Glycobiology vol. 19 no. 8 pp. 816–828, 2009
doi:10.1093/glycob/cwp066
Advance Access publication on May 9, 2009

REVIEW

Protein *O*-mannosylation: Conserved from bacteria to humans*

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Received on March 18, 2009; revised on May 6, 2009; accepted on May 6, 2009

Protein *O*-mannosylation is an essential modification in fungi and animals. Different from most other types of *O*-glycosylation, protein *O*-mannosylation is initiated in the endoplasmic reticulum by the transfer of mannose from dolichol monophosphate-activated mannose to serine and threonine residues of secretory proteins. In recent years, it has emerged that even bacteria are capable of *O*-mannosylation and that the biosynthetic pathway of *O*-mannosyl glycans is conserved between pro- and eukaryotes. In this review, we summarize the observations that have opened up the field and highlight characteristics of *O*-mannosylation in the different domains/kingdoms of life.

Keywords: congenital muscular dystrophy/glycosylation/mannosyltransferase/*O*-mannosylation

Introduction

Glycosylation is a highly abundant posttranslational protein modification in eukarya that also occurs in bacteria and archea (Abu-Qarn et al. 2008; Lehle et al. 2006; Yurist-Doutsch et al. 2008). Among the various modifications a protein can undergo in a living cell, the covalent attachment of carbohydrates is the most common and the most diverse. Thirteen monosaccharides and eight different amino acids have been reported to be involved in at least 37 different carbohydrate–protein linkages. A further magnitude of complexity is achieved by the formation of linear and branched oligosaccharides of varying length, composition, and anomeric configurations (reviewed in Spiro 2002). In this way, an enormous variety of glycan structures is generated. Being far more than mere decoration, carbohydrate moieties not only alter the physico–chemical properties of a protein but are also involved in various functional aspects of a protein such as cellular localization, turnover, protein quality control, and ligand interaction (for reviews, see Lehle et al. 2006; Marth and Grewal 2008; Trombetta and Parodi 2003).

Based on the nature of the glycosidic bond, distinct types of glycosylation reactions are defined. In the case of *O*-glycosylation, glycans get attached via an *O*-glycosidic linkage to the hydroxy group of hydroxy amino acids. All hydroxy amino acids (serine (Ser), threonine (Thr), tyrosine, hydroxyproline, and hydroxylysine) have been implicated in *O*-linked glycosylation, although the most commonly modified ones are Ser and Thr residues. A variety of reducing terminal sugar residues can be *O*-glycosidically attached to Ser and Thr, such as *N*-acetylgalactosamine, fucose (Fuc), glucose, *N*-acetylglucosamine (GlcNAc), xylose, galactose (Gal), arabinose, and mannose (Man) (reviewed in Spiro 2002).

In this review, we particularly address protein *O*-mannosylation, which for a long time was believed to be restricted to fungi. However, it is now clear that this type of *O*-glycosylation is conserved throughout the fungal and animal kingdoms and that bacteria are also capable of *O*-mannosylation. Mainly focusing on protein *O*-mannosyltransferases, we summarize the main features of the biosynthetic pathway of *O*-mannosyl glycans and address their biological significance in pro- and eukaryotes.

Protein *O*-mannosylation in eukaryotes

Occurrence and structures of O-mannosyl glycans

O-Mannosyl glycans linked to Ser and Thr residues were first identified in the late 1960s by Sentandreu and Northcote (1968) in bakers' yeast. Today we know that in yeasts and fungi the majority of secreted and cell wall proteins are substantially *O*-mannosylated (De Groot et al. 2005; Strahl-Bolsinger et al. 1999). In all yeasts and fungi studied so far, the reducing terminal mannose residue of *O*-mannosyl glycans is α-linked to Ser or Thr and may be extended to form an α1,2-linked mannotriose Manα1-2Manα1-2Manα1-Ser/Thr (Gemmill and Trimble 1999). This core structure is further processed according to the yeast/fungi species. In *Saccharomyces cerevisiae*, further mannose residues are added in α1,3-linkage to form short linear oligosaccharides that are up to five mannosyl residues long and can be modified by mannosyl phosphate (Figure 1A). The characterization of heterologously expressed leech-derived tryptase inhibitor revealed that *S. cerevisiae* is also capable of synthesizing *O*-linked Manα1-3-Manα1-2Man mannotriose and mannooligosaccharides of up to 13 mannose moieties. These high degrees of polymerization might be achieved by the incorporation of mannosyl phosphate that can occur at any position of the mannosyl backbone (Bergwerff et al. 1998). *O*-Glycans of the human fungal pathogen *Candida albicans* consist of α1,2-linked mannose polymers of up to five residues (Herrero et al. 2002). In the methylotrophic yeast *Pichia pastoris*, the most abundant *O*-mannosyl glycan structures are α1,2-Man polymers of two, three, or rarely four mannose residues

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*Dedicated to Professor Widmar Tanner on the occasion of his 70th birthday.

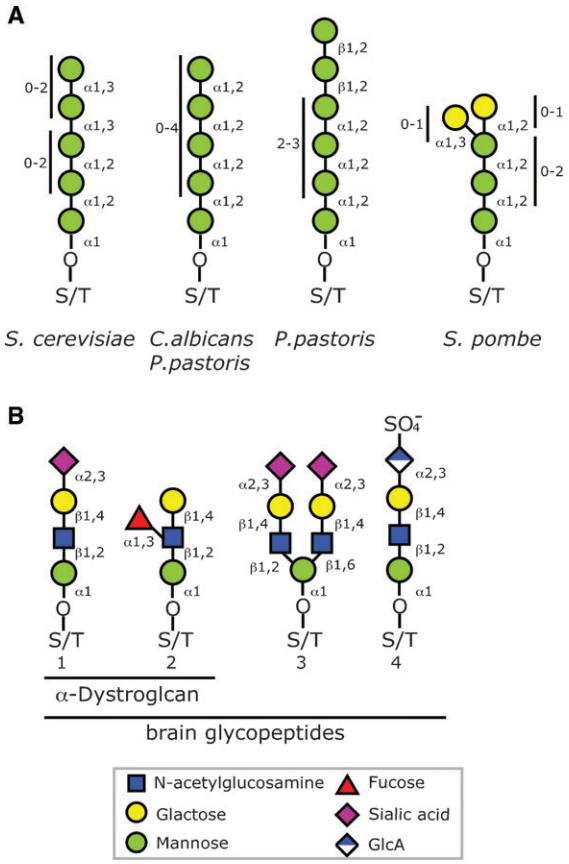

Fig. 1. Schematic representation of *O*-mannosyl glycans. (**A**) An α1,2-linked core structure Man_{0-2}-Man-Ser/Thr is common in all yeasts. In *S. cerevisiae*, mannosylphophate can additionally be incorporated at any position of the oligomannose backbone. (**B**) In mammals the core structure Galβ1-4GlcNAcβ1-2Man-Ser/Thr is common in all *O*-mannosyl glycans. On α-DG this core structure can be sialylated (1) or fucosylated (2). From brain glycopeptides, branched structures (3) and *O*-mannosidically linked HNK-1 (4) epitopes have been described.

