

Competition between a sterol biosynthetic enzyme and tRNA modification in addition to changes in the protein synthesis machinery causes altered nonsense suppression

Ann L. Benko*, Gabriela Vaduva*, Nancy C. Martin[†], and Anita K. Hopper**

*Department of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, H171, 500 University Drive, Hershey, PA 17033; and [†]Department of Biochemistry, University of Louisville School of Medicine, 312 Abraham Flexner Way, Louisville, KY 40292

Edited by Fred Sherman, University of Rochester School of Medicine and Dentistry, Rochester, NY, and approved November 15, 1999 (received for review June 2, 1999)

The *Saccharomyces cerevisiae* Mod5p protein catalyzes isopentenylolation of A to i⁶A on tRNAs in the nucleus, cytosol, and mitochondria. The substrate for Mod5p, dimethylallyl pyrophosphate, is also a substrate for Erg20p that catalyzes an essential step in sterol biosynthesis. Changing the distribution of Mod5p so that less Mod5p is present in the cytosol decreases i⁶A on cytosolic tRNAs and alters tRNA-mediated nonsense suppression. We devised a colony color/growth assay to assess tRNA-mediated nonsense suppression and used it to search for genes, which, when overexpressed, affect nonsense suppression. We identified *SAL6*, *TEF4*, and *YDL219w*, all of which likely affect nonsense suppression via alteration of the protein synthesis machinery. We also identified *ARC1*, whose product interacts with aminoacyl synthetases. Interestingly, we identified *ERG20*. Midwestern analysis showed that yeast cells overproducing Erg20p have reduced levels of i⁶A on tRNAs. Thus, Erg20p appears to affect nonsense suppression by competing with Mod5p for substrate. Identification of *ERG20* reveals that yeast have a limited pool of dimethylallyl pyrophosphate. It also demonstrates that disrupting the balance between enzymes that use dimethylallyl pyrophosphate as substrate affects translation.

N⁶-(Δ^2 -isopentenyl)adenosine | sterol biosynthesis | assay for flux in sterol pathway

The *Saccharomyces cerevisiae* protein Mod5p catalyzes the addition of an isopentenyl group to adenosine (i⁶A) at position 37 of the anticodon loop of some tRNAs (1–5). There are two isoforms of Mod5p, Mod5p-I and Mod5p-II, that differ in the site of their translation initiation codon and in their distribution in the cell. Mod5p-I is translated starting at codon 1 of the *MOD5* ORF and is localized to mitochondria and the cytoplasm. Mod5p-II, translated from codon 12 of the *MOD5* ORF, is located in the nucleus and the cytoplasm (6, 7).

The i⁶A modification promotes the efficiency of *SUP7* tRNA in cytosolic suppression of UAA nonsense mutations by the insertion of tyrosine (8). Cells possessing only Mod5p-I have limiting cytosolic amounts of isozyme, and changes in the subcellular distribution and/or the activity of this isozyme alter nonsense suppression. Hence, genetic screens/selections based on nonsense suppression can identify cells with altered cytosolic Mod5p-I activity (9).

Here we employed the genetic strategy of using overexpression to perturb a pathway (10) and developed a protocol for the selection of cells with lower than normal levels of cytosolic Mod5p-I activity. Using this strategy we were able to sample the entire yeast genome and identify genes that, when overexpressed, lead to lower than normal levels of cytosolic Mod5p-I activity.

As a result of that screen, we identified two categories of genes. The first category includes genes that affect nonsense

suppression via alteration of the protein synthetic machinery. Our studies suggest that the yeast gene product encoded by *YDL219w* may function in protein synthesis and that the translation elongation factor EF1- γ may function in translational proofreading. *SAL6* and *ARC1*, with previously established effects on protein synthesis, also were recovered.

The second category includes *ERG20*, involved in sterol biosynthesis. The mevalonate pathway generates sterols, prenylated proteins, heme A, dolichol, ubiquinone, and the substrate required for isopentenylolation of tRNAs (Fig. 1). Both Mod5p and Erg20p use the same substrate, dimethylallyl pyrophosphate (DMAPP). Recovery of *ERG20* coupled with subsequent biochemical assays demonstrating a reduction of i⁶A on tRNA is most easily explained by a model in which Mod5p and Erg20p compete for a limited pool of DMAPP. When more DMAPP is used to make sterols, less is available for modification of tRNA and a reduction in the efficiency of nonsense suppression results. Thus, we have demonstrated that the tRNA biosynthetic pathway and the sterol biosynthetic pathway are in apparent competition for substrate and that Erg20p and Mod5p must be balanced to optimally maintain the protein synthetic machinery. A practical consequence is that it should be possible to adapt the selection we developed to assess the effect of mutations and/or drugs that change the distribution of DMAPP between the sterol pathway and the tRNA biosynthetic pathway.

