SYR2, a gene necessary for syringomycin growth inhibition of Saccharomyces cerevisiae

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The Pseudomonas syringae cyclic lipodepsipeptide syringomycin inhibits the growth of Saccharomyces cerevisiae. A novel yeast gene, SYR2, was found to complement two syringomycin-resistant S. cerevisiae mutants. SYR2 was cloned, sequenced, and shown to encode a 349 amino acid protein located in the endoplasmic reticulum. SYR2 was identical to SUR2, which is involved in survival during nutritional starvation. Gene disruption or overexpression of SYR2 did not affect cell viability or ergosterol levels, but did influence cellular phospholipid levels. The findings suggest that phospholipids are important for the growth inhibitory action of syringomycin.

Keywords: syringomycin, SYR2, Saccharomyces cerevisiae, SUR2, phospholipid biosynthesis

INTRODUCTION

Syringomycin is a small cyclic lipodepsipeptide produced by stone-fruit and grass isolates of Pseudomonas syringae pv. syringae (Segre et al., 1989; Fukuchi et al., 1990; Takemoto, 1992). Generally considered a non-host specific phytotoxin (Gross & DeVay, 1977), syringomycin also inhibits the growth of fungi, particularly yeasts such as Saccharomyces cerevisiae and Rhodotorula pilimanae (Zhang & Takemoto, 1986, 1987). Thus, it may play dual roles as a plant virulence factor and an antifungal agent against fungal competitors on plant surfaces (Takemoto, 1992). Several structurally related cyclic lipodepsipeptides produced by P. syringae strains have been identified. These include the syringostatins produced by a lilac isolate (Fukuchi et al., 1992), syringotoxin produced by citrus isolates (Ballio et al., 1990), and the pseudomycins produced from a barley isolate mutated with a transposon (Ballio et al., 1994).

The cellular site of action of syringomycin is the plasma membrane. It alters several membrane functions such as membrane potential, protein phosphorylation, H+-ATPase activity and cation-transport fluxes (Zhang & Takemoto, 1987, 1989; Bidwai et al., 1987; Bidwai & Takemoto, 1987; Suzuki et al., 1992). The relationships of these activities and their relative importance in the response to syringomycin exposure are not clear. To learn more about the mode of action of syringomycin, recessive syringomycin-resistant strains of S. cerevisiae were isolated and characterized (Takemoto et al., 1991, 1993). One class of mutants (yr7) were deficient in membrane ergosterol and sensitive to high external Ca2+ levels. yr1 mutants were complemented by gene SYR1 (identical to ERG3) that encodes the ergosterol biosynthetic enzyme C-5 sterol desaturase (Taguchi et al., 1994). Therefore, ergosterol is necessary for syringomycin action, and the binding of syringomycin to this lipid in the plasma membrane has been proposed (Julmanop et al., 1993). Other classes of syringomycin-resistant mutants with normal ergosterol levels have been identified (Takemoto et al., 1993). Such mutants presumably have defects in other genes whose products are necessary for syringomycin action.

In this paper, we describe the cloning and characterization of yeast gene SYR2 that complements certain syringomycin-resistant mutants with normal ergosterol levels. SYR2 is shown to be identical to SUR2 (Desfarges et al., 1993), which is involved in survival during nutritional starvation. The SYR2-encoded protein is shown to associate with the endoplasmic reticulum and to have a role in phospholipid metabolism. The findings suggest that phospholipids are important for syringomycin action.

METHODS

Organisms and general genetic methods. S. cerevisiae syringomycin-resistant mutants 13N-F2 and 3N-H1 were isolated from haploid strain KZ1–1C (MATa his3 leu2 trp1 ura3), and were previously described by Takemoto et al. (1993). Syringomycin-

Abbreviation: GST, glutathione S-transferase.