(Duman et al. 1998). Some of the α1,2-Man_3 and α1,2-Man_4 structures can be capped by a Manβ1-2Manβ1-2-disaccharide (Trimble et al. 2004; Figure 1A). In the fission yeast *Schizosaccharomyces pombe*, linear α1,2-linked mannobiose and mannotriose are formed. In addition, up to two galactosyl residues may be added to the α1,2-linked mannose backbone to generate branched *O*-linked oligosaccharides of the $Gal_{0-2}Man_{1-3}$ structure (Gemmill and Trimble 1999; Figure 1A). Short linear α1,2-linked mannose polymers (Man_{1-3}) have been isolated from glucoamylase of the filamentous fungus *Aspergillus awamori* var. *kawachi* (Oka et al. 2005). Besides, branched *O*-linked oligomannoses containing galactose, glucose, and galactopentose have been described from different *Aspergillus* species that have been summarized in a recent review by Goto (2007).

Although highly abundant in fungi, protein *O*-mannosylation is less frequent in metazoa. *O*-Linked mannose has been identified among oligosaccharides on skin collagen of the clam worm (Spiro and Bhoyroo 1980). In mammals, *O*-mannosyl glycans were isolated from chondroitin sulfate proteoglycans and total glycopeptides from brain tissue (Finne et al. 1979; Krusius et al. 1986, 1987; Yuen et al. 1997), on α-dystroglycan (α-DG) originating from nerve and muscular tissues (Chiba et al. 1997; Sasaki et al. 1998; Smalheiser et al. 1998), and recently on neuron-specific receptor protein–tyrosine phosphatase β (RPTPβ; Abbott et al. 2008). Different from fungal *O*-mannosyl glycans that mainly consist of mannosyl residues, the vast majority of mammalian *O*-mannosyl glycans represent variations of the tetrasaccharide NeuAcα2-3Galβ1-4GlcNAcβ1-2Manα1-Ser/Thr with different lengths (e.g., asialo) and fucose (α1,3-linked to GlcNAc) contents (Chiba et al. 1997; Sasaki et al. 1998; Smalheiser et al. 1998). Further, branched structures with 2,6-di-substituted mannose (GlcNAc-linked β1,2 and β1,6) have been reported (Chai et al. 1999; Figure 1B). Interestingly, IIH6 monoclonal antibodies that recognize an *O*-mannosidically linked glycoepitope on α-DG also react with α-DG from zebrafish (Thornhill et al. 2008), suggesting that *O*-mannosyl glycan structures are conserved in vertebrates. In addition to the conserved tetrasaccharide, *O*-mannosyl glycans containing the human natural killer-1 (HNK-1) epitope HSO_4-3GlcAβ1-3Galβ1-4GlcNacβ1-2Man-S/T (Figure 1B) have been detected in significant amounts among total rabbit brain glycopeptides (Yuen et al. 1997), and recently on RPTPβ from neuroblastoma cells (Abbott et al. 2008).

Biosynthesis of *O*-mannosyl glycans

Initiation of O-mannosylation in the endoplasmic reticulum

The biosynthesis of *O*-mannosyl glycans has been uncovered in *S. cerevisiae* by the pioneering work of Tanner and co-workers. The elucidation of the biosynthetic pathway started with the discovery of dolichyl phosphate-activated monosaccharides in eukaryotic cells (Behrens 1970; Tanner 1969; Tanner and Behrens 1971). Babczinski and Tanner (1973) then demonstrated that in vitro a majority of the mannosyl residues derived from dolichyl phosphate-bound mannose (Dol-P-Man) are directly linked to protein (Sharma et al. 1974). Orlean (1990) finally confirmed that Dol-P-Man serves as the mannosyl donor for the initial transfer reaction in the *O*-mannosylation pathway in vivo. Dol-P-Man is synthesized from GDP-Man and Dol-P on the cytosolic face of the endoplasmic reticulum (ER) membrane (Figure 2). In yeast, this reaction is catalyzed by the GDP-Man:Dol-P mannosyltransferase Dpm1p (Haselbeck 1989; Orlean et al. 1988). Subsequently, Dol-P-Man flip-flops into the ER lumen through a putative flippase machinery. There it serves as the sole mannose donor for the ER resident glycosyltransferases. For structure, function, and regulation of Dol-P-Man and Dol-P-Man synthase in yeasts and mammals, we refer to a recent review by Maeda and Kinoshita (2008).

Tanner and co-workers further demonstrated that biosynthesis of *O*-mannosyl glycans in *S. cerevisiae* is initiated at the ER (Haselbeck and Tanner 1983). Dolichyl phosphate-D-mannose:protein *O*-mannosyltransferases catalyze the transfer of a mannosyl residue from Dol-P-ß-D-Man (Lehle and Tanner 1978) to Ser and Thr residues of secretory proteins (Gentzsch et al. 1995; Gentzsch and Tanner 1996; Strahl-Bolsinger et al. 1993; Strahl-Bolsinger and Tanner 1991; Figure 2). While inverting the anomeric configuration of the mannose moiety an α-D-mannosidic linkage is formed (Bause and Lehle 1979). Early experiments suggested that *O*-mannosylation occurs exclusively during the translocation of secretory proteins into the ER (Elorza et al. 1977). Yet, more recent work showed that the transfer of

