Functional complementation of yeast ribosomal P0 protein with Plasmodium falciparum P0

K. Aruna\textsuperscript{a}, Tirtha Chakraborty\textsuperscript{a,1}, Pavitra N. Rao\textsuperscript{a}, Cruz Santos\textsuperscript{b}, Juan P.G. Ballesta\textsuperscript{b}, Shobhona Sharma\textsuperscript{a,*}

\textsuperscript{a}Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai 400 005, India
\textsuperscript{b}Centro de Biologia Molecular 'Severo Ochoa', Universidad Autonoma de Madrid and Consejo Superior de Investigaciones Cientificas, Cantoblanco, 28049 Madrid, Spain

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Abstract

A complex of three phosphoproteins (P0, P1 and P2) constitutes the stalk region at the GTPase center of the eukaryotic large ribosomal subunit, amongst which the protein P0 plays the most crucial role. Earlier studies have shown the functional complementation of the conditional P0-null mutant of \textit{Saccharomyces cerevisiae} (W303dGP0) with orthologous P0 genes from fungal and mammalian organisms, but not the protozoan parasite \textit{Leishmania infantum}. In this paper we show that the \textit{PfP0} gene from the protozoan malaria parasite \textit{Plasmodium falciparum} can functionally complement the conditional P0-null W303dGP0 mutant of \textit{S. cerevisiae}. Unlike the above orthologous genes, \textit{PfP0} gene could also rescue the D67dGP0 strain, which in addition to being a conditional null for \textit{ScP0} gene, is a null-mutant for both \textit{ScP1a} and \textit{ScP1h} genes. However, under stress conditions such as high temperature, salt and osmolarity, \textit{PfP0} gene could not rescue D67dGP0 strain. Ribosomes purified from W303dGP0 carrying \textit{PfP0} gene did not contain \textit{ScP1} protein, indicating a lack of binding of \textit{ScP1} to \textit{PfP0} protein. Yeast 2-hybrid analysis further confirmed the lack of binding of \textit{ScP1} to \textit{PfP0} protein. The polymerizing activities of ribosomes with \textit{ScP0} or \textit{PfP0} protein, in the absence of \textit{ScP1} protein, were found to be about 40–45% that of ribosomes with all the yeast P-proteins. In its sensitivity to the inhibitor sordarin, \textit{PfP0} was similar to the P0 protein from the fungus \textit{Aspergillus fumigatus}. These results indicate a closer functional relationship of \textit{P. falciparum} P0 gene to fungal P0 genes.

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1. Introduction

The neutral protein P0 and the acidic proteins P1 and P2 make up a pentameric complex P0(P1)\textsubscript{2}(P2)\textsubscript{2}, which constitutes the eukaryotic stalk (Uchiumi et al., 1987), a ribosomal protuberance of the large ribosomal subunit, functionally equivalent to the bacterial complex L10(L7/L12) (Liljas et al., 1986). The stalk interacts with the elongation factor EF-2 and plays an important role in the regulation of protein synthesis. In \textit{Saccharomyces cerevisiae}, the stalk consists of two P1 proteins (ScP1\textsubscript{a} and \textsubscript{b}) and two P2 proteins (ScP2\textsubscript{a} and \textsubscript{b}) in addition to ScP0 (Remacha et al., 1995). The P0 protein, but not P1 or P2 protein, is essential for ribosomal activity and cell viability (Santos and Ballesta, 1994, 1995).

Study of the capacity of orthologous P0 proteins to complement the endogenous protein in conditional P0-null mutants of \textit{S. cerevisiae} has been performed earlier...
Several orthologous P0 genes from fungi, cellular slime mold, insects and mammals allowed growth under restrictive conditions of conditional P0-null yeast strains, which carried the ScP0 gene under the inducible GAL promoter. However, the P0 gene from the protozoan parasite *Leishmania infantum* did not complement the ScP0 protein. Chimeric constructs of aminoterminal domain from the mammalian P0 and carboxy-terminal domain from ScP0 could complement the ScP0 conditional null-mutant as well as rescue certain thermal and osmolar sensitivity (Rodriguez-Gabriel et al., 2000).

