Mutant farnesyltransferase β subunit of *Saccharomyces cerevisiae* that can substitute for geranylgeranyltransferase type I β subunit

(yeast/CAAX motifs/*DPR1*/cross-specificity/peptidomimetic inhibitors)

HIROSHI MITSUZAWA, KENNETH ESSON, AND FUYUHIKO TAMANOI*

Department of Microbiology and Molecular Genetics, University of California, Los Angeles, 405 Hilgard Avenue, Los Angeles, CA 90024-1489

Communicated by John A. Glomset, University of Washington, Seattle, WA, November 21, 1994 (received for review October 3, 1994)

ABSTRACT The protein farnesyltransferase (PFT) β -subunit gene of *Saccharomyces cerevisiae*, *DPR1*, was randomly mutagenized by PCR to construct a mutant *DPR1* gene library on a high-copy plasmid. The library was screened for suppression of the temperature sensitivity conferred by a mutation in the protein geranylgeranyltransferase type I (PGGT-I) β -subunit gene, *CAL1*. A mutant *DPR1* gene was identified whose product contained a single amino acid change of Ser-159 to Asn. This mutant gene also suppressed a *cal1* disruption even on a low-copy plasmid, suggesting that the product (designated S159N) can substitute for PGGT-I β subunit *in vivo*. Its ability to act as a PFT is not drastically reduced, since the mutant gene still complemented a *dpr1* disruption. Results of *in vitro* assays demonstrate that the mutant enzyme has increased activity to farnesylate, a substrate for PGGT-I. On the other hand, the ability to farnesylate its own substrate is reduced. The increased ability to utilize the PGGT-I substrate is due to its increased affinity for the protein substrate. In addition, the mutant enzyme shows a severalfold increase in the sensitivity to a peptidomimetic inhibitor that acts as a competitor of the protein substrate. These results point to the importance of the β subunit of PFT for the binding of a protein substrate and demonstrate that Ser-159 of *DPR1* product is critical for its substrate specificity.

Farnesylation plays a critical role in the localization and function of proteins such as Ras (1, 2). This modification is a crucial step in a series of C-terminal modification events that facilitate membrane association. Because membrane association is important for the function of Ras protein including its transforming capability, protein farnesyltransferase (PFT), which catalyzes the reaction, has been studied extensively as a possible target of antitumor drugs (3, 4).

PFT is a heterodimer composed of α and β subunits (5, 6). The α subunit is shared by another member of protein prenyltransferases, protein geranylgeranyltransferase type I (PGGT-I). PFT transfers a C15 farnesyl group from farnesyl diphosphate (FPP) to proteins ending with CAAX (C is cysteine, A is usually an aliphatic amino acid, and X is the C-terminal amino acid, which is predominantly methionine, serine, cysteine, alanine, or glutamine), whereas PGGT-I transfers a C20 geranylgeranyl group from geranylgeranyl diphosphate (GGPP) to a protein ending with CAAL (L is leucine, but phenylalanine also can function). The β subunit of PGGT-I is distinct from the β subunit of PFT, but significant sequence similarity is found between the two (7). Thus, the substrate specificity appears to be conferred by the β subunit. Consistent with this idea is the suggestion by cross-linking studies that both FPP (8) and protein substrates (9, 10) bind

to the β subunit of PFT. When two different-sized photoaffinity-labeling peptide substrates were used, a shorter peptide substrate cross-linked to the β subunit, whereas a longer peptide substrate cross-linked to both α and β subunits (10), which raises the possibility that the peptide binding site is at the interface between the two subunits.

To gain information concerning the region of PFT β subunit involved in substrate specificity, we have used the yeast *Saccharomyces cerevisiae* system in which genetic screens can be applied. Yeast PFT β subunit and PGGT-I β subunit are encoded by *DPR1*/*RAM1* (referred to as *DPR1* throughout) and *CAL1*/*CDC43*, respectively (11–13). PFT and PGGT-I share an α subunit encoded by *RAM2* (12, 13). We have attempted to isolate mutant PFT β subunit that can substitute for PGGT-I β subunit by screening randomly mutagenized *DPR1* genes for suppression of a temperature-sensitive mutation in the *CAL1* gene. We reasoned from the following observations that such mutant *DPR1* genes could be obtained: (i) *DPR1* and *CAL1* products share a significant sequence similarity (14), and (ii) like mammalian PFT (15), yeast enzyme shows low but significant cross-specificity to substrates for PGGT-I (16–18).

