



Use of a synthetic lethal screen to identify genes related to *TIF51A* in *Saccharomyces cerevisiae*

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ABSTRACT. The putative eukaryotic translation initiation factor 5A (eIF5A) is an essential protein for cell viability and the only cellular protein known to contain the unusual amino acid residue hypusine. eIF5A has been implicated in translation initiation, cell proliferation, nucleocytoplasmic transport, mRNA decay, and actin polarization, but the precise biological function of this protein is not clear. However, eIF5A was recently shown to be directly involved with the translational machinery. A screen for synthetic lethal mutations was carried out with one of the temperature-sensitive alleles of *TIF51A* (*tif51A-3*) to identify factors that functionally interact with eIF5A and revealed the essential gene *YPT1*. This gene encodes a small GTPase, a member of the rab family involved with secretion, acting in the vesicular trafficking between endoplasmatic reticulum and the Golgi. Thus, the synthetic lethality between *TIF51A* and *YPT1* may reveal the connection between translation and the polarized distribution of membrane components, suggesting that these proteins work together in the cell to guarantee proper protein

synthesis and secretion necessary for correct bud formation during G1/S transition. Future studies will investigate the functional interaction between eIF5A and Ypt1 in order to clarify this involvement of eIF5A with vesicular trafficking.

Key words: eIF5A, Ypt1, Synthetic lethality, Genetic interaction, Vesicular trafficking

INTRODUCTION

The putative eukaryotic translation initiation factor 5A (eIF5A) was isolated from ribosomes of rabbit reticulocyte lysates (Benne and Hershey, 1978). eIF5A was classified as a translation initiation factor because of its stimulatory effect in the methionyl-puromycin assay used to evaluate factors involved in the first peptide bond formation (Benne and Hershey, 1978).

eIF5A is a small, abundant and highly conserved protein, present in all organisms from archeobacteria to mammals but not in eubacteria (Schnier et al., 1991; Chen and Liu, 1997). In *Saccharomyces cerevisiae*, eIF5A is encoded by the essential gene *TIF51A* in aerobic conditions. This protein is post-translationally modified and thus activated by the conversion of a specific lysine into the unusual amino acid residue hypusine (Park et al., 1998).

eIF5A has been implicated in many cellular processes, but the role played by this essential factor remains unclear. Early studies suggested its involvement with initiation of protein synthesis (Benne and Hershey, 1978). However, depletion of eIF5A in yeast showed only a slight decrease in protein synthesis (Kang and Hershey, 1994). Therefore, it was hypothesized that eIF5A may function in the translation of a specific subset of mRNAs. As depletion of eIF5A in yeast also causes an increase of G1 arrested cells, it was proposed that eIF5A may be important for translation of mRNAs encoding proteins required for cell cycle progression (Kang and Hershey, 1994). This involvement of eIF5A with cellular proliferation is corroborated by the observation that blocking any step of hypusine formation in mammalian cells inhibits cell proliferation (Caraglia et al., 2001) and conditional mutants of eIF5A show a defect in actin polymerization essential for G1/S transition in yeast (Zanelli and Valentini, 2005).

eIF5A has also been associated with nucleocytoplasmic export of Rev-dependent HIV-1 transcripts (Ruhl et al., 1993; Bevec et al., 1996; Bevec and Hauber, 1997; Zuk and Jacobson, 1998). However, other studies do not confirm the association of eIF5A with Rev-dependent nuclear export in both mammalian and yeast systems (Shi et al., 1996, 1997; Henderson and Percipalle, 1997; Lipowsky et al., 2000; Jao and Yu Chen, 2002; Valentini et al., 2002). Furthermore, using different conditional mutants, it was suggested that eIF5A is involved with mRNA decay (Zuk and Jacobson, 1998; Valentini et al., 2002), but this role seems to be secondary (Valentini et al., 2002).

More recently, it was demonstrated that eIF5A is associated with the translational machinery and affects protein synthesis in yeast (Zanelli et al., 2006). Although these results do not conclusively define a function for eIF5A, they provide new evidence for its role in translation.

Genetic interactions have contributed to the identification of key relationships that lead to the determination of a protein function in the cell (Guarente, 1993). Synthetic lethality is one of such genetic interactions that can be used to screen genes encoding functionally related proteins through the isolation of mutations which are lethal in combination. This genetic approach has been successfully used in the functional characterization of proteins (Bender and Pringle, 1991; Costigan et al., 1992; Shen et al., 1998; Costa and Arndt, 2000; Care et al., 2004).

In an attempt to find cellular partners for eIF5A, a synthetic lethal screen was performed using the temperature-sensitive mutant *tif51A-3* of eIF5A. In this screen, we revealed that eIF5A is synthetic lethal with the essential gene *YPT1* which encodes for an Ypt-GTPase involved with secretion, acting in the vesicular trafficking between the endoplasmatic reticulum and the Golgi.