Strain D67dGP0, in addition to being a conditional null for ScP0, is a null-mutant for both ScP1 α and β genes (Remacha et al., 1992). When grown in non-restrictive conditions, such as in galactose (Remacha et al., 1992), or complemented with ectopic ScP0 in restrictive conditions (Santos and Ballesta, 1994), the D67dGP0 strain contains ribosomes, which are devoid of both the acidic P1 and P2 proteins. Detailed in vitro studies in yeast have further established that in the absence of P1 protein, P2 does not bind to P0 protein (Zurdo et al., 2000), establishing that P1 is critical for the formation of the pentameric stalk P-protein complex. The strain D67dGP0 grows slowly and the protein expression pattern is different (Remacha et al., 1995). Therefore, in addition to the structural contribution to the stalk region of the 60S ribosomal subunit, the acidic proteins are hypothesized to play a role in the translation regulatory mechanism in yeast (Remacha et al., 1995). Amongst all the orthologous P0 genes used for the complementation analysis, only the insect P0 proteins could complement the strain D67dGP0 (Gagou et al., 2000). Based on these results, it has been postulated that the amino terminal rRNA binding domain of most of the eukaryotic P0 proteins is conserved, while the carboxy-terminal located acid protein binding domain has diverged considerably. The carboxy-terminal regions of the insect P0 proteins, however, appear to be structurally closer to that of ScP0 (Kouyanou et al., 2003). In this paper we show that the protozoan human malarial parasite PfP0 protein could replace the yeast ScP0 protein in the presence as well as absence of ScP1 proteins. The ribosomal activity of PfP0 with or without ScP1 was comparable but the cells were differentially stress sensitive. The results also showed that unlike all other orthologous RPP0 proteins tested so far, PfP0 did not bind to the ScP1 protein.

2. Materials and methods

2.1. Yeast and bacterial strains and growth media

*S. cerevisiae* W303dGP0 (MAT α, leu2-3, 112, ura3-1, trp1-1, his3-11, 15, ade2-1, can1-100, RPP0::URA3-GAL1-RPP0) and *S. cerevisiae* D67dGP0 (MAT α, leu2-3, 112, ura3-1, trp1-1, his3-11, 15, ade2-1, can1-100, RpY1α::RpY1β::TRP1, RPP0::URA3-GAL1-RPP0) were derived from *S. cerevisiae* W303 and D67, respectively, by integration through homologous recombination in the RPP0 locus of a construction carrying the P0 coding region fused to the GAL1 promoter (Santos and Ballesta, 1995). Yeasts were grown in either YEP medium (1% yeast extract, 2% peptone) or minimal YNB medium, supplemented with the necessary nutritional requirements. In both cases, the carbon source was either 2% glucose or 2% galactose as indicated. Stock cultures of all yeast strains were maintained in galactose medium. When required, cells were shifted to glucose medium and allowed to grow for at least 20 generations to reach steady state growth conditions. *Escherichia coli* DH5α was used as a host for the routine maintenance and preparation of plasmids and was grown in LB medium. Strains were tested for growth under osmolar stress in minimal medium containing 2% glucose and 0.3 M NaCl or 0.8 M sorbitol.

2.2. Enzymes and reagents

Restriction endonucleases were purchased from Roche Molecular Biochemicals, MBI Fermentas, New England Biolabs, and Amersham Pharmacia Biotech, and were used as recommended by the suppliers. T4 DNA ligase, calf intestinal alkaline phosphatase, and the DNA polymerase I Klenow fragment were from Roche Molecular Biochemicals.

DNA manipulations of *E. coli* and yeast were performed using standard protocols (Sambrook et al., 1989; Adams et al., 1997). Monoclonal antibodies against PfP0 were raised in the laboratory (Rajeshwari et al., 2004). Polyclonal rabbit sera against ScP0 was obtained as described previously (Santos and Ballesta, 1994).

