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Suppression of Yeast Geranylgeranyl Transferase I Defect by Alternative Prenylation of Two Target GTPases, Rho1p and Cdc42p

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Geranylgeranyl transferase I (GGTase I), which modifies proteins containing the sequence Cys-Ali-Ali-Leu (Ali: aliphatic) at their C-termini, is indispensable for growth in the budding yeast *Saccharomyces cerevisiae*. We report here that GGTase I is no longer essential when Rho1p and Cdc42p are simultaneously overproduced. The lethality of a GGTase I deletion is most efficiently suppressed by provision of both Rho1p and Cdc42p with altered C-terminal sequences (Cys-Ali-Ali-Met) corresponding to the C-termini of substrates of farnesyl transferase (FTase). Under these circumstances, the FTase, normally not essential for growth of yeast, becomes essential.

INTRODUCTION

More than 50 proteins in eukaryotic cells are known to be posttranslationally modified by addition of 15-carbon farnesyl or 20-carbon geranylgeranyl isoprenoids (Farnsworth *et al.*, 1990; Havel *et al.*, 1992) (for review see Schafer and Rine [1992] and Clarke [1992]). Genetic and biochemical studies on protein prenylation have revealed three distinct enzymes, each consisting of evolutionarily conserved subunits (Chen *et al.*, 1991b; Kohl *et al.*, 1991). Geranylgeranyl transferase I (GGTase I or CAAX GGTase) (Moores *et al.*, 1991; Seabra *et al.*, 1991; Yokoyama *et al.*, 1991; Moomaw and Casey, 1992) and farnesyl transferase (FTase) (Reiss *et al.*, 1990, 1991a; Chen *et al.*, 1991a; Seabra *et al.*, 1991) modify proteins containing the Cys-Ali-Ali-X sequence (Moores *et al.*, 1991; Yokoyama *et al.*, 1991; Reiss *et al.*, 1991b; Kinsella *et al.*, 1991), form heterodimers, and share a common α subunit (Seabra *et al.*, 1991). The third prenyltransferase, named GGTase II (Moores *et al.*, 1991) or Rab GGTase (Seabra *et al.*, 1992), modifies proteins that terminate with GGCC or CXC and consists of three nonidentical subunits (Seabra *et al.*, 1992). The β subunits of the yeast GGTase I (Mayer *et al.*, 1992) and FTase (He *et al.*, 1991) are encoded by the genes *CAL1* (Ohya *et al.*, 1991) (also known as *CDC43* [Johnson *et al.*, 1991; Finegold *et al.*, 1991]) and *DPR1* (Goodman *et al.*, 1988) (also known as *RAM1* [He *et al.*, 1991]), respectively, and the common α subunit is encoded by *RAM2* (He *et al.*, 1991). The genes *BET2* (Rossi *et al.*, 1991) and *MAD2* (Li and Murray, 1991) apparently encode two of the yeast GGTase II subunits (Boguski *et al.*, 1992).

A number of isoprenoid-containing proteins with the Cys-Ali-Ali-X sequence feature have been analyzed chemically, confirming the initial suggestion that the C-terminal residue largely specifies the isoprenoid species (reviewed in Clarke, 1992). In all known farnesylated proteins, the C-terminal residue is serine, methionine, cysteine, glutamine, or alanine, whereas the geranylgeranylated proteins end with leucine (or perhaps phenylalanine). In vitro study of substrate specificity using partially purified FTase and GGTase I is basically consistent with this rule. However, the FTase can modify proteins with C-terminal leucine to a limited extent when a high concentration of the protein substrate is used (Yokoyama *et al.*, 1991). This in vitro cross-specificity may be explained by the fact that the two enzymes not only share a common α subunit but have β subunits that, though not identical, are nevertheless similar to each other. The yeast FTase and GGTase I β subunits are 32% identical in amino acid sequence (Ohya *et al.*, 1991). There are also two genetic results that suggest a functional similarity between FTase and GGTase I. First, overproduction of GGTase I partially suppresses the functional defect of an FTase null mutant, and second,

