ARTICULO

Expression of the acidic ribosomal stalk P proteins is affected differently by the growth conditions in *Saccharomyces cerevisiae*

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ABSTRACT

The ribosomal acidic proteins, called P proteins in eukaryotes, accumulate free in the cytoplasm due to the fact that they escape from the strict control that assure the synthesis of equimolecular amounts of the ribosomal components. In addition, it has been proposed that in yeast, translation may be regulated by the composition of the ribosome with respect to the four P proteins present in this organism. As a step forward in the analysis of the mechanisms controlling the expression of the four yeast P proteins in *Saccharomyces cerevisiae*, the effect of different metabolic alterations on their expression has been studied using the lacZ gene as a reporter. The first thing was to establish the characteristics of the constructs, the size of the promoter and encoding regions, for maximum expression of the P protein-lacZ fusions. Then, using the best possible constructs, the expression of the protein chimeras was monitored in these different metabolic situations: during the growth cycle, when using different carbon sources and upon temperature shifts. The results indicate that the P proteins stop being synthesized before the cells enter stationary phase. Moreover, the expression of each one of the four P proteins is affected differently by the metabolic conditions, either by nutritional changes or by temperature alterations. In this way, the pattern of expression of the four proteins, and therefore their relative proportion in the cell, is specific for a given metabolic situation. These results are compatible with the proposal that the yeast, by regulating the ribosomal P protein composition, can adjust the translational capacity of the particles to make it optimal in each metabolic condition.

Key words: *Sacchacromyces cerevisiae*, ribosomal stalk, P proteins, Protein synthesis

RESUMEN

La expresión de las proteínas ácidas P del tallo ribosomal es afectada diferencialmente por las condiciones de crecimiento en *Saccharomyces cerevisiae*

Las proteínas ácidas del tallo ribosomal, llamadas proteínas P en eucariotes, se acumulan en forma libre en el citoplasma porque escapan del control estricto que asegura la síntesis de cantidades equimoleculares de los componentes ribosomales. En adición, se ha propuesto que en levadura, la traducción puede ser regulada por la composición del ribosoma con respecto a las cuatro proteínas P presentes en este organismo. Para avanzar en el análisis de los mecanismos de control de las cuatro proteínas P en *Saccharomyces cerevisiae*, se han estudiado los efectos de diferentes alteraciones

metabólicas en su expresión usando el gen lacZ como reportero. En primer lugar se establecieron las características de los diferentes constructos, el tamaño de las regiones promotoras y codificantes, para expresión máxima de fusiones proteínaP-LacZ. Entones, usando los mejores constructos, se examinó la expresión de las proteínas quiméricas en diferentes situaciones metabólicas: durante el ciclo de crecimiento, cuando se usaron diferentes fuentes de carbono y frente a cambios de temperatura. Los resultados indican que las proteínas P dejan de ser sintetizadas antes de que las células entren en fase estacionaria. Además, la expresión de cada una de las proteínas P es afectada en forma diferente por las condiciones metabólicas, ya sea por cambio de nutrientes o por alteraciones de temperatura. En esta forma, el patrón de expresión de las cuatro proteínas, por tanto, su proporción relativa en la célula, son específicas para una situación metabólica dada. Estos resultados son compatibles con la propuesta de que la levadura, mediante regulación de la conposición de las proteínas ribosomales P, puede ajustar la capacidad traduccional de sus ribosomas para hacerla óptima en cada situación metabólica.

Palabras clave: *Sacchacromyces cerevisiae*, tallo ribosomal, proteínas P, Síntesis de proteínas

INTRODUCTION

The synthesis of ribosomes is a tightly controlled process that requires the coordinate expression of the multiple ribosomal components, among them about 80 r-proteins in the case of eukaryotes [1, 2]. In yeast, it has been shown that the regulation of r-protein synthesis takes place mostly at the level of transcription (see [3-5] for reviews). All the r-protein genes so far analyzed contain a variable number of cis-acting UAS elements in the promoter region [6] that bind either ABF1 [7] or RAP1 [8], two well-characterized multifunctional transcriptional regulatory factors, and control their transcription [9]. In addition, it is probable that other still no well known factors also participate in the regulatory process [10-12] [13]. In some cases, regulation of r-protein synthesis at the level of mRNA splicing [14] and mRNA stability [15] has been reported. The coordinate action of these regulatory processes results in the synthesis of roughly similar amounts of r-proteins [16], which are immediately transported to the nucleolus and assembled into the new ribosomes. Moreover, the cells have a scavenging mechanism that degrades any ribosomal component that is overexpressed under certain unusual conditions [17-19].