In this paper, we describe isolation and characterization of mutant PFT, which can function in place of PGGT-I *in vivo*. The mutant enzyme has increased affinity to a PGGT-I substrate, while exhibiting reduced affinity for its own substrate. Interestingly, a single amino acid alteration is responsible for this change in substrate specificity.

MATERIALS AND METHODS

Yeast Strains, Media, and Transformation. *S. cerevisiae* strains used in this study were the following: YOT559-3C (*MATa cal1-1' leu2 trp1 ura3 ade2*), YOT359-12C (*MATa $\Delta cal1::ura3 lys2 leu2 trp1 ura3 ade2 his3$ pRB1601*) (16), KMY200-sgp2-No.2-12A [*MATa dpr1(sgp2)::URA3 ade8 trp1 ura3 his3*] (19), and CJ198-2B (*MATa cdc43-2 trp1 ura3 gal2*) (13). Complex medium (YPD; yeast extract/peptone/dextrose) was prepared as described (20). Synthetic complete media lacking tryptophan (SC-Trp) were prepared by supplementing the synthetic minimal medium (20) with 0.5% Casamino acids and uracil and/or adenine. The yeast transformation was carried out by a modified lithium acetate method (21).

Plasmids. pSK(*DPR1*) was constructed by ligating a 2.4-kb *Sal I/Xba I DPR1* fragment (22) into the *Sal I/Xba I* site of pBluescript SK+ (Stratagene). The 1.3-kb *Pst I/Hpa I* region of *DPR1* (Fig. 1A) on pSK(*DPR1*) was amplified by PCR using

Abbreviations: PFT, protein farnesyltransferase; PGGT-I, protein geranylgeranyltransferase type I; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; GST-CIIS, glutathione *S*-transferase fused to the C-terminal 12 amino acids of yeast RAS2 protein; GST-CIIL, the same as GST-CIIS except the C-terminal serine is changed to leucine.

*To whom reprint requests should be addressed.

FIG. 1. Schematic representation of wild-type and chimeric *DPR1* genes used in this study. A 2.4-kb *Sal* I/*Xba* I region containing the *DPR1* gene (22) is shown. A, *Afl* II; B, *Bam*HI; H, *Hpa* I; N, *Nco* I; P, *Pst* I. (A) Wild-type *DPR1* gene. Arrows indicate primers DPR1#1 and DPR1#2. (B) *DPR1* gene in which *Bam*HI and *Afl* II sites are introduced. (C) Chimeric *DPR1* gene consisting of 5' portion of mutant gene and 3' portion of wild-type gene. (D) Chimeric *DPR1* gene consisting of 5' portion of wild-type gene and 3' portion of mutant gene.

the primers DPR1#1 (5'-ACAAGTGCAGGGATCCTA-AAGCTATAGG-3') and DPR1#2 (5'-TATGTGGTTA-ACTTGGAGAACTTAAGTTGG-3'). These primers were designed to introduce a *Bam*HI site at the 5' upstream region of *DPR1* and an *Afl* II site near the 3' terminus of *DPR1* (base changes introduced in PCR primers are underlined) (Fig. 1B). The base changes near the 3' terminus of *DPR1* do not affect the coding sequence. The amplified product was digested with *Pst* I and *Hpa* I and ligated with *Pst* I/*Hpa* I-digested pSK-*DPR1* to construct pSK(*DPR1*)BA, whose *DPR1* has *Bam*HI and *Afl* II sites. The high-copy (YE) plasmids pYO324(*DPR1*) and pYO324(*DPR1*)BA were constructed by ligating 2.4-kb *Xho* I/*Sac* I fragments from pSK(*DPR1*) and pSK(*DPR1*)BA into the *Xho* I/*Sac* I site of pYO324 (23). pYO324(*DPR1*)BA, like pYO324(*DPR1*), complemented a *dpr1* mutation. To construct chimeric plasmids, a 1.3-kb *Xho* I/*Nco* I fragment containing the 5' portion of *DPR1* from a mutant plasmid and a 1.1-kb *Nco* I/*Sac* I fragment containing the 3' portion of *DPR1* from a mutant plasmid were used to replace the corresponding region of pYO324(*DPR1*). The low-copy (YC) plasmids pRS314(*DPR1*)BA and pRS314[1-2] were constructed by ligating 2.4-kb *Xho* I/*Sac* I fragments from pYO324(*DPR1*)BA and mutant clone 1-2 into the *Xho* I/*Sac* I site of pRS314 (24). The plasmid DNAs were sequenced by the dideoxynucleotide chain-termination method.

