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Aspergillus nidulans polarity mutant *swoA* is complemented by protein *O*-mannosyltransferase *pmtA*

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Abstract

Previously *swoA* was identified in *Aspergillus nidulans* as a single locus, temperature-sensitive (ts) mutant aberrant in polarity maintenance. *swoA* was complemented by transformation with a plasmid genomic library. The sequence of the complementing gene was identical to a previously submitted GenBank sequence for *pmtA*, a protein *O*-mannosyltransferase. The *pmtA/swoA-2* gene hybridized to three cosmids that are located on chromosome V and the *swoA* mutation was mitotically mapped to chromosome V. PMTs are endoplasmic reticulum-resident proteins responsible for the first step of *O*-glycosylation. Structural predictions suggest that PmtA contains seven membrane spans similar to PMTs from *Saccharomyces cerevisiae* and other organisms. Phylogenetic analysis indicates that PmtA is most closely related to the *S. cerevisiae* sub-family of PMTs containing Pmt2, Pmt3 and Pmt6. The mutant *pmtA/swoA-2* locus contained a lesion that changed Y662 to a stop codon, eliminating the final membrane spanning domain and interrupting a domain essential for function in other PMTs.

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IDT: *Emericella nidulans*; Mannosylation; Polar growth; Polarity

1. Introduction

Polar growth is a fundamental process in filamentous fungi where the selective addition of new material exclusively at the growing tip is responsible for hyphal morphology. Many plant and animal pathogens rely on polar growth for disease initiation and invasion of substrates. In *Aspergillus nidulans* two distinct growth modes occur during the process of spore germination and early development. When dormancy is broken, the spore first expands isotropically, adding new cell wall material uniformly in every direction. A switch to polarized extension follows the first round of mitosis in *A. nidulans*, with new cell wall deposition occurring only at the hyphal tip (Momany and Taylor, 2000). Two processes are involved in the switch to polar growth; polarity establishment, or choosing the location where new material will be deposited, and polarity maintenance,

the continued deposition of wall material at the extending tip (Momany et al., 1999).

Among the *A. nidulans* mutants defective in polar growth are the ts *swo* (swollen cell) mutants, which are characterized by either continued isotropic growth without establishment of polarity or the inability to maintain polar growth at restrictive temperature. Temperature-shift experiments have shown that polarity establishment and polarity maintenance are genetically separable (Momany et al., 1999). The N-myristoyl transferase SwoF is involved in both establishment and maintenance of cellular polarity (Momany et al., 1999; Shaw et al., 2002).

swoA was previously identified as a single locus ts mutant that failed to switch from isotropic to polar growth (Momany et al., 1999). At restrictive temperature (42 °C) each cell grew to a diameter of >20 µm and contained 64 or more nuclei over a period of 14 h. Temperature downshift experiments revealed that multiple points of polarity were established during growth at restrictive temperature, but that polar growth was not maintained unless the cell was shifted to permissive temperature. These multiple points of polarity were all

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located in one hemisphere of the mutant cell (Momany et al., 1999).

In this paper we describe the complementation of *swaA*. The gene that complements *swaA* is a dolichylphosphate-mannose: protein *O*-mannosyltransferase (PMT) that was previously deposited in GenBank as *pmtA* (Accession No. AF225551). We will refer to the mutant as *swaA* and the gene as *pmtA/swaA*. At the time of this writing, the depositors have not published their characterization of the gene. Protein mannosyltransferases are a seven-gene family in *Saccharomyces cerevisiae* with some degree of functional redundancy (Strahl-Bolsinger et al., 1999). PMTs have multiple membrane spanning domains and are found in the endoplasmic reticulum. They are responsible for the first step in *O*-glycosylation, the co-translational transfer of a mannose group from dolichol-P-mannose to a serine or threonine residue near the N-terminus of the target protein.

2. Materials and methods

2.1. Strains and media used

Strain A773 (*pyrG89*, *pyroA* 4, and *wA3*) was crossed with strain AGA7 (*swaA-2*, *pabaA*, and *biA1*) using standard methodologies (Harris et al., 1994; Kafer, 1977) to produce strain AXL4 (*pyrG89*, *swaA-2*, and *biA1*). All experiments reported herein used strain AXL4 as *swaA* and strain A773 as wild-type. Media used were as previously reported (Momany et al., 1999).

