MOLECULAR AND CELLULAR BIOLOGY, July 1990, p. 3397–3404
0270-7306/90/073397-08$02.00/0

Vol. 10, No. 7

Expression of a Proteolipid Gene from a High-Copy-Number Plasmid Confers Trifluoperazine Resistance to *Saccharomyces cerevisiae*

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Received 22 December/Accepted 30 March 1990

A wild-type haploid yeast strain was transformed with a library of wild-type yeast DNA fragments ligated into a high-copy-number plasmid vector (YEp24). The pooled URA$^+$ transformants were plated on rich medium containing a lethal concentration of trifluoperazine (TFP). Plasmids rescued into *Escherichia coli* from TFP-resistant yeast colonies contained overlapping DNA fragments from a unique region of yeast chromosome XVI. Deletion and disruption experiments, mini-Tn*10* LUK hop analysis, and DNA sequencing defined a novel gene with significant amino acid identity to bovine and yeast vacuoletype proteolipid subunits. This is the second locus identified that can be altered to confer TFP resistance to *Saccharomyces cerevisiae* and that has significant amino acid identity to a vacuolar ATPase subunit. This suggests that a target for TFP in *S. cerevisiae* is the electrogenic membranes of the vacuolar network and that alteration of expression or activity of vacuolar proton ATPase subunits is a general mechanism for TFP resistance in this yeast.

The vacuolar network of eucaryotic cells is a complex and dynamic organelle. This endomembrane network contains a variety of intracellular vesicles and hydrolytic processes (16). One important activity of this network is to collect and integrate signals between the secretory and the endocytic pathways. A driving force for the vacuolar network appears to be the electrochemical gradients of protons across the membranes of vesicles and endomembrane compartments (16). The major vacuolar proton ATPase complex is responsible for generating this transmembrane pH gradient (1, 3, 16, 18).

The structure of the major ATPase complex of the vacuolar network is analogous to the F_0F_1 ATP synthase of bacterial, mitochondrial, and chloroplast membranes (1–3, 11, 18, 24). The more soluble, hydrophilic F_1 portion of the complex hydrolyzes ATP and sits on the cytoplasmic surface of the vacuolar membrane. The proton channel through the membrane is formed by the very hydrophobic F_0 portion, which is an aggregate of small hydrophobic proteins called proteolipids (13, 24). The predicted amino acid sequences of proteolipid genes from bovine chromaffin granules and *Saccharomyces cerevisiae* have been published recently and show a very high degree of amino acid identity (15, 17). A more distantly related proteolipid gene from *Sulfolobus acidocaldarius* has also been cloned and sequenced recently (8).

The present article reports the cloning and sequencing of a second predicted proteolipid gene from *S. cerevisiae*. The strategy used to isolate this gene is now a standard experiment in yeast research and uses a library of wild-type sequences in a high-copy-number plasmid vector to overexpress specific gene products. The overexpression of a gene product can confer resistance to drugs and inhibitors (20) or can suppress or bypass other mutations (6). This work characterizes the *TFP3*$^+$ gene, which confers resistance to the antipsychotic drug trifluoperazine (TFP) when it is present in yeast cells on a high-copy-number plasmid.

