DOI: 10.1534/genetics.105.048082

Pkc1 Acts Through Zds1 and Gic1 to Suppress Growth and Cell Polarity Defects of a Yeast eIF5A Mutant

Cleslei F. Zanelli and Sandro R. Valentini[1]

Department of Biological Sciences, School of Pharmaceutical Sciences, São Paulo State University, UNESP, Araraquara, São Paulo 14801-902, Brazil

Manuscript received July 11, 2005
Accepted for publication August 30, 2005

ABSTRACT

eIF5A is a highly conserved putative eukaryotic translation initiation factor that has been implicated in translation initiation, nucleocytoplasmic transport, mRNA decay, and cell proliferation, but with no precise function assigned so far. We have previously shown that high-copy *PKC1* suppresses the phenotype of *tif51A-1*, a temperature-sensitive mutant of eIF5A in *S. cerevisiae*. Here, in an attempt to further understand how Pkc1 functionally interacts with eIF-5A, it was determined that *PKC1* suppression of *tif51A-1* is independent of the cell integrity MAP kinase cascade. Furthermore, two new suppressor genes, *ZDS1* and *GIC1*, were identified. We demonstrated that *ZDS1* and *ZDS2* are necessary for *PKC1*, but not for *GIC1* suppression. Moreover, high-copy *GIC1* also suppresses the growth defect of a *PKC1* mutant (*stt1*), suggesting the existence of a Pkc1-Zds1-Gic1 pathway. Consistent with the function of Gic1 in actin organization, the *tif51A-1* strain shows an actin polarity defect that is partially recovered by overexpression of Pkc1 and Zds1 as well as Gic1. Additionally, *PCL1* and *BNI1*, important regulators of yeast cell polarity, also suppress *tif51A-1* temperature sensitivity. Taken together, these data strongly support the correlated involvement of Pkc1 and eIF5A in establishing actin polarity, which is essential for bud formation and G1/S transition in *S. cerevisiae*.

THE putative translation initiation factor 5A (eIF5A) is a highly conserved and essential protein present in all organisms from archebacteria to mammals but not in eubacteria (Schnier *et al.* 1991; Chen and Liu 1997). Despite its highly conserved essential function, the critical cellular role of eIF5A is not known. A number of studies implicate eIF5A in a variety of cellular processes.

The involvement of eIF5A with translation initiation was proposed due to its purification from ribosomes of reticulocyte lysates and to its stimulatory effect in the methionyl-puromycin assay used to implicate factors in the first peptide bond formation (Benne and Hershey 1978). However, depletion of eIF5A in yeast caused only a slight decrease in the protein synthesis rate, arguing against a role as a general translation initiation factor. Therefore, it was hypothesized that eIF5A may function in the translation of a specific subset of mRNAs. Since depletion of eIF5A in yeast also causes an increase of G1-arrested cells, judged by cell morphology, it was proposed that eIF5A may be important for translating mRNAs encoding proteins required for cell cycle progression (Kang and Hershey 1994). This connection between eIF5A and cell cycle progression is further supported by the observation that blocking any step of hypusination, its essential post-translational modification, in mammalian cells inhibits cell proliferation (Park *et al.* 1997), placing eIF5A among the potential targets for cancer therapy (Caraglia *et al.* 2001).

eIF5A has also been implicated in nucleocytoplasmic export of Rev-dependent HIV-1 transcripts and mRNA decay (Ruhl *et al.* 1993; Bevec *et al.* 1996; Bevec and Hauber 1997; Zuk and Jacobson 1998). However, subsequent studies have not confirmed the involvement of eIF5A with Rev-dependent nuclear export in either mammalian or yeast systems (Shi *et al.* 1996, 1997; Henderson and Percipalle 1997; Lipowsky *et al.* 2000; Li-En Jao and Chen 2002; Valentini *et al.* 2002). Moreover, the effect of eIF5A on mRNA decay seems to be secondary, as arrest of cell growth of eIF5A temperature-sensitive mutants does not directly correlate with mRNA accumulation (Valentini *et al.* 2002).

Thus, although eIF5A has been associated with different cellular events, the role played by this essential factor remains unclear. In an attempt to identify cellular partners for eIF5A and understand its critical cellular function, a temperature-sensitive mutant of *TIF51A* (*tif51A-1*), one of the genes encoding eIF5A in *Saccharomyces cerevisiae*, was used in a high-copy suppressor screen. *PKC1*, encoding for the only yeast protein kinase C, was one of the suppressors isolated together with three members of the cell integrity pathway, *WSC1*, *WSC2*, and *WSC3* (Valentini *et al.* 2002).

[1]*Corresponding author:* School of Pharmaceutical Sciences, São Paulo State University, UNESP, Rodovia Araraquara-Jaú, Km 01, Araraquara, SP 14801-902, Brazil. E-mail: valentsr@fcfar.unesp.br

TABLE 1

Yeast strains used in this study

Strain	Genotype	Source
SVL14	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 ssd1-d tif51A-1*	Valentini *et al.* (2002)
SVL26	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 ssd1-d tif51A-2*	Valentini *et al.* (2002)
SVL32	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 ssd1-d tif51A-3*	Valentini *et al.* (2002)
SVL248	*MAT***a** *ade2 his3 leu2 trp1 ura3 tif51A(ts1159)*	This study
SVL82 (W303)	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 ssd1-d*	Pamela Silver
SVL170 (DLY486)	*MATα ade8 his3 leu2 met3 ura3 stt1 (pkc1)*	David Levin
SVL230 (YEF877)	*MATα his3 leu2 lys2 trp1 ura3 zds1ΔHIS3*	Bi and Pringle (1996)
SVL321 (CY832)	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 ura3-1 can1-100 trp1-1 ssd1ΔLEU2*	Rosenwald *et al.* (2002)
SVL327	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 zds2ΔkanMX4*	Anita Corbett
SVL411	*MAT***a** *ade2 his3 leu2 ura3 trp1 zds1ΔHIS3 zds2ΔkanMX4 ssd1-d tif51A-1*	This study
SVL412	*MAT***a** *ade2 his3 leu2 ura3 trp1 ssd1ΔLEU2 tif51A-1*	This study
SVL531	*MATα ade2 his3 leu2 ura3 trp1 zds1ΔHIS3 ssd1ΔLEU2*	This study
SVL532	*MATα ura3 leu2 his3 trp1 zds1ΔHIS3 zds2ΔkanMX4 ssd1ΔLEU2*	This study

Pkc1 in *S. cerevisiae* controls a variety of cellular processes such as cell cycle progression, mating, nutrient sensing, and the structural organization of the cytoskeleton (Heinisch *et al.* 1999). Pkc1 is activated by a small GTPase of the Rho family, Rho1, which receives upstream signals from the Slg1 (Wsc1) and Mid2 transmembrane sensors (Philip and Levin 2001). The downstream *PKC1*-mitogen-activated protein kinase (MAPK) cascade, consisting of Bck1, Mkk1/Mkk2, and Mpk1 kinases, phosphorylates transcription factors that regulate cell wall remodeling and cytoskeleton organization in polarized cell growth (Heinisch *et al.* 1999). Although less well defined, several studies report evidence for other biochemical pathways branching out from Pkc1 (Ketela *et al.* 1999; Andrews and Stark 2000; Li *et al.* 2000; Nanduri and Tartakoff 2001; Chai *et al.* 2002; Valdivia and Schekman 2003; Vilella *et al.* 2005).

In this work, we further analyzed the functional interaction between eIF5A and the Pkc1-cell integrity pathway. Our results demonstrate that *PKC1* suppression of *tif51A-1* is MAP kinase independent. Furthermore, we present data that suggest the existence of a novel pathway independent of the MAP kinases. This pathway links Pkc1 to the Cdc42 effector, Gic1, in a Zds1/Zds2-dependent manner. The results herein favor a more direct involvement of Pkc1 in actin polarization, which is necessary for bud formation during G1/S transition. Finally, we discuss a possible role for eIF5A in this process.

MATERIALS AND METHODS

Yeast strains manipulation and plasmids: *S. cerevisiae* strains used in this work are listed in Table 1. Procedures for cell growth and genetic manipulations were carried out according to standard protocols (Guthrie and Fink 1991). Plasmids used in this work are listed in Table 2. The functionality of all plasmids showing negative results in the high-copy suppression analysis (Table 3) was confirmed by complementation or phenotypic suppression of known mutants (supplemental material at http://www.genetics.org/supplemental/). Cloning by PCR was performed with *Pfx* DNA polymerase (Invitrogen, San Diego) or with Vent DNA polymerase (New England Biolabs, Beverly, MA) following standard molecular biology procedures (Ausubel *et al.* 2005).