The GenBank accession number for the sequence reported in this paper is SCU10427.
resistant mutant 28N-D6 was also derived from KZ1-1C and was shown by diploid analysis to be in the same genetic complementation group as 13N-F2. Resistant mutant 3N-H1 was defective in SYR1 (also designated ERG3), which encodes the sterol biosynthetic enzyme C-5 sterol desaturase (Taguchi et al., 1994). For gene cloning, Escherichia coli strains DH5α and HB101 were used (Sambrook et al., 1989). Yeast plasmid shuttle vectors YCpN1 (Liu et al., 1991), YEp50 (Rose et al., 1987), YEp24 (Rose & Broach, 1991) and pYES2 (Invitrogen) were used. Genomic libraries of KZ1-1C were prepared in YCp50 and YEp24 as described by Rose &负债 (1991). Yeast extract-peptone-dextrose (YPD), minimal salts-dextrose minus uracil (SD-Ura) and minimal salts-galactose minus uracil (SG-Ura) growth media were prepared as described by Rose et al. (1990). Syringomycin was added to growth media at concentrations ranging from 0.5 to 2.0 μg ml⁻¹. Yeast transformations were done using the lithium acetate method (Ito et al., 1983) or by electroporation (Becker & Guarente, 1991). After transformation, yeast cells from single colonies growing on selective agar media were suspended in 100 μl water in microtitre plate wells. Cells were replica-plated onto YPD agar medium and YPD agar medium containing syringomycin. After 48 h the plates were visually inspected for clones showing little or no growth on syringomycin-containing plates but normal growth on plates without syringomycin. Genetic complementation tests of syringomycin-resistant strains were performed as previously described by Takemoto et al. (1993).

Syringomycin purification. Syringomycin (E form) was purified as described previously by Bidwai & Takemoto (1987).

Nested deletion subcloning of SYR2. Nested deletions were performed using the Erase-a-Base system (Promega). A 4.8 kb SYR2 clone (Spbl-Spbl) contained in pBluescript II (Stratagene) was used. The plasmid was linearized at KpnI and Spbl and Spbl restriction sites, and then subjected to exonuclease III digestion for varying lengths of time to unidirectionally excise portions of insert DNA. After exonuclease III excision, the DNA was treated in DNA ligase according to the manufacturer's protocol. The resulting SYR2 subclones were transferred to YCpN1 to test for functional complementation of yr2 strains.

SYR2 nucleotide sequence determination. DNA sequencing was performed using the dideoxynucleotide chain-termination method (Sanger et al., 1977). Sequences were determined on both strands from clones prepared by nested deletion or from clones prepared with suitable restriction enzymes in pGEM-3Z (Promega).

Northern blot analysis. Yeast total RNA was isolated using TRI-Reagent (Molecular Research Center). RNA was treated with glyoxal and electrophoresed on 1.4% (w/v) agarose gels as previously described (Franke et al., 1987). RNA fragments were transferred to nylon membranes as described by Sambrook et al. (1989). DNA probes were labelled with the ECL direct labelling and detection system (Amersham) and used for hybridization to the RNA fragments. A 1.2 kb PstI–Spbl SYR2 fragment created using PCR was used as the probe.

Cell fractionation and Western blot analysis. Two cell fractionation methods were employed. For small-scale preparations, the glass-bead lysis method of Harlow & Lane (1988) was used. For large-scale preparations, a procedure similar to one described by Serrano (1983) was used. Yeast cells were grown to late exponential stage in 500 ml YPD medium. Cells were centrifuged in a Beckman JA10 rotor at 5000 r.p.m. for 10 min, and the cell pellets were washed with 1.5 M sorbitol. The cells were resuspended in 40 ml homogenizing buffer (0.1 M Tris/HCl, pH 7.5, 5 mM EDTA, 2 mM DTT) and 6% (w/v) sorbitol. Zymolyase 100T (Seikagaku America; 200 U) was added. Cells were incubated at 30 °C for 60–90 min. Spheroplasts were collected, resuspended in 40 ml ice-cold homogenizing buffer, and lysed using 15–20 strokes in a chilled Dounce glass homogenizer. Lysates from both preparations were centrifuged at 100000 g for 30 min at 4 °C to isolate the pellet crude membranes. Crude membranes from the large-scale isolation were fractionated using a sucrose step gradient containing 3 ml 53% (w/v) sucrose, 3.5 ml 43% (w/v) sucrose and 3.5 ml 26% (w/v) sucrose. The gradient was centrifuged overnight at 68000 g in a Beckman SW41 rotor at 4 °C. Plasma and endoplasmic reticulum membrane fractions were collected from the lower and upper interfaces, respectively. Vanadate-sensitive H⁺-ATPase and NADPH-cytochrome-c oxidoreductase activities were used to verify the identification of the plasma and endoplasmic reticulum membrane fractions, respectively (Marriot & Tanner, 1979). Samples (30–50 μg protein per lane) were analysed by SDS-PAGE. After electrophoresis, the proteins were transferred to nitrocellulose membranes and Western-blotted as described by Harlow & Lane (1988).

