Mutation of the Rab6 Homologue of Saccharomyces cerevisiae, YPT6, Inhibits Both Early Golgi Function and Ribosome Biosynthesis*

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A screen was designed to identify temperature-sensitive mutants of Saccharomyces cerevisiae, whose transcription of both ribosomal RNA and ribosomal protein genes is repressed at the nonpermissive temperature. The gene from one such mutant was cloned by complementation. The gene encodes a predicted product that is nearly 65% identical to the human GTPase, Rab6, and is likely to be identical to the yeast gene YPT6. It is essential for growth only at elevated temperatures. The mutant strain is partially defective in the maturation of the vacuolar protein carboxypeptidase Y, as well as in the secretion of invertase, which accumulates as a core-glycosylated form characteristic of the endoplasmic reticulum or the cis-Golgi, suggesting that Ypt6p is involved in an early step of the secretory pathway, earlier than that reported for the mammalian Rab6. The mutant protein, a truncation at codon 64 of 215, has a stronger phenotype than the null allele of YPT6. Four other mutants selected for defective ribosome synthesis at the nonpermissive temperature were also found to have defects in carboxypeptidase Y maturation, giving emphasis to our previous finding that a functional secretory pathway is essential for continued ribosome synthesis. Cloning of extragenic suppressors of the ts allele of YPT6 has revealed two additional proteins that influence the secretory pathway: Ssd1p, a suppressor of many mutations, and Imh1p, which bears some homology to the C-terminal portion of the cytoskeletal proteins integrin and myosin.

The doubling of a cell requires the balanced doubling of all components of the cell. Most attention is generally placed on the doubling of the genetic apparatus, with its complex set of controls, based largely on the cyclins and associated kinases, as well as specific checkpoints to ensure accurate transmission of the genomes to the daughter cells.

Less understood but no less important is the doubling of the other components of the cell, among them the variety of different membranes and the complement of ribosomes. Balance must be maintained. In a complex system of parallel reactions, balance can only be ensured if there is cross-talk between the parallel reactions. An example of such cross-talk is apparent in the recent finding that the synthesis of ribosomes is dependent on the continued functioning of the secretory pathway (1, 2).

Inactivation of any tested component of the secretory pathway, from the ER, e.g., Sec63p, to the plasma membrane, e.g., Sec1p, leads to rapid repression of the transcription of both ribosomal RNA and ribosomal protein genes (1). Indeed, the coupling is sufficiently tight that one can distinguish a tight from a leaky sec mutant by their relative effects on the level of mRNA encoding ribosomal proteins. A similar repression occurs when the secretory pathway is inhibited by the drugs tunicamycin or brefeldin A (1).

Although the secretory pathway carries out a variety of functions, a major one is the production of the cell’s membranes, in particular the plasma membrane. We surmise that the observations described above reflect the cell’s attempt to maintain balance between the synthesis of cell membranes and the synthesis of ribosomes. This repression prevents the runaway production of ribosomes and therefore of protein synthesis capacity when the cell membrane system is under stress.

Several of the many proteins that participate in the secretory pathway are small GTPases that function as molecular switches in protein trafficking. They can be divided into two groups, the ARF/Sar family and the Sec4/Ypt/Rab family, which in mammals has at least 30 members (reviewed in Refs. 3 and 4). They are localized at distinct places along the secretory and endocytosis pathways, suggesting a quite general role in protein trafficking. In Saccharomyces cerevisiae, the most studied examples are Sec4p and Ypt1p (3). Ypt1p is required for ER to Golgi transport, apparently to facilitate the attachment and/or fusion of ER-derived vesicles to Golgi membranes (4, 5), and perhaps also in cis-medial Golgi transport (6). Sec4p is involved in transport from the Golgi to the plasma membrane (4, 5). One of the mammalian members of this family is Rab6, which by immunolocalization and functional studies has been implicated both in late intra-Golgi transport (7) and in budding from the TGN (8) (reviewed in Ref. 9).