High-copy suppressor screen: A *URA3*/2μ genomic yeast library (Connelly and Hieter 1996) was transformed into a *tif51A-1* strain (SVL14). Approximately 40,000 transformants were selected by plating on uracil dropout plates, incubating at 25° overnight, and shifting plates to 36° for 3–4 days. Plasmids were rescued from temperature-resistant clones and retransformed into SVL14 to test for plasmid linkage. The genomic segment present in each of the selected clones was determined by sequencing the ends with T3 and T7 primers and using these sequences to search the *S. cerevisiae* genome database. High-copy suppressor genes were characterized by subcloning different segments of the original clone into pSV65 (pRS426) and testing them in SVL14. *GIC1* and *ZDS1* were also cloned into pSV65 (pRS426) using the following primers: GIC1-A, 5′-CGG GGT ACC AAT ACG TAC CCG GGT AGT AG-3′; GIC1-B, 5′-CGG GGT ACC GTC TGA GCA GGA ATA AAG AG-3′; and ZDS1-A, 5′-CGC GGA TCC TGG AAT TCT ATC GAG CGA CC-3′; ZDS1-B, 5′-CGC GGA TCC CTC TGT TCT TAT ACG GTT CC-5′. The plasmid pSV501 was constructed by subcloning the *Bam*HI fragment containing *TIF51A* from pSV107 into pSV58 (pRS314).

Phalloidin staining: Staining of actin filaments was carried out essentially as described (Amberg 1998). Cells from exponential phase cultures were fixed at room temperature for 1 hr with 3.7% formaldehyde in the culture medium plus 1 hr incubation in PBS with 3.7% formaldehyde. Cells were subsequently washed twice with PBS and resuspended in 500 μl of PBS. Staining was performed by adding 10 μl of rhodamine-phalloidin (Molecular Probes, Eugene, OR) to 100 μl of cell suspension. Cells were incubated in the dark for 1 hr, washed five times with 1 ml of PBS, and finally suspended in 100 μl of mount solution [90% glycerol, 0.1× PBS, 92.5 mm *p*-phenylenediamine (Sigma, St. Louis), pH adjusted to 0.8 with 0.5 m sodium carbonate, pH 9.0]. Stained cells were stored at −20° until microscopic analysis. Rhodamine-phalloidin-stained cells (2.5 μl) were visualized by fluorescence microscopy using a rhodamine filter and a Nikon TE300 inverted microscope. Images were captured with a MicroMax 5-MHz CCD (Princeton Instruments, Princeton, NJ) and the software Image-Pro Plus (Media Cybernetics).

TABLE 2

Plasmids used in this study

Plasmid	Feature	Source
pSV58 (pRS314)	*TRP1, CEN*	Sikorski and Hieter (1989)
pSV64 (pRS425)	*LEU2,* 2μ	Christianson *et al.* (1992)
pSV65 (pRS426)	*URA3*, 2μ	Christianson *et al.* (1992)
pSV107	*TIF51A, URA3,* 2μ	Valentini *et al.* (2002)
pSV108	*TIF51A, LEU2,* 2μ	Valentini *et al.* (2002)
pSV115	*WSC1, URA3,* 2μ	Valentini *et al.* (2002)
pSV116	*WSC2, URA3,* 2μ	Valentini *et al.* (2002)
pSV117	*WSC3, URA3,* 2μ	Valentini *et al.* (2002)
pSV181	*PKC1, URA3,* 2μ	Valentini *et al.* (2002)
pSV292 (p*ROM2*)	*ROM2, TRP1,* 2μ	Helliwell *et al.* (1998)
pSV293 (p*RHO2*)	*RHO2, TRP1,* 2μ	Helliwell *et al.* (1998)
pSV294 (*pBCK1-20*)	*BCK1-20, URA3, CEN*	Helliwell *et al.* (1998)
pSV295 (p*MKK1*)	*MKK1, URA3,* 2μ	Helliwell *et al.* (1998)
pSV296 (p*MPK1*)	*MPK1 (SLT2), URA3,* 2μ	Helliwell *et al.* (1998)
pSV304	*GIC1, URA3,* 2μ	This study
pSV352 (YEp24-*BNI1*)	*BNI1, URA3,* 2μ	Helliwell *et al.* (1998)
pSV376 (YEp351-*CDC24*)	*CDC24, LEU2,* 2μ	Richman *et al.* (1999)
pSV377 (YEp351-*CDC42*)	*CDC42, LEU2,* 2μ	Richman *et al.* (1999)
pSV381 (YEp13-*BEM3*)	*BEM3, LEU2,* 2μ	Richman *et al.* (1999)
pSV383 (YEp13-*RGA1*)	*RGA1, LEU2,* 2μ	Richman *et al.* (1999)
pSV384 (pFK2CU)	*SSD1, URA3, CEN*	Uesono *et al.* (1994)
pSV387 (YEp13-*MID2*)	*MID2, LEU2,* 2μ	de Bettignies *et al.* (2001)
pSV388 (YEp13-*MTL1*)	*MTL1, LEU2,* 2μ	de Bettignies *et al.* (2001)
pSV396 (EBO459)	*CLN2, URA3,* 2μ	Lenburg and O'Shea (2001)
pSV401 (pDLB678)	*BEM1, URA3,* 2μ	Gladfelter *et al.* (2002)
pSV403 (pDLB722)	*CLA4, URA3,* 2μ	Gladfelter *et al.* (2002)
pSV407 (YEp352-*STE20*)	*STE20, URA3,* 2μ	Gladfelter *et al.* (2002)
pSV411 (MPO120)	*PCL1, URA3*, 2μ	Lenburg and O'Shea (2001)
pSV412 (MPO121)	*PCL2, URA3,* 2μ	Lenburg and O'Shea (2001)
pSV454 (pKT1057)	*BEM2, LEU2,* 2μ	Kawasaki *et al.* (2003)
pSV455 (pKT1130)	*BUD1, LEU2,* 2μ	Kawasaki *et al.* (2003)
pSV501	*TIF51A, TRP1, CEN*	This study
pSV531 (pTH123)	*GIC1, LEU2,* 2μ	Höfken and Schiebel (2004)
pSV532 (pTH124)	*GIC2, LEU2,* 2μ	Höfken and Schiebel (2004)
pSV534 (pTH179)	$GIC1^{CRIB-}$, *LEU2,* 2μ	Höfken and Schiebel (2004)
pSV535 (pTH185)	*GIC1-pr, LEU2,* 2μ	Höfken and Schiebel (2004)
pSV588 (YEp-*ZDS2*)	*ZDS2, URA3,* 2μ	Yu *et al.* (1996)
pSV589	*ZDS1, URA3,* 2μ	This study

RESULTS

***PKC1* suppression of a temperature-sensitive mutant of eIF5A occurs in a MAP kinase-independent pathway:** In a previous study, three temperature-sensitive alleles of *TIF51A*, *tif51A-1*, *tif51A-2*, and *tif51A-3* were characterized and used to further investigate eIF5A function in yeast (Valentini *et al.* 2002). As *PKC1* and *WSC1-3* were identified as high-copy suppressors of the *tif51A-1* mutant (Valentini *et al.* 2002), we decided to analyze the mechanism governing this suppression. Pkc1, the yeast protein kinase C counterpart, with its putative upstream regulators Wsc1 to -3, plays an important role in cell integrity maintenance. This function is performed by Pkc1 signaling through different downstream effectors to achieve cell wall remodeling and actin reorganization in response to several stimuli, including heat shock, pheromone, low osmolarity, nutrient starvation, and cell cycle progression (Heinisch *et al.* 1999). A well-established effector cascade downstream of Pkc1 is the MAP kinase pathway, which is composed of the kinases Bck1, Mkk1/Mkk2, and Mpk1. Activation of Mpk1 upregulates transcription of a series of genes involved in cell cycle progression and cell wall synthesis (Gustin *et al.* 1998). While transcription of a great number of genes is mediated by the MAP kinases cascade in response to different cell wall impairments, little is known about other Pkc1 downstream effectors (Ketela *et al.* 1999; Andrews and Stark 2000; Li *et al.* 2000; Nanduri and Tartakoff 2001; Chai *et al.* 2002; Valdivia and Schekman 2003).