Although the interaction between eIF5A and Ypt1 by itself does not further clarify the role played by eIF5A, it suggests that these proteins work together in the cell to allow proper protein synthesis and secretion necessary for bud formation during G1/S transition.

MATERIAL AND METHODS

Yeast strains and manipulation

S. cerevisiae strains used in this study are listed in Table 1. Strains SVL103, SVL104 and SVL114 were initially obtained by crossing the SVL32 with the SVL83 to generate SVL102 which was then crossed with the SVL73. The strain SVL422, containing *YPT1* single mutant was first obtained by crossing the SVL398 and SVL82 strains to generate SVL401 and then SVL401 was crossed with the synthetic lethal mutant strain (SVL338). Procedures for cell growth and genetic manipulations were carried out according to standard protocols (Guthrie and Fink, 1991). Transformation of yeast cells with genomic library was performed using a high-efficiency method (Gietz and Woods, 2002).

Table 1. Yeast strains used in the present study.

Strain	Genotype	Source
SVL32	<i>MAT_a ade2 his3 leu2 trp1 ura3 can1 tif51A-3</i>	Valentini et al., 2002
SVL73 (CH1305)	<i>MAT_a ade2 ade3 leu2 lys2 ura3 can1</i>	Kranz and Holm, 1990
SVL82 (W303)	<i>MAT_a ade2 his3 leu2 trp1 ura3 can1</i>	Pamela Silver
SVL83 (W303)	<i>MAT_α ade2 his3 leu2 trp1 ura3 can1</i>	Pamela Silver
SVL102	<i>MAT_α ade2 his3 leu2 trp1 ura3 can1 tif51A-3</i>	Present study
SVL103	<i>MAT_a ade2 ade3 his3 leu2 lys2 ura3 can1 tif51A-3</i>	Present study
SVL104	<i>MAT_α ade2 ade3 leu2 trp1 ura3 can1 tif51A-3</i>	Present study
SVL114	<i>MAT_α ade2 ade3 his3 leu2 trp1 ura3 can1 tif51A-3</i>	Present study
SVL132	<i>MAT_α his3 leu2 ura3 tif51A::HIS3</i> [pSV138]	Valentini et al., 2002
SVL338	<i>MAT_α ade2 his3 leu2 ura3 lys2 tif51A-3 ypt1</i> [pSV99]	Present study
SVL398	<i>MAT_α ade2 his3 leu2 ura3 trp1 YPT1::LEU2</i>	Present study
SVL401	<i>MAT_a ade2 his3 leu2 ura3 trp1 YPT1::LEU2</i>	Present study
SVL422	<i>MAT_α ade2 his3 leu2 ura3 trp1 ypt1</i>	Present study

Plasmids

Plasmids used in this study are listed in Table 2. Plasmids pSV99, pSV146, pSV254, and pSV398 were constructed by inserting a 1.5-kb *Bam*HI fragment carrying *TIF51A* from pSV108 into pSV28, pSV59, pSV75, and pSV347, respectively. Cloning by PCR was performed with *Hifi* DNA polymerase (Invitrogen) following standard molecular biology procedures (Ausubel et al., 2005). *TUB2* and *YPT1* were amplified by PCR using primers SVO189 (5'-TCC CCG CGG TTT CAA CCT GGG CCT AAC AG-3') and SVO190 (5'-TCC CCG CGG TAG AAT ACC ATA AGC ACC GC-3') and SVO193 (5'-GCT CTA GAT GTA GTA GCT GCT ATG TCA-3') and SVO194 (5'-GCT CTA GAG CAA GAG GAC GTG GAA GAA A-3'), respectively. The PCR fragments were cloned into the *Sac*II or *Xba*I site of pSV60, resulting in the plasmids pSV427 (*TUB2*) and pSV428 (*YPT1*), respectively. The *YPT1* PCR fragment was cloned into the *Xba*I site of pSV71 and pSV65, resulting in the plasmids pSV468 and pSV451, respectively.

Table 2. Plasmids used in the present study.