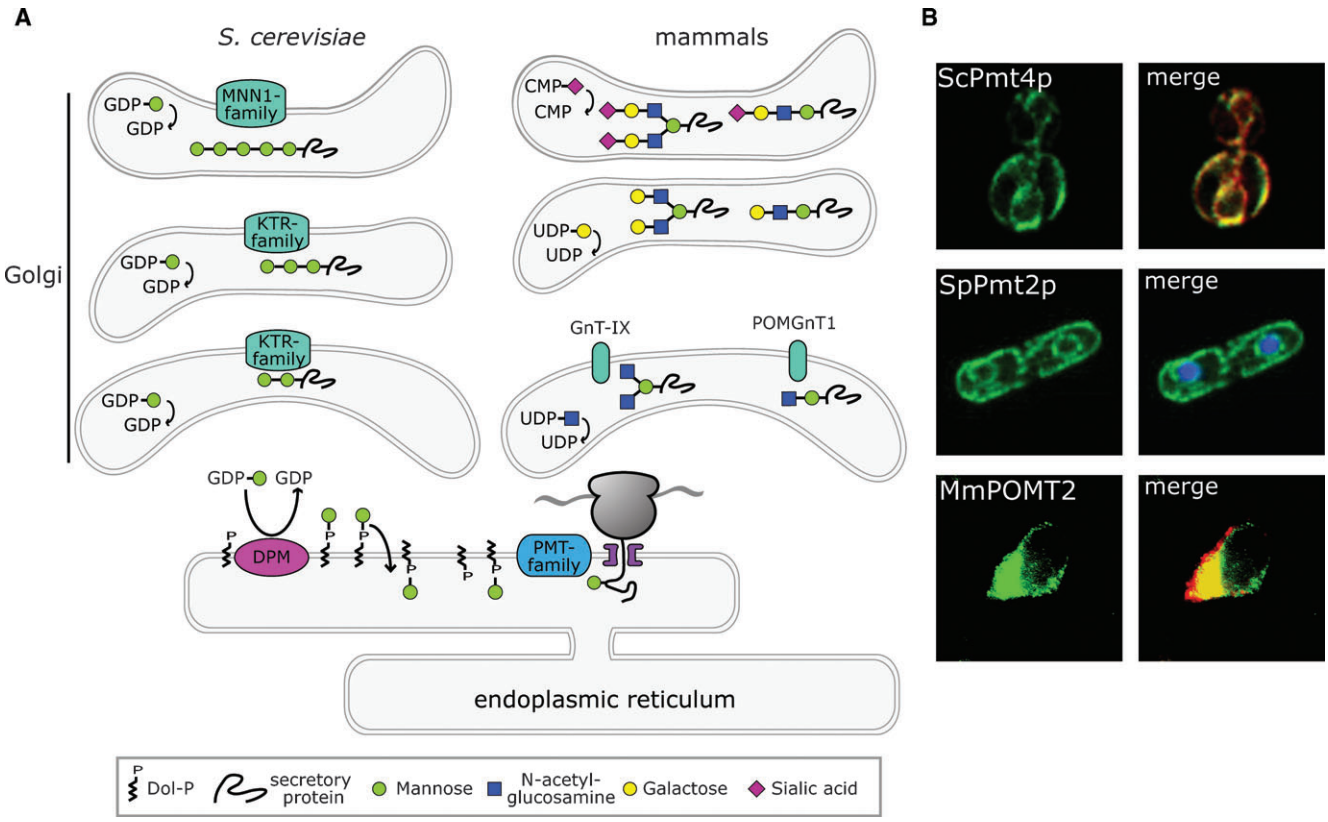

Fig. 2. Biosynthesis of *O*-mannosyl glycans in eukaryotes. (**A**) Schematic representation of the *O*-mannosylation pathway. The initial steps in the ER are conserved between fungi, animals, and humans. Dol-P-Man is synthesized on the cytosolic face of the ER membrane and flip-flops into the ER lumen. Mannose is further transferred to proteins entering the secretory pathway by the members of the PMT-family. Diversification occurs in the Golgi apparatus where further chain elongation takes place as detailed in the text. (**B**) ER localization of PMT proteins. Upper panel: co-localization of Pmt4p and the oligosaccharyltransferase subunit Ost1p in the ER of bakers' yeast (realized by J. Hutzler). Middle panel: In fission yeast Oma2p (PMT2 type) localizes to the perinuclear and peripheral ER. The nucleus is visualized with DAPI (realized by F. Hutzler). Lower panel: Mouse POMT2 and the ER marker protein ERp72 co-localize in cultured fibroblast cells.

O-linked mannose can also occur after proteins have entered the ER (Harty et al. 2001).

Five years ago, the transfer reaction catalyzed by Dol-P-Man:protein *O*-mannosyltransferases has also been demonstrated in mammals and insects (Ichimiya et al. 2004; Manya et al. 2004) affirming that the initial steps of *O*-mannosylation are conserved between the fungal and the animal kingdom (Figure 2). Hence, metazoan *O*-mannosylation is distinct from most other *O*-glycosylation reactions that take place exclusively in the Golgi apparatus (Lehle et al. 2006).

Chain elongation in the Golgi apparatus

Further extension of the *O*-linked mannose residue takes place in the Golgi apparatus. In *S. cerevisiae*, three α1,2-mannosyltrasferases of the KTR family (ScKre2p, ScKtr1p, and ScKtr3p) catalyze the stepwise addition of the first and second α1,2-linked mannose in the medial Golgi compartment (reviewed in Lussier et al. (1999); Strahl-Bolsinger et al. (1999); Figure 2A). Another member of the KTR family, ScMnn6p, is a mannosylphosphate transferase implicated in the mannosylphosphorylation of *O*-linked glycans (Jigami and Odani 1999). Further α1,3-linked mannose residues are added by α1,3-mannosyltrasferases including ScMnn1p, ScMnt2p, and ScMnt3p (Lussier et al. 1999; Romero et al. 1999) which reside in the medial and *trans*-Golgi (Graham et al. 1994). All Golgi glycosyltransferases are type-II membrane proteins with large C-terminal catalytic domains facing the Golgi lumen. In the Golgi, GDP-Man serves as the mannosyl donor.

So far, the elongation of *O*-mannosyl glycans in mammals is only partially understood. The transfer of GlcNAc to mannose in the 2-OH position is catalyzed by the protein *O*-mannose *N*-acetylglucosaminyltransferase 1 (POMGnT1; Takahashi et al. 2001; Zhang et al. 2002), while β1,6-*N*-acetylglucosaminyl transferase (GnT-IX) links GlcNAc to mannose in the 6-OH position in vitro (Inamori et al. 2004; Figure 2A). The addition of GlcNAc in β1,2-Man linkage by PomGnT1 is required before GnT-IX can add GlcNAc, strongly suggesting that GnT-IX is responsible for the formation of 2,6-branched structures in brain *O*-mannosyl glycans (Inamori et al. 2004). Further enzymes directly involved in the elongation of *O*-mannose saccharides remain to be identified among families of β1,4-galactosyltransferases, α2,3-sialyltransferases, α1,3-fucosyltransferases, and HNK-1-specific glucuronyltransferasese that have been found in mammals. Three genes, *fukutin*, *fukutin-related protein,* and *Large*, seem to be involved in the formation of *O*-mannosyl glycans on α-DG (Barresi and Campbell 2006). The corresponding proteins show substantial similarities to known glycosyltransferases (Aravind and Koonin 1999; Esapa et al. 2002; Grewal et al. 2001; Kobayashi et al. 1998); however, their specific functions remain to be determined.