Our continuing study of the regulation of ribosome synthesis has led us to clone the gene encoding the S. cerevisiae homologue of Rab6, which complements a temperature-sensitive mutant in which ribosome synthesis is repressed at the nonpermissive temperature. (While this paper was in preparation, a new release to GenBank revealed that this gene is identical to YPT6 (accession number X59598, which had been described (8, 10) but whose sequence had not been made public.) Ypt6p is essential only at elevated temperatures. Assays of invertase secretion and carboxypeptidase Y (CPY) maturation suggested that Ypt6p is involved either in ER to Golgi or in cis- to medial Golgi transport, in distinct contrast to its apparent role in mammalian cells. Isolation of second site suppressors implicated two additional proteins, Ssd1p and Imh1p, in the secretory pathway. Four additional mutants identified by the repression of ribosome synthesis were also found to be defective

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1 The abbreviations used are: ER, endoplasmic reticulum; CPY, carboxypeptidase Y; kb, kilobase pair(s); ts, temperature-sensitive.
in CPY maturation, suggesting that they, too, are in genes encoding components of the secretory pathway.

**MATERIALS AND METHODS**

Strains and Plasmids—All the strains used are derivatives of W303 (11). J1003.6A (MATa, leu2-3, 112, ura3-1, can1-100, ade2-1, ssd1-1) is a haploid derivative of W303. 169ts is a temperature-sensitive strain derived by EMS mutagenesis (MATa, leu2-3, 112, ura3-1, can1-100, ade2-1, his3-11, 15, ssd1-1) (12).

Mutagenesis and Mutant Isolation—1006 cells were treated with ethyl-methane sulfonate to an approximate killing ratio of 45%. 517 temperature-sensitive strains were generated that can grow at 23°C but not at 37°C. Northern analysis was applied to screen for the mutants that show repressed transcription of ribosomal protein genes (1, 12). In this report, we focus on 169ts, one of several with this phenotype.

Cloning the Gene by Complementation—169ts cells were transformed with a CEN library carrying a URA3 marker (13) and selected on drop-out Ura plates at 37°C. Screening for cells in which growth at 37°C and on drop-out Ura plates and for integration of the right locus. The positive cells were crossed to wildtype cells. The diploid shifted to 37°C, whereas the rest remained at 23°C. After 90 min cells were harvested and resuspended in 0.1% dextrose. Half of the culture was put at 37°C; half remained at 23°C. After 2 h of growth, 2 units of Act1 gene by Southern analysis.

MATERIALS AND METHODS

**Strain 169ts Is a Temperature-sensitive Mutant in Which Transcription of Ribosomal Protein and Ribosomal RNA Genes Is Repressed at 37°C—169ts is one of several mutants isolated from the screen of a bank of 517 ts mutants for those in which the levels of mRNA encoding ribosomal proteins appeared to be preferentially repressed at the nonpermissive temperature (see Ref. 1 and "Materials and Methods"). The mutant has been back-crossed twice with wild type to confirm the co-segregation of the recessive ts and ribosomal RNA phenotypes. The basis for choosing ts169 is shown in Fig. 1A, in which it is clear that the transcription of ribosomal protein genes is repressed at nonpermissive temperature. Although wild type cells show a transient repression of transcription of ribosomal protein genes that has been well documented (20, 21), mutant cells show a rapid and total loss of ribosomal protein mRNAs. To ask whether rRNA transcription was similarly affected, we carried out pulse labeling studies (Fig. 1B). In wild type cells, one can see the 35 S initial transcript, as well as the 27 and 20 S intermediates and the 25 and 18 S final RNA products. In 169ts cells at the permissive temperature, transcription and processing occur, albeit at a reduced rate. At 37°C, however, the transcription of rRNA is severely repressed, and the few transcripts made are not processed. Based upon the phenotypes described, 169ts appeared likely to be carrying a mutant allele of a gene controlling the formation of ribosomes in S. cerevisiae.