The only ribosomal components that escape from this strict control and accumulate free in the cytoplasm are the stalk acidic proteins. These proteins are small, about 12 kDa, although they are usually present as dimers, and have a pI lower than 4. The acidic proteins have been located in the ribosomal stalk, a typical structure of the large ribosomal subunit detected in all organisms [20 for a review]. The stalk is a pentameric complex, made up of two acidic protein dimers and one non-acidic protein, that is involved in the interaction of the supernatant factor with the ribosome during protein synthesis [21]. The acidic proteins are called L7 and L12 in bacteria, and they have been well characterized, both functionally and structurally [22]. In eukaryotes the equivalent proteins are called P proteins because they were found phosphorylated in the ribosome [23]. The P protein family has a variable number of members depending on the species, but they can be associated in two groups called P1 and P2. In yeast, there are four acidic proteins, two of the P1 type, YP1α and YP1β, and two of the P2 type, YP2□ and YP2□, which carry a phosphorylated serine near the carboxyl end [24]. Phosphorylation of the stalk components is important but essential for activity [25].

About two thirds of the total acidic P proteins have been reported in the supernatant of *S. cerevisiae* [26] and *A. salina* [27], although immunoblotting has shown considerably lower amounts in mammalian cells [28]. Differences in the amount of the different acidic proteins in the cytoplasm have also been estimated using specific monoclonal antibodies. Thus, protein YP2□ seems to be the most abundant in the cytoplasm, followed by YP2α and YP1β (Vilella, et al., 1991). Estimations of YP1□ are not available. However, differences in both the total P protein pool sizes and the relative proportion of the different proteins seem to be affected by the metabolic state of the cells as well as by their genetic background. Thus, variations have been found in cells collected at different stages in the growth curve as well as in different yeast strains (B.L. Ortiz-Reyes M. Remacha, and J.P.G.Ballesta, unpublished results).

In *S. cerevisiae* the stalk is formed by two P1/P2 heterodimers (P1α/P2β and P1β/P2α) that bind to protein P0 [29]. The P1 and P2 proteins are not essential for ribosome activity [30], due to the presence of protein P0, a larger (38 kDa) and less acidic polypeptide, that partially substitutes for them in the ribosome function [31]. The acidic proteins are, however, required for maximal translation activity. Moreover, acidic protein deficient cells express an altered pattern of proteins, indicating that the presence of different P proteins differentially affects the capacity of the ribosomes to translate certain mRNAs [30]. In fact, since the ribosomes from stationary phase contains fewer acidic proteins than those from exponential phase [32], it has been suggested that the acidic protein deficient ribosomes might preferentially translate stationary phase specific mRNAs [33]. Moreover, since stalk-defective ribosomes are found even in exponentially growing cells [34], the proposed regulatory mechanisms may be working in all metabolic conditions.

According to this proposal, yeast might control the expression of some proteins by regulating the P protein composition of the ribosomes [1]. However, since they have four acidic proteins and only two of them, one of each group [35, 36], can form the stalk, the ribosomal acidic protein composition in yeast can change qualitatively as well as quantitatively. Moreover, a qualitative change in the ribosomal P protein composition can take place without "de novo" synthesis of ribosomes since an exchange has been shown to exist between the proteins in the cytoplasmic pool and those in the ribosomes [37, 38]. Therefore, the ribosome composition can be altered rapidly by changing the proportion of the free acidic proteins in the cytoplasm, which can be carried out easily through the regulation of the P protein expression.

Qualitative changes in the P protein expression may be the result of metabolic alterations, and according to the proposed model, can affect the capacity of the ribosomes to translate certain mRNAs. In other words, the regulation of the ribosomal P protein composition might be one of the mechanisms working in the adaptation of the cell to metabolic changes.

Obviously, a necessary requirement for this model is the existence of a mechanism which would regulate the relative expression of the P proteins to provide the optimal proportion of each one for the given metabolic conditions. To test this possibility, the expression of the four P proteins was explored using lacZ gene fusions in different situations that alter the cell metabolism. The

results, which show a different effect of the metabolic changes on the expression of the four P proteins is reported here.

MATERIALS AND METHODS

Strains and growth conditions. *Saccharomyces cerevisiae* W303-1B (MAT α, *leu2-3, trp1-1, ura3-1, ade2-1, his3-11,15, can 1-100*) was used throughout the study. Yeasts were grown either in rich YEPD medium (5% yeast extract, 10% peptone and 2% glucose) or SD minimal complete medium (0.67% yeast nitrogen base without aminoacids, 40 mg/ml each of the strain nutritional requirements and 2% glucose). When required, agar was added up to 2% to prepare solid YEPD and SD media.

The host for vector and plasmid amplification was *E. coli* strain C600 (F-, *thi*-1, *thr*-1, *leu*B6, *Lac*Y1, *Ton*a21, *sup*E44) which was grown at 37º C in liquid LB (per liter: 10g Bacto peptone, 5g yeast extract, 5g NaCl and 2g glucose) or solid (LB + 1.5% agar) broth.