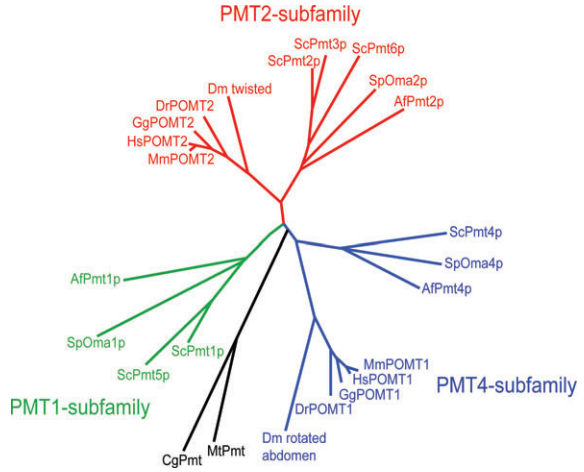

Fig. 3. Phylogeny of the three PMT subfamilies. Protein sequences were analyzed using the ClustalW (Thompson et al. 1994) algorithm and displayed graphically using the Phylodendron software. Members of the PMT1, PMT2, and PMT4 subfamily are depicted in green, red, and blue, respectively. Pmts from bacteria are shown in black. Af, *A. fumigatus*; Cg, *C. glutamicum*; Dm, *D. melanogaster*; Dr, *Danio rerio*; Gg, *Gallus gallus*; Hs, *Homo sapiens*; Mm, *Mus musculus*; Mt, *M. tuberculosis;* Sc, *S. cerevisiae*; Sp, *S. pombe*.

The PMT family of protein *O*-mannosyltransferases

Dol-P-Man:protein *O*-mannosyltransferases (PMTs) are the key enzymes initiating *O*-mannosylation in the ER. The first PMT has been identified in bakers' yeast. Based on its enzymatic activity, ScPmt1p was purified and the corresponding gene was cloned (Strahl-Bolsinger and Tanner 1991; Strahl-Bolsinger et al. 1993). Due to the homology to *ScPMT1*, the PMT family was identified in *S. cerevisiae* (Gentzsch et al. 1995; Gentzsch and Tanner 1996; Lussier et al. 1995) and subsequently also in other yeasts and filamentous fungi such as *C. albicans* (Timpel et al. 1998), *Aspergillus fumigatus* (Shaw and Momany 2002), *Trichoderma reesei* (Zakrzewska et al. 2003), *S. pombe* (Willer et al. 2005), and *Cryptococcus neoformans* (Olson et al. 2007). In metazoa, PMTs were first identified in *Drosophila melanogaster* (Martin-Blanco and Garcia-Bellido 1996) and in humans (Jurado et al. 1999). Whole genome sequences of various organisms revealed that PMTs are not limited to fungi, but are conserved throughout the animal kingdom. To date all PMT family members are classified as glycosyltransferase family 39 (GT39) according to the carbohydrate-active enzymes (CAZy) database (http://www.cazy.org). Interestingly, PMT genes are absent in the genomes of *Caenorhabditis elegans* and plants (e.g., *Arabidopsis thaliana*; *Oryza sativa*).

Phylogenetic analyses showed that the PMT family is divided in three subfamilies: the PMT1, PMT2, and PMT4 subfamily (Girrbach et al. 2000; Willer et al. 2002; Figure 3). In *S. cerevisiae* and *C. albicans*, the PMT1 and PMT2 subfamilies are redundant (two or more members of each subfamily) whereas only a single member of the PMT4 subfamily is present (Gentzsch and Tanner 1996; Prill et al. 2005). The same is true for other yeasts of the class *Saccharomycetes*. Redundancy of PMT1 and PMT2 subfamily members might have evolved by gene duplication (Girrbach and Strahl 2003). In *S. cerevisiae*, the PMT1 subfamily members Pmt1p and Pmt5p feature an overall amino acid sequence identity of 53% (72% similarity), and the PMT2 subfamily members Pmt2p and Pmt3p of 65% (81% similarity). In view of this, high degree of conservation tandem gene duplication of *PMT1* is supported by the fact that *PMT1* (YDL095w) and *PMT5* (YDL093w) are located directly next to each other on chromosome IV. Further, the report of Wolfe and Shields (1997) suggests that *PMT2* (YAL023c; chromosome I) and *PMT3* (YOR321w; chromosome XV) constitute a gene pair that derives from an ancient duplication of the entire yeast genome (Wolfe and Shields 1997). Different from *Saccharomycetes*, in the *Schizosaccharomycete S. pombe*, in the basidiomycetous yeast *C. neoformans*, and in all filamentous fungi analyzed so far, only a single member of each PMT subfamily is present (Goto 2007; Olson et al. 2007; Willer et al. 2005). In contrast to fungi, in animals a single member of both the PMT2 and the PMT4 subfamily is present, whereas PMT1 homologs have not been found (Figure 3).

Yeast protein O-mannosyltransferases

As mentioned earlier, PMTs have been extensively characterized in bakers' yeast where at least six PMT family members (Pmt1p–Pmt6p) are present (Gentzsch and Tanner 1996; Strahl-Bolsinger et al. 1993; Strahl-Bolsinger and Tanner 1991; Figure 3). The members of the PMT1 subfamily (Pmt1p, Pmt5p) form heterodimeric complexes with members of the PMT2 subfamily (Pmt2p, Pmt3p; Girrbach and Strahl 2003). In wild-type yeast, Pmt1p–Pmt2p and Pmt5p–Pmt3p complexes exist, yet the vast majority of mannosyl transfer activity is due to Pmt1p–Pmt2p complexes. Under certain physiological conditions, however, Pmt1p interacts also with Pmt3p, and Pmt5p with Pmt2p, suggesting a compensatory cooperation that guarantees the maintenance of *O*-mannosylation. Unlike the PMT1/PMT2 subfamily members, Pmt4p acts as a homomeric complex. Mutational analyses showed that the same conserved protein domains underlie both heteromeric and homomeric interactions (Girrbach and Strahl 2003). Pmt1p–Pmt2p complexes have also been demonstrated in fission yeast (Willer et al. 2005) suggesting that complex formation is mandatory for PMT function. There might be a number of reasons why PMTs form homo- or heteromeric protein complexes. One is that these complexes ensure efficient *O*-mannosylation of a wide range of target proteins. A common feature of *O*-glycosylated proteins is that *O*-linked carbohydrate chains are clustered in distinct Ser/Thr-rich regions (Jentoft 1990). Such areas are thought to adopt rod-like structures important for protein function. *O*-Mannosylation can occur while proteins are translocated in the lumen of the ER. Thus, the clustering of *O*-linked sugars requires highly efficient sugar transfer, which could be provided by PMT complexes.