Plasmid	Feature	Source
pSV28	<i>ADE3, URA3, 2μ</i>	Pamela Silver
pSV59 (pRS315)	<i>LEU2, CEN</i>	Sikorski and Hieter, 1989
pSV60 (pRS316)	<i>URA3, CEN</i>	Sikorski and Hieter, 1989
pSV64 (pRS425)	<i>LEU2, 2μ</i>	Christianson et al., 1992
pSV65 (pRS426)	<i>URA3, 2μ</i>	Christianson et al., 1992
pSV71 (pRS305)	<i>LEU2</i>	Sikorski and Hieter, 1989
pSV75	<i>ADE3, LEU2, 2μ</i>	Pamela Silver
pSV99	<i>TIF51A, ADE3, URA3, 2μ</i>	Present study
pSV108	<i>TIF51A, LEU2, 2μ</i>	Valentini et al., 2002
pSV138	<i>TIF51A, URA3, CEN</i>	Valentini et al., 2002
pSV146	<i>TIF51A, LEU2, CEN</i>	Present study
pSV254	<i>TIF51A, ADE3, LEU2, 2μ</i>	Present study
pSV347 (YCp50)	<i>URA3, CEN</i>	Rose et al., 1987
pSV398	<i>TIF51A, URA3, CEN</i>	Present study
pSV427	<i>TUB2, URA3, CEN</i>	Present study
pSV428	<i>YPT1, URA3, CEN</i>	Present study
pSV451	<i>YPT1, URA3, 2μ</i>	Present study
pSV468	<i>YPT1, LEU2</i>	Present study
pSV837	#370-5	Present study
pSV838	#370-21	Present study
pSV839	<i>FETS</i> partial, <i>YFL040W, ACT1, URA3, CEN</i>	Present study
pSV840	<i>YPT1</i> and <i>TUB2</i> partial, <i>URA3, CEN</i>	Present study

In vivo mutagenesis and mutant selection

The mutant strains SVL103, SVL104 and SVL114 carrying a *TIF51A/ADE3/URA3/2μ* plasmid (pSV99) were mutagenized with ethyl methanesulfonate at a concentration high

enough to promote ~50% cell death (modified from Lawrence, 1991). The mutagenized cells were plated on YPD and incubated at 25°C for at least 15 days, in order to distinguish the color of the colonies. Red colonies lacking white sectors (*sec*⁻ phenotype) were selected. The *sec*⁻ phenotype was confirmed three times by growing the candidates on YPD plates. The *sec*⁻ strains were then grown on 5-fluoroorotic acid (5-FOA) plates, in order to confirm the presence of a functional *URA3* gene (Rose et al., 1987). Strains producing red colonies on YPD (*sec*⁻) and showing 5-FOA sensitivity (5-FOA^s) were selected for further study.

Identification of the synthetic lethal genes

Strains harboring a recessive extragenic single mutation had their *TIF51A/ADE3/URA3/2μ* plasmid (pSV99) substituted for a *TIF51A/ADE3/LEU2/2μ* plasmid (pSV254) using a plasmid shuffling technique, and were then transformed with a genomic centromeric library constructed in the YCp50 vector (Rose et al., 1987). Wild-type genes that complemented the plasmid-dependent mutations (*sec*⁺ phenotype) were isolated using low adenine medium, containing 6 mg/L adenine rather than the normal concentration of 10 mg/L (Nigavekar and Cannon, 2002), as high levels of adenine repress purine biosynthesis (Appling, 1999). This condition still supports growth of *ade*⁻ cells and allows pigment formation, facilitating the *sec*⁺ phenotype observation (Appling, 1999). The complementing plasmids were isolated and subjected to DNA sequencing using primers SVO133 (5'-CAC TATC GAC TAC GCG ATC A-3') and SVO134 (5'-ATG CGT CCG GCG TAG A-3').

Sequences of the DNA fragment were used to characterize the cloned yeast genome segment in the *Saccharomyces* Genome Database. Subsequently, the genes present on the library fragment were subcloned or cloned by PCR into an *URA3/CEN* plasmid (pSV60 or pSV347) and used to define the synthetic lethal gene.

RESULTS

Isolation of synthetic lethal mutations in combination with *tif51A-3* mutation

To identify genes that are functionally related to the essential gene *TIF51A*, a synthetic lethal screen was conducted using the temperature-sensitive mutant *tif51A-3*. This mutant produces eIF5A containing the C39Y and G118D mutations (Valentini et al., 2002).

The synthetic lethal screen used is based on a plasmid-dependent assay which promotes changes in yeast colony phenotype (Bender and Pringle, 1991). Mutations in the *ADE2* gene cause yeast cells to accumulate a red pigment derived from the intermediate 5-aminoimidazole ribonucleotide (AIR), which is the substrate of the Ade2 enzyme (5-aminoimidazole ribonucleotide carboxylase). Moreover, mutations in the *ADE3* gene, which encodes an enzyme acting upstream to Ade2, prevent the accumulation of AIR. Thus, *ade2* strains are red, while *ade2 ade3* strains are white (Appling, 1999). The screen uses an *ade2 ade3* strain with a mutation in the gene of interest. This strain carries a plasmid containing a wild-type copy of the gene of interest, the cell viability recovering plasmid, which also contains the color differentiating *ADE3* gene, in addition to a selectable marker, usually *URA3*. At the permissive temperature on YPD, without plasmid selection, this plasmid is lost at a certain frequency per cell division, generating a mixture of colonies with white sectors on a red back-

ground and completely white ones (sec^+ phenotype). These cells are mutagenized and screened for mutants that have become unable to survive in the absence of the plasmid. The mutants now require the recovering plasmid to survive and are recognized as solid red colonies (sec^- phenotype).