2.3. Cloning of PfP0 in yeast expression vector

A pBluescript vector, pBSHsP0, containing the full-length human P0 gene (Rodriguez-Gabriel et al., 2000), cloned between the upstream (1 kb) and downstream (0.7 kb) flanking regions of yeast P0 gene, was digested with *NdeI* and *NheI* enzymes to remove the human P0 gene, and to replace with PfP0 full length gene. However, as PfP0 gene had an *NdeI* site in its sequence, it had to be cloned in two parts. The entire gene was amplified using primers containing *NdeI* and *NheI* sites (5′-GCTAAGCTATGATGGCGAATTATCCAAAG3′; 5′-TCGCTAGCCTAACACATTCAAAT3′). This fragment was restricted with *NdeI* and *NheI*, and the 3′−400 bp of *NdeI−NheI* fragment of PfP0 was cloned into pIP0pBSHsP0/NdeI/NheI. The recombinant clone, pBSPiP0C, was then digested with NdeI and the 5′−551 bp *NdeI* fragment of PfP0 was cloned into this, resulting into pBSPiP0 with the entire PfP0 in proper orientation. The PfP0 insert was then PCR amplified from pBSPiP0 using T3 and T7 primers, digested with *NdeI* and *SalI*, and ligated into the vector pFL37 (Rodriguez-Gabriel et al., 2000), resulting in the plasmid pFL37PfP0. The
sequence of the PfP0 DNA fragment cloned in this vector was confirmed by sequencing. For the yeast two-hybrid screening, plasmids, pEG202, pSH18-34, pJG4-5 and the yeast strain EGY48 were obtained from Roger Brent’s lab and have been described earlier (Gyuris et al., 1993). The entire PfP0 (316 amino acids) was amplified from λ12 clone (Goswami et al., 1996), and the entire yeast P0 (312 amino acids) was amplified from genomic DNA using primers YP0FR1 (5’-CTCTGAATTCATGGGAGGCATTCGTGAAAAG3’) and YP0RX1 (5’-CGGCCCTCAGATCCTGTTGCCACTTCC3’). These were ligated into pEG202 in frame resulting in plasmids pEGPfP0 and pEGScP0 respectively. ScP1α gene was amplified using (5’-CTCTGAATTCATGGGAGGCATTCGTGAAAAG3’) and (5’-CTCTGAATTCATGTCTACTGAAACCGGCCTCGAGTCATGTTGCACTTC3’) primers, and cloned in the pJG4-5 vector in the correct reading frame. The screen was performed as described elsewhere (Gyuris et al., 1993). Briefly, the yeast strain EGY48 (MATa, his3, trpl, ural-3-52, leu2::pLEU2-LexAop6) was pre-transformed by the lithium acetate method, with the bait pEGPfP0/pEGScP0 and the lacZ LexAop6. These transformants were tested for the lack of growth on Leu− plates. These cells were then transformed with pJG4-5-ScP1α plasmid DNA and grown on glucose Leu− Ura− His− Trp− plates. Colonies were replica plated onto β-galactosidase assay plates containing either galactose or glucose as the carbon source. Only Leu+ colonies that grew and demonstrated β-galactosidase activity on galactose, but not dextrose, were considered positives. Quantitative β-galactosidase measurements were carried out with yeast liquid cultures, using the procedure described by Clonetech. Typically, 0.1 ml of cells were harvested in 200 μl of phosphate buffer, pH 7.0, permeabilized by liquid nitrogen and broken with glass beads. The extract was centrifuged at 90,000 rpm for 30 min at 4°C, yielding the supernatant 30 fractions, which was centrifuged at 90,000 rpm for 30 min at 4°C in a Beckman TL100.3 rotor. The supernatant S100 fraction was stored at −80°C and the crude ribosome pellet was resuspended in buffer 2 (20 mM Tris–HCl, pH 7.4, 500 mM AcNH4, 100 mM MgCl2, 5 mM β-mercaptoethanol) and centrifuged through a discontinuous sucrose gradient (20/40%) in buffer 2 at 90,000 rpm for 120 min at 4°C in a TL100.3 rotor. The pellet of washed ribosomes was dissolved in buffer 1 and stored at 20°C.