Among all PMTs, *S. cerevisiae* Pmtp1 is characterized best. Pmt1p is an integral ER membrane glycoprotein with seven transmembrane spanning domains (Strahl-Bolsinger and Scheinost 1999; Figure 4). Since hydropathy profiles are conserved between PMTs, it is highly likely that the proposed topology also applies to all of them. An invariant arginine residue of the transmembrane domain 2 is essential for the formation and/or stability of PMT complexes (Girrbach and Strahl 2003; Figure 4B). The Pmt1p amino terminus faces the cytoplasm whereas the carboxyl terminus faces the lumen of the ER. Two major hydrophilic domains that are located between transmembrane spans 1 and 2 (loop 1) and transmembrane spans 5 and 6 (loop 5), respectively, are oriented toward the ER lumen.

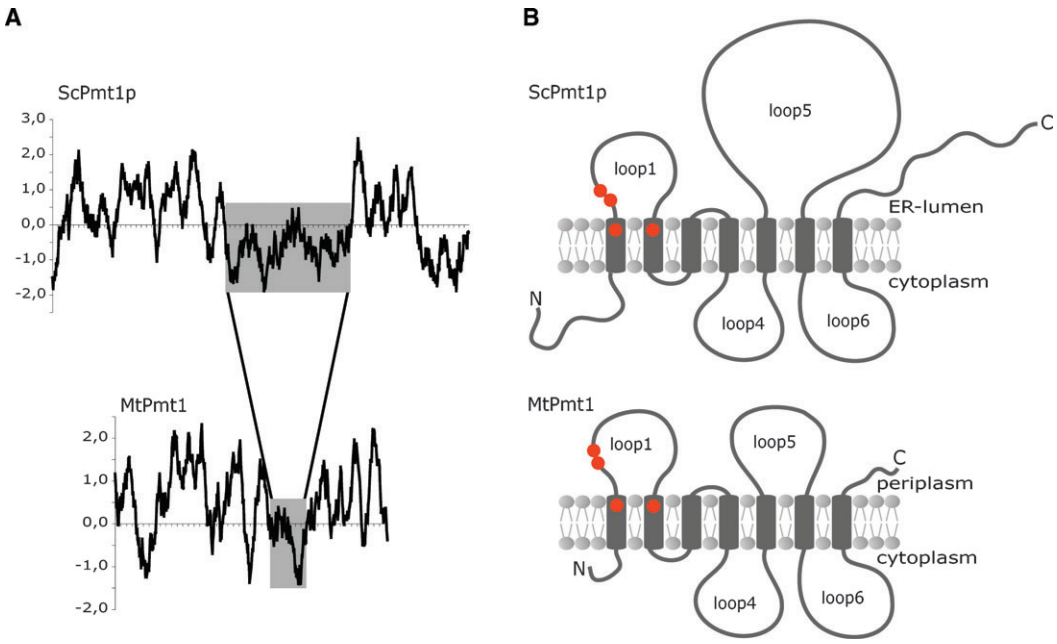

Fig. 4. Structure of PMT proteins. (**A**) PMTs from eukaryotes and bacteria share similar hydropathy profiles. Hydropathy profiles of ScPmt1 and Mt-Pmt were generated according to Kyte and Doolittle (1982). Loop 5 domains are shaded in gray. (**B**) Topology models of ScPmt1 and Mt-Pmt according to Strahl-Bolsinger and Scheinost (1999). Conserved Arg residues within transmembrane domain one and two and conserved Asp-Glu motif in loop 1 that are crucial for enzymatic activity are indicated in red (Girrbach et al. 2000; VanderVen et al. 2005).

Site-directed mutagenesis of conserved amino acids and affinity labeling with a synthetic acceptor peptide showed that amino acids Asp77 and Glu78 (located in loop 1; Figure 4B) are crucial for substrate binding and enzyme activity (Girrbach et al. 2000; A. Schott and S. Strahl, unpublished data). These DEx/Dex-like motifs play a key role for catalysis in many glycosyltransferases, suggesting that loop 1 represents the catalytic domain. Although deletion of the loop 5 domain does not affect binding of an artificial acceptor peptide to Pmt1p (V. Girrbach and S. Strahl, unpublished data), it results in the complete loss of transferase activity (Girrbach et al. 2000). Furthermore, exchange of highly conserved leucine residues to alanine in loop 5 causes a dramatic decrease in enzymatic activity (Girrbach et al. 2000). This loop 5 domain is not present in bacterial PMTs (see below; Figure 4), suggesting that it fulfills a – yet unknown – function specific to eukaryotes. Comparison of PMTs from different organisms defined highly conserved peptide motifs within the loop 5 region (Girrbach et al. 2000). These so-called MIR motifs are also found in inositol triphosphate and ryanodine receptors (Ponting 2000). Moreover, mammalian SDF2 (stromal-cell-derived factor 2) and SDF2-like1 (Fukuda et al. 2001; Hamada et al. 1996) are soluble proteins residing in the ER that show significant homology to the MIR motifs of the PMT-loop 5 domain. SDF2-like1 has been identified as a component of a multiprotein complex of chaperons and folding enzymes in the ER, suggesting a function in protein folding (Meunier et al. 2002). A similar role might be envisaged for the loop 5 domain of PMTs, which yet needs to be demonstrated. Two other major domains that are located between transmembrane spans 4 and 5 (loop 4) and transmembrane spans 6 and 7 (loop 6), respectively, are oriented toward the cytosol and, like loop 5, are also essential for Pmt1p activity (Girrbach et al. 2000). Loop 4 is partially hydrophobic, but does not span the ER membrane (Strahl-Bolsinger and Scheinost 1999). PSI-BLAST analyses revealed weak homologies between loop 4 and regions in other Dol-P-Man utilizing enzymes (S. Strahl, unpublished data); however, functions of the cytoplasmic loops in Pmt1p remain to be elucidated.

Due to the architecture described above, PMTs are categorized as GT-C fold glycosyltransferases. The predicted GT-C fold is that of a large hydrophobic integral membrane protein located in the ER or the plasma membrane having several transmembrane helices and an active site located on a loop region. Interestingly, most of the GT-C enzymes utilize phosphate-activated donor sugar substrates (reviewed in Lairson et al. (2008)).

Animal protein O-mannosyltransferases

In recent years, PMTs have been identified within different animal phyla. Two PMT homologs have been characterized from *D. melanogaster*. The genes, *rotated abdomen* (*rt*; Dm*POMT1*) and *twisted* (*tw*; Dm*POMT2*), encode members of the PMT4- and PMT2-type enzymes, respectively (Jurado et al. 1999; Martin-Blanco and Garcia-Bellido 1996; Willer et al. 2002). As demonstrated by RNA interference, DmPOMT1 and DmPOMT2 act as *O*-mannosyltransferases in vivo (Ichimiya et al. 2004). Coexpression of both genes is necessary to increase POMT activity in insect cells suggesting the formation of DmPOMT1–DmPOMT2 complexes (Ichimiya et al. 2004). In situ hybridization revealed high levels of Dm*POMT1* and Dm*POMT2* transcripts at stage 14 of larvae development that corresponds to the period of active muscle differentiation (Lyalin et al. 2006).