Protein determination. Protein concentrations were determined using Coomassie Protein Assay Reagent (Pierce) and BSA as standard.

Alkaline phosphatase treatment of crude membranes. Crude membranes (50 μg protein) in 200 μl phosphatase buffer (10 mM Tris/HCl, pH 9, 1 mM ZnCl₂, 1 mM MgCl₂) were treated with 17 U alkaline phosphatase (Boehringer Mannheim) at 37 °C for 30 min.

Preparation of Syr2 fusion protein and antisera. The bacterial vector pGEX-KG (Guan & Dixon, 1991) was used to prepare a Syr2–glutathione-S-transferase (GST) fusion protein. Initially, an EcoRI–SalI fragment containing intact SYR2 was cloned into the vector. Expression of this GST–Syr2 fusion protein severely restricted the growth of E. coli and the fusion protein was not isolated in sufficient quantities. Subsequently, the HpaI–Spbl fragment from SYR2 (encoding the C-terminal 124 amino acids) (Fig. 2b) was inserted in-frame with the GST sequences to produce a GST–Syr2 fusion protein. The 5′-end of the fragment (HpaI) was ligated with blunt ends to the SalI site of the pGEX-KG vector. A HindIII site was borrowed from pBluescript II phagemid (Stratagene) and used to clone the 3′-end of SYR2 into pGEX-KG at the corresponding site. E. coli HB101 was transformed with the construct. Upon IPTG induction, a 40 kDa polypeptide presumed to be the fusion product was produced. The fusion protein was purified and the Syr2 peptide was cleaved with thrombin as described by Smith & Johnson (1988). Peptide Syr2 was purified by SDS-PAGE using a 8–15% (w/v) gradient polyacrylamide gel. After staining with Coomassie Blue, the Syr2 band was cut from the gel. The gel slice was fragmented and prepared for injection as described by Harlow & Lane (1988). Rabbit antisera were prepared by immunizing rabbits with 75 μg protein in Freund’s complete adjuvant. Booster injections of 40 μg protein in Freund’s incomplete adjuvant were given 10–14 d before collection of sera. The sera were used directly for Western blot analysis.

To confirm the Syr2 peptide sequence, the peptide was transferred to Immobilon-P membrane (Millipore) using the method of LeGendre & Matsudaira (1989). The membrane was stained with Coomassie Brilliant Blue, the Syr2 fragment was excised, and the N-terminus (31 amino acids) was sequenced at the Utah State University Biotechnology Center (Logan, UT, USA). The sequence included two residues (Gly-Ser) encoded by vector sequences remaining after thrombin cleavage.
**SYR2 disruption.** A 2.3 kb pBluescript clone of *SYR2* created by nested deletion was used to construct the *SYR2*-disruptant strain. Plasmid C1 was constructed by replacing the *HpaI*-EcoRV segment of *SYR2* with a 1.2 kb *HindIII* fragment containing UR.A3 from plasmid YEp24. The C1 plasmid was digested with *SacI* and *SpI*, and the resulting 3.14 kb linear fragment was purified and used to transform diploid strain W303–1A (MATa/MATα his3 trpl ura3::LEU2). The one-step gene disruption method was used (Rothstein, 1983). Transformants were induced to sporulate, and the spores were dissected for tetrad analysis (Rose et al., 1990). A typical *SYR2*-disrupted segregant was selected and designated strain W303C.

**SYR2 overexpression.** The *PstI*-*SpI* fragment of *SYR2* was cloned into the GAL1 promoter-containing vector pYES2 to yield pYSYR2. pYSYR2 allowed overexpression of *SYR2* in SG-URA growth medium. The *PstI*-*SpI* fragment was missing the first ATG start codon and the following 24 codons, yielding an expressed protein that was shorter than intact Syr2.