2.5. Western blotting

Proteins in gels were transferred to membranes by electrophoresis in a semi-dry system using Novablot LKB buffer. The membranes were treated with 5% non-fat milk dissolved in TBS (10 mM Tris–HCl, pH 7.4, 200 mM NaCl) for 30 min and afterward they were incubated for 1 h with the antibody diluted in the same buffer. Subsequently, the membranes were washed 15 min in TBS and 0.1% Tween 20. Then, the second antibody (anti rabbit HRP conjugated and anti mouse HRP conjugated secondary antibodies were used), diluted in the former buffer, was added and the membranes were incubated for 30 min. Finally, they were washed 15 min with 0.1% Tween 20 in TBS. Bound antibodies were located by detecting peroxidase activity.

2.6. Polyphenylalanine synthesis

The reaction was performed as described earlier (Remacha et al., 1995). Briefly 50-μl samples containing 10 pmol of 80 S ribosomes, 5 μl of S-100, 0.5 mg/ml tRNA, 0.3 mg/ml polyuridylic acid, 40 μM [3H]phenylalanine (120 cpm/μmol), 0.5 mM GTP, 1 mM ATP, 2 mM phosphocreatine, and 40 μg/ml creatine phosphokinase in 50 mM Tris–HCl, pH 7.6, 15 mM MgCl2, 90 mM KCl, 5 mM β-mercaptoethanol. After incubation at 30°C for 30 min, samples were precipitated with 10% trichloroacetic acid, boiled for 10 min, and filtered through glass fiber filters. Radioactivity incorporated in blanks lacking ribosomes was subtracted from all samples. In assays to test the relative activity of ribosomes from different transformants, the same S-100 fraction from wild-type strain W303 was used.

2.7. In vivo sordarin resistance assay

The assay was carried out in 96-well plates with 10^5 cells per well in 100 μl of rich medium supplemented with different amount of sordarin derivative GM193663 (a gift of Glaxo-Smith-Kline Research Center, Tres Cantos, Madrid). Ten serial dilutions of the antibiotic were done starting with the concentration of 5 μg/ml. After 48 h incubation at 30°C the A600 was measured and the IC50 (minimal concentration of compound that inhibits 50% of growth) was calculated.

3. Results

3.1. Complementation of the conditional P0 null phenotype by orthologous proteins at 30°C and 37°C

Both the S. cerevisiae W303dGP0 and D67dGP0 strains carry the essential RPP0 gene under the control of the
GAL1 promoter and are viable only when grown in galactose as a carbon source. In addition, the strain D67dGP0 is a mutant in which both the P1α/P1β have been disrupted and the ribosomes are totally depleted from acidic proteins (Remacha et al., 1992). These strains were transformed with the plasmid pFL37-PfP0, which contained the entire coding sequence of the PfP0 gene from Plasmodium falciparum under the control of the 5′ regulatory region of the yeast RPP0 gene to assure the same level of expression. For comparison, these strains were also transformed with the plasmids pFL37-ScP0, pFL37-AfP0 and pFL37-HsP0 carrying the coding sequence of the RPP0 genes from *Saccharomyces cerevisiae*, *Aspergillus fumigatus* and *Homo sapiens*, respectively. These transformed strains were then analyzed for their ability to grow on glucose. Since there is a residual amount of ScP0 remaining from the growth under galactose, the cells were grown for at least two generations before they were scored for their growth on glucose under normal as well as different stress conditions (Fig. 1 and Table 1). W303dGP0 cells transformed with pFL37 containing the RPP0 gene of each of these sources were able to grow at 30 °C on glucose plates (Fig. 1A). Therefore, like ScP0, AfP0 and HsP0, the PfP0 gene could rescue the null-phenotype. The RPP0 genes from each of these sources could rescue W303dGP0 under most of the stress conditions, but the rescue by PfP0 at high temperature as well as high salt concentration was weaker (Table 1).

In the case of D67dGP0 strain, only ScP0 and PfP0 genes could rescue the inability to grow in glucose containing media, showing that unlike HsP0 and AfP0, PfP0 gene could rescue growth even in the absence of P1 proteins (Fig. 1B). However, PfP0 gene could not rescue growth under high temperature, salt or osmolar stress. Even the native ScP0 protein, could not support growth at 37 °C in the absence of ScP1 protein.

