Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 8164–8168, September 1993
Biochemistry

PMT1, the gene for a key enzyme of protein O-glycosylation in *Saccharomyces cerevisiae*

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Communicated by Phillips W. Robbins, May 17, 1993

ABSTRACT **The integral endoplasmic reticulum membrane protein catalyzing the initial reaction of protein O-glycosylation in *Saccharomyces cerevisiae* has been purified to homogeneity. The 92-kDa N-glycosylated protein transfers mannose residues from dolichyl phosphate-D-mannose to specific serine/threonine residues of proteins entering the secretory pathway. This type of mannosyl transfer reaction has so far been observed only in fungal cells. Oligonucleotides derived from peptide sequences of the transferase were used to screen a genomic yeast library. A clone was isolated which contains an open reading frame of 2451 bp corresponding to an mRNA transcript of 3 kb. The predicted protein consists of 817 amino acids including three potential N-glycosylation sites. The hydropathy plot indicates a tripartite structure of the protein: an amino-terminal third and a carboxyl-terminal third, both with multiple potential transmembrane helices, and a central hydrophilic part. Expression of the clone in *Escherichia coli* resulted in mannosyltransferase activity. Gene disruption led to a complete loss of *in vitro* mannosyltransferase activity from dolichyl phosphate-D-mannose to a peptide used as acceptor in the enzymatic assay. *In vivo* it was observed, however, that protein O-mannosylation in the disruptant had decreased only to about 40–50%, indicating the existence of an additional transferase which had not been measured by the *in vitro* enzyme assay.**

Whereas the initial reactions of protein N-glycosylation proceed identically in all eukaryotic cells, protein O-glycosylation differs considerably among various organisms (1–3). For example, serine and threonine residues of proteins are glycosylated in fungal cells via dolichyl phosphate-D-mannose (Dol-*P*-Man) as an intermediate, a reaction which so far has not been observed in higher eukaryotes (4–9). The following reactions have been established for *Saccharomyces cerevisiae* (4, 5).

(I) GDP-Man + Dol-*P* $\overset{Mg^{2+}}{\rightleftharpoons}$ Dol-*P*-Man + GDP

(II) Dol-*P*-Man + protein(Ser/Thr) $\xrightarrow{Mg^{2+}}$ protein(Ser/Thr)-Man + Dol-*P*

(III) Protein(Ser/Thr)-Man + *n*GDP-Man $\xrightarrow{Mn^{2+}}$ protein(Ser/Thr)-Man_{n+1} + *n*GDP.

Evidence has been presented that reactions I and II proceed in the endoplasmic reticulum (ER) (10–12), whereas those reactions summed up in III (mannosyltransferases for α1,2- and α1,3-linked mannoses) most likely take place in the Golgi apparatus (12, 13). Strong *in vivo* evidence that reactions I and II represent the only pathway for protein O-mannosylation in *S. cerevisiae* has been presented by Orlean (14).

The enzyme catalyzing reaction I, a membrane protein of 30 kDa, has been purified (15, 16). The corresponding gene (*DPM1*) has been cloned and gene disruption resulted in a lethal phenotype (17). The enzyme catalyzing reaction II has been purified (18, 19) and an antibody precipitating the enzyme activity was shown to react with a membrane protein of 92 kDa (18). A membrane-bound α-1,2-mannosyltransferase from *S. cerevisiae* originally described by Lehle and Tanner (20) has been purified, cloned, and sequenced (21). The *MNT1* gene codes for a 41-kDa Golgi protein which is required for the attachment of the third mannose residue of O-linked saccharides (13).

Besides O-mannosylation of proteins, Dol-*P*-Man is required also for N-glycosylation and for the synthesis of glycosyl-phosphatidylinositol membrane anchors (14, 22). As has been discussed by Orlean *et al.* (17) it seems unlikely that the requirement of Dol-*P*-Man for the latter two reactions is vital for a yeast cell, since the loss of the ability to synthesize Dol-*P*-Man in mammalian cells did not cause loss of viability of these cells (23). The type of O-mannosylation described by reaction II, on the other hand, has so far not been found to take place in higher eukaryotes (2). To definitely prove, however, that protein O-mannosylation is essential for yeast growth it is necessary to specifically prevent this reaction. Since no inhibitors for O-glycosylation are known, we decided to clone the gene for the Dol-*P*-Man:protein mannosyltransferase (*PMT1*; reaction II) and study the effects of disruption of this gene.