Thus, the strains SVL103, SVL104 and SVL114, bearing the mutations *ade2*, *ade3* and *tif51A-3* and containing the wild-type copy of *TIF51A* in an *URA3/ADE3* plasmid (recovering plasmid - pSV99), produce sectoring colonies (sec^+) at 25°C on YPD medium (data not shown). At this temperature, the plasmid is lost as the *TIF51A* gene is not essential for *tif51A-3* cells at the permissive condition. If a new chromosomal mutation is produced on a synthetically lethal gene, the plasmid containing the wild-type copy of *TIF51A* gene would be then required for survival at 25°C, generating red colonies (sec^-) due to pigment accumulation.

These strains were mutagenized with ethyl methanesulfonate. Survivors were selected based on the sec^- phenotype after 15 days of incubation. Figure 1 shows the possible colony phenotypes in this type of screen and some sec^- candidates are indicated. The candidates showing solid red colonies were grown three times on YPD plates to confirm plasmid-dependent growth. Moreover, the candidates were tested for sensitivity to 5-FOA. A number of 3.2×10^5 were screened and 464 sec^- candidates were selected. However, only 54 candidates confirmed the sec^- phenotype and were 5-FOA^S (Table 3).

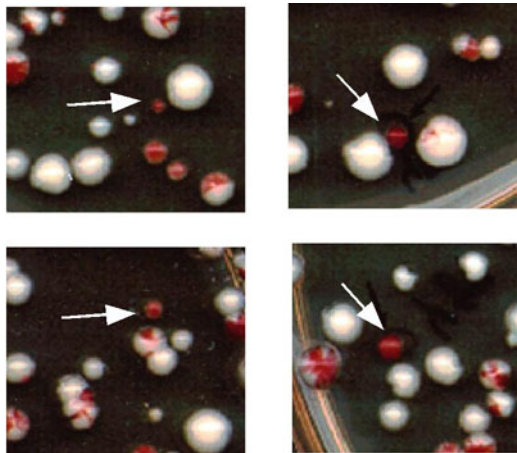


Figure 1. Isolation of synthetic lethal mutant candidates with *tif51A-3*. Ethyl methanesulfonate-treated cells were plated on YPD and incubated at 25°C for 15 days. Synthetic lethal mutant candidates retain the recovering plasmid and grow as solid red nonsectoring (sec^-) colonies as indicated by arrows.

Characterization of synthetic lethal candidates

First, to determine if the pSV99-dependent growth was due to *TIF51A* gene, the 54 $sec^-/5\text{-FOA}^S$ candidates were individually transformed with the plasmid pSV146 (*TIF51A/LEU2/CEN*) or the empty vector pSV59 (*LEU2/CEN*). If the synthetic lethality were specific to wild-type *TIF51A* expression, pSV146 should allow recovery of the sec^+ phenotype and 5-FOA resistance (5-FOA^R). As shown in Table 3 only 15 candidates became $sec^+/5\text{-FOA}^R$ in the presence of *TIF51A* wild-type copy and remained $sec^-/5\text{-FOA}^S$ in the presence of vector alone.

Table 3. Results of genetic screen for synthetic lethal gene using the temperature-sensitive mutant *tif51A-3*.

Strain SVL	Number of clones screened	Sec ⁻	Sec ⁻ 5-FOA ^S (pSV99)	Sec ⁺ 5-FOA ^R (pSV146)	Recessive mutation	Unique mutation	Extragenic mutation
103	1.5 x 10 ⁵	203	18	-	-	-	-
104	1.3 x 10 ⁵	142	18	6	6	4	1
114	4.5 x 10 ⁴	119	18	9	9	7	1
Total	3.2 x 10 ⁵	464	54	15	15	11	2

5-FOA^S, 5-FOA^R = 5-fluoroorotic acid sensitivity and resistance, respectively.

To facilitate the identification of the synthetic lethal gene, which will involve complementation of the sec⁻ phenotype with a yeast genomic centromeric library, candidates with dominant or multiple mutations were eliminated. Initially, the 15 selected candidates were crossed with the parental strain of opposite mating type (SVL32). The diploids were tested for sectoring phenotype on YPD medium. Diploids harboring recessive mutations may lose the recovering *URA3* plasmid (pSV99) due to the presence of the chromosomal wild-type copy of the synthetic lethal gene, changing the sec⁻ phenotype to the sec⁺ one. Diploids with dominant mutations retain the plasmid and maintain the sec⁻ phenotype. All diploids were sec⁺, indicating the presence of recessive mutations in the candidates. These diploids were then sporulated and dissected, and 11 candidates exhibiting segregation 2:2 of the sec⁺ phenotype were then selected.