![Fig. 1. Growth of *S. cerevisiae* W303dGP0 cells (A) and D67dGP0 (B) transformed with plasmid pFL37 carrying various P0 genes on minimal medium containing different osmotic conditions (0.3 M NaCl and 0.8 M sorbitol) at 30° and 37 °C. Hs—*Homo sapiens*; Af—*Aspergillus fumigatus*; Pf—*Plasmodium falciparum*; Sc—*Saccharomyces cerevisiae*; Control: W303dGP0 (A); D67dGP0 (B) cells.](image-url)
3.2. Growth curve and expression of PfP0 protein in yeast strains

Figs. 2A and B show the growth profile for W303dGP0 strain containing various P0 genes from different sources at 30°C and 37°C, respectively. All orthologous genes conferred slower growth rates, and the doubling times for cells containing ScP0, PfP0, AfP0 and HsP0 genes at 30°C were 95, 140, 133 and 170 min, respectively (Fig. 3C). The doubling times for cells containing ScP0, AfP0 and HsP0 genes were similar to the values reported earlier (Rodriguez-Gabriel et al., 2000) and comparable to those containing PfP0 gene. However, at 37°C the doubling time of cells containing PfP0 gene is 370 min, considerably higher than 255–265 min for cells with AfP0 and HsP0 genes (Fig. 2C). Cells with ScP0 genes doubled at 105 min at 37°C. The D67dGP0 strain containing PfP0 grew very slowly with a doubling time of > 540 min, as opposed to 180–200 min of D67dGP0 with ScP0 (data not shown). It was also observed that freshly after transformation, W303dGP0 cells containing PfP0 showed long lag periods of about 4 to 6 h in liquid cultures, and we postulate that the lag-period as well as slower growth may be partly due to the differential codon usage in yeast and Plasmodium (Vinkenoog et al., 1998).

In order to assess that the complementation was due to PfP0 protein, and not due to the reversion of ScP0 protein production, immunoblots were probed with antibodies specific for these P0 proteins (Fig. 3). PfP0 and ScP0 proteins are 38 and 34 kDa in size respectively, and were exclusively detected in protein extracts from cells containing the respective RPP0 genes. No reversion was detected in W303dGP0 strain containing PfP0 gene even after several generations in liquid culture. However, upon continuous culturing of D67dGP0 strain containing PfP0 gene in liquid glucose medium, reversion to shorter doubling time concomitant with expression of ScP0 protein was observed frequently. Clearly the rescue afforded by PfP0 gene in the absence of ScP1 is inefficient, resulting in a stress to produce ScP0 protein. It is a conjecture that
the GAL1 promoter undergoes mutation(s) under such stress and becomes leaky.

3.3. Effect of PfP0 on yeast ribosome activity, composition and sensitivity to sordarin

The polymerizing activities of ribosomes from the W303dGP0 and D67dGP0 strains containing PfP0 gene were found to be comparable, around 40–45% of the W303dGP0 strain with ScP0; this value is also similar to the activity of ribosomes from control D67dGP0 expressing the endogenous ScP0 (Fig. 4A). It has been demonstrated earlier that P0 interacts primarily with P1 proteins (Lalioti et al., 2002). In order to test whether P1 protein is bound to the ribosome stalk in W303dGP0 strain containing PfP0, an immunoblot of purified ribosomes were probed with antibody specific for ScP1\(h\) protein, and it was observed that it did not bind to yeast ribosomes containing PfP0 protein (Fig. 4B).

The lack of interaction between PfP0 and ScP1 proteins was independently confirmed with yeast two-hybrid assay using P0 as the bait fusion, and ScP1 as the trap fusion partner. Earlier in vitro studies in yeast have established that it is ScP1\(\alpha\) which interacts with maximum efficiency with ScP0 protein (Zurdo et al., 2000), and therefore ScP1\(\alpha\) was used as the trap fusion partner. When the cells were subjected to selection conditions, the cells containing the control ScP0 protein as the bait fusion protein grew as Leu\(^{+}\), establishing interaction of ScP0 with ScP1\(\alpha\) (Fig. 5). \(\beta\)-galactosidase activity of cells with ScP0 and ScP1\(\alpha\) was measured as 417 Miller units (Miller, 1972), indicating interaction between ScP0 and ScP1\(\alpha\). However, the PfP0 bait-fusion protein did not grow under these selection conditions, demonstrating an absence of interaction with ScP1\(\alpha\) (Fig. 5B).

