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Activity of the yeast *MNN1* α-1,3-mannosyltransferase requires a motif conserved in many other families of glycosyltransferases

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ABSTRACT **A wide diversity of biological molecules are modified by the addition of sugar residues, and a large number of glycosyltransferases have been identified that are responsible for these reactions. Despite catalyzing closely related reactions, many of these transferases show little apparent sequence homology. By comparing two apparently unrelated families of yeast Golgi mannosyltransferases, a short motif containing two aspartate residues was observed that was conserved in both groups of proteins. Mutagenesis of one of the members of these families, the α-1,3-mannosyltransferase Mnn1p, showed that altering either of these aspartates eliminates all enzymatic activity. These changes do not appear to affect the overall folding and assembly of Mnn1p. A similar aspartate-containing sequence was found to be conserved in a diverse range of other glycosyltransferase families, much more frequently than would be expected by chance, suggesting that it is a feature of the catalytic site, or an element of a structural fold, shared by many glycosyltransferases.**

The attachment of specific sugar residues to substrates is of fundamental importance to both prokaryotes and eukaryotes. Proteins and lipids can be covalently modified by the addition of carbohydrate groups, and further sugar residues can then be added to form complex oligosaccharide structures. These glycosylated molecules have a wide range of roles, including those of determining protein structure and folding, maintenance of the structure of cell membranes and cell walls, construction of extracellular matrix, storage of food, and serving as ligands for cell–cell interactions (1). In addition, some bacterial toxins irreversibly inhibit target proteins by glycosylation (2). This wide range of glycosylation reactions is catalyzed by a correspondingly large number of specific glycosyltransferases (3). In most cases the sugar residue is transferred to an acceptor hydroxyl on the target protein, lipid, or oligosaccharide. The most common donors are nucleotide sugars, although some enzymes use lipid-based donors such as dolichol phosphate sugars.

Genome sequencing is identifying an increasing number of open reading frames (ORFs), encoding proteins of unknown function but with homologs in other species. Identification of sequence motifs shared by enzymes with related functions provides a means to propose functions for such ORFs. Glycosyltransferases are often members of families of related proteins that catalyze closely related reactions, but there is a striking lack of sequence conservation between these families. This situation is in contrast to other large groups of diverse enzymes, such as nucleotide polymerases or proteases, for which it has been possible to identify conserved residues, which are often involved in catalysis (4, 5). This lack of discernible homology between glycosyltransferases is clearly apparent among those of the eukaryotic secretory pathway. These enzymes are responsible for modifying the N- and O-linked glycans of glycoproteins and glycolipids, by the addition of a wide range of oligosaccharides. Most of these enzymes act in the Golgi apparatus and use nucleotide sugars as donors, yet enzymes responsible for attaching the same sugar to different acceptors, or different sugars to the same acceptor, can show no apparent sequence homology. Joziasse *et al.* (6) reported a short region of identity between bovine α-1,3-galactosyltransferase and human β-1,4-galactosyltransferase, but this identity is not conserved in the members of this family examined subsequently.

Such a lack of homology need not imply a lack of structural similarity, and indeed structural determination can identify key conserved motifs missed by comparison of primary sequences. However, at present no structure of a nucleotide-sugar-using glycosyltransferase has been reported. Attempts have been made to use secondary structure prediction to look for structural relatedness between glycosyltransferase families. On this basis it has been suggested that bacterial mannosyltransferases have a structure related to the cellulose synthases of bacteria and plants (7, 8). However, this still leaves many other families of transferases. In this paper we report a short sequence motif that we initially observed as being conserved in two protein families related to the mannosyltransferases Mnn1p and Och1p from the yeast *Saccharomyces cerevisiae.* *MNN1* encodes a type II membrane protein localized to the medial Golgi apparatus (9). *mnn1* mutants lack the terminal α-1,3-mannose residues on the outer chains of both O- and N-linked sugars, and Mnn1p has been shown to have α-1,3-mannosyltransferase activity *in vitro* (10). Mutagenesis of Mnn1p shows that two conserved aspartate residues of the motif are absolutely essential for Mnn1p activity both *in vivo* and *in vitro*. Moreover, this aspartate-containing motif appears to be conserved in many families of glycosyltransferases, suggesting that it could be a general feature of either their structure or their active sites.

MATERIALS AND METHODS

Yeast Strains and Media. *S. cerevisiae* strain SEY6210 (*MATα ura3-52 leu2-3,112 his3-Δ200 trp1-Δ901 lys2-801 suc2Δ9*) was used (11). Growth of yeast strains and plasmid transformation were by standard procedures (12). The *MNN1* gene was knocked out, or tagged with the simian virus 5 (SV5) or hemagglutinin (HA) epitope, by using *Schizosaccharomyces pombe HIS5*, and insertions were checked by PCR (13). *MNN1* from plasmid pZV236 (9) was tagged directly at the C terminus with three copies of the myc epitope, and it was expressed under its own promoter in vector pRS414. Single point mutations were introduced into *MNN1* by PCR, and all were checked by sequencing.

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Abbreviation: ER, endoplasmic reticulum.

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Immunoprecipitation. Yeast grown to an OD_{600} of 0.9–1.0 were harvested, treated with 10 mM sodium azide, resuspended in 100 mM Tris·HCl, pH 9.4/10 mM DTT, and incubated for 10 min at 30°C. Cells were then spheroplasted in 1 M sorbitol/50 mM Tris·HCl, pH 7.4/2 mM $MgCl_2$/10 mM DTT/0.2 mg/ml lyticase for 40 min at 30°C. Spheroplasts were lysed in lysis buffer (1% digitonin/10 mM triethanolamine·HCl, pH 7.5/0.15 M NaCl/1 mM EDTA/0.01% phenylmethylsulfonyl fluoride/5 μg/ml pepstatin A/5 μg/ml chymostatin) by inversion at 4°C for 30 min. Unlysed cells were removed by centrifugation at 13,000 $\times$ *g* for 10 min, and tagged protein was precipitated from the supernatant by using 30 μg/ml anti-myc monoclonal antibody 9E10, and 60 μl/ml protein G-Sepharose, overnight at 4°C. The protein G beads were washed twice with lysis buffer and once with 0.4% digitonin/50 mM Hepes, pH 7.2, prior to assaying mannosyltransferase activity.

Mannosyltransferase Assays. Samples were assayed for α-1,3-mannosyltransferase activity by using a modification of established methods (14). Reaction mixtures (50 μl) containing 50 mM Hepes at pH 7.2, 10 mM $MnCl_2$, 0.4% digitonin, 10 mM α-1,2-mannobiose (Dextra Laboratories, Reading, U.K.) as acceptor, 0.6 mM GDP-mannose, and 4 μM guanosine diphospho-D-[U-^{14}C]mannose (305 mCi/mmol, Amersham; 1 mCi = 37 MBq) were incubated for 1 hr at 30°C with gentle shaking. Neutral products were eluted from a Dowex 1-X8 (acetate) column with 1 ml of water, 9 ml of Ecoscint (National Diagnostics) was added, and radioactivity was quantified by scintillation counting.

Immunofluorescence and Immunoblotting. Immunofluorescence, preparation of whole-cell protein extracts, and immunoblotting were carried out as described previously (15). Rabbit anti-myc (Santa Cruz Biotechnology), anti-myc monoclonal antibody 9E10, rat anti-HA monoclonal antibody (Boehringer Mannheim), and anti-HDEL monoclonal antibody 2E7 were used for immunofluorescence (16). Rabbit anti-myc, anti-SV5 monoclonal antibody, and rabbit anti-α-1,3-mannose antiserum (gift of Rainer Duden, University of Cambridge, U.K.) were used for immunoblotting and were detected by enhanced chemiluminescence (ECL; Amersham) (17).

RESULTS AND DISCUSSION

Identification of Homologs of the Mannosyltransferases Mnn1p and Och1p. Mnn1p, an α-1,3-mannosyltransferase in the Golgi apparatus of *S. cerevisiae*, has a type II topology with a short cytoplasmic tail and a large catalytic domain in the lumen, a structure typical of Golgi glycosyltransferases. When the luminal portion of Mnn1p is used to search the complete *S. cerevisiae* genome, five further sequence relatives can be identified, all with the same predicted topology. Three of these genes encode proteins quite closely related to Mnn1p, with homology all along their luminal portions and significance score calculated by using the BLAST algorithm of $P < 10^{-6}$ (18). Two further genes, YBR015c and YJL186w, are highly homologous to each other, but more distantly related to *MNN1,* with homology restricted to three sections of the proteins. Fig. 1 shows an alignment of all six proteins in the central and longest of these related sections, together with an ORF from *Pichia pastoris* that is most closely related to YBR015c. At present the functions of these Mnn1p relatives are unknown, although the sequence homology suggests that they are glycosyltransferases in the secretory pathway. It is known that in addition to the α-1,3-linked mannose added by Mnn1p, additional α-1,3-mannose residues are added to O-linked glycans and to glycosyl-phosphatidylinositol anchors in the Golgi apparatus, so it is possible that they are added by Mnn1p relatives.

Och1p is an α-1,6-mannosyltransferase of the yeast cis-Golgi (19). Searching the yeast genome has revealed one closely related protein, Hoc1p, and two more distantly related proteins, Sur1p and YBR161w. In addition, there are two genes in *Sch. pombe* that encode relatives of Sur1p (Fig. 1). All of these Och1p relatives are predicted to be membrane proteins. Although the function of none is known, Hoc1p is a Golgi protein, and Sur1p is required for the mannosylation of sphingolipids, which in yeast occurs in the Golgi apparatus (20–22). The homology between Och1p and Sur1p is restricted to a region of approximately 100 residues, and comparison of this region to the longest conserved region of the Mnn1p family showed that, despite the general lack of similarity between the two families, they do share one short conserved motif (Fig. 1). This motif is a spaced pair of aspartates, with hydrophobic residues on both sides, particularly the N-terminal side. Because of the striking conservation of this motif, and, as will be discussed later, its appearance in further families of glycosyltransferases, site-directed mutagenesis was used to examine the importance of these residues for the function of Mnn1p.

Tagging and Expression of *MNN1*. To allow isolation and localization of Mnn1p, the *MNN1* gene was tagged at its C terminus with three copies of the myc epitope (Fig. 2*A*). The tagged gene was expressed under its own promoter in a CEN vector both in wild type SEY6210 and in a strain derived from this in which the endogenous *MNN1* gene had been deleted. To confirm that the tagged protein was still functional, the addition of α-1,3-mannose residues to total yeast proteins was analyzed by immunoblotting. Fig. 2*B* shows that the tagged *MNN1* gene restored addition of α-1,3-mannose residues to the Δ*mnn1* strain, demonstrating that the tagged enzyme is still active. In addition, immunoprecipitation of the tagged Mnn1p allowed its mannosyltransferase activity to be assayed *in vitro*. The tagged protein used α-1,2-mannobiose as a substrate, in preference to α-methyl α-D-mannoside or α-1,6-mannobiose, and the reaction was linear for at least an hour under the conditions used (data not shown).

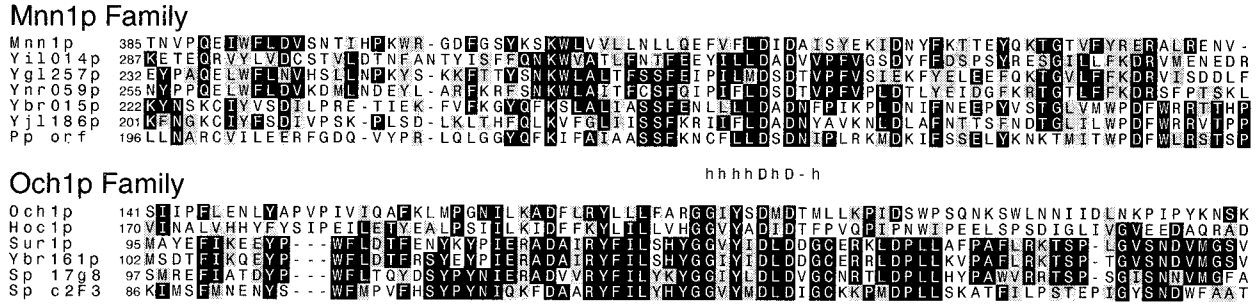

FIG. 1. Alignment of yeast proteins related to Mnn1p and to Och1p. Homologs identified by BLAST were aligned with CLUSTAL W. ORFs are from *S. cerevisiae*, unless indicated (Pp, *P. pastoris*; Sp, *Sch. pombe*. "Pp orf" is SwissProt accession no. Q92264). Residues conserved (black) or related (gray) in three or more sequences are boxed. The conserved DXD motif is indicated.

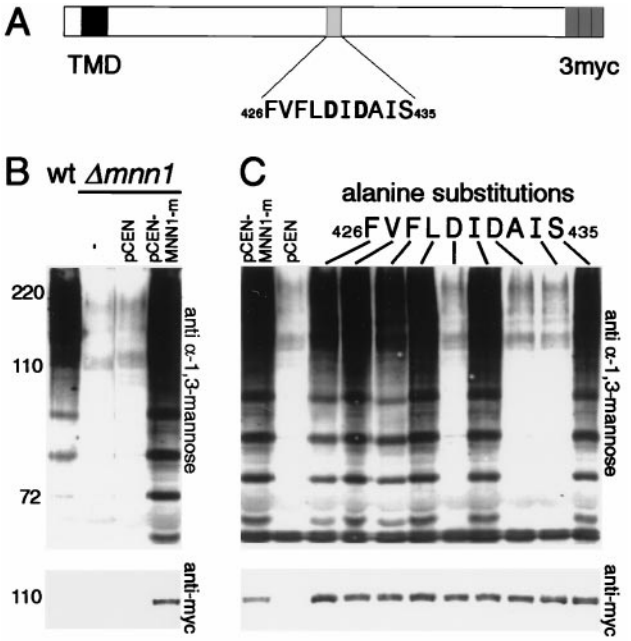

FIG. 2. Expression and *in vivo* activity of mutated versions of Mnn1p. (*A*) Organization of Mnn1p, showing the transmembrane domain (TMD) and the myc-epitope tags. (*B*) myc-tagged Mnn1p is active. Shown are blots of total protein from wild type (wt) or from Δ*mnn1* strains containing no plasmid, a control CEN plasmid, or a CEN plasmid expressing myc-tagged Mnn1p, probed with antibodies to α-1,3-mannose or to the myc epitope. (*C*) Activity of alanine scanning mutations of Mnn1p. As *B*, but proteins were prepared from Δ*mnn1* yeast containing Mnn1p-myc on a CEN plasmid with the indicated residues changed to alanine.

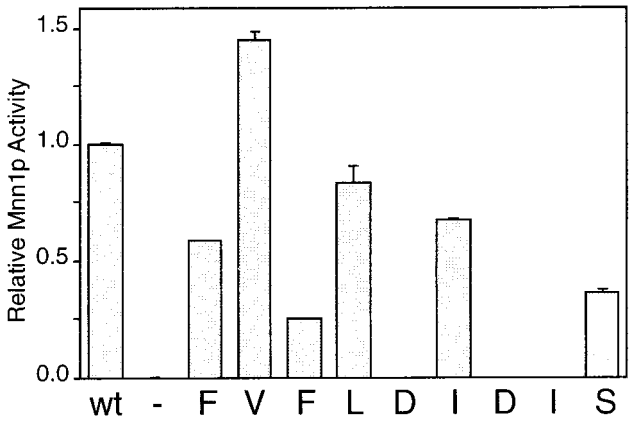

FIG. 3. *In vitro* mannosyltransferase activity of mutated versions of Mnn1p. Myc-tagged proteins were immunoprecipitated from the yeast strains in Fig. 2, and the beads were assayed for transfer of [^{14}C]mannose from GDP-mannose to α-1,2-mannobiose. The results are means of duplicates normalized to the amount of Mnn1p on the beads as determined by quantitative protein blotting. Background obtained from a strain with no myc-tagged protein (" - ", 3% of wild-type signal) was subtracted.

Mutants in Mnn1p That Affect Enzymatic Activity. To determine the importance of the residues within the conserved motif, each residue in this region of Mnn1p was mutated individually to alanine. Mutant proteins were expressed in the Δ*mnn1* strain, and total protein was immunoblotted for α-1,3-linked mannose. As can be seen in Fig. 2*C*, the only residues essential for α-1,3-mannosyltransferase activity are the pair of aspartate residues, together with the isoleucine two residues C-terminal to them. All other mutants exhibited at least some activity, although at a reduced level in some cases (e.g., F428A, Fig. 2*C*). This finding suggests that the highly conserved aspartate residues identified by sequence comparisons are critical for Mnn1p activity. Each strain was also immunoblotted for the myc-tagged Mnn1p protein. The blots showed that all mutant proteins were expressed at comparable levels, indicating that the reduction in activity *in vivo* was not because of a reduction in protein expression because of misfolding and hence degradation of Mnn1p.

The mannosyltransferase activity of the mutants was also assayed *in vitro* by immunoprecipitating Mnn1p-myc from each strain (Fig. 3). Mutations to the two aspartates and the second isoleucine residue result in loss of all detectable activity (<0.2% of wild type). The other residues are apparently not essential for activity, but the lower activity with F426A and F428A suggests that alternating residues in this hydrophobic region are required for full activity. These results are consistent with the apparent *in vivo* activities.

Intracellular Localization of the Mnn1p Mutants. Mnn1p is a resident protein of the yeast Golgi apparatus. If the inactivating mutations identified above are seriously disrupting the folding of Mnn1p, the resulting aberrant proteins would be unlikely to escape the quality control system of the endoplasmic reticulum (ER). Thus the intracellular localization of the wild-type and mutant myc-tagged proteins was examined by immunofluorescence. It has been shown previously that epitope tagging Mnn1p at the C terminus does not perturb its localization (24), and Fig. 4*A* shows that, as expected, myc-tagged Mnn1p is localized to punctate structures characteristic of the yeast Golgi apparatus. A similar punctate pattern was seen with the D430A and D432A mutants, demonstrating that these altered proteins were not accumulating in the ER (Fig. 4*A*). When these myc-tagged proteins were expressed in a strain in which the endogenous Mnn1p was tagged with the SV5 epitope (see below), double staining showed colocalization of the two epitopes (not shown). A similar result was seen with all of the other alanine mutations (not shown) except for the other inactive mutation, I434A. This latter mutant showed a different staining pattern that was coincident with the ER, indicating that it is unable to leave the ER after synthesis even though the endogenous Mnn1p was unaffected (Fig. 4*A*). It therefore seems likely that its loss of activity may be because of misfolding of the protein. In contrast, mutation of either aspartate does not prevent Mnn1p exiting the ER, suggesting that the residues constituting this DXD motif may not be essential for the overall folding of Mnn1p.

Mnn1p Forms Multimers. Several glycosyltransferases of the mammalian Golgi apparatus have been shown to dimerize, although this has not previously been demonstrated for Mnn1p (25, 26). If Mnn1p also forms such dimers, it is possible that the DXD motif has a role in dimerization. To investigate this possibility, strains were constructed expressing two separate copies of the *MNN1* gene, each tagged at the C terminus with a different epitope. Immunoprecipitation of one of these tagged proteins showed that the other tagged version was also coprecipitated, suggesting that Mnn1p assembles into at least a dimer (Fig. 4*B*). This was also the case when the D430A mutant protein was precipitated, indicating that the lack of activity in this mutant is not because of the disruption of multimer formation. Consistent with this interpretation, when the aspartate mutants were immunoprecipitated from cells also expressing an untagged wild-type Mnn1p, the oligosaccharide showed mannosyltransferase activity, presumably because of the coimmunoprecipitation of the wild-type enzyme (data not shown). Taken together, the above results show that mutations in the conserved aspartate residues eliminate enzymatic activity but do not affect protein multimerization. This finding suggests that the aspartates might have a catalytic rather than a structural role.

Mnn1p Requires Divalent Metal Ions for Activity. Conserved acidic residues have been found in other proteins to

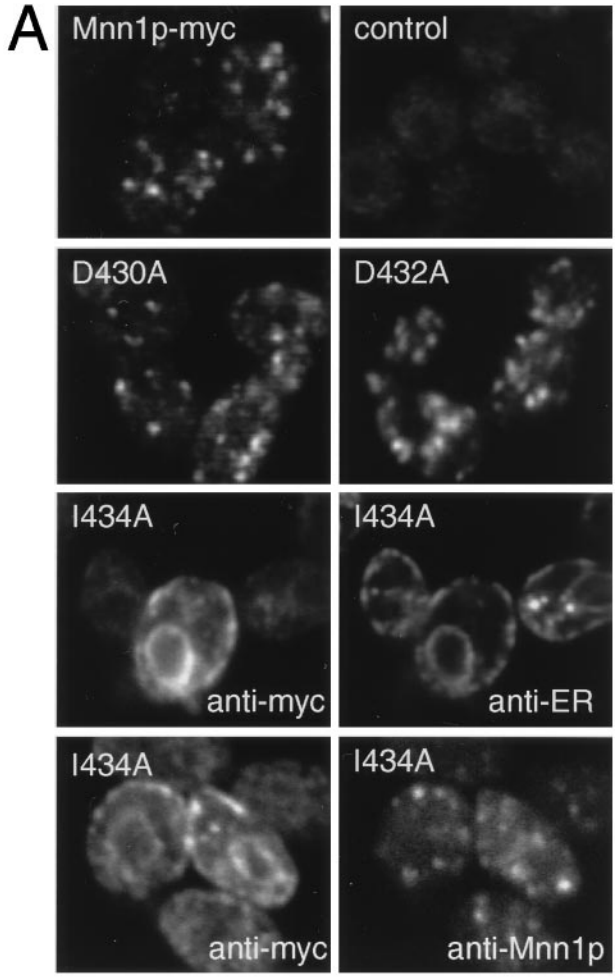

FIG. 4. Localization and oligomerization of the Mnn1p mutants. (*A*) Immunofluorescence micrographs of Δ*mnn1* yeast containing CEN plasmids expressing myc-tagged Mnn1p, no protein (control), or the indicated myc-tagged Mnn1p mutants, analyzed with the anti-myc monoclonal antibody 9E10. For I434A, cells were double labeled with rabbit anti-myc and an ER marker (anti-HDEL monoclonal antibody 2E7). The expression level of all constructs varied somewhat between cells, presumably reflecting variations in CEN plasmid copy number, or the known cell cycle regulation of *MNN1* (23). However, the intracellular distributions of the proteins were unaffected by expression level. I434A was also expressed in a strain in which the endogenous Mnn1p was HA tagged, and the cells were double labeled with anti-myc and anti-HA. (*B*) Multimerization of Mnn1p. Anti-SV5 tag protein blots of total protein, or of anti-myc immunoprecipitates, from yeast with the genomic copy of *MNN1* tagged with SV5, and with CEN plasmids expressing no protein, or myc-tagged Mnn1p as wild type or D430A. The SV5-tagged Mnn1p is precipitated by the anti-myc tag only when a myc-tagged Mnn1p is present, and this interaction is not affected by the D430A mutation.

have a role in metal ion coordination, and it has previously been shown that several Golgi mannosyltransferases require the divalent cation manganese for activity (14, 27, 28). To assess the metal ion requirements of Mnn1p, its activity was assayed *in vitro* with various divalent cations. Analysis of the wild-type protein showed that manganese is indeed the ion preferred for enzyme activity *in vitro*. Half-maximal Mnn1p activity was observed at 0.1 mM manganese chloride, 100-fold lower than the 10 mM normally used in the enzyme assay (data not shown). All other metal ions were also tested at 0.1 mM, and partial activity was observed with iron, cobalt, and zinc (Fig. 5).

Examination of the Effects of Conservative Substitutions of the Conserved Aspartate Residues. To analyze further the importance of the aspartate residues, they were replaced individually by glutamate, asparagine, and histidine residues. All of the resulting six mutants exited the ER as judged by immunofluorescence but were inactive both *in vivo* and *in vitro* (data not shown). This suggests that both the charge and size of these residues are important for activity. In those manganese-utilizing proteins for which atomic structures are available, the metal ion is often coordinated by aspartate residues, but in some cases by glutamates or histidines (29). For none of the mutants could activity be restored by replacing the manganese with any of the cations assayed above (data not shown).

The DXD Motif Is Conserved in Other Families of Glycosyltransferases. To determine whether this DXD motif was found in other glycosyltransferases we examined alignments of the different families of these enzymes. Fig. 6 shows that a DXD is found conserved in several glycosyltransferase families from both prokaryotes and eukaryotes, even though these families do not show any other obvious sequence relationship. In almost all cases the pair of aspartates is flanked by four hydrophobic residues on the N-terminal side, with the third of these often being an aromatic residue. In several cases the protein families include members from widely divergent eukaryotes, and in the case of dolichol-phosphate mannosyltransferase both human and *E. coli* proteins, implying maintenance of the sequence over long evolutionary periods.

The probability that DXD is conserved by chance in the n residues that are invariant in a family of length N can be closely approximated as $f^2 \times [n/N \times (n - 1)/(N - 1)]N$, where f is the expected frequency of an aspartate (5% in the total yeast genome). When values for n and N derived from CLUSTAL alignments of full-length proteins are used, the probability that DXD has occurred by chance is <0.01 for each of families a, c, d, e, f, g, i, and k in Fig. 6. Because there are only some 30 families of nucleotide-sugar-using glycosyltransferases (3), finding a motif in eight at $P < 0.01$ by chance alone has a

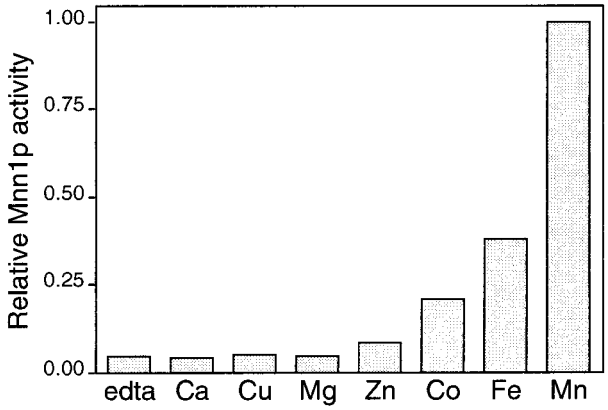

FIG. 5. Metal ion dependence of the *in vitro* activity of Mnn1p. myc-tagged Mnn1p was immunoprecipitated and assayed as in Fig. 3 but in the presence of the indicated divalent cations at 0.1 mM. The background was not subtracted and is the same as the EDTA signal.

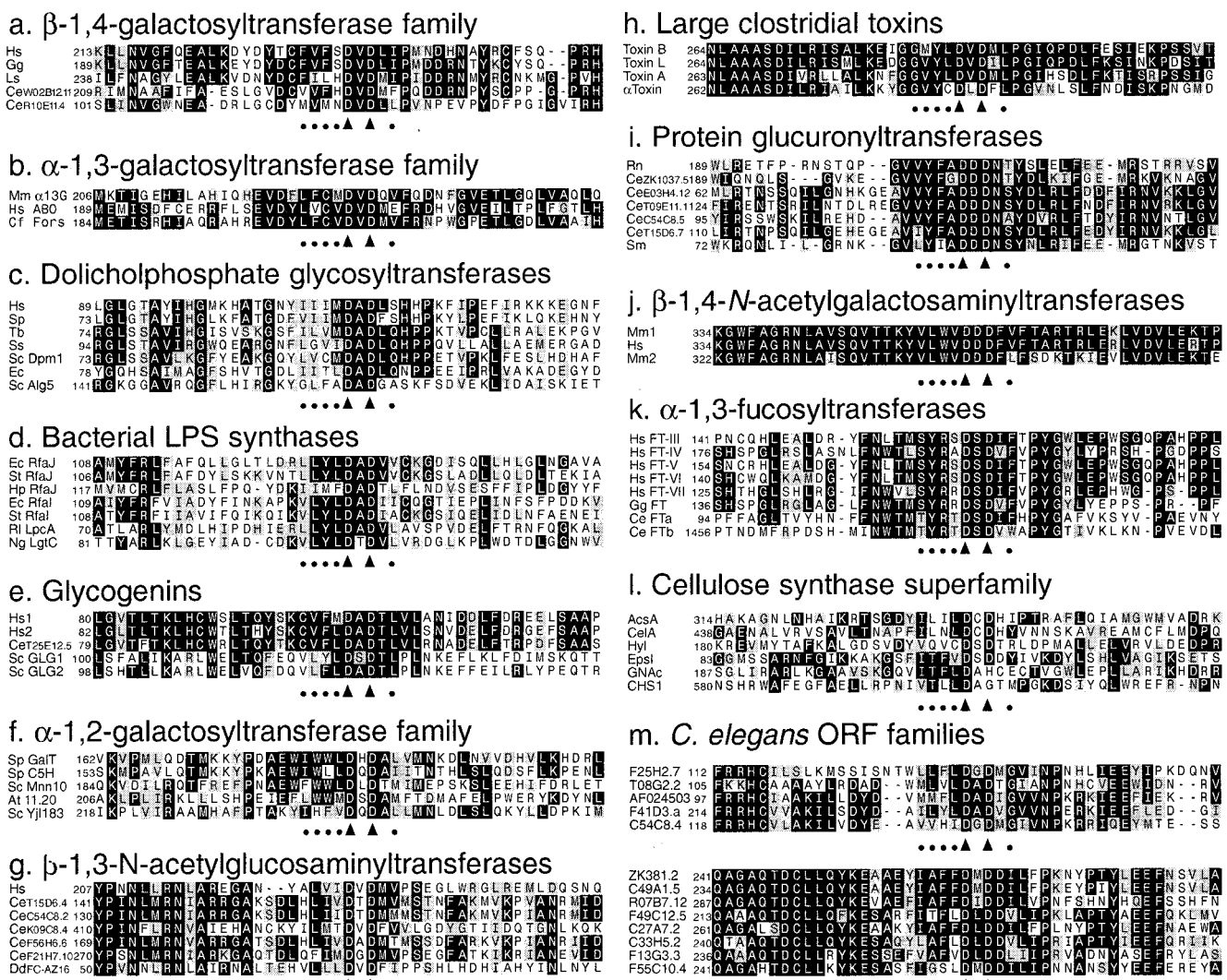

FIG. 6. Conserved DXD motifs in families of glycosyltransferases. Known families of glycosyltransferases were initially aligned by using BLAST. Where a DXD motif is conserved, the regions surrounding it were aligned with CLUSTAL. Residues identical (black) or related (gray) in a majority of the family are boxed. Species are indicated as: At, *Arabidopsis thaliana*; Ce, *Caenorhabditis elegans*; Cf, *Canis familiaris*; Dd, *Dictyostelium discoideum*; Ec, *Escherichia coli*; Gg, *Gallus gallus*; Hp, *Helicobacter pylori*; Hs, *Homo sapiens*; Ls, *Lymnaea stagnalis*; Mm, *Mus musculus*; Ng, *Neisseria gonorrhoeae*; Rl, *Rhizobium leguminosarum*; Rn, *Rattus norvegicus*; Sc, *S. cerevisiae*; Sm, *Schistosoma mansoni*; Sp, *Sch. pombe*; Ss, *Synechocystis* sp.; St, *Salmonella typhimurium*; Tb, *Trypanosoma brucei*. The members of the cellulose synthase superfamily are: *Acetobacter xylinus* cellulose synthase *acsAB* (acsA); *Gossypium hirsutum* cellulose synthase A (CelA); Hs hyaluronan synthase 1 (Hyl); *Streptococcus thermophilus epsI* (EpsI); Hs *N*-acetylgalactosaminyltransferase I (GNAc); and Sc chitin synthase 1 (CHS1).

probability of $<10^{-9}$. For the less divergent families b, h, and j, $0.2 > P > 0.07$, and so the presence of the motif is of less clear significance at present. However, this analysis underestimates the significance of the conservation, as it does not include the residues flanking the DXD, which share a hydrophobic character in all but family k (hhhhDxDxh). To demonstrate the significance of this whole motif, the DXDs plus five residues on either side from the families in Fig. 6 that have no homologs in the yeast genome (a, b, d, g, h, i, j) were aligned, and we used this to build a weight matrix by using a hidden Markov model (30). When all 6,000 proteins in yeast were ranked by the odds probability score (which indicated how closely they matched this motif), 9 of the top 30 were known DXD-containing glycosyltransferases (out of a total of 20), including members of five of the seven families. The remaining transferases are found somewhat further down in the list [14 (from all seven families) of 20 in the top 4%], but assimilation of further DXD sequences may allow improvement of the search matrix. Thus although the motif is perhaps too short to be perfectly accurate, it may be of use to suggest functions for unidentified ORFs, particularly if the DXD is also conserved in any homologs. Indeed, investigation of the other high-scoring proteins from yeast has revealed a Golgi protein (Yor320p), with homologs in plants, whose function is currently under investigation (unpublished results). In addition, PSI-BLAST searches with the DXD motif and surrounding sequence from the yeast mannosyltransferase Mnn10p led us to identify several large families of ORFs of unknown function from *C. elegans* (22, 31). These proteins have a single TMD near the N terminus and conserved DXD motifs, suggesting that they may be novel Golgi glycosyltransferases (Fig. 6, family m). Indeed, some of these ORFs are found clustered in the *C. elegans* genome, interspersed with homologs of mammalian Golgi glycosyltransferases (e.g., GenBank accession no. Z83125; S.M., unpublished observation).

At present no atomic structure of any of these transferases is available, so it is possible only to speculate as to the role of the DXD motif. Our results do suggest that mutations in the DXD motif do not cause complete unfolding of Mnn1p, although it is still possible that the DXD motif is involved in

the folding of a small region of the protein required for catalysis but not dimerization. Alternatively, the motif could have a role in the catalytic site. In this context it is worth noting that although the families in which it is found include both inverting and noninverting transferases, which add a range of different sugars (glucose, mannose, galactose, *N*-acetylglucosamine, etc.) to other sugars, phosphates (dolichol-phosphate mannosyltransferase), and proteins (clostridial toxins), all these enzymes use nucleoside diphosphate sugars and, where investigated, require divalent cations, usually manganese. Although the sugar and nucleoside used vary between enzymes, the diphosphate is invariant, and so if the motif is in the catalytic site it could be involved in binding a metal ion that is used to coordinate the phosphates in the active site. A similar motif, DXD with surrounding hydrophobic residues, is found conserved in the α and β families of DNA polymerases, and crystal structures show that these aspartates are in the active site, coordinating manganese and magnesium ions that hold the phosphates of, in this case, a nucleoside triphosphate (5). The only nucleotide-sugar-using glycosyltransferase for which a structure is known is a T4 phage enzyme that glucosylates DNA. Although is does not have a DXD motif, it has an aspartate (Asp-100) in the active site that was proposed to bind magnesium, although the ion was not actually present in the crystal structure (32). Interestingly, an analysis of the predicted fold of the α-1,3-fucosyltransferases suggested that they could adopt a structure similar to this T4 enzyme, in which the aspartate corresponding to the first in the DXD shown in Fig. 6 family k was suggested to correspond to Asp-100 in the T4 protein (33).

In addition to the families described here, there are other families of glycosyltransferases that do not have a conserved DXD motif, such as the glucuronyltransferases of the ER, the glycogen synthases, and the α-1,2-fucosyltransferases and sialyltransferases of the Golgi apparatus. These enzymes may instead have unrelated structures and enzymatic mechanisms, and indeed the sialyltransferases use a nucleoside monophosphate sugar and do not require divalent metals (34). However, it is possible that if the DXD-containing glycosyltransferases do share a common structure, this could be extended to other families of glycosyltransferases where conserved aspartates can be found, if not in the same configuration. For example, there are further nucleotide polymerases that share a common structural fold with the α and β families of DNA polymerases, but have the pair of spaced aspartates replaced with adjacent aspartates, or even a single acidic residue (5). Such structural diversity could also explain the existence of glycosyltransferase families in which the DXD motif is frequent but not invariant. For example, the bacterial exopolysaccharide and cellulose synthases, and the eukaryotic cellulose, hyaluronan, and chitin synthases constitute a large superfamily of related proteins (3, 7). A conserved DXD motif can be found in all of these except the chitin synthases, which have DXD or DXG, and the *N*-acetylgalactosaminyltransferases, which have DXH (Fig. 6 family l). Thus although variation of the aspartates is not compatible with activity in the case of Mnn1p, this need not be the case if there are sufficient compensating changes elsewhere in a protein. However, the resolution of these issues concerning the function of the motif will ultimately require structural information from a DXD-containing glycosyltransferase, with such information having the potential to be relevant to the other families containing the motif.

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1. Dwek, R. A. (1996) *Chem. Rev.* **96,** 683–720.
2. Just, I., Selzer, J., Wilm, M., Voneichelstreiber, C., Mann, M. & Aktories, K. (1995) *Nature (London)* **375,** 500–503.
3. Campbell, J. A., Davies, G. J., Bulone, V. & Henrissat, B. (1997) *Biochem. J.* **326,** 929–939.
4. Rawlings, N. D. & Barrett, A. J. (1993) *Biochem. J.* **290,** 205–218.
5. Joyce, C. M. & Steitz, T. A. (1994) *Annu. Rev. Biochem.* **63,** 777–822.
6. Joziasse, D. H., Shaper, J. H., Van den Eijnden, D. H., Vantunen, A. J. & Shaper, N. L. (1989) *J. Biol. Chem.* **264,** 14290–14297.
7. Saxena, I. M., Kudlicka, K., Okuda, K. & Brown, R. M. (1994) *J. Bacteriol.* **176,** 5735–5752.
8. Saxena, I. M., Brown, R. M., Fevre, M., Geremia, R. A. & Henrissat, B. (1995) *J. Bacteriol.* **177,** 1419–1424.
9. Yip, C. L., Welch, S. K., Klebl, F., Gilbert, T., Seidel, P., Grant, F. J., O'Hara, P. J. & MacKay, V. L. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 2723–2727.
10. Graham, T. R., Verosteck, M. F., MacKay, V., Trimble, R. & Emr, S. D. (1992) *Yeast* **8,** S458 (abstr.).
11. Robinson, J. S., Klionsky, D. J., Banta, L. M. & Emr, S. D. (1988) *Mol. Cell. Biol.* **8,** 4936–4948.
12. Guthrie, C. & Fink, G. R. (1991) *Guide to Yeast Genetics and Molecular Biology* (Academic, San Diego).
13. Wach, A., Brachat, A., Alberti-Segui, C., Rebischung, C. & Philippsen, P. (1997) *Yeast* **13,** 1065–1075.
14. Nakajima, T. & Ballou, C. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3912–3916.
15. Sweet, D. J. & Pelham, H. R. B. (1993) *EMBO J.* **12,** 2831–2840.
16. Napier, R. M., Fowke, L. C., Hawes, C., Lewis, M. & Pelham, H. R. B. (1992) *J. Cell Sci.* **102,** 261–271.
17. Hanke, T., Szawlowski, P. & Randall, R. E. (1992) *J. Gen. Virol.* **73,** 653–660.
18. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
19. Nakayama, K., Nagasu, T., Shimma, Y., Kuromitsu, J. & Jigami, Y. (1992) *EMBO J.* **11,** 2511–2519.
20. Dean, N. & Poster, J. B. (1996) *Glycobiology* **6,** 73–81.
21. Beeler, T. J., Fu, D., Rivera, J., Monaghan, E., Gable, K. & Dunn, T. M. (1997) *Mol. Gen. Genet.* **255,** 570–579.
22. Jungmann, J. & Munro, S. (1998) *EMBO J.* **17,** 423–434.
23. Igual, J. C., Johnson, A. L. & Johnston, L. H. (1996) *EMBO J.* **15,** 5001–5013.
24. Lussier, M., Sdicu, A. M., Ketela, T. & Bussey, H. (1995) *J. Cell Biol.* **131,** 913–927.
25. Fleischer, B., McIntyre, J. O. & Kempner, E. S. (1993) *Biochemistry* **32,** 2076–2081.
26. Jaskiewicz, E., Zhu, G. F., Bassi, R., Darling, D. S. & Young, W. W. (1996) *J. Biol. Chem.* **271,** 26395–26403.
27. Kaufman, R. J., Swaroop, M. & Murthariel, P. (1994) *Biochemistry* **33,** 9813–9819.
28. Kuhn, N. J., Ward, S. & Leong, W. S. (1991) *Eur. J. Biochem.* **195,** 243–250.
29. Karlin, S., Zhu, Z. Y. & Karlin, K. D. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 14225–14230.
30. Eddy, S. R. (1996) *Curr. Opin. Struct. Biol.* **6,** 361–365.
31. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J. H., Zhang, Z., Miller, W. & Lipman, D. J. (1997) *Nucleic Acids Res.* **25,** 3389–3402.
32. Vrielink, A., Ruger, W., Driessen, H. P. C. & Freemont, P. S. (1994) *EMBO J.* **13,** 3413–3422.
33. Breton, C., Oriol, R. & Imberty, A. (1996) *Glycobiology* **6,** R7–R12.
34. Datta, R. K. & Paulson, J. C. (1995) *J. Biol. Chem.* **270,** 1497–1500.