Materials and Methods

Strains and Media. Strain ALB1 (*MAT α mod5-M2 SUP7 ade2-1 can1-100 leu2-3, -112 lys1-1 lys2-1 trp1 ura3-1*) was created from MD14A (6) by the replacement of the genomic *mod5-1* through a pop-in/pop-out procedure (12). Plasmid YCf-mod5-M2 (6) was used as the donor of the M2 mutation. The *ARS1* and *CEN4* sequences were removed from YCfmod5-M2 by a *Bgl*II/*Sma*I digestion. The *Bgl*II end of the remaining plasmid was filled in by using Klenow DNA Polymerase and then ligated to the *Sma*I blunt end. The resulting plasmid was linearized by *Sna*BI just upstream of the *mod5-M2* ORF and then transformed into MD14A. Integration of the plasmid was selected for by growth on medium lacking uracil. Resulting cells contained two *mod5* alleles: *mod5-1* and *mod5-M2*. Cells in which intrachromosomal homologous recombination and loss of the *mod5-1* mutation occurred were selected for by growth on medium

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Abbreviations: i⁶A, N⁶-(Δ^2 -isopentenyl)adenosine; DMAPP, dimethylallyl pyrophosphate; EF-1, elongation factor 1; SC medium, synthetic complete medium.

*To whom reprint requests should be addressed. E-mail: ahopper@psu.edu.

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containing 5-fluoro-orotic acid (5-FOA) and for the ability to grow on medium lacking adenine. The presence of the remaining *mod5-M2* allele was confirmed by DNA blot hybridization.

Strain ALB8 (*MAT α SUP7 can1-100 ade2-1 leu2-3, -112 lys1-1 lys2-1 trp1 mod5::TRP1 ura3-1::MOD5*) was created through integration of a plasmid containing *MOD5* at the *ura3-1* locus of strain MT-8 (6). Plasmid YCMOD5 (6) was used as the donor of *MOD5*. The *ARS1* and *CEN4* sequences were removed as described above, and the resulting plasmid was linearized with *NcoI* to target it to *ura3-1*. MT-8 cells that acquired the plasmid were selected on medium lacking uracil. Correct integrants were assessed by DNA blot hybridization.

Strain SL680-9C (*MAT α asu9-1 sup45-2 ura3-52 can1-132 leu2-1 lys2-1 met8-1 trp1-1 or trp1-289*) (13) was obtained from S. Liebman (University of Illinois, Chicago).

Yeast strains were propagated in YEPD (dextrose) medium or synthetic complete (SC) medium lacking certain nutrients to select for the presence of plasmid and to assay suppression. Canavanine sulfate salt (Sigma) was added to medium lacking arginine to a final concentration of 30 μ g/ml. Yeast strains were transformed by following a modified TRAF0 protocol (14).

Library DNAs and Plasmids. The following DNA libraries were used: a genomic DNA library based in YEp13 (15), a genomic DNA library based in YEp24 (16), a cDNA library based in pMac561, a 2 μ vector with the *ADHI* promoter (17), and a cDNA library based in pRS316 (18).

YEpMOD5 contains the 1.8-kb DNA fragment encoding wild-type *MOD5* gene and regulatory regions in the YEp24 vector (5). pRH127-3, a gift from R. Wright (University of Washington, Seattle), is a 2 μ -based plasmid that contains a truncated *HMG1* gene that causes increased HMG-CoA reductase activity and increased levels (\approx 10 times greater) of intermediates in the mevalonate pathway (19).

Sequencing of Inserts of Library Clones. Library plasmids were isolated from yeast by the method of Ward (20). DNAs were sequenced by either the chain termination method (21) with Sequenase Version 2.0 DNA Sequencing Kit (United States Biochemical) or by automated cycle sequencing performed in the Pennsylvania State University College of Medicine Macromolecular Core Facility. Nucleotide sequences were identified by a BLAST (22) search at the *Saccharomyces* Genome Database BLAST server (<http://genome-www2.stanford.edu/cgi-bin/SGD/nph-blast2sgd/>).