Protein P0 plays an important role in determining the cell susceptibility to antifungal sordarin derivatives (Gomez-Lorenzo and Garcia-Bustos, 1998; Justice et al., 1999). It

![Fig. 4. A: Table showing polymerizing activity of ribosomes derived from *S. cerevisiae* carrying PfP0 or ScP0 proteins. B: Western blot probed with anti-ScP1\(h\) monoclonal antibodies. Lanes contain ribosomes extracted from W303dGP0 cells transformed with ScP0 and PfP0 genes respectively. Arrow shows the 12 kDa ScP1\(h\) protein.](image)

![Fig. 5. Yeast 2-hybrid interaction of P0 with ScP1 protein. Growth of yeast reporter strain EGY48 (pSH18-34) transformed with ScP0 or PfP0 in pEG202 vector on A) U–H– T– Glucose medium and B) U– H– T– L– Gal/raf medium containing ScP1\(\alpha\) in pG4-5 vector.](image)

![Fig. 6. Inhibition of cell growth by sordarin derivative GM193663. Increasing concentrations of antibiotic were added to cells from *S. cerevisiae* W303dGP0/ScP0 (■), W303dGP0/PfP0 (△) and W303dGP0/ AtP0 (○) growing in glucose rich medium as indicated in Section 2. 100% corresponds to the \(A_{600}\) of sample growing in the absence of drug.](image)
has been shown that expression in *S. cerevisiae* of P0 protein from *A. fumigatus*, a sordarin resistant organism, increases the resistance of yeast to the antifungal (Santos and Ballesta, 2002). The effect of PfP0 on susceptibility of strain W303dGP0-PfP0 to sordarin was tested and PfP0 was found to be comparable to AfP0 in its resistance to sordarin (Fig. 6). The IC50 for strain W303dGP0 expressing ScP0, PfP0 or AfP0 was found to 0.03 μM, 0.8 μM and 0.96 μM respectively. Thus > 25-fold increase in resistance was observed in cells containing either PfP0 or AfP0.

4. Discussion

Amongst all the orthologous genes tested for complementation earlier, the *RPP0* genes from mammalian organisms such as *H. sapiens* and *Rattus norvegicus*, cellular slime mold *D. discoideum* and the fungus *A. fumigatus* complemented the conditional null-phenotype for growth on glucose, but that from protozoan parasite *L. infantum* did not (Rodriguez-Gabriel et al., 2000). Homology mapping has indicated PfP0 gene to be more closely related to yeast and mammalian genes, as compared to the kinetoplastid protozoan *Leishmania* and *Trypanosoma* genes (Goswami et al., 1996). The complementation results confirm that PfP0 protein is functionally closer to yeast P0 protein, as compared to the kinetoplastid protozoan P0 protein. The results presented in this paper also show that in the absence of stress conditions, *PfP0* gene allows for growth of cells comparable to the other orthologous *RPP0* genes listed above. However, under stress conditions the *PfP0* gene performs comparatively poorly. Unlike these orthologous *RPP0* genes, *PfP0* gene could support the growth of yeast cells in the absence of ScP1 proteins.

One of the major differences between other P0 and PfP0 proteins lies in the absence of interactions between PfP0 and ScP1 proteins. This would imply that the ribosomal stalks of both W303dGP0 and D67dGP0 cells containing PfP0 protein would be devoid of both P1 and P2 proteins, since the yeast P2 proteins bind to P0 protein only through the P1 proteins (Zurdo et al., 2000). The comparable polymerizing activities of ribosomes from the W303dGP0 and D67dGP0 strains containing *PfP0* gene are therefore not unexpected.