2.2. Growth of germlings and microscopic observation

Conditions for growth and preparation of germlings for observation were as previously reported (Momany et al., 1999). Microscopic observations were made using a Zeiss Axioplan microscope (Thornwood, NY, USA) and digital images were acquired using an Optronics digital imaging system (Goleta, CA, USA). Image preparation used Photoshop 5.5 (Adobe, Mountain View, CA, USA).

2.3. Complementation, plasmid recovery

A genomic library was generously provided by Greg May (University of Texas, M.D. Anderson Cancer Center, Houston, TX, USA). This library was constructed by ligating *Sau*3A fragments of genomic DNA into the *Bam*HI site of pRG3AMA1 (Oshero and May, 2000). Protoplasts were produced and transformation conducted using standard *A. nidulans* protocols (Yelton et al., 1984). Transformants were selected by assaying for restoration to *pyrG* prototrophy. Complementation was judged by restoration of wild-type growth at re-

strictive temp (42 °C). The complementing plasmid replicated autonomously (e.g., extra-chromosomally) due to the AMA1 sequence (Aleksenko and Clutterbuck, 1997) contained in the library vector. The complementing plasmid was recovered by transformation of *Escherichia coli* XL1-blue. Complementing plasmid p4c1 was chosen for sequencing.

2.4. Sequencing

p4c1 was transposon tagged using the GPS-1 kit (New England Biolabs, Beverly, MA, USA), and transformed into *E. coli* strain XL1-blue. Colonies representing individual randomly tagged plasmids were arrayed in a 96 well format. Plasmid preparation used the R.E.A.L. 96-well kit (Qiagen, Valencia, CA, USA). Label was incorporated using Big Dye 2.0 (Perkin Elmer Applied Biosystems, Boston, MA, USA). Sequencing used outward facing primers designed to the transposon, provided with the GPS-1 kit. Unincorporated dyes were removed using the DyeEx 96 Kit (Qiagen). Sequencing in a 96 well format was performed on an ABI Prism 3700 robotic sequencer (Foster City, CA, USA). Subsequent analysis used the programs Phred version 0.000925c and Phrap version 0.990319 for assembly and quality determination, and Consed version 11.0 for sequence viewing and recovery (all three Unix based programs available from <http://depts.washington.edu/ventures/collabtr/direct/ppccombo.htm>). All sequences contained at least four-fold redundancy with a quality rating of at least 20.

2.5. Identification of the complementing gene

Complete sequence for complementing genomic DNA was blasted against the National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov) using the blastX protocol. Two open reading frames were found within the genomic sequence. Transposon tagged plasmids with strategically placed transposon insertions were chosen from the plasmid array to test for complementation. Transposon tagged plasmids were transformed into AXL4. Transformants were replica plated to permissive and restrictive temp. The open reading frame which when disrupted by transposon insertion lost the ability to restore AXL4 to wild-type growth at 42 °C was identified as the complementing gene.

2.6. Protein alignment and structural prediction

Sequences for orthologues of PMTs were obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>). Protein sequence alignment was carried out using the program GeneDoc version 2.6.001 (www.psc.edu/biomed/genedoc) with default parameters and minimal manual adjustment to align sequences with secondary structural

features. Membrane spans were predicted using TMpred version 2.0 (http://www.ch.embnet.org/software/TMPRED_form.html) an algorithm designed to predict transmembrane helices from protein sequence, as well as by comparison to other known PMTs. The phylogenetic tree was built using ClustalX version 1.81 (www-igbmc.u-strasbg.fr/BioInfo/) bootstrap neighbor joining tree utility with the following parameters: exclude gap positions, correct for multiple substitutions, and with 1000 bootstrap trials. The tree was exported in New Hampshire format for viewing using Treeview version 1.6.6 (<http://taxonomy.zoology.gla.ac.uk/rod/rod.html>). The tree was then exported to Photoshop for image preparation.