Determination of the Levels of i⁶A in tRNA. RNA was obtained (23) from overnight log-phase cultures of the following strains: ALB8, MD14A, and ALB1 with the pRS316 library plasmids containing *SAL6*, *ARCI*, *TEF4*, *ERG20*, YDL219w, or no cDNA insert. The RNA concentration was calculated from optical density measurement.

RNAs were resolved on a 12% polyacrylamide/7 M urea gel. The RNA was transferred to Hybond N+ Nylon Membrane (Amersham Pharmacia) in a Hoefer TE Series Transphor Apparatus containing TAE buffer (40 mM Tris-acetate, 1 mM EDTA). The RNA was fixed onto the membrane by UV crosslinking. The membrane was subjected to immunoblotting by following the protocol of the Renaissance Western Blot Chemiluminescence Kit (NEN) with slight variation. The primary antibody, anti-i⁶A isolated from rabbit (R. Clark and N.C.M., unpublished results) prepared by the method of Senapathy and Jacob (24), was diluted either 1:33.3 or 1:72.5 before use. Horseradish peroxidase-conjugated anti-rabbit IgG from donkey (Amersham Pharmacia) diluted 1:5,000 or 1:2,500 served as secondary antibody. The membrane also was utilized for RNA detection by using radiolabeled oligonucleotide complementary to a region of mature tRNA^{Tr}.

The intensities of the fluorescent and radioactive signals for each specimen on the immunoblots and RNA blots, respectively, were determined by densitometry using a Molecular Dynamics laser scanner with the Discovery Series Quantity One computer software (Bio-Rad). For each sample, the ratio of its signal with respect to that of the signal from the ALB1 with vector-only sample was calculated from the immunoblots (ratio 1) and from the RNA blots (ratio 2). The average values for ratio 1 to ratio 2 for samples from two membranes were calculated.

Results

Identification of Plasmids Causing Reduced Nonsense Suppression.

We employed the *can1-100* and *ade2-1* alleles in a *mod5-M2* background (strain ALB1) to identify plasmids causing alterations in nonsense suppression. *Mod5-M2* is an allele of *MOD5* that has normal levels of mitochondrial but reduced levels of cytosolic Mod5p activity. In this strain, cytosolic tRNAs are only partially modified with i⁶A (60%; ref. 6) and further alterations in Mod5p levels are readily assessed by changes in nonsense suppression (9). The *can1-100* and *ade2-1* alleles contain UAA nonsense mutations. *CAN1* encodes an arginine permease that allows the uptake of the arginine analog canavanine (25), and cells cannot grow in its presence. Therefore, cells with wild-type *CAN1* are sensitive to canavanine (Can^s), but cells with the mutant *can1-100* are resistant to canavanine (Can^r). Cells containing *SUP7* tRNAs completely modified with i⁶A have functional arginine permease and, therefore, cannot grow in the presence of canavanine, whereas cells lacking i⁶A on their tRNAs are able to grow in the presence of this drug. Cells with intermediate levels of i⁶A show intermediate levels of growth in the presence of the drug.

ADE2 encodes an enzyme involved in the synthesis of adenine. Cells with *ade2-1* turn red in color and fail to grow on defined medium lacking adenine (Ade⁻), whereas cells producing functional Ade2p can grow on such medium (Ade⁺) and are white (26). Cells with the *ade2-1* allele and sufficient i⁶A-modified suppressor tRNA can grow in the absence of exogenous adenine and generate white colonies on rich medium, whereas cells with insufficient i⁶A-modified tRNA are unable to grow in the absence of exogenous adenine and generate red colonies on rich medium. Cells with intermediate levels of i⁶A modified tRNA have intermediate phenotypes in colony color and intermediate rates of growth in the absence of exogenous adenine.

Transformants able to grow on canavanine were selected from approximately 1.5 million independent ALB1 (relevant genotype: *SUP7 ade2-1 can1-100 mod5-M2*) transformed cells. One hundred and fifty-eight candidates that exhibited growth on medium containing canavanine were tested for lack of growth on medium lacking adenine to eliminate those gaining canavanine resistance because of additional nonsuppressible mutations in *can1-100*.

