Schizosaccharomyces pombe och1$^+$ encodes α-1,6-mannosyltransferase that is involved in outer chain elongation of *N*-linked oligosaccharides

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Abstract The fission yeast *Schizosaccharomyces pombe* attaches an outer chain containing mannose and galactose to the *N*-linked oligosaccharides on many of its glycoproteins. We identified an *S. pombe och1* mutant that did not synthesize the outer chains on acid phosphatase. The *S. pombe och1*$^+$ gene was a functional homolog of *Saccharomyces cerevisiae OCH1*, and its gene product (SpOch1p) incorporated α-1,6-linked mannose into pyridylaminated $Man_9GlcNAc_2$, indicating that *och1*$^+$ encodes an α-1,6-mannosyltransferase. Our results indicate that SpOch1p is a key enzyme of outer chain elongation. The substrate specificity of SpOch1p was different from that of *S. cerevisiae OCH1* gene product (ScOch1p), suggesting that SpOch1p may have a wider substrate specificity than that of ScOch1p.

Key words: Mannosyltransferase; Oligosaccharide; *Schizosaccharomyces pombe*

1. Introduction

N-linked oligosaccharide is one of the major forms of protein glycosylation in eukaryotes. Yeasts elongate the *N*-linked oligosaccharides on many of their proteins, forming the large structure called outer chain [1]. The elongated form in the budding yeast *Saccharomyces cerevisiae* is called mannan, and is exclusively comprised of mannose [2]. The α-1,6-mannosyltransferase encoded by *OCH1* is a key enzyme of outer chain elongation in *S. cerevisiae* [3,4]. Mannosyltransferase activity of *OCH1* gene product [3] and the substrate specificity [5] have been investigated in vitro. *HOC1*, a homolog of *OCH1*, is reported as a high copy suppressor of a protein kinase C mutant in *S. cerevisiae* [6]. Although *HOC1* gene product is a member of Mnn9p–Anp1p complex that is involved in the elongation of outer chain [7], there are no reports on the enzymatic activity of *HOC1* gene product.

The fission yeast *Schizosaccharomyces pombe* synthesizes an outer chain which contains both mannose and galactose residues [8]. However, the key enzymes of outer chain elongation remain unidentified. In this paper, we report that an *S. pombe* gene, *och1*$^+$, that is homologous to *S. cerevisiae OCH1* and *HOC1*, encodes an α1,6-mannosyltransferase that is involved in the elongation of outer chain.

2. Materials and methods

2.1. Strains, media and genetic methods

The fission yeast strains used in this study are JY741 (*h*$^-$ *ura4-D18 leu1-32 ade6-M216*; used as wild-type), 7X4 (*h*$^-$ *leu1-32 och1-1*) and KT97 (*h*$^-$ *leu1-32 ura4-D18 och1*Δ*::ura4*$^+$). The media used for culture of the fission yeast cells and the genetic methods of the fission yeast were as described by Moreno et al. [9]. The preparation of YES-P medium, which contains a reduced amount of inorganic phosphate, was described previously [10]. Transformation of the fission yeast was performed by the lithium acetate method of Okazaki et al. [11]. Standard techniques for molecular cloning were adopted from those of Sambrook et al. [12].

2.2. Library and construction of plasmids

The *S. pombe* genomic library was constructed by inserting *Xba*I-digested wild-type (L972) genomic DNA into the pALSK$^+$ vector [11]. Plasmid p7X4 was isolated from the library. p7X4 was digested by *Pst*I and self-ligated to construct plasmid p7X4ΔPstI. This plasmid carried a 5.0-kb *Xba*I–*Pst*I fragment containing *och1*$^+$ gene. The 0.7-kb *Hin*dIII fragment, located in the *och1*$^+$ open reading frame of p7X4ΔPstI, was removed and a 1.8-kb *Hin*dIII fragment carrying *ura4*$^+$ was inserted to construct plasmid p7X4::ura4. It was digested with *Xho*I and *Pst*I, and used for *och1*$^+$ gene disruption. The disruption of genomic *och1* gene was confirmed by PCR using oligonucleotide primers 5′-TTTCCATTCTGAGCTCCATT-3′ and 5′-AGCAAG-GGCATTAAGGCTTA-3′.