Cell fractionation. The method was previously described [39] with slight modifications. Yeast cells were grown in uracil-free SD liquid medium up to the mid -log phase. They were washed twice with ice-cold sterile water and resuspended (1g/mL) in 80 mmol/L KCl, 12 mmol/L $MgCl_2$, 100 mmol/L Tris-HCl, 5 mmol/L β-mercaptoethanol and 1mmol/L PMSF, pH 7.4. They were then mechanically disrupted as described above and centrifuged at 30,000 x g at 4ºC for 20 min. The pellet was discarded and the supernatant (S30 cell fraction) was centrifuged at 50,000 rpm (rotor 60 Ti; Beckman) at 4ºC for 3 hours.

Cell transformation and plasmid amplification. *E. coli* cells were transformed [40], and transformants were simultaneously selected forampicillin resistance and ß-galactosidase expression on X-gal LB plates. Plasmids were obtained from transformants grown at 37º C in ampicillin supplemented (100 μg/mL) LB liquid medium by standard methods [41]. Yeasts were transformed following the method of Hinnen et. al. [42]. Yeast transformants were selected as blue colonies on uracil-free M 63 solid medium.

Plasmids. All constructs have been carried out on the centromeric plasmid series YCp356, 357, 356R, 357R as vector. The YCp series was derived from the equivalent integrative YIp series [43] by inserting an ARS-CEN element obtained from the pFL39 plasmid [44] as summarized in **Figure 1** for YCp356. A similar approach was used to obtain the R series, but in this case the ARS-CEN was retrieved as a HindIII-NcoI 3,71 Kbp fragment from pFL39X, which is derived from pFL39 by substituting its MCS for a unique *Hin*dIII site.

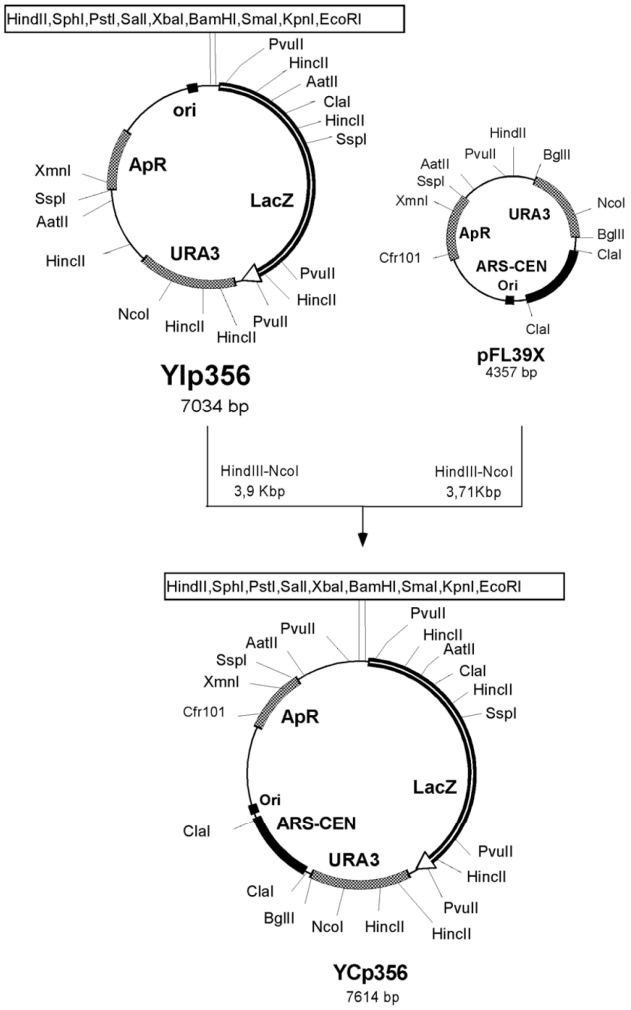

Figure 1. Construction of YCp356.

Plasmids YCFp47ep, YCFp47eh, YCFp46ph, YCFp44ep, YCFp45bb, YCFp45ph, were constructed as previously reported [45] for their homologues in the episomic YEp series [43]. YCFp45bh and YCFp45bp were constructed in the same way as YCFp45bb, but inserting instead YP2ß gene fragments containing the same part of the encoding region and a larger portion of the 5' region. The total insert size was of 0,46 Kbp, 0,56 Kbp and 2,46 Kbp for YCFp45bb, YCFp45bp and YCFp45bh, respectively.