In mammals, *POMT1* (PMT4-type) and *POMT2* (PMT2-type) have been identified (Jurado et al. 1999; Willer et al. 2002). Analogous to the yeast system, complex formation between POMT1 and POMT2 proteins has been demonstrated.

These POMT1–POMT2 complexes are crucial for mannosyltransferase enzyme activity in vitro (Akasaka-Manya et al. 2006), confirming that complex formation is mandatory for PMT function also in metazoa. During embryonic development in mice, *Pomt1* is expressed in brain, muscle, and eye (Prados et al. 2007; Willer et al. 2004). In adult rodents and humans, *POMT1* and *POMT2* are expressed in all tissues investigated, predominantly in testis (Manya et al. 2006; Willer et al. 2004, 2002). Interestingly, *Pomt2* of mice is transcribed from two different transcription initiation sites leading to transcripts of different length in certain tissues. The shorter transcript (somatic *Pomt2*; *sPomt2*) is present in all tissues examined. The longer transcript (testis *Pomt2*; *tPomt2*) is highly abundant in testis and encodes a testis-specific tPOMT2 protein (Willer et al. 2002). Specific antibodies were used to directly prove that tPOMT2 contains a 70-aa N-terminal extension that is highly conserved among mammals and humans. This testis-specific tPOMT2 isoform is restricted to the acrosome (Lommel et al. 2008), a sperm-specific organelle that is indispensable for successful fertilization (Abou-Haila and Tulsiani 2000). Interestingly, several lines of evidence indicate that tPOMT2 does not act as *O*-mannosyltransferase in the acrosome in vivo, while POMT1–sPOMT2 complexes in the ER of spermatogenic and nonspermatogenic cells are enzymatically active (Lommel et al. 2008). Even if its function is not clear at the moment, the high degree of conservation of the testis-specific tPOMT2 isoform between mammals and human points toward a crucial role in acrosome maturation or even fertilization.

Substrate specificity of protein *O*-mannosyltransferases

In contrast to other types of glycosylation, signals causing *O*-mannosylation of Ser and Thr residues appear to be complex both in fungal and in animal cells. Early in vitro studies in *S. cerevisiae* using synthetic acceptor peptides suggested that in general Thr is more frequently modified than Ser and that this Thr/Ser mannosylation is influenced by the neighboring amino acids (Sharma et al. 1991; Strahl-Bolsinger and Tanner 1991). However, these in vitro observations could not be verified in vivo. After the identification of the PMT family in *S. cerevisiae*, it became obvious that Pmt1p–Pmt2p complexes and Pmt4p complexes mannosylate different target proteins (Ecker et al. 2003; Gentzsch and Tanner 1997). It was shown that a subset of *O*-mannosylated proteins such as Kre9p, Cts1p, Bar1p, Pir2p, and Aga2p is exclusively mannosylated by Pmt1p–Pmt2p complexes, while Kex2p, Gas1p, Axl2p, and Fus1p are *O*-mannosylated by Pmt4p only (Ecker et al. 2003; Gentzsch and Tanner 1997; Proszynski et al. 2004; Sanders et al. 1999). A third group of proteins including Mid2p, the WSC family members, and Ccw5p is glycosylated by both complexes. However, different domains within these proteins are mannosylated by the different complexes (Ecker et al. 2003; Lommel et al. 2004). Recently, first signals determining Pmt4p-dependent *O*-mannosylation in *S. cerevisiae* in vivo were identified. Hutzler et al. (2007) proved that Pmt4p mannosylates exclusively membrane-bound proteins whereas Pmt1p/Pmt2p complexes act on both soluble and membrane proteins. Analysis of specific model proteins demonstrated that the nature of the membrane anchoring sequence is not relevant for recognition, as long as it is flanked by a Ser/Thr-rich domain facing the ER lumen. The implementation of these findings in a bioinformatics approach identified several novel Pmt4p substrate proteins (Hutzler et al. 2007).

In mammals the only well-characterized PMT substrate is α-DG, a membrane-associated protein of the dystrophin glycoprotein complex (Barresi and Campbell 2006). Several peptides deduced from α-DG have been identified as useful in vitro substrates of POMT1–POMT2 complexes (Manya et al. 2007). Based on these in vitro peptide substrates, the consensus sequence IXPT(P/X)TXPXXXXPT(T/X)XX was proposed as a sufficient trigger for *O*-mannosylation in mammals (Manya et al. 2007). Yet, the consensus sequence established in vitro could not be verified in vivo. Breloy et al. (2008) used recombinant fragments of α-DG to monitor POMT activity in vivo. This way, preferred target sites of POMT1–POMT2 complexes were localized within a region of α-DG that contains Ser/Thr-rich clusters and single Thr residues that are flanked by one or two basic amino acids. Furthermore, initiation of *O*-mannosylation was shown to be dependent on the presence of two structural elements (located between aa 346–367 and aa 377–417 of human α-DG) upstream of the target site (Breloy et al. 2008). All together, the in vivo results obtained by Hutzler et al. (2007) and Breloy et al. (2008) suggest that *O*-mannosylation signals are not just linear sequences of a protein's primary structure but rather represent structural determinants in the flanking regions of Ser/Thr-rich domains.

Biological significance of protein *O*-mannosylation in fungi

The isolation and characterization of *pmt*Δ mutants clearly demonstrated that protein *O*-mannosylation represents an essential modification in fungi. Tanner and co-workers first showed that in *S. cerevisiae* the simultaneous knockout of particular combinations of three PMT family members (*pmt1pmt2pmt4*Δ and *pmt2pmt3pmt4*Δ) is lethal (Gentzsch and Tanner 1996). Similarly, in *S. pombe* the simultaneous knockout of the PMT1 and PMT4 subfamily members, but even more drastically, also the single knockout of the PMT2 subfamily member is lethal (Willer et al. 2005). Similar results were obtained for *C. albicans* (Prill et al. 2005) and *C. neoformans* (Olson et al. 2007). However, the question remains why the lack of *O*-mannosylation results in lethality. As summarized below, protein *O*-mannosylation affects numerous important cellular processes. However, its major role seems to be the maintenance of an intact cell wall, an essential structure in fungi.