For each of the 71 canavanine-resistant candidates that also were unable to grow well on medium lacking adenine or were red-pink in color, an assay was performed to determine whether these phenotypes required the presence of the library plasmid. Thirty-three candidates showed dependence of suppression levels upon the presence of a plasmid. Analysis of the plasmids in these cells resulted in the identification of 11 with unique restriction patterns. These plasmids were retransformed into ALB1 and the transformants were reassayed for canavanine resistance and adenine prototrophy. One plasmid did not confer either of these phenotypes. Four pRS316 library clones were still able to prevent growth on medium lacking adenine, and six of the remaining clones still could produce both desired phenotypes in ALB1 as confirmed by a second set of co-loss experiments (Table 1). Sequencing of the inserts of these latter 10 clones led to the identification of five different genes: *SAL6*, *ARCI*, *TEF4*, YDL219W, and *ERG20*.

Table 1. Growth of strains containing plasmids

Strain	Growth on canavanine		Growth/colony color on SC-adenine	
	Galactose	Glucose	Glucose	Galactose
MD14A	++	- r	- r	- r
pRSvector/ALB1	-	+ w	+ w	+ w
pRSSAL6/ALB1	+	+ w	+/- p	+/- p
pRSERG20/ALB1	+	+ w	+/- p	+/- p
pRSARC1/ALB1	+/-	+ w	+/- p	+/- p
pRSYDL219w/ALB1	+/-	+ w	+/- p	+/- p
pRSTEF4/ALB1	+/-	+w	+/- p	+/- p

++, Very strong growth; +, strong growth; +/-, poor growth; -, no growth; r, red; p, pink; w, white.

ERG20, which encodes farnesyl diphosphate synthetase, was found once in each of the YEp13, pMac561, and pRS316 libraries. *SAL6* cDNA, encoding a protein phosphatase, was discovered four times in the pRS316 library. *ARC1*, *TEF4*, and the putative ORF YDL219w were single candidates from the pRS316 library (Table 1). The pRS316 library is constructed such that the cloned genes are regulated by the *GAL1* promoter. Thus, transcription is induced by galactose and repressed by glucose in the media. The effects of the identified genes upon nonsense suppression were dependent on having galactose in the medium (Table 1), showing that the phenotypes are a result of their expression.

SAL6, TEF4, and YDL219w Confer Antisuppressor Phenotypes. *SUP45* encodes a translation termination factor, and mutation of this gene can result in omnipotent suppression (27, 28). When *SAL6* is overexpressed in a yeast strain containing *sup45-2*, an anti-suppressor phenotype occurs (13). This suggested that the canavanine resistance conferred upon ALB1 by high levels of Sal6p also may be due to antisuppression. Therefore, all of the candidate genes were tested for effects upon translation by assessing whether they scored as antisuppressors of *sup45-2*.

SL680-9C (relevant genotype *sup45-2 asu9-1 leu2-1 met8-1 ura3-52*) is capable of growing on medium lacking methionine because the mutant *sup45* permits translation through nonsense mutations such as *met8-1* (13, 27, 28). The *asu9* antisuppressor allele serves to weaken the effects of *sup45* and permits better growth of cells with both mutations (13, 27).

SL680-9C cells transformed by each of the candidate genes in the pRS316 vector were analyzed for growth on galactose-containing or glucose-containing medium lacking uracil and methionine. The data (Table 2) indicate that overexpressed *SAL6* and, to a lesser degree, *TEF4* and YDL219w cause

Table 2. Growth of SL680-9C cells overexpressing candidate genes

Candidate gene/ORF	Growth on SC-uracil-methionine (galactose)	Growth on SC-uracil-methionine (glucose)
None (vector only)	+	+
<i>SAL6</i>	-	+
<i>ARC1</i>	+	+
<i>TEF4</i>	+/-*	+
<i>ERG20</i>	+	+
YDL219w	+/-	+

Cells were grown at 28°C for 5–8 days; results are for three experiments. +, strong growth; +/-, poor growth; -, no growth.

*Galactose vs. glucose growth difference sometimes not apparent.