The protein mannosyltransferase (Pmt1p) has been purified to homogeneity and the corresponding *S. cerevisiae* gene, *PMT1*, has been cloned. The open reading frame codes for an integral membrane protein of 817 amino acids,§ containing three potential N-glycosylation sites and multiple potential transmembrane helices. Gene disruption leads to a complete loss of enzyme activity *in vitro; in vivo*, protein O-mannosylation is decreased to about 40%, indicating the existence of at least one additional protein mannosyltransferase.

MATERIALS AND METHODS

Strains and Growth Conditions. *S. cerevisiae* SEY2101 (*MAT***a**, *ura3-52, leu2,3-112, ade2-1, suc2-Δ9*), SEYD2112 (*MAT***a**/*MATα*, *ura3-52/ura3-52, leu2,3-112/leu2,3-112, +/ade2-1, +/his4-519, suc2-Δ9/suc2-Δ9*) (24), Oll (*MATα*, *ura3-52, leu2,3-112, his3*) (obtained from E. Hurt, European Molecular Biology Laboratory, Heidelberg), and BYS232-31-4 (*Mat***a**, *prb1-1, prc1-3, cps1-1, ade2-1, ura3-52, leu2,3-112*) (obtained from T. Achstetter, Transgene, Strasbourg) were grown in YPD medium (1% yeast extract/2% bactopeptone/2% glucose) or minimal medium [0.67% yeast nitrogen base without amino acids (Difco)] supplemented with the required substrates (leucine, 30 μg/ml; adenine, 30 μg/ml; uracil, 30 μg/ml) and 2% glucose at 29°C. *Escherichia coli*

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Abbreviations: Dol-*P*-Man, dolichyl phosphate-D-mannose; ER, endoplasmic reticulum.

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§The sequence presented in this paper has been deposited in the GenBank database (accession no. L19169).

DH5α (BRL) was grown in LB medium (25). To test sensitivity toward killer toxin, the *S. cerevisiae* killer strain RC1777 (*MATα, ade, his4c, KILK1*) was used.

Purification of the 92-kDa Protein. The mannosyltransferase was solubilized from total yeast membranes and partially purified on hydroxylapatite (18). One hundred milliliters of fractions from the hydroxylapatite column containing mannosyltransferase activity was made 30% saturated with $(NH_4)_2SO_4$. After centrifugation for 30 min at 8000 × *g*, the resulting pellet was redissolved in 8 ml of AB buffer [10 mM Tris/HCl, pH 7.5/15% (vol/vol) glycerol/0.1% (vol/vol) Lubrol PX/150 mM NaCl] and dialyzed for 1 hr against the same buffer.

The antibodies raised against the 92-kDa protein were covalently coupled (26) to protein A-Sepharose CL-4B. Dimethyl suberimidate was used as coupling reagent. The column material was equilibrated with AB buffer. The $(NH_4)_2SO_4$-precipitated and dialyzed protein was incubated with the affinity resin for 16 hr at 4°C. A column (2 cm × 0.5 cm) was filled with the resin, which was washed with AB buffer, and bound material was eluted with 100 mM glycine/HCl, pH 3.0/0.05% Lubrol PX/15% glycerol.

The fractions containing the 92-kDa protein were detected by Western blot analysis. The protein was precipitated with 10 volumes of ethanol. After centrifugation and drying the pellet was suspended in 150 μl of SDS sample buffer (0.07 M Na_2CO_3/0.07% 2-mercaptoethanol/2% SDS/12% sucrose/0.07% bromphenol blue). SDS/PAGE was carried out according to Laemmli and Favre (27).

Peptide Preparation and Sequence Analysis. After SDS/PAGE the 92-kDa band was cut out (≈10 μg of protein). The gel fragment was cut up, washed in 50% methanol/10% acetic acid and in 50% methanol, and lyophilized. Digestion with trypsin (2 μg) was carried out in 0.3 ml of 0.2 M NH_4HCO_3 for 16 hr at 37°C. Peptides were eluted three times for 1 hr at 37°C in 0.2 ml of 0.2 M NH_4HCO_3 and once in 0.2 ml of 0.2 M NH_4HCO_3/30% acetonitrile. The eluted material was pooled, lyophilized, and dissolved in 6 M guanidinium hydrochloride/50 mM Tris/HCl, pH 7.5. Peptides were chromatographed on a Vydac 218TP column (250 mm × 2.1 mm; MZ Analysentechnik/Mainz). Several peptides were selected for sequencing by automated Edman degradation on a gas-phase protein sequencer (model 477A, Applied Biosystems) with an on-line phenythiohydantoin analyzer (model 120A).