Construction of Mutant *DPR1* Library. The 1.3-kb *Pst* I/*Hpa* I region of pYO324(*DPR1*)BA was amplified by PCR using the primers DPR1#1 and DPR1#2 with 5 ng of pYO324(*DPR1*)BA (10 mM Tris-HCl, pH 8.3/50 mM KCl/2.5 mM MgCl₂/0.001% gelatin/200 μM each dNTP/1 μM primer/2.5 units of *Taq* DNA polymerase). The reaction was 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min. The PCR process generated random mutations due to the inherent infidelity of *Taq* DNA polymerase. The amplified fragment was digested with *Bam*HI and *Afl* II and ligated with *Bam*HI/*Afl* II-digested pYO324(*DPR1*)BA. Approximately 45,000 *Escherichia coli* transformants were collected to construct a mutant *DPR1* gene library.

Protein Prenyltransferase Assays and Preparation of Crude Soluble Extracts. Protein prenyltransferase assays were carried out essentially as described (13, 25). The reaction mixture contained 50 mM Tris-HCl (pH 7.4), 10 mM MgCl₂, 5 μM ZnCl₂, 5 mM dithiothreitol, GST-CIIS (glutathione S-transferase fused to the C-terminal 12 amino acids of yeast RAS2 protein) or GST-CIIL (the same as GST-CIIS except

the C-terminal serine is changed to leucine) (13), [³H]FPP (22.5 Ci/mmol; 1 Ci = 37 GBq) or [³H]GGPP (19.3 Ci/mmol), and crude soluble extract or partially purified enzyme. Other conditions are described in the figure and table legends. Crude soluble extracts were prepared from yeast cells as described (25). Cells were grown in 50 ml of SC-Trp medium at 25°C and were harvested when the OD₆₀₀ of the culture was ≈ 1.

Partial Purification of PFT. Wild-type and mutant PFTs were partially purified from KMY200-sgp2-No.2-12A carrying pYO324(*DPR1*)BA or 1-2N (chimeric plasmid containing 5' portion of the mutant gene shown in Fig. 1C) as described (11). Briefly, extracts of yeast cells grown in 5 liters of SC-Trp medium at 30°C were subjected to ammonium sulfate fractionation (30–60% saturation), and the sample was applied to a Mono Q column. The peak fractions eluting at ≈ 0.35 M NaCl were pooled, concentrated, and kept frozen at –80°C after addition of *n*-octyl glucoside at a final concentration of 0.2%. The activity to incorporate radioactive farnesyl into GST-CIIS substrate was used as an assay to follow purification. For the mutant enzyme, Mono Q fractions were also tested for their activity to incorporate radioactive farnesyl into GST-CIIL substrate.

RESULTS

Mutant *DPR1* Genes That Suppress *cal1*^{ts}. *cal1-1*, a mutation in the yeast PGGT-I β-subunit gene, confers a temperature-sensitive growth phenotype. This defect is not suppressed by the yeast PFT β-subunit gene *DPR1* on a high-copy plasmid (ref. 14; see also Fig. 2A Right, streak 2). To isolate mutant *DPR1* genes that can substitute for the *CAL1* gene, a *cal1-1* strain (YOT559-3C) was transformed with randomly mutagenized *DPR1* genes on a high-copy YE plasmid (mutant *DPR1* library; see Materials and Methods). Transformants selected on SC-Trp plates at 25°C were replica-plated onto YPD