As the present screen was carried out with an essential gene, additional mutations in the *tif51A-3* mutant could make this allele completely non-functional, causing the recovering plasmid to become essential for cell viability. Thus, it was necessary to distinguish extragenic mutations from intragenic ones. In order to test that, the 11 remaining candidates were crossed with a *tif51A::HIS3* strain (SVL132) bearing a *TIF51A/URA3/CEN* plasmid (pSV138) and the diploids were tested for 5-FOA^S. If the synthetic lethal mutation is extragenic to the functional *tif51A-3* allele, the resulting diploid can lose the plasmid containing wild-type *TIF51A* (pSV138) and become resistant to 5-FOA. Only two 5-FOA^R candidates, bearing extragenic mutations were selected (Table 3). The two synthetic lethal candidates (#1 and #370) were crossed with each other and the diploids were tested for sectoring phenotype on YPD. Due to the sec⁺ phenotype of the diploids, these mutants were categorized into two separate complementation groups, indicating that they possess different extragenic mutations. However, only one mutant (#370) was used so far to identify the synthetic lethal gene.

Identification of the synthetic lethal gene

Using plasmid shuffling, the recovering *TIF51A/ADE3/URA3/2μ* plasmid (pSV99) was replaced by a different recovering plasmid (*TIF51A/ADE3/LEU2/2μ* - pSV254), changing the sec⁻/5-FOA^S phenotype of candidate #370 to sec⁻/5-FOA^R and allowing complementation of the sec⁻ phenotype in the cloning step as the yeast genomic library used was constructed in a *URA3/CEN* vector. After transformation with the genomic library, 25 sec⁺ clones were selected and had their *URA3* plasmids isolated. These plasmids were reintroduced into candidate #370 to

confirm plasmid linkage of sec^+ phenotype. Five clones (#370-5, #370-9, #370-10, #370-18, and #370-21) confirmed the sec^+ phenotype. As shown in Figure 2, two patterns of sec^+ phenotypes were observed: a mixture of white colonies and red colonies with white sectors (#370-5, #370-10 and #370-21) or only white colonies (#370-9 and #370-18). The negative and positive controls used in the cloning step and shown in Figure 2 correspond to the candidate #370 transformed with vector alone or plasmid containing a wild-type copy of *TIF51A* (pSV398), respectively.

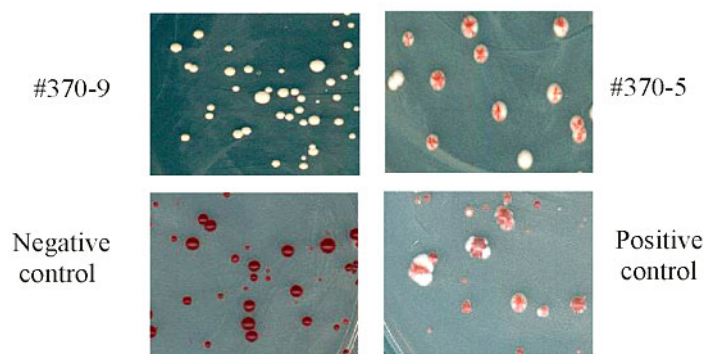


Figure 2. Patterns of sec^+ phenotype revealed by the complementing plasmid. Clone #370-9 developed only white colonies. Clone #370-5 developed a mixture of sectoring and white colonies. The negative and positive controls correspond to the candidate #370 transformed with empty vector (pSV347) or pSV398 (*TIF51A/URA3/CEN*), respectively.

The genomic fragments present in all five library plasmids were determined by DNA sequencing and search in the *Saccharomyces* Genome Database. The *ADE2* gene was revealed in the genomic fragment of clones #370-9 and #370-18. The presence of *ADE2* gene product in these clones causes a complete degradation of AIR, preventing red pigment accumulation and resulting in a pattern of only white colonies (Figure 2). Thus, *ADE2* does not complement the synthetic lethality and can be considered as an artifact of the cloning step. The genomic fragment isolated from clone #370-10 contains, among other genes and ORFs, the *TIF51A* gene. Identification of the wild-type gene was expected and indicated that cloning by phenotypic complementation was working properly. Finally, the two genomic fragments obtained from clones #370-5 (pSV837) and #370-21 (pSV838) revealed overlapping regions of chromosome VI, encompassing the following full length genes/ORF: *YFL040W*, *ACT1*, *YPT1*, and *TUB2*.

In order to identify the gene responsible for the synthetic lethality, the genomic fragment present in the plasmid from clone #370-21 (pSV838) was subcloned using digestion with *Bam*HI (Figure 3A). The *Bam*HI fragment containing *YPT1* and approximately 1 kb of *TUB2* was subcloned into vector YCp50 to generate pSV840 plasmid. The remaining backbone, containing *ACT1* and *YFL040W*, was religated to generate pSV839 plasmid. Both plasmids were introduced into candidate #370 and tested for sec^+ phenotype. As shown in Figure 3B, the plasmid containing *ACT1* and *YFL040W* maintained the sec^- phenotype and was eliminated. The plasmid containing *YPT1* and part of *TUB2*, restored the sec^+ phenotype indicating the presence of the synthetic lethal gene.