Isolation of the *PMT1* Gene. Two oligodeoxynucleotides, A [5′-G(T/C)GTCACCGTCGAANCC-3′] and B [5′-(C/T)TCGTAGAC(G/A)TG(A/T)GG(T/C)TC-3′], were used to screen a genomic DNA library of *S. cerevisiae* in plasmid pCS19 (28). The oligonucleotides were labeled by the kinase reaction using [γ-^{32}P]dATP (29). Colony hybridization was performed according to Grunstein and Hogness (30). Plasmids were isolated according to Birnboim and Doly (31). Southern blot analysis was performed using oligonucleotides A, B, and C [5′-ATTTC(T/A)TA(T/C)AA(A/G)CC(A/T)GCT-3′]. The hybridization temperature for oligonucleotides A and B was 25°C, and that for oligonucleotide C was 45°C. Washing was performed at 35°C for oligonucleotides A and B, and at 55°C for oligonucleotide C. Clone pDM3 reacted with oligonucleotides A, B, and C.

DNA Sequence Analysis. DNA sequencing was carried out according to Sanger *et al.* (32). Sequence data were obtained from successive exonuclease III deletions (33, 34) or by using synthetic oligodeoxynucleotides. Sequence information was obtained from both DNA strands for the complete open reading frame.

Northern Analysis. RNA was isolated according to Domdey *et al.* (35). Total RNA was electrophoresed in a 2.2 M formaldehyde/0.8% agarose gel and transferred to a nitrocellulose filter as described (29). As probe the *Eco*RI–*Xho* I fragment (Fig. 1) was labeled with [α-^{32}P]dCTP by using the "megaprime" labeling kit (Amersham) according to the instruction manual.

Primer Extension. Primer extension was performed (29) with oligonucleotides 5′-CCGTGCTTGTAGCTGTTAGCTG-3′ and 5′-CGTAACGAGGCCAATTCGGC-3′ as primers.

Gene Disruption. For the first gene disruption the 1.15-kb *Eco*RI–*Xho* I fragment (Fig. 1) was subcloned into pUC18 without a *Hin*dIII restriction site in the polylinker. The 1.1-kb *Hin*dIII fragment of YEp24 (36) containing the *URA3* gene of *S. cerevisiae* was isolated and subcloned into the *Hin*dIII site of this vector. For the second gene disruption the 2.37-kb *Nco* I–*Xho* I fragment was replaced by the *HIS3* gene (Fig. 1). Linear fragments containing the *pmt1::URA3* or *pmt1::HIS3* allele were used to make gene disruptions in SEY2101, Ol1, and SEYD2112 by the procedure of Rothstein (37). Disruption was tested by Southern blot and Western blot analysis. Genomic DNA was isolated (38), digested with several restriction endonucleases, electrophoresed in an agarose gel, and blotted to nitrocellulose (29). The blot was hybridized at 42°C in 5× Denhardt's solution/0.3 M NaCl/0.03 M sodium citrate/0.1% SDS/50% formamide containing salmon sperm DNA at 0.1 mg/ml. As probe the *Hin*dIII or the *Hin*dIII–*Xho* I fragment (Fig. 1) was labeled as described above.

Assay of Enzyme Activity *in Vitro*. The assay for Dol-*P*-Man:protein *O*-D-mannosyltransferase was performed as described (18).

Assay of Enzyme Activity *in Vivo*. Wild-type and mutant cells were grown in minimal medium containing 2% sucrose. Cells in 5 ml of the culture (OD_{578} 1–2) were pelleted and washed with 5 ml of water. The cells were grown in 5 ml of YP with 0.5% sucrose and 250 μCi of [2-H^3]mannose (1 μCi = 37 kBq) for 2 hr at 30°C. Five OD units of the [2-H^3]mannose-labeled cells were washed with 50 mM Tris/HCl, pH 7.5/50 mM $MgCl_2$ and broken with glass beads. Total radioactivity incorporated was determined from an aliquot of the homogenate. The extract was centrifuged for 15 min at 10,000 × *g*, and the pellet (cell wall and membranes) was suspended for β-elimination in 0.1 M NaOH and incubated for 24 hr at 30°C. β-Eliminated material was separated by thin-layer chromatography.