Table 1. Plasmids

Plasmids (other name)	Characteristics	Source or reference
pRB1589	*RHO1 (L209M), CDC42 (L191M), TRP1*, 2 μ ori (high-copy)	This study
pRB1590	*RHO1*$^+$, *CDC42*$^+$, *TRP1*, 2 μ ori (high-copy)	This study
pRB1591	*RHO1 (L209M), TRP1*, 2 μ ori (high-copy)	This study
pRB1592	*RHO1*$^+$, *TRP1*, 2 μ ori (high-copy)	This study
pRB1593	*RHO1 (C206S), TRP1*, 2 μ ori (high-copy)	This study
pRB1594	*RHO2*$^+$, *TRP1*, 2 μ ori (high-copy)	This study
pRB1595	*CDC42 (L191M), LEU2*, 2 μ ori (high-copy)	This study
pRB1596	*CDC42*$^+$, *LEU2*, 2 μ ori (high-copy)	This study
pRB1597	*CDC42 (C188S), LEU2*, 2 μ ori (high-copy)	This study
pRB1598	*RHO1 (L209M), CDC42 (L191M), TRP1, CEN6* (low-copy)	This study
pRB1599	*ade3::HIS3*	This study
pRB1600	*RHO1 (L209M), CDC42 (L191M), ade3::HIS3*	This study
pRB1601	*CAL1 (CDC43), URA3*, 2 μ ori (high-copy)	This study
pRB1602 (YEpT-CAL1)	*CAL1 (CDC43), TRP1*, 2 μ ori (high-copy)	Ohya *et al.*, (1991)
pRB1603 (YEpU-DPR1)	*DPR1, URA3*, 2 μ ori (high-copy)	Ohya *et al.*, (1991)
pRB1604 (pYO324)	*TRP1*, 2 μ ori (high-copy)	Ohya *et al.*, (1991)
pRB1605 (pCAL101-URA3)	*cal1::URA3*	Ohya *et al.*, (1991)
pRB1606 (pJR868)	*dpr1::HIS3*	Schafer *et al.*, (1990)
pRB1620	*RHO1*$^+$, *LEU2*, 2 μ ori (high-copy)	This study

synthetic lethality was observed between a point mutation affecting the β subunit of GGTase I and a deletion removing the β subunit of FTase (Ohya *et al.*, 1991).

A mutation in either subunit of the yeast GGTase I (deletion of *CAL1/CDC43* or deletion of *RAM2*) results in lethality (He *et al.*, 1991; Ohya *et al.*, 1991). In contrast, deletions in the gene (*DPR1/RAM1*) specifying the β subunit of yeast FTase are not lethal (Schafer *et al.*, 1990). These results indicate that prenylation of GGTase I substrate proteins has a critical function for cell growth; presumably, unlike yeast FTase, yeast GGTase I is required to prenylate certain substrate proteins that are themselves essential in their prenylated forms. To test this hypothesis straightforwardly, one could provide prenylation of all the essential GGTase I substrate proteins by some alternative method; the lethality of the GGTase I deletion should then be suppressed. Among known yeast GGTase I substrate proteins, two GTPases, Rho1p (Madaule *et al.*, 1987) and Cdc42p (Johnson and Pringle, 1990), are known to be essential for cell growth. In this paper, we report that overproduction of these two GTPases suppresses the GGTase I defect in a way that depends on FTase, raising the possibility that prenylation of Rho1p and Cdc42p is the primary, possibly even the only, essential function of yeast GGTase I.

MATERIALS AND METHODS

Genetics and Molecular Constructions

Yeast were grown on minimal medium (YNBD) and rich medium (YPD) and subjected to sporulation and tetrad dissection according to standard methods (Rose *et al.*, 1990). Lithium acetate yeast transformation was performed using a modification (Schiestl and Gietz, 1989) of the original method (Ito *et al.*, 1983). Standard techniques were used to construct plasmids and propagate them in *Escherichia coli* strain DH5αF' (Sambrook *et al.*, 1989; Ausubel *et al.*, 1991).

The polymerase chain reaction (PCR) (Ho *et al.*, 1989) was used to introduce C-terminal mutations of *RHO1* and *CDC42*. Each mutation was confirmed by DNA sequencing. The plasmids used in this study are listed in Table 1. Plasmids pRB1589 and pRB1590 (containing both *RHO1* and *CDC42* with Met [pRB1589] and the normal Leu [pRB1590] C-termini) were derived from pRB1604 (Table 1), as were pRB1591 (*RHO1* with terminal Met), pRB1592 (*RHO1*), pRB1593 (*RHO1* C206S), and pRB1594 (*RHO2*). The plasmids carrying wild-type and mutant *CDC42* genes (pRB1595, pRB1596, and pRB1597) were derived from YEp351 (*LEU2*, 2 μori). The Δ*cal1* plasmid (pRB1605), which retains only the N-terminal 19 amino acid residues of the open reading frame and *URA3* marker, was described previously (Ohya *et al.*, 1991).

Yeast strains are listed in Table 2. YPH501 was the parental strain (Sikorsky and Hieter, 1989). To construct an isogenic Δ*cal1* strain, YOT359-12C, a haploid strain (Δ*cal1::ura3* [pRB1601]) was obtained by tetrad analysis of YOT359, which was obtained by deleting *cal1*, introducing a *ura3* mutation and transforming with pRB1601 (*CAL1 URA3*). Because the YOT359-12C strain contains the wild-type *CAL1* gene on a *URA3* plasmid, a Δ*cal1* strain could be obtained after loss of the *CAL1* plasmid, which we selected on 5-fluoro orotic acid (FOA) plates (Rose *et al.*, 1990). Strain YOT359-12C was transformed again with pRB1589 to make strain YOT35920, and YOT35953 was obtained again as an FOA-resistant derivative. To make a strain with the *RHO1* and *CDC42* genes integrated into the genome, both genes from pRB1589 were cloned as a single fragment into pRB1599 (an *ade3::HIS3*, YIp plasmid). The resultant plasmid (pRB1600) was linearized and used to transform YOT359-12C to construct YOT35922.