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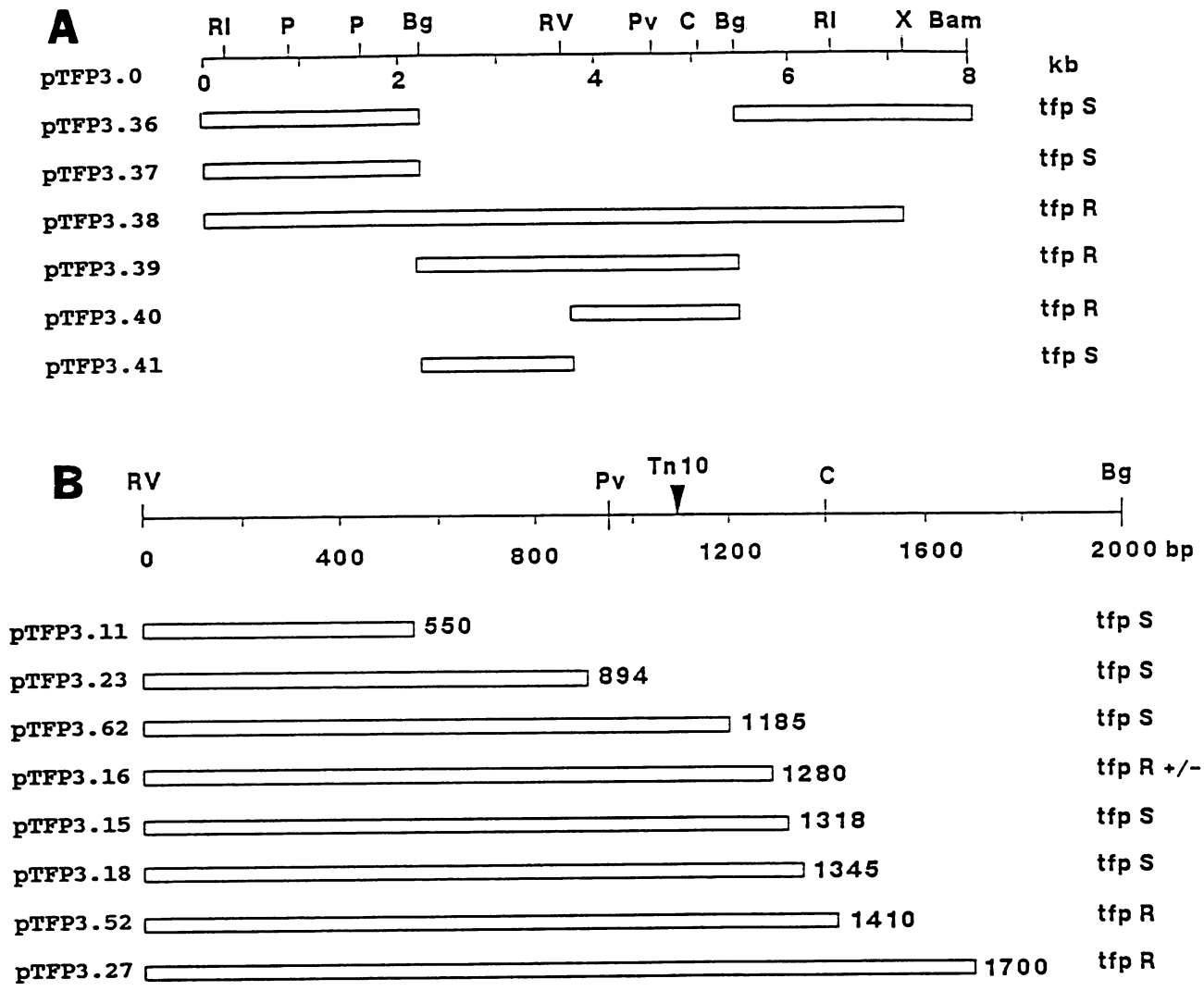
MATERIALS AND METHODS

Strains and plasmids. The wild-type TFP-sensitive *S. cerevisiae* strains NF134 (*MAT***a** *his4-539 lys2-801 ura3-52*), NF147 (*MAT*α *ade2 ura3-52*), and NF182 (*MAT***a** *ade2 his3-Δ200 leu2-3,112 lys2-801 trp1-1 ura3-52*) are of the S288c background (23, 26). The yeast genomic library in YEp24 contains DNA from the S288c background (5). Bacterial strains and phage used for the Tn*10* LUK hop were provided by W. Raymond and N. Kleckner (14). Other bacterial strains, plasmid vectors, media, and methods were as described before (26).

Construction of deletions and DNA sequencing. Restriction enzyme reactions, ligations, and DNA preparations were done as described before (26). Unidirectional deletions were generated by using exonuclease III (Exo-III) (19). Deletions for DNA sequencing were generated by the method of Dale et al. (7). Single-stranded and double-stranded DNA sequencing was performed by the U.S. Biochemical Sequenase system according to the manufacturer's instructions. The sequencing primer used to determine the insertion site of the transposon was provided by C. Clark-Adams and F. Winston (6). DNA sequence information was compiled and analyzed as described before (26).

Gel transfer and hybridization and disruption and insertion analysis. Total RNA and polyadenylated [poly(A)$^+$] RNA were prepared from yeast cells as described before (5) and transferred to a nylon filter membrane. Chromosomes from wild-type yeast cells were separated on a CHEF gel system and transferred to a nylon filter membrane as described before (4, 26). The 530-base-pair (bp) *Pvu*II-*Cla*I fragment (Fig. 1) was used as the hybridization probe for the *TFP3* locus. The probe for chromosome XIII was the *ILV2* gene, provided by S. Carl Falco (10).