Cloning the Mutant Gene in 169ts—Cloning the mutant gene of 169ts. Genomic DNA was isolated from 169ts and was used as template for polymerase chain reaction (PCR). Oligonucleotides 

**MATERIALS AND METHODS**

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Cloning the Mutant Gene in 169ts—The gene whose muta-
Ypt6p in Early Golgi Function and Ribosome Biosynthesis

Fig. 1. A, Northern analysis showing transcription of ribosomal protein genes is repressed in 169ts and the YPT6 null strain. Cells were grown in YPD at 23°C overnight to log phase. An aliquot was harvested, the rest of the culture shifted to 37°C, and aliquots harvested at 20, 60, and 90 min. Total RNA was isolated, and 10 μg was fractionated on 1.5% agarose-formaldehyde gel (1). RNA derived from ACT1 (Actin) and PYK1 (Pyr. Kin.) was detected using labeled antisense RNA. Labeled oligonucleotides were used to detect RNA derived from ENO1 (Enolase), the four ribosomal protein genes, TCM (encoding L3), L4, L32, and S10, as well as U3 small nuclear RNA. The latter, a stable RNA, was used as a loading control. B, synthesis and processing of rRNA are repressed in 169ts. W303 and 169ts were grown at 23°C overnight to log-phase and a portion of the culture was shifted to 37°C. After 90 min each culture was labeled with 60 μCi/ml [3H]methionine for 2.5 min. RNA was isolated and fractionated on an agarose gel, and the radioactivity was visualized by autoradiography after treatment with RNase A (Dupont NEN). Note that the lane corresponding to the mutant was loaded with about five times as much RNA as the other lanes (1). At the permissive temperature the 35 S primary transcript of the rRNA genes and the 27 and 20 S intermediates are readily seen, and a small amount of mature 25 and 18 S RNA has been formed. At 37°C the wild-type cells process the rRNA more rapidly, but the mutant cells have synthesized almost no rRNA at all.

ribosomal protein mRNAs

TCM

wild type 169ts ΔYpt6p

min after shift
from 23°C to 37°C

0 20 60 90 0 20 60 90 0 20 60 90

L4

S10

Pyr. Kin. Enolase Actin

stable RNAs

U3 snRNA

B

WT 169ts

23 30min 23 30min

26S 27S 28S 18S

26S 27S 28S 18S

Fig. 2. Three different genes support the growth of 169ts at 37°C. W303 transformed with YCp50 and 169ts transformed with YCp50, or the plasmids A, B, or C (see text) were grown to log-phase at 23°C in Ura- medium and then streaked onto Ura- plates and grown at 37°C for 3 days.

Table

| ribosomal protein mRNAs | wild type | 169ts | ΔYpt6p |
|-------------------------|-----------|-------|--------|
| TCM                     |           |       |        |
| L4                      |           |       |        |
| L32                     |           |       |        |
| S10                     |           |       |        |
| Pyr. Kin. Enolase       |           |       |        |
| stable RNAs             |           |       |        |
| U3 snRNA                |           |       |        |

back into 169ts, the growth of the transformed cells and wild type cells were compared at 37°C (Fig. 2). Group A permits 169ts to grow nearly as well as wild type; Group B and especially Group C support the growth of 169ts less well. We assumed that A carried the wild type allele of 169ts mutant gene and that B and C were single-copy suppressors. A 2.5-kb EcorV fragment of group A was found to rescue the growth of 169ts at 37°C (Fig. 3A). This DNA fragment, found on cosmid 8479 from Chromosome XII (accession number U17244) carries only one complete gene. This gene encodes a protein 62.5% identical to the human Rab6 (Fig. 3B) and 68.6% identical to the Schizosaccharomyces pombe Rhyl (22), both implicated in the secretory pathway. It has subsequently become clear that it is identical to the S. cerevisiae gene designated YPT6 (8, 10); to avoid confusion, we have adopted that nomenclature. This gene encodes a protein of 215 amino acids and has the consensus C-terminal sequence, CXC, used for post-translational addition of geranylgeranyl groups (8, 23). The sequence similarity suggests that Ypt6p is involved in the secretory pathway in S. cerevisiae.

To confirm that ypt6 is the mutant gene responsible for the phenotype shown in 169ts, wild type YPT6 was cloned into the URA3 integration vector Yip5. The plasmid was linearized within the YPT6 gene and used to transform competent 169ts cells. Ura+ cells, shown by Southern analysis to have undergone homologous recombination at the YPT6 locus, can grow at 37°C. They were crossed to W303, the diploid cells were dissected, and the spores were grown on YPD plates at 23°C. Among the spores derived from the 12 tetrads checked, none were ts, suggesting that YPT6 is or is very tightly linked to the mutant gene.