The *DPR1* gene was deleted in strain YOT35919 with the pJR868 plasmid (Schafer *et al.*, 1990), kindly provided by Dr. C. Trueblood (University of California, Berkeley), yielding strain YOT35924. Strain YOT35925 was constructed after elimination of pRB1590 from strain YOT35924 and retransformation with pRB1589. To examine the effect of Dpr1p overproduction in the Δ*cal1* strains, pRB1601 (*URA3, CAL1*, 2 μori), pRB1603 (*URA3, DPR1*, 2 μori), and YEp24 (*URA3*, 2 μori) were used to transform strains YOT35952, YOT35953, and YOT35954. Plasmid pRB1603 consists of a 2.4-kilobase (kb) *Bam*HI-*Sal* I fragment containing *DPR1* cloned into the multicopy YEp24 vector.

Table 2. *S. cerevisiae* strains used in this study

Strain	Relevant genotype	Source
YPH499	a *ade2 lys2 his3 trp1 leu2 ura3*	Sikorsky and Hieter (1989)
YPH501	a/α *ade2/ade2 lys2/lys2 his3/his3 trp1/trp1 leu2/leu2 ura3/ura3*	Sikorsky and Hieter (1989)
YOT359	a/α *ade2/ade2 lys2/lys2 his3/his3 trp1/trp1 leu2/leu2 ura3/ura3 CAL1/Δcall::ura3* [pRB1601]	This study
YOT359-12C	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601]	This study
YOT35902	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1604]	This study
YOT35903	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1592]	This study
YOT35904	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1591]	This study
YOT35905	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1593]	This study
YOT35906	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1594]	This study
YOT35907	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1596]	This study
YOT35908	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1595]	This study
YOT35909	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1597]	This study
YOT35910	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1592,pRB1596]	This study
YOT35912	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1592,pRB1597]	This study
YOT35914	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1591,pRB1595]	This study
YOT35915	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1591,pRB1597]	This study
YOT35916	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1593,pRB1596]	This study
YOT35917	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1593,pRB1595]	This study
YOT35918	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1593,pRB1597]	This study
YOT35919	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1590]	This study
YOT35920	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1589]	This study
YOT35921	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1598]	This study
YOT35922	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3 ade3::HIS3-(RHO1^{M}+CDC42^{M})* [pRB1601]	This study
YOT35924	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3 Δdpr1::HIS3* [pRB1601,pRB1590]	This study
YOT35925	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3 Δdpr1::HIS3* [pRB1601,pRB1589]	This study
YOT35935	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1594,pRB1596]	This study
YOT35936	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1601,pRB1594,pRB1620]	This study
YOT35950	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1602]	This study
YOT35952	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1590]	This study
YOT35953	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1589]	This study
YOT35954	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1592]	This study
YOT35956	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1592,pRB1595]	This study
YOT35957	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1592,pRB1597]	This study
YOT35958	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1591]	This study
YOT35959	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1591, pRB1596]	This study
YOT35961	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1591, pRB1597]	This study
YOT35962	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1594, pRB1620]	This study
YOT35963[a]	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3 ade3::HIS3-(RHO1^{M}+CDC42^{M})*	This study
YOT35964	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1590,pRB1601]	This study
YOT35965	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1590,pRB1603]	This study
YOT35966	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1590,pRB1604]	This study
YOT35967	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1589,pRB1601]	This study
YOT35968	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1589,pRB1603]	This study
YOT35969	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1589,pRB1604]	This study
YOT35970	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1592,pRB1601]	This study
YOT35971	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1592,pRB1603]	This study
YOT35972	a *ade2 lys2 his3 trp1 leu2 ura3 Δcall::ura3* [pRB1592,pRB1604]	This study

[a] YOT35963 contains spontaneous mutation(s) to suppress the GGTase I defect (see text).

For analysis of the wild-type *CAL1* allele and the *Δcal1* allele by PCR (Figure 2C), three primers were used: Primers 1 (ATGTGTCAAGCTACCAATGGC) and 2 (ATCAAAGTCTTCTTTGGATCG) amplify a 560-base pair (bp) fragment of the wild-type *CAL1* gene, and primers 1 and 3 (ACCCGGTGTGGGTTTAGATGA) amplify a 710-bp fragment of the *cal1* disruption. The three primers were combined to visualize both alleles at the same time. After 30 cycles of PCR reaction (94°C for 45 s, 50°C for 1 min, 72°C for 2 min), products were analyzed by agarose electrophoresis.

Growth Rate Measurements

Strains were inoculated into 15-ml glass tubes containing 10 ml YPD medium and grown at the indicated temperature in a model TC7 rollerdrum (New Brunswick Scientific, Edison, NJ) at 70 rpm. Doubling times were calculated using the exponential curve fit function of Cricket Graph (Cricket Software, Malvern, PA), on four to seven absorbance (600 nm) readings taken during the exponential growth phase. Each doubling time (Table 2 and Table 3) was the average of two independent cultures; growth rates for independent cultures differed by less than 10%.