An insertion and disruption construction was made by digesting pTFP3-40 at the unique *Pvu*II site at amino acid 24


A
RI P P Bg RV Pv C Bg RI X Bam
pTFP3.0
0 2 4 6 8
kb
pTFP3.36
tfp S
pTFP3.37
tfp S
pTFP3.38
tfp R
pTFP3.39
tfp R
pTFP3.40
tfp R
pTFP3.41
tfp S
B
RV Pv Tn10 C Bg
0 400 800 1200 1600 2000 bp
pTFP3.11 550 tfp S
pTFP3.23 894 tfp S
pTFP3.62 1185 tfp S
pTFP3.16 1280 tfp R +/-
pTFP3.15 1318 tfp S
pTFP3.18 1345 tfp S
pTFP3.52 1410 tfp R
pTFP3.27 1700 tfp R
C
Pv Tn10 C
0 200 400 600 800 bp

of the *TFP3* open reading frame and at the *Cla*I site (Fig. 1). The 1.1-kilobase (kb) *Hin*dIII fragment containing the *URA3*$^+$ gene (21) from YEp24 was inserted into this deletion by blunt-end ligation. A second disruption construction was made by blunt-end ligation of the 1-kb *Bam*HI restriction fragment containing the *HIS3*$^+$ gene (28) into the *Pvu*II site. Yeast genomic DNA was prepared from cells containing wild-type and/or null alleles of the *TFP3* gene, digested with *Bgl*II, and analyzed by gel transfer and hybridization as described before (26). The hybridization probe for the *TFP3* locus was the 3.3-kb *Bgl*II fragment (Fig. 1).

RESULTS

Expression of a wild-type sequence from a 2μm vector confers TFP resistance to wild-type cells. A library of random genomic DNA fragments on a 2μm plasmid vector (5) were transformed into wild-type haploid strain NF134. Approximately 10,000 URA$^+$ transformant colonies were pooled, and cells were plated out at low density on YPD plates containing 12.5 μM TFP (26). Eight candidate transformants with a consistently TFP-resistant phenotype were grown overnight in YPD medium to allow segregation of the plasmids. These cultures were plated on YPD plates for single colonies. Each culture had approximately 80% URA$^+$ colonies and 20% Ura$^-$ colonies. All of the URA$^+$ colonies were TFP resistant, and all of the Ura$^-$ colonies were TFP sensitive for all eight transformants tested. This result demonstrates that the TFP-resistant phenotype is due to the presence of the plasmid in the cells.

After rescue of the ampicillin-resistant plasmids into *Escherichia coli*, plasmid DNA was prepared and the yeast transformation was repeated. All of the resulting URA$^+$ colonies from each of the eight plasmids were TFP resistant (42 of 42). Transformation of the vector DNA alone (YEp24) gave no TFP-resistant colonies (0 of 42).

Each of the eight plasmids recovered was analyzed by restriction enzyme analysis. Four unique plasmids were recovered which had restriction fragments in common. The plasmid containing the smallest insert of yeast genomic DNA was chosen for detailed analysis (Fig. 1A, pTFP3.0).

The plasmid pTFP3-0 contains a 7.8-kb *Sau*3A fragment cloned into the *Bam*HI site of vector YEp24. A unique *Bam*HI site is recreated at one end of the insert. Three drop-out deletions were constructed (Fig. 1A, pTFP3.36, pTFP3.37, and pTFP3.38). The cells containing pTFP3.36 and pTFP3.37 were TFP sensitive, and those containing pTFP3.38 were TFP resistant. A plasmid was made by ligating the 3.3-kb *Bgl*II fragment into the *Bam*HI site of YEp24 (pTFP3-39). The resulting URA$^+$ transformants were TFP resistant, demonstrating that this fragment was sufficient to encode the drug resistance phenotype.

The 3.3-kb *Bgl*II fragment was ligated into the *Bam*HI site of the integrating plasmid vector YIp5 (pTFP3.5). The resulting URA$^+$ transformants were TFP sensitive (42 of 42), demonstrating that two copies of the DNA sequence are not sufficient for TFP resistance. These results indicate that the high copy number of the DNA insert in the cell or overexpression of a gene product from this 3.3-kb *Bgl*II fragment is necessary to confer TFP resistance to wild-type cells.