The YCF47ee, YCF46ep, YCF45ph and YCF44ee constructs, containing the larger portion of the respective acidic protein gene, were prepared by introducing a new restriction site at the appropriate position in the 3' end of the genes by PCR mutagenesis. An *Eco*RI site was created in the YP1α, YP1ß and YP2α genes and a *Hin*cII site in YP2ß. The acidic protein genes [46], subcloned in suitable vectors [45], as templates, and the mutagenic and the universal oligonucleotide as primers, were used for amplification. The restricted PCR products, (0.77 Kbp EcoRI-EcoRI for YP1α, 1,1 Kbp PstI-EcoRI for YP1ß, 1,35 Kbp EcoRI-EcoRI for YP2α and 0,67 Kbp PstI-HincII for YP2ß) containing the promoter and the truncated genes, were inserted in the adequate YCp vector, which was YCp356R in all four cases. The truncated genes encoded proteins lacking only the last 11 amino acids.

β-galactosidase activity assay.- The ß-galactosidase activity in the cells was measured as reported [47]. Cells were collected by centrifugation and resuspended in 400 µL of 100 mmol/L potassium phosphate buffer pH 7.0 were mixed with an equal volume of buffer Z (65 mmol/L Na_2HPO_4, 40 mmol/L NaH_2PO_4, 10 mmol/L KCl, 1 mmol/L $MgSO_4$, and 40 mmol/L 2-mercaptoethanol, pH 7.0) plus 10 µL of 1% SDS and 10 µL of chloroform, and incubated at 28ºC. Then 200 µL of ONPG (4 mg/mL in potassium buffer, pH 7.0) were added and

the samples were again incubated at 28ºC. The reaction was stopped with 500 µL of 1.0 mol/L Na_2CO_3 and the A_{420} in the samples was estimated. The enzymatic activity was expressed as A_{420}/incubation time in min.

RESULTS

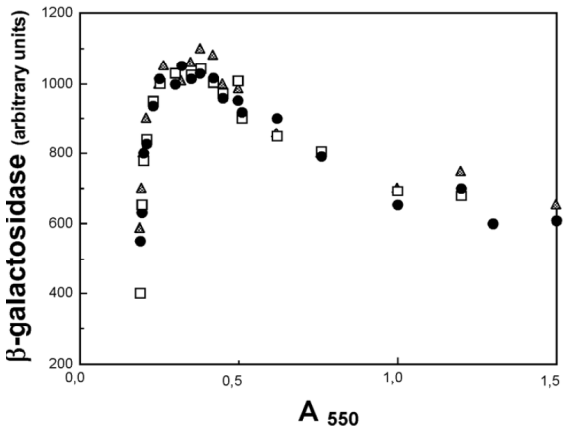

Figure 2. Expression of Yp2ß-lacZ constructs carrying 5' leader regions of different lengths. YCFp45bb (▲), YCFp45bp (□), and YCFp45bh (●) have 5' end regions of 0.3 kbp, 0.4 kbp and 2.3 kbb, respectively. The encoding region fragment is the same in the three constructs.

Effect of 5' leader region on the expression of acidic protein-ß-galactosidase fusion proteins. To determine the influence of the 5' flanking region on the expression of the acidic protein-ß-galactosidase chimera, inserts including the fused gene carrying different 5' extension sizes were subcloned in YEp357R. Leader regions ranging from 2.5 Kbp to 0.3 Kbp were tested. The shorter one, YCFp45bb, included a UAS for ABF1, located -232 [48]; the 5' region in YCFp45bp also included an additional potential UAS at -366 [46] and YCFp45bh included a long 5' leader of 2.2 Kbp. The ß-galactosidase activity was estimated in *S.cerevisiae* W303 transformed with these plasmids grown at different cell cycle stages.

The results, summarized in **Figure 2** for the YP2ß gene fusions, show a similar level of activity for all the constructs at the equivalent cell stage, regardless their 5' flanking region.

These results indicate that the ABF1 site is the essential regulatory cis-element in YP2ß, confirming a previous report [48].

Similar results with the other proteins support the idea that there are no other upstream cis-acting activating elements in the promoter region of the acidic ribosomal proteins apart form the RAP1 and ABF-1 sites, usually located no further than 500 nucleotides up from the transcription initiation site [49].

Based on these results, the 5' flanking region in the constructs was large enough to include all the reported UAS elements in the promoter region [46, 50].

Effect of the acidic protein fused fragment on the expression of the protein chimera. A number of constructs have been prepared containing increasing fragments of the acidic protein gene encoding region fused to the LacZ gene. In all cases the 5' flanking region is the same for all the constructs from the same gene. The enzymatic activity in the total cell extracts (S30 extracts) from the different transformed yeast strains, grown to the same cell density (A550= 0.5), was estimated and is included in **Table 1.**

Table 1. ß-Galactosidase activity expressed from different acidic protein-LacZ constructs

