The amino acid sequence of the specific α-mannosidase involved in N-oligosaccharide processing in Saccharomyces cerevisiae was found to have a high degree of similarity to the deduced amino acid sequence of a rabbit liver α-mannosidase partial cDNA, demonstrating that processing mannosidases have been conserved through eukaryotic evolution. Regions of sequence identity were chosen to design degenerate oligonucleotide primers that can be used to prepare probes using the genomic DNA using PCR, and PCR as probes revealed a cDNA library. It contains an open reading frame which is identical with the corresponding sequence of the rabbit enzyme, respectively. Southern blot analysis of mouse genomic DNA using PCR, and PCR as probes revealed that they are derived from two different genes, indicating the existence of a mammalian mannosidase gene family with at least two members. Using PCR as a probe, a novel mouse cDNA was isolated from a 3T3 cell line. It contains an open reading frame which encodes a type II membrane protein of 73 kDa with a cytoplasmic region of about 35 amino acids, a Ca²⁺ binding consensus sequence, and a single N-glycosylation site. Northern blot analysis of mouse tissue and cell lines revealed tissue-specific expression of multiple transcripts, ranging in size from 4.2 to 8.5 kilobases, that interferences with the development of capillaries and tumor cell metastasis. The α,2-mannosidase inhibitors, 1-deoxymannojirimycin and kifunensine, which inhibit the synthesis of N-complex oligosaccharides and cause the accumulation of oligomannose oligosaccharides, have been shown to interfere with the development of capillaries in vitro (Nguyen et al., 1992). The α-mannosidase II inhibitor, swainsonine, which causes the formation of hybrid structures instead of complex oligosaccharides, is able to reverse the transformed phenotype of NIH 3T3 cells in vitro (DeSantis et al., 1987), and to inhibit tumor cell metastasis in vivo (Dennis, 1986; Newton et al., 1989). Although little is known of the molecular genetics of human processing mannosidases, one form of the human hereditary anemia, HEMPAS is caused by a deficiency in α-mannosidase II expression (Fukuda, 1990). As a result, hybrid oligosaccharides are found on HEMPAS erythrocyte glycoproteins in the place of the normal poly-N-acetylated structures, an alteration that causes increased susceptibility to lysis. Biochemical studies indicate that several processing α,2-mannosidases exist in mammalian cells with different molecular properties, specificities, and subcellular localization, but the number of distinct mannosidases and their respective role in the processing pathway is not known (for review, see Moremen et al., 1994). Cloning of mammalian α,2-mannosidases is therefore necessary to determine how many of these enzymes are involved, to establish their specific role in the maturation process and their intracellular localization, and to elucidate the genetic control of the early stages of N-oligosaccharide processing.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) U03457 and U03458.

Pathway begins with the transfer of a dolichol-linked oligosaccharide precursor, usually Glc₃Man₃GlcNAc₂, to Asn/X to newly formed polypeptide chains (for review, see Kornfeld and Kornfeld (1985)). Glc₃Man₃GlcNAc₂ is then trimmed by two specific glucosidases which remove the glucose residues, and by several ER and Golgi α,2-mannosidases that can cleave up to 4 mannose residues to yield Man₃GlcNAc₂. The action of α,2-mannosidases at this point in the pathway is an essential step in the maturation process, for the resulting Man₃GlcNAc₂ can be modified by GlcNAc transferase I, the first glycosyltransferase leading to the synthesis of hybrid or complex oligosaccharides. Subsequently, Golgi α-mannosidase II can remove the terminal α,3- and α,6-linked mannose residues from GlcNAcMan₃GlcNAc₂ to form GlcNAcMan₃- GlcNAc₂. This oligosaccharide is then further modified by Golgi glycosyltransferases to generate the variety of N-complex structures found on glycoproteins.