To determine whether the suppression of *tif51A-1* promoted by high-copy *PKC1* occurs via the MAP kinases, we examined whether high-copy *MKK1*, *MPK1*, or an

TABLE 3

Genes analyzed for their ability to suppress the temperature-sensitive phenotype of the *tif51A-1* mutant

Gene	Functional information	Suppression
TIF51A	Putative translation initiation factor eIF5A	+
WSC1	Sensor for cell wall integrity signaling, plays a role in activation of the Pkc1-MAPK pathway; Wsc family	+
WSC2	Putative sensor for cell wall integrity signaling; Wsc family	+
WSC3	Putative sensor for cell wall integrity signaling; Wsc family	+
MID2	Sensor for cell wall integrity signaling, plays a role in activation of the Pkc1-MAPK pathway; Mtl1 homolog	+
MTL1	Protein that acts in signal transduction of cell wall stress, plays a role in regulation of cell integrity pathway; Mid2 homolog	+
ROM2	GDP/GTP exchange factor (GEF) for Rho1 and Rho2	+
RHO2	GTP-binding protein, member of the rho subfamily of ras-like proteins	+
PKC1	Protein kinase C, regulates MAP kinase cascade involved in regulating cell wall metabolism (cell integrity pathway)	+
BCK1-20[a]	MAP kinase kinase kinase component of cell integrity pathway	−
MKK1	MAP kinase kinase component of cell integrity pathway	−
MPK1	MAP kinase component of cell integrity pathway	−
ZDS1	Protein with effects on cell polarity and transcriptional silencing, homolog of Zds2	+
GIC1	Effector of Cdc42, important for bud emergence; Gic2 homolog	+
PCL1	G1/S-specific Pho85 cyclin	+
PCL2	G1/S-specific Pho85 cyclin	−
CLN2	G1/S-specific Cdc28 cyclin	−
GIC2	Effector of Cdc42, important for bud emergence; Gic1 homolog	−
CDC42	Rho-type GTPase involved in bud site assembly and cell polarity	−
CLA4	PAK kinase required for cytokinesis, effector of Cdc42	−
STE20	PAK kinase of the pheromone pathway; also regulates polarized growth, effector of Cdc42	−
CDC24	GEF for Cdc42, involved in bud emergence, bud site selection, and growth of mating projection	−
BUD1	GTP-binding protein of the ras superfamily involved in bud site selection	−
BEM1	Scaffold protein for complexes involving cell polarity establishment and morphogenesis factors such as Cdc24 and Bud1	+
BEM2	Rho-type GTPase-activating protein (GAP) for Rho1	−
BNI1	Formin protein involved in cytoskeletal polarization and cytokinesis	+
ZDS2	Protein with effects on cell polarity and transcriptional silencing, homolog of Zds1	+
RGA1	Rho-type GAP for Cdc42	−
BEM3	Rho-type GAP for Cdc42	−
SSD1	mRNA binding protein; plays a role in maintenance of cellular integrity	+

[a] Encodes a constitutively activated version of Bck1 (Lee and Levin 1992).

activated allele of *BCK1*, *BCK1-20*, could suppress the temperature-sensitive phenotype of *tif51A-1*. Unlike *PKC1*, none of the MAP kinase genes was able to suppress the *tif51A-1* growth defect at the nonpermissive temperature of 37° (Figure 1A). However, as a control, the activated allele *BCK1-20* successfully overcame the defect of a *PKC1* temperature-sensitive mutant, *stt1* (Yoshida *et al.* 1994; Figure 1B). This result suggests that, although *BCK1-20* can suppress the temperature-sensitive phenotype of the *pkc1* mutant (*stt1*), activation of the cell integrity MAP kinase pathway is not sufficient to overcome the *tif51A-1* growth phenotype. In contrast to the results with genes that function downstream of Pkc1, high-copy plasmids containing genes for known and putative upstream activators of Pkc1 (including *WSC1-3*, *MID2*, *MTL1*, *ROM2*, and *RHO2*) do suppress the *tif51A-1* mutant phenotype (Hohmann 2002; Table 3). Taken together, these results strongly suggest that Pkc1 does not act through its downstream MAP kinase cascade to promote suppression of the *tif51A-1* temperature-sensitive phenotype.

New suppressors of a temperature-sensitive mutant of eIF5A suggest a novel pathway linking Pkc1 to cell polarity: Since enhanced activity of cell integrity MAP kinase cascade members failed to suppress the temperature-sensitive phenotype of the *tif51A-1* mutant, we screened for new suppressors that could help us to understand the functional relationship between Pkc1 and eIF5A. Two additional high-copy suppressors were identified: *GIC1* and *ZDS1* (Figure 2A).

The mutant used in the screen above is one of the four yeast eIF5A mutants described so far: *ts1159* (Zuk and Jacobson 1998), *tif51A-1*, *tif51A-2*, and *tif51A-3* (Valentini *et al.* 2002). To analyze whether *PKC1*, *ZDS1*, and *GIC1* suppression is allele specific, we tested the temperature sensitivity of eIF5A mutants other than

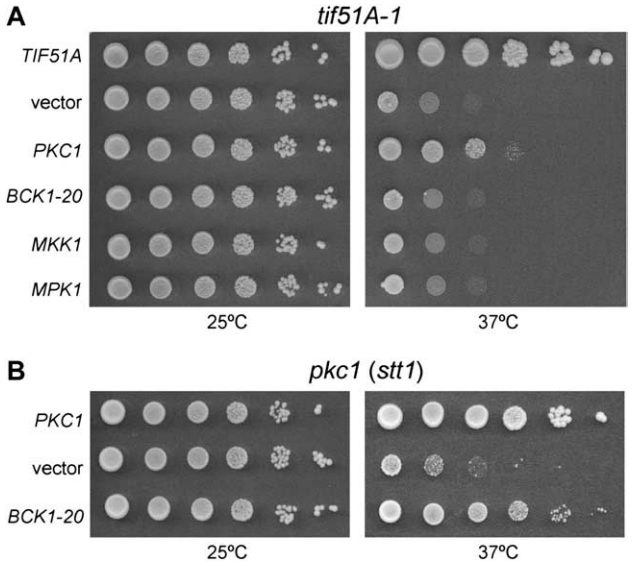

Figure 1.—*PKC1* suppression of the *tif51A-1* mutant is independent of its downstream MAP kinase effectors. (A) Tenfold serial dilutions of early saturated *tif51A-1 cells* (SVL14) harboring pSV107 (*TIF51A*), pRS426 (vector), pSV181 (*PKC1*), pSV294 (*BCK1-20*), pSV295 (*MKK1*), or pSV296 (*MPK1*) were plated onto SC plates to determine growth at permissive (25°) or nonpermissive (37°) temperatures. (B) *PKC1* temperature-sensitive mutant cells (SVL170) harboring pSV181 (*PKC1*), pRS426 (vector), or pSV294 (*BCK1-20*) were assayed as described in A. The plates were photographed after 3–4 days of growth.

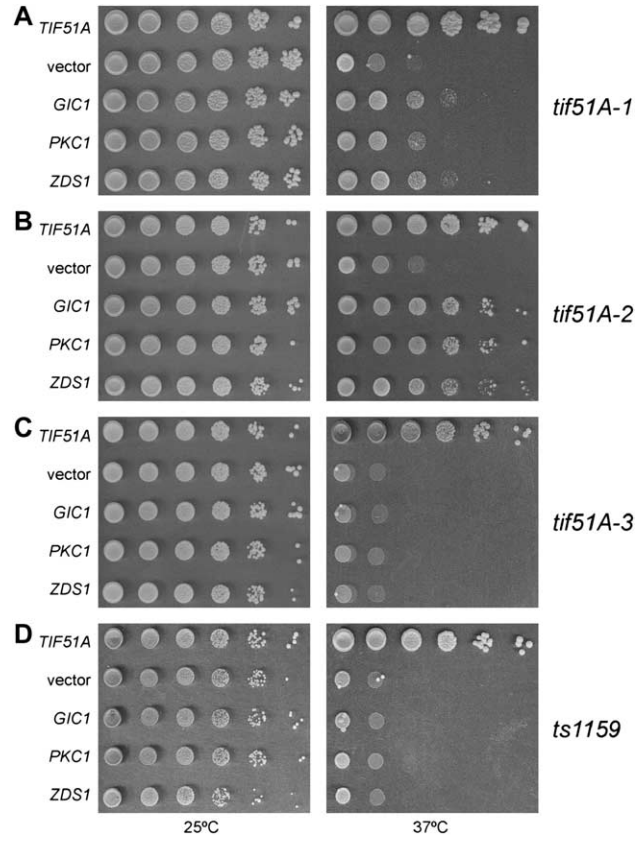

Figure 2.—High-copy suppressors of eIF5A mutants. Dilutions of the *tif51A-1* (A, SVL14), *tif51A-2* (B, SVL26), *tif51A-3* (C, SVL32), and *ts1159* strains (D, SVL248) bearing the plasmids pSV107 (*TIF51A*), pRS426 (vector), pSV304 (*GIC1*), pSV181 (*PKC1*), and pSV589 (*ZDS1*) were grown for 3–4 days on SC plates at 25° and 37°.

tif51A-1 in the presence of the suppressors (Figure 2, B–D). As observed, only *tif51A-2* is also suppressed by *PKC1*, *ZDS1*, and *GIC1*, while no growth improvement is conferred to the alleles *ts1159* and *tif51A-3*. Interestingly, *tif51A-1* and *tif51A-2* mutants contain amino acid substitutions in the same residue (P83S and P83L, respectively). On the other hand, the mutants that were not suppressed harbor amino acid changes in different points of eIF5A: *ts1159* (S149P) and *tif51A-3* (C39Y and G118D). Therefore, this allele-specific suppression may reflect the presence of similar defects in *tif51A-1* and *tif51A-2* mutants that can be bypassed by *PKC1*, *ZDS1*, and *GIC1*. However, as mutants *ts1159* and *tif51A-3* are much more sick than the others (data not shown; Valentini *et al.* 2002), the lack of suppression by these genes may not reflect the occurrence of completely different phenotypes between *ts1159*/*tif51A-3* and *tif51A-1*/*tif51A-2*, but rather may be due to a broader range of defects in the former mutants. In agreement with this hypothesis is the fact that *PKC1*, *ZDS1*, and *GIC1* are not able to suppress *tif51A*Δ, or *tif51A-1* and *tif51A-2* at higher temperatures (data not shown), demonstrating that these suppressors can only partially correct the defects of the mutants analyzed.