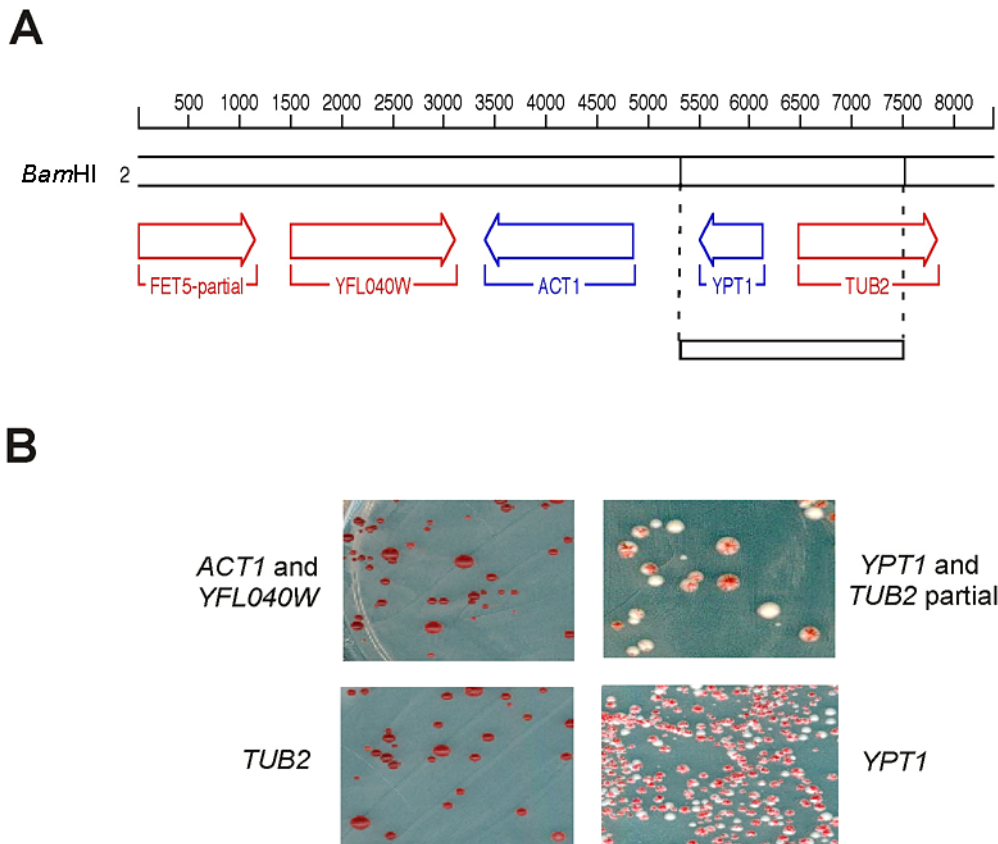


Figure 3. Identification of the synthetic lethal gene with *TIF51A*. **A.** Schematic of the genomic fragment from clone #370 used for subcloning. **B.** The clone #370 was transformed with pSV839 (*ACT1* and *YFL040W*), pSV840 (*YPT1* and partial *TUB2*), pSV427 (*TUB2*) or pSV428 (*YPT1*) and plated on medium lacking uracil.

Finally, to conclusively define the synthetic lethal gene, both *YPT1* and *TUB2* were cloned by PCR and the resulting plasmids pSV427 (*TUB2*) and pSV428 (*YPT1*) were introduced into candidate #370. As shown in Figure 3B, the plasmid containing *TUB2* maintained the *sec*⁻ phenotype, and the *sec*⁺ phenotype was restored by the plasmid containing *YPT1*, revealing the identity of the synthetic lethal gene with *TIF51A*.

Generation of a *YPT1* single mutant strain

To generate a strain containing only the *YPT1* mutation, the synthetic lethal mutant strain (candidate #370, named SVL338) harboring the recovering plasmid (*TIF51A/ADE3/URA3/2μ*) was crossed with a wild-type strain containing the *LEU2* gene integrated next to *YPT1* (SVL401). The resulting diploid was grown on 5-FOA medium to eliminate the recovering plasmid. Before sporulation, the diploid was transformed with pSV451 plasmid (*YPT1/URA3/2μ*), as wild-type *YPT1* gene product is required for spore viability (Segev and Botstein, 1987). The diploid was then sporulated and subjected to tetrad analysis. Figure 4A shows the analysis