Studies with inhibitors clearly demonstrate the essential role played by mannosidases in the maturation of N-oligosaccharides (for review, see Elbein (1991)) and show that preventing the activity of processing mannosidases may have important biological consequences. The α,2-mannosidase inhibitors, 1-deoxymannojirimycin and kifunensine, which inhibit the synthesis of N-complex oligosaccharides and cause the accumulation of oligomannose oligosaccharides, have been shown to interfere with the development of capillaries in vitro (Nguyen et al., 1992). The α-mannosidase II inhibitor, swainsonine, which causes the formation of hybrid structures instead of complex oligosaccharides, is able to reverse the transformed phenotype of NIH 3T3 cells in vitro (DeSantis et al., 1987), and to inhibit tumor cell metastasis in vivo (Dennis, 1986; Newton et al., 1989). Although little is known of the molecular genetics of human processing mannosidases, one form of the human hereditary anemia, HEMPAS is caused by a deficiency in α-mannosidase II expression (Fukuda, 1990). As a result, hybrid oligosaccharides are found on HEMPAS erythrocyte glycoproteins in the place of the normal poly-N-acetylated structures, an alteration that causes increased susceptibility to lysis.

Mannosidases play an important role at different stages in the maturation of N-oligosaccharides in mammalian cells. This

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This paper is dedicated to the memory of Gersz Nejman (1906-1942).

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In contrast to mammalian cells, the trimming process is much simpler in the yeast, Saccharomyces cerevisiae. The early stages of N-oligosaccharide biosynthesis, including glucose removal, are identical to those observed in mammalian cells, but there is only one processing α,2-mannosidase that removes a single mannose residue from the middle arm of the precursor oligosaccharide to form a single isomer of Man,GlcNAc$_2$. The oligosaccharide precursor is then elongated by Golgi mannosyltransferases to form the mature mannoproteins characteristic of S. cerevisiae (for review, see Herscovics and Orlean (1993)). The yeast specific α-mannosidase has been purified (Jelínek-Kelly et al., 1988; Jelínek-Kelly and Herscovics, 1988; Ziegler and Trimble, 1991) and its gene (MNS1) has been isolated (Camirand et al., 1991). It encodes a type II membrane protein of 63 kDa containing a very short cytoplasmic region of 2-3 amino acids, three N-glycosylation sites, a calcium-binding consensus sequence (Camirand et al., 1991), and a catalytic domain facing the lumen of the endoplasmic reticulum (Grondin and Herscovics, 1992). In contrast to mammalian cells where preventing mannosidase activity interferes with the formation of complex glycosyltransferases, disruption of the yeast processing mannosidase gene is of little consequence to the subsequent maturation of N-oligosaccharides in S. cerevisiae (Puccia et al., 1993).

Although in recent years, an increasing number of mammalian glycosyltransferase genes and cDNAs have been cloned and characterized (for reviews, see Schachter (1991), Shaper and Shaper (1992), and Joziasse (1992)), little is known of the molecular genetics of processing glycosidases. No processing glucosidase and only two full-length mannosidase cDNAs have been isolated from mammalian cells. The first is a rat liver cDNA encoding a cystosolic/ER α-mannosidase that can remove mannose residues from Man$_n$GlcNAC$_2$ (Shoup and Tobster, 1976; Bischoff and Kornfeld, 1983, 1986). The role of this cystosolic/ER enzyme in the processing pathway needs to be clarified since no hydrophobic region which could serve as a transmembrane domain or signal sequence is found in the deduced amino acid sequence (Bischoff et al., 1990). This absence is unusual for an enzyme expected to act on oligosaccharide processing on the luminal side of the ER. In fact, this cDNA is homologous to the yeast vacuolar α-mannosidase, a membrane-bound enzyme acting in the lumen of the yeast vacuole, that gains access to this compartment by a signal sequence-independent mechanism (Yoshiihsa and Anraku, 1990). The other processing mannosidase cDNA clone that has been reported encodes Golgi α-mannosidase II (Moremen and Robbins, 1991). The cDNA contains an unusually long 3′-untranslated region and encodes a protein whose deduced amino acid sequence exhibits the type II membrane topology characteristic of Golgi glycosyltransferases.

