Purified yeast protein farnesyltransferase is structurally and functionally similar to its mammalian counterpart

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Protein farnesyltransferase (FTase) catalyses the addition of a farnesyl group to a cysteine within the so-called ‘CAAX box’ at the C-terminus of various proteins. In the present paper we report purification of Saccharomyces cerevisiae FTase to near-homogeneity. This was accomplished by constructing a yeast strain overproducing FTase approx. 100-fold. The purified enzyme was a heterodimer of approx. 90 kDa and consisted of 43 kDa and 34 kDa subunits. The 43 kDa subunit was shown to be the product of the DPRI gene by using antibody raised against baculovirus-produced DPRI polypeptide. The purified enzyme required Mg²⁺, showed a pH optimum of 7.8 and was most active at 50 °C. The Kₘ values for farnesyl pyrophosphate and GST-CIIS (glutathione S-transferase fused to the C-terminal 12 amino acids of yeast RAS2 protein), Kₘ⁻FPP and Kₘ⁻GST-CIIS, were 8.1 and 5.1 μM respectively. The enzyme was capable of farnesylating GST-CIIS (the same as GST-CIIS, except that the C-terminal serine is changed to leucine), a substrate protein for the enzyme geranylgeranyltransferase, although with a higher apparent Kₘ than for GST-CIIS. Like its mammalian counterpart, yeast FTase activity was inhibited by peptides containing the C-terminal CAAX sequence (that is, one where C = cysteine, A = aliphatic amino acid and X = any amino acid). These results provide direct evidence for the idea that the yeast and mammalian FTases are structurally and functionally very similar.

INTRODUCTION

Protein farnesyltransferase (FTase) is a key enzyme that is responsible for the post-translational modification of a number of proteins involved in cell growth. These proteins include ras proteins, nuclear lamins and the yeast a-mating factor, which end with a unique C-terminal sequence, CysAAX (A is an aliphatic amino acid and X is the C-terminal amino acid) [1-4]. This CysAAX sequence is termed the ‘CAAX box’ [5] and the FTase catalyses the addition of a farnesyl group to the cysteine. Another type of protein prenylation involves the addition of a geranylgeranyl group, and protein geranylgeranyltransferases (GGTases) are responsible for this type of modification [6-11].

One of the interests in FTase is its potential as a target for drugs which inhibit the action of ras proteins [12,13]. In the case of ras proteins, farnesylation contributes to an increase in their hydrophobicity, thus facilitating their membrane localization [14,15]. Because a proper membrane localization is required for the function of ras proteins, action of oncogenic forms of the ras protein can be blocked by inhibiting their membrane localization. Peptide inhibitors of FTase have been reported [13].

FTase from rat brain has been extensively characterized. This enzyme has strong affinity to the CAAX sequence; it binds to a column containing peptides having the CAAX sequence and is inhibited by tetrapeptides containing the CAAX sequence [1]. The tetrapeptide inhibition enabled the determination of sequence requirements for the CAAX box [16]. The mammalian enzyme is a heterodimer consisting of two similar-size subunits termed α and β [7,17]. The β subunit can be cross-linked to ras proteins, suggesting that the site of CAAX recognition is within the β-subunit [17].

An FTase similar to the mammalian enzyme has been detected in crude extracts of yeast cells [2,18]. The requirement of two genes, DPRI/RAMI (referred to here as DPRI) and RAM2 for the yeast FTase activity suggested that the enzyme consisted of two subunits encoded by these genes. This idea was further strengthened by the detection of a significant (approx. 30%) identity between DPRI and the β-subunit of the mammalian FTase as well as between RAM2 and the α-subunit of the mammalian FTase [19,20]. In addition, FTase activity was detected after simultaneous expression of DPRI and RAM2 genes in Escherichia coli [21]. Although these results point to the possibility that the yeast FTase consists of DPRI and RAM2 gene products, direct evidence to support this idea has been lacking. Moreover, the yeast FTase has not been purified.