Further subclones into YEP24 were made by using the unique *Eco*RV site within the middle of the *Bgl*II fragment (Fig. 1A). The *Eco*RV-*Bgl*II fragment containing the *Pvu*II and *Cla*I restriction sites (pTFP3-40) was sufficient to confer TFP resistance. This plasmid was used for subsequent analysis.

Three types of experiments were performed to further define the region conferring TFP resistance: mini-Tn*10* insertion mutagenesis (14), unidirectional Exo-III deletions (19), and construction of specific insertion and deletion mutations. Eighty kanamycin-resistant, ampicillin-resistant plasmids from the LUK mini-Tn*10* insertion mutagenesis were transformed into NF134, and URA$^+$ transformants were tested for TFP resistance. Two plasmids had become TFP sensitive. One insertion was found approximately 200 bp from the *Pvu*II site (Fig. 1B and C) by sequencing (6). The sequence of the insertion in the other plasmid was ambiguous.

Unidirectional Exo-III deletions were made in pTFP3-40. Seventy-five plasmids with small deletions of the yeast insert were isolated and transformed into NF134, and URA$^+$ transformants were tested for TFP resistance. The locations of deletion endpoints of representative TFP-resistant and TFP-sensitive plasmids were determined by sequencing (Fig. 1B). The plasmid with the smallest insert that showed a consistent TFP-resistant phenotype included the *Cla*I site in the construction.

A specific insertion mutation was constructed by ligating the *HIS3*$^+$ gene (28) into the unique *Pvu*II site in pTFP3-40. All of the transformants of this plasmid (pTFP3-30) into NF182 were HIS$^+$ URA$^+$ TFP sensitive (42 of 42). The results of these three experiments define a region to the left of the *Cla*I site and including the *Pvu*II site (Fig. 1) as necessary to confer TFP resistance.

Specific transcript overexpressed in plasmid-containing cells which are TFP resistant. Poly(A)$^+$ RNA was prepared from wild-type cells in the log phase and stationary phase of growth. After agarose gel electrophoresis and transfer to a nylon filter, the RNA was hybridized with the *Pvu*II-*Cla*I fragment. In poly(A)$^+$ RNA from NF134 cells, there was one transcript of approximately 950 bp that hybridized with the probe (Fig. 2, lane 1). The amount of this transcript was greatly reduced in stationary-phase cells (Fig. 2, lane 2). This specific transcript was overexpressed in cells containing plasmids which conferred TFP resistance (Fig. 2, lanes 3 and 4). This RNA species was reduced in cells containing deleted plasmids which were TFP sensitive (Fig. 2, lane 5). This

FIG. 1. Restriction, deletion, and sequencing of the *TFP3* gene. (A) YEp24-based plasmids were constructed, and URA$^+$ transformants of these plasmids into wild-type NF134 or NF147 cells were tested for TFP resistance. The plasmid designations are indicated on the left. The resulting phenotype is indicated on the right. A partial restriction map of the yeast DNA insert from the original library plasmid (pTFP3.0) is shown. The restriction fragments in the plasmids (pTFP3.36 through pTFP3.41) are indicated by the bars. (B) Plasmid pTFP3.40 was cut at unique *Sph*I and *Sal*I sites in the vector, and a series of unidirectional Exo-III deletions were constructed. The deletion endpoints were determined by sequencing with an oligonucleotide (5′-CATGAGCGCTTGTTTCGGCG-3′) that hybridizes to the vector, and the distance (in nucleotides) from the *Eco*RV site is shown. URA$^+$ transformants of these plasmids into NF134 or NF147 cells were tested for TFP resistance, and the phenotypes are indicated on the right. The position of one mini-Tn*10* insertion-disruption determined by sequencing is shown. The plasmid indicated +/− gave a consistent partial or weak resistance phenotype. (C) The DNA sequence of the *Eco*RV-*Bgl*II fragment in pTFP3.40 was determined, and a plot to stop codons in all six open reading frames is shown for the region containing the *Pvu*II site. The open reading frame for the *TFP3* gene is indicated by the arrow. Abbreviations for restriction enzyme sites: RI, *Eco*RI; P, *Pst*I; Bg, *Bgl*II; RV, *Eco*RV; Pv, *Pvu*II; C, *Cla*I; X, *Xho*I; Bam, *Bam*HI.