The role of *O*-mannosyl glycans is best understood in *S. cerevisiae.* Analyses of viable *pmt*Δ single and conditional lethal *pmt*Δ double mutants showed that *O*-mannosylation is crucial for the stability, localization and/or function of plasma membrane proteins and thus affects various cellular processes such as mating and maintenance of cell polarity (Lommel et al. 2004; Proszynski et al. 2004; Sanders et al. 1999). For example, haploid *pmt4*Δ mutants exhibit unipolar, rather than axial budding that is characteristic for haploid cells. This change in cell polarity is caused by aberrant *O*-mannosylation of the landmark protein Axl2p, which is required for the axial budding pattern. In cells lacking Pmt4p, Axl2p is incompletely *O*-mannosylated, unstable, and mislocalized (Sanders et al. 1999).

Another function of *O*-mannosylation in *S. cerevisiae* is the solubilization of misfolded proteins in the ER. When misfolded

proteins accumulate in the ER upon stress conditions, the unfolded protein response is triggered to remove these proteins by the ER-associated degradation (ERAD; Ellgaard and Helenius 2003). Upon induction of ER stress, transcription of PMT1 (*PMT1* and *PMT5*) and PMT2 (*PMT2* and *PMT3*) subfamily members is upregulated (Travers et al. 2000). Several mutant secretory proteins that are unable to fold properly in the ER are *O*-mannosylated by Pmt1p and/or Pmt2p whereas their wild-type counterparts are not (Coughlan et al. 2004; Harty et al. 2001; Hirayama et al. 2008; Nakatsukasa et al. 2004; Vashist et al. 2001). In *pmt1pmt2*Δ mutant strains, these mutant proteins tend to aggregate indicating that the modification by Pmt1p and Pmt2p prevents aggregation that might in turn be cytotoxic (Nakatsukasa et al. 2004). Therefore, it was suggested that *O*-mannosylation might function as a fail-safe mechanism for ERAD by solubilizing misfolded proteins and thereby reducing the load for ER chaperones (Nakatsukasa et al. 2004). Very recently, it was shown that Pmt4p-dependent *O*-mannosylation of heterologously expressed human β-amyloid precursor protein counteracts aggregation of the protein in the ER (Murakami-Sekimata et al. 2009). Thus, *O*-mannosylation might play a general role in the solubilization of proteins in the ER.

Probably, the most important function of protein *O*-mannosylation in fungi is its role in formation/maintenance of a stable cell wall. Analyses of viable *pmt*Δ mutants from different yeasts and filamentous fungi revealed that most of these mutants are susceptible toward cell wall disturbing agents and display aberrant cell wall morphologies (Gentzsch and Tanner 1996; Goto 2007; Olson et al. 2007; Timpel et al. 1998, 2000; Willer et al. 2005). For example, several *S. cerevisiae pmt*Δ mutants show increased sensitivity toward Calcofluor white and form large cell clumps during vegetative growth. These phenotypes resemble those of yeast mutants with defects in the assembly, organization, or maintenance of a rigid cell wall (Lorberg et al. 1999). Similarly, *S. pombe oma1*Δ (PMT1 orthologue) and *oma4*Δ (PMT4 orthologue) mutants are rounded, irregular shaped, and form aggregates. Ultrastructural analysis revealed dramatic changes in cell wall architecture and defective septum formation (Willer et al. 2005). Again, the molecular basis of cell wall defects in *pmt*Δ mutants is best known in *S. cerevisiae*, as summarized below.

In bakers' yeast, many cell wall proteins are highly *N*-glycosylated, *O*-mannosylated, or both. These mannoproteins are structurally and/or functionally important cell wall components, which determine cell wall permeability, hydrophilic properties, and accessibility of cell wall glucan, and are also involved in cell–cell recognition processes (for reviews, see Klis et al. 2006; Lesage and Bussey 2006). They are crucial for cell wall biogenesis and/or remodeling in order to maintain stability of this essential structure in yeast and fungal cells. In addition, a number of highly *O*-mannosylated plasma membrane receptors/sensors of different MAPK pathways have been identified which serve as key functions in the sensing of environmental changes and different stress conditions. Among those are the sensors of the cell wall integrity pathway (CWI) – the WSC family members and Mid2p – that control cell wall integrity during vegetative growth, periods of environmental stress, and pheromone-induced morphogenesis (Levin 2005). Analyses of conditionally lethal *pmt2pmt4*Δ mutants revealed that *O*-mannosyl glycans are indispensable for stability and processing of the CWI sensors Wsc1p, Wsc2p, and Mid2p. Reduced *O*-mannosylation leads to incorrect proteolytic processing of these proteins, which in turn results in impaired activation of CWI and finally causes cell death in the absence of osmotic stabilization (Lommel et al. 2004). However, all CWI mutants are viable when osmotically stabilized (Levin 2005). Thus, the loss of CWI sensor function does not completely explain why *pmt2pmt4*Δ double mutants can be rescued by osmotic stabilizers while the additional deletion of *PMT1* in *pmt1pmt2pmt4*Δ mutants causes cell death even under osmotically stabilizing conditions (Gentzsch and Tanner 1996). Besides the CWI sensors, many other cell wall mannoproteins are affected in the absence of *O*-mannosylation (summarized in Lengeler et al. 2008). Further, it was suggested that *O*-mannosylation precedes and potentially controls the *N*-glycosylation of glycoproteins (Ecker et al. 2003). Thus, diminished *O*-mannosylation might change general glycosylation patterns of cell wall mannoproteins thereby further affecting protein maturation and function. Considering these various aspects, it seems likely that once the *O*-mannosylating capacity falls below a critical level, folding, maturation, stability, and/or function of too many glycoproteins is perturbed and cells are no longer able to compensate for all resulting defects.

Phenotypes of *pmt*Δ mutants in other yeasts and filamentous fungi are highly similar to those observed in *S. cerevisiae pmt*Δ strains. In addition, it has been demonstrated that *O*-mannosylation of surface mannoproteins of fungal pathogens contributes significantly to virulence, as recently summarized by Goto (2007) and Lengeler et al. (2008).

Biological significance of protein *O*-mannosylation in animals and humans

To date, the best-studied *O*-mannosylated protein in metazoa is α-DG. It comprises two globular domains separated by a Ser/Thr-rich mucin-like region that is substantially *O*-mannosylated (Chiba et al. 1997; Sasaki et al. 1998). α-DG is an essential component of the dystrophin–glycoprotein complex (DGC) in skeletal muscle (Barresi and Campbell, 2006). Deficiencies within DGC result in inherited forms of muscular dystrophy (Blake et al. 2002). Remarkably, in flies, mice, and humans, most of the defects associated with impaired *O*-mannosylation can be explained by diminished functionality of α-DG and are similarly observed in dystroglycan mutants.