Here we report the purification of yeast FTase and show that the enzyme is a heterodimer consisting of two polypeptides having approximate molecular masses of 43 and 34 kDa. The chromatographic behaviour as well as characteristics of the yeast enzyme are very similar to those of the mammalian enzyme.

EXPERIMENTAL

Strains, plasmid DNAs and peptides

SP1 (MATα leu2 trp1 his3 ade8 can1) [22] has been used for most experiments. Other Saccharomyces cerevisiae strains are
RS16-4C (MATa ura3 trpl ade8 can1 SUP84l) and UC100 (MATa leu2 trpl ura3 pep4 prb) [2]. Cells were grown in synthetic media containing 2% glucose and lacking appropriate amino acids [23]. YEpDPRI contains 4.8 kbp BamHI-to-SalI fragment of DPRI cloned into the BamHI to SalI region of YEp24 [24,25]. pBH28 (kindly provided by Scott Powers, Robert Wood Johnson Medical School, Piscataway, NJ, U.S.A.) contains RAM2 gene on the multicopy plasmid YEp13. Synthetic peptides [MG13 (NNSVWCTLM), MG14 (SGSGGCCCIS) and P119 (YPYDVPDYAS)] were kindly provided by Malcolm Whiteway (NRC Biotechnology Research Institute, Montreal, Quebec, Canada). LHRH (luteinizing-hormone-releasing factor) peptide (EHWSYGLRPG) was from Boeringer Mannheim. pVL1392 and AcNPV DNAs were gifts from Max D. Summers (Texas A & M University, College Station, TX, U.S.A.). Spodoptera frugiperda Sf9 insect cells were from the American Type Culture Collection.

Prenyltransferase assays and preparation of crude extracts

Prenyltransferase assays were performed essentially as described [2,6]. Briefly, the reaction mixture (20 μl) contained 50 mM potassium phosphate, pH 7.4 (or 50 mM Tris/HCl, pH 8.0), 10 mM MgCl₂, 5 mM DTT, 5 μM ZnCl₂, 0.8–1.12 μM [H]farnesyl pyrophosphate (Fpp) (20 μM/mmol; New England Nuclear) or [3H]geranylgeranyl pyrophosphate (GGpp) (20 μM/mmol; New England Nuclear), 19 μg of GST-ClIIS or 30 μg of GST-ClIIL protein and enzymes. Incubation was carried out at 37 °C, and the radioactivity incorporated was determined by spotting the reaction mixture onto a filter paper, treating with 10% trichloroacetic acid, followed by ethanol and acetone washing. The variability between different FTase assays was generally within 8% of the values shown. For example, the values shown in Figures 1 and 4 (below) had the variability of 7.2% and 5.1% respectively between different assays. Crude extracts of yeast cells were prepared by suspending cells in Buffer A [0.1 M Mes/NaOH (pH 6.5) 0.1 M MgCl₂/0.1 M EGTA/1 mM DTT] containing 2 mM phenylmethylsulphonyl fluoride (PMSF) and disrupting cells using glass beads. The extracts were centrifuged at 100000 × g for 1 h after low-speed centrifugation. Protein concentrations of the crude extract as determined by the bicinchoninic acid (‘BCA’) method [26] were approx. 10 mg/ml.