Microscopy and Immunofluorescence Localization

Actin was visualized using rhodamine phalloidin (Sigma, St. Louis, MO) as described by Pringle *et al.* (1989). Cells were fixed by adding

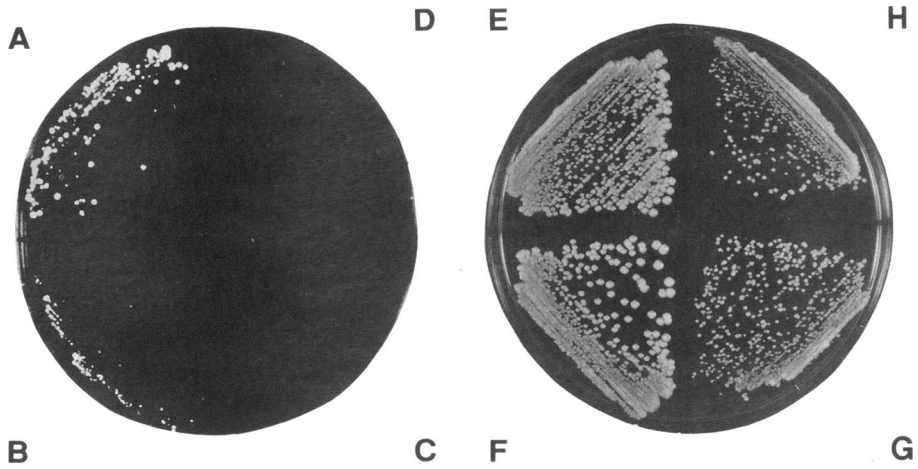

Figure 1. Suppression of the GGTase I defect was tested with the wild-type *DPR1* strains, YOT35920 (Δ*cal1* [pRB1601, pRB1589]) (A and E) and YOT35919 (Δ*cal1* [pRB1601, pRB1590]) (B and F) and the Δ*dpr1* strains, YOT35924 (Δ*cal1* Δ*dpr1* [pRB1601, pRB1590]) (C and G) and YOT35925 (Δ*cal1* Δ*dpr1* [pRB1601, pRB1589]) (D and H). The four strains were streaked on a FOA plate (A–D) and incubated for 5 d at 25°C to lose the *CAL1* plasmid. YNBD (-Trp) plate was used as a control (E–H).

formaldehyde directly to culture medium to a final concentration of 3.7% (vol/vol). Cell morphologies (using Nomarski optics) and localization of actin were observed and photographed using a Zeiss Axioskop microscope (Thornwood, NJ).

RESULTS

To examine whether overproduction of *RHO1* and *CDC42* suppresses a GGTase I defect, we constructed a strain that contained a GGTase I deletion (Δ*cal1/cdc43*), the wild-type *CAL1/CDC43* gene on a *URA3* plasmid (pRB1601), and a multicopy plasmid harboring the wild-type *RHO1* and *CDC42* genes (pRB1590). The ability of this strain (YOT35919) to lose pRB1601 was then tested on FOA plates. A number of colonies appeared on FOA (Figure 1B), but the frequency of FOA-resistant colonies was relatively low ($<10^{-3}$). To exclude the possibility of the introduction of mutations during loss of the *CAL1* plasmid, we retransformed FOA-resistant derivatives with pRB1601 and rechecked the frequency of plasmid loss. The frequency of loss was again the same, indicating that no mutations had been introduced during the operation. Absence of the wild-type copy of *CAL1/CDC43* in the FOA-resistant cells was confirmed by PCR (Figure 2B).

The growth rate of strain YOT35952 (Δ*cal1/cdc43* with high-copy-plasmid-borne *RHO1* and *CDC42*) was