FIG. 2. Suppression of *cal1* and complementation of *dpr1* by mutant *DPR1*. (A) YOT559-3C (*cal1-1* strain) cells transformed with pYO324 (streak 1), pYO324(*DPR1*)BA (streak 2), or mutant clone 1-2 (streak 3) were grown on SC-Trp medium at 25°C for 3 days or on YPD medium at 37°C for 2 days. (B) *cal1*: YOT359-12C (Δ *cal1::ura3* strain carrying *CAL1* gene on *URA3* plasmid) cells transformed with pRS314 (streak 1), pRS314(*DPR1*)BA (streak 2), or pRS314[1-2] (streak 3) were grown on SC-Trp medium containing 1 mg of 5-fluoroorotic acid per ml at 30°C for 2 days, after growth on SC-Trp medium, which allows cells to lose the *CAL1* plasmid. *dpr1*: KMY200-sgp2-No.2-12A (*dpr1::URA3* strain) cells transformed with pRS314 (streak 1), pRS314(*DPR1*)BA (streak 2), or pRS314[1-2] (streak 3) were grown on YPD medium at 37°C for 2 days.

plates and screened for growth at 37°C (the restrictive temperature for the *cal1-1* strain). Approximately 10,000 colonies were screened, and plasmids were recovered from 10 colonies that grew well at 37°C. These plasmids were used to retransform the *cal1-1* strain. Fig. 2A shows the results with one of the mutant *DPR1* plasmids (clone 1-2). The other nine mutant plasmids gave similar results. The mutant *DPR1* on the high-copy plasmid suppressed both the slow growth at 25°C as well as the temperature-sensitive growth at 37°C of *cal1-1* cells (Fig. 2A *Left* and *Right*, streak 3). In contrast, the wild-type *DPR1* did not suppress the inability of *cal1-1* cells to grow at 37°C and furthermore impaired the growth at 25°C (compare streak 1 and streak 2 in Fig. 2A *Left*). The latter observation may result from competition between overproduced *DPR1* protein and *cal1-1* protein for RAM2 protein, which reduces the amount of active PGGT-I. The suppression by mutant *DPR1* is not limited to the *cal1-1* allele, since the mutant genes also suppressed the temperature sensitivity caused by another allele of *CAL1*, *cdc43-2* (data not shown).

The mutant *DPR1* gene also suppressed *cal1* or complemented *dpr1* on a low-copy plasmid. This was shown by using the clone 1-2, which has a single amino acid change (see below). Transformation of the *cal1-1* strain with the mutant *DPR1* gene placed on a YCp vector showed that the temperature-sensitive growth of the strain was suppressed by the mutant *DPR1* (data not shown). The low-copy mutant *DPR1* plasmid was also introduced into a *cal1* disruption strain YOT359-12C. This strain is viable, since it carries *CAL1* on a *URA3* plasmid (pRB1601). Transformants obtained were then grown on a plate containing 5-fluoroorotic acid to select for the growth of cells that had lost the *CAL1* plasmid. As shown in Fig. 2B (*Left*), the *cal1* transformant in the absence of the *CAL1* plasmid was capable of growth. Thus, the mutant *DPR1* gene on a low-copy plasmid suppressed the lethality conferred by *cal1* disruption. The experiments with the *dpr1* strain demonstrated that the low-copy mutant *DPR1* plasmid is also capable of complementing *dpr1* disruption for temperature sensitivity (Fig. 2B *Right*). These results indicate that the product of the mutant *DPR1* gene can substitute for either PGGT-I β subunit or PFT β subunit *in vivo*.

Amino Acid Change Responsible for Suppression. To examine whether the mutation(s) responsible for *cal1* suppression is located on the N-terminal or C-terminal portion of the gene product, we prepared two kinds of chimeric plasmids from each mutant plasmid clone, 1-1, 1-2, 1-5, and 3-5 (Fig. 1 C and D). This was possible by using a *Nco* I site, which cuts the *DPR1* product into the N-terminal two-thirds and the C-terminal one-third. All four chimeras containing the region of mutant gene upstream of the *Nco* I site suppressed *cal1-1*, whereas the constructs containing the 3' portion of the mutant gene did not (data not shown). Thus, the mutation(s) responsible for the suppression is present in the N-terminal portion of the gene product.