Purification and characterization of FTase

Yeast FTase was purified as follows. SP1 cells carrying YEpDPRI and pBH28 plasmids were grown in a synthetic medium lacking leucine and uracil to late-exponential phase. A 20 g (wet weight) portion of cells was suspended in 60 ml of buffer A containing 3.1 μg/ml bestatin, 1 mM PMSF, 6.7 μg/ml pepstatin, 37 μg/ml tosyl-lysychloromethane (‘TLCK’), 35 μg/ml tosylphenylalanine chloromethane (‘TPCK’) and were disrupted with glass beads using a Bead Beater (Biospec). After low-speed centrifugation at 1500 g for 10 min, crude soluble extracts were prepared by centrifuging at 100000 g for 1 h. (NH₄)₂SO₄ was added to the extracts to make 30% saturation and the pellet was removed by centrifugation. Additional (NH₄)₂SO₄ was added to the supernatant to make 60% saturation, and the pellet was collected. The pellet was suspended in a small volume of 25 mM Tris/HCl (pH 7.4)/1 mM DTT/0.1 mM MgCl₂/0.1 mM EGTA and was dialysed against the same buffer. The sample was applied to a Mono Q column (HR 16/10; Pharmacia), and the elution was carried out with a salt gradient of 0.1–0.4 M NaCl in Mono Q buffer consisting of 25 mM Tris/HCl (pH 7.4)/1 mM MgCl₂/1 mM DTT. The activity was eluted at a salt concentration of approx. 250 mM. The peak fractions were pooled, n-octyl β-D-glucopyranoside was added to 0.2%, and stored frozen at −80 °C. When necessary, rechromatography on Mono Q (HR5/5) was carried out by diluting the pooled fractions with the Mono Q buffer and reapplying to the column.

The (NH₄)₂SO₄-fractionation step could be replaced by an alternative step which involved DEAEE-Sepharose followed by Sephacryl S-300. High-speed supernatants from 15 g of cells were applied to DEAEE-Sepharose CL-4B (Pharmacia) (column volume 130 ml) equilibrated by Mono Q buffer. Elution was carried out by increasing NaCl concentration from 0 to 0.4 M. The peak fractions eluting with a salt concentration of 0.17 M were concentrated by adding (NH₄)₂SO₄ to 80% saturation and were applied to a Sephacryl S-300 (column volume 100 ml) which had been equilibrated in 25 mM Tris/HCl (pH 7.4)/1 mM MgCl₂/50 mM NaCl. The peak fractions were pooled and applied to a Mono Q column (HR5/5) as described above.

Expression of DPR1 protein in baculovirus-infected insect cells and production of antibody against DPR1 protein

PCR was used to generate a DNA sequence containing the DPRI coding sequence of YCpDPR2.24 [24] and restriction sites suitable for subcloning into a baculovirus transfer vector. The primers used for PCR were 5'-GGGCCCCGTCGACTTAACTTGGAGAAGATAAATTGG-3' and 3'-GGGGGAAAACATGGGACAGAAGAGATGAAAGG-3'. The PCR product was treated with T4 DNA polymerase in the presence of dNTPs, digested with EcoRI and ligated into the baculovirus transfer vector pVL1392, which had been digested with SmaI and EcoRI. The sequence of the junctions at the 5' and 3' end of the DPR1 gene was confirmed. The resulting plasmid, called pAcS13, was co-transfected along with DNA of strain E2 of Autographa californica nuclear polyhedrosis virus into Spodoptera frugiperda Sf9 insect cells and recombinant viruses were isolated as described in [27].

Expression of DPR1 in the baculovirus system was accomplished by growing Sf9 cells at 1.5 × 10⁶/ml in 50 ml or 500 ml of suspension cultures in Ex-Cell 400 medium (JHR Biosciences, Lenexa, KS, U.S.A.) at 27 °C, infected with recombinant viruses at a multiplicity of infection of 10. At 2 days after infection, the Sf9 cells were centrifuged at 10000 g for 10 min, re-suspended in ice-cold lysis buffer [10 mM Hepes (pH 7.4)/1 mM MgCl₂/1 mM EGTA/1 mM PMSF] at 4 × 10⁷ cells/ml, homogenized with 20 strokes in a Dounce-type grinder with a ‘B’ pestle and centrifuged at 100000 g for 1 h at 4 °C. The supernatant, referred to as the ‘S100 fraction’, contained DPR1 protein as determined by Coomassie Blue staining of the SDS/polyacrylamide gel.