Gic1 is an effector of Cdc42 that is important for bud emergence and contains a Cdc42/Rac-interactive-binding (CRIB) domain, which mediates interaction with GTP-bound Cdc42 (Brown *et al.* 1997). Gic1, together with its homolog Gic2, seems to exert its function during the G1/S cell cycle transition by linking the major polarization organizer Cdc42 and the formin Bni1, which is responsible for induction of actin polymerization (Pruyne *et al.* 2004). Zds1 has also been implicated in cell cycle progression but with a less well-defined function (Ma *et al.* 1996). *ZDS1* and *ZDS2*, its functionally redundant homolog, have been isolated in a series of high-copy suppressor screens (Schwer and Shuman 1996; Tsuchiya *et al.* 1996; Bourbonnais *et al.* 2001; Sekiya-Kawasaki *et al.* 2002). Interestingly, high levels of Zds1 can decrease Cdc42 activity (Bi and Pringle 1996) and high-throughput two-hybrid data have shown physical interactions among Pkc1, Zds2, Zds1, and Gic1 (Drees *et al.* 2001).

To further investigate the relationship among Pkc1, Zds1, and Gic1, we tested whether Zds function is important for the suppression mediated by high-copy *PKC1* or *GIC1*. To address this question, a *tif51A-1* strain lacking both *ZDS1* and *ZDS2* was generated (SVL411), and the ability of *PKC1* or *GIC1* to suppress the temperature-sensitive growth phenotype of this mutant was examined (Figure 3). To avoid possible influences of the

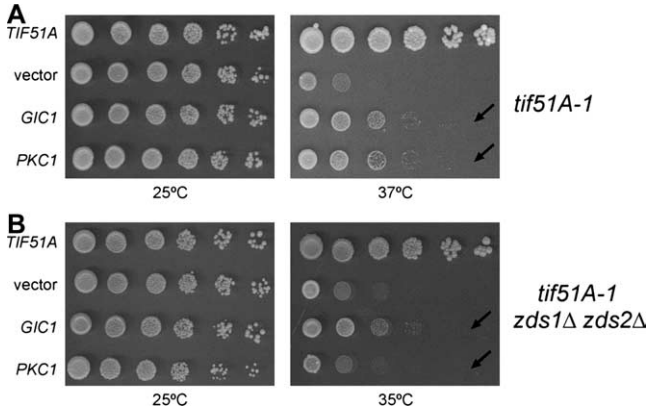

FIGURE 3.—Suppression of the *tif51A-1* mutant by *PKC1* is dependent on the *ZDS1* and *ZDS2* genes. Dilutions of the *tif51A-1* (A, SVL412) and *tif51A-1 zds1*Δ *zds2*Δ (B, SVL411) strains harboring plasmids pSV107 (*TIF51A*), pRS426 (vector), pSV304 (*GIC1*), or pSV181 (*PKC1*) were grown for 3–4 days on SC plates at 25°, 35°, or 37°.

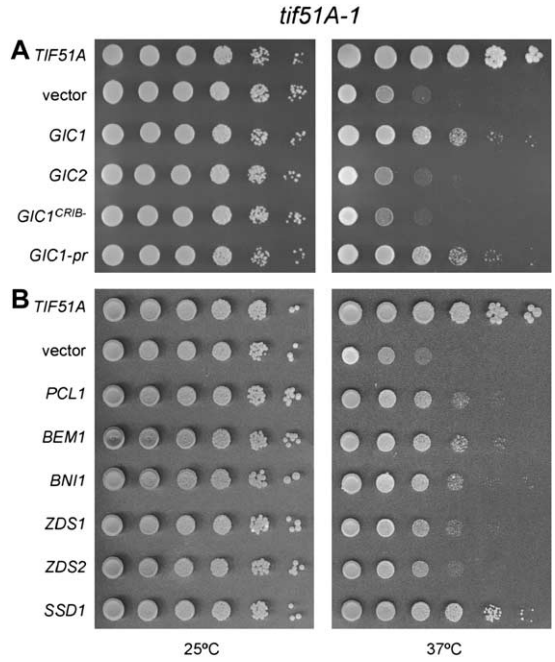

FIGURE 4.—Suppression of the *tif51A-1* mutant requires different factors related to cell cycle progression. Serial dilutions of *tif51A-1* cells (SVL14) carrying the indicated genes on *LEU2* (A) or *URA3* (B) plasmids were spotted on SC plates and incubated for 3–5 days at 25° and 37°.

genetic background on *PKC1* suppression, this assay was performed in parallel with the strain SVL412, obtained from the same tetrad as SVL411, but containing only the *tif51A-1* mutation. Interestingly, *PKC1* could not suppress *tif51A-1* in the absence of *ZDS1* and *ZDS2*, while *GIC1* could (Figure 3, A and B, compare rows indicated by arrows). Also, the *tif51A-1 zds1*Δ *zds2*Δ strain demonstrates enhanced temperature sensitivity, lowering from 37° to 35° the restrictive temperatures of the triple mutant (Figure 3, A and B, right). This synthetic sickness between these genes strengthens their functional connection. These data, together with the previous physical interactions described (DREES *et al.* 2001), strongly support a model in which Pkc1 acts through a downstream pathway different from the MAP kinase cascade. Furthermore, these data suggest that both Zds1 and Gic1 participate in this signaling pathway. Thus, as all these factors act in the same pathway to promote eIF5A mutant suppression, it is possible that eIF5A plays a role in cell polarity.

The eIF5A mutant *tif51A-1* genetically interacts with G1/S transition factors that are involved with cell polarity and important for proper actin organization: To better understand the function of eIF5A, other genes functionally related to those suppressors identified in the screen were tested to determine whether they could also suppress the growth defect of the *tif51A-1* mutant (Table 3). As mentioned before, for those genes related to the cell integrity pathway, only members proposed to act upstream of Pkc1 (Wsc1-3, Mid2, Mtl1, Rom2, and Rho2) (HOHMANN 2002) were able to suppress the temperature-sensitive phenotype of the *tif51A-1* mutant. In addition to the role of Pkc1 in the cell integrity pathway, this kinase is also genetically linked to G1 cyclins via an interaction network (LENBURG and O'SHEA 2001). Thus, we tested whether overexpression of the G1 cyclins *PCL1*, *PCL2*, and *CLN2* would suppress the *tif51A-1* mutant. Notably, only Pcl1 could suppress the *tif51A-1* mutant (Figure 4B; Table 3).

Then, genes functionally linked to Gic1 were tested for suppression of the *tif51A-1* mutant. Initially, the *GIC1* homolog, *GIC2*, and the gene encoding the Gic1 recruiter, *CDC42*, were tested. Unexpectedly, neither one was able to suppress the *tif51A-1* mutant phenotype, suggesting that the suppression is dependent on a Gic1-specific function (Figure 4A; Table 3). Genes encoding other effectors of Cdc42, *CLA4* and *STE20*, and the Cdc42 activator, *CDC24*, also could not suppress the *tif51A-1* phenotype (Table 3). These findings reinforce the hypothesis that a Gic1-specific function is required for *tif51A-1* suppression.

To determine whether the interaction between Gic1 and Cdc42 is required for *GIC1*-mediated suppression of *tif51A-1*, it was tested if $GIC1^{CRIB-}$, an allele known to disrupt protein-protein interactions with Cdc42 (BROWN *et al.* 1997), can suppress the *tif51A-1* mutant. As shown in Figure 4A, *GIC1* lacking the CRIB domain could not suppress *tif51A-1*, suggesting that although *CDC42* does not suppress by itself, a Gic1-Cdc42 physical interaction may be necessary for high-copy *GIC1* suppression of the *tif51A-1* temperature-sensitive phenotype.