The region upstream of the *Nco* I site has been completely sequenced for all 10 mutant plasmids. The results, summarized in Fig. 3, demonstrate that there are four classes of mutant plasmids. Note that in the previous experiment chimeras were made from representatives of each class (1-1, 1-2, 1-5, and 3-5). A change of Ser-159 to Asn is common to all the mutant plasmids and is the only change in 1-2 and 3-6, indicating that this change is responsible for the suppression of *cal1*. Interestingly, Asn, to which Ser-159 has changed, is the amino acid present in the corresponding position of *CAL1* protein (see *Discussion*).

PFT Activity in Crude Extracts. To gain insights into the biochemical basis for suppression of the *cal1* strains, protein prenyltransferase activity was determined in extracts prepared from the *cal1-1* strain carrying the high-copy mutant *DPR1* gene. Examination of PGGT-I activity using GST-CIIL and GGPP as substrates showed that this PGGT-I activity re-

FIG. 3. Amino acid change found in mutant *DPR1* plasmids. Nucleotide changes were T to C at 245 (F82S), T to C at 326 (M109T), C to T at 437 (P146S), G to A at 476 (S159N), A to G at 535 (R179G), and A to G at 538 (I180V). All the mutations are transitions. Sequencing confirmed that the *Pst* I/*Nco* I region of *DPR1* on pYO324(*DPR1*)BA has no mutation except for the *Bam*HI site. The sequence downstream of the *Nco* I site (arrowhead corresponds to this site) was not determined.

mained low (data not shown). However, when FPP was used instead of GGPP as a prenyl substrate, we observed that the mutant extracts incorporated significant radioactivity into GST-CIIL. This suggests that the suppression is due to the ability of the mutant enzyme to farnesylate PGGT-I substrates.

To more precisely compare the activity to farnesylate GST-CIIL substrate between the wild-type and the mutant activity, we utilized a *dpr1* disruption strain that does not contain any detectable PFT activity. Crude soluble extracts from *dpr1* cells carrying a high-copy wild-type or mutant *DPR1* gene were assayed for PFT activity with GST-CIIS and GST-CIIL as substrates (Fig. 4). Extracts from *dpr1* disruption cells carrying vector alone showed little activity. Wild-type *DPR1* product showed ≈ 7 -fold higher PFT activity with GST-CIIS than with GST-CIIL under the conditions used. In contrast, the mutant 1-2 (S159N) showed a PFT activity higher with GST-CIIL than with GST-CIIS. Other mutants, 1-1 (P146S, S159N) and 1-5 (F82S, M109T, S159N, R179G), also showed greater PFT activities with GST-CIIL than with GST-CIIS. The ratio of these activities with GST-CIIL and GST-CIIS is similar to that seen with mutant 1-2. These latter results suggest that the additional amino acid changes in 1-1 and 1-5 do not affect specificity. The mutant 3-5 (S159N, I180V) showed a slight change in specificity.

Characterization of Mutant PFT. To further characterize the mutant enzyme, PFT was partially purified from *dpr1* cells carrying a high-copy wild-type or mutant *DPR1* gene. For this purpose, we used the construct derived from clone 1-2, which contains a single amino acid change, as a source of the mutant *DPR1* gene. PFT was purified by ammonium sulfate fractionation followed by Mono Q column chromatography. Both the

FIG. 4. PFT activity in crude soluble extracts. Crude soluble extracts prepared from KMY200-sgp2-No.2-12A carrying pYO324 (vector), pYO324(*DPR1*)BA (wt), 1-1, 1-2, 1-5, or 3-5 were assayed for PFT activity by using the substrates 0.88 μ M [3 H]FPP and 7.6 μ M GST-CIIS (open bars) or 7.5 μ M GST-CIIL (solid bars). The reaction mixture contained 0.2 mg of crude extract per ml. The reaction was carried out at 30°C for 30 min. The experiment was carried out twice with similar results.