The baculovirus-expressed DPR1 from the S100 fraction was partially purified as follows. The S100 fraction was applied to a DE52 column equilibrated in 20 mM Tris/HCl (pH 7.4)/1 mM DTT/5%/glycerol/0.1 mM EDTA/0.5 mM PMSF. Elution was carried out by increasing NaCl concentration from 0 to 0.6 M. DPR1 polypeptide, as determined by SDS/PAGE, was eluted with 0.16 M NaCl and was completely separated from insect-cell FTase, which was eluted by 0.2 M NaCl. The DPR1 peak fractions were pooled, concentrated and loaded on to a Sephacryl S200 HR column using 50 mM sodium phosphate (pH 7.0)/1 mM DTT/200 mM NaCl/5% glycerol as a buffer. The fractions containing the peak of DPR1 were pooled and further purified by a preparative SDS/PAGE. The region of the
RESULTS

Overexpression of DPR1 and RAM2 results in a dramatic increase of FTase activity

Cloning of the DPR1 gene has previously been reported. The gene encodes a protein of 431 residues and the cell-free translation product migrates as a protein of approx. 43 kDa [24]. The RAM2 gene encodes a protein of 316 amino acid residues [21]. These genes were placed on multicopy plasmids and transfected into wild-type yeast cells, either together or individually. As Figure 1 shows, extracts of cells carrying both DPR1 and RAM2 plasmids exhibited a significantly higher level of FTase activity compared with the cells carrying the vectors. Comparison of the rate of the reaction suggested that the overexpressor had approx. 100-fold higher FTase activity. In contrast, overexpression of DPR1 alone resulted in an approx. 10-fold increase in activity. Similarly, overexpression of RAM2 alone resulted in a 6-fold increase.

Thus both genes are needed to obtain the highest level of FTase activity. The slight increase of the activity seen with single overexpressors could be due to the presence of free subunits in these cells. Essentially similar results were obtained when overexpression of DPR1 and/or RAM2 genes was carried out in another yeast strain, UC100; a significant increase of FTase activity was seen only when both DPR1 and RAM2 were expressed.

Purification of FTase

Increased FTase activity in the overexpressor provided a convenient source to purify FTase. Extracts of the cells overexpressing both DPR1 and RAM2 were subjected to (NH4)2SO4 fractionation followed by Mono Q column chromatography. The FTase activity was eluted at a salt concentration of approx. 250 mM. Occasionally the activity was split into two peaks. Analysis by SDS/PAGE did not reveal any differences between the two peaks. Both contained the two bands discussed below. As shown in Table 1, this scheme resulted in 3108-fold purification of the enzyme activity, with 73% recovery. The enzyme kept frozen at −80 °C in the presence of n-octyl β-D-glucopyranoside was stable for at least 2 months.

Molecular mass and subunits

A Superose 12 column profile of the FTase activity is shown in Figure 2(a). We observed a single peak of activity that was eluted in fractions corresponding to proteins of 80–100 kDa. Comparison with the marker proteins suggested that the native molecular mass of the yeast FTase is approx. 90 kDa. SDS/PAGE of the purified FTase revealed the presence of two major bands, as shown in Figure 2(b). The two bands corresponded to proteins having apparent molecular masses of 43 and 34 kDa. The bands seen at 60 kDa were present even in lanes only containing sample buffer (results not shown) and thus appear to represent keratins, common artifacts of silver staining [29]. To demonstrate that the 43 and 34 kDa bands represented polypeptides associated with the FTase activity, the sample was applied to a Superose 12 column and the column fractions were analysed by SDS/PAGE. As Figure 3 shows, the two bands were detected in the fractions where the FTase activity was detected. Co-elution of these two bands with the FTase activity was also seen when the sample was analysed by means of a Mono Q column (results not shown). Thus the yeast FTase consists of two polypeptides of molecular mass 43 and 34 kDa.