In addition to a Gic1 function in actin polarization, a high-copy suppressor screen with *let1*Δ, a mitotic exit network component mutant, has recently implicated Gic1 in this process. This new role for Gic1 is separable

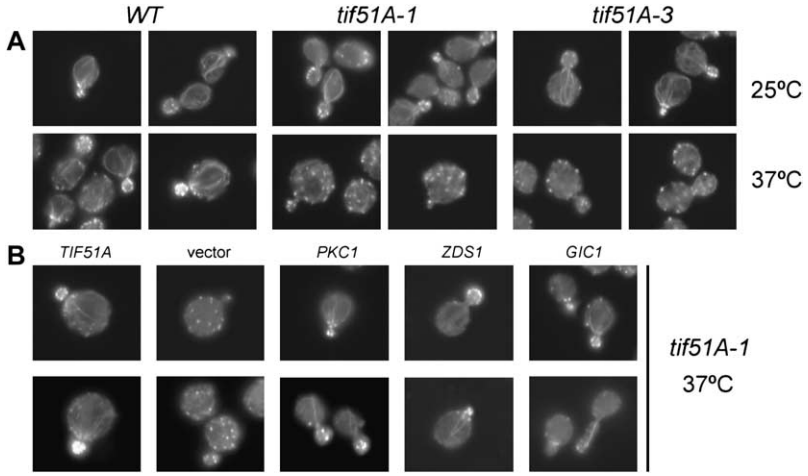

FIGURE 5.—The actin cytoskeleton defect of the *tif51A-1* mutant is also suppressed by the temperature-sensitive phenotype suppressors *PKC1*, *ZDS1*, and *GIC1*. (A) Wild-type strain SVL82 (W303) and *tif51A-1* and *tif51A-3* mutant (SVL14 and SVL32) cells were grown at 25° and then shifted to 37° for 4 hr. Actin was stained with rhodamin-conjugated phalloidin and visualized by fluorescence microscopy. (B) *tif51A-1* cells (SVL14) bearing pSV107 (*TIF51A*), pRS426 (vector), pSV181 (*PKC1*), pSV589 (*ZDS1*), and pSV304 (*GIC1*) were analyzed as described in A.

from that in the G1/S transition, since the function in mitotic exit requires a pool of Gic1 not associated with the cell cortex, as a prenylated form of Gic1 (Gic1-pr) cannot work in the mitotic exit (HÖFKEN and SCHIEBEL 2004). To determine which Gic1 function is necessary to suppress the *tif51A-1* mutant phenotype, we tested the cortex-restricted Gic1 for suppression of *tif51A-1*. As shown in Figure 4A, this form of Gic1 promotes growth of the *tif51A-1* mutant at the nonpermissive temperature. This result supports the model that the G1/S transition function of Gic1 is responsible for the *tif51A-1* mutant suppression.

The last set of genes functionally linked to *GIC1* includes *BEM2*, *BEM1*, *BNI1*, and *BUD1*. These genes are involved with polarized cell growth and show genetic defects with *gic1*Δ *gic2*Δ (CHEN *et al.* 1997; JAQUENOUD and PETER 2000; KAWASAKI *et al.* 2003). We tested whether any of these genes could suppress the phenotype of the *tif51A-1* mutant. This analysis revealed that both *BEM1* and *BNI1* are high-copy suppressors of *tif51A-1* (Figure 4B; Table 3). These results show that other factors important for G1/S transition are also able to suppress the *tif51A-1* mutant.

Finally, we tested whether the following *ZDS1*-correlated genes could suppress *tif51A-1*: *ZDS2*, its homolog; *RGA1* and *BEM3*, encoding negative regulators of Cdc42; and *SSD1*, a gene of unknown function that suppresses defects in the cell integrity pathway and has its null mutant suppressed by *ZDS1* (TSUCHIYA *et al.* 1996; KAEBERLEIN and GUARENTE 2002). Among these genes, only *ZDS2* and *SSD1* suppress the *tif51A*-1 phenotype (Figure 4B; Table 3). These results show that *ZDS2* shares the *ZDS1* function necessary for *tif51A-1* mutant suppression. The fact that presence of *SSD1* can greatly compensate for loss of eIF5A function is considered later (see DISCUSSION). The observation that neither *RGA1* nor *BEM3* was identified as suppressors suggests that the Zds1 function important for suppression is, most probably, not related to its role in negative regulation of Cdc42 (BI and PRINGLE 1996).

Taken together, the results point to a G1/S defect in the *tif51A-1* mutant as most of the suppressors described above act in cell polarity during cell cycle progression. An important event in G1/S transition in *S. cerevisiae* is the establishment of an axis of polarity. Immediately after the positioning of the Cdc42-related factors at the bud site, the assembly of a polarized actin cytoskeleton is crucial for progression of the cell cycle (PRUYNE *et al.* 2004). Therefore, we tested whether two mutants of *TIF51A*, *tif51A-1* and *tif51A-3*, exibit any actin polarization defect. Interestingly, both mutants showed marked defects in actin cytoskeleton organization in budding cells at the nonpermissive temperature. In contrast, actin cables and patches appeared normal during growth at the permissive temperature (Figure 5A).

To test if *PKC1* and the newly identified suppressors, *GIC1* and *ZDS1*, can suppress the actin organization defect as well as the growth phenotype of the *tif51A-1* mutant at the nonpermissive temperature, the actin cytoskeleton organization of this *tif51A-1* mutant in the presence of high-copy *PKC1*, *GIC1*, and *ZDS1* was analyzed (Figure 5B). The results demonstrate that suppression of the temperature-sensitive growth phenotype is correlated with a suppression of the actin organization defect at the restrictive temperature and suggest that eIF5A plays a role in the establishment of cell polarity.

***GIC1* overexpression can cause cell hyperpolarization:** While analyzing the effect of overexpression of *GIC1* on the actin cytoskeleton defect of the *tif51A-1* mutant, it was noted that ~10–20% of cells exhibited elongated buds with a hyperpolarized actin cytoskeleton (Figure 5B, *GIC1*, bottom). To investigate if this hyperpolarization was correlated with the *tif51A-1* mutant suppression by *GIC1*, the morphology of cells growing on a plate at the restrictive temperature was inspected. We observed that both elongated and normal morphologies were present, but elongated ones occurred at a much lower number, apparently at the same frequency as that seen during actin cytoskeleton observation (data

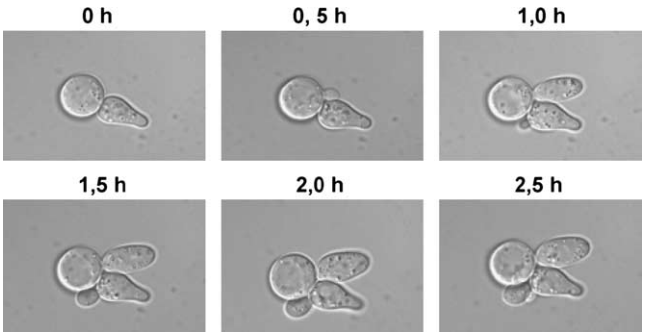

FIGURE 6.—Expression of *GIC1* from a high-copy plasmid generates elongated viable cells at low frequency. Time-lapse images of *tif51A-1* cells (SVL14) containing pSV304 (*GIC1*) are shown. An aliquot of midlog phase cells growing in SC liquid medium was collected and monitored by time-lapse microscopy after immobilizing cells on SC pads. Cells were photographed at 30-min intervals for 150 min at room temperature.

not shown). Furthermore, to check if cells acquiring elongated morphology are able to successfully progress through the cell cycle and thus are not sick or dead, time-lapse microscopy was performed. This analysis revealed no apparent defect in growth and, moreover, the generation of new buds was also detected (Figure 6). These data imply that actin hyperpolarization is not necessary for *GIC1* suppression and that the resulting elongated cells can properly progress through the cell cycle.

***GIC1* and *BNI1* can bypass the growth defect of a *PKC1* temperature-sensitive mutant:** The results presented above suggest a pathway branching from Pkc1 to Gic1. Thus, we hypothesized that increased levels of Gic1 would suppress a *PKC1* mutant. To test this hypothesis, the ability of high-copy *GIC1*, as well as of the downstream acting formin *BNI1*, to suppress the temperature-sensitive phenotype of the *PKC1* mutant *stt1* was evaluated (Figure 7). In agreement with our hypothesis, high-copy *GIC1* and *BNI1* could improve the *PKC1* mutant (*stt1*) growth at the restrictive temperature. This finding further supports the existence of a novel Gic1 pathway downstream of Pkc1.