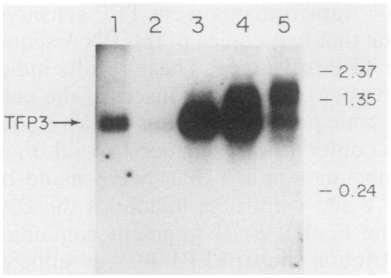


FIG. 2. Gel transfer and hybridization of total RNA and poly(A)$^+$ RNA from wild-type and plasmid-containing yeast cells. Lane 1, 1 μg of poly(A)$^+$ RNA from log-phase NF134 cells. Lane 2, 1 μg of poly(A)$^+$ RNA from stationary-phase NF134 cells. Lane 3, 10 μg of total RNA from NF134 cells carrying pTFP3.40. Lane 4, 10 μg of total RNA from NF134 cells carrying pTFP3.52. Lane 5, 10 μg of total RNA from NF134 cells carrying pTFP3.62. The *Pvu*II-*Cla*I fragment (Fig. 1C) was used as the hybridization probe. The positions of RNA molecular size standards (Bethesda Research Laboratories) are indicated on the right (in kilobases). The position of the *TFP3* mRNA is indicated on the left.

950-bp mRNA probably codes for the *TFP3* gene product. Hybridization of strand-specific probes generated from M13 constructions (data not shown) showed that the direction of transcription is as indicated in Fig. 1C.

This analysis was complicated by the appearance of a new transcript in Exo-III deletion plasmids (Fig. 2, lanes 4 and 5). Hybridization with strand-specific probes showed that this new larger transcript was derived from the opposite strand of the plasmid relative to the *TFP3* mRNA species (data not shown). The deletions appear to have removed a transcription termination block, allowing an adjacent and opposing mRNA to be continued into the *TFP3* locus. The exact nature of this second transcription unit is not known.

DNA sequence of *TFP3* predicts a hydrophobic protein with significant amino acid identity to two proteolipids. The DNA sequence of the 2-kb insert of pTFP3-40 revealed several small open reading frames in the *Pvu*II-*Cla*I region (Fig. 1C). The largest of these (which includes the *Pvu*II site) predicts a hydrophobic protein of 103 amino acids (Fig. 3). A search of known amino acid sequences revealed a significant identity of the *TFP3* gene with two proteolipid gene sequences (15, 17) (Fig. 4). The two published proteolipid gene sequences predict larger proteins with four membrane-spanning regions. The predicted *TFP3* protein appears to have only two transmembrane regions (amino acids 5 to 30 and 49 to 79) by analogy to the predicted hydrophobicity of the published sequences.

Haploid cells containing a deletion of the *TFP3* gene are viable. A deletion allele of the *TFP3* gene was constructed by removing the *Pvu*II-*Cla*I fragment and replacing it with the *URA3*$^+$ gene (21). The plasmid was cut with restriction enzymes to create double-strand breaks within the yeast sequences flanking the selectable marker (22). This construction was transformed into NF134 and into a diploid strain constructed by mating NF134 and NF147. There was a fivefold difference in the transformation frequency between the haploid strain (85 URA$^+$ colonies per μg of DNA) and the diploid strain (425 URA$^+$ colonies per μg of DNA). Sporulation and dissection of several independent diploid transformants showed a 2:2 segregation of all auxotrophic markers, and most tetrads had four viable spores (15 of 20).

DNA was made from a wild-type haploid strain, a diploid transformant strain, and two complete tetrads. A gel transfer-hybridization analysis of *Bgl*II-digested DNA preparations (Fig. 5) showed a 2:2 segregation of the wild-type locus (3.3-kb *Bgl*II fragment) and the slightly larger disrupted locus (3.9 kb). By this analysis, the *TFP3* gene is dispensable for spore germination and mitotic growth of haploid cells on YPD medium. The cells containing the disrupted allele of the *TFP3* gene did not show any temperature-sensitive or carbon source-dependent phenotypes either.

***TFP3* is located on chromosome XVI.** Separated yeast chromosomes (4) transferred to a nylon membrane were hybridized with a probe made from the *Pvu*II-*Cla*I fragment of the *TFP3* gene. The probe hybridized to the chromosome XVI-XIII region (Fig. 6). By ethidium bromide staining of the separated chromosomes, the labeled chromosome was determined to be XVI. To verify the assignment of the location as XVI, a probe for chromosome XIII was prepared from the *ILV2* gene (10). Separated yeast chromosomes were hybridized with each probe individually (lanes A and C) or mixed (lane B). This localized the *TFP3* gene to chromosome XVI.