**Chromosomal localization.** Yeast chromosomes were separated by PFGE using a Bio-Rad CHEF DR-II system. Chromosomal DNA was electrophoresed in 1% (w/v) agarose at 200 V. Pulse time was 60 s for 15 h followed by 90 s for 9 h. DNA for Southern blotting was digested with appropriate restriction enzymes and separated on 0.7% (w/v) agarose gels. DNA was transferred to nylon membranes as described by Sambrook et al. (1989). The *SYR2* probe was the same as described above for Northern blot analysis. A *Dictyostelium* actin-8 probe was also used, prepared by digesting an actin-8 clone contained in pMB9 (Cooper, 1977). Individual phospholipids were resolved by one-dimensional TLC (silica gel G-60, Merck; 20 x 20 cm) using chloroform/methanol/acetonic acid/water (25:15:4:2, by vol.) as the developing solvent. For phospholipid detection, plates were sprayed with molybdenum blue reagent (Dittmer & Lester, 1964). Authentic standards of phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol were used as standards. For ergosterol analysis, non-saponifiable lipid extracts (Radin, 1969) were chromatographed on silica gel G-60 (Merck; 20 x 20 cm) thin-layer plates using n-hexane/diethyl ether/acetone (70:30:1, by vol.). After chromatography, the plates were sprayed with phosphomolybdate spray reagent (Sigma) for visualization of ergosterol.

**RESULTS**

**SYR2 cloning**

Genetic complementation analyses showed that syringomycin-resistant strains 13N–F2 (Take moto et al., 1993) and 28N–D6 had closely linked mutant alleles designated *syr2*. In contrast to other mutations that conferred syringomycin resistance, *syr2* mutations did not affect ergosterol biosynthesis (Take moto et al., 1993; Taguchi et al., 1994). Strains 13N–F2 and 28N–D6 were transformed with genomic libraries prepared in yeast centromeric vectors YCpN1 and YCp50. Inserts for the two libraries averaged 12 kb. Approximately 5000 transformants of each strain were screened for phenotypic complementation of *syr2*. Transformants were replicated onto YPD medium containing 1–2 μg syringomycin ml⁻¹ and inspected for growth after 48 h. Plasmids were isolated from transformants that showed little or no growth with syringomycin. Five clones were isolated with inserts ranging from 10.5 to 14 kb. Restriction fragment analysis revealed that the clones were allelic, containing large overlap regions (data not shown). The smallest clone, 246–D6 (derived from a YCPN1 library), was selected for isolation of the *SYR2* gene (Fig. 1). The gene was subcloned on a 2.5 kb BstBI fragment with restriction endonucleases and then on a 1.68 kb fragment by nested deletion. Smaller subclones generated by S1 nuclease treatment were not expressed in yeast, suggesting that coding or upstream regulatory sequences had been excised.

A portion of the 1.68 kb fragment was used as a probe for chromosomal mapping of *SYR2*. Yeast chromosomes of strain KZ1–1C were separated by CHEF gel electrophoresis and blotted onto nylon membranes. Hybridization of the *SYR2* probe to chromosomal blots revealed that *SYR2* is located on chromosome IV (Fig. 2). To look for genes having similar or overlapping function with *SYR2*, the strain KZ1–1C genome was hybridized to the *SYR2* probe using low stringency conditions. No similar genes were found under hybridization conditions that allowed *Dictyostelium* actin-8 (Romans & Firtel, 1985) to bind yeast actin genes (approximately 78% identity) (data not shown).

**Sequence analyses**

A 2 kb fragment that included the 1.68 kb fragment was sequenced. An ORF sequence was located, beginning at ATG position 270, containing 1047 nucleotides and
predicted to encode a protein of 349 amino acids (Fig. 3). The ORF was preceded by regulatory sequences (CAAT) and (TATAA) beginning at positions -259 and -128, respectively. Northern blot analysis showed a transcript of approximately 1-4 kb (data not shown), consistent in size with a polyA-modified transcript encoded by the ORF. The predicted amino acid sequence was confirmed by sequencing the N-terminal 31 amino acids of a TH2 fusion protein. The determined sequence (Thr227-Ala253) corresponded to the predicted sequence.

SYR2 (GenBank accession no. SCU10427) was identical to SUR2 (GenBank accession no. SCU07171), identified in S. cerevisiae and related to survival during nutrient starvation (Desfarges et al., 1993). No other homologues of the encoded Syr2/Sur2 protein were found in the standard protein computer databases.