Ypt6p Is Essential Only at Elevated Temperatures—To study further the function of Ypt6p in S. cerevisiae, a null YPT6 strain was constructed (see "Methods and Materials"). In a plasmid containing the 4-kb EcoRI/ClaI fragment, a 1-kb HindIII fragment containing the promoter and part of the coding region of YPT6 was replaced by a 1.1-kb fragment carrying the URA3 gene. The DNA fragment containing URA3 was used to transform wild type diploid cells. Homologous recombinants were identified by Southern blotting. The Ura+ diploid cells were sporulated, the asci were dissected, and the spores were grown on YPD plates at 23°C. Although all four spores were viable, the two Ura+ colonies are distinctly smaller. Southern analysis confirmed that the two smaller colonies derive from spores containing the disrupted gene. Like 169ts, the null strain grows well at temperatures up to 34°C, but at 37°C it doubles only once. Based upon these results, we expected 169ts to carry a nonsense mutation in YPT6. The mutant gene was sequenced from a polymerase chain reaction fragment derived from amplification of the ypt6 gene of 169ts using a pair of

conditions is responsible for temperature sensitivity and repression of ribosomal protein synthesis was done using a library based on the CEN plasmid YCp50 (13) to complement the temperature sensitivity of 169ts. From about 40,000 Ura+ transformants, seven were able to grow at 37°C. Using restriction map and Southern analysis (data not shown), they can be divided into groups A, B, and C. After re-transforming these plasmids
oligonucleotides that flank the gene (see “Materials and Methods”). A single mutation was found: a transition at codon 64 of TGG (Trp) to TGA (amber), resulting in a truncated Ypt6p (see Fig. 3B). This result further proves that Ypt6p is essential only at elevated temperatures.

In the Null Strain the Transcription of Ribosomal Protein Genes Is Repressed at High Temperature—Because the YPT6 null strain behaves like 169ts in terms of growth, we tested the transcription of ribosomal protein genes at 23°C and 37°C. The results are shown in Fig. 1A. As expected, the null strain shows repressed transcription of ribosomal protein genes at 37°C, while transcription of nonribosomal protein genes is relatively unaffected. Surprisingly, the repression of transcription at 37°C is not as severe as that in 169ts, suggesting that the truncated Ypt6p has some negative activity.

The Secretion of Invertase Is Impaired in 169ts and the Null Strain—Based upon the sequence homology with Rab6, as well as on our previous observation that continued secretion is essential for ribosome synthesis (1), Ypt6p seemed likely to be involved in protein transport in S. cerevisiae. We therefore asked if 169ts was impaired in secretion. Suc2, encoding invertase, has two transcripts. The minor one is transcribed constitutively and its protein is soluble and stays in the cytoplasm; the major one is repressed by glucose, and its protein is transported to the cell surface along the secretory pathway. In derepressed wild type cells, invertase is mainly outside the cell. In mutants of the secretion pathway, the invertase remains largely within the cell (18, 24).

Mutant and wild type cultures were grown at 23°C to log phase and then transferred to 37°C in derepression medium for 2 h. The external and internal invertase activity were measured (18, 24) (Fig. 4). At the nonpermissive temperature, invertase secretion is reduced to 15% of normal in the mutant strain, suggesting that Ypt6p is indeed involved in the secretory pathway. Invertase secretion is considerably less impaired in the null strain than in 169ts, suggesting again that truncated Ypt6p can have a negative effect on secretion.

Ypt6p Acts at an Early Step of the Secretory Pathway—To confirm the secretion defect and to determine the site at which Ypt6p functions, the glycosylation of CPY was analyzed in 169ts and the null strain. CPY is a vacuolar protein, synthesized as a proenzyme that is core-glycosylated in the ER (form p1), further glycosylated in the Golgi (form p2), and cleaved to mature CPY (form m) by Pep4p in the vacuole (Ref. 25 and Fig. 5). Analysis of the accumulation of isoforms of CPY can suggest the functional location of a mutant protein within the pathway (26). Thus in a pulse-chase experiment (Fig. 5), wild type cells show mostly mature CPY, whereas pep4 cells accumulate the p2 form within the vacuole.

