Disruption of the processing α -mannosidase gene does not prevent outer chain synthesis in *Saccharomyces cerevisiae*

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Processing of N-linked oligosaccharides in *Saccharomyces cerevisiae* begins with the removal of glucose and mannose residues from Glc₃Man₉GlcNAc₂ to form a single isomer of Man₈GlcNAc₂. The importance of mannose removal for subsequent outer chain synthesis was examined in strains of *S. cerevisiae* disrupted in the *MNS1* gene encoding a specific α 1,2-mannosidase responsible for Man₈GlcNAc₂ synthesis [Camirand, Heysen, Grondin and Herscovics (1991) *J. Biol. Chem.* 266, 15120–15127]. Both *MNS1* transcripts of 1.85 kb and 1.7 kb were not observed in Northern blots of *mns1* cells (i.e. cells containing the disrupted gene). Analysis on Bio-Gel P-6 of endo- β -N-acetylglucosaminidase-H-sensitive oligosaccharides following a 10 min pulse with [2-³H]mannose revealed similar amounts of labelled outer chains excluded from the gel in both control and *mns1* cells. H.p.l.c. of the included oligosaccharides

showed that a Man₈GlcNAc₂, rather than a Man₉GlcNAc₂ intermediate was formed in *mns1* cells. Analysis of [³H]mannose-labelled core oligosaccharides from immunoprecipitated CPY and invertase by h.p.l.c. showed a similar size distribution in *mns1* and control cells. Invertase immunoprecipitated from [³⁵S]methionine-labelled *mns1* cells was highly glycosylated, but migrated slightly faster than that from control cells on denaturing PAGE, indicating a small difference in glycosylation. A similar difference in mobility was observed for invertase activity stain following non-denaturing gel electrophoresis. It is concluded that the α -mannosidase encoded by *MNS1* is the only enzyme responsible for mannose removal *in vivo*, and that this processing step is not essential for outer chain synthesis.

INTRODUCTION

The process of N-linked protein glycosylation starts in the lumen of the endoplasmic reticulum (ER) with the transfer of Glc₃Man₉GlcNAc₂ from dolichyl pyrophosphate to nascent protein, followed by rapid removal of the three glucose residues. In mammalian cells, Man₈GlcNAc₂ is converted to a glycan containing five mannose residues by the action of ER and Golgi α -mannosidases, and synthesis of complex oligosaccharides proceeds by the stepwise addition of different monosaccharides (Kornfeld and Kornfeld, 1985). In *Saccharomyces cerevisiae*, a specific ER α 1,2-mannosidase converts Man₈GlcNAc₂ into Man₉GlcNAc₂, which is elongated in the Golgi to form mannose chains containing from 8–14 (core) to up to 200 (outer chains) mannose residues (Kukuruzinska et al., 1987; Tanner and Lehle, 1987). Oligosaccharide outer chains attached to the core consist of a backbone of α 1,6-linked mannose residues substituted with α 1,2- and α 1,3-linked mannose-containing side chains. Outer chain synthesis is initiated by the transfer of an α 1,6-mannose to the α 1,3-linked mannose which is itself attached to the β 1,4-mannose of the Man₈GlcNAc₂ core (Hernandez et al., 1989; Reason et al., 1991; Trimble and Atkinson, 1992).

The mechanisms involved in the regulation of yeast outer chain synthesis and elongation are still poorly understood (Kukuruzinska et al., 1987; Ballou, 1990). Byrd et al. (1982) proposed that removal of the specific α 1,2-mannose to form the Man₈GlcNAc₂ intermediate is an essential step for chain elongation to occur. On the other hand, Tsai et al. (1984) detected small amounts of this mannose residue in Man₁₁GlcNAc₂ oligosaccharides isolated from the *S. cerevisiae* *mnn1/mnn9* double mutant. In subsequent studies, Trimble and

Atkinson (1986, 1992) considered the possibility that the α 1,2-mannose residue may have been added back to the precursor Man₁₀GlcNAc₂, but no direct evidence for this hypothesis was provided. We have characterized (Romero and Herscovics, 1989; Reason et al., 1991) and purified (Romero and Herscovics, 1992) the α -1,6-mannosyltransferase responsible for initiation of outer chain synthesis and shown that both lipid-derived Man₈GlcNAc₂ and processed Man₈GlcNAc₂ intermediates were equally good acceptors for this enzyme. These observations suggest that removal of the specific mannose residue from Man₈GlcNAc₂ is not essential for outer chain synthesis, at least in a cell-free system, but do not address the role of mannose removal *in vivo*.