Plasmid	Hybrid protein	aa in the fused ORF	ß-galactosidase Units/mg of S30
YEFp47ep	YP1□(69aa)-ßgal	69	19,4
YEFp47eh	YP1□(80aa)-ßgal	80	26,0
YEFp47ee	YP1□(95aa)-ßgal	95	29,3
YEFp46ph	YP1ß(36aa)-ßgal	36	4,3
YEFp46ep	YP1ß(95aa)-ßgal	95	28,2
YEFp44ep	YP2□(65aa)-ßgal	65	44,8
YEFp44ee	YP2□(95aa)-ßgal	95	49,1
YEFp45bb	YP2ß(50aa)-ßgal	50	32,1
YEFp45ph	YP2ß(75aa)-ßgal	75	39,5
YEFp45ee	YP2ß(99aa)-ßgal	99	37,9

In general, the activity tends to be lower as the size of the acidic protein fragment is shorter, although the difference is only notable when the P protein fragment is shorter than 50 amino acids. The difference in enzymatic activity resulting from the various constructs of the same protein probably reflects a different stability of the fused protein rather than differences in the level of expression, since all the constructs carry exactly the same promoter region. This interpretation is supported by the very low activity displayed by the cells transformed with YEFp46ph, the resulting YP1□(36aa)-□gal protein being the only one among the different chimeras tested that was unable to bind to *S. cerevisiae* W303 ribosomes [45] and, therefore, susceptible to faster degradation.

In view of the effect of the acidic protein fragment size on the final enzymatic activity, all the consecutive tests were performed with the constructs carrying the larger fragment, namely YEFp47ee, YEFp46ep, YEFp45ee and YEFp44ee. In the four constructs, β-galactosidase is fused to acidic proteins which only lack the last five amino acids.

Expression of acidic proteins along the *S. cerevisiae* growth curve. The previous experiments showed a close relationship between the growth stage and the expressed β-gal activity (Figure 2). Therefore, the enzymatic activity expressed by the constructs along the growth curve was followed in a rich as well as in a minimal medium.

In **Figure 3** the data obtained in the case of the YP2ß construct are shown as a representative example. The results clearly indicated that when the cells are growing in a YEPG medium with a generation time of around 90 min, the activity increases very fast and reaches a maximum roughly at the middle of the exponential phase. Afterwards, the enzyme decreases quickly, and when the cells enter stationary phase it is reduced to about half of its maximal activity **(Figure 3A)**. On the other hand, in a minimal medium using glucose as a carbon source, the cells double every 130 min, and the β-galactosidase activity keeps increasing until late exponential phase and peaks when cells are close to the stationary phase. In this case, the activity decreases more slowly than in the rich medium **(Figure 3B).**

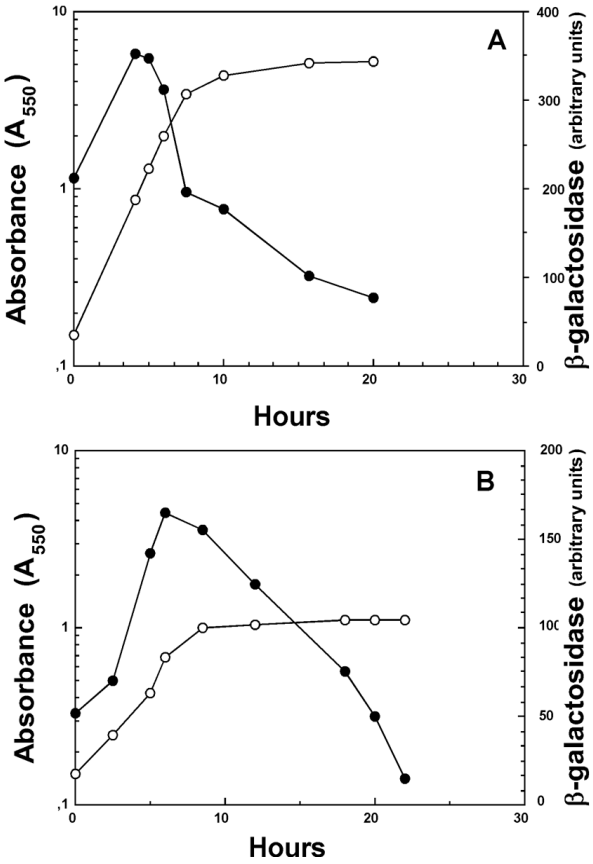

Figure 3. Expression of YP2ß-LacZ fusion throughout the cell growth cycle. *S. cerevisiae* W303 transformed with YCP45ee was grown in minimal (A) and in rich (B) medium. Aliquots were taken at different times and tested for β-galactosidase activity (●). Absorbance (O),

Effect of the carbon source on the expression of the different acidic ribosomal proteins. The relative expression of the four acidic P proteins is affected by the cell growth rate. To analyze this effect, *S. cerevisiae* strains transformed with the constructs encoding the larger protein chimeras were grown in minimal medium using different carbon sources, including glucose, galactose, glycerol and acetate. The maximal ß-galactosidase activity estimated in the total extracts of each transformant grown in the four carbon sources is presented in **Figure 4.**

