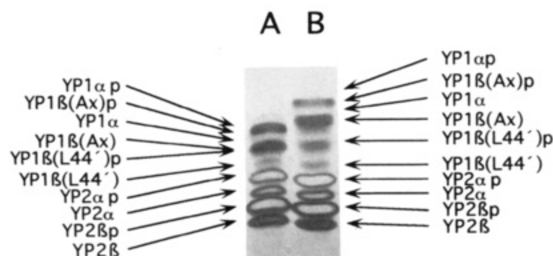

FIGURE 5: Isoelectrofocusing of acidic ribosomal proteins from *S. cerevisiae* NAT1 (A) and *S. cerevisiae* W303 (B). Electrofocusing conditions as in previous figures.

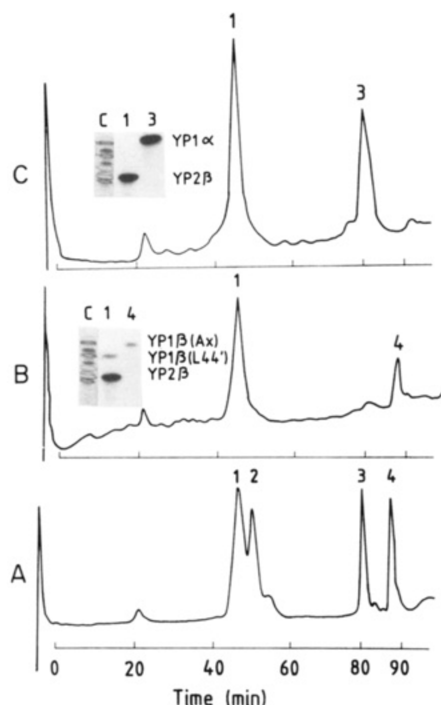

FIGURE 6: HPLC of acidic proteins from *S. cerevisiae* W303 (A), *S. cerevisiae* D46 (B), and *S. cerevisiae* D47 (C) extracted from ^{32}P-labeled cells. Peaks were collected and analyzed either by ELISA using monoclonal antibodies or by isoelectrofocusing as in previous figures (inset).

appear as a double band in the electrofocusing gels (Juan-Vidales et al., 1984). The fact that the Ax and YP1α proteins appear as double bands suggests that these two polypeptides are also present in phosphorylated and nonphosphorylated forms.

Phosphorylation of YP1α and Ax bands was confirmed by in vivo labeling of the cells with $^{32}PO_4$. When the acidic proteins of the labeled ribosomes were analyzed, five radioactive bands were detected by autoradiography (Figure 3D,E). The most acidic band corresponds to protein YP1α, which is more clearly noted in the sample obtained from YP1β-defective *S. cerevisiae* W303D6. The phosphorylated form of Ax is detected closely below the YP1α band in the wild-type sample and is absent in the *S. cerevisiae* D6 proteins.

Characterization of YP1α and Ax Proteins by HPLC. The acidic proteins can also be resolved using reverse-phase HPLC (Saenz-Robles et al., 1988). The chromatography of acidic protein preparations in a C3-alkyl-chain column results in four main peaks (Figure 6). The use of specific monoclonal antibodies confirmed the presence of proteins YP2β(L45) and YP2α(L44) in peaks 1 and 2, respectively. A variable cross-reaction with anti-YP1β(L44′), usually low, can also be detected in peak 1, but most of the anti-YP1β-reactive material is in peak 4. On the other hand, peak 3 showed cross-reaction with mAbs specific for the conserved carboxyl common to the four acidic polypeptides, but not with any of the mAbs specific for YP1β, YP2α, and YP2β. By exclusion, peak 3 must correspond, therefore, to protein YP1α for which no mAb is available. This peak is also detected in extracts from *S. cerevisiae* Y166.

These results were confirmed when extracts from strain D46, defective in YP1β and YP2α, and strain D47, lacking YP1α and YP2α, were HPLC-analyzed. Peak 2 (YP2α) is missing from both samples, and, in addition, peaks 3 and 4 are absent from the YP1α and YP1β-defective samples, respectively (Figure 6). To identify more precisely the protein present in the different HPLC peaks, $^{32}PO_4$-labeled proteins of the same strains were resolved and analyzed by electrofocusing. The results confirmed that the peak 3 component moves to the most acidic position of the gels, corresponding to protein YP1α, while peak 4 is made of the Ax form of YP1β. In the case of the disruptant D47, peak 1 contains YP2β and a small amount of the L44′ form of YP1β, which is absent in the YP1β-defective D46 sample, as expected.

DISCUSSION

Our results complete the characterization of the different products derived from the four genes encoding acidic ribosomal proteins in *S. cerevisiae* which is summarized in Table II (Mitsui & Tsurugi, 1988; Newton et al., 1990; Remacha et al., 1988).

The proteins previously designated L44′, L44, and L45 had been clearly identified as the product of three different genes presently called rpYP1β, rpYP2α, and rpYP2β, respectively (Remacha et al., 1988). Now, in vitro coupled transcription–translation of the cloned gene, analysis of extracts from acidic protein-deficient strains, and, more unequivocally, protein sequencing have allowed the unambiguous identification of the product of the fourth acidic protein gene, rpYP1α, as the most acidic band in the electrofocusing gels of acidic proteins. Moreover, like the other acidic proteins (Juan-Vidales et al., 1984; Sanchez-Madrid et al., 1981), protein YP1α is present in two bands which correspond to the phosphorylated and dephosphorylated forms of the polypeptide.