We have previously characterized and purified (Jelinek-Kelly et al., 1985; Jelinek-Kelly and Herscovics, 1988) the specific processing α 1,2-mannosidase. More recently, the gene encoding this enzyme has been isolated and sequenced, and was designated *MNS1* (Camirand et al., 1991). Overexpression of *MNS1* caused an 8–10-fold increase in enzyme activity. Strains disrupted in the *MNS1* gene had no detectable specific α -mannosidase activity measured *in vitro*, but the mutation did not affect yeast viability. The aim of the present work was to study the effects of *MNS1* disruption on the synthesis of yeast glycoproteins *in vivo*, to determine whether removal of mannose is essential to outer chain synthesis.

MATERIALS AND METHODS

Materials

Yeast strain MK1-11B (*MAT α lys2-801 his4-619 ura3-52 ams1::LEU2*), disrupted in the vacuolar α -mannosidase, was

Abbreviations used: ER, endoplasmic reticulum; CPY, carboxypeptidase Y; Endo H, endo- β -N-acetylglucosaminidase H; R, retention time of each oligosaccharide divided by the retention time of standard Glc₃Man₉GlcNAc₂.

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kindly provided by M. Kuranda (Massachusetts Institute of Technology, Cambridge, MA, U.S.A.). Strains 9.16.a (*his4-619 mns1::URA3 ams1::LEU2*), 9.16.b (*lys2-801 ura3-52 ams1::LEU2*), 9.16.c (*ura3-52 ams1::LEU2*) and 9.16.d (*lys2-801 his4-619 mns1::URA3 ams1::LEU2*) were the haploids of the tetrad obtained in this laboratory in the MK1-11B background (Camirand et al., 1991). Anti-invertase rabbit serum (raised against SUC2 fusion protein) was kindly provided by Dr. H. Bussey (McGill University, Montreal, Canada). The yeast actin used as probe was a 350 bp fragment containing the actin part of exon 2 and part of the actin intron (Fouser and Friesen, 1986). [^{14}C]Glucose-labelled $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ oligosaccharide standard for h.p.l.c. was prepared as described (Kilker et al., 1981). Rainbow ^{14}C -labelled high-molecular-mass marker proteins were from Amersham Canada Ltd., Oakville, Ontario, Canada. [$2\text{-}^3\text{H}$]Mannose (28 Ci/mmol), Tran[^{35}S]-Label (1136 Ci/mmol, containing 70% L-[^{35}S]methionine), endo- β -N-acetylglucosaminidase H (Endo H) and Universol were from ICN Radiochemicals, Irvine, CA, U.S.A. EN 3 HANCE was from Du Pont Inc., Lachine, Quebec, Canada. Protein A-Sepharose CL-4B was from Pharmacia LKB Biotechnology, Uppsala, Sweden. BSA and Triton X-100 were from Sigma, St. Louis, MO, U.S.A. Bio-Gel P-6 and all reagents for electrophoresis were from Bio-Rad Laboratories, Richmond, CA, U.S.A. 2,3,5-Triphenyl-2H-tetrazolium chloride, used for invertase staining, was from Aldrich, Milwaukee, WI, U.S.A. Bacto-extract, Bacto-peptone and yeast nitrogen base were from Difco Laboratories, Detroit, MI, U.S.A. All other chemicals were of reagent grade.

RNA blot analysis

Northern blot analysis was performed essentially as described before. Poly(A) $^+$ RNA was isolated from exponentially growing cells and the blot was hybridized with a random-labelled 832 bp *NcoI*-*NcoI* probe from the *MNS1* gene (Camirand et al., 1991). The blot was then hybridized with a random-labelled yeast actin probe.