The expressed enzymatic activity is clearly affected in different ways depending on the transforming plasmid. However, no a uniform pattern of variation associated with the growth rate emerges from these data. Thus, while the expression of the YP2 fused protein is barely affected in glucose, galactose and glycerol, it is substantially reduced in acetate grown cells. On the other hand, the levels of activity expressed from YP1β increase with the doubling time, going from glucose to glycerol, but are less affected when grow in acetate

(Figure 4A). These differences are more clearly displayed when the ratios of the activities in the different media are plotted **(Figure 3B**). The notable increase of YP1β/YP2β ratio becomes very obvious as the growth rate decreases going from glucose to acetate. The YP1α/YP1β ratio, on the other hand, is barely affected in the same conditions.

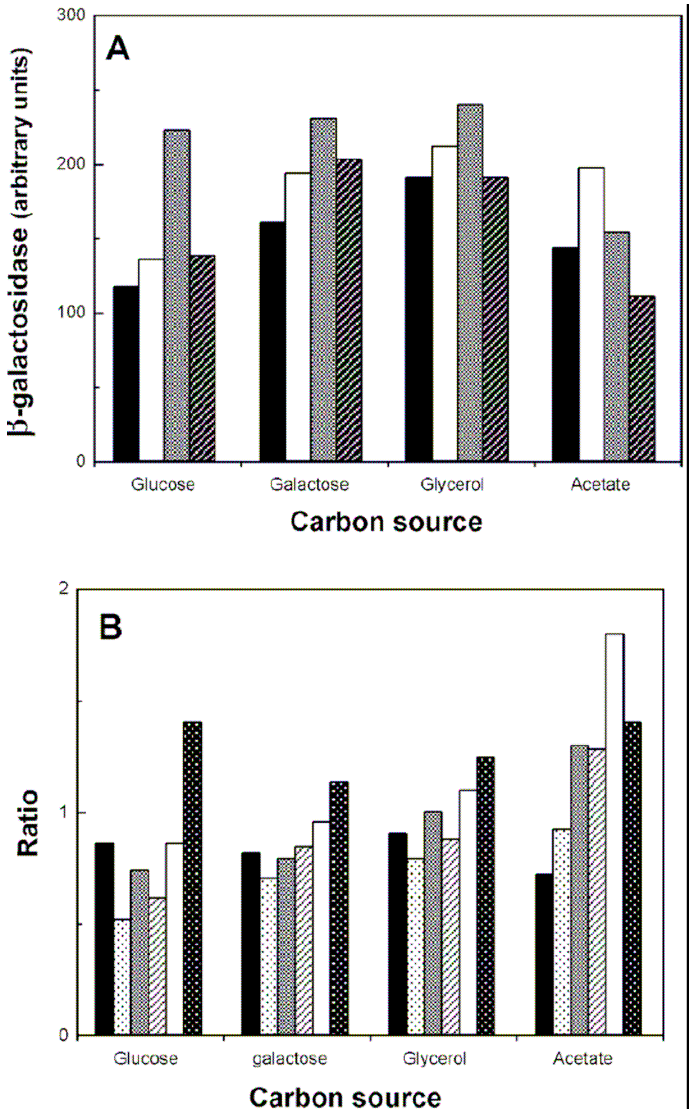

Figure 4. Expression of the four P-protein-lacZ in minimal medium using different metabolic conditions. Panel A. *S. cerevisiae* W303 transformed with YCFp47eh (■), YCFp46ep (□), YCFp45ee (▨) and YCFp44ee (▩) were grown in minimal medium using different carbon sources as indicated. The □-galactosidase activity was estimated at different points in the growth curve and the maximum value in each case is shown in panel A. Panel B. Te ratio of the activity expressed from the different P protein constructs in the four media used is plotted.YP1α/YP1β (■), YP1α/YP2α (▩), YP1α/YP2β (▨), YP1β/YP2α (▧), YP1β/YP2β (□), YP2α/YP2β (▨).

It seems that each protein responds in a different way to the changes in the growth conditions, and as a result the relative proportion of the four components

of the P protein family expressed seems to be specific for each growth condition.

Effect of a temperature shift on the expression of the P protein-LacZ fusions. P protein-LacZ plasmid-transformed *S. cerevisiae* strains growing exponentially in minimal medium at 30ºC were transferred to 37ºC and the ß-galactosidase activity in the cell extracts was estimated at different times after the shift **(Figure 5)**. The enzymatic activity expressed from the YP1ß fusion decreases notably after the shift and is reduced by about 40% only 10 min after the temperature change. Similarly, the YP2α protein is reduced, though to a less extent. On the contrary, the activity from the YP2β and YP1α fusion is stimulated by the heat-shock. The effect is especially notable in the second case where the enzymatic activity is almost doubled in only five minutes at 37ºC.