2.7. Sequencing of the *pmtA*/*swoA-2* mutant allele

Genomic DNA from AXL4 was isolated using standard methods (Sambrook et al., 1989). This genomic DNA was used as template in a PCR reaction with the Expand High Fidelity PCR System (Roche Diagnostics, Indianapolis, IN, USA). The following primers were used for PCR amplification *swoA* 53 1–20 5-ATGGCTGAAA TTGGCTTTGC-3 and *swoA* 35 2460–2440 5-TTAGTT AGCGATTCGCCAAC-3. PCR products were cloned into the pGEM-T Vector System (Promega, Madison, WI, USA), transformed into *E. coli* XL1-Blue, and selected on ampicillin medium with blue/white selection. Clones were verified by restriction analysis. Three clones were sequenced using the strategy described above. A second round of PCR cloning and sequencing was carried out to verify the mutant lesion and used primers *swoA* 35 2460–2440 and *swoA* 53 1651–1670 5-CACTAATCTTC CTCAGTGGG-3. Three clones were selected, verified by PCR and sequenced. Sequences of all six clones were compared to the wild-type to determine the mutant lesion. All six clones showed the same change relative to the wild-type sequence.

2.8. Mapping of *pmtA*/*swoA-2*

Aspergillus nidulans chromosome specific cosmid libraries pWE15 and pLORIST2 (<http://www.fgsc.net>) were arrayed and transferred to nylon membranes and a ³²P-labeled *Clal* fragment of p4c1 containing the *pmtA*/*swoA* gene was hybridized to the membrane using standard methods (Sambrook et al., 1989). The *pmtA*/*swoA* probe hybridized to five cosmids from chromosome V. Cosmids which hybridized with the *pmtA*/*swoA*-containing fragment were identified on the *A. nidulans* physical map website (<http://gene.genetics.uga.edu/>).

To verify the chromosome V location of *swoA* gene, we took advantage of the parasexual cycle in *A. nidulans* (Kafer, 1977) to mitotically map *swoA*. A heterozygous diploid was made between AGA7 (*swoA*, *pabaA*, and *biA1*) and the mitotic mapping strain A104 (*yA2*, *ade20*, *AcrA1*, *phenA2*, *pyroA4*, *lysB5*, *sB3*, and *coA1*), which

has a marker on each chromosome. Diploid conidia were plated on complete medium, treated with the microtubule destabilizing drug benomyl (60 µg/ml) for two days to stimulate chromosome loss and transferred to complete medium without benomyl for 10 days. Conidia from the resulting haploid sectors were tested for their genotypes. In 28 sectors analyzed, the *ts swoA-2* allele segregated in repulsion to chromosome V markers supporting the assignment of *swoA* to chromosome V. No other markers segregated in repulsion to *swoA*.

Meiotic mapping of *swoA* used standard methodologies (Kafer, 1977) and crossed AXL4 (*pyrG* 89, *swoA-2*, and *biA1*) with several chromosome V mapping strains from the Fungal Genetics Stock Center (<http://www.fgsc.net/>) including: A258 (*nicA2*, *hxA1*, *facA303*, and *riboD5*), A296 (*biA1*, *lysE13*, and *sB3*), A491 (*acrA1*, *lysB5*, *pA2*, *facA303*, *hxA2*, and *riboD5*), and A495 (*lysB5*, *nicA2*, and *pA2*). Progeny were replicated on appropriate selective media and the number that were recombinant for any two markers was divided by the total number of progeny to give the map unit (centiMorgan) distance. Any value equal to or greater than 50 cM indicates unlinked markers. In analysis of 610 progeny *swoA* was weakly linked to *nicA* (36.9 centiMorgans). No linkage was found to markers *lysB*, *pA*, *facA*, *lysE*, or *riboD*. Scoring of recombination with *hxA* was not possible due to difficulties excluding nitrate from the medium.

3. Results

The *swoA* mutant was previously identified as a *ts* mutant that was unable to maintain polarity (Momany et al., 1999). At restrictive temperature wild-type germlings are characterized by long primary and secondary germ tubes and an even distribution of nuclei along the hyphae. Each hypha is typically 4–5 µm in diameter and at least 100 µm long after 14 h (Figs. 1a and c). *swoA* cells at restrictive temperature typically grow isotropically and do not send out germ tubes. Each large round cell can reach 20–30 µm in diameter and contain at least 64 nuclei by 14 h (Figs. 1b and e).

3.1. Complementation, plasmid recovery

Strain AXL 4 (*swoA-2*, *pyrG*, *biA1*) was transformed with a genomic library constructed in the vector pRG3AMA1, which carries the *pyr-4* gene from *Neurospora crassa* and the AMA1 sequence for plasmid autonomous replication. A total of 912 transformants were screened for growth at restrictive temp (42 °C). Transformant 4c1 showed a wild-type phenotype at restrictive temp (Figs. 1d and f). The complementing plasmid, p4c1, was selected for sequencing by transposon insertion.