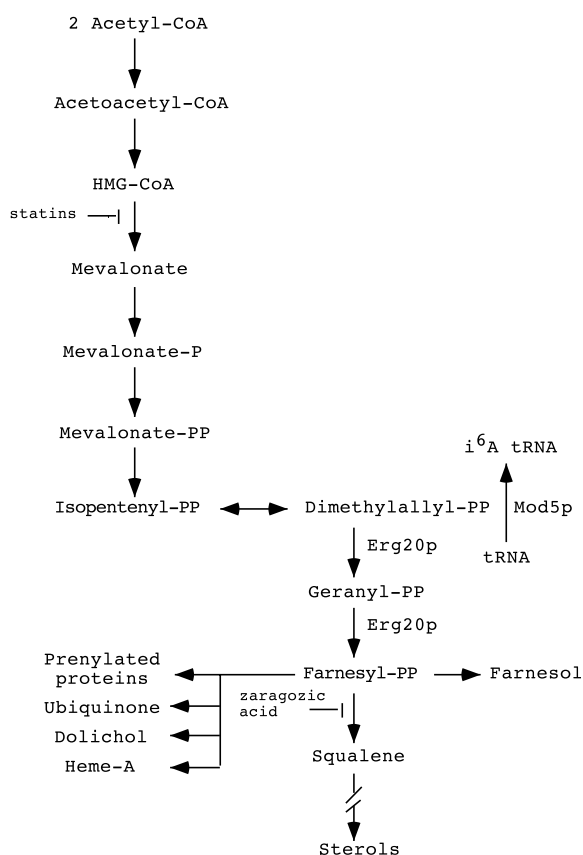


Fig. 1. The mevalonate pathway (11).

antisuppression, and, therefore, loss of suppression of the *can1-100* and *ade2-1* in ALB1 is likely Mod5p-I-independent. This phenotype was not observed consistently in the cells containing large amounts of Tef4p. Neither Arc1p nor Erg20p in high levels affected the suppression of *met8-1* in SL680-9C, and, therefore, each probably affects *can1-100* and *ade2-1* suppression in ALB1 via another route, likely by alteration of Mod5p-I or tRNA activities or location.

Overexpression of *ERG20* Results in the Reduction of Isopentenyl Adenosine Present in tRNA. The protein encoded by *ERG20*, farnesyl diphosphate synthetase, catalyzes the formation of geranyl pyrophosphate and farnesyl pyrophosphate by the condensation of units of DMAPP and its isomer isopentenyl pyrophosphate (IPP). DMAPP is the same molecule used by Mod5p as a substrate in the synthesis of *i*⁶A in tRNA (refs. 29 and 30; Fig. 1). It was possible that the large pool of Erg20p produced from the library clones used the DMAPP normally utilized for tRNA modification. This would cause reduced *i*⁶A-modified tRNA and, hence, reduced function of these tRNAs in nonsense suppression. It was also possible that others of the candidate genes, when overexpressed, inhibited the formation of *i*⁶A tRNA.

To determine the effect of overexpression of the candidate genes upon *i*⁶A modification of tRNA, we employed the Mid-western procedure (31) by using anti-*i*⁶A antibody. Low-molecular-weight RNA was prepared from cells with each plasmid and from control strains of ALB1 with vector only, ALB8, and MD14A. The RNAs were resolved on urea-polyacrylamide gels, transferred to membranes, and probed with anti-*i*⁶A antibody (Fig. 2A Upper). Hybridization with a radio-labeled oligonucleotide complementary to part of mature