In both 169ts and ypt6-null strains subjected at nonpermissive temperature to a pulse-chase with radioactive amino acids, there was accumulation of two forms of CPY (Fig. 5), one migrating as p1 and the other as m. Although unprocessed proCPY comigrates with the m form, treatment with endoglycosidase H demonstrated the band to represent authentic mature CPY (data not shown). We conclude that CPY maturation is partially defective at 37°C and that Ypt6p is probably involved either in ER to Golgi transport or in a cis-to-medial-Golgi step. In the case of CPY, 169ts and the null strain appear equivalent. YPT6 in a CEN plasmid complements the secretion defect in both 169ts (Fig. 5) and the null strain (not shown).

To determine more precisely the step of the secretory pathway at which Ypt6p is required, the glycosylation of invertase was analyzed. A mc-tagged invertase gene under the TPI1 promoter (kindly provided by N. Dean) was integrated by homologous recombination into the URA3 locus of each of the indicated strains. Extracts were made from cultures growing at 23°C and after 120 min at 37°C were subjected to Western

Fig. 3A, identification of YPT6 by subcloning. The fragments shown in the figure were cloned into pRS316 and the resulting constructs were used to transform 169ts. Growth or no growth of the transformed cells at 37°C is expressed as + and —; B, comparison of human Rab6 and Ypt6p. This is a modified BLAST program from GCG (44). The vertical bars represent identity; Colons and periods represent similarity. △ indicates the truncation site in Ypt6p of 169ts.
at a step between the ER and the Golgi or at a very early step.

CPY experiment. We conclude that Ypt6p acts primarily either... presumably due to the leakiness of the phenotype as seen in the mnn10 cells, although there is some intermediate material...

.. the gene that can suppress 169ts was identified as ORF 60318). The insert contains a single open reading frame, relatively good growth of 169ts at 37°C. Determination of sequence at the ends of the insert in this plasmid revealed that it was derived from chromosome IV (accession number M60318). The insert contains a single open reading frame...

analysis using the myc-specific monoclonal antibody 9E10 (Fig. 6).

Because the TPI1 promoter is constitutively on, the invertase visualized in each lane represents molecules synthesized at both the permissive and nonpermissive temperatures. Wild type cells accumulate a broad band of polyglycosylated invertase secreted into the periplasmic space. In the mutant sec18 at the nonpermissive temperature, a more rapidly migrating form accumulates that has core-glycosylated in the ER but has not been transported to the Golgi (27, 28). In the mutant mnn10 at both temperatures, invertase accumulates in the medial Golgi due to a deficiency of an glycosyl transferase (29) whose precise activity has not been determined (30). (Note that MNN10 has recently been identified in a screen for cell cycle mutants and rechristened BED1 (31).) Because some Golgi modifications have occurred, the molecules migrate more slowly than the invertase from the sec18 strain but still much faster than that from wild type.

Fig. 6 shows that at the nonpermissive temperature the 169ts cells, carrying the ypt6 truncation, accumulated invertase that has a mobility similar to that of the ER form (sec18ts) and greater than that of the medial Golgi form seen in the mnn10 cells, although there is some intermediate material, presumably due to the leakiness of the phenotype as seen in the CPY experiment. We conclude that Ypt6p acts primarily either at a step between the ER and the Golgi or at a very early step within the Golgi, certainly before the MNN10 step.

typt6 is Synthetic... ssd1-d—During the cloning of YPT6, we isolated two second site suppressors. Plasmid B supports relative growth of 169ts at 37°C. Determination of sequences at the ends of the insert in this plasmid revealed that it was derived from chromosome IV (accession number M60318). The insert contains a single open reading frame, SSD1 (Fig. 7A) (32). This gene has been isolated in numerous suppressor screens and is also known as MCS1 (33), SRK1 (34), and SSL1 (35). SSD1 on a CEN plasmid can complement the secretion defect of 169ts or of the null as tested by CPY accumulation (Fig. 5). Presumably as a result, the repression of ribosomal protein genes is partially suppressed (Fig. 8).