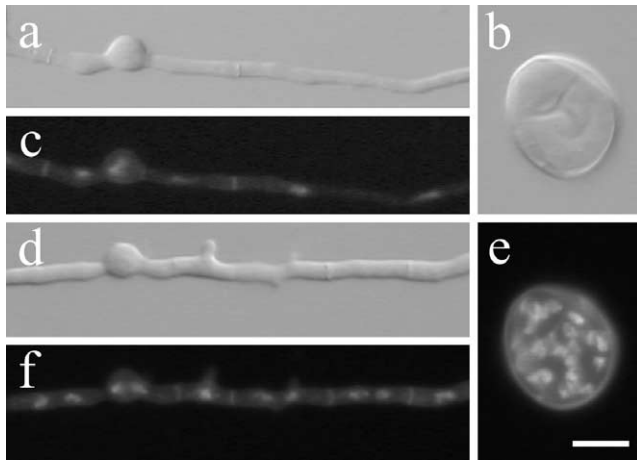


Fig. 1. Wild-type, *swoA* and complemented cells grown in minimal medium for 14 h at restrictive temp (42 °C). (a), (b), and (d) Differential interference contrast. (c), (e), and (f) Fluorescence images of cells costained with Calcofluor to reveal cell walls and septa and Hoechst to reveal nuclei. (a) and (c) Wild-type strain A773. (b) and (e) *swoA* strain AXL4. (d) and (f) AXL4 transformed with p4c1 containing wild-type *pmtA/swoA*. Bar = 10 μ m.

3.2. Sequencing

Sequencing of the genomic insert in p4c1 returned two contigs of 6037 bp and 4237 bp. GenBank searches with each sequence revealed that the 6 kb contig contained two open reading frames, a protein *O*-mannosyltransferase (PMT) and a glucose transporter (Fig. 2a). The 4.2 kb contig contained one open reading frame, a mitochondrial transport protein (data not

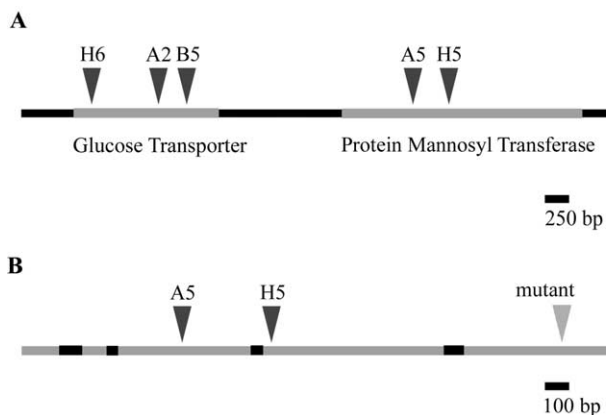


Fig. 2. Complementing genomic DNA and *pmtA/swoA*. (a) Schematic representation of 6037 bp genomic fragment that complements *swoA*. Two open reading frames were revealed by a search of GenBank, the mannosyltransferase gene and a glucose transporter. Locations of five transposon insertions are denoted by gray arrowheads. Transposon A5 and H5 disrupted the complementation of *swoA*. Bar = 250 bp. (b) Schematic representation of *pmtA/swoA* (protein mannosyltransferase), a 2464 bp open reading frame containing four introns represented by black boxes. Light gray arrowhead shows the location of the mutant lesion that changed Y662 to a stop codon. Bar = 100 bp.

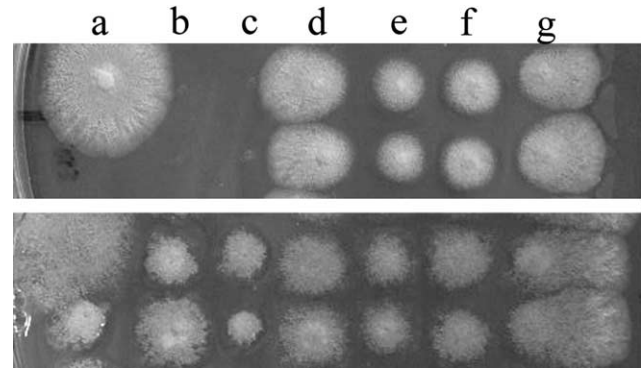


Fig. 3. Transposon insertion verifies that the protein mannosyltransferase complements *swoA*. Colonies of *swoA* cells grown at restrictive temp (42 °C) above or permissive temp (30 °C) below the line. (a) Wild-type, top and *swoA*, below. (b)–(g) *swoA* transformed with plasmid 4c1 transposon insertion A5 (b) H5 (c), H6 (d), A2 (e), B5 (f), or containing no transposon insertion (g).