Purification of a-Agglutinin and of Chitinase. a-Agglutinin (39) and chitinase (40) were purified as described. Chitinase was detected by immunoblotting with an antibody specific for the O-linked saccharides of a-agglutinin (39).

Assay for Killer Toxin K1. SEY2101 and SEY2101 *pmt1::URA3* strains were tested for sensitivity to killer toxin K1 (41).

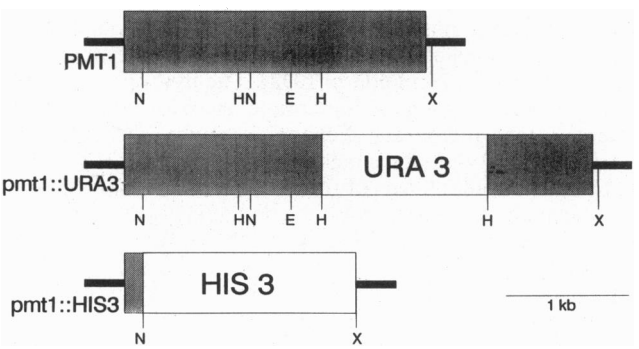

FIG. 1. Restriction sites within the *PMT1* gene and construction of the disrupted alleles. Gray bar represents the open reading frame of *PMT1*; open bar represents *URA3* or *HIS3*. Restriction sites: *N*, *Nco* I; H, *Hin*dIII; E, *Eco*RI; X, *Xho* I.

Expression of Pmt1p in *E. coli*. *PMT1* was cloned into the *E. coli* expression vector PTrc99A (Pharmacia). *E. coli* JM109 cells carrying the plasmid were grown to OD_{550} of 0.5, induced by addition of 2 mM isopropyl β-D-thiogalactopyranoside, and incubated for 30 min to 2 hr. The cells were broken by sonification and then centrifuged at 10,000 × *g* for 15 min. The pellet was incubated with 100 μl of SDS sample buffer for 15 min at room temperature and centrifuged, and the resulting supernatant was subjected to SDS/PAGE followed by immunoblot analysis.

Deglycosylation of Pmt1p. Pmt1p was partially purified up to the deoxycholate extract (18). Seventy microliters of the deoxycholate extract was deglycosylated in 100 μl containing 50 mM potassium phosphate buffer (pH 6.5), 10 mM EDTA (pH 8.0), 1% (wt/vol) octyl glucoside, 1% (vol/vol) 2-mercaptoethanol, and 0.5 unit of endo-β-*N*-acetylglucosaminidase F (Boehringer Mannheim) for 2 hr at room temperature.

RESULTS

Purification of the Protein Mannosyltransferase. The two main features of the purification protocol were (*a*) an analytical procedure that gave rise to an antibody precipitating the enzyme activity, which correlated with a 92-kDa membrane protein [this has been detailed previously (18)] and (*b*) a preparative purification procedure (see *Materials and Methods*) whereby the 92-kDa immunoreactive protein and not the enzyme activity was followed during the purification. In this way it was possible to purify 10–20 μg of an integral ER membrane protein that is present within *S. cerevisiae* cells at ≈0.01% of total protein.

Preparation of Peptides. Since the 92-kDa protein was N-terminally blocked, internal peptides had to be prepared. The peptide mixture was separated by HPLC on an RP18 column, which yielded up to 40 different peptide peaks. Five peptides were sequenced and partial sequences were used for designing oligonucleotides.

Identification and Characterization of the *PMT1* Gene. A genomic DNA library of *S. cerevisiae* was screened with a mixture of oligonucleotides A and B. Analysis of the positive clones by Southern blotting using three oligonucleotides individually (see *Materials and Methods*) resulted in clone pDM3, which reacted with all three probes (data not shown). Restriction and Southern blot analysis of pDM3 resulted in the identification of the region of a possible open reading frame; sequence analysis uncovered an open reading frame of 2451 bp. Northern analysis revealed a single mRNA transcript, of 3 kb (data not shown).