of five tetrads on YPD, synthetic complete medium lacking leucine and 5-FOA medium, which allows the determination of the segregation type. In this figure, it is possible to observe parental ditype and tetratype segregations and it is important to point out that the spore containing the *YPT1* mutation alone will only be present in the tetratype segregation. Thus, tetrad number 4 was chosen for further analysis. The spore 4c is nonviable on 5-FOA medium due to the combination of the synthetic lethal mutated alleles of *TIF51A* and *YPT1*. The three viable spores on 5-FOA were streaked on YPD and incubated at permissive (25°C) and nonpermissive (36°C) temperatures to define the strain containing the *tif51A-3* mutation and to test if the *YPT1* mutant alone generated here shows temperature-sensitivity (Figure 4B). As shown in this figure, the strain from spore 4a contains the wild-type copy of *TIF51A* and *YPT1* and the strain from spore 4d contains only the temperature-sensitive allele of *TIF51A* (*tif51A-3*). Finally, the strain from spore 4b contains only the mutated allele of *YPT1*, which has also shown to be a temperature-sensitive phenotype. This mutant will be further characterized and used to understand the genetic interaction between eIF5A and Ypt1.

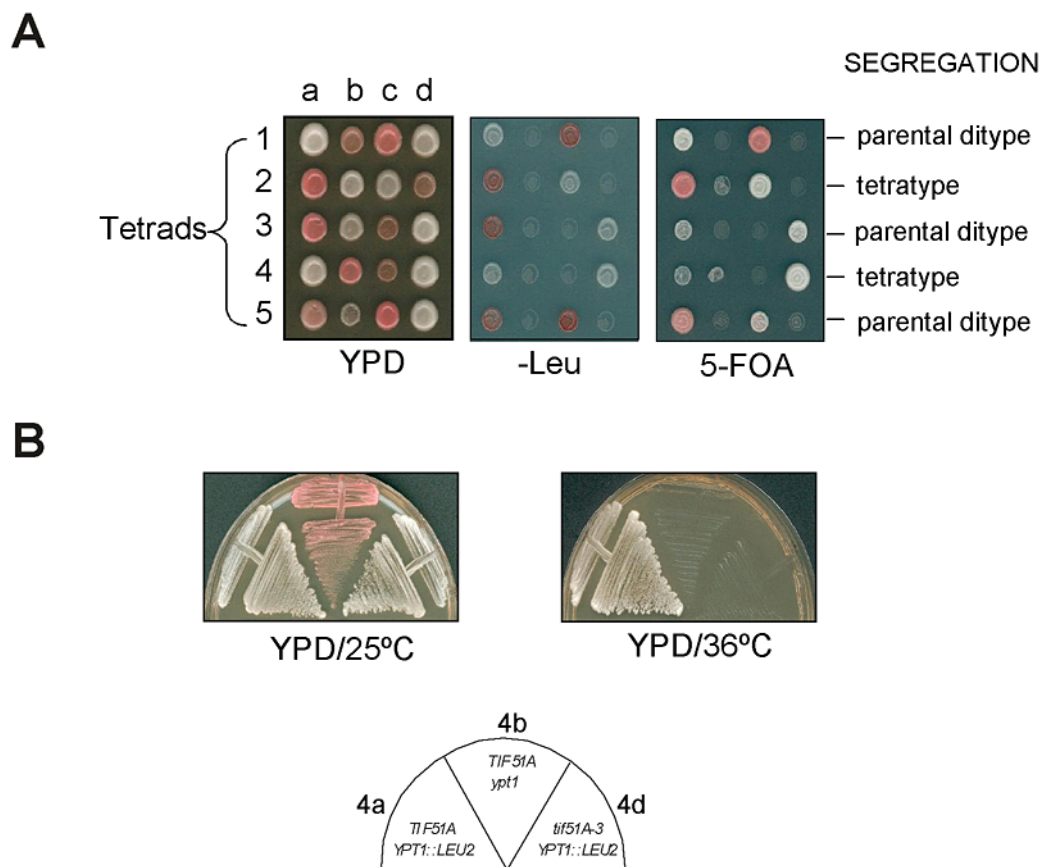


Figure 4. Generation of *YPT1* single mutant strain. **A.** The synthetic lethal strain was crossed to a strain containing the *YPT1* marked allele (*YPT1::LEU2*) and the resulting diploid strain was submitted to tetrad analysis after sporulation. Viable haploid cells from tetrad 4 were selected for further analysis. **B.** Haploid cells were grown on -Leu plates and YPD at 25°C and 36°C to confirm the separation of the *YPT1* mutated allele (4b) from *tif51A-3* allele (4d).

DISCUSSION

eIF5A is a mysterious protein. Although it has been associated with several steps of mRNA metabolism (translation initiation, nucleocytoplasmic transport and mRNA decay), cell proliferation and actin polarization, the function of this protein remains an open question.

The power of genetic strategies relies on the ability to search huge populations for rare events (Appling, 1999). Furthermore, genetic screens can reveal interactions that are unstable or transient *in vitro*. The genetic interaction does not imply a physical association between the two gene products, as regulatory pathway or other indirect interactions may be responsible for an observed genetic relationship (Appling, 1999).