shown). Transformation of *swoA* cells with plasmids containing transposon disruptions in the PMT (A5 and H5), the glucose transporter (H6, A2, and B5), and the mitochondrial transport protein (not shown) revealed that only disruption of the PMT eliminated the ability of p4c1 to complement *swoA* temperature sensitivity (Fig. 3). Both tested transposon insertions within the PMT homologue failed to complement *swoA* (Figs. 3b and c). The complete sequence of this gene was recently independently submitted to GenBank as *pmtA* (Accession No. AF225551).

3.3. Alignment

Alignment of the PmtA/SwoA sequence with several known PMT proteins revealed a high degree of conservation (Table 1; Fig. 4). The highest identity and similarity (79% and 86%, respectively) is to a predicted PMT from *A. niger* var. *awamorrhii* found in GenBank (AAK77607). The highest identity and similarity to experimentally verified PMTs are to *S. cerevisiae* Pmt3p and Pmt2p (50%, 68%, and 50%, 66%, respectively). The N-terminal 60 residues of these proteins are poorly conserved, however, the remaining 680–700 residues are highly conserved. The algorithm TMpred predicts 11 possible transmembrane helices (Fig. 4). Similarly, Strahl-Bolsinger and Scheinost (1999) used TMpred to predict that *S. cerevisiae* Pmt1p also contains 11 possible transmembrane helices. Further characterization using a topology-sensitive monitor protein domain led to the model that ScPmt1p contains 7 membrane passes. The placement of the seven verified membrane spanning regions from ScPmt1 are indicated (Fig. 4). Transposon insertions that disrupted PmtA/SwoA function are indicated at W229 and K346. Additionally, the locations of three

Table 1
Protein identity and similarity of PmtA/SwoA with other protein mannosyl transferases

Species	Protein	GenBank Accession no.	Percent identity	Percent similarity	e Value
<i>Aspergillus niger</i> var. <i>awamorrhii</i>	PmtA	AAK77607	79	86	0.0
<i>Neurospora crassa</i>	Pmt	CAB99175	69	79	0.0
<i>Saccharomyces cerevisiae</i>	Pmt3	NP 014966	50	68	0.0
<i>Saccharomyces cerevisiae</i>	Pmt2	NP 009379	50	66	0.0
<i>Saccharomyces cerevisiae</i>	Pmt6	NP 0117158	44	61	e ⁻¹⁷³
<i>Saccharomyces cerevisiae</i>	Pmt1	>NP 010188	34	51	e ⁻¹⁰³