Intact O-mannosylation is essential for viability of flies and mice

In *D. melanogaster*, recessive mutations in the *rt/*Dm*POMT1* gene (PMT4-type) lead to poorly viable flies with defects in muscle development that result in a 60–90° twist of the abdomen relative to the body axis (Martin-Blanco and Garcia-Bellido 1996). A similar phenotype is observed upon the RNAi knockdown of Dm*POMT1* (Ichimiya et al. 2004). Likewise, recessive mutant alleles and RNAi knockdown of the *tw/*Dm*POMT2* gene (PMT2-type) induce the same twisted abdomen phenotype (Ichimiya et al. 2004; Lyalin et al. 2006). Already during larval development, mutations in either Dm*POMT1* or Dm*POMT2* result in defects in muscle attachment and changes in muscle contraction (Haines et al. 2007). Remarkably, these phenotypes are even more pronounced upon combination of either Dm*POMT1* or Dm*POMT2* mutations with a dystroglycan mutant allele,

suggesting that PMTs are required for the proper function of dystroglycan in flies (Haines et al. 2007).

In mice, during embryogenesis, the m*Pomt1* and m*Pomt2* genes are prominently expressed in the neural tube, the developing eye, and the mesenchyme (Lommel et al. 2008; Willer et al. 2004). Interestingly, these expression patterns in mice correlate with the sites of main tissue alterations observed in patients with mutations in h*POMT1* or h*POMT2* (see below). The disruption of murine *Pomt1* results in embryonic lethality around embryonic day 7.5–10.5 which is due to structural and functional perturbations of the first basal membrane (Reichert's membrane) separating the embryo from the surrounding maternal tissue (Willer et al. 2004). It was shown that in *pomt1*$^{-/-}$ embryos, *O*-mannosylation of α-DG is abolished indicating that α-DG-mannosylation is essential for the proper formation of the Reichert's membrane. Accordingly, a similar phenotype is observed in dystroglycan-deficient mice (Williamson et al. 1997). In contrast to *pomt1*$^{-/-}$ mice, *POMGnT* null-mutant mice are viable but show severe developmental defects. Skeletal muscle shows dystrophic changes such as small rounded fibers and the infiltration of fat cells (Liu et al. 2006). The cerebellum in brains of mutant mice is smaller and the cerebral cortex is thinner. Severe perturbations of the brain architecture are observed such as lamination defects of the cortex and breaches or even absence of the basal membrane that separates the neuronal tissue from the menningen (Hu et al. 2007; Liu et al. 2006). Additionally, eye abnormalities are described in *pomGnT*$^{-/-}$ mice that include reduced size of the optic nerve and retinal abnormities (Liu et al. 2006).

Impaired protein O-mannosylation causes congenital muscular dystrophies

In humans, impaired *O*-mannosylation is associated with a clinically and genetically heterogeneous group of autosomal recessive muscular dystrophies associated with variable brain and ocular abnormalities (Yoshida et al. 2001; Muntoni et al. 2004). These congenital muscular dystrophies (CMD) are also referred to as secondary α-dystroglycanopathies since a common pathological feature is the hypoglycosylation of α-DG. Phenotypic severity of dystroglycanopathy patients is highly variable. The most severe disorder is Walker–Warburg syndrome (WWS) followed by muscle–eye–brain disease (MEB) and Fukuyama congenital muscular dystrophy (FCMD; reviewed in Muntoni et al. 2004). These diseases are characterized by CMD associated with severe brain malformations (cobblestone lissencephaly) and abnormalities of the eye. Due to multiple malformations, WWS patients often die within the first year of life. In contrast, the mildest disorders may not present until adulthood such as limb-girdle muscular dystrophy (LGMD) where neither brain nor eyes are affected (Biancheri et al. 2007). Between these extremes, intermediate phenotypes with CMD but mild mental retardation and microcephaly have been described (van Reeuwijk et al. 2006).

To date, mutations in six known or putative glycosyltransferase genes have been identified to cause dystroglycanopathies with overlapping phenotypes. Among these are h*POMT1* (OMIM 607423), h*POMT2* (OMIM 607439), and h*POMGnT1* (OMIM 606822) that are directly involved in the synthesis of *O*-mannosyl glycans linked to α-DG. Association of POMTs with WWS was first established by Bertran-Valero de Bernabe and coworkers, who identified *POMT1* mutations in 6 out of 30 WWS patients (Beltran-Valero de Bernabe et al. 2002). In addition, mutations in *POMT1* have been reported that result in MEB (Godfrey et al. 2007), as well as milder forms of CMD such as congenital muscular dystrophy with mental retardation (CMD/MR; van Reeuwijk et al. 2006) and LGMD-2K (Balci et al. 2005). Mutations in *POMT2* have been detected in WWS patients, a milder MEB-like patient, and CMD/MR patients (Mercuri et al. 2006; van Reeuwijk et al. 2005; Yanagisawa et al. 2007). Further, a *POMT2* mutation has been described in a patient with an LGMD-2N phenotype, a mild clinical form of CMD with no neurological involvement (Biancheri et al. 2007). A recent study with a large cohort of dystroglycanopathy patients identified mutations in either *POMT1* or *POMT2* in about 20 % of the cases, finally establishing POMTs as causatives for CMD associated with clinical heterogeneity (Godfrey et al. 2007).

Pathologies arising from POMT mutations

As emphasized above, phenotypes observed in POMT mutants in animals and humans correlate with α-DG deficiencies. Within the DGC, α-DG is noncovalently associated with β-dystroglycan, a membrane protein of the sarcolemma that interacts directly with subsarcolemma proteins such as dystrophin. α-DG interacts with components of the extracellular matrix (ECM) such as laminin, providing a physical link between the subsarcolemma cytoskeleton and basement membranes (Barresi and Campbell 2006). It is believed that DGC confers structural stability to the sarcolemma during contraction, although precise mechanisms are not yet understood (Petrof et al. 1993). Inherited deficiencies in most of the proteins within DGC result in CMD (Blake et al. 2002), probably due to membrane microlesions and compromised muscle membrane function that might be induced by mechanical stress from muscle contraction (Blake et al. 2002; Deconinck and Dan 2007). Hypoglycosylated α-DG loses the ability to interact with its ECM-ligands in muscle (e.g., laminin, agrin, and perlecan) and in brain (e.g., neurexin; Michele et al. 2002; Michele and Campbell 2003; Muntoni et al. 2004). Thus, loss of α-DG *O*-mannosyl glycans reduces the association between DGC and ECM, therefore leading to muscular dystrophy. Aside from skeletal muscle, dystroglycan is found in several other tissues were it is abundantly expressed on the basal side of most epithelia (Durbeej et al. 1998; Durbeej et al. 1995). Thus, a general role in the assembly and organization of basal membranes was proposed (Barresi and Campbell 2006