On the basis of extensive analysis on the interaction sites, a putative leucine zipper site on ScP0 has been predicted to bind the ScP1 proteins (Lalioti et al., 2002). This leucine zipper site is entirely missing in *PfP0* gene as compared to the other orthologous P0 genes (Fig. 7). This difference may explain the lack of binding of ScP1 proteins with PfP0. Like the *PfP0* gene, the P0 genes from the insects *Ceratitis capitata* and *Bombyx mori* have been reported to complement *D67dGP0* strain. However, the putative leucine zipper is present in all the insect P0 proteins, and insect P0 proteins do bind to the acidic proteins (Gagou et al., 2000; Kouyanou et al., 2003). The genome database for *P. falciparum* predicts putative single genes for orthologous PfP1 and PfP2 acidic proteins. PfP2 gene has been cloned and the gene expression has been reported (Fidock et al., 1998). However, no information exists regarding the properties of these *P. falciparum* acidic P-proteins.

P1/P2 proteins distinctly play a role in the ribosomal efficiency in yeast, since a drop of about 50–60% of
polymerizing activity is observed in the absence of P1 protein. The presence of ScP1 proteins also appears to be vital under several stress conditions. Although P-proteins play a structural role in the composition of the yeast 60S ribosomal subunit, heterogeneity of ribosomal composition has been observed. Ribosomes from stationary phase are deficient in P1/P2 proteins, as compared to ribosomes from exponential phase of growth (Saenz-Robles et al., 1990).

The pattern of protein expression in the absence of P1 and P2 proteins is distinct from that in the presence of these acidic proteins (Remacha et al., 1995). It was documented that the distinct pattern of expression was not due to translation error or termination suppression, but was postulated to be due to differential translation modulation, or/and due to extra-ribosomal properties of these acidic proteins (Remacha et al., 1995). Recently the ribosomal protein L13a was shown to bind to the 3′-UTR of ceruloplasmin, a protein involved in the inflammatory response of human monocytes, and inhibit its translation (Mazumder et al., 2003). A regulatory role of ribosomes, through differential binding of selective mRNAs to ribosomes, has been postulated to play an important part in translational control (Ballesta and Remacha, 1996; Mauro and Edelman, 2002). The level of P1 protein also appears to be regulated, since P1 has been detected as a fusion protein with ubiquitin in the chlorarachniophyte algae (Archibald et al., 2003).

Resistance to the antifungal sordarin has been mapped earlier to the P0 domain associated with elongation factor binding site. Sordinar resistant strains showed point mutations clustered in the region around 130–147 amino acid position of the P0 protein (Gomez-Lorenzo and Garcia-Bustos, 1998; Justice et al., 1999). The sordinar resistance of W303dGP0 strain carrying the orthologous *A. fumigatus* P0 (AfP0) protein, has been shown earlier to be several fold higher than that of W303dGP0-ScP0 (Santos and Ballesta, 2002). Amino acids 130–147 were found to be identical between AfP0 and ScP0, and by this new analysis three point mutations at the positions E117A, E134A, and R122P were demonstrated to be responsible for the sordinar resistance. PfP0 is very similar to AfP0 protein in these three positions. The charged glutamic acid residue is replaced with isoleucine at 117 position, while the residues 122 and 124 are proline and glycine, same as in AfP0 (Fig. 7). Thus the higher resistance of W303dGP0-Pfp0 to sordinar is consistent with the changes in this conserved domain, implicated in translation elongation.

Amongst protozoans, *Plasmodium* is an Apicomplexan parasite, whereas *Leishmania* is a kinetoplastid, and their evolutionary distance has been postulated recently based on the currently available genome information (Aravind et al., 2003). The protozoans have a wide spread, and it is apparent from the genomic data that the kinetoplastids are quite separated from Apicomplexans. In several metabolic pathways, *Plasmodium* appears to be similar to the free-living yeasts (Aravind et al., 2003). It possesses a yeast-like mRNA capping apparatus, structurally and mechanistically unrelated to that found in metazoans and plants (Ho and Shuman, 2001). The complementation capability of PfP0 gene in *S. cerevisiae*, and its similarity in the sordinar resistance properties with *A. fumigatus*, further strengthens the evolutionary linkage of Plasmodium to fungi. Although it has now become possible to carry out genetic manipulations in *P. falciparum*, disruption of vital genes continue to be difficult to manipulate. The functional complementation of ScP0 with PfP0 protein, as well as the closeness of Apicomplexans to fungi, makes it possible to use *S. cerevisiae* as a genetic system to dissect out some of the multiple functions of PfP0 protein.

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