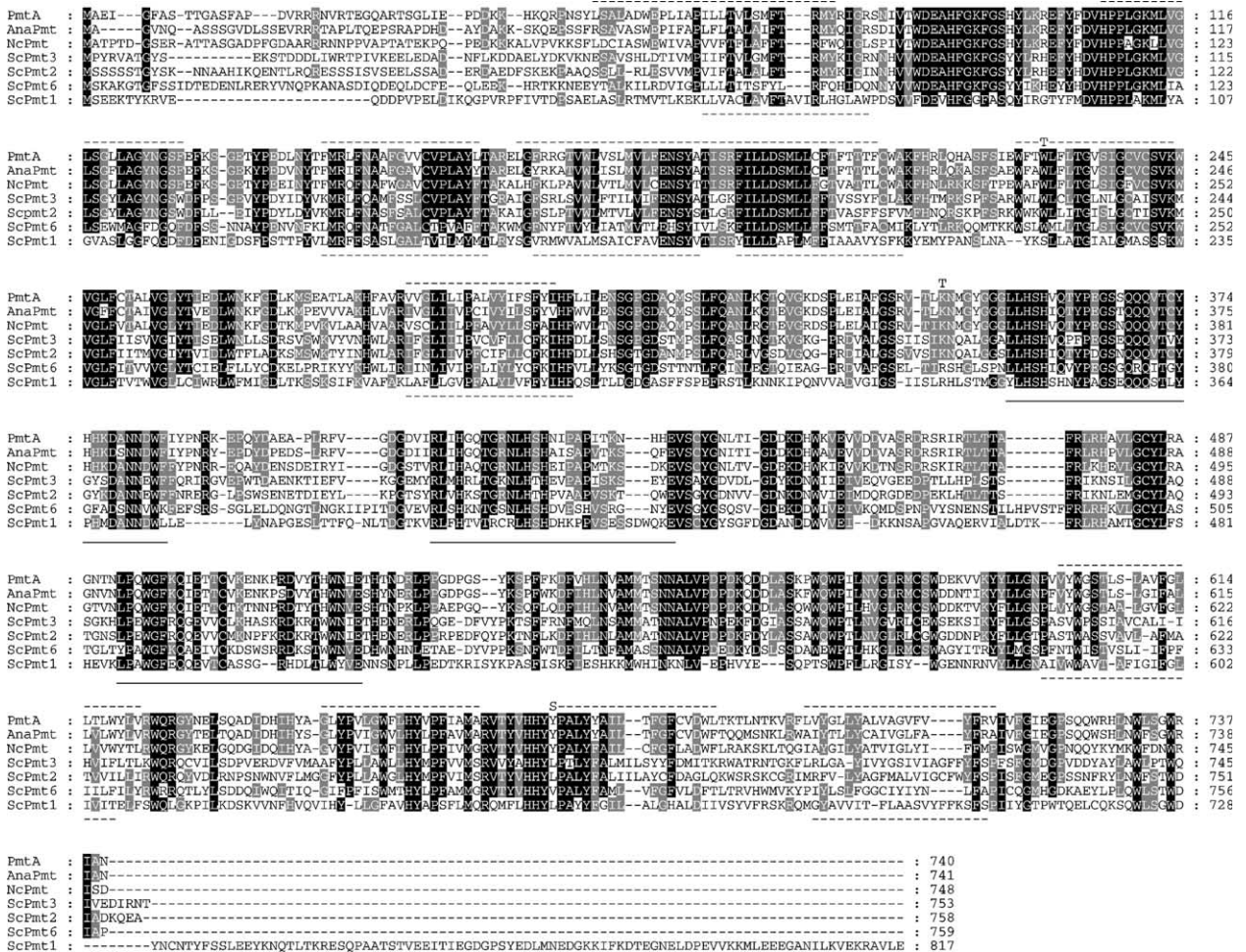


Fig. 4. Alignment of *A. nidulans* PmtA/SwoA, putative PMT from *A. niger* var. *awamorrhii* (AnaPmt), putative PMT from *N. crassa* (NcPmt), Pmt3 from *S. cerevisiae* (ScPmt3), Pmt2 from *S. cerevisiae* (ScPmt2), Pmt6 from *S. cerevisiae* (ScPmt6), and Pmt1 from *S. cerevisiae* (ScPmt1). Black shading shows residue similarity in at least five proteins. Gray shading shows similarity in at least three residues. Eleven predicted membrane spans are indicated by dashes above the sequence. The location of the seven verified membrane spans from *S. cerevisiae* Pmt1 are denoted by dashes below the SwoA sequence. The location of three predicted MIR domains is indicated by a solid line below the alignment. Two disruptive transposon insertions are marked with a T above the sequence. The mutant lesion in *sowA2* introducing a stop codon is marked above the sequence with an S.

active motifs previously identified in *S. cerevisiae* (Girrbach et al., 2000) and predicted in PmtA/SwoA by the NCBI conserved domain search (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) are indicated in a large region void of predicted membrane

spans. An unrooted phylogenetic tree was built to compare PmtA/SwoA to seven *S. cerevisiae* PMTs and two *Candida albicans* PMTs. PmtA/SwoA groups with the *S. cerevisiae* subfamily that includes Pmt2, Pmt3, and Pmt6, as well as with *C. albicans* Pmt6 (Fig. 5).