The open reading frame predicts a peptide sequence of 817 amino acids with three potential N-glycosylation sites (Fig. 2), which agrees with the previously observed change in apparent molecular mass of the 92-kDa protein to 84 kDa due to endoglycosidase F treatment (18). The sequence does not show significant homology to any sequence available from the software package HUSAR Release 2.1 (German Cancer Research Center, Heidelberg, 1992). The hydropathy profile (42) predicts an integral membrane protein with multiple potential transmembrane helices, whereby the lipophilic parts of the protein are concentrated within the N-terminal and the C-terminal thirds of the protein, whereas the central part corresponds to a hydrophylic domain (Fig. 3). The latter would have to face the ER lumen for the first and second potential N-glycosylation site to be glycosylated. All tryptic peptides were found within the sequence; they are all part of the hydrophilic domain of the protein.

To prove that translation initiated at the first in-frame ATG, Pmt1p was expressed in *E. coli*. The *E. coli* product showed a molecular mass corresponding to that of the nonglycosylated yeast protein (Fig. 4). The deglycosylated yeast protein partially aggregated (Fig. 4, lane 1); the protein

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1
MSEEKTYKRV EQDDPVPELD IKQGPVRPFI VTDPSAELAS LRTMVTLKEK

LLVACLAVFT AVIRLHGLAW PDSVVFDEVH FGGFASQYIR GTYFMDVHPP
101
LAKMLYAGVA SLGGFQGDFD FENIGDSFPS TTPYVLMRFF SASLGALTVI

LMYMTLRYSG VRMWVALMSA ICFAVENSYV TISRYILLDA PLMFFIAAAV
201
YSFKKYEMYP ANSLNAYKSL LATGIALGMA SSSKWVGLFT VTWVGLLCIW

RLWFMIGDLT KSSKSIFKVA FAKLAFLLGV PFALYLVFFY IHFQSLTLDG
301
DGASFFSPEF RSTLKNNKIP QNVVADVGIG SIISLRHLST MGGYLHSHSH

NYPAGSEQQQ STLYPHMDAN NDWLLELYNA PGESLTTFQN LTDGTKVRLF
401
HTVTRCRLHS HDHKPPVSES SDWQKEVSCY GYSGFDGDAN DDWVVEIDKK

NSAPGVAQER VIALDTKFRL RHAMTGCYLF SHEVKLPAWG FEQQEVTCAS
501
SGRHDLTLWY VENNSNPLLP EDTKRISYKP ASFISKFIES HKKMWHINKN

LVEPHVYESQ PTSWPFLLRG ISYWGENNRN VYLLGNAIVW WAVTAFIGIF
601
GLIVITELFS WQLGKPILKD SKVVNFHVQV IHYLLGFAVH YAPSFLMQRQ

MFLHHYLPAY YFGILALGHA LDIIVSYVFR SKRQMGYAVV ITFLAASVYF
701
FKSFSPIIYG TPWTQELCQK SQWLSGWDYN CNTYFSSLEE YKNQTLTKRE

SQPAATSTVE EITIEGDGPS YEDLMNEDGK KIFKDTEGNE LDPEVVKKML
801
EEEGANILKV EKRAVLE
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FIG. 2. Predicted amino acid sequence of the yeast *PMT1* gene. Experimentally determined tryptic peptide sequences are underscored. Three potential N-glycosylation sites are shown in bold (NLT at 390–392, NNS at 513–515, and NQT at 743–745).

heterologously expressed in *E. coli* showed enzymatic activity, but considerably less than was expected from the Western signal (Table 1). Primer extension (data not shown) showed a transcription initiation at position −98 relative to the translation start site.

Disruption of the *PMT1* Gene. Disruption of *PMT1* in the yeast strains SEY2101 and SEYD2112 is described in *Materials and Methods*. Southern analysis demonstrated correct disruption of the gene (data not shown).

When crude membranes were prepared from haploid SEY2101 cells and analyzed by immunoblotting, the 92-kDa protein was absent from disruptants (data not shown). Also the enzymatic transfer of [^{14}C]mannose residues from Dol-*P*-[^{14}C]Man to the hexapeptide Tyr-Asn-Pro-Thr-Ser-Val did not take place with extracts of the disruptant (Table 1). The mutant showed the same growth rate in liquid culture as wild-type cells. The only phenotypic difference was a distinct tendency of the mutant cells to aggregate, which was reversed, however, by short ultrasonic treatment. To exclude the possibility that the *URA3* insertion into the C-terminal end did not destroy total enzyme activity, a second gene disruption was carried out by replacing 95% of the coding region with the *HIS3* gene (Fig. 1). This disruptant behaved identically to the first one. From these results it was concluded that either protein O-mannosylation is not a vital