Effects of pH and temperature on FTase activity

Effect of pH on enzyme activity was examined from pH 3–10 using citrate/phosphate, phosphate, ammidol and Tris/HCl buffers. A broad pH optimum between 7 and 9 was observed (results not shown). Figure 4(a) shows the results with Tris/HCl buffer, which demonstrated that the maximum activity was obtained at pH 7.8 with Tris/HCl buffer. The temperature-dependence of the enzyme activity is shown in Figure 4(b). The enzyme was highly active above 37 °C, and little activity was detected below 24 °C. The activity increased up to 50 °C and rapidly decreased above 55 °C. Preincubation of the FTase at 55 °C for 10 min resulted in 88% decrease of the activity. The
presence of Fpp during the preincubation reduced this decrease to 67% of that of untreated enzyme. In contrast, addition of GST-CIIS protein during the preincubation did not have any significant effect (results not shown).

**Effects of bivalent and univalent cations**

Addition of salts inhibited the FTase activity. Approx. 50% inhibition was seen with 100 mM NaCl, LiCl or KCl. Of the three, KCl showed the most inhibitory effect (results not shown). The enzyme showed absolute requirement for bivalent cations. EDTA addition resulted in a dramatic inhibition of the FTase activity. Addition of 5 mM Mg²⁺ to the EDTA-inhibited enzyme fully restored the activity. Further addition of zinc resulted in a slight increase of the activity (Figure 5). Addition of 0.5–1 mM zinc alone was partially effective in the recovery of the activity of EDTA-treated enzyme; zinc alone recovered approx. 50% of the activity obtained with Mg²⁺ alone (results not shown).

**Substrate specificity**

Substrate specificity of yeast FTase was investigated by comparing incorporation of Fpp or Gppp into two different substrate proteins, GST-CIIS and GST-CIIL. GST-CIIL was previously shown to be an efficient substrate for GGTase [6]. As expected,
The reaction (20 μl) for results shown in open squares (□) contained 1.12 μM [3H]Fpp, 4.38 ng of FTase, 19 μM GST-CIIS, 0.1 mM EDTA and the indicated concentration of MgCl2. Reaction mixtures (20 μl) for results shown in closed diamonds (●) contained 0.1 mM ZnCl2 in addition to the above. Incubation was carried out for 10 min at 37 °C, and the radioactivity incorporated was determined as described in the Experimental section.

Figure 5  Bivalent-cation requirement for FTase

Figure 6  Incorporation of Fpp into two different substrate proteins, GST-CIIS or GST-CIIL

The reaction mixture (20 μl) contained 1.12 μM [3H]Fpp, 20 ng of FTase and the indicated concentrations of either GST-CIIS (□) or GST-CIIL (●) protein. Incubations were carried out for 10 min at 37 °C and the radioactivity incorporated was measured as described in the Experimental section.

Fpp was efficiently incorporated into GST-CIIS protein, whereas the incorporation of Fpp into GST-CIIL protein was much less efficient (Figure 6). Surprisingly, however, a significant level of Fpp incorporation was seen with GST-CIIL protein at high GST-CIIL concentrations. An apparent K_m obtained for GST-CIIL protein was 39 μM at an Fpp concentration of 1.1 μM. Virtually no incorporation of GGpp was seen with either substrate protein (results not shown). Effects of changing concentration of substrates, Fpp and GST-CIIS protein, are shown in Figure 7. Secondary plots from these plots, showed the K_m for Fpp and K_m for GST-CIIS to be 8.1 and 5.1 μM respectively.

Peptide inhibition

Like its mammalian counterpart, the yeast FTase activity was strongly inhibited by the addition of peptides having a C-terminal CAAX sequence. As Figure 8 shows, the activity was inhibited by the addition of a peptide, MG14, which contained ten residues of RAS2 C-terminal sequence, SGSGGCCIS; 50% inhibition was seen with 4 μM peptide. Another peptide, MG13, which contained ten residues of STE18 C-terminal sequence, NSNSVCCSTLM, also inhibited FTase, with 50% inhibition observed in the presence of 10 μM peptide. By contrast, little inhibition was seen with two control peptides of the same size, P119 and LHRH, having unrelated sequences.