Syr2 possessed the C-terminal dilysyl motif for retrieval to the endoplasmic reticulum (Letourneau et al., 1994). Syr2 hydropathy profiles suggested six potential membrane-spanning regions with hydrophobicity exceeding 1.25 using the parameters of Kyte & Doolittle (1982) (Fig. 4). The C-terminus contained 26% lysine residues within a stretch of 50 residues, giving Syr2 an overall basic character. A pI of 9.32 was calculated. Potential protein phosphorylation and N-glycosylation sites were revealed using computer programs ProFLiEscan and Motif (GCG Corporation, Madison, WI, USA) (Altschul et al., 1990).

SYR2 disruption
All four meiotic segregants recovered after SYR2 disruption in diploid strain W303-1A were viable in YPD growth medium. A 2:2 cosegregation of syringomycin...
Syringomycin inhibition of *S. cerevisiae*

**Fig. 5.** Disruption of SYR2. (a) Structure of the DNA fragment used for the construction of the SYR2 null allele. (b) Tetrad analysis of the disrupted diploid showing cosegregation of syringomycin resistance and the URA3 marker. (c) Southern blot analysis of *PstI*-digested genomic DNA from diploid strain W303-1A (lane 1) and SYR2-disruptant WASYR2 (lane 2). The URA3 marker contains a *PstI* site 1.6 kb downstream of the SYR2 *PstI* and creates two bands in the null allele.

Resistance and ura* phenotypes occurred, indicating *URA3* replacement in the wild-type SYR2 allele (Fig. 5). Disruption was confirmed by Southern blot hybridization (Fig. 5c). No SYR2 transcripts were detected by Northern blot analyses in the disruptant strains. The WASYR2 segregants were viable in SD media and insensitive to ethanol, heat shock and high concentrations of sorbitol (1.25 M) and Na⁺ (1 M). They grew with maltose, galactose, glycerol and acetate as carbon sources.

**Syr2 detection and cell localization**

Western blot analysis using antisera directed against Syr2 identified an approximately 43 kDa immunoreactive protein, which was slightly larger than the predicted mass of Syr2. The protein was detected in whole-cell extracts, microsomal fractions and endoplasmic reticulum membranes, but not the plasma membrane (Fig. 6). The strongest immunoreaction occurred with the cellular fractions enriched for endoplasmic reticulum markers. Syr2 was not detected in plasma membrane fractions (lane 4) or microsomal fractions prepared from SYR2-disrupted mutants (lane 5). Pre-immune sera did not react with microsomal extracts (lane 5).

In extracts from SYR2-overexpression strains, two equally intense immunoreactive bands on Western blots were often observed. This observation raised the possibility that Syr2 exists in two forms. To test the possibility that one form may be phosphorylated, crude membrane preparations were treated with alkaline phosphatase before SDS-PAGE and Western blotting. Both forms were still detected in the phosphatase-treated samples (data not shown). Likewise, no glycosylated form of Syr2 was evident after staining equivalent gels and blots with periodic acid/Schiff base reagent (data not shown).
Fig. 7. Ergosterol levels in SYR2-overexpressing and disruption strains. Equal amounts (1 mg) of total cell nonsaponifiable lipid extracts were chromatographed on thin-layer silica gel plates and stained with phosphomolybdic acid reagent. Authentic ergosterol (1 mg) was applied for comparison. The chromatographic origin (0) is indicated.

Table 1. Total cell phospholipid levels of SYR2-overexpression and disruption strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth medium</th>
<th>Lipid phosphate [ug (mg dry wt cells)-1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>KZ1-1C(pYSYR2)</td>
<td>SD-URA</td>
<td>1.35 (0.31)</td>
</tr>
<tr>
<td>KZ1-1C(pYSYR2)</td>
<td>SG-URA</td>
<td>2.47 (1.19)</td>
</tr>
<tr>
<td>WASYR2</td>
<td>YPD</td>
<td>1.71 (0.73)</td>
</tr>
<tr>
<td>W303C</td>
<td>YPD</td>
<td>3.88 (1.03)</td>
</tr>
</tbody>
</table>

*Values are means from three separate experiments. Standard deviations (n-weighted) are indicated in parentheses.