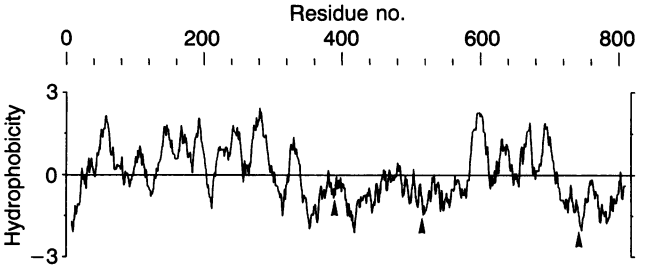

FIG. 3. Hydropathy profile according to Kyte and Doolittle (42). A window of 16 amino acids was used. Arrowheads indicate potential N-glycosylation sites.

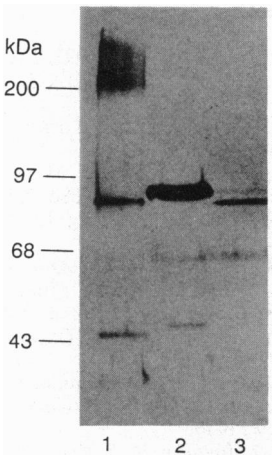

FIG. 4. Deglycosylation of yeast Pmt1p compared to the *PMT1* gene product expressed in *E. coli*. Lanes: 1, 20 μl of deoxycholate extract treated with endo-β-*N*-acetylglucosaminidase F; 2, 20 μl of deoxycholate extract (control); 3, Pmt1p expressed in *E. coli* JM109 (membrane proteins corresponding to 5 OD units of cells).

reaction in *S. cerevisiae* or that another protein mannosyltransferase exists which is not measured with the enzymatic assay *in vitro*. It had to be checked, therefore, whether mutant cells were still able to O-mannosylate proteins.

***In Vivo* Protein O-Mannosylation in Wild-Type Cells and Disruptants.** Wild-type and mutant cells were grown for 2 hr in the presence of [2-^{3}H]mannose. β-Eliminable radioactivity was indeed obtained from mutant cells, although it amounted to only about 50% of that of wild-type cells (Table 2). When the β-eliminable material from both cell types was separated by thin-layer chromatography, a very similar pattern of labeled oligosaccharides was obtained. However, the amounts from the null mutant again were only about 50% of those in control cells (data not shown). From these results it was concluded that at least one additional protein mannosyltransferase gene must be present in *S. cerevisiae* and that the corresponding gene product is not assayed when the transfer of [^{14}C]mannose from Dol-*P*-[^{14}C]Man to the hexapeptide is measured.

This conclusion is further supported by the following observations. When the synthesis of the exclusively O-mannosylated a-agglutinin (39, 43) was followed in the disruptant, the amount was significantly lower and on SDS/PAGE the agglutinin moved as if slightly underglycosylated (Fig. 5*A*). A shift due to underglycosylation was also clearly visible with chitinase (Fig. 5*B*), an O-glycosylated extracellular protein of *S. cerevisiae* (40). Finally, the sensitivity of yeast cells toward the killer toxin K1 depends on the extension of O-linked cell wall saccharides beyond the disaccharide (13, 44). Since the sensitivity of the *PMT1* disruptant toward the K1 toxin clearly decreased by about 50% compared with an isogenic control strain (data not shown), the *PMT1* gene product obviously is responsible for the synthesis of only about half the O-linked saccharides.

Table 1. O-mannosylation activity *in vitro*

Source	^{14}C transferred, cpm
E. coli(vector + insert)	938
E. coli(vector)	66
S. cerevisiae wild type (SEY2101)	2280
S. cerevisiae disruptant (SEY2101 *pmt1*::*URA3*)	57

Crude membranes of *S. cerevisiae* (10 μg of protein) or of *E. coli* (400 μg of protein) were incubated with 0.02 μCi of Dol-*P*-[^{14}C]Man (303 Ci/mol) and 3 mM hexapeptide (Tyr-Asn-Pro-Thr-Ser-Val) for 30 min. Radioactivity transferred to the peptide was determined. Control values (without peptide) have been subtracted. For details see ref. 18.

Table 2. O-Mannosylation of proteins *in vivo*

	^{3}H, cpm × 10^{-7}	
	Wild type	Disruptant
Total radioactivity incorporated	1.46	1.12
Radioactivity in cell wall and membrane mannoproteins	1.20	0.79
Radioactivity released by β-elimination	0.12 (10.0%)*	0.04 (5.1%)*

Wild-type (SEY2101) and disruptant (SEY2101 *pmt1*::*URA3*) cells were radiolabeled with [2-^{3}H]mannose for 2 hr. Subsequently the β-eliminable radioactivity incorporated into proteins was determined.