Production of antibody against DPR1 protein and identification of 43 kDa protein as DPR1 gene product

Polyclonal antibody was raised against baculovirus-produced DPR1 protein. To accomplish this, DPR1 protein was first expressed in baculovirus-infected insect cells as described in the Experimental section. Extracts were made, then cleared by a high-speed centrifugation. The resulting supernatant was analysed by SDS/PAGE. As Figure 9(a) shows, a prominent band
Figure 9 (a) Expression of DPR1 in baculovirus-infected Sf9 cells and (b) immunoblot analysis of purified FTase

(a) Portions (32 μg) of extracts prepared from Sf9 cells infected with the recombinant virus carrying the DPR1 gene (lane 2) or the control virus (lane 1) were analysed on an SDS/12.5%-polyacrylamide gel. (b) Portions (0.57 μg for lane 4) and 1 μg for lane 3) of FTase, as well as 2.3 μg of DPR1 polypeptide purified from baculovirus-infected Sf9 cells (lane 1), were electrophoresed on an SDS/12.5%-polyacrylamide gel, transferred to nitrocellulose membrane and probed with anti-DPR1 antibody as described in the Experimental section. Lane 2 is blank. Abbreviation: M, molecular mass.

of approx. 43 kDa was observed in Coomassie Blue-stained gels in S100 fraction derived from Sf9 cells infected with the recombinant viruses (lane 2). This band was not present in the S100 fraction derived from cells infected with the control viruses (lane 1).

DPR1 protein could be separated from the insect cell FTase by DE52 cellulose column chromatography, since it was eluted from the column at a lower salt concentration than was FTase (results not shown). The DPR1 protein, thus purified, was used to immunize rabbits to produce polyclonal antibody against DPR1. The antibody was identified by immunoblotting.

The rabbit sera containing the DPR1 antibody was used to probe the purified yeast FTase. As shown in Figure 9(b), a band corresponding to 43 kDa was detected by immunoblotting of the purified yeast FTase with the anti-DPR1 antibody (lanes 3 and 4). This band co-migrated with the DPR1 protein purified from baculovirus-infected cells (lane 1). The 34 kDa band, in contrast, did not cross-react with the DPR1 antibody. Thus the 43 kDa polypeptide is the product of the DPR1 gene. The antibody did not neutralize FTase activity, nor did it precipitate the activity.

**DISCUSSION**

In the present study we have shown that the purified yeast FTase is a heterodimer of approx. 90 kDa and consists of 43 kDa and 34 kDa subunits. By using antibody against DPR1 we have shown that the 43 kDa polypeptide is the product of the DPR1 gene. The 34 kDa polypeptide is most likely to be the product of the RAM2 gene, since the RAM2 gene encodes a protein of 316 amino acid residues [21]. A possible structure of this enzyme has been proposed previously, on the basis of the following observations: (i) mutations in either the DPR1 or RAM2 gene almost completely abolish FTase activity [2,18]; (ii) DPR1 protein purified from E. coli can reconstitute FTase when added to the extracts prepared from dpr1 mutant cells [18]; (iii) sequences of DPR1 and RAM2 proteins share a low, but significant, similarity with β- and α-subunits respectively of the mammalian FTase [19,20]; (iv) extracts of E. coli cells expressing both DPR1 and RAM2 exhibit FTase activity [21]. What has been lacking in these studies is the direct demonstration of the structure of yeast FTase. Our results present the first report on the purification of the yeast FTase which demonstrate its structure. Taken together, these results firmly establish that the yeast FTase consists of the DPR1 and RAM2 gene products.