DISCUSSION

Deduced amino acid sequence of the *TFP3* gene predicts a novel proteolipid. A comparison of the predicted amino acid sequence of the *TFP3* gene with two published proteolipid sequences (15, 17) shows significant identity. All three sequences predict very hydrophobic proteins with very few charged amino acid residues and multiple potential transmembrane domains. The *TFP3* gene predicts a protein of 103 amino acids, whereas the predicted gene products of the two published genes are larger (155 and 160 amino acids).

By analogy to the structure predicted for the two larger proteolipids (15, 17), the *TFP3* gene product has the potential to form two transmembrane domains, compared with the four proposed for the two larger proteolipids. The two larger proteolipids contain a predicted *N*,*N*′-dicyclohexylcarbodiimide (DCCD)-binding domain in the final transmembrane segment which is not found in the deduced *TFP3* product sequence. By analogy to the F_0 proteolipids (11–13, 24), the DCCD-binding site is defined by an aspartic acid or glutamic acid residue embedded in the transmembrane domain surrounded by several conserved hydrophobic amino acids. This negatively charged residue is critical for proton translocation, as determined by biochemical and genetic studies. The size and amino acid sequence of the *TFP3* gene suggest that the *TFP3* gene product is not likely to be the proton-conducting subunit for the major vacuolar ATPase and that the yeast proteolipid gene previously identified (17) is likely to be.

The regions of the greatest identity between the *TFP3* product and the two published proteolipid sequences are where the first predicted transmembrane segment exits the membrane and forms a predicted cytoplasmic segment, and the second predicted transmembrane segment. The number and arrangement of charged amino acids in this predicted hydrophilic region are the same among all three products (Fig. 4). This predicted cytoplasmic domain (15) is the same size in all three products and is delineated by conserved lysine residues at the beginning and end of the region. The region where the *TFP3* product has the greatest identity with the bovine proteolipid is in the second predicted transmembrane segment (21 of 27 amino acids). Therefore, the *TFP3* product appears to have the amino-terminal half of the two larger proteolipids with a longer C-terminal hydrophilic tail.

Normal function and location of the *TFP3* gene are not known. The DNA sequence of the *TFP3* gene predicts a