wild-type and mutant enzymes were eluted at approximately the same salt concentration (0.35 M NaCl). With the mutant enzyme, the profile of the activity to farnesylate the GST-CIIL substrate was identical to the profile of the activity to farnesylate the GST-CIIS substrate. Peak fractions did not contain any PGGT-I activity as assayed by using GGPP and GST-CIIL. These fractions also did not contain activity to geranylgeranilate GST-CIIS. The peak fractions were pooled and used as the source of PFT. PFT activity was examined for wild-type and mutant enzymes by using various concentrations of GST-CIIS or GST-CIIL in the presence of excess FPP. K_m values for either GST-CIIL or GST-CIIS are shown in Table 1. With the mutant enzyme (S159N), the K_m for GST-CIIL is decreased by >20-fold and the K_m for GST-CIIS is increased 6-fold. Thus, the mutant enzyme has an increased affinity for the PGGT-I substrate and a decreased affinity for its own substrate.

We have also noticed that the mutant enzyme is more sensitive to a peptidomimetic inhibitor than the wild-type enzyme. This was shown by using the CAAX peptide analogue, B581 (26). This compound is known to inhibit PFT specifically and to block activated *ras* phenotypes by using *Xenopus* oocytes (26). As shown in Fig. 5, PFT activity of the wild-type enzyme was inhibited with an IC_{50} of 200 nM. In contrast, PFT activity of the mutant enzyme was inhibited with an IC_{50} of 30 nM. Thus, the mutant enzyme is severalfold more sensitive to B581.

DISCUSSION

Our attempt to convert PFT to PGGT-I by *in vitro* mutagenesis led to the identification of a mutant with a single amino acid change in the β subunit. The mutant DPR1 protein can replace the physiological function of PGGT-I β subunit (CAL1/CDC43 protein). The suppression is quite efficient, since it is observed with the mutant gene on a low-copy vector. The suppression is due to an altered substrate affinity of the mutant PFT; the mutant enzyme can now accommodate PGGT-I protein substrates, while the affinity for the normal PFT protein substrate is decreased. Calculation of k_{cat} values based on the rough estimate of the amount of enzyme in the partially purified preparations revealed that the values for the wild-type and mutant enzymes are not substantially different (unpublished data). This suggests that the difference in the catalytic efficiency (k_{cat}/K_m) reflects the difference in K_m . However, more detailed analyses with purified enzyme, are needed. The mutant PFT (S159N) is a type of enzyme different from previously characterized mutants, which are defective mutants with reduced substrate affinity and catalytic activity (8).

It is of interest to elucidate which of the two subunits of PFT contains the substrate binding sites. Previous studies with cross-linkers support the idea that the β subunit is important for binding the protein substrate (9, 10). Our finding that the alteration of an amino acid in the β subunit affects the substrate specificity of PFT further supports the importance of the β subunit for protein substrate binding. Since a FPP analogue also cross-links to the β subunit of PFT (8), it may be the case that the β subunit contains the substrate binding sites for both protein and FPP. In this model, the β subunits of PFT and

FIG. 5. Effect of B581 on wild-type and mutant PFTs. Partially purified enzymes from KMY200-sgp2-No.2-12A carrying pYO324(DPR1)BA (solid squares) or 1-2N (solid circles) were assayed for PFT activity in the presence of various concentrations of B581 with the substrates 0.88 μ M [3 H]FPP and 7.6 μ M GST-CIIS. The reaction was carried out at 37°C for 30 min. The 100% value corresponds to the activity in the absence of B581 (39,046 cpm and 55,504 cpm for wild-type and mutant enzymes, respectively). The experiment was carried out twice with similar results.

PGGT-I determine the substrate specificity of the enzyme. The α subunit could then have an auxiliary role, such as bringing the two substrate binding sites into proximity.

It was surprising to find that a single amino acid alteration was responsible for changing the specificity for protein substrates. The change is not simply due to decreased specificity, since the mutant enzyme shows an increased affinity to the PGGT-I substrate and a decreased affinity to the PFT substrate. Thus, it appears that the amino acid change induced a conformational change from a form favorable for CIIS substrates to a form favorable for CIIL substrates. The conformational change is unlikely to involve gross changes in the protein structure, since the wild-type and mutant enzymes behaved similarly during their purification. The mutant enzyme also exhibited increased sensitivity to a peptidomimetic inhibitor. This is consistent with the idea that the mutation affects the binding of protein substrates. Since PGGT-I substrates differ from PFT substrates in having leucine or phenylalanine at their C termini, the mutant enzyme might have higher affinity for these amino acids. The peptidomimetic inhibitor is derived from CVFM (26), and the phenylalanine moiety may have been recognized by the mutant enzyme. Alternatively, the mutant enzyme may have increased affinity for substrates having C-terminal methionine. Further characterization of the substrate specificity of the mutant enzyme by using a battery of peptides may be revealing.