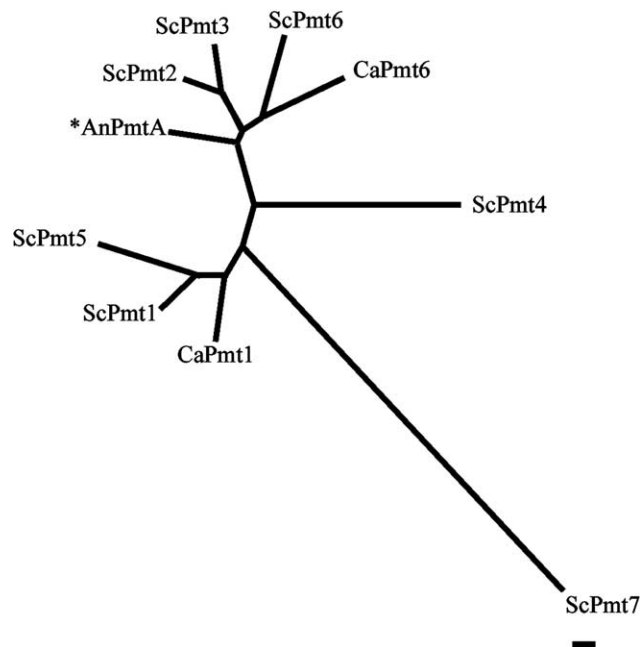


Fig. 5. An unrooted phylogenetic tree of PmtA/SwoA with other known PMTs. PmtA/SwoA (AnPmtA), as well as seven PMTs from *S. cerevisiae* (ScPMT1-7) and two PMTs from *C. albicans* (CaPmt1, 6) are included. PmtA/SwoA groups with the *S. cerevisiae* subfamily including Pmt2, Pmt3, and Pmt6. Bar = 0.1 nucleotide substitutions per site.

3.4. Sequencing of mutant *pmtA/swoA-2*

pmtA/swoA-2 was amplified by high fidelity PCR from *swoA-2* strain AXL4 and cloned. Six independent clones were sequenced. A single base substitution was detected (C2248A). This substitution is predicted to change amino acid residue Y662 in PmtA/SwoA to a stop codon. This lesion occurs between the predicted sixth and seventh membrane spans of the protein (Fig. 4).

3.5. Mapping of *pmtA/swoA-2*

Probing the *A. nidulans* chromosome specific cosmid libraries pWE15 and pLORIST2 with ^{32}P -labeled *pmtA/swoA* showed that the gene is resident on chromosome V. The probe hybridized to five cosmids from chromosome V: L23B03, L23H05, W10G09, W10G10, and W27C12. Three of these cosmids, L23H05, W10G09, and W27C12, are placed on the *A. nidulans* physical map (<http://www.genetics.uga.edu/>), within 50 kb of *hxA* on the right arm of chromosome V. Limited mitotic mapping placed *swoA* on chromosome V, though this result is based on a small number of ts^- isolates ($n = 28$ of 300 isolates scored). Based on the *A. nidulans* physical map, *swoA* should be linked to *hxA*. Difficulties excluding nitrates from the growth medium, however, precluded direct scoring of *hxA*. *swoA* showed no linkage to markers on the right arm of chromosome V and was

weakly linked to *nicA* (36.9 centiMorgans $n = 610$) on the left arm of chromosome V.

4. Discussion

It is likely that *pmtA* is *swoA*. Previously, it was shown that *swoA* is a single gene mutation (Momany et al., 1999). Sequencing of the *pmtA* gene from a *ts swoA-2* strain revealed that the gene contains a mutant lesion introducing a stop codon. This stop codon is expected to eliminate a loop region shown to be critical for function in *S. cerevisiae* (Girrbach et al., 2000). Both *swoA* and *pmtA* are on chromosome V. It remains a formal possibility, however, that *pmtA* is a suppressor of *swoA-2* and not the authentic *swoA* gene, as we were unable to show linkage between the physical map position of *pmtA* and the genetic map position of *swoA*. The linkage of *pmtA* to *hxA* on the physical map is strongly supported as all three *pmtA* hybridizing cosmids L23H05, W10G09, and W27C12 are linked through cross-hybridization with two *hxA* hybridizing cosmids, W14B05 and W17A06. Though we were unable to score the *hxA* marker because of technical difficulties excluding nitrates from the medium, our meiotic mapping shows no linkage of *swoA* with other markers in the region of *hxA*. It should be noted that the position of *hxA* relative to the centromere on the physical map (<http://gene.genetics.uga.edu/>) and the current genetic map (<http://www.fgsc.net>) are in disagreement. In fact the physical map does show *hxA* to be on the left arm of the chromosome similar to our placement of *swoA*, and not on the right arm as the genetic map suggests.