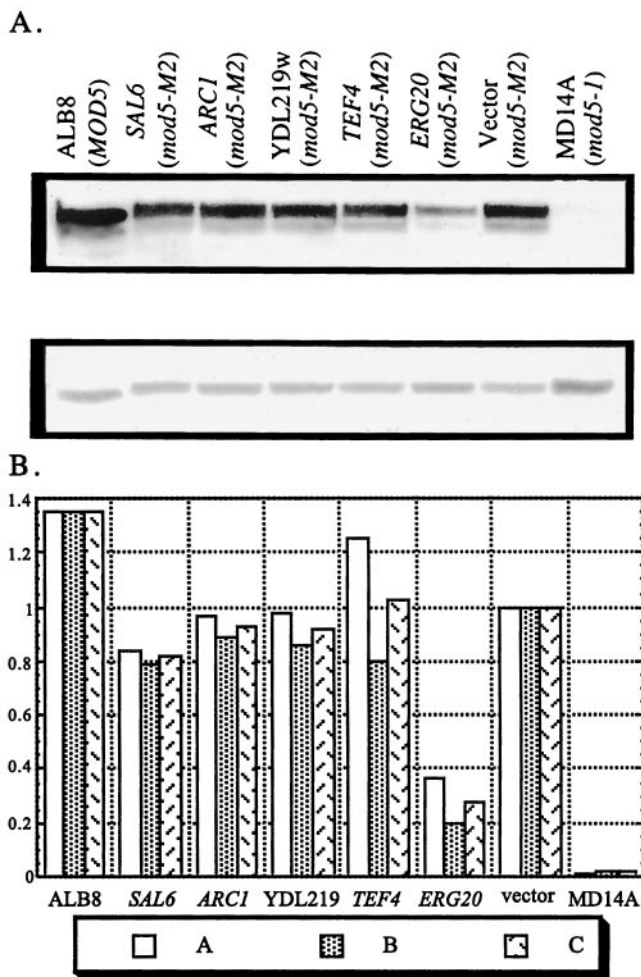


Fig. 2. The level of isopentenylated tRNA found in ALB1 overexpressing *ERG20* is reduced substantially. (A) Low-molecular-weight RNA was prepared from ALB1 (*mod5-M2*) with each of the candidate genes or vector alone, ALB8 (*MOD5*), or MD14A (*mod5-1*). The RNAs were resolved on polyacrylamide gels, transferred to membranes, and probed with anti-isopentenyl adenosine antibody (Upper) or radiolabeled oligonucleotide complementary to mature tRNA^{Tyr} (Lower). (B) The levels of isopentenyl adenosine tRNA found in ALB1 with each of the candidate genes or vector only or in the strain ALB8 or MD14A were assessed by densitometric analysis of two immunoblots and expressed as a fraction of the level found in the "vector" control. A, membrane 1 values; B, membrane 2 values; C, average values.

tRNA^{Tyr} was used to assess the relative amount of tRNA in each lane (Fig. 2A Lower). Through densitometric analysis, the amount of i⁶A formed in tRNA of each strain as a fraction of the amount formed in the vector-only control strain was determined (Fig. 2B).

As expected (32), MD14A cells with the *mod5-1* allele have barely detectable levels of modified tRNA. Also as anticipated, ALB8 cells with a wild-type *MOD5* that encodes both Mod5p-I and Mod5p-II contained higher levels ($\approx 35\%$ more) of i⁶A-modified tRNA than ALB1 cells expressing only Mod5p-I (ref. 6; Fig. 2). The level of i⁶A tRNA in ALB1 overexpressing *SAL6*, *ARC1*, *YDL219w*, or *TEF4* is similar to the level of i⁶A from ALB1 cells with vector only. However, the amount of i⁶A tRNA in ALB1 with excess *Erg20p* is significantly reduced to approximately 30% of that in ALB1 with vector alone. Overproduction of *Erg20p* results in reduced levels of i⁶A tRNA and causes a decrease in nonsense suppression.

If overproduction of *Erg20p* acts via competition with

Mod5p-I for a common substrate (Fig. 3), then providing additional Mod5p should reverse the loss of suppression. To test this, we transformed ALB1 cells containing YEp*ERG20* with YEp*MOD5* and assessed suppression of *ade2-1* and *can1-100*. The results (Table 3) show that suppression indeed is restored by additional Mod5p. Likewise, if *Erg20p* and Mod5p are in competition for the same substrate, then alterations that increase the substrate should dampen the competition. To test this, we introduced a multicopy vector harboring a mutant *HMG1* gene, pRH127-3, that causes elevated levels of intermediates of the mevalonate pathway (19) into ALB1 containing YEp*ERG20*. We found that increased levels of the intermediates also restored nonsense suppression (Table 3). The data support the model that the mevalonate pathway and the i⁶A tRNA modification pathways are in competition.

Discussion

Our interest in the distribution of sorting isozymes led us to design a screen for gene products that function in the subcellular distribution of Mod5p or affect its ability to modify cytosolic tRNAs. We anticipated that we would uncover genes affecting transcription of *MOD5* and/or tRNAs, nuclear export of tRNA, and the translation process, in addition to those involved in protein distribution. As anticipated, three gene products, *Sal6p*, *Tef4p*, and *YDL219w*, appear to affect nonsense suppression via function in protein translation and *Arc1p* could play a role in nuclear export of tRNA (see below). Surprisingly, no gene products affecting *MOD5* or tRNA transcription or Mod5p-I subcellular distribution were uncovered. Rather, *ERG20*, important to sterol biosynthesis, was found.