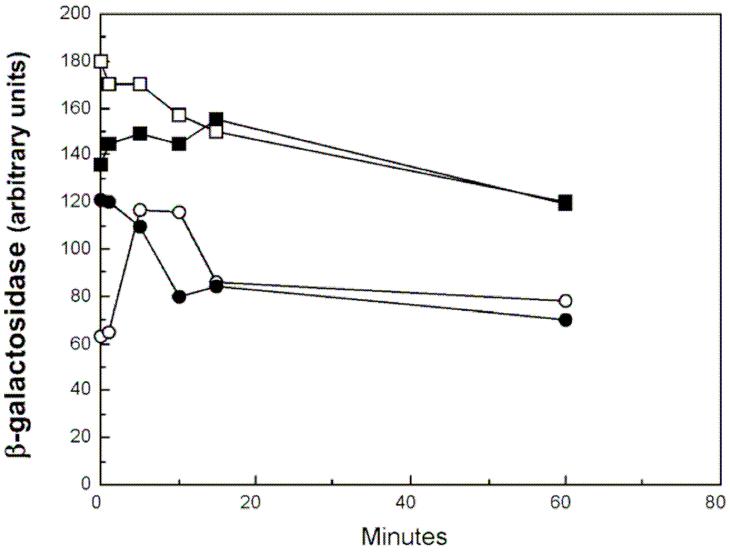

Figure 5. Expression of P-protein -lacZ fusion after a temperature shift. *S. cerevisiae* W303 transformed with YCFp47eh (○), YCFp46ep (●), YCFp45ee (■), and YCFp44ee (□) growing in minimal-glucose medium at 30ºC were shifted to 37ºC and the ß-galactosidase activity tested in samples taken at different times after the shift.

DISCUSSION

It was previously shown that yeast acidic protein deficient ribosomes, that accumulate in stationary phase [51] but are also present in exponentially growing cells [34] preferentially translate some mRNAs which might encode stationary phase-specific proteins [30, 33]. This data led to the proposal of a translation control mechanism that by quantitatively regulating the amount of acidic P proteins in the ribosome, can modulate its capacity to translate specific mRNAs [1, 30]. However, in organisms that have more than two P proteins, like *S. cerevisiae*, the cell might regulate also the ribosomal acidic protein content qualitatively, and the proposed translation control mechanism could be regulated in these cases by the qualitative as well as by the quantitative composition of the ribosome. However, to achieve a qualitative change of the ribosomal P protein composition, the cell must be able to regulate the expression of the different acidic proteins independently in order to adapt their relative concentrations to the translational requirements.

To study the way the expression of the four *S. cerevisiae* P proteins is affected by the metabolic changes, yeasts carrying a series of P protein-lacZ fusions have been constructed and tested under different metabolic conditions.
First, the optimal characteristics of the fusions for maximal expression were explored. Using fusions carrying 5' regions of different sizes it was confirmed that, as in other r-protein genes [52, 53]), the promoter region in the P protein genes is limited to the fragment including the UAS elements previously described [46, 50]. This is especially clear in the case of the YP2□ protein; the inclusion of 5' regions larger than about 300 nucleotides, which is roughly the minimal size to include the known ABF1 site [48], did not improve the expression of the fusion proteins in any of the cases. It seems, therefore, that all the transacting elements required for the expression of this protein, obviously including ABF1, must interact with this minimal 5' end region of the gene.

Another important parameter that affects the expression of the LacZ fusions is the size of the P protein included in the construct. The P protein fragment definitely modifies the kinetic parameters of the ß-galactosidase reaction. Thus, the Vmax but not the Km, seems to be reduced with the P-protein fragment size (Payo, et al., 1995). Here it is clearly shown that the expression of the protein chimera is considerably reduced when the P-protein fragment is shorter than about 50 amino acids. It has previously been shown that in these cases the polypeptide is unable to bind to wild-type ribosomes [45], and, probably, the low level of enzymatic activity in the cell might be due to degradation of the unbound protein. To avoid these negative effects, fusions carrying almost the complete P protein have been used for the comparative studies discussed later.

Another interesting conclusion from these results is that the maximal β-galactosidase activity, as well as its place in the growth cycle, depends on the growth rate. The maximal activity is almost two fold higher in cells growing in rich medium than in those growing in minimal medium, indicating that synthesis of the P proteins is proportional to the growth rate. This is,
in fact, in agreement with previous reports showing that in yeast [54] as in bacteria [55], the synthesis of ribosomes is directly related to the cell growth. In minimal medium the β-galactosidase maximum is reached at late exponential phase and decreases rather slowly afterwards. On the contrary, the maximal activity peak is sharper and declines faster in rich medium; moreover, it occurs earlier in the growth cycle. The decrease in the β-galactosidase activity suggests that the expression of the fusion protein is stopped and the existent protein is degraded. P protein synthesis stops before the cells enter stationary phase while they are still growing exponentially. This is especially evident in rich medium where the maximal activity is detected in the middle log phase.