Identification of processing intermediates

Yeast cultures in exponential phase ($A_{600} = 4.0$) at 30 °C in YPD [1% Bacto-yeast extract, 2% Bacto-peptone and 1% (rather than 2%) glucose] were concentrated 4-fold in the same medium (0.5 ml) and labelled for 10 min with 400 μCi of [$2\text{-}^3\text{H}$]mannose. Cell pellets were obtained by centrifugation (2000 g, 5 min) and processed as described by Byrd et al. (1982). Briefly, cell pellets were sequentially extracted twice with 1 ml of chloroform/methanol (2:1, v/v), five times with water, and seven times with chloroform/methanol/water (10:10:3, by vol.) to remove lipid-linked oligosaccharides. Pellets were washed with methanol, dried in air and proteins were dissolved by boiling in 10% SDS. O-linked oligosaccharides were eliminated by treatment with 0.1 M NaOH for 3 h at room temperature. The products were exhaustively dialysed against 10 mM sodium acetate buffer, pH 5.8, and treated with Endo H for 48 h (25 units/ml added every 24 h). Protein was precipitated with methanol (67%, v/v), and Endo H-released oligosaccharides present in the supernatant were dried, resuspended in 0.1 M pyridine acetate, pH 5.0 (1 ml), and fractionated on a Bio-Gel P-6 column (100 cm $\times$ 1 cm) in the same buffer, with BSA and mannose as internal standards. Included fractions were pooled and further analysed by h.p.l.c. with [^{14}C]glucose-labelled standard, on a column of 5 μm -particle-size Aminospherisorb (Phase

Separations; packed by Chromatography Sciences Co., Ville Mont Royal, Quebec, Canada) with a Varian model 5000 liquid chromatograph, as detailed elsewhere (Romero et al., 1985). Since the retention times of the oligosaccharides vary in different runs using the same column, an internal standard of [^{14}C]Glc $_3$ Man $_9$ GlcNAc was included in each chromatography. Consequently *R* values, calculated as a ratio of the retention time of that of [^{14}C]Glc $_3$ Man $_9$ GlcNAc, were used to compare peaks between different chromatograms. In previous work (Romero and Herscovics, 1989; Reason et al., 1991), it was demonstrated by both n.m.r. and fast-atom bombardment analysis that the peaks with *R* values of 0.67–0.68, 0.83 and 0.96 correspond to Man $_8$ GlcNAc, Man $_9$ GlcNAc and Man $_{10}$ GlcNAc respectively.

Labelling and immunoprecipitation of invertase

Yeast cells, grown with shaking at 30 °C in minimal medium (0.17% yeast nitrogen base, 0.5% NH_4SO_4 , 2% sucrose) supplemented with amino acid requirements, were harvested in exponential phase and washed with minimal medium containing 0.1% glucose. Cell pellets were concentrated to $A_{600} = 8$ in the same medium, preincubated for 20 min, and labelled for 30 min with Tran[^{35}S]-Label (50 $\mu\text{Ci}/\text{ml}$). Labelling was interrupted by cooling and washing the cells twice with 20 mM sodium azide. Cell pellets ($A_{600} = 4$) were mechanically broken by vortex-mixing (six pulses of 30 s; cooling in ice for 30 s between pulses) with 100 μl of acid-washed glass beads (0.45–0.5 mm) in 200 μl of PBS [0.01 M phosphate buffer, pH 7.2, and 0.85% (v/v) NaCl] containing 2% SDS. Cell debris and glass beads were removed by centrifugation at 2000 g (5 min), and the supernatants were boiled (5 min) and centrifuged at 15600 g for 5 min in a microcentrifuge. For immunoprecipitation, lysates (20 $\times$ 10 6 c.p.m.) were diluted to 0.4% SDS with PBS containing 1 mg/ml BSA and 1% Triton X-100 (immunoprecipitation buffer) and pre-cleared by incubation with pre-immune serum (30 μl) for 4 h at 4 °C, and for another 30 min in the presence of Protein A-Sepharose [100 μl of a 20% (v/v) suspension, equilibrated in the same buffer]. Pre-cleared supernatants were incubated overnight at 4 °C with anti-invertase rabbit serum (30 μl). Immune complexes were immobilized with Protein A-Sepharose as described above, washed four times with immunoprecipitation buffer containing 0.4% SDS and once with PBS, resuspended in 60 μl of 150 mM citrate buffer, pH 5.2, containing BSA (1 mg/ml), and divided into two aliquots. Endo H (66 units/ml) was added to one of the aliquots and the mixtures were incubated overnight at 37 °C. The pellets obtained by centrifugation in a microcentrifuge (5 min, 15600 g) were boiled for 5 min in 25 μl of SDS/PAGE sample buffer and analysed by SDS/PAGE, along with ^{14}C -labelled marker proteins. After electrophoresis, the gels were stained with Coomassie Brilliant Blue, fluorographed with EN 3 HANCE, dried and exposed to Kodak X-OMAT AR film at –70 °C.