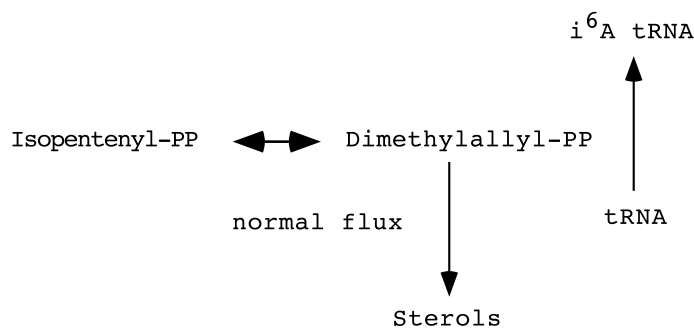
Sal6p/*Ppq1p* is a serine-threonine protein phosphatase very similar to mammalian phosphatase *PP1* (13, 33). Multiple copies of *SAL6* cause antisuppression of nonsense mutations (13), and the *sal6-1* allele acts as an allosuppressor (13, 27). Yeast cells containing a disrupted *PPQ1* gene exhibit a slowed translation rate and hypersensitivity to inhibitors of protein synthesis (33). These findings suggest a role for *Sal6p* in the regulation of the fidelity of translation (13, 33).

Tef4p is the γ -subunit of elongation factor 1 (EF-1). Eukaryotic EF-1 functions in delivering incoming tRNAs to the ribosome and is composed of at least three subunits. EF-1 α binds GTP and then the proper aminoacyl-tRNA and positions the aminoacyl tRNA in the ribosomal A site. Subsequently, GTP is hydrolyzed to GDP and EF-1 β catalyzes the exchange of GDP for GTP to restore EF-1 α to its initial state (34, 35). The exact function of EF-1 γ has not been determined; however, in *Artemia salina* this subunit was observed to enhance the function of EF-1 β catalysis. In the same study EF-1 γ was found to interact with cell membranes and tubulin, suggesting that it might mediate the association of the translational machinery with the cell framework (36).

In bacteria, the EF-1 counterpart functions in translational proofreading. Incorrectly inserted aminoacyl tRNAs can be removed before GTP hydrolysis and before the departure of EF-1 α from the ribosome (35). If the yeast EF-1 serves a similar function as the bacterial counterpart, it is unlikely that the α -subunit participates in this function as extra copies of the genes encoding EF-1 α decrease fidelity (i.e., they enhance suppression of nonsense mutations; ref. 37). We show that overexpression of *Tef4p* reduces nonsense suppression and, therefore, it is possible that it is the EF-1 γ subunit that functions in EF-1 proofreading.

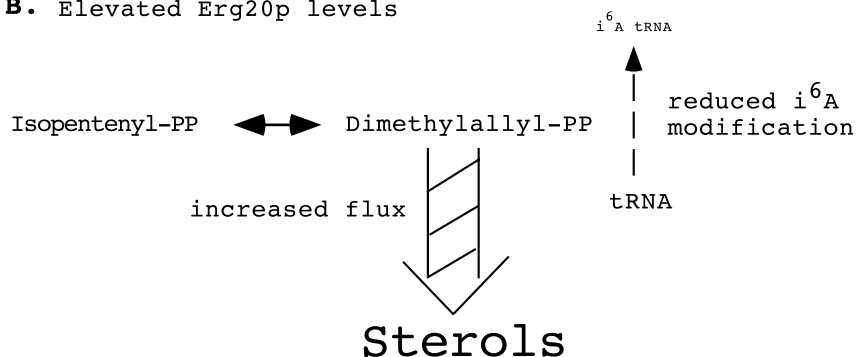
ORF *YDL219w* is predicted to code for a 150-aa protein with no significant homology to any characterized protein. However, two lines of evidence indicate that this protein may function in the translation process. First, the gene possesses an intron (*Saccharomyces* Genome Database, <http://genome-www.stanford.edu/cgi-bin/dbrun>). Because introns are rare in yeast other than for approximately half of the genes encoding ribo-

A. Normal Erg20p levels



white/light pink colonies; growth on -ade media; no growth on canavanine-containing media

B. Elevated Erg20p levels



pink/red colonies; poor growth on -ade media; able to grow on canavanine-containing media

Fig. 3. Model of competition between i^6A modification of tRNA and sterol biosynthesis.

somal proteins (38), the presence of the intron is suggestive of a role in translation. Second, we show that overexpression of YDL219w affects tRNA-mediated nonsense suppression.