There are seven PMTs in *S. cerevisiae* and five in *C. albicans* (Ernst and Prill, 2001; Gentzsch and Tanner, 1996). Searches of the *A. nidulans* EST and the Cereon genomic databases indicate that *A. nidulans* has at least five PMTs (Shaw and Momany, unpublished). There are three major subfamilies of *Saccharomyces* PMTs: (1) Pmt1 and Pmt5, (2) Pmt2, Pmt3, and Pmt6, (3) Pmt4 (Ernst and Prill, 2001). *A. nidulans* PmtA/SwoA has highest identity and similarity to Pmt2, Pmt3, and Pmt6 (Table 1, Fig. 4) and groups in phylogenetic analysis with the same proteins (Fig. 5). In *S. cerevisiae* PMTs have some functional redundancy within the three main subgroups.

In addition to the alignment data two characteristic structural motifs help identify PmtA/SwoA as a PMT. *S. cerevisiae* Pmt1 has seven transmembrane helices with a central luminal loop occurring between spans 5 and 6 containing the catalytic domain (Girrbach et al., 2000; Strahl-Bolsinger and Scheinost, 1999). All *S. cerevisiae* and *C. albicans* PMTs appear to have the same topology (Ernst and Prill, 2001; Strahl-Bolsinger et al., 1999). Our analysis of PmtA/SwoA with TMpred also predicts

multiple membrane spanning regions (Fig. 4). Alignment with other PMTs suggests that the seven membrane-spanning model is also valid for SwoA (Fig. 4). The second characteristic structural motif is three MIR domains (Girrbach et al., 2000). These motifs are designated MIR domains because they are found in: protein *O*-Mannosyltransferases, Inositol trisphosphate receptors, and Ryanodine. PmtA/SwoA also contains all three MIR motifs in the region that by alignment with other PMTs is likely to be the large endoplasmic reticulum lumenal loop (Fig. 4).

The *ts* mutation in *pmtA/swoA-2* is caused by the introduction of a stop codon at Y662. This stop codon appears between the sixth and the seventh predicted membrane spans of the protein (Fig. 4). This lesion is similar to one tested in *S. cerevisiae* (Girrbach et al., 2000) also occurring between the sixth and seventh membrane span but 39 residues earlier in the protein. ScPmt1 Δ 617–817 loses its ability to mannosylate two test substrates, chitinase and Hsp150. Additionally, ScPmt1 Δ 617–817 appears to effect the complex formation of ScPmt1 and ScPmt2 (Girrbach et al., 2000). ScPmt1 and ScPmt2 need to form a complex to be fully functional (Strahl-Bolsinger et al., 1999). Since PmtA/SwoA appears to be in the ScPmt2 sub-family (Fig. 5, Table 1), we hypothesize that the *swoA2* phenotype is due to loss of PMT function from abnormal complex formation between PmtA/SwoA and an as yet unknown ScPmt1 homologue in *A. nidulans*.

It is not surprising that a PMT mutant would cause a polarity defective phenotype. In *C. albicans* on certain media hyphal formation is blocked in a *pmt1/pmt6* double mutant (Timpel et al., 1998; Timpel et al., 2000). This phenotype is very similar to *swoA* cells that grow isotropically, with no hyphal formation, indefinitely at restrictive temperature (Fig. 1). Also in *C. albicans*, *pmt1* mutants appear to have cell wall defects as they are hypersensitive to Calcofluor, congo red, and SDS (Timpel et al., 1998). *swoA* is also hypersensitive to Calcofluor (Momany et al., 1999). In *S. cerevisiae*, Pmt4 activity is required for proper function of Axl2 and thus for proper axial budding, a form of polarized growth (Sanders et al., 1999).

Several proteins are known to be modified by PMTs in *S. cerevisiae* including: chitinase, Bar1 protease, Hsp150, α -agglutinin, Kre1, Kre9, Kex2, Gas1, Fus1, and Axl2 (Strahl-Bolsinger et al., 1999) as well as Wsc1 and Mid2 (Philip and Levin, 2001). The substrates of *O*-glycosylation are generally either secreted or cell wall resident. Because mannosylated proteins play a variety of roles in the cell an understanding of the role of PMTs in polar growth will depend on identification of targets of *pmtA/swoA* and their role in the cell. It is our hypothesis that a substrate of *pmtA/swoA* requires *O*-mannosylation to be

properly targeted to the cell wall and this target is involved in directing polarized growth.

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