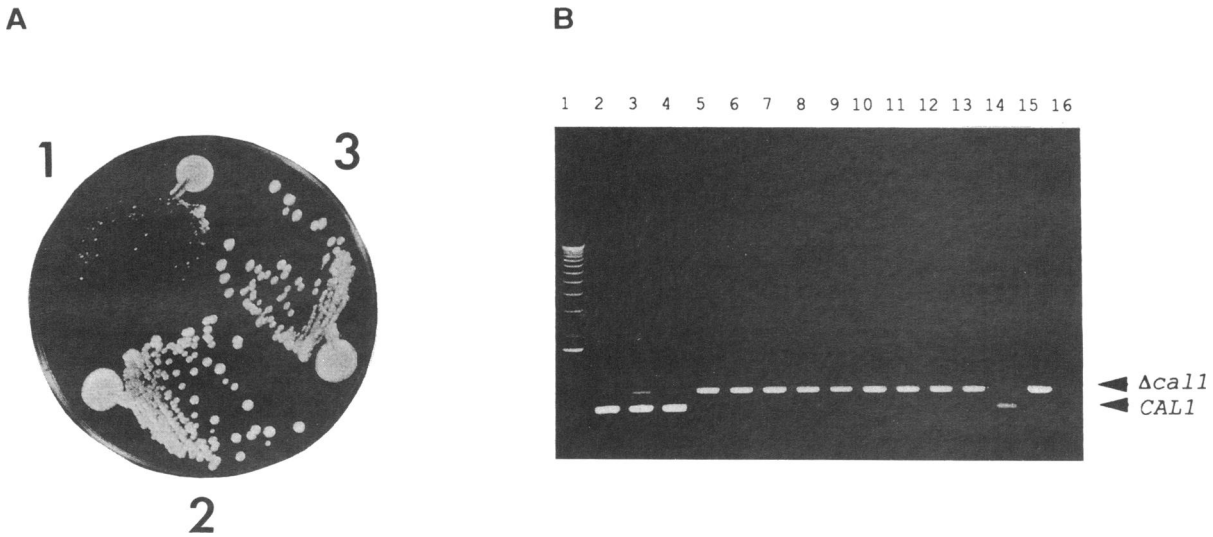

Figure 2. *RHO1* (L209M) and *CDC42* (L191M) suppress GGTase I defect. (A) Growth of the YOT35953 strain harboring Δ*cal1* and pRB1590 (1), the YOT35952 strain harboring Δ*cal1* and pRB1589 (2), and a wild-type YPH499 strain with pRB1604 vector (3) were analyzed after 2 d incubation on YPD at 30°C. (B) The DNAs from wild-type YPH499 (2), Δ*cal1* [pRB1601, pRB1604] (YOT35902) (3), Δ*cal1* [pRB1602] (YOT35950) (4), Δ*cal1* [pRB1590] (YOT35952) (5), Δ*cal1* [pRB1589] (YOT35953) (6), Δ*cal1* [pRB1592] (YOT35954) (7), Δ*cal1* [pRB1591] (YOT35958) (8), Δ*cal1* [pRB1592, pRB1595] (YOT35956) (9), Δ*cal1* [pRB1592, pRB1597] (YOT35957) (10), Δ*cal1* [pRB1591, pRB1596] (YOT35959) (11), Δ*cal1* [pRB1591, pRB1597] (YOT35961) (12), and YOT35963 (13) were analyzed after PCR. PCR reactions with the pRB1601 plasmid (14), the pRB1605 plasmid (15), and no DNA (16) served as controls. Lane 1 contains 1-kb ladder DNA marker.

slower than that of wild-type controls at any temperature (Figure 2A, Table 3); nevertheless, it is clear that the *cal1/cdc43* deletion is suppressed. It seemed possible that this suppression might depend on prenylation of these GTPases by the FTase, because it is known that the FTase can modify GGTase I target proteins to a limited extent given a high concentration of the protein substrate. The suppression might be incomplete either because of insufficient prenylation by the FTase, because of the provision of the wrong isoprenoid by FTase, or both. To test the effect of more efficient prenylation of these two GTPases, we constructed mutations of *RHO1* and *CDC42* with alterations at the C-termini (Cys-Ali-Ali-Leu to Cys-Ali-Ali-Met) that should serve as better substrates for the FTase. We made a multicopy plasmid (pRB1589) that contains both Rho1p and Cdc42p with this alteration at their C-termini. The Δ*cal1/cdc43* strain (YOT35953) with this plasmid grows much better than the strain with a comparable plasmid containing wild-type *RHO1* and *CDC42* genes (Figure 1, A and B); indeed, it grows as well as the wild-type control (Figure 2A, Table 3). We observed similarly robust growth at 14, 25, 30, and 37.5°C. We examined cell morphology (Figure 3B) and actin distribution, both of which appear to be the same in strain YOT35953 and its wild-type parent. These observations show that yeast GGTase I is no longer essential when Rho1p and Cdc42p with C-terminal mutations (L209M and L191M, respectively) are overproduced.

When either *RHO1* (L209M) or *CDC42* (L191M) was not present on the plasmid, the results were different. The *CDC42* (L191M) gene alone did not detectably suppress the GGTase I defect (Table 3). The *RHO1* (L209M) gene alone allowed the Δ*cal1/cdc43* cells (strain YOT35958) to grow slowly at 25°C but did not allow growth at 37.5°C (Table 3). In the microscope, these cells are bigger and rounder even at 25°C than the wild-type cells (Figure 3C). At 37.5°C, cells of strain YOT35958 show a cell cycle arrest with enlarged unbudded cells, closely resembling temperature-sensitive *cdc42* mutant cells (Figure 3D). Finally, immunofluorescence microscopy showed that cells of strain

Table 3. Suppression of GGTase I defect by overexpression of wild-type and mutant *RHO1* and *CDC42*