Residue 159 is serine in DPR1 protein and the corresponding residue in CAL1 protein is asparagine. Therefore, the change of serine to asparagine observed in the mutant PFT is consistent with the idea that the residue at this position defines, at least in part, the difference in protein substrate specificity between PFT and PGGT-I. The requirement for a particular amino acid at this position, however, may not be limited to serine, since proline is present at this position in mammalian PFTs. Further experiments are needed to understand the specific requirements for particular amino acids at this residue. It is of interest to note that residue 159 is located in a region highly conserved in protein prenyltransferases (8). The next residue, Thr-160, is conserved in all known protein prenyltransferases and residues 144–153 are conserved in all known PFTs. It is tempting to speculate that this region forms a part of the protein substrate binding site. Alternatively, the protein binding site may be located away from this region and the region around residue 159 may play a critical role in the folding of the binding pocket. Further identification of mutant PFTs with altered substrate specificity may provide more information regarding the binding of protein substrates.

Table 1. Protein substrate affinity of wild-type and mutant PFTs

Enzyme	K_m , μ M	
	CIIS	CIIL
Wild type	1.5 $\pm$ 0.1	40 $\pm$ 3.3
Mutant (S159N)	9.0 $\pm$ 1.9	1.9 $\pm$ 0.4

PFT assays were carried out as described. Partially purified PFT (Mono Q fraction) was used for the assays. Initial velocities were measured with 4 μ M [3 H]FPP and various concentrations of GST-CIIS (1.2–19 μ M) or GST-CIIL (1.9–60 μ M). K_m values were determined by nonlinear curve fitting of plots of initial velocity vs. substrate concentration. SE is shown.

In vitro results demonstrate that the mutant PFT farnesylates a substrate for PGGT-I (Fig. 4). This altered property of the enzyme is most likely to be the reason for the suppression of *cal1* mutations. Low but significant substrate cross-specificity between PFT and PGGT-I has been reported (15–18). The mutant enzyme described here appears to exaggerate this cross-specificity. Our results also suggest that PGGT-I substrates that are normally geranylgeranylated are functional when they are farnesylated. This is in line with the recent observation by Ohya *et al.* (16). These investigators reported both that the lethality of a *cal1* disruption strain can be suppressed by overexpressing *CDC42* and *RHO1* and that the suppression is much more efficient when their C termini are changed to methionine to permit farnesylation. However, in this latter case, the suppression occurred only when these genes were overexpressed by using high-copy plasmids. In our mutant, all the potential PGGT-I substrates are expected to be farnesylated, and this may obviate the need for overexpression. Our results also agree with the recent observation on the mating pheromone, a factor, which could be modified with a geranylgeranyl group without any significant effect on its function (27).

The yeast system described here provides a powerful tool for investigating the structure and function of PFT. In addition to mutants with altered substrate specificity described here, other types of mutant could be obtained by using yeast genetic screens. Given the sequence and biochemical similarities between yeast and mammalian PFTs (8, 11), results from yeast PFT would be useful for the understanding of human PFT, which is a target for anti-*ras*-mediated tumor drugs (3, 4).

We thank Dr. Yoshikazu Ohya for providing yeast strains and plasmids, and Dr. Anna Maria Garcia for B581. We are grateful to Drs. Peter Edwards and Steve Clarke for critical reading of the manuscript and Donald Hawken for discussions. This work was supported by National Institutes of Health Grant CA41996.