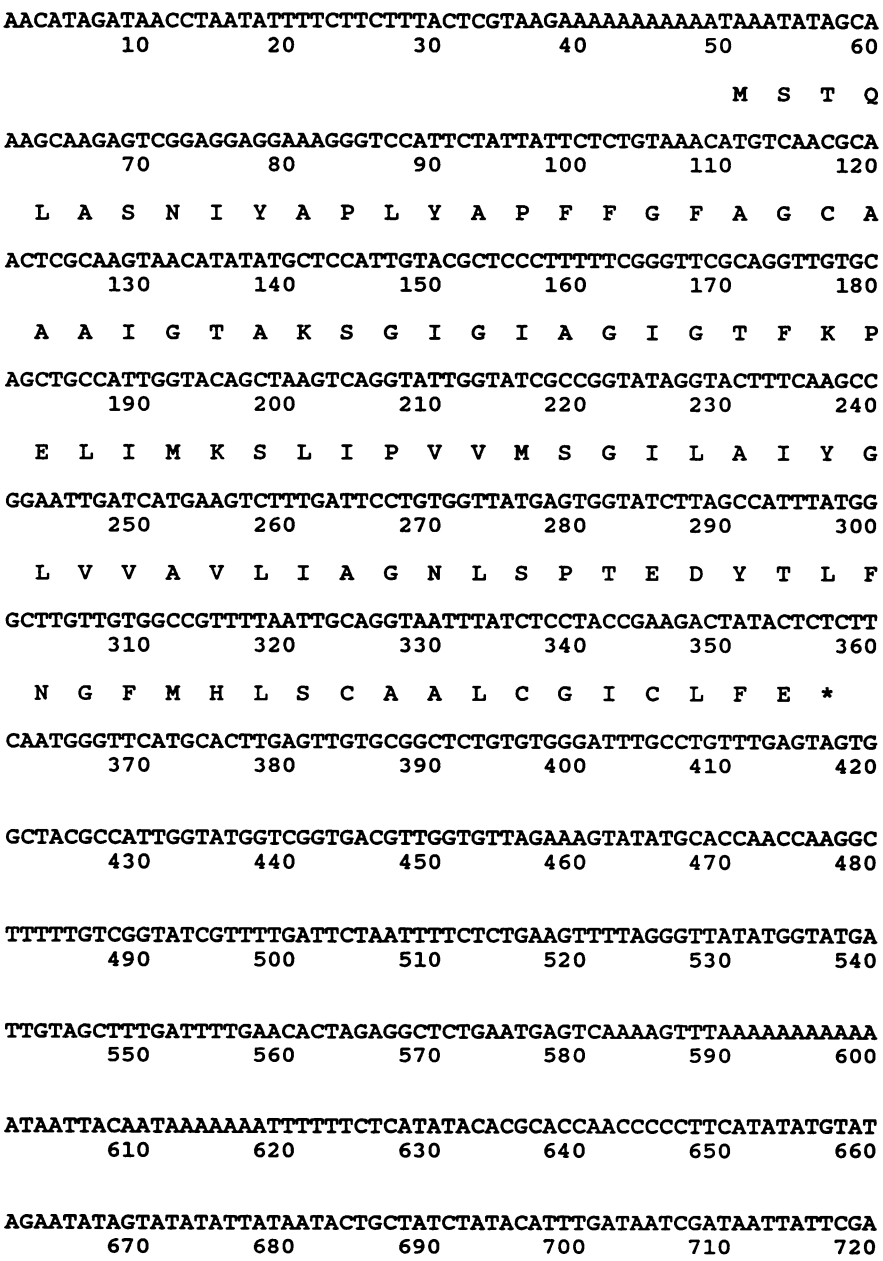


FIG. 3. DNA sequence of the *TFP3* gene. The translation of the open reading frame is shown in the one-letter amino acid code. The *Pvu*II site is at nucleotide 180, the position of the mini-Tn*10* insertion-disruption is at 390, and the *Cla*I site is at 706. The GenBank accession number for this sequence is M32736.

novel proteolipid gene. This gene is not essential for growth of a haploid cell on rich medium or for spore germination. The exact in vivo location and function of this small hydrophobic protein are not known. The *TFP3* gene may be nonessential, as there may be a family of proteolipid genes in yeast cells with redundant functions. The *TFP3* gene might play a role in assembly or function of a vacuolar ATPase complex under some nutritional or other stress conditions.

The amino acid identity with vacuolar ATPase proteolipid genes suggests a role for it in the vacuolar network. The *TFP3* gene has significant amino acid identity to the amino-terminal half of the bovine chromaffin granule gene (15) and much less identity with the proteolipid from *S. acidocaldarius* (8) or with the F_0 subunits (11, 13, 24). In contrast, the proteolipid gene from *S. acidocaldarius* shows the greatest similarity to the two carboxyl-terminal transmembrane domains of the bovine gene product (8, 15). There may be another gene in *S. cerevisiae* homologous to the *S. acidocaldarius* gene such that the *TFP3* gene product and this hypothetical second gene product would interact to form a functional proton channel. The *TFP3* gene product may be required for assembly or regulation of proton-conducting channels under certain environmental conditions.

Biochemical purification of vacuolar ATPase complexes in several laboratories (2, 3, 18, 30) has identified the major proteolipid as being 16 kilodaltons, which is similar to the size predicted by the bovine and yeast genes sequenced previously (15, 17). A protein with significant identity to the

FIG. 4. Amino acid identity of the predicted *TFP3* gene product with eucaryotic proteolipid sequences. Amino acid identity of the predicted *TFP3* gene product with the amino acid sequence of two sequenced proteolipid gene products (15, 17). A gap has been introduced into the *TFP3* sequence to show a conserved motif between the two *S. cerevisiae* genes. The positions of conserved charged amino acid residues in the predicted hydrophilic domain are indicated. Two predicted transmembrane domains are underlined. + indicates a positively charged amino acid residue; − indicates a negatively charged amino acid residue; * marks the position of the stop codon.

bovine proteolipid has been isolated from gap junction preparations (9), suggesting that proteolipids could have multiple roles in the cell. The exact functional relationship between these predicted yeast proteolipid genes and the vacuolar network remains to be determined by further genetic and biochemical analysis.

The mRNA for the *TFP3* gene is large (950 bp) compared with the size necessary to encode a 103-amino-acid protein. The Exo-III deletion data are consistent with a long mRNA. The exact endpoints of the mRNA have not been determined. The untranslated regions of the mRNA may play a role in regulation of the expression of this gene via some type of translational control.