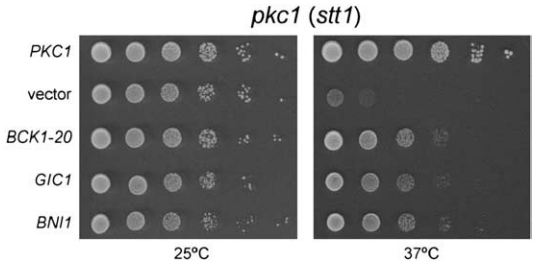

FIGURE 7.—High-copy *GIC1* and *BNI1* suppress the temperature-sensitive phenotype of a *pkc1* (*stt1*) mutant. Tenfold dilutions of the *pkc1* (*stt1*) strain (SVL170) harboring plasmids pSV181 (*PKC1*), pRS426 (vector), pSV294 (*BCK1-20*), or pSV304 (*GIC1*) were grown for 3–4 days on SC plates at 25° and 37°.

DISCUSSION

***PKC1* suppression of the eIF5A mutant is MAP kinase independent:** In this work, we have analyzed the mechanism that underlies the high-copy *PKC1* suppression of the temperature-sensitive eIF5A mutant *tif51A-1*. Although *PKC1* and some of its putative upstream activators show genetic interactions with the *tif51A-1* mutant, our genetic analysis indicates that the downstream MAP kinase cascade members of the cell integrity pathway are not able to suppress the *tif51A-1* mutant, suggesting that a less well-characterized different branch of Pkc1 signaling is involved in this suppression. It has been proposed that Pkc1 has other downstream effectors, as the *pkc1* null mutant phenotype is more severe than those associated with the lack of the genes encoding the downstream components of the MAP kinase cascade (HEINISCH *et al.* 1999). In fact, several cellular mechanisms in which Pkc1 plays a role are MAP kinase independent (KETELA *et al.* 1999; ANDREWS and STARK 2000; LI *et al.* 2000; NANDURI and TARTAKOFF 2001; CHAI *et al.* 2002; VALDIVIA and SCHEKMAN 2003; VILELLA *et al.* 2005).

Moreover, consistent with the suppression data, although eIF5A mutants show enhanced growth on media containing 1 M sorbitol (VALENTINI *et al.* 2002), they do not demonstrate other phenotypes associated with defects in cell integrity of MAP kinase mutants such as cell lysis at the restrictive temperature and sensitivity to caffeine, staurosporine (a specific inhibitor of PKC isozymes), and calcofluor white (HEINISCH *et al.* 1999) (data not shown). Thus, considering the fact that *PKC1* suppression is MAP kinase independent, we searched for new suppressors of the eIF5A mutant *tif51A-1* that could unveil the effectors downstream of Pkc1 that are rescuing the growth impairment of this eIF5A mutant.

A novel pathway links Pkc1 to the Cdc42 effector Gic1: In addition to the well-known *tif51A-1* mutant suppressor *PKC1*, we show here that *ZDS1* and *GIC1* are also suppressors of *tif51A-1*. As the proteins encoded by these three genes participate in a network of physical interactions (DREES *et al.* 2001), we tested the hypothesis that Pkc1-Zds1-Gic1 constitutes a new signaling pathway, illustrated in the model in Figure 8. In agreement with this model, *PKC1* suppression is abolished in *tif51A-1* cells lacking the redundant genes *ZDS1* and *ZDS2*. In contrast, high-copy *GIC1* still suppresses the *tif51A-1 zds1*Δ *zds2*Δ triple mutant. Considering that Pkc1 acts in response to Rho1 (HEINISCH *et al.* 1999), this is the first study demonstrating functional data linking Rho1- and Cdc42-regulated pathways through Zds1 and Zds2.

In addition to the fact that the Zds proteins physically interact with factors important for polarized growth, septin organization, and cytokinesis (DREES *et al.* 2001),

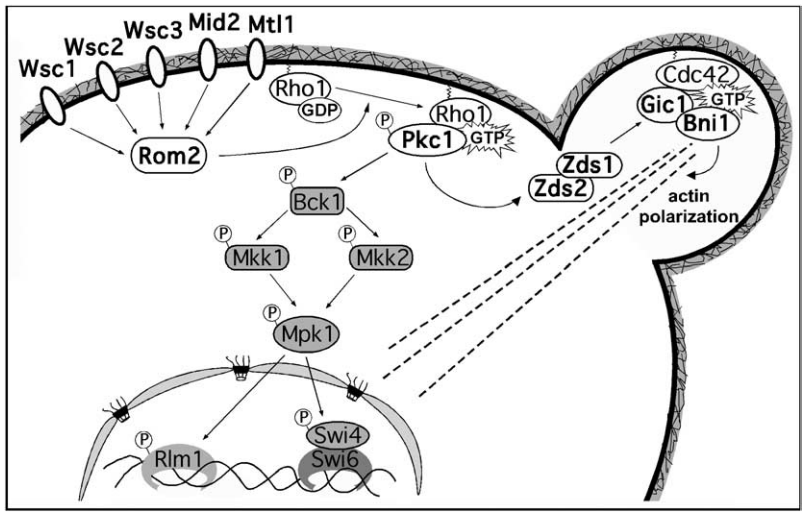

FIGURE 8.—Schematic of pathways acting downstream of Pkc1. In addition to the well-known MAP kinase cascade, we propose a link between Pkc1 and Gic1 via Zds1 and Zds2. This novel pathway connects Pkc1 directly to cell polarity factors and appears to be responsible for high-copy suppression of the eIF5A mutant *tif51A-1*.

ZDS1 and *ZDS2* genetically interact with *CDC42* and *SWE1*, important factors regulating cell cycle progression and morphogenesis (BI and PRINGLE 1996; MA *et al.* 1996). Thus, Zds proteins may act as an integration point for distinct signaling pathways that helps maintain a balance among different signals. This model could explain why Zds1 downregulates both Pkc1-MAP kinase and Cdc42 activity (BI and PRINGLE 1996; GRIFFIOEN *et al.* 2003). In fact, high-copy *ZDS1* does not suppress the temperature-sensitive phenotype of the *PKC1* mutant *stt1* (data not shown), as high-copy *GIC1* does (discussed below). However, although overexpression of *ZDS1* causes a cell integrity defect at elevated temperatures (GRIFFIOEN *et al.* 2003), high-copy *PKC1* is not toxic to *zds1*Δ *zds2*Δ cells at any temperature, and it does not exacerbate even the cold sensitivity of this double mutant (data not shown). Therefore, the inability of *PKC1* to act as a suppressor in the strain *tif51A-1 zds1*Δ *zds2*Δ is not likely the result of Pkc1 overactivation and toxicity. Thus, Zds1 may exert Pkc1-MAP kinase negative feedback and also act as a downstream member of a pathway leading to the Cdc42 effector Gic1.

Furthermore, the influence of Zds1 and Zds2 over Cdc42 may not occur only as negative regulation. Two pieces of data support this idea: (1) high-copy *ZDS1* is able to induce actin polarization in a *tif51A-1* mutant at the restrictive temperature; and (2) decreasing Cdc42 activity through overexpression of its known GTPase-activating proteins (GAPs) Rga1 and Bem3 does not suppress the *tif51A-1* mutant, suggesting that Zds1 is acting to promote suppression through a mechanism that does not involve Cdc42 inhibition.

It is important to note that high-copy *CDC42* does not suppress the *tif51A-1* mutant, but its function may be necessary for *GIC1* suppression, since abolishing the Cdc42-Gic1 interaction, via CRIB domain mutations, also abolishes *GIC1* suppression. This indication of the involvement of a specific Gic1 function in *tif51A-1* suppression is reinforced by the fact that no other Cdc42 effectors were identified as suppressors of this eIF5A mutant. Also consistent with this observation, overexpression of Bni1, a formin proposed to act after and in a manner dependent on Gic1 (JAQUENOUD and PETER 2000), also suppresses the *tif51A-1* temperature-sensitive phenotype.

Finally, we demonstrated that high levels of Gic1 and Bni1 can bypass the temperature-sensitive growth defect of the *PKC1* mutant *stt1*, indicating that these factors may function downstream of Pkc1. On the other hand, *ZDS1* does not suppress the *stt1* mutant (data not shown), but this fact could be due to its proposed role in maintaining the balance of different signaling pathways, as mentioned above. Taken together, these data strongly support the existence of the proposed pathway linking Pkc1 to Gic1 through Zds1 and Zds2 (Figure 8) and connecting Pkc1 signaling to polarized growth.