Analysis of core oligosaccharides

For the analysis of core oligosaccharides of carboxypeptidase Y (CPY) and invertase, exponential-phase yeast cells ($A_{600} = 3$), growing at 30 °C in YPS (1% yeast extract, 2% peptone, 2% sucrose), were resuspended in YPS containing 0.5% sucrose and 1 mCi of [$2\text{-}^3\text{H}$]mannose (2 ml) and incubated for 90 min. Cell pellets were washed twice with cold 20 mM NaN_3 and lysates were prepared for immunoprecipitation as described above, but without the pre-clearing step. Total lysates were boiled and centrifuged (15600 g, 5 min), diluted to 0.4% SDS (1 ml final) and immunoprecipitated first with anti-CPY rabbit serum

(2 μ l) and Protein A-Sepharose. The supernatant was then immunoprecipitated with anti-invertase rabbit serum (15 μ l). Immunoprecipitates immobilized on Protein A-Sepharose were washed as described above, resuspended in 0.15 M sodium acetate buffer, pH 5.2 (50 μ l), containing BSA (1 mg/ml) and treated with Endo H (100 munits/ml) overnight at 37 °C. The suspension was centrifuged (15600 *g*, 5 min) and the released oligosaccharides in the supernatants were fractionated on Bio-Gel P-6 and h.p.l.c.

Staining for invertase activity

Exponential-phase cells growing in YPD ($A_{600} = 2$) were centrifuged (2000 *g*, 5 min), washed with YPD containing 0.05 % glucose, and grown for 3 h in an equal volume of the same medium to de-repress invertase. The cultures were then washed twice with cold 15 mM Na₂S₂O₃. Cell pellets ($A_{600} = 2$) were kept at -85 °C until use. Conditions for cell breakage, PAGE and staining for invertase activity were as described by Ballou (1990), except that the lysates were heated at 50 °C for 30 min prior to PAGE to convert all of the invertase to a dimeric form (Esmon et al., 1987) and the gels (0.75 mm thick and 15 cm long) were run overnight at 3 mA.

Analytical methods

Protein determinations were carried out with a modification of Lowry's method (Markwell et al., 1981), using BSA as standard. Mannose was determined with the phenol sulphuric acid method (Dubois et al., 1956), using mannose as standard. Radioactivity was detected in an LKB scintillation spectrophotometer (model 1212 or 1218) using 4 ml of Universol in minivials. SDS/PAGE was performed according to Laemmli (1970) using 0.75 mm-thick gels in a Mini-Protein II apparatus (Bio-Rad).

RESULTS

Northern blot analysis

In previous work (Camirand et al., 1991) disruption of the *MNS1* gene, as shown in Southern blots, eliminated specific α -mannosidase activity in cell-free extracts. It remained to be established that the transcription of the *MNS1* gene reflected this disruption in *mns1* cells (i.e. cells containing the disrupted gene).

Figure 1 shows the Northern blot analysis of poly(A)⁺ RNA extracted from control (MK1-11B) and *mns1* mutant (9.16.a) strains probed with a random-labelled fragment of the *MNS1* gene. Two transcripts of 1.85 kb and 1.7 kb can be seen in the control, with the faster-migrating band being the more abundant. The same bands were not seen in the *mns1* strain, showing that the two transcripts present in the control are related to *MNS1* and that the gene has been effectively disrupted in the mutant.