Although we purified FTase from the overproducer, this enzyme is indistinguishable from the enzyme purified from the non-overproducer. This point was addressed by carrying out purification of FTase from yeast cells which had not been transformed with overexpression plasmids. FTase activity from these cells eluted as a single peak from a Mono Q column with a salt concentration of approx. 250 mM [30] (L. E. Goodman and F. Tamanoi, unpublished work), which was the same salt concentration that we observed with the preparation from the overproducer. This enzyme had an apparent native molecular mass of 80–100 kDa as determined by Superose 12 column chromatography [30] (L. E. Goodman and F. Tamanoi, unpublished work). The enzyme bound to a column containing a CAAX sequence, GTPRASNRSCAI. Elution of the enzyme from this column by changing the pH to 5 resulted in the appearance of two prominent bands having apparent molecular masses of 43 and 34 kDa [30] (L. E. Goodman and F. Tamanoi, unpublished work). In addition, we found similarities between the overproduced enzyme and non-overproduced enzyme in their properties such as pH optimum, Mg²⁺ requirement and optimum temperature.

The yeast FTase continued to be active up to 50 °C, but rapidly decreased in activity above 55 °C. One possible explanation for the temperature-dependence curve not being bell-shaped could be alterations in the physical properties of the substrates, Fpp and GST-CIIL. Preincubation at 55 °C inactivated the FTase activity, but partial protection could be achieved by the addition of Fpp. On the other hand, the FTase was sensitive to the addition of salts. Another intriguing characteristic of the yeast FTase is its ability to utilize a GGTase substrate, GST-CIIL, protein, albeit at a reduced efficiency. A similar observation was made with the mammalian FTase purified from porcine brain (S. S. Saini and V. Manne, unpublished work). Yokoyama et al. [10] reported similar findings using the mammalian FTase and peptide substrates. Thus changing the C-terminal amino acid of an FTase substrate to leucine alters the substrate to be preferentially utilized by GGTase, but does not exclude it being used as an FTase substrate. This point may have to be taken into consideration when interpreting results of experiments looking at the effects of changing C-terminus of ras proteins to leucine.

Because FTase is a bisubstrate enzyme, the question arises as to the mechanism of action: ordered Bi Bi, random Bi Bi or Ping Pong. Our plot (Figure 7) appears to exclude the Ping Pong mechanism, since the plot does not show parallel slopes at different substrate concentrations. In regard to the ordered Bi Bi or random Bi Bi mechanism, our results reported in this paper appear to favour the ordered Bi Bi mechanism. The double-reciprocal plot (Figure 7) shows that Kₘ for GST-CIIL substrate is decreasing with increasing concentration of Fpp. Furthermore, the protection from heat denaturation by preincubation with Fpp, but not with GST-CIIL protein, is consistent with the idea that Fpp is the first substrate to bind. Recently, Pompliano et al. [31] reported that bovine brain FTase proceeds through a random Bi Bi mechanism. It is unclear whether this means that yeast and mammalian FTases have different mechanisms. More rigorous analyses of the mechanism of action of yeast FTase need to be carried out to address this point. We also determined the Kₘ values extrapolated to infinite substrate concentrations. The value of 8.1 μM obtained for Fpp is significantly higher than the
value reported for the mammalian FTase [1]. This may explain our failure to isolate an enzyme–Fpp complex by gel filtration (R. Gomez and F. Tamanoi, unpublished work).

Purification and characterization of the yeast FTase strongly suggest that the yeast enzyme is very similar to its mammalian counterpart. First, their physical structure appears to be similar. Both the mammalian and yeast enzymes are heterodimers of approx. 100 kDa. Furthermore, both enzymes are eluted from the Mono Q column by a very similar salt concentration, suggesting their similar overall charge. In addition, both enzymes have strong affinity for the CAAX sequence. We have shown that peptides containing the C-terminal ten residues of RAS2 or STE18 inhibit yeast FTase activity. These results suggest strong evolutionary conservation of the FTase enzyme.

In summary, we have shown directly that yeast FTase is a heterodimer consisting of DPR1 and RAM2 subunits. Because there are mutants of DPR1 and RAM2 already available and because more mutants can be generated by yeast genetics, the yeast system provides a suitable system to carry out a structure–function study of FTase. Furthermore, the similarity between the yeast and the mammalian enzymes raises the possibility that the knowledge obtained with the yeast enzyme might contribute to our understanding of the mammalian enzyme.

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