The synthetic lethal screen is an important tool to reveal these genetic interactions. Approximately 20% of genes in the yeast *Saccharomyces cerevisiae* are individually essential, but genetic screens suggest that synthetic lethal interactions are common among the remaining 80% (Kaelin Jr, 2005). Synthetic lethal screens have been used to identify genes involved in cell polarity, secretion, DNA repair, and other numerous cellular processes (Bender and Pringle, 1991; Costigan et al., 1992; Shen et al., 1998; Costa and Arndt, 2000; Care et al., 2004). In yeast, the synthetic lethal screen method is commonly based on a colony color assay that enables the visual identification of plasmid-loss events. The use of a colony color assay for this type of screen has the advantages of simplicity and extreme sensitivity. Furthermore, mutants that have very low viability can still be isolated.

In order to identify cellular partners of eIF5A, a genetic screen for synthetic lethal genes was performed using the temperature-sensitive mutant *tif51A-3*. In this study, 3.2×10^5 clones were screened and 464 candidates were initially selected. The candidates were submitted to the characterization steps to help eliminate false-positives. During this stage, it was confirmed if the synthetic lethality was specific to *TIF51A*. Unique and recessive mutations were selected to facilitate the identification of the synthetic lethal gene by phenotypic complementation with a wild-type yeast genomic library. Moreover, it was necessary to verify if the synthetic lethal mutation was extragenic; this is important for screens using essential genes. From the 464 candidates initially selected, only 2 (#1 and #370) passed through all the characterization steps. Finally, the definition of complementation groups indicates that the mutants were affected in different genes. In this study, we pursued the identification of the plasmid-dependent mutation of candidate #370 (SVL338). The genes/ORF *YFL040W*, *ACT1*, *YPT1*, and *TUB2* were common in both genomic fragments obtained. Finally, further analysis revealed that mutation in *YPT1* is responsible for the synthetic lethality with *tif51A-3*.

The *YPT1* gene (yeast protein transport 1) was identified in 1983 (Gallwitz et al., 1983). This gene is present in chromosome VI and encodes a protein of 206 amino acids that shows a significant homology with Ras proteins from mammals (Segev and Botstein, 1987). The proteins encoded by *YPT* genes are also known as “Ras-like GTPases”. The *YPT* genes have homologs in mammals, termed rab (*ras*-like in *rat brain*) genes, which are involved in vesicular transport and organelle dynamics (Martinez and Goud, 1998).

In the secretory and endocytic pathways of eukaryotic cells, proteins and membranes are transported through a series of compartments. The vesicles bud from a donor compartment and fuse with an acceptor compartment (Segev, 2001). The transported vesicle must specifically recognize the correct target membrane. The products of *YPT* genes are involved in this stage of vesicle transport. The specific function of *YPT1* gene product is related to vesicular traffick-

ing, acting in the fusion of endoplasmic reticulum vesicles to the Golgi apparatus (Clague, 1998).

The finding of *YPT1* gene as synthetic lethal with *TIF51A* indicates an involvement of eIF5A with the secretory pathway. The synthetic lethality can indicate that the genes encode proteins that perform redundant functions in an essential pathway (Appling, 1999). However, both *YPT1* and *TIF51A* are essential by themselves and eIF5A shows no similarity to Ypt1. A synthetic lethal interaction also indicates that each of the gene products could be part of an essential multiprotein complex or that they could work together in an essential linear pathway, which ceases to function when two steps have reduced activity. Finally, there is the possibility that the gene products can participate in parallel pathways that are together essential for cell survival (Appling, 1999).

Recent data have implicated eIF5A in cell integrity and cell polarity (Valentini et al., 2002; Zanelli and Valentini, 2005). It was shown that eIF5A is necessary for establishment of actin polarity, which is essential for bud formation and G1/S transition in *S. cerevisiae* (Zanelli and Valentini, 2005). Cytoskeletal reorganization, secretion and cell wall deposition are all required to produce a new bud. These activities must be orchestrated, and transcriptional as well as post-transcriptional regulatory mechanisms are expected to be important for regulating the different processes involved in polarized cell growth in yeast (Pruyne and Bretscher, 2000). It would not be appropriate to propose a direct function for eIF5A in the establishment of cell polarity in *S. cerevisiae* as eIF5A function has been conserved throughout evolution, but budding is not a mechanism ubiquitously used for eukaryotes to progress in the cell cycle. However, eIF5A was initially characterized as a translation initiation factor (Benne and Hershey, 1978) and the association of this protein with the translation machinery was recently reconsidered (Zanelli et al., 2006) suggesting that eIF5A may have a role in translational control. Thus, the synthetic lethality between *TIF51A* and *YPT1* may reveal the connection between translation and the polarized distribution of membrane components, suggesting that these proteins work together in the cell to guarantee proper protein synthesis and secretion necessary for correct bud formation during G1/S transition. Future studies will be necessary to clarify this genetic interaction and may contribute to the elucidation of eIF5A function in the vesicular trafficking pathway.

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