Fig. 8. Phospholipid profiles of SYR2-overexpressing and disruption strains. Equal amounts (10 mg) of total cell lipid extracts of strains KZ1-1C(pYES2) grown in SD-URA medium (lane 1) and SG-URA medium (lane 2), KZ1-1C(pYSYR2) grown in SD-URA (lane 3) and SG-URA medium (lane 4), WASYR2 (lane 5) and W303C (lane 6) were chromatographed on thin-layer silica gel G-60 plates and stained with molybdenum blue reagent. Positions of authentic lysophosphatidylcholine (LP), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) are indicated. The chromatographic origin (0) is indicated.

SYR2 overexpression

To further analyse SYR2 function, overexpression vector pYSYR2, containing the PstI-SphI portion of SYR2 under the regulation of the GAL7 promoter, was constructed. When yr2 mutant strains 13N-F2 and 28N-D6 were transformed with pYSYR2 and grown in SG-URA medium, sensitivity to syringomycin was restored. Transformants W303C(pYSYR2) and KZ1-1C(pYSYR2) displayed syringomycin sensitivities comparable to the corresponding nontransformed strains, W303C and KZ1-1C, when grown in galactose-containing media. Also, pYSYR2 transformants of yr1 mutant 3N-H1 maintained levels of resistance to syringomycin in SG-URA medium similar to those of nontransformants. SYR2 overexpression was confirmed by Northern blot and Western blot analyses.

Sterol and phospholipid compositions

The cellular ergosterol levels of disruptant strain WASYR2 and strain W303C were similar (Fig. 7). Also, the ergosterol levels of KZ1-1C(pYSYR2) cells grown in minimal medium with either galactose or glucose were similar (Fig. 7). In contrast, WASYR2 had lower levels of total cellular phospholipid (Table 1) and of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol (Fig. 8) than W303C. On the other hand, KZ1-1C(pYSYR2) cells grown with galactose had higher total cellular levels of phospholipids (Table 1) and of phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol when compared to cells grown with glucose (Fig. 8).

DISCUSSION

It was determined in the present study that a novel gene, SYR2, is required for yeast growth inhibition by syringomycin. SYR2 differs from other identified syringomycin response genes since it does not involve ergosterol biosynthesis (Takemoto et al., 1993). Although its precise biochemical function is not known, SYR2 appears to have a role in cellular phospholipid biosynthesis. SYR2 is not essential for viability and, therefore, it is unlikely that it catalyses a critical step in the phospholipid biosynthetic pathways. Mutant yr2 alleles, however, lead to syringomycin resistance, indicating that normal phospholipid
levels are required for syringomycin action. Previous work showed that the plasma membrane is the site of action of this lipodepsipeptide (Takemoto, 1992), and we suggest that the two major lipid classes of the yeast plasma membrane, ergosterol and phospholipids, are important for its action.

SYR2 is identical to the yeast gene SUR2. Mutant syr2/sur2 alleles, as well as sur1, sur3 and sur4 mutations, are suppressors of rvs161 (Desfarges et al., 1993), which impairs survival of yeast during nutrient starvation. Like syr2/sur2, sur1 and sur4 lead to alterations in the relative levels of various phospholipids. Desfarges et al. (1993) have hypothesized that all four identified SUR genes function in the same biochemical pathway.

Sequence analysis of Syr2 revealed several potential sites for phosphorylation and N-glycosylation. However, evidence for covalent modification of Syr2 was not observed. Syr2 was often seen as a doublet on Western blots of crude extracts obtained from SYR2-overexpression strains. The doublet may reflect a modified or degraded form of Syr2. The overexpressed Syr2 did not possess an N-terminal signal sequence, eliminating the possibility that one form resulted from cleavage of a signal peptide.

Hydropathy profiles suggested that Syr2 is a membrane protein. As expected for an enzyme involved in phospholipid biosynthesis, an association with the endoplasmic reticulum was demonstrated. This location is consistent with the dilysyl motif at the C-terminus, which is an endoplasmic reticulum retrieval and coatamer interacting signal (Letourneur et al., 1994). Yeast proteins Wbp1p and C-5 sterol desaturase (encoded by ERG3/SYR1) also contain this signal (Taguchi et al., 1994; Arthington et al., 1991; Te Heesen et al., 1992; Letourneur et al., 1994), and in the former, the dilysyl motif occurs in the cytoplasmic domain (Townsley & Pelham, 1994; Gaynor et al., 1994). By analogy, we suggest that the Syr2 hydrophilic C-terminus is exposed on the cytoplasmic side of the endoplasmic reticulum.

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