In the present work we show that the derived amino acid sequence of the yeast processing mannosidase (Camirand et al., 1991) exhibits significant similarity (37% identity, 58% similarity) to the rabbit liver Ca$^{2+}$-dependent α,2-mannosidase partial cDNA briefly described by Moremen et al. (1990). This cDNA was isolated using amino acid sequence obtained from the rabbit liver α,2-mannosidase purified by Forsee et al. (1989). The yeast and rabbit α,2-mannosidases have no apparent similarity with the amino acid sequences of either Golgi α-mannosidase II (Moremen and Robbins, 1991) or the cystosolic/ER α-mannosidase (Bischoff et al., 1990). Regions of identical amino acid sequences between the yeast and the rabbit enzyme were chosen to design degenerate oligonucleotides for PCR on mouse liver cDNA as template. Using the resulting PCR products, we present evidence for the existence of two related mouse mannosidase genes, and report the isolation of a novel mouse mannosidase cDNA that exhibits tissue-specific expression. We also show, using epitope tagging, that this mannosidase is localized to a juxtanuclear position corresponding to the Golgi following transient expression in COS cells.

**EXPERIMENTAL PROCEDURES**

*Materials.*—Materials were obtained from the following sources: restriction enzymes, New England Biolabs, Life Technologies Inc., or Pharmacia LKB Biotechnology Inc. (Base DF-400, Quebec); the GenAmpDNA amplification reagent kit, Perkin Elmer Cetus; Sequenase, U. S. Biochemical Corp.; T7 polymerase sequencing kit, Sepharose 6B, IgG Sepharose 6FF, Pharmacia, LKB Biotechnology Inc.; the Cyclone Biosystem M13 deletion kit, International Biotechnologies Inc.; the random primed DNA labeling kit, U. S. Biochemical Corp.; Tetrapeptide membranes, Bio-Rad; Hybrid-N nylon membrane, Amersham Corp. All other reagents were at least reagent grade. Synthetic oligonucleotides were prepared at the MIT Biopolymers Laboratory on an Applied Biosystems (Model 380B) DNA synthesizer, or at the Sheldon Biotechnology Centre, McGill University, on a Gene-Assembler Plus from Pharmacia according to the manufacturer’s instructions. Plasmid preparations were obtained using columns obtained from Qiagen Inc. Bovine serum albumin (highest grade) was obtained from Boehringer-Mannheim (Laval, Quebec). All procedures were performed according to Sambrook et al. (1989) unless otherwise specified.

**Polymerase Chain Reaction Experiments.**—Degenerate oligonucleotides corresponding to two regions that were completely conserved between the yeast (Camirand et al., 1991) and the rabbit (Moremen et al., 1990) mannosidases were designed as shown in Table I. The sense oligonucleotide contained 2 deoxynucleosine residues. First strand cDNA was synthesized from oligo(dT)-selected mouse liver RNA using murine leukemia virus reverse transcriptase and random primers, as described previously (Moremen, 1989). This cDNA served as a template in the polymerase chain reaction using the Perkin Elmer Cetus reagents in a final volume of 50 ml containing 50 mm Tris-HCl, pH 8.3, 50 mm KCl, 1.5 mm MgCl$_2$, 0.001% gelatin, 200 μg of each dNTP, 1 μg of each oligonucleotide primer, and 2.5 units of Taq polymerase, overlaid with 50 μl of mineral oil. 35 automated step cycles were conducted as follows: 1 min at 92°C, 1 min at 50°C, and 3 min at 72°C. The last cycle was

| Oligonucleotide 1 (sense) | CCG | CAA | TTC | D | GTT | S | TCT | F | Y | E | TAT | L | TTG | CAG | CCG | TTA | AA |
|--------------------------|----|----|-----|---|-----|---|-----|---|---|---|-----|---|-----|-----|----|-----|---|
| Oligonucleotide 2 (antisense) | CCG | AAG | CTT | H | AAG | E | TTC | TAT | N | F | TTA | G | TTT | G | G | TTA | C |

---

**Table I**

**Oligonucleotide primers used for PCR reactions**

Oligonucleotides were designed to amino acid sequences conserved between the yeast and rabbit mannosidases. Oligonucleotide 1 corresponds to the yeast amino acid residues 275–283, and oligonucleotide 2 to amino acid residues 528–523 (Camirand et al., 1991). Amino acids are above the nucleotide sequences in one-letter code, with the sequence reversed for the antisense oligonucleotide. Nucleotide sequences are written 5′ to 3′.
followed by a 3-mm extension at 72 °C. Control PCR reactions were also done with rabbit liver cDNA and yeast genomic DNA as templates. The products were fractionated by electrophoresis in 1.6% agarose. A major amplification product of about 760 bp was obtained with all three templates. The PCR product obtained from mouse liver cDNA was subcloned into Bluescript pSK (+) and M13 mp19 for sequencing of 10 random clones in each orientation. Two populations of sequences, named PCR1, and PCR2, were obtained.

Isolation of cDNA Clones—A BALB/c 3T3 cell line primed with a mixture of oligo(dT) and random hexamers and packaged into a AZAP II vector (Stratagene, La Jolla, CA) was obtained from D. J. G. Rees (Massachusetts Institute of Technology) (Rees et al., 1990). The 672-bp PCR product was random-labeled (specific activity, 2-4 x 10^6 cpm/μg of DNA) and used to screen 2.6 x 10^8 plaque forming units of the cDNA library spread on a lawn of XL1-Blue cells (35 x 150-mm Petri dishes). Plaque lifts were carried out in duplicate using Hybond-N nylon membranes, followed by prehybridization and hybridization with labeled PCR, (6-8 x 10^6 cpm/ml) as probe, as previously described (Moremen and Robbins, 1991). Positive clones were purified by three additional rounds of screening as described above. Excision of the phage insert DNA was done in M13 using T4 polymerase (Cyclone Biosystem M13 sequencing kit) (Sanchez-Lopez et al., 1988) and used to screen 2.6 x 10^8 plaque forming units. Sequences, named PCR1, and PCR2, were obtained. Sequence assembly was done using the SeqMan program of DNASTAR (Madison, WI). The filters were washed twice for 15 min at room temperature, followed by a wash in 0.2% Tween 20, PBS, and then incubated for 1 h at room temperature in 0.2% Tween 20, PBS, and 0.4% SDS. The gel was then equilibrated in 10 mM Na phosphate, pH 7, containing 150 mM NaCl, and transferred to 150-mm Petri dishes. The day before transfection, the cells were trypsinized and seeded onto four-chamber glass slides (Nunc, Naperville, IL). Cells at 50-70% confluency were transfected by the DEAE-dextran plus chloroquine method (Ausubel et al., 1989) with 1 μg/chamber of mammnosidase cDNA in pXm-139. Expression was allowed to proceed for 20-64 h before the cells were processed for immunofluorescence, essentially as described (Lebkowitz et al., 1992). Cells were washed twice in PBS, fixed for 30 min at room temperature in 4% formaldehyde in PBS, permeabilized for 30 min at room temperature in 0.2% Tween 20, 4% formaldehyde, PBS. After washing with 0.2% Tween 20, PBS, nonspecific sites were blocked by 5% nonfat dry milk in 0.2% Tween 20, PBS, and then incubated for 1 h at room temperature in 0.2% Tween 20, PBS, and 0.2% bovine serum albumin, 0.2% NaCl. After washing with 0.2% Tween 20, PBS, the cells were incubated overnight at 4 °C with a 1:1000 dilution of mouse ascites fluid containing 125I-conjugated antibody directed against the influenza HA epitope. The cells were washed five times with 0.2% Tween 20, PBS, and then incubated for 1 h at room temperature in the dark with a 1:100 dilution of tetramethyl rhodamine isothiocyanate-conjugated goat anti-mouse IgG (H+L) (Jackson Immunoresearch, West Grove, PA). Following seven washes in 0.2% Tween 20, PBS, slides were mounted in 30% glycerol, 0.02% NaN3, PBS, sealed with nail polish. The cells were kept in the dark. The slides were observed with a different Planapochromat inverted microscope with epifluorescence. Photographs were taken on Kodak Tmax 400 film with an MC 100 camera at either 100 or 400 x magnification.
Sequence Similarity between Yeast and Rabbit Mannosidase—The deduced amino acid sequence of the MNS1 gene (accession number, M63598) encoding the yeast processing α-mannosidase that removes a single specific mannosic residue from Man₉GlcNAc (Camirand et al., 1991) was found to be similar to that of a partial cDNA encoding an α,1,2-mannosidase purified from rabbit liver (Forsee et al., 1989; Moremen et al., 1990, accession number V04301). The deduced amino acid sequences of these two enzymes exhibited 37% identity and 58% similarity when analyzed with the Bestfit (version 7) sequence analysis program from the University of Wisconsin Genetics Computer Group. Four regions of 7–10 amino acids (amino acids 139–145, 274–283, 501–509, and 523–528 of the yeast mannosidase, see Camirand et al. (1991)) are identical in the yeast and rabbit mannosidases. Two of these conserved peptide sequences were used to design degenerate oligonucleotides as the two primers. Restriction analysis, however, suggested that corresponding to the size expected for amplification between the two primers. Restriction analysis, however, suggested that PCR product obtained using mouse liver cDNA as template might be heterogeneous. The mouse liver PCR product was therefore subcloned into M13 in both orientations and random clones were sequenced. Two PCR populations with different sequences were obtained: the deduced amino acid sequence of PCR, was 88% identical with the corresponding region in the rabbit mannosidase cDNA clone and 98% identical with that of a mannosidase cDNA clone isolated from a 3T3 cDNA library using the rabbit mannosidase cDNA as a probe. On the other hand, the amino acid sequence of PCR, exhibited only 65 and 72% amino acid sequence identity with the rabbit and its corresponding 3T3 mannosidase cDNA, respectively. Both PCR, and PCR, contained a third conserved region corresponding to amino acids 501–509 of the yeast mannosidase sequence.

Southern Blot Analysis—Since the isolation of two distinct PCR products suggested that there may be two different mouse mannosidase genes, Southern blots of mouse genomic DNA hybridized with labeled PCR, and PCR, were compared (Fig. 1). It is evident that the pattern of labeled restriction fragments obtained with these two probes is quite different, thereby demonstrating the existence of two distinct mannosidase genes.

Isolation of cDNA Clones Encoding Murine Mannosidase—Labeled PCR, was used as a probe to screen a 3T3 cDNA li-

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men, manuscript submitted for publication.
between clone 4 and clone 16 are indicated by while those in mannosidases are very different. The mouse protein has a cytoplasmic region of about 35 amino acids and about 100 amino acids between its putative transmembrane domain and the majority of positive cells (Fig. 7) 24-64 h after transfection. In their relative expression (Fig. 6). The highest level of expression was observed in L cells, followed by colon, ovary, thymus, and brain; lower levels were observed in kidney, uterus, liver, and lung. In most cases the major transcripts were 4.2, 5.1, 6.4, 7.4, 8.7 kb, with the notable exception of brain in which the major transcript was 8.7 kb, and ovary which also had a high level of expression of the epitope-tagged mannosidase cDNA in COS cells some cells there was also a fine reticular pattern of immunostaining. The nucleotide sequence of the combined clone 4 and clone 16 DNAs is shown. On the right the numbers in normal type refer to the nucleotides with position 1 corresponding to the initiation codon. The nucleotide sequence of the combined clone 4 and clone 16 DNAs is shown. On the right the numbers in normal type refer to the nucleotides with position 1 corresponding to the initiation codon.

Expression of Mannosidase in COS Cells—Transient expression of the epitope-tagged mannosidase cDNA in COS cells followed by indirect immunofluorescence using monoclonal antibody 12C5 to the influenza hemagglutinin epitope showed strong immunofluorescence in a juxtanuclear position in a majority of positive cells (Fig. 7) 24–64 h after transfection. In some cells there was also a fine reticular pattern of immuno-
Mouse Golgi Mannosidase cDNA

Fig. 4. Alignment of mouse mannosidase corresponding to probe PCR, and yeast processing mannosidase protein sequences. The Bestfit (Version 7) sequence analysis program from the University of Wisconsin Genetics Computer Group was used for the alignment (gap weight = 3.0, gap length weight = 0.1), except that the putative transmembrane domains of the two proteins (underlined) were lined up. The mouse mannosidase amino acid sequence is on top and the yeast processing mannosidase is on the bottom. Bars indicate amino acid identities, double dots indicate conservative amino acid substitutions, and single dots indicate similar amino acid substitutions. ●, conserved cysteine residues; rectangles surround regions conserved between species, the solid one being the Ca²⁺-binding consensus sequence.

Mouse 1  http://plspgrriplnlqpsfphixafyrlseky
Yeast  1  NYS56

Fig. 5. DOTPLOT analysis. The mouse α-mannosidase corresponding to probe PCR, was compared to the mouse α-mannosidase corresponding to probe PCR, (Lal et al., Fig. 2; accession number V04299) Comparisons were made with the DOTPLOT program from the University of Wisconsin Genetics Computer Group, Version 7, using a window of 30 and a stringency of 15.

fluorescence corresponding to the ER, indicating an accumulation of the mannosidase in the ER most likely due to overexpression. There was no apparent difference in the immunofluorescence pattern observed with clone 4 or a reconstituted clone 4/16.

To establish that the isolated cDNA encodes a catalytically active mannosidase, the C-terminal region of clone 4/16 lacking the putative transmembrane domain was expressed in COS cells as a secreted fusion protein with the IgG binding domain of S. aureus Protein A in the mammalian expression vector pPROTA (Sanchez-Lopez et al., 1988) essentially as described previously for the expression of glycosyltransferases (Larsen et al., 1989; Kukowska-Latallo et al., 1990). The medium of cells transfected with either the control vector pPak, or the vector encoding the fusion protein pPakman 416/106 was treated with IgG-Sepharose, and the α-mannosidase activity of the beads was assayed directly using uniformly labeled [³H]Man₉GlcNAc (8200 cpm) as substrate, as described previously (Herscovics and Jelinek-Kelly, 1987). In separate transfections, no significant release of [³H]Man was observed in the samples obtained from cells transfected with the control vector compared to about

Fig. 6. Northern blot of mouse tissues and L cells. Poly(A⁺) RNA (10 μg) from mouse tissues and L cells were probed with random labeled coding region of clone 4. L cells showed a similar pattern of transcripts using random labeled PCR, 5′-untranslated region (nucleotides −558 to −226), or 3′-untranslated region (nucleotides 1995 to 2656) as probe (see Fig. 3). Exposure for radioautography to Kodak X-AR5 film was 2 days, except for the RNA from L cells in the last lane which was re-exposed overnight. G3PDH, the blot was probed with glyceraldehyde-3-phosphate dehydrogenase cDNA.

Fig. 7. Localization of mannosidase. Epitope-tagged mouse mannosidase cDNA was localized in COS 7 cells by immunofluorescence using monoclonal antibody 12CA5 to the influenza hemagglutinin epitope, as described under “Experimental Procedures.” The top panel shows immunofluorescence (left) and phase-contrast of the same cell (right) 42 h post-transfection. The bottom panel shows immunofluorescence of a cell 42 h post-transfection. The bars indicate 3 μm.
This report is the first demonstration of the existence of a mannosidase gene family conserved through eukaryotic evolution. The similarity observed between the amino acid sequence of the processing α-mannosidase from S. cerevisiae and the rabbit liver Ca⁺⁺-dependent α-mannosidase allowed us to design degenerate oligonucleotide primers for PCR which are useful to isolate members of this gene family from different species. Using these degenerate primers for PCR with mouse liver cDNA as template, two distinct but related PCR products (PCR₁ and PCR₂) were obtained. Southern blot analysis of mouse genomic DNA using PCR₁ and PCR₂ as probes revealed that the mouse genome contains at least two members of the mannosidase gene family.

A novel mouse mannosidase cDNA was isolated using PCR₂ as a probe. It encodes a type II membrane protein of 73 kDa which localizes to the Golgi following transient expression in COS cells. Its derived amino acid sequence is very similar to that of the mouse mannosidase cDNA corresponding to PCR₁, but that was isolated using the rabbit liver cDNA clone as a probe (Moremen et al., 1990). The two mouse enzymes are highly similar in sequence (64% identity, 77% similarity), size, and topology. They both have a Ca⁺⁺ binding consensus sequence. The N-terminal region of the mouse mannosidases is completely different from that of the yeast mannosidase. The yeast protein has a different transmembrane region and lacks a significant cytoplasmic region as well as the region of about 100 amino acids immediately following the transmembrane domain. It will be of interest to determine whether the differences in the N-terminal region of the mouse and yeast enzymes are related to different subcellular localization or to differences in enzyme specificity. It has been demonstrated that the transmembrane domain is essential for targeting of glycosyltransferases to the Golgi (for review, see Shaper and Shaper (1992)). The yeast processing mannosidase, unlike the two mouse mannosidases, is thought to be located in the ER or in an intermediate pre-Golgi compartment, since the sec18 mutant, which is blocked in ER to Golgi transport, is capable of trimming N-oligosaccharides to Man, GlcNAc₂ at the nonpermissive temperature (Emson et al., 1984).

The complex pattern and tissue-specific expression of the mouse mannosidase observed upon Northern blot analysis may be due to a combination of different factors including alternate splicing, the use of different polyadenylation sites, and of alternate tissue-specific promoters. In some tissues, there is an inverse relationship between the expression of the two mannosidase genes. For example, liver which expresses the highest level of mRNA hybridizing with PCR₁, has a low level of expression with PCR₂, whereas L cells which express high levels of PCR₂ have no detectable PCR₁ transcripts. Recent studies on the regulation of expression of the mouse α,2-mannosidase suggest that the regulation of expression of the mouse mannosidase gene family may be determined by the presence of Ca⁺⁺ and possibly by the presence of a Ca⁺⁺-dependent mannosidase.

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REFERENCES

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Kroh, R. (1989) Current Protocols in Molecular Biology, John Wiley and Sons, New York.

Bause, E., Breuer, W., Schweden, J., Roesser, R., and Geyer, R. (1992) Eur. J. Biochem. 208, 451–457.

Bischoff, J., and Kornfeld, R. (1983) J. Biol. Chem. 258, 7907–7910.

Bischoff, J., and Kornfeld, R. (1996) J. Biol. Chem. 271, 4768–4767.

Bischoff, J., Moremen, K., and Lodish, H. F. (1990) J. Biol. Chem. 265, 17110-17117.

Bonay, P., and Hughes, R. C. (1991) Eur J. Biochem. 197, 229–238.

Camirand, A., Heysen, A., Grendin, B., and Herscovics, A. (1991) J. Biol. Chem. 266, 15120–15127.

Chingwin, J. M., Przybylea, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299.

Dennis, J. W. (1986) Cancer Res. 46, 5131–5136.

Del Cass, P. A., and Slichter, J. J. (1957) Biochim. Biophys. Res. Commun. 142, 348–353.

Elbein, A. D. (1991) FASEB J. 5, 3055–3060.

Esmon, B., Esmon, P. C., and Schekman, R. (1984) J. Biol. Chem. 259, 10322–10327.

Field, J., Nikawa, J.-I., Broek, D., MacDonald, B., Rodgers, L., Wilson, I. A., Lerner, R. A., and Wiegler, M. (1988) Mol. Cell. Biol. 8, 2159–2165.

Forse, W. T., and Schutz, R. J. (1981) J. Biol. Chem. 256, 6577–6582.

Forsee, W. T., Palmor, C. F., and Schutz, R. J. (1989) J. Biol. Chem. 264, 3869–3876.

Fukuda, M. N. (1990) Glycobiology 1, 9–15.

Grendin, B., and Herscovics, A. (1992) Glycoconjugate J. 9, 369–372.

Harduin-Lepers, A., Shaper, J. H., and Shaper, N. L. (1993) J. Biol. Chem. 268, 14348–14350.

Hartman, E., Rapoport, T. A., and Lodish, H. F. (1989) Proc Natl. Acad. Sci. U. S. A. 86, 5786–5790.

Herzog, A., and Jelinek-Horowitz, S. (1987) Anal. Biochem. 166, 85–89.

Herzog, A., and Orlean, P. (1993) FASEB J. 7, 540–550.

Jelinek-Kelly, S., and Herscovics, A. (1988) J. Biol. Chem. 263, 14757–14762.

Jelinek-Kelly, S., Akiyama, T., Sonnier, B., Tkacz, J. S., and Herscovics, A. (1985) J. Biol. Chem. 260, 2253–2257.

Joziasse, D. H. (1992) Glycobiology 2, 271–277.

Kolodziej, P. A., and Young, B. A. (1989) Mol. Cell. Biol. 9, 5387–5394.

Kornfeld, R., and Kornfeld, S. (1985) Annu. Rev. Biochem. 54, 631–664.

Kozak, M. (1989) J. Cell Biol. 106, 229–241.

Kukowska-Latallo, J. F., Larsen, R. D., Nair, R. P., and Lowe, J. B. (1990) Genes & Dev. 4, 1288–1303.

Larsen, R. D., Bajaj, V. P., Ruff, M. M., Kukowska-Latallo, J., Cummings, R. D., and Lowe, J. B. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8227–8231.

Lepikowicz, F., Goyer, C., Darveau, A., Neron, S., Lemieux, R., and Sorenberg, N. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 9612–9616.

Lubas, W. A., and Siro, R. G. (1987) J. Biol. Chem. 262, 3990–3996.

Marsden, R. J., Shaw, G. S., and Sykes, B. D. (1990) Biochem. Cell Biol. 68, 587–601.

Moremen, K. W. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5278–5280.

Moremen, K. W., and Robbins, P. W. (1991) J. Cell Biol. 114, 1521–1534.

Moremen, K. W., Schutz, R. J., Forsee, W. T., Neame, P., Bischoff, J., and Lodish, H. F. (1990) Glycoconjugate J. 7, 401.

Moremen, K. W., Trumble, R. B., and Herscovics, A. (1994) Glycobiology, in press.

Newton, S. A., White, S. L., Humphries, M. J., and Olden, K. (1989) J. Natl. Cancer Inst. 81, 1024–1028.

Nguyen, M., Folkman, J., and Bischoff, J. (1992) J. Biol. Chem. 267, 26157–26167.

Puccia, R., Grendin, B., and Herscovics, A. (1993) Biochem. J. 290, 21–26.

Rees, D. J. G., Aitken, S. J., and Hynes, R. O. (1990) Nature 347, 685–689.

Roth, J., Brada, D., Lackie, P. M., Schweden, J., and Bause, E. (1990) Eur. J. Cell Biol. 53, 131–141.

Sanfilippo, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Second ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanchez-Lopez, R., Nicholson, R., Gonzal, M. C., Matrisian, L. M., and Breathnach, R. (1988) J. Biol. Chem. 263, 11892–11899.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467.

Schachter, H. (1991) Curr. Opin. Struct. Biol. 1, 755–765.

Schwed, J., and Bause, E. (1989) Biochem. J. 264, 347–355.

Schwed, J., Leag, G., and Baus, E. (1986) Eur. J. Biochem. 157, 563–570.

Shaper, J. H., and Shaper, N. L. (1993) Curr. Opin. Struct. Biol. 3, 701–709.

Shoup, A. V., and Touster, O. (1976) J. Biol. Chem. 251, 3845–3852.

Tabas, I., and Kornfeld, S. (1990) J. Biol. Chem. 265, 11655–11663.

Tusiani, D. R. P., Hubbard, S. C., Robbins, P. W., and Touster, O. (1992) J. Biol. Chem. 267, 3660–3668.

Tusiani, D. R. P., and Touster, O. (1986) J. Biol. Chem. 261, 13081–13087.

Tusiani, D. R. P., and Touster, O. (1988) J. Biol. Chem. 263, 5408–5417.

Velasco, A., Hendricks, L., Moremen, K. W., Tusiani, D. R. P., Touster, O., and Farquhar, M. G. (1993) J. Cell Biol. 122, 39–51.

von Heijne, G., and Gavel, Y. (1988) Eur. J. Biochem. 174, 671–679.

Yang, Y.-C., Carlstedt, A. B., Temple, P. A., Chung, M. P., Kovacic, S., Witek-Giannotti, J. S., Leary, A. C., Kriz, R., Denahue, R. E., Wong, G. C., and Clark, S. C. (1986) Cell 47, 3–10.

Yoshihisa, T., and Anraku, Y. (1990) J. Biol. Chem. 266, 22418–22425.

Ziegler, F. D., and Trimble, R. B. (1991) Glycobiology, 605–614.