		Growth			
		25°C		37.5°C	
Strain		Plates	Rate (h)	Plates	Rate (h)
Wild-type		+++	2.3	+++	2.7
Δ*cal1*		−		−	
Δ*cal1* (*RHO1* multicopy)					
RHO1$^+$		+	7.2	−	
RHO1 (L209M)		++	5.2	−	
rho1 (C206S)		−		−	
Δ*cal1* (*CDC42* multicopy)					
CDC42$^+$		−		−	
CDC42 (L191M)		−		−	
cdc42 (C188S)		−		−	
Δ*cal1* (both multicopy)					
RHO1$^+$	*CDC42*$^+$	+	6.7	+	10.4
RHO1$^+$ *cdc42*	*(C188S)*	+	7.3	−	
RHO1 (L209M)	*CDC42 (L191M)*	+++	2.2	+++	2.6
RHO1 (L209M)	*cdc42 (C188S)*	++	4.9	−	
rho1 (C206S)	*CDC42*$^+$	−		−	
rho1 (C206S)	*CDC42 (L191M)*	−		−	
rho1 (C206S)	*cdc42 (C188S)*	−		−	
Δ*cal1* (both on *CEN* plasmid)					
RHO1 (L209M)	*CDC42 (L191M)*	−		−	
Δ*cal1* (both integration)					
RHO1 (L209M)	*CDC42 (L191M)*	−		−	
Δ*cal1* (*RHO2* multicopy)					
RHO2$^+$	*CDC42*$^+$	−		−	
RHO2$^+$	*RHO1*$^+$	+	7.0	−	

The experiments employed the plasmids and strains described in Tables 1 and 2. All the strains having the *CAL1* gene on a *URA3* plasmid were tested for growth on FOA plates at 25°C and 37.5°C. Relative growth rates on agar are indicated as follows: +++, robust growth after 4 d at 25°C or 2 d at 37.5°C; ++ and +, less vigorous growth; −, no growth. Exponential growth rates (expressed as time [h] required to double turbidity at 600 nm) of the wild-type strain and several Δ*cal1* strains were measured in rich (YPD) medium at 25°C and at 37.5°C using two independent colonies. Experimental errors were <10%.

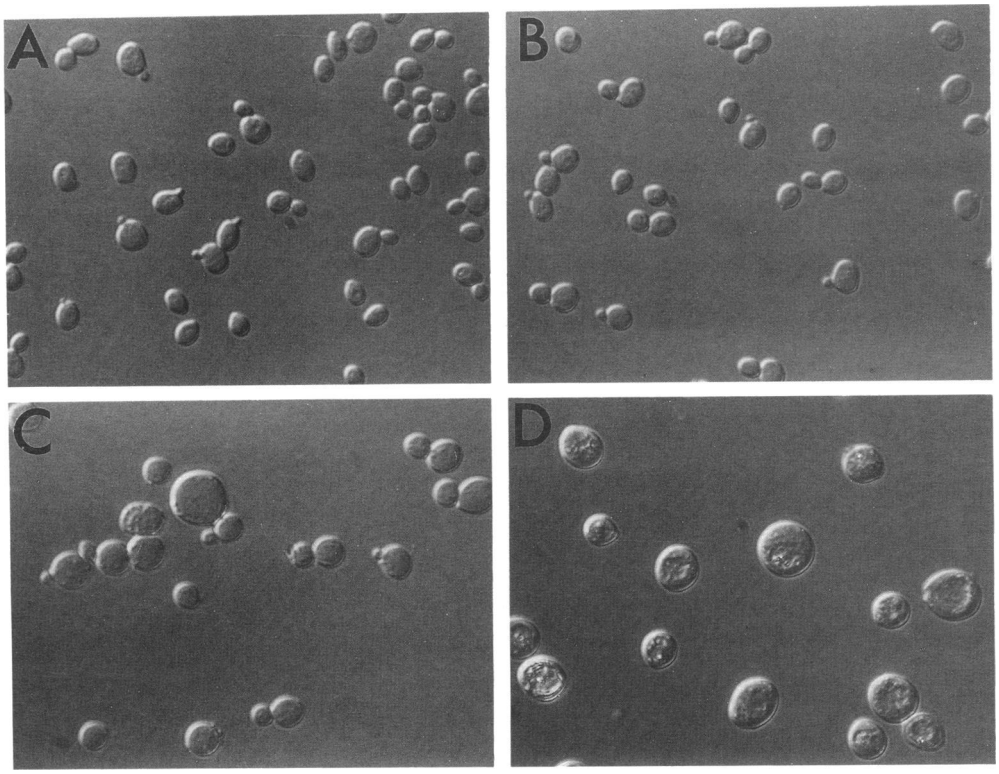

Figure 3. Nomarski images of cells grown at 25°C (A–C) or incubated for 6 h at 37.5°C (D). YPH499, a wild-type *CAL1* strain (A), YOT35953, Δ*cal1* with pRB1589 (B), and YOT35958, Δ*cal1* with pRB1591 (C and D) are shown.

YOT35958 frequently contained delocalized actin, as do *cdc42-ts* strains (Adams *et al.*, 1990).

We verified that prenylation at the C-terminal cysteine residue of *RHO1* and *CDC42* is essential to the suppression by changing this residue to serine. A *rho1* (C206S) mutation results in no suppression (Table 3), whereas either the *RHO1* wild-type gene or the *RHO1* (L209M) gene partially suppresses Δ*cal1/cdc43* at 25°C. Introduction of the *cdc42* (C188S) mutation resulted in no additional suppression beyond that seen with either *RHO1* or *RHO1* (L209M) alone (Table 3). Another rho-related gene, *RHO2*, which is dispensable for cell growth, could be replaced with neither *RHO1* nor *CDC42* (Table 3).