Analysis of processing intermediates

In order to determine how the *mns1* mutation affects the synthesis of oligosaccharide processing intermediates *in vivo*, cells of the parental type MK1-11B and of *mns1* strain (9.16.a) were labelled with [2-³H]mannose for 10 min, during which time the Man₈GlcNAc intermediate is usually observed in wild-type cells (Byrd et al., 1982). Labelled oligosaccharides sensitive to Endo H treatment were first fractionated on a Bio-Gel P-6 column. The profiles, shown in Figure 2, were very similar for both strains:

51–55 % of the total radioactivity was eluted in the V_0 , demonstrating that elongation of oligosaccharide outer chains occurred in *mns1* cells. The included peak corresponding to core oligosaccharides represented 23–26 % of the total radioactivity;

Figure 1 Northern blot analysis

Poly(A)⁺ RNA (about 20 μ g) extracted from MK1-11B (control) and 9.16.a (*mns1*) cells was analysed by hybridization with a random-labelled 832 bp *NcoI*–*NcoI* probe from the *MNS1* gene and exposed for radioautography for 10 days (a). The same blot was then reprobed with a labelled 350 bp yeast actin fragment, and exposed for only 75 min (b). Quantification with a Bio Image (MilliGen/Biosearch; Millipore) showed a 3-fold difference in actin transcription, but no detectable signal for the two mannosidase transcripts in *mns1*. The size of the *MNS1* transcripts is indicated.

Figure 2 Effect of the *mns1* mutation on size distribution of total Endo H-sensitive oligosaccharides

Bio-Gel P-6 (200–400 mesh) chromatography of Endo H-released total oligosaccharides metabolically labelled with [2-³H]mannose for 10 min: (a) control strain (MK1-11B); (b) strain 9.16.a (*mns1*). Chromatography was performed at 6 ml/h in 0.1 M pyridine acetate buffer, pH 5.0; fractions (1: ml) were collected and aliquots (50 μ l) were assayed for radioactivity. Elution of BSA (V_0) and mannose (Man) are indicated. K_{av} values are given above the peaks.

Figure 3 Effect of the *mns1* mutation on Endo H-sensitive intermediates of oligosaccharide processing

Included oligosaccharides (K_{av} 0.58–0.61) from Bio-Gel P-6 (Figure 2) were fractionated by h.p.l.c.: (a) control strain (MK1-11B); (b) strain 9.16.a (*mns1*). The R values are indicated above the peaks. The position of ^{14}C -labelled $\text{Glc}_3\text{Man}_9\text{GlcNAc}$, used as an internal standard, is indicated by the arrow.

it can be seen, however, that this peak is eluted slightly earlier in the *mns1* mutant (K_{av} 0.58) compared with the control (K_{av} 0.61).

The fractions corresponding to the peaks included on Bio-Gel P-6 (K_{av} 0.58–0.61) were pooled and analysed by h.p.l.c. (Figure 3). The profiles showed the accumulation of a major peak of $\text{Man}_8\text{GlcNAc}$ ($R = 0.69$) in the control (Figure 3a) and of $\text{Man}_9\text{GlcNAc}$ ($R = 0.8$) in the *mns1* mutant (Figure 3b). The complete absence of $\text{Man}_8\text{GlcNAc}$ in the mutant strain demonstrates that, *in vivo*, no other $\alpha 1,2$ -mannosidase with similar specificity to that encoded by the *MNS1* gene is expressed in *S. cerevisiae*. The presence of small peaks which are eluted after the major component also shows that elongation of inner core oligosaccharides is occurring in the *mns1* strain.

The fractions corresponding to the V_0 of Bio-Gel P-6 (Figure 2) were pooled and chromatographed on Bio-Gel P-60, but no apparent difference in profiles was observed (results not shown).

Electrophoretic mobility of invertase

The effect of the specific α -mannosidase gene disruption on invertase synthesis was analysed by electrophoresis. As shown in Figure 4, under both denaturing (Figure 4a) and non-denaturing (Figure 4b) conditions, invertase produced by the *mns1* cells (Figure 4a, lane c; Figure 4b, strains a and d) of the tetrad migrated slightly faster than the controls bearing intact *MNS1* (Figure 4a, lanes a and b; Figure 4b, strains b and c). Similar differences in mobility between control and mutant was observed when equal amounts of labelled invertase were applied to SDS/PAGE. When treated with Endo H, invertase from *mns1*

Figure 4 Effect of the *mns1* mutation on the electrophoretic mobility of invertase