- Glomset, J. A., Gelb, M. H. & Farnsworth, C. C. (1990) *Trends Biochem. Sci.* **15**, 139–142.
- Clarke, S. (1992) *Annu. Rev. Biochem.* **61**, 355–386.
- Gibbs, J. B., Oliff, A. & Kohl, N. E. (1994) *Cell* **77**, 175–178.
- Tamanoi, F. (1993) *Trends Biochem. Sci.* **18**, 349–353.
- Reiss, Y., Goldstein, J. L., Seabra, M. C., Casey, P. J. & Brown, M. S. (1990) *Cell* **62**, 81–88.
- Kohl, N. E., Diehl, R. E., Schaber, M. D., Rands, E., Soderman, D. D., He, B., Moores, S. L., Pompliano, D. L., Ferro-Novick, S., Powers, S., Thomas, K. A. & Gibbs, J. B. (1991) *J. Biol. Chem.* **266**, 18884–18888.
- Zhang, F. L., Diehl, R. E., Kohl, N. E., Gibbs, J. B., Giros, B., Casey, P. J. & Omer, C. A. (1994) *J. Biol. Chem.* **269**, 3175–3180.
- Omer, C. A., Kral, A. M., Diehl, R. E., Prendergast, G. C., Powers, S., Allen, C. M., Gibbs, J. B. & Kohl, N. E. (1993) *Biochemistry* **32**, 5167–5176.
- Reiss, Y., Seabra, M. C., Armstrong, S. A., Slaughter, C. A., Goldstein, J. L. & Brown, M. S. (1991) *J. Biol. Chem.* **266**, 10672–10677.
- Ying, W., Sepp-Lorenzino, L., Cai, K., Aloise, P. & Coleman, P. S. (1994) *J. Biol. Chem.* **269**, 470–477.
- Gomez, R., Goodman, L. E., Tripathy, S. K., O'Rourke, E., Manne, V. & Tamanoi, F. (1993) *Biochem. J.* **289**, 25–31.
- He, B., Chen, P., Chen, S., Vancura, K. L., Michaelis, S. & Powers, S. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11373–11377.
- Finogold, A. A., Johnson, D. I., Farnsworth, C. C., Gelb, M. H., Judd, S. R., Glomset, J. A. & Tamanoi, F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 4448–4452.
- Ohya, Y., Goebel, M., Goodman, L. E., Petersen-Björn, S., Friesen, J. D., Tamanoi, F. & Anraku, Y. (1991) *J. Biol. Chem.* **266**, 12356–12360.
- Yokoyama, K., Goodwin, G. W., Ghomashchi, F., Glomset, J. A. & Gelb, M. H. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5302–5306.
- Ohya, Y., Qadota, H., Anraku, Y., Pringle, J. R. & Botstein, D. (1993) *Mol. Biol. Cell* **4**, 1017–1025.
- Trueblood, C. E., Ohya, Y. & Rine, J. (1993) *Mol. Cell. Biol.* **13**, 4260–4275.
- Caplin, B. E., Hettich, L. A. & Marshall, M. S. (1994) *Biochem. Biophys. Acta* **1205**, 39–48.
- Nakayama, N., Arai, K. & Matsumoto, K. (1988) *Mol. Cell. Biol.* **8**, 5410–5416.
- Sherman, F. (1991) *Methods Enzymol.* **194**, 3–21.
- Gietz, D., St. Jean, A., Woods, R. A. & Schiestl, R. H. (1992) *Nucleic Acids Res.* **20**, 1425.
- Goodman, L. E., Perou, C. M., Fujiyama, A. & Tamanoi, F. (1988) *Yeast* **4**, 271–281.
- Qadota, H., Ishii, I., Fujiyama, A., Ohya, Y. & Anraku, Y. (1992) *Yeast* **8**, 735–741.
- Sikorski, R. S. & Hieter, P. (1989) *Genetics* **122**, 19–27.
- Goodman, L. E., Judd, S. R., Farnsworth, C. C., Powers, S., Gelb, M. H., Glomset, J. A. & Tamanoi, F. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 9665–9669.
- Garcia, A. M., Rowell, C., Ackermann, K., Kowalczyk, J. J. & Lewis, M. D. (1993) *J. Biol. Chem.* **268**, 18415–18418.
- Caldwell, G. A., Wang, S.-H., Naider, F. & Becker, J. M. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 1275–1279.