Possible relationship between TFP resistance and the vacuolar network. A novel predicted proteolipid gene has been cloned from *S. cerevisiae*. When overexpressed from a high-copy-number plasmid, the gene confers TFP resistance to drug-sensitive wild-type cells. This is the second gene isolated by using selection for TFP resistance in *S. cerevisiae* and the second gene to have significant amino acid identity to a vacuolar ATPase subunit (26; unpublished).

TFP is the most hydrophobic of the medically effective anti-psychotic drugs (25, 27). The pharmacology literature reports that TFP is active as an antischizophrenic drug by its affinity for one of the two types of dopamine receptors (25,

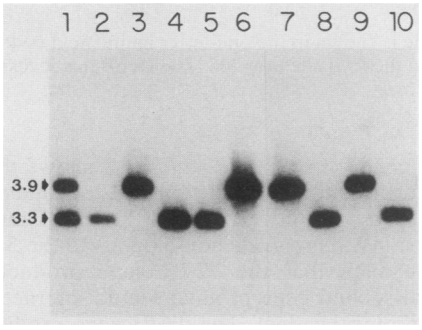


FIG. 5. Gel transfer and hybridization of *Bgl*II-digested genomic DNA from wild-type cells and cells containing a disrupted allele of the *TFP3* gene. Lane 1 contains DNA from a diploid URA$^+$ transformant strain. Lane 2 contains DNA from a wild-type haploid strain. Lanes 3 to 6 and 7 to 10 contain DNA from two different complete tetrads from the sporulation and dissection of the diploid transformant. The *Bgl*II fragment containing the wild-type gene is 3.3 kb. The *Bgl*II fragment containing the null allele is 3.9 kb.

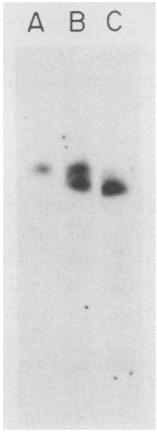


FIG. 6. Localization of the *TFP3* gene to chromosome XVI. Separated yeast chromosomes (4) were transferred to a nylon membrane and hybridized with the *Pvu*II-*Cla*I fragment of the *TFP3* gene (lane A), the *ILV2* gene (9) (lane C), or both (lane B).

27). The biochemical literature has proposed that TFP binds to calmodulin in a calcium-dependent manner and irreversibly inhibits it (31). To date there is no direct evidence that TFP binds to calmodulin in yeast cells or that inhibition of the activity of calmodulin is responsible for the lethal effects of the drug on the cells.

Another possible model is that TFP is a membrane-intercalating agent that disrupts or alters transmembrane events. The presence of a rigid planar molecule in the membrane would be expected to affect the fluidity of the membrane and assembly of membrane-associated complexes. By virtue of the fact that two genes that can be altered to confer TFP resistance to yeast cells encode predicted vacuolar ATPase subunits, it is possible that TFP has a particular affinity for the electrogenic membranes of the vacuolar network, and its effects as an antipsychotic drug are related to an inhibition of receptor recycling and vesicle traffic in neurons.

The proteolipids form proton channels via assembly of six or more subunits (2). The exact subunit composition of these vacuolar proton channels is not known, and little is known about the regulation of the assembly of the channels (18, 25). A purified DCCD-binding 16-kilodalton proteolipid has been shown to be capable of forming a passive proton channel in reconstituted liposomes (29). These studies suggest that overexpression of the *TFP3* gene could lead to a passive channel, discharging the pH gradient created by the regulated normal ATPase complex. Overexpression could also interfere with assembly or promote improper assembly of the subunits of an ATPase complex. A third possible model would be nonspecific hydrophobic sequestration, so that overproduction of a hydrophobic protein would titrate out the hydrophobic drug.

We favor the model that TFP intercalates into the electrogenic membranes of the vacuolar network and disrupts normal transmembrane functions. Resistance to TFP occurs when yeast cells have an alteration in the expression or activity of the vacuolar ATPase complex subunits.

ACKNOWLEDGMENTS

We thank Tom Stevens and Philip Nagley for advice and encouragement, S. Carl Falco for the plasmid containing the *ILV2* gene, Nancy Kleckner for strains and phage, Fred Winston for providing the Tn*10*-complementary oligonucleotide, and David Botstein, in whose laboratory this work was begun.

C.-K. Shih was supported by a postdoctoral fellowship from the Exxon Corporation. N.N. was supported by the American Cancer Society (JRA-189 and MV-357).

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