Taken all together, the results suggest that cells are able to sense P protein content and to stop their synthesis when the amount reaches a maximal value that seems to be proportional to the growth rate. Apparently, the faster the cells grow, the sooner the maximum is reached.Interestingly enough, it has previously been reported that the synthesis of ribosomes as a whole is also stooped well before stationary phase. Thus, in minimal medium, the transcription of rRNA and different ribosomal proteins stops roughly at the same time that it does for P proteins [56]. No data are available on ribosome synthesis during the growth

cycle in rich medium, but our results suggest that in this case the synthesis will stop earlier, as found for the P proteins. In any case, our results confirm that cells growing exponentially have a ribosome content that exceeds their requirements for protein synthesis, and the protein synthesis machinery can function at the required efficiency for a relatively extended period of time in the absence of "de novo" synthesis of ribosomes. The length of time that cells keep growing exponentially after abolishing the expression of an essential ribosomal component [57] also leads to the conclusion that there is an excess of ribosomes.

On the other hand, it seems that although the P proteins are synthesized in excess and accumulate free in the cytoplasm, they are controlled by the same overall mechanisms acting on the rest of the ribosomal components as has also been shown in the case of nutritional shift-up experiments for protein YP2β. The transcription of this protein is stimulated in parallel with that of other r-proteins upon addition of glucose to an ethanol medium, and as indicated previously, this transcription depends on the presence of an ABF1 binding UAS [48].

Nevertheless, in spite of the fact that the P proteins seem to respond to the general mechanisms that control ribosome synthesis, our results clearly indicate that yeast can also regulate the expression of the four polypeptides in a coordinate but independent way. Thus, the expression of the four P protein-lacZ fusions does not change in the same way when the nutritional conditions are changed. Depending on the carbon source the ratio of the expressed P protein fusions is different. Similarly, the response of the four proteins to a temperature shift is not identical. Therefore, while YP1β and YP2α expression notably decrease with this shift, YP1α and YP2β respond in the opposite way. In all cases, the change in the metabolic conditions results in a change in the relative expression of the four acidic proteins that makes the final P protein composition of the cell different from the previous one.

Little is known about the mechanisms by which the cell can regulate the expression of the P proteins separately from the rest of the ribosomal components and how these mechanisms are superimposed to those controlling the overall ribosome synthesis. The available data obtained from gene disruption studies indicate that the P proteins themselves play an important role in these regulatory processes. Thus, a strong stimulation of the P2 protein expression has been observed, and especially of YP2β, upon inactivation of the P1 type proteins [58]. On the contrary, inactivation of the P2 proteins results in a strong inhibition of the P1 proteins [58]. Apparently, there is a mutual and opposite effect of the protein of each group on the expression of the other group. Interestingly enough, the amount of YP2β mRNA is increased in conditions of over-expression of this protein; however, the mRNA of YP1 proteins is not substantially affected when the protein expression is strongly inhibited. It seems, therefore, that the regulatory mechanism controlling the relative expression of the different proteins works at different levels [59].

It is difficult at this moment to understand the physiological meaning of the P protein ratio changes in the cell metabolism. However, since a quantitative alteration of the P protein ribosome composition has been shown to affect the

pattern of protein expression and, therefore, the capacity of the ribosomes to translate some mRNAs [30, 33]a qualitative change may have a similar effect. Our results are compatible with this proposal and suggest that the cell, by changing the ratio of the P proteins in the ribosome, might modulate its capacity to translate some mRNAs better than others. In this way, the level of expression of the different cellular proteins, the pattern of expressed proteins, might be optimized for the given metabolic conditions. Moreover, the changes in the P protein expression can easily be translated to the overall ribosomal population through an exchange between the proteins in the ribosome and those in the cytoplasmic pool as previously reported.

On the other hand, the proposed modulation of the ribosomal activity by the P proteins provides an explanation for the existence of more than one P protein of the same type in many organisms, including lower eukaryotes (yeast and fungi), protozoa and plants [60]. Indeed, if the function of the P proteins is limited to their role in the interaction of the elongation factors, the reason for the existence of more than one protein of each type to form the stalk is not obvious. In fact, the data showing that the absence of one of the P proteins cannot be fully complemented by the presence of the other protein of the same type [35, 59] strongly suggests that they play some additional role which can be related to the optimization of the ribosome activity in order to adapt the cell to the metabolic changes, as suggested previously.

To confirm this working model, the "in vitro" protein synthesizing capacity of ribosomes from cells grown in different conditions will shortly be analyzed to try to relate it to the P protein composition of the particles.

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