Low doses of the C-terminal mutant proteins, *RHO1* (L209M) and *CDC42* (L191M), carried either on a centromere plasmid (pRB1598) or in a chromosomal integration did not suppress the GGTase I defect (Table 3). Although we saw FOA-resistant colonies with the *RHO1* (L209M) and *CDC42* (L191M) integrant at very low frequency ($<10^{-5}$), these colonies appeared because of spontaneous mutations introduced during loss of the *CAL1/CDC43* plasmid. When four such colonies were isolated, retransformed with pRB1601, and rechecked for plasmid loss efficiency, the efficiency with the new transformants was very much higher than that of the original transformant, indicating the introduction of mutation(s) during selection. This result confirmed that suppression of Δ*cal1/cdc43* requires overproduction of *RHO1* and *CDC42*.

The preceding results suggest that the FTase is responsible for modifying the two essential GTPases, Rho1p and Cdc42p, when the GGTase I is absent. This idea predicts that the presence of the FTase becomes essential in GGTase I mutants. Indeed, we found that suppression of GGTase I mutants was enhanced by increases in the dose of the β subunit of FTase (Table 4). The increase in the activity of the FTase under similar circumstances has been measured to be about threefold (Schafer *et al.*, 1990). We observed also that strains carrying a deletion of the gene specifying this subunit (Δ*dpr1/ram1*, strains YOT35924 and YOT35925) could not lose the plasmid carrying the gene specifying the β subunit of GGTase I (Figure 1).

DISCUSSION

Although a large number of eukaryotic proteins are modified by the farnesyl and geranylgeranyl groups, we found here that lethality caused by a yeast GGTase I defect was suppressed by overproduction of just two proteins. Rho1p, Cdc42p, Rho2p (Madaule *et al.*, 1987), and Rsr1p (Bender and Pringle, 1989) are yeast GTP-binding proteins that have Cys-Ali-Ali-Leu sequences: CVLL, CTIL, CIVL, and CTIL, respectively. Each of these proteins is implicated as a GGTase I substrate in vitro (Caplin *et al.*, 1993). Gene disruption experiments have shown that Rho1p and Cdc42p are essential proteins (Madaule *et al.*, 1987; Johnson and Pringle, 1990), whereas Rho2p and Rsr1p are not. Overproduction of

Table 4. Overexpression of FTase in GGTase I deficient cells

Strains	Growth rate (h) 25°C	37.5°C
Δ*cal1* (multicopy *RHO1*$^+$ + *CDC42*$^+$)		
+ *CAL1* multicopy	2.2	2.5
+ *DPR1* multicopy	5.1	8.7
+ no additional gene	9.9	11.7
Δ*cal1* (multicopy *RHO1 (L209M)* + *CDC42 (L191M)*)		
+ *CAL1* multicopy	2.2	2.4
+ *DPR1* multicopy	2.5	2.8
+ no additional gene	2.3	2.7
Δ*cal1* (multicopy *RHO1*$^+$)		
+ *CAL1* multicopy	2.2	2.5
+ *DPR1* multicopy	3.8	—
+ no additional gene	7.8	—

pRB1602 (+ *CAL1* multicopy), pRB1603 (+ *DPR1* multicopy), and YEp24 (+ no additional gene) plasmids (see Table 1) were introduced into strains of the indicated genotypes (see Table 2). −, indicates no growth.

Rho1p and Cdc42p, especially when mutated to become better prenylation substrates for the FTase, suffices to make fully viable strains carrying deletions removing yeast GGTase I.

We have provided three lines of evidence that implicate the FTase in the C-terminal modification of Rho1p and Cdc42p in GGTase I-deficient cells. First, the C-terminally altered forms of the two GTPases suitable for the FTase gave the best suppression, whereas removal of the C-terminal Cys eliminated suppression (Table 3). Second, the GGTase I defect was no longer suppressed in the Δ*dpr1* background by overproduction of the GTPases, regardless of the C-terminal amino acids (Figure 1). Third, overproduction of *DPR1* improved suppression of the GGTase I deficiency when only wild-type *RHO1* was overproduced or when both wild-type GTPases were overproduced (Table 4). These results support strongly the hypothesis that FTase is responsible for prenylation of the essential GTPases in the GGTase I deletion strain.

C-terminal alteration of Rho1p and Cdc42p to methionine, however, is insufficient for suppression of the GGTase I defect; we found that provision at low copy number of these altered GTPases could not suppress the GGTase I defect. We think that this is because modification by an inappropriate isoprenoid, in this case the C15 farnesyl instead of the C20 geranylgeranyl, does not allow Rho1p and Cdc42p to function perfectly. Cox *et al.* (1992) have proposed that the difference in length between the farnesyl group and the geranylgeranyl group can affect Ras function in this way. Normal H-ras protein contains serine at its C-terminus and is modified with a farnesyl group. Ras with a C-terminal leucine, unlike wild-type H-ras, which is geranylgeranylated, has a dominant-negative phenotype when moderately expressed, presumably by perturbing normal Ras function (Cox *et al.*, 1992).