It has recently been shown that W303, our wild type strain, carries the defective ssd1-d allele (32). The suppression of the growth defect of 169ts demonstrates that a mutant or null ypt6 shows synthetic lethality with ssd1-d at temperatures above 34°C. The wild type, SSD1-v, was first isolated as a suppressor of the deletion of a putative protein phosphatase implicated in cell cycle progression (32) and later was found to suppress mutant alleles of a number of genes including PDE2, BCY1, INS1, and BEM1 (32, 34, 36). Although the mechanism by which Ssd1p functions is not clear, evidence suggests that it is probably involved in cell growth and morphogenesis (19). Fig. 5 is, however, the first evidence that Ssd1p can influence the secretory pathway and in turn ribosomal protein synthesis (Fig. 8).

IMH1 Is a Single Copy Suppressor—A second, weaker suppressor of the ypt6 mutation in 169ts, plasmid C, was found to reside on chromosome XII (cosmid L2142, accession number U17247). Within this insert are six complete genes including CDC25 (Fig. 7B). By multiple-step subcloning, we identified the gene that can suppress 169ts was identified as ORF L2142.5, not previously observed biologically. Since it shares with integrins and myosins significant homology, we term it IMH1. Although IMH1 on a CEN plasmid has little apparent effect on the leaky inhibition of CPY maturation in a ypt6 mutant or null strain (Fig. 5), it nevertheless restores growth...
and substantially rescues the transcription of ribosomal protein genes from ypt6-induced repression (Fig. 8). Because it was conceivable that Imh1p exerted its effect by suppressing the mutant phenotype of ssd1-d, we transformed the plasmid containing IMH1 into strains containing mutant alleles of SIT4 and LAS1, which grow normally in SSD1-v strains but not in ssd1-d strains. The presence of IMH1 on a CEN plasmid rescued neither the sit4 nor las1 mutants. Therefore Imh1p is unlikely to act through Ssd1p.

Other Mutants Identified by Their Repression of Ribosomal Protein mRNA Transcription Are Defective in the Secretory Apparatus—The original screen for ts mutants that appeared to be specifically repressed for their expression of mRNA for ribosomal proteins uncovered only a few (1). Because two of these were in fact defective in genes of the secretory pathway, SLY1 (1) and YPT6, we asked if others might also be involved in secretion. Fig. 9 shows that by the criteria of CPY glycosylation and maturation, 257ts, 271ts, and 367ts are clearly defective in secretion, whereas 394ts is moderately defective. Furthermore each of them induces KAR2 (data not shown), a characteristic of mutants in the early secretory pathway (37). No clearer evidence is needed for the close interlocking of the secretory pathway with ribosome synthesis than the observation that four more mutants, selected for insufficient ribosomal biosynthesis, have serious defects in the secretory pathway.

**DISCUSSION**

This report describes the cloning of YPT6, encoding the Rab6 homologue of *S. cerevisiae*, by complementing a temperature-sensitive mutant identified by the repression of ribosome synthesis at the restrictive temperature. Our studies provide three lines of evidence that Ypt6p is involved in the secretory pathway of *S. cerevisiae* at elevated temperatures. The maturation and transport to the vacuole of carboxypeptidase Y is impaired in the absence of functional Ypt6p (Fig. 5), although this phenotype is rather leaky (compare Fig. 5 with Fig. 9). The secretion of invertase is inhibited in the absence of Ypt6p (Fig. 4), the molecules accumulating apparently in the ER or the cis-Golgi (Fig. 6). Finally, electron microscopic examination of 169ts cells maintained at the restrictive temperature for 90 min revealed the development of many vesicular structures and an apparent loss of vacuolar contents (data not shown). Surprisingly, Ypt6p is essential only at elevated temperatures, as is its homologue in *S. pombe* (22). Is this a characteristic of the secretory pathway, or is there a protein with redundant function that itself is temperature-sensitive?