Arc1p, or G4p1, originally was discovered as a quadruplex nucleic acid-binding protein (39). Subsequently, Arc1p was demonstrated to bind tRNA and to complex with methionyl and glutamyl tRNA synthetases (40, 41). Recent studies have shown that aminoacylation is important for tRNA nuclear export (42, 43). Moreover, Simos *et al.* (40) uncovered *ARC1* by synthetic interactions with Los1p, the yeast tRNA exportin (41, 44–46). Thus, it is possible that overexpression of Arc1p interferes with the appropriate nucleus/cytosol distribution of tRNA. Alternatively, overexpressed Arc1p could interfere with the interaction of tRNA and ribosomes. Incubation of tRNA^{Met} with large amounts of Arc1p *in vitro* resulted in the creation of supershifted complexes in a gel mobility assay, indicative of higher-order complexes (40). Perhaps large quantities of cellular Arc1p form complexes with tRNA, preventing the tRNA from functioning normally in translation.

Table 3. Growth of strains containing multicopy plasmids

ALB1 with plasmids	Growth on canavanine	Growth/colony color on SC-adenine
YEp13 + YEpMOD5	–	+ w
YEpERG20 + YEp24	+/-	+/- p
YEpERG20 + YEpMOD5	–	+ w
YEp13 + YEp24	–	+ w
YEp13 + pRH127-3	–	+ w
YEpERG20 + YEp24	+/-	+/- p
YEpERG20 + pRH127-3	+/-	+ w

+ , strong growth; +/- , poor growth; – , no growth; p, pink; w, white.

Overexpression of Erg20p causes a decrease in i^6A tRNA-mediated suppression in yeast and an approximately 70% decrease in i^6A modification of tRNA. These effects are most likely due to the loss of Mod5p substrate to Erg20p. The data demonstrate the dependence of tRNA processing upon changes in components of the sterol pathway. The data also indicate that, at least in yeast, Erg20p may catalyze a rate-limiting step in sterol biosynthesis. Hence, the tRNA and the sterol biosynthetic pathways are in apparent competition, and a delicate balance in protein levels is required to maintain proper functioning of translation and mevalonate metabolism.

The mevalonate pathway generates sterols, prenylated proteins, heme A, dolichol, ubiquinone, and isopentenyl tRNAs. Inhibitors of HMG-CoA reductase, catalyzing a rate-limiting step of the pathway, have been developed for administration to patients with hypercholesterolemia (47, 48). The “statin” class (e.g., compactin and lovastatin) of reductase inhibitors is safe and effective in lowering total cholesterol levels and preventing the progression and reducing the occurrence of coronary disease events (48–51). Zargozic acid inhibits squalene synthetase, catalyzing the first step of the pathway committed solely to sterol biosynthesis, but appears not to be used currently in the clinical setting. A novel type of zargozic acid, ZAD3, also inhibits farnesyl protein transferase and, therefore, protein prenylation as well (52). There appears to be no drug in current clinical use that affects solely cholesterol biosynthesis without affecting other farnesyl-PP-derived products.

We have demonstrated that increased levels of an enzyme of the sterol biosynthetic pathway can be assessed indirectly simply by colony color and/or growth on particular medium because changes in nonsense suppression occur. Thus, additional mutations and/or drugs that affect the pathway could be identified by a modification of the screening protocol we describe. The effect of prospective new regulators of pathway enzymes on the

suppressor ability of a strain with the ALB1 genotype (*mod5-M2 SUP7 can1-100 ade2-1*) could be assessed by adding them to canavanine-containing or adenine-lacking medium and monitoring cell growth. If the presence of a substance enhances colony growth on canavanine or deters colony formation on medium lacking adenine, in comparison with the growth observed on medium without the test substance, then the substance may be increasing the flow of DMAPP through the mevalonate pathway and decreasing tRNA modification. Likewise, by using the *mod5-M2, KR6* allele, we previously have shown (9) that

increased cytosolic Mod5p activity results in acquisition of the ability to grow on medium lacking lysine. Therefore, compounds that decrease the use of DMAPP by the sterol pathway also may be assessed easily. In this way, potential enhancers and inhibitors of the mevalonate pathway could be screened initially for their efficacy quickly and inexpensively.

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