Interestingly, another protein, Tos2, also connects Pkc1 to Cdc42, but via Cdc24 (DREES *et al.* 2001), and, recently, this protein has been shown to have a possible role in anchoring Cdc24 to the plasma membrane (TOENJES *et al.* 2004). Like Zds1 and Zds2, Tos2 contains multiple protein kinase C consensus phosphorylation sites and physically interacts with Pkc1 (DREES *et al.* 2001; TOENJES *et al.* 2004). Moreover, Skg6, which also interacts with both Zds1 and Zds2, shows interesting homology (35% identity, 48% similarity) to Tos2 (DREES *et al.* 2001; TOENJES *et al.* 2004). These protein interactions suggest a connection between Pkc1 and members of cell polarity determination in a common protein-protein network and further support the link between Pkc1 and Gic1 mediated by Zds1 and Zds2 as a novel pathway.

Curiously, here we also characterized *SSD1* as a *tif51A-1* suppressor in a low-copy plasmid. *SSD1* is a polymorphic gene that encodes a protein that may or may not be functional, depending on the allele present in the

genetic background of the yeast strain considered (Stettler *et al.* 1993). Although its specific role has not been determined, a functional allele of *SSD1* can suppress different mutants related to cell integrity and also the *gic1*Δ *gic2*Δ double mutant. Futhermore, *ssd1*Δ phenotypes can be suppressed by *ZDS1* (Tsuchiya *et al.* 1996; Chen *et al.* 1997; Kaeberlein and Guarente 2002). Therefore, the isolation of *SSD1* as a *tif51A-1* suppressor strengthens the functional interaction between eIF5A and the Pkc1-Zds1-Gic1 pathway.

eIF5A function is important for actin cytoskeleton organization: The identity of the eIF5A mutant suppressors described herein raised the hypothesis that a defect in establishment of cell polarity occurs in the *tif51A-1* mutant. Subsequent analysis of the *tif51A-1* strain actin cytoskeleton confirmed that this mutant shows defects in actin organization at the restrictive temperature. These data agree with a previous study, in which eIF5A was proposed to be important for translation of a subset of mRNAs involved in the G1/S transition, since depletion of this factor in yeast causes only a minor defect of total translation rate and an increase of enlarged cells with G1 morphology (Kang and Hershey 1994). The question of how eIF5A acts to assure correct polarized growth in *S. cerevisiae* is being investigated currently.

eIF5A is highly conserved throughout evolution, from archeabacteria to mammals, and this may reflect at some level a conservation of function. Therefore, as budding is not a mechanism ubiquitously used for eukaryotes to progress in the cell cycle, it would not be appropriate to propose a direct function for eIF5A in establishment of cell polarity in *S. cerevisiae*. Moreover, as mentioned before, overexpression of Pkc1, Zds1, and Gic1 cannot completely rescue eIF5A mutant defects, demonstrating that actin polarization is not the only function of this essential protein. Thus, it is unwise to assume that these proteins, including eIF5A, act directly in the same pathway. Conversely, eIF5A could control the expression of some factors important for G1/S transition such as the suppressors of the eIF5A mutant. Therefore, future studies involving the factors revealed herein may contribute to the elucidation of the role played by eIF5A toward specific gene expression.

We are grateful to Anita Corbett, Daniel Lew, David Levin, Douglas Johnson, Elmar Schiebel, Enrique Herrero, Erin O'Shea, John Pringle, Kazuma Tanaka, Keith Kozminski, Marc Crouzet, Michael Hall, Pamela Silver, and Yoshiko Kikuchi for strains and plasmids. We also thank Anita Corbett, Anne McBride, and Michelle Harreman for the critical reading of this manuscript. This work was supported by grants to S.R.V. and a Ph.D. fellowship to C.F.Z., both from Fundação de Amparo à Pesquisa do Estado de São Paulo.

LITERATURE CITED

Amberg, D. C., 1998 Three-dimensional imaging of the yeast actin cytoskeleton through the budding cell cycle. Mol. Biol. Cell **9:** 3259–3262.

Andrews, P. D., and M. J. Stark, 2000 Type 1 protein phosphatase is required for maintenance of cell wall integrity, morphogenesis and cell cycle progression in Saccharomyces cerevisiae. J. Cell Sci. **113:** 507–520.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman *et al.*, 2005 *Current Protocols in Molecular Biology*. John Wiley & Sons, New York.

Benne, R., and J. W. Hershey, 1978 The mechanism of action of protein synthesis initiation factors from rabbit reticulocytes. J. Biol. Chem. **253:** 3078–3087.

Bevec, D., H. Jaksche, M. Oft, T. Wohl, M. Himmelspach *et al.*, 1996 Inhibition of HIV-1 replication in lymphocytes by mutants of the Rev cofactor eIF-5A. Science **271:** 1858–1860.

Bevec, D., and J. Hauber, 1997 Eukaryotic initiation factor 5A activity and HIV-1 Rev function. Biol. Signals **6:** 124–133.

Bi, E., and J. R. Pringle, 1996 ZDS1 and ZDS2, genes whose products may regulate Cdc42 in Saccharomyces cerevisiae. Mol. Cell. Biol. **16:** 5264–5275.

Bourbonnais, Y., N. Faucher, D. Pallotta and C. Larouche, 2001 Multiple cellular processes affected by the absence of the Rpb4 subunit of RNA polymerase II contribute to the deficiency in the stress response of the yeast rpb4delta mutant. Mol. Gen. Genet. **264:** 763–772.

Brown, J. L., M. Jaquenoud, M. P. Gulli, J. Chant and M. Peter, 1997 Novel Cdc42-binding proteins Gic1 and Gic2 control cell polarity in yeast. Genes Dev. **11:** 2972–2982.

Caraglia, M., M. Marra, G. Giuberti, A. M. D'Alessandro, A. Budillon *et al.*, 2001 The role of eukaryotic initiation factor 5A in the control of cell proliferation and apoptosis. Amino Acids **20:** 91–104.

Chai, B., J. M. Hsu, J. Du and B. C. Laurent, 2002 Yeast RSC function is required for organization of the cellular cytoskeleton via an alternative PKC1 pathway. Genetics **161:** 575–584.

Chen, G. C, Y. J. Kim and C. S. Chan, 1997 The Cdc42 GTPase-associated proteins Gic1 and Gic2 are required for polarized cell growth in Saccharomyces cerevisiae. Genes Dev. **11:** 2958–2971.

Chen, K. Y., and A. Y. Liu, 1997 Biochemistry and function of hypusine formation on eukaryotic initiation factor 5A. Biol. Signals **6:** 105–109.

Christianson, T. W., R. S. Sikorski, M. Dante, J. H. Shero and P. Hieter, 1992 Multifunctional yeast high-copy-number shuttle vectors. Gene **110:** 119–122.

Connelly, C., and P. Hieter, 1996 Budding yeast SKP1 encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. Cell **86:** 275–285.

de Bettignies, G., D. Thoraval, C. Morel, M. F. Peypouquet and M. Crouzet, 2001 Overactivation of the protein kinase C-signaling pathway suppresses the defects of cells lacking the Rho3/Rho4-GAP Rgd1p in *Saccharomyces cerevisiae*. Genetics **159:** 1435–1448.

Drees, B. L., B. Sundin, E. Brazeau, J. P. Caviston, G. C. Chen *et al.*, 2001 A protein interaction map for cell polarity development. J. Cell Biol. **154:** 549–571.

Gladfelter, A. S., I. Bose, T. R. Zyla, E. S. Bardes and D. J. Lew, 2002 Septin ring assembly involves cycles of GTP loading and hydrolysis by Cdc42p. J. Cell Biol. **156:** 315–326.

Griffioen, G., S. Swinnen and J. M. Thevelein, 2003 Feedback inhibition on cell wall integrity signaling by Zds1 involves Gsk3 phosphorylation of a cAMP-dependent protein kinase regulatory subunit. J. Biol. Chem. **278:** 23460–23471.

Gustin, M. C., J. Albertyn, M. Alexander and K. Davenport, 1998 MAP kinase pathways in the yeast Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. **62:** 1264–1300.

Guthrie, C., and G. R. Fink, 1991 *Guide to Yeast Genetics*. Academic Press, New York.

Heinisch, J. J., A. Lorberg, H. P. Schmitz and J. J. Jacoby, 1999 The protein kinase C-mediated MAP kinase pathway involved in the maintenance of cellular integrity in Saccharomyces cerevisiae. Mol. Microbiol. **32:** 671–680.

Helliwell, S. B., A. Schmidt, Y. Ohya and M. N. Hall, 1998 The Rho1 effector Pkc1, but not Bni1, mediates signalling from Tor2 to the actin cytoskeleton. Curr. Biol. **8:** 1211–1214.

Henderson, B. R., and P. Percipalle, 1997 Interactions between HIV Rev and nuclear import and export factors: the Rev nuclear localisation signal mediates specific binding to human importin-beta. J. Mol. Biol. **274:** 693–707.

Höfken, T., and E. Schiebel, 2004 Novel regulation of mitotic exit by the Cdc42 effectors Gic1 and Gic2. J. Cell Biol. **164:** 219–231.