The DNAs corresponding to the open reading frames of *S. pombe och1*$^+$, *S. cerevisiae OCH1* and *S. cerevisiae HOC1* were amplified by PCR using oligonucleotide primers 5′-CCCCCGAATTCCATATGTTGA-GACTCCGATTGAGAAGTATTGTA3′ and 5′-CCCCCGTCGACAGATCTTCAATCATCTTTCCATGAACCGGCAAAGAA-3′ (for *S. pombe och1*$^+$), 5′-CCCCCCTCGAGCATATGTCTAGGAAGTTGTCCCACCTGATCGCT-3′ and 5′-CCCCCGCGGCCGCAGATCTTTATTTATGACCTGCATTTTTATCAGCATC-3′ (for *S. cerevisiae OCH1*), and 5′-CCCCCGAATTCCATATGGCCAAAACAACAAAAAAGAGCCTCCAGT-3′ and 5′-CCCCCGTCGACGGATCCTTATTTCTGCTCCACCTTTGGAGTATTTTT-3′ (for *S. cerevisiae HOC1*). The genomic DNAs of *S. pombe* or *S. cerevisiae* wild-type cells (L972 and X2180-1A, respectively) were used as templates. After confirmation of nucleotide sequence, they were inserted between *Nde*I and *Bam*HI site of pREP1 *S. pombe* expression vector [13] to construct pREP1+Spoch1, pREP1+ScOCH1 and pREP1+HOC1, respectively. In these plasmids, the inserted genes are under the control of the *nmt1*$^+$ promoter.

2.3. Isolation of 7X4 strain

K150-A6 (*h*$^-$ *leu1-32*) cells [14] were mutagenized by nitrosoguanidine as described by Moreno et al. [9]. After colony formation on

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Abbreviations: Man, mannose; GlcNAc, *N*-acetylglucosamine; SpOch1p, *Schizosaccharomyces pombe och1*$^+$ gene product; ScOch1p, *Saccharomyces cerevisiae OCH1* gene product; PA, pyridylamino

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YES plates, the mutagenized cells were replica-plated to MM plates containing 100 μg/ml L-leucine, 1% polypeptone and 50 μg/ml E7070, an anticancer drug [15]. The cells that did not form colonies on the plates containing E7070 were isolated and backcrossed three times.

2.4. Cloning of och1$^+$ gene

The 7X4 strain was transformed with *S. pombe* genomic library and spread on MM plates at 25°C. After 5 days, the Leu$^+$ colonies were recovered, and replated on YPD agar plates containing 100 μg/ml of E7070 at 25°C for 3–4 days to select complemented cells.

2.5. Analysis of acid phosphatase

Analysis of acid phosphatase from the fission yeast was as described previously [10].

2.6. Preparation of solubilized microsomal proteins

Solubilized enzymes from various cells were prepared as described previously [16]. The resultant 100 000×*g* precipitates enriched for Golgi membrane fractions were suspended in TMS buffer (20 mM Tris–HCl, 5 mM $MgCl_2$ and 0.25 M sucrose, pH 7.5) to a protein concentration of 20 mg/ml and microsomes were disrupted and solubilized by adding Triton X-100 to a final concentration of 2% (by vol). After mild mixing at 4°C for 1 h, the mixture was used as the source of solubilized enzyme for the mannosyltransferase assay.

2.7. Mannosyltransferase assay by pyridylamino (PA)-labeled oligosaccharides as an acceptor

The mannosyltransferase activity was assayed as described by Nakayama et al. [5] with some modifications. The reaction mixture in a total volume of 50 μl contained 10 μg (for *S. cerevisiae* Och1p) or 200 μg (for *S. pombe* Och1p) of solubilized proteins in 50 mM Tris–HCl (pH 7.5), 10 mM $MnCl_2$, 1 mM GDP-mannose, 0.5 mM 1-deoxymannojirimycin, 100 pmol oligosaccharide-PA acceptor (Takara, Shiga, Japan). The reaction mixture was incubated at 30°C for 10–30 min and terminated by incubation at 99°C for 5 min. After adding 200 μl ice-cold water, the mixture was filtered through an Ultrafree-MC membrane (10K cut; Millipore, Bedford, MA, USA). The filtrate was subjected to HPLC analysis.

2.8. HPLC analysis of N-linked oligosaccharides

PA-labeled oligosaccharides were analyzed as described previously [10]. Each peak was collected and digested with either α-1,2-mannosidase (from *Aspergillus saitoi*, Oxford Glycosystems, Bedford, MA, USA) or α-1,6-mannosidase (from *Xanthomonas manihotis*, New England Biolabs, Beverly, MA, USA) according to manufacturers' protocols.

3. Results and discussion

3.1. Isolation of S. pombe och1$^+$ gene

We generated several *S. pombe* mutants that were hypersensitive to an anticancer drug E7070 [15]. Among them, a mutant strain 7X4 was hypersensitive not only to E7070 but also to hygromycin B, and in addition showed temperature-sensitive growth (Fig. 1). The hygromycin B- and temperature-sensitive phenotypes are characteristic features of cell wall-defective mutants in *S. cerevisiae* [17]. Therefore, we were interested in identifying genes and molecules that are involved in cell wall integrity, and so investigated 7X4 further. To isolate the gene mutated in the 7X4 strain, an *S. pombe* genomic DNA library was screened for genes that could suppress the E7070 sensitivity. Two distinct clones were isolated, and they were found to also suppress the hygromycin B and temperature sensitivity of the 7X4 mutant. They were subcloned, and DNA sequences were determined. One subclone p7X4ΔPst, carrying 5.0-kb insert DNA, included a gene encoding a putative mannosyltransferase with similarity to *S. cerevisiae OCH1*. Therefore, we designated the identified gene as *och1*$^+$. Another suppressor was the tryptophanyl tRNA gene. The nucleotide sequence of *och1*$^+$ is now available from GenBank database as gene SPAC1006.05c from the *S. pombe* genome sequencing project (accession number AL132828), although the reported amino acid sequence is 30 amino acids shorter than that deduced by us, due to a difference in the position of methionine for translational initiation. An alignment of the amino acid sequences of *S. pombe och1*$^+$, *S. cerevisiae OCH1* and *HOC1* is shown in Fig. 2. Overall, the *S. pombe och1*$^+$ gene product (SpOch1p) is 32% identical and 42% similar to the *S. cerevisiae HOC1* gene product (ScHoc1p), and 31% identical and 39% similar to the *S. cerevisiae OCH1* gene product (ScOch1p). ScOch1p has three regions that are absent in SpOch1p and ScHoc1p (Fig. 2). When the conserved region that is shared by all three proteins (194th amino acid to 396th for SpOch1p) is compared, SpOch1p was 57% identical to ScOch1p, and 40% identical to ScHoc1p. The identity between ScOch1p and ScHoc1p in this region was

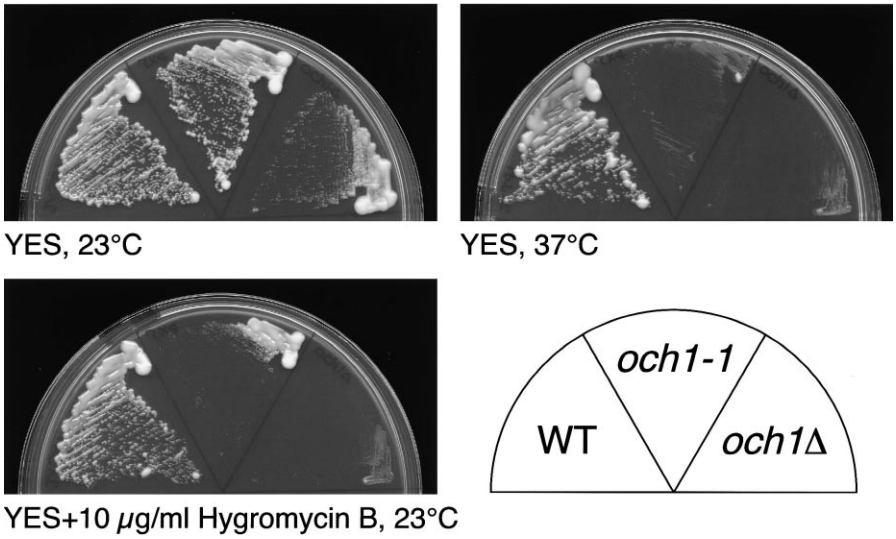


Fig. 1. 7X4 (*och1-1*) and *och1*Δ cells are temperature- and hygromycin B-sensitive. Cells were streaked on the plates indicated in the figure, and incubated at 37°C for 3 days or at 23°C for 4 days.

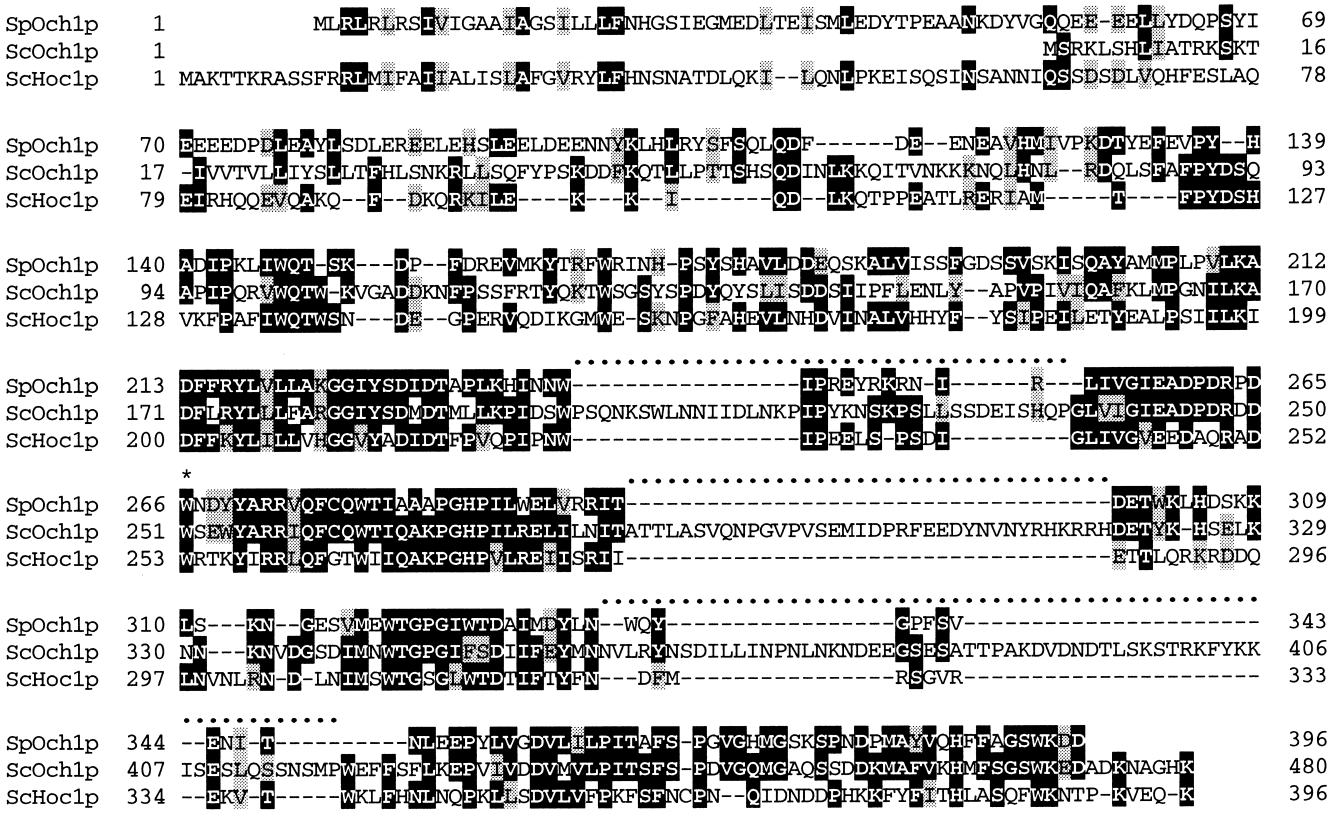


Fig. 2. Overall sequence comparison of the *S. pombe och1*$^{+}$ gene product (SpOch1p), the *S. cerevisiae OCH1* gene product (ScOch1p; GenBank accession number D11095) and the *S. cerevisiae HOC1* gene product (ScHoc1p; accession number U62942). Identical residues are shown as white on black letters, and conservative changes are shaded. The conservative amino acid groups are as follows: A and G; D and E; F, W and Y; H, K and R; I, L and V; N and Q; and S and T. Dots show three regions that are specific to ScOch1p. An asterisk above tryptophan 266 of SpOch1p shows the point of the non-sense mutation in the 7X4 (*och1-1*) strain.

43%. This comparison indicates that SpOch1p is more similar to ScOch1p than to ScHoc1p in the conserved region.

DNA sequencing of the *och1* locus on the genome of 7X4 strain revealed that the *och1* gene in this mutant had a non-sense mutation in codon 266 ($TGG^{trp} \rightarrow TGA^{stop}$; shown in Fig. 2 by asterisk), which results in a truncated Och1 protein lacking the C-terminal region (33%) of the protein. We designated the *och1* mutation in 7X4 strain as *och1-1*. The non-sense mutation in the Trp-266 codon is presumably translated in the strain containing the multicopy Trp-tRNA gene.

We constructed a disruption mutant of *S. pombe och1*$^{+}$. The *och1*Δ disruption mutant was viable and showed a slow-growing phenotype (Fig. 1). Not only *och1-1* cells but also *och1*Δ cells were temperature- and hygromycin B-sensitive (Fig. 1). Slow-growing and temperature-sensitive phenotypes are also reported in *S. cerevisiae och1*Δ cells [3]. However, the disruption of *S. cerevisiae HOC1* causes no effect on vegetative growth [6]. Therefore, the phenotype of *S. pombe och1*Δ cells is more similar to *S. cerevisiae och1*Δ cells than to *hoc1*Δ cells.

3.2. *S. pombe och1*$^{+}$ *is involved in N-glycosylation*

To investigate the effect of *och1*$^{+}$ gene disruption on protein glycosylation, the electrophoretic mobility of acid phosphatase, a typical glycoprotein in *S. pombe*, was analyzed. Cell extracts from wild-type, *och1-1* and *och1*Δ cells were subjected to native polyacrylamide gel electrophoresis, and the position of acid phosphatase was detected by activity staining (Fig. 3). Whilst the wild-type cells produced a smear band with a higher molecular weight (Fig. 3, lane 1), both the *och1-1* and *och1*Δ cells produced a discrete band with an increased electrophoretic mobility (Fig. 3, lanes 2, 3), suggesting that outer

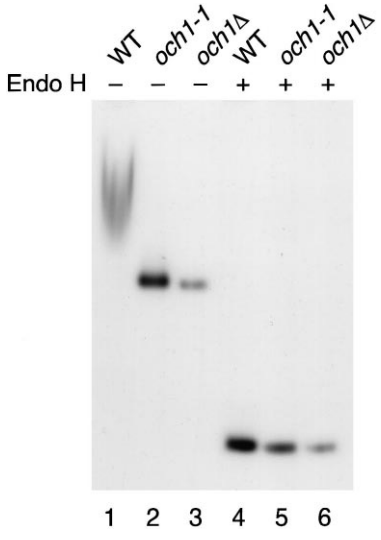


Fig. 3. Activity staining of acid phosphatase. Lysates from wild-type, *och1-1* and *och1*Δ cells induced for acid phosphatase expression were subjected to electrophoresis on a 6% native polyacrylamide gel, and stained for acid phosphatase activity, as described in Section 2. Lanes 1–3, untreated lysates; lanes 4–6, lysates treated with endoglycosidase H.

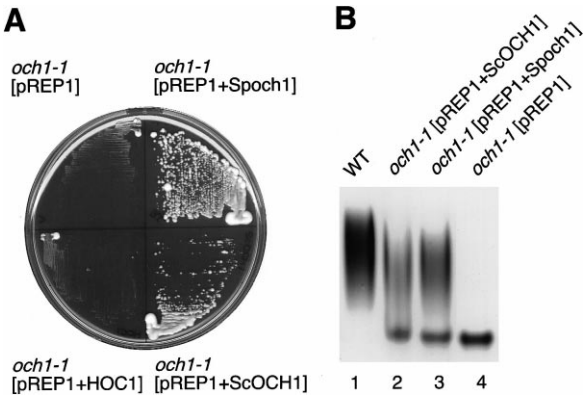


Fig. 4. Suppression of the phenotypes of *S. pombe och1-1* cells by the *S. cerevisiae OCH1* gene. A: *och1-1* cells that were transformed with plasmids pREP1+Spoch1, pREP1+ScOCH1, pREP1+HOC1 and pREP1 (indicated by brackets) were streaked on a YES plate and incubated at 37°C for 3 days. B: Activity staining of acid phosphatase. Lysates from wild-type and *och1-1* cells that were transformed with the plasmids indicated by brackets and induced for acid phosphatase expression were subjected to the 6% native polyacrylamide gel electrophoresis.

chains were not elongated in these mutant strains. After digestion with endoglycosidase H, the acid phosphatase from all strains exhibited the same mobility (Fig. 3, lanes 4–6), indicating that the difference in electrophoretic mobility was due to the difference in the extent of glycosylation of *N*-linked oligosaccharides. While *S. cerevisiae hoc1* disruption mutant shows no glycosylation defects [6], the invertase of *S. cerevisiae och1* disruption mutant exhibits the increased mobility [3]. Our result suggests that the function of *S. pombe och1*$^+$ is more similar to that of *S. cerevisiae OCH1* rather than that of *HOC1*.

3.3. *S. pombe och1*$^+$ *is a functional homolog of S. cerevisiae OCH1*

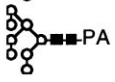
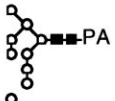
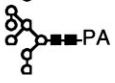
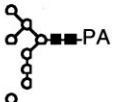
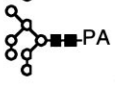
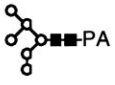
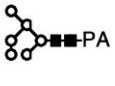
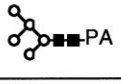
S. pombe och1 disrupted cells showed extremely slow growth phenotype (Fig. 1) and we could not introduce plasmids into the *och1* disruption mutant. Since outer chain was not elongated in *och1-1* cells (Fig. 3), it is possible that the mutant Och1p may lose its mannosyltransferase activity. Therefore, we used *och1-1* cells for further characterization.

S. cerevisiae OCH1 or *HOC1* was expressed in *S. pombe och1-1* mutant cells. The *och1-1* cells containing the *S. cerevisiae OCH1* expression plasmid (pREP1+ScOCH1) grew well at 37°C (Fig. 4A), indicating that the temperature sensitivity of *och1-1* cells was suppressed by *S. cerevisiae OCH1*. In contrast, overexpression of *S. cerevisiae HOC1* (pREP1+HOC1) did not suppress the temperature sensitivity of *och1-1* cells (Fig. 4A). These results again suggest that the function of *S. pombe och1*$^+$ is more similar to that of *S. cerevisiae OCH1* than that of *HOC1*. To examine whether *S. cerevisiae OCH1* rescues the glycosylation defect in *och1-1* cells, the mobility of acid phosphatase was investigated. The mobility of acid phosphatase from the *och1-1* cells containing the *S. cerevisiae OCH1* expression plasmid was decreased to the same extent as that seen in *och1-1* cells containing the *S. pombe och1*$^+$ expression plasmid (Fig. 4B). We observed unmodified acid phosphatase in the mutant carrying *S. pombe och1*$^+$ and *S. cerevisiae OCH1* (Fig. 4B). This might be due to the differences of promoter strength used for acid phosphatase production.

3.4. *S. pombe Och1p has α-1,6-mannosyltransferase activity*

We next investigated the mannosyltransferase activity of the SpOch1p. Solubilized membrane fractions from wild-type cells, *och1-1* cells containing the *och1*$^+$ expression plasmid pREP1+Spoch1 and *och1-1* cells containing the control plasmid pREP1 were used as the sources of enzyme. PA-labeled $Man_9GlcNAc_2$, which is the core oligosaccharide formed in the endoplasmic reticulum of *S. pombe* [8,18], was used as an acceptor, and the product was analyzed by HPLC. A peak corresponding to $Man_{10}GlcNAc_2$-PA was produced by the extract from wild-type and *och1-1* (pREP1+Spoch1) cells (Fig. 5A, C, panel a), showing mannosyltransferase activity of the *och1*$^+$ gene product. In this assay system, we did not observe any larger products formed by other enzymes elongating the $Man_{10}GlcNAc_2$-PA to higher mannose structures. The

Table 1
Substrate specificity of SpOch1p and ScOch1p

Acceptor		Relative activity (%)	
		SpOch1p	ScOch1p
Man9A		100	100
Man8A		117	118
Man8B		217	69
Man8C		23	34
Man7A		245	78
Man7B		25	34
Man7D		96	17
Man6B		111	17
Man6C		6.9	0
Man5A		7.2	0

The *och1-1* cells containing the plasmid pREP1+Spoch1 or the plasmid pREP1+ScOCH1 were cultured in MM medium, and 200 μg (SpOch1p) or 10 μg (ScOch1p) proteins of solubilized membrane fractions were used as the enzyme source. The reaction mixture was incubated at 30°C for 30 min (SpOch1p) or 10 min (ScOch1p). 100% corresponds to 5.47 pmol min^{-1} mg protein^{-1} for SpOch1p and 294 pmol min^{-1} mg protein^{-1} for ScOch1p under the assay conditions described in Section 2.

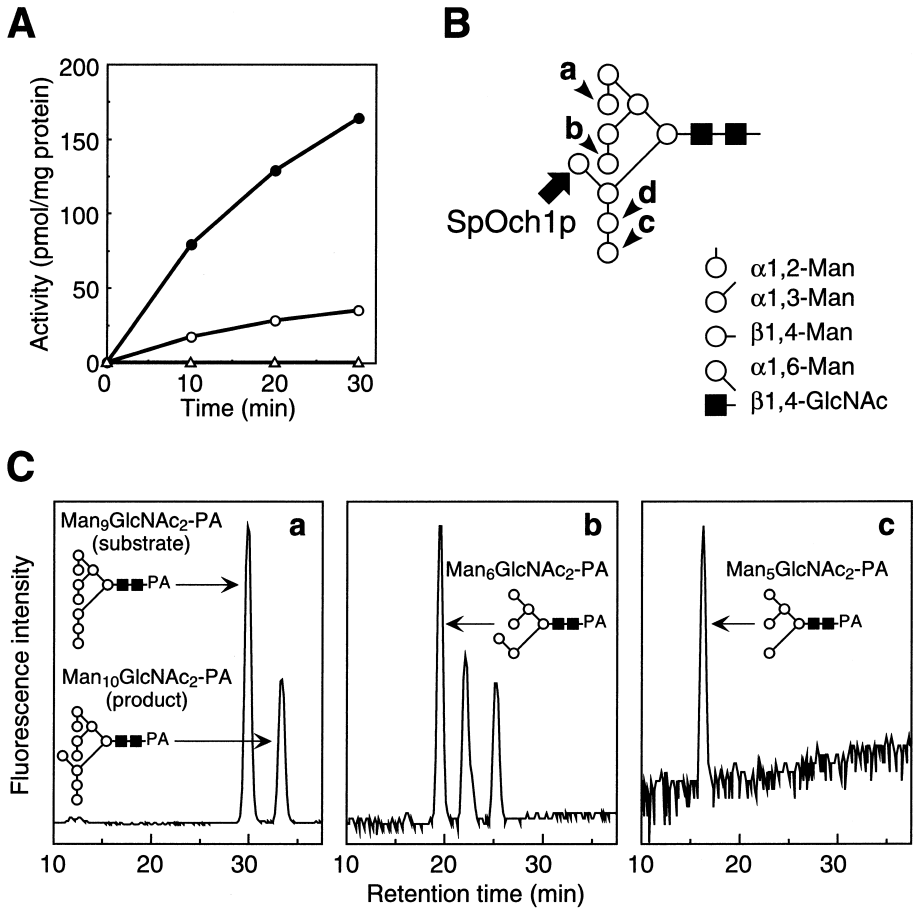


Fig. 5. Mannosyltransferase activity of SpOch1p. A: Time-course of mannosyltransferase activity. Wild-type cells (open circles), *och1-1* cells containing the plasmid pREP1+Spoch1 (closed circles) and *och1-1* cells containing the control plasmid pREP1 (open triangles) were cultured in MM medium, and solubilized membrane fractions were used as the enzyme source. $Man_9GlcNAc_2$-PA was used as an acceptor. The products were analyzed by HPLC, and the amount of $Man_{10}GlcNAc_2$-PA was calculated from the peak area of the HPLC profile. B: Structure of $Man_{10}GlcNAc_2$ reported in *S. pombe* glycoproteins. The α-1,6-mannose that appears to be incorporated by SpOch1p is shown by an arrow. Four α-1,2-mannoses are shown by arrowheads a, b, c and d. C: Confirmation of the structure produced in vitro by SpOch1p. Solubilized membrane fractions from *och1-1* cells containing the plasmid pREP1+Spoch1 were used as the enzyme source. The reaction mixture was incubated at 30°C for 30 min. The $Man_{10}GlcNAc_2$-PA product shown in panel a was collected, digested by α-1,2-mannosidase and analyzed by HPLC (panel b). The peak corresponding to $Man_6GlcNAc_2$-PA, as deduced from standard PA-labeled oligosaccharides, is marked by an arrow and the deduced structure. Two unmarked peaks corresponding to $Man_7GlcNAc_2$-PA and $Man_8GlcNAc_2$-PA seem to be incompletely digested products. The peak corresponding to $Man_6GlcNAc_2$-PA in panel b was collected again, digested by α-1,6-mannosidase and analyzed (panel c). The single peak corresponding to $Man_5GlcNAc_2$-PA, as deduced from standard PA-labeled oligosaccharides, is indicated by an arrow and the deduced structure. The α-1,6-linked mannose in the upper arm still remains after digestion, because the α-1,6-mannosidase used in this experiment only hydrolyzes terminal Man-1,6-linkages that are linked to a non-branched sugar.

activity in *och1-1* (pREP1+Spoch1) cells was approximately 5-fold higher than that in wild-type cells (Fig. 5A), suggesting that the amount of SpOch1p itself was important for the mannosyltransferase activity. No mannosyltransferase activity was detected in *och1-1* (pREP1) cells (Fig. 5A), indicating that the truncated version of SpOch1p in *och1-1* cells retained no activity.

The suppression of the glycosylation defect in *S. pombe och1-1* cells by the *S. cerevisiae OCH1* gene (Fig. 4B) suggests that SpOch1p may incorporate mannose in the same fashion as ScOch1p does. This would mean that SpOch1p incorporates α-1,6-linked mannose into the α-1,3-linked mannose branch that is attached to the β-1,4-linked mannose (Fig. 5B). Ziegler et al. reported the structure of $Man_{10}GlcNAc_2$ in *S. pombe*, as shown in Fig. 5B [19], supporting the notion that SpOch1p incorporates the α-1,6-linked mannose as shown in Fig. 5B.

To confirm the position of mannose incorporation, the product $Man_{10}GlcNAc_2$-PA was digested by α-1,2-mannosidase and subsequently by α-1,6-mannosidase. A peak that corresponded to $Man_6GlcNAc_2$-PA was observed after the α-1,2-mannosidase digestion (Fig. 5C, panel b). The putative $Man_6GlcNAc_2$-PA was changed to $Man_5GlcNAc_2$-PA by α-1,6-mannosidase digestion (Fig. 5C, panel c). These results are in a good agreement with the hypothesis that SpOch1p incorporates the α-1,6-linked mannose residue as shown in Fig. 5B. The results on the acid phosphatase mobility (Fig. 3) and mannosyltransferase activity assay (Fig. 5) suggest that SpOch1p is the key enzyme for the initiation of outer chain elongation, as reported for ScOch1p [4].

3.5. Substrate specificity of S. pombe Och1p

We investigated the difference in substrate specificity between SpOch1p and ScOch1p by measuring their mannosyl-

transferase activity using various PA-oligosaccharides as acceptors (Table 1). The activity of ScOch1p was approximately 50-fold higher than that of SpOch1p when $Man_9GlcNAc_2$-PA was used as an acceptor. Since both were expressed under the control of the same promoter, no significant difference may exist in the expression levels. It is possible that the difference in activity is caused by the difference in the stability of the Och1 proteins. The removal of α-1,2-mannose at the upper arm (shown by arrowhead a in Fig. 5B) decreased the mannosyltransferase activity of both SpOch1p and ScOch1p (compare Man9A with Man8C, Man8A with Man7B, Man8B with Man7D, and Man7A with Man6B in Table 1). α-1,2-Mannose at the middle arm (shown by arrowhead b in Fig. 5B) seems to exhibit no effect on both mannosyltransferase activities (compare Man9A with Man8A, Man8B with Man7A, Man8C with Man7B, Man7D with Man6B, and Man6C with Man5A in Table 1). In these points, the substrate specificity of SpOch1p is similar to that of ScOch1p.

Interestingly, the removal of α-1,2-mannose at the lower arm (shown by arrowhead c in Fig. 5B) increased the mannosyltransferase activity of SpOch1p while it decreased the activity of ScOch1p (compare Man9A with Man8B, Man8A with Man7A, Man8C with Man7D, and Man7B with Man6B in Table 1). Although Man6B shown in Table 1 is a poor acceptor for ScOch1p, it acted as a good acceptor for SpOch1p. The removal of the second α-1,2-mannose at the lower arm (shown by arrowhead d in Fig. 5B) completely inhibited the activity of ScOch1p while SpOch1p still incorporated mannose to Man6C and Man5A (Table 1). Therefore, it is clear that the substrate specificity of SpOch1p is different from that of ScOch1p.

This difference in substrate specificity seems likely to be due to a difference in the precise way the enzymes recognize their large acceptor substrate. As described above, SpOch1p lacks three regions that are present in ScOch1p (Fig. 2). Therefore, it is conceivable that these regions are involved in the difference in their substrate specificity. Further characterization of *S. cerevisiae OCH1*, *HOC1* and *S. pombe och1*$^+$ gene products, including three-dimensional structure analysis, will provide a better understanding of how mannosyltransferases recognize their specific acceptor substrates.

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