An alternative explanation for the failure of low-copy suppression is that one or more additional essential geranylgeranylated protein(s) may exist whose function is dependent on prenyl modification but which can be substituted for by overproduction of *RHO1* and *CDC42*. The *cdc24-4* mutation was found to be suppressed by overproduction of *CDC42* (Bender and Pringle, 1989), but Cdc24p is not a member of the small GTPase family and its primary structure does not contain the Cys-Ali-Ali-Leu sequence that is a substrate for GGTase I (Miyamoto *et al.*, 1987, 1991). We have begun to isolate spontaneous mutations that allow the integrated copies of *RHO1* (L209M) and *CDC42* (L191M) to suppress the GGTase I deletion. Further study of these mutations may be helpful in understanding the failure of low-copy suppression.

The possibility that Cdc42p and Rho1p are the only essential target proteins of the yeast GGTase I is consistent with analysis of conditional lethal *cal1/cdc43*, *cdc42*, and *rho1* mutants. The *cal1/cdc43* point mutants are deficient in establishment of cell polarity (Adams *et al.*, 1990) and/or in nuclear division (Ohya *et al.*, 1984). *cdc42* mutants (Adams *et al.*, 1990; Ziman *et al.*, 1991) have defects in cell polarity, whereas *rho1* mutants have both defects (Qadota *et al.*, unpublished data). However, it should also be noted that yeast cells may contain other proteins that are predominantly geranylgeranylated in vivo by GGTase I and are essential for viability. Such proteins would not appear as essential substrates in our assay if the prenylation were not essential for function or if the level of alternative prenylation (e.g., by FTase) were sufficient to allow the essential function. For example, Rsr1p is a nonessential protein but important for determination of budding pattern (Bender and Pringle, 1989). It is likely that the GGTase I deletion results in loss of Rsr1p function, but we could not test this possibility because overproduction of Cdc42p itself causes random budding pattern (Johnson and Pringle, 1990).

The partial suppression by *RHO1*$^+$ or *RHO1* (L209M) alone (Table 3) was an unexpected result, because prenylation of Cdc42p is thought to be necessary for its function (Ziman *et al.*, 1991). Given the fact that wild-type Cdc42p can be slightly prenylated by the FTase in vitro (Caplin *et al.*, 1993), we speculate that a small amount of wild-type Cdc42p prenylated by the normal amount of FTase in vivo may be sufficient for *CDC42* function at low temperature. This is consistent with the observation that the GGTase I cells with multicopy *RHO1* (L209M) have a bud emergence defect at high temperature similar to that of *cdc42* mutants (Figure 3D). Overproduction of *RHO1* alone cannot suppress the GGTase I deletion mutation sufficiently to allow growth

from spores (Qadota *et al.*, 1992), suggesting that prenylation of Cdc42p is important at germination.

Although overproduction of neither *RHO1* nor *RHO2* can suppress a deletion of *cal1/cdc43* at high temperature (Table 3), overexpression of either *RHO* gene suppresses the temperature-sensitive growth defect of the *cal1-1* mutation (Qadota *et al.*, 1992). These results clearly indicate that the suppression by the *RHO* genes at high temperature requires the *cal1-1* mutant gene product. Because overproduction of these Rho proteins stimulates *CAL1/CDC43*-dependent prenylation of Ras2p in vivo (Qadota *et al.*, 1992), we would argue that Rho1p has two functions. One of these functions is essential for cell growth, cell cycle progression, and secretion (McCaffrey *et al.*, 1991); the other is an apparently nonessential feedback regulation of GGTase I activity.

Although the yeast GGTase I is essential for growth, the yeast FTase deletion mutant is viable. As we have shown above, the FTase becomes essential when it is needed to substitute for the GGTase I and prenylate Rho1p and Cdc42p (Figure 1). Even in ordinary yeast strains, there is a hint of an essential role for FTase. Trueblood *et al.* (1993) recently reported that FTase deletions might be viable because GGTase I can prenylate FTase-substrate proteins in the FTase deletion strains. Consistently, synthetic lethal interactions are observed between *cal1* mutations and the *dpr1* deletion mutation (Ohya *et al.*, 1991; Trueblood *et al.*, 1993). Ras2p is likely the most important normal FTase substrate, because overproduction of Ras2p suppresses the *cdc43-2 Δdpr1* synthetic lethality. FTase deletion mutants also do not grow at temperatures above 35°C. FTase-dependent prenylation of YDJ1/Mas5p (Atencio and Yaffe, 1992; Caplan *et al.*, 1992) and possibly Rho3p (Matsui and Toh-e, 1992) may be required for yeast growth at high temperature.

To date, nine mammalian GGTase I substrate proteins including rhoA, G25K, rap1A, rap1B, ralA, rac1, rac2, cGMP phosphodiesterase, and the γ subunit of G-protein are known. It should be noted that the mammalian proteins rhoA and G25K are functional counterparts of the yeast Rho1p and Cdc42p. It is not yet known which mammalian proteins play crucial roles as GGTase I substrates. To dissect the signaling events that involve prenylated proteins as well as to apply prenylation inhibitors to ras-related oncogenic diseases, it would be helpful to know the crucial target proteins of each of the prenyltransferases also in mammalian cells.

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