Hohmann, S., 2002 Osmotic stress signaling and osmoadaptation in yeasts. Microbiol. Mol. Biol. Rev. **66:** 300–372.

Jaquenoud, M., and M. Peter, 2000 Gic2p may link activated Cdc42p to components involved in actin polarization, including Bni1p and Bud6p (Aip3p). Mol. Cell. Biol. **20:** 6244–6258.

Kaeberlein, M., and L. Guarente, 2002 Saccharomyces cerevisiae MPT5 and SSD1 function in parallel pathways to promote cell wall integrity. Genetics **60:** 83–95.

Kang, H. A., and J. W. Hershey, 1994 Effect of initiation factor eIF-5A depletion on protein synthesis and proliferation of Saccharomyces cerevisiae. J. Biol. Chem. **269:** 3934–3940.

Kawasaki, R., K. Fujimura-Kamada, H. Toi, H. Kato and K. Tanaka, 2003 The upstream regulator, Rsr1p, and downstream effectors, Gic1p and Gic2p, of the Cdc42p small GTPase coordinately regulate initiation of budding in Saccharomyces cerevisiae. Genes Cells **8:** 235–250.

Ketela, T., R. Green and H. Bussey, 1999 Saccharomyces cerevisiae Mid2p is a potential cell wall stress sensor and upstream activator of the PKC1–MPK1 cell integrity pathway. J. Bacteriol. **181:** 3330–3340.

Lee, K. S., and D. E. Levin, 1992 Dominant mutations in a gene encoding a putative protein kinase (BCK1) bypass the requirement for a Saccharomyces cerevisiae protein kinase C homolog. Mol. Cell. Biol. **12:** 172–182.

Lenburg, M. E, and E. K. O'Shea, 2001 Genetic evidence for a morphogenetic function of the Saccharomyces cerevisiae Pho85 cyclin-dependent kinase. Genetics **157:** 39–51.

Li, Y., R. D. Moir, I. K. Sethy-Coraci, J. R. Warner and I. M. Willis, 2000 Repression of ribosome and tRNA synthesis in secretion-defective cells is signaled by a novel branch of the cell integrity pathway. Mol. Cell. Biol. **20:** 3843–3851.

Li-En Jao, D., and K. Yu Chen, 2002 Subcellular localization of the hypusine-containing eukaryotic initiation factor 5A by immunofluorescent staining and green fluorescent protein tagging. J. Cell Biochem. **86:** 590–600.

Lipowsky, G., F. R. Bischoff, P. Schwarzmaier, R. Kraft, S. Kostka *et al.*, 2000 Exportin 4: a mediator of a novel nuclear export pathway in higher eukaryotes. EMBO J. **19:** 4362–4371.

Ma, X. J., Q. Lu and M. Grunstein, 1996 A search for proteins that interact genetically with histone H3 and H4 amino termini uncovers novel regulators of the Swe1 kinase in Saccharomyces cerevisiae. Genes Dev. **10:** 1327–1340.

Nanduri, J., and A. M. Tartakoff, 2001 Perturbation of the nucleus: a novel Hog1p-independent, Pkc1p-dependent consequence of hypertonic shock in yeast. Mol. Biol. Cell **12:** 1835–1841.

Park, M. H., Y. B. Lee and A. Joe, 1997 Hypusine is essential for eukaryotic cell proliferation. Biol. Signals **6:** 15–23.

Philip, B., and D. E. Levin, 2001 Wsc1 and Mid2 are cell surface sensors for cell wall integrity signaling that act through Rom2, a guanine nucleotide exchange factor for Rho1. Mol. Cell. Biol. **21:** 271–280.

Pruyne, D., A. Legesse-Miller, L. Gao, Y. Dong and A. Bretscher, 2004 Mechanisms of polarized growth and organelle segregation in yeast. Annu. Rev. Cell Dev. Biol. **20:** 559–591.

Richman, T. J., M. M. Sawyer and D. I. Johnson, 1999 The Cdc42p GTPase is involved in a G2/M morphogenetic checkpoint regulating the apical-isotropic switch and nuclear division in yeast. J. Biol. Chem. **274:** 16861–16870.

Rosenwald, A. G., M. A. Rhodes, H. Van Valkenburgh, V. Palanivel, G. Chapman *et al.*, 2002 ARL1 and membrane traffic in Saccharomyces cerevisiae. Yeast **19:** 1039–1056.

Ruhl, M., M. Himmelspach, G. M. Bahr, F. Hammerschmid, H. Jaksche *et al.*, 1993 Eukaryotic initiation factor 5A is a cellular target of the human immunodeficiency virus type 1 Rev activation domain mediating trans-activation. J. Cell Biol. **123:** 1309–1320.

Schnier, J., H. G. Schwelberger, Z. Smit-McBride, H. A. Kang and J. W. Hershey, 1991 Translation initiation factor 5A and its hypusine modification are essential for cell viability in the yeast Saccharomyces cerevisiae. Mol. Cell. Biol. **11:** 3105–3114.

Schwer, B., and S. Shuman, 1996 Multicopy suppressors of temperature-sensitive mutations of yeast mRNA capping enzyme. Gene Exp. **5:** 331–344.

Sekiya-Kawasaki, M., M. Abe, A. Saka, D. Watanabe, K. Kono *et al.*, 2002 Dissection of upstream regulatory components of the Rho1p effector, 1,3-beta-glucan synthase, in *Saccharomyces cerevisiae*. Genetics **162:** 663–676.

Shi, X. P., K. C. Yin, Z. A. Zimolo, A. M. Stern and L. Waxman, 1996 The subcellular distribution of eukaryotic translation initiation factor, eIF-5A, in cultured cells. Exp. Cell Res. **225:** 348–356.

Shi, X. P., K. C. Yin and L. Waxman, 1997 Effects of inhibitors of RNA and protein synthesis on the subcellular distribution of the eukaryotic translation initiation factor, eIF-5A, and the HIV-1 Rev protein. Biol. Signals **6:** 143–149.

Sikorski, R. S., and P. Hieter, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:** 19–27.

Stettler, S., N. Chiannilkulchai, S. Hermann-Le Denmat, D. Lalo, F. Lacroute *et al.*, 1993 A general suppressor of RNA polymerase I, II and III mutations in Saccharomyces cerevisiae. Mol. Gen. Genet. **239:** 169–176.

Toenjes, K. A., D. Simpson and D. I. Johnson, 2004 Separate membrane targeting and anchoring domains function in the localization of the S. cerevisiae Cdc24p guanine nucleotide exchange factor. Curr. Genet. **45:** 257–264.

Tsuchiya, E., G. Matsuzaki, K. Kurano, T. Fukuchi, A. Tsukao *et al.*, 1996 The Saccharomyces cerevisiae SSD1 gene is involved in the tolerance to high concentration of Ca2+ with the participation of HST1/NRC1/BFR1. Gene **176:** 35–38.

Uesono, Y., A. Fujita, A. Toh-E and Y. Kikuchi, 1994 The MCS1/SSD1/SRK1/SSL1 gene is involved in stable maintenance of the chromosome in yeast. Gene **143:** 135–138.

Valdivia, R. H, and R. Schekman, 2003 The yeasts Rho1p and Pkc1p regulate the transport of chitin synthase III (Chs3p) from internal stores to the plasma membrane. Proc. Natl. Acad. Sci. USA **100:** 10287–10292.

Valentini, S. R., J. M. Casolari, C. C. Oliveira, P. A. Silver and A. McBride, 2002 Genetic interactions of yeast eukaryotic translation initiation factor 5A (eIF5A) reveal connections to poly(A)-binding protein and protein kinase C signaling. Genetics **160:** 393–405.

Vilella, F., E. Herrero, J. Torres and M. A. de la Torre-Ruiz, 2005 Pkc1 and the upstream elements of the cell integrity pathway in Saccharomyces cerevisiae, Rom2 and Mtl1, are required for cellular responses to oxidative stress. J. Biol. Chem. **280:** 9149–9159.

Yoshida, S., Y. Ohya, A. Nakano and Y. Anraku, 1994 Genetic interactions among genes involved in the STT4–PKC1 pathway of Saccharomyces cerevisiae. Mol. Gen. Genet. **242:** 631–640.

Yu, Y., Y. W. Jiang, R. J. Wellinger, K. Carlson, J. M. Roberts *et al.*, 1996 Mutations in the homologous ZDS1 and ZDS2 genes affect cell cycle progression. Mol. Cell. Biol. **16:** 5254–5263.

Zuk, D., and A. Jacobson, 1998 A single amino acid substitution in yeast eIF-5A results in mRNA stabilization. EMBO J. **17:** 2914–2925.

Communicating editor: L. Pillus