The failure to detect this protein in ribosomes from certain yeast strains (Juan-Vidales et al., 1984) suggests that this polypeptide may not always be either expressed or bound to the particles. Alternatively, the very strong acidic character of this protein may cause a selective loss of the polypeptide during extraction and manipulation. In fact, the amount of protein detected in isoelectrofocusing gels is not always reproducible, even in strains in which the YP1α gene is positively expressed, which suggests the existence of some methodological problem in recovering of the protein. YP1α can, on the other hand, be easily resolved from the other acidic proteins by HPLC, which is probably the most convenient method of recovering this polypeptide.

Attempts to sequence the YP1α bands directly were unsuccessful, indicating the existence of an N^{α}-blocked amino terminus. However, using proteins obtained from the NH_2-terminal acetyltransferase-defective *S. cerevisiae* NAT1 (Mullen et al., 1989), an amino acid sequence starting at the serine at position 2 was deduced. It seems that, after translation, the first methionine is removed and N^{α}-acetylation of the next serine takes place, as has been shown to occur in several other ribosomal proteins having a serine in the second position (Takakura et al., 1992).

The bands previously called L44′ and Ax in the isoelectrofocusing gels of yeast acidic protein preparations (Jaun-

Table II

Protein			Gene	Protein structure
Name	Previous name	Reference[a]		
YP1α	A1 L12eIIA	1 2	rpYP1α	MSTESALSYA ALILADSEIE ISSEKLLTLT NAANVPDENI WADIFAKALD GQNLKDLLVN FSAGAAAPAG VAGGVAGGEA GEAEAEKEEE EAKEESDDDM GFGLFD
YP1β	Ax L12eIIB	3 2	rpYP1β	MSDSIISFAA FILADAGLEI TSDNLLTITK AAGANVDNVW ADVYAKALEG KDLKEILSGF HNAGPVAGAG AASGAAAAGG DAAAEEEKEE EAAEESDDDM GFGLFD
YP1β′	L44′	3	rpYP1β	AA FILADAGLEI TSDNLLTITK AAGANVDNVW ADVYAKALEG KDLKEILSGF HNAGPVAGAG AASGAAAAGG DAAAEEEKEE EAAEESDDDM GFGLFD
YP2α	L44 L35 A2 L12eIB	4 5 6 2	rpYP2α	MKYLAAYLLL NAAGNTPDAT KIKAILESVG IEIEDEKVSS VLSALEGKSV DELITEGNEK LAAVPAAGPA SAGGAAAASG DAAAEEEKEE EAAEESDDDM GFGLFD
YP2β	YPA1 L45 L36 L12eIA	7 4 5 2	rpYP2β	MKYLAAYLLL VQGGNAAPSA ADIKAVVESV GAEVDEARIN ELLSSLEGKG SLEEIIAEGQ KKFATVPTGG ASSAAAGAAG AAAGGDAAEE EKEEEAKEES DDDMGFGLFD

[a] References: 1, Mitsui & Tsurugi, 1988a; 2, Newton et al., 1990; 3, Juan-Vidales et al., 1984; 4, Kruiswilk & Planta, 1975; 5, Zinker & Warner, 1976; 6, Mitsui & Tsurugi, 1988b; 7, Itoh, 1981.

Vidales et al., 1984) have been shown to be products of the same rpYP1β gene. Protein L44′, which has been previously purified and considered a functional ribosomal component (Juan-Vidales et al., 1984), turned out to be a truncated form of protein YP1β lacking the first eight amino acid residues. We propose to call this truncated polypeptide YP1β′.

The truncation of YP1β, yielding L44′ (YP1β′), takes place during preparation of the acidic proteins by a protease which seems to be removed from the particles together with the acidic proteins by the ammonium chloride–ethanol wash. However, the L44′ bands begin to appear when proteins are extracted in the presence of NH_4Cl concentrations higher than 0.3 M, indicating that either the activity or the release from the ribosomes of the degrading enzyme is stimulated by high salt concentrations.

It is noteworthy about this degradation process that mutations substituting serine in position 2 strongly stimulate the formation of the L44′ bands in the acidic protein preparations and dramatically decrease the accumulation of protein YP1β in the cytoplasmic pool (Naranda et al., 1993), indicating that the removal of the first amino acids may be the initial step in the total degradation of the protein. These data suggest that the accumulation of acidic proteins in the cytoplasm (Saenz-Robles et al., 1990), as opposed to the rest of ribosomal components, may be related to their resistance to protease degradation, which is determined mostly by the structure of the amino termini.

The amino-terminal deletion found in YP1β′ provides an explanation for the previously reported inability of this protein to dimerize like the other acidic proteins YP2β(L45) and YP2α(L44) (Juan-Vidales et al., 1984). The missing first amino acids probably play an important role in the dimerization process.

On the other hand, band Ax seems to correspond to the functional form of protein YP1β in the ribosomes and, like protein YP1α, is the result of the posttranslational elimination of the first methionine and the NH_2-terminal acetylation of the second serine residue as also shown by amino acid sequencing of the protein obtained from *S. cerevisiae* NAT1.

It seems, therefore, that both members of the P1 family have N^{α}-blocked amino termini. On the contrary, both P2 proteins have unprocessed free amino ends starting with the first methionine and can be directly sequenced from wild-type strains. A similar situation has been reported in *Artemia salina* (van Agthoven et al., 1978). Rat liver protein P2 has also been shown not to be posttranslationally processed, and it carries a free methionine at the amino end (Lin et al., 1982), although in this organism there are no data on protein P1.

Interestingly, the only structural difference between the otherwise functionally identical bacterial acidic proteins L7 and L12 is precisely the state of the amino end. This difference, which apparently is functionally irrelevant in prokaryotes (Brot et al., 1973; Isono & Isono, 1981), may have been the first step in the evolutionary divergence that eventually resulted in the two structurally and functionally different P1 and P2 eukaryotic acidic proteins.

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