Functional studies of Rab6p in mammalian systems suggest that it participates in late intra-Golgi transport, i.e., between the cis-Golgi → medial/trans-Golgi compartments (7), and perhaps also in budding of exocytic vesicles from the TGN (38). Our data summarized above show that in *S. cerevisiae* deficiency of Ypt6p causes a block at an earlier step in the secretory pathway. The CPY and invertase molecules that escape this block, however, are matured normally, suggesting that Ypt6p does not participate in transport of secretory proteins beyond the medial Golgi. Yet mammalian Rab6 can support the growth of a YPT6 null strain (8). It remains to be seen if this molecule has different roles in mammalian compared with yeast secretion, or, more likely, if the assays used in the two systems are not fully congruent.

The ypt6 gene of 169ts carries a nonsense mutation at codon 64, generating a truncated Ypt6p. Genetically, this mutation is recessive; wild type YPT6 on a CEN plasmid can rescue the growth, the secretion defects, and the repression of ribosomal protein synthesis at nonpermissive temperature. Nevertheless, the truncated form of Ypt6p has detectable negative effects in comparison with a null strain, both in secretion of invertase (Fig. 4) and in the repression of ribosomal protein gene transcription (Fig. 1A). It is unlikely that 169ts carries a mutation in a second gene because it has been repeatedly back-crossed to...
Ypt6p in Early Golgi Function and Ribosome Biosynthesis

C. R. Nierras and J. R. Warner, unpublished observations.

The post-translational modification of carboxypeptidase Y in ts mutants 257ts, 271ts, 367ts, and 394ts. The protocol is the same as that described in the legend to Fig. 5. Wild type, sly1, and pep4 strains have been included for markers of the m, p1, and p2 forms of CPY, respectively.

The wild type parental strain. Ypt6p, like other members of the small G-proteins, has five highly conserved regions, termed G-1 to G-5, that are critical for GTP-induced conformational change, GTP hydrolysis, and GTP/GDP exchange (39). The truncation in the ypt6 allele of 169ts occurs in the middle of domain G-3. Perhaps the truncated Ypt6p sequesters proteins that interact with its N-terminal domain.

Ypt6p is essential for growth at high temperatures only in the absence of functional Ssd1p. W303, the parental strain from which 169ts was derived, was shown to contain the ssd1-d allele (32). In 169ts SSD1-v on a CEN plasmid can suppress the growth defect (Fig. 2), the secretion defects (Fig. 5), and the repression of ribosomal protein transcription (Fig. 1A). SSD1 was isolated originally as a suppressor of the deletion of IMH1 (36), and SSD1 is required for the start of S phase (32). Later, it was shown that 169ts was derived from which 169ts was isolated, and was shown to contain the IMH1 allele (32). In 169ts, the secretion defects (Fig. 5), and the growth defect (Fig. 2), the secretion defects (Fig. 5), and the repression of ribosomal protein transcription (Fig. 1A). SSD1 was isolated originally as a suppressor of the deletion of IMH1 (36), and SSD1 is required for the start of S phase (32). Later, it was shown that 169ts was derived from which 169ts was isolated, and was shown to contain the IMH1 allele (32). In 169ts, the secretion defects (Fig. 5), and the growth defect (Fig. 2), the secretion defects (Fig. 5), and the repression of ribosomal protein transcription (Fig. 1A). SSD1 was isolated originally as a suppressor of the deletion of IMH1 (36), and SSD1 is required for the start of S phase (32). Later, it was shown that 169ts was derived from which 169ts was isolated, and was shown to contain the IMH1 allele (32).

What is the basis for the dependence of ribosome synthesis on the secretory pathway? In experiments to be reported elsewhere, we show that the coupling is neither through the Ire1-mediated pathway by which KAR2 is induced by accumulation of imperfect proteins in the ER (37), nor through known pathways regulating ribosome synthesis, such as the stringent response to amino acid deprivation (42), nor through the protein kinase A response to carbon source (43). Thus we surmise that an as yet unidentified regulatory pathway connects ribosome synthesis to the secretory pathway. It remains to be seen whether the repression of ribosome synthesis represents a stress response to insufficient plasma membrane synthesis or a monitoring of the health of the secretory pathway in some more direct way.

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