(a) Denaturing conditions. Cells de-repressed for the production of external invertase were labelled with [^{35}S]methionine. Labelled invertase was immunoprecipitated, incubated with (lanes d–f) or without (lanes a–c) Endo H and analysed by SDS/PAGE (8.5%), as detailed in the Materials and methods section. Lanes a and d, control (MK1-11B); b and e, strain overexpressing *MNS1*; c and f, 9.16.a (*mns1*). The positions of the molecular mass markers are indicated. (b) Non-denaturing conditions. Crude extracts of cells ($A_{600} = 2$) de-repressed for the production of external invertase were heated at 50 °C for 30 min and analysed by PAGE (8%), as described in the Materials and methods section. Invertase produced by strains 9.16.a and 9.16.d (*mns1*), and 9.16.b and 9.16.c (intact for *MNS1*), of a tetrad are shown.

and control strains migrated with the same molecular mass of ~ 62 kDa (Figure 4a, lanes d–f), confirming that the difference in the migration of intact invertase from the mutant cells is due exclusively to an alteration in the carbohydrate chains.

Analysis of core oligosaccharides

In order to verify whether the difference in electrophoretic mobility of invertase isolated from *mns1* cells was due to an alteration in elongation of the inner core, CPY and invertase core oligosaccharides were analysed by h.p.l.c. Exponentially growing cells of the control (MK1-11B) and *mns1* (9.16.a) strains were labelled with [$2\text{-}^3\text{H}$]mannose for 90 min and preparations enriched for CPY and invertase were obtained by immunoprecipitation of total lysates with specific antibodies. Endo H-sensitive oligosaccharides from these preparations were fractionated on Bio-Gel P-6 and the included peaks (K_{av} 0.52–0.55) were analysed by h.p.l.c. The profiles of core oligosaccharides from both CPY and invertase were similar for the two strains analysed, and only the profiles of CPY are shown (Figure 5). When the R values of the peaks shown in Figure 5 were compared with those of labelled oligosaccharides synthesized in cell lysates from *S. cerevisiae mnn1*, peaks

Figure 5 Effect of the *mns1* mutation on the fractionation of CPY core oligosaccharides by h.p.l.c.

An enriched preparation of CPY was obtained by immunoprecipitation with specific antibodies of cell lysates labelled with [^3H]mannose for 90 min, as described in the Materials and Methods section. Endo H-sensitive labelled oligosaccharides were chromatographed on Bio-Gel P-6 and the included fractions were pooled, freeze-dried and fractionated on h.p.l.c. (a) Control (MK1-11B); (b) strain 9.16.a (*mns1*). The main peaks of CPY oligosaccharides isolated from the control (a) were numbered I–VII. Peaks of similar *R* values in the mutant (b) were given the same number as in the control. A peak of *R* = 0.68 (peak I), corresponding to $\text{Man}_9\text{GlcNAc}$, is absent from *mns1*. The arrow indicates the position of ^{14}C -labelled $\text{Glc}_3\text{Man}_9\text{GlcNAc}$ used as internal standard.

I–III corresponded to the migration of $\text{Man}_{8-10}\text{GlcNAc}$ oligosaccharides (Romero and Herscovics, 1989), and peaks IV–V corresponded to the migration of $\text{Man}_{11-12}\text{GlcNAc}$ oligosaccharides in cell lysates of the wild type (P. Romero, unpublished work).

$\text{Man}_9\text{GlcNAc}$ (*R* = 0.68) is not present in the CPY from the mutant strain (Figure 5b). In CPY from *mns1* cells (Figure 5b), the peaks of $\text{Man}_9\text{GlcNAc}$ (II) and $\text{Man}_{11-14}\text{GlcNAc}$ (IV–VII) have similar mobilities to those in the control, indicating that the different isomers of each oligosaccharide size could not be resolved in this h.p.l.c. system. However, these results demonstrate that in *mns1* cells the inner core is elongated to give oligosaccharide lengths comparable with those in the control.

DISCUSSION

Analysis of glycoproteins from strains disrupted in the *MNS1* gene, encoding the specific $\alpha 1,2$ -mannosidase processing enzyme, unequivocally demonstrates that removal of the $\alpha 1,2$ -mannose from $\text{Man}_9\text{GlcNAc}_2$ is not an essential step for elongation of the mannose chains of glycoproteins in *S. cerevisiae*. These studies have been carried out in strains disrupted in the *AMS1* gene (Kuranda and Robbins, 1987) to avoid the formation of products of the non-specific vacuolar α -mannosidase.