*Radioactivity in cell wall and membrane mannoproteins was taken as 100%.

DISCUSSION

Cell wall proteins of *S. cerevisiae* containing O-linked saccharides were first described by Sentandreu and Northcote (45). Although O-linked saccharides with mannose as the initial hexose have been described as rare exceptions from mammalian tissues (46, 47), the participation of Dol-*P*-Man as mannosyl donor was shown to be unlikely (47, 48). The fungal pathway may be unique, therefore, and if protein O-glycosylation were essential for fungal cells, the protein mannosyltransferase would be a promising target for antifungal compounds. Cloning and disruption of the *PMT1* gene have uncovered the existence of at least one additional protein mannosyltransferase gene. Since not only the total *in vivo* O-mannosylation activity but also the O-mannosylation of a-agglutinin and of chitinase and that contributing to the susceptibility toward killer toxin K1 were decreased to about the same extent in the *PMT1* disruptant, it seems likely that the various protein mannosyltransferases do not show specificity *in vivo* for different protein substrates. Differences have to be postulated, however, to exist for peptides *in vitro*.

The specificity for protein O-glycosylation is understood neither in mammalian nor in fungal cells. Sugars get attached only to specific serine and threonine residues within a protein. Depending on the protein, the sequence information for O-glycosylation may differ or may be identical in mammalian and fungal cells (49–51). The observation that in the case of

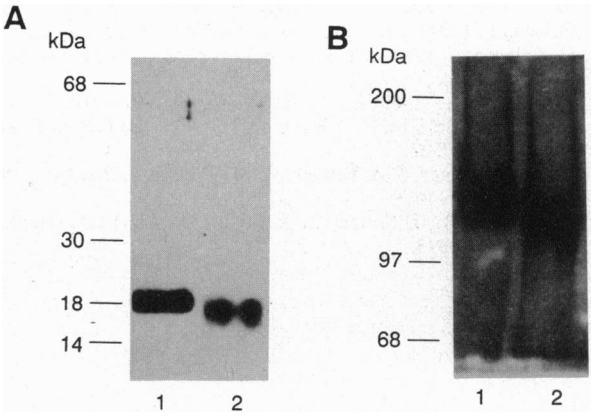

FIG. 5. (*A*) a-Agglutinin was isolated from 3 OD units of cells, separated by SDS/15% PAGE, and detected by immunoblotting with the antibody described previously (39). Protein was isolated from SEY2101 (lane 1) and SEY2101 *pmt1*::*URA3* (lane 2). (*B*) Chitinase was isolated from 15 ml of medium of a stationary culture, separated by SDS/6% PAGE, and detected by immunoblot. Protein was isolated from SEY2101 (lane 1) and SEY2101 *pmt1*::*URA3* (lane 2).

the human granulocyte/macrophage colony-stimulating factor the *in vivo* O-glycosylation patterns were similar, but the *in vitro* ones different, between yeast and rat liver (51, 52) may well be related to findings in this study. Most likely the *in vitro* conditions in yeast resulted in only one mannosyltransferase being active.

The ER localization of the *PMT1* gene product is mainly deduced from *in vivo* O-mannosylation experiments (12). Direct evidence has also been obtained by immunofluorescence. Antibodies against Pmt1p were found to stain an internal structure resembling the ER and the staining pattern was identical to that obtained with an antibody against the *DPM1* gene product (R. Laub, personal communication).

The potential ER retention signals are not found in Pmt1p. Neither the His-Asp-Glu-Leu sequence, recently described for type I membrane proteins (53), nor the previously proposed C-terminal Lys-Lys motif (54) is present in the *PMT1* gene product. An additional ER retention signal has to be postulated, therefore.

This paper is dedicated to Prof. Dr. Meinhart Zenk (Munich) on the occasion of his 60th birthday. We are grateful to Brigitte Zieschank, Maria Reithmeier, and Alexandra Scheinost for expert technical assistance; to Martina Gentzsch for carrying out the killer toxin experiment; to Dr. J. Ernst, Düsseldorf, for helpful discussions; and to Dr. M. Payton, Glaxo Genf, for oligonucleotides. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 43) and by the Fonds der Chemischen Industrie.

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