In previous work (Camirand et al., 1991), and in the current

study of *MNS1* transcripts by Northern blot analysis, we have provided good evidence for complete disruption of the *MNS1* gene. We have shown that *mns1* cells accumulate $\text{Man}_9\text{GlcNAc}$ processing intermediate *in vivo*. Moreover, no $\text{Man}_9\text{GlcNAc}$ oligosaccharide is present, indicating that the α -mannosidase encoded by *MNS1* has a unique specificity in *S. cerevisiae*.

We observed by h.p.l.c. analysis that invertase and CPY core oligosaccharides isolated from *mns1* strains have a similar distribution of lengths ($\text{Man}_{9-14}\text{GlcNAc}$) to that of the controls. In a recent publication, Trimble and Atkinson (1992) studied the structural heterogeneity of $\text{Man}_{9-13}\text{GlcNAc}$ oligosaccharides isolated from total glycoproteins obtained from exponential-phase yeast cells. It was observed that the main pathway of core synthesis was similar to that already proposed for invertase (Trimble and Atkinson, 1986; Alvarado et al., 1990) and CPY (Ballou et al., 1990), and consists of the successive addition of an $\alpha 1,6$ -mannose to the $\alpha 1,3$ -linked mannose of the $\text{Man}_9\text{GlcNAc}$, followed by substitution of this residue with an $\alpha 1,2$ -mannose, and the stepwise addition of $\alpha 1,3$ -mannose residues to each of the $\alpha 1,2$ -linked non-reducing end units. However, the authors also found a significant amount of $\text{Man}_{9-13}\text{GlcNAc}$ oligosaccharide structures derived from the $\text{Man}_9\text{GlcNAc}$ lipid intermediate isomer. Structural analysis suggested that the synthesis of these oligosaccharides followed the same stepwise addition of residues described above, despite the presence of the extra $\alpha 1,2$ -mannose. The results shown in the present paper indicate that the minor pathway in wild-type cells is probably the same as in *mns1* strains, and is likely to result from escape from α -mannosidase action rather than re-addition of mannose.

Although mutation in the *MNS1* gene did not appear to prevent core and outer chain elongation, the electrophoretic mobility of invertase in mutant strains was slightly altered under both denaturing and non-denaturing conditions. It is possible that the extra mannose in oligosaccharides of the *mns1* cells causes some minor changes in the distribution of isomers which could account for this difference in mobility by preventing the addition of some residues to the outer chain. Although fractionation on Bio-Gel P-60 of outer chains isolated from total glycoproteins did not reveal any difference between mutant and control, this method was probably not sensitive enough to detect minor differences.

In a previous publication (Camirand et al., 1991), Northern blot analysis showed a major *MNS1* mRNA transcript of about 1.7 kb when wild-type and MK1-11B strains were analysed. At the time, it was not clear whether the minor band was also a transcript, since it migrated in the same position as 18 S ribosomal RNA. The current Northern blot analysis demonstrates that both 1.85 kb and 1.7 kb bands are transcripts of *MNS1*. Transcript heterogeneity has previously been observed for the *ALG1* and *ALG7* yeast genes, which are involved in the formation of lipid-linked oligosaccharide precursors of protein N-glycosylation. *ALG1* codes for two transcripts differing by about 100 bp at their 5' end and their expression seems to be modulated as the cells switch from an actively growing to a stationary phase (Albright and Robbins, 1990). *ALG7* is transcribed into two mRNAs differing in their 3' ends, and their expression is modulated with gene dosage and in mutants defective in lipid-linked sugar-donor biosynthesis (Kukuruzinska et al., 1987). The role of the untranslated regions in the expression of these genes associated with N-linked glycosylation is not known. With respect to *MNS1*, heterogeneity is not likely to be located at the 3' end since only one putative consensus termination sequence, TAG...TAGT...TTT, is present after the stop codon (Zaret and Sherman, 1982). Heterogeneity is therefore probably located at the 5' end.

This work was supported by United States Public Health Service Grant GM-31265 from the National Institutes of Health. R.P. was supported by a post-doctoral fellowship from Fundação de Amparo à Pesquisa do Estado de São Paulo, and B.G. was supported by a studentship from the Fonds de la Recherche en Santé du Québec.

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Received 28 May 1992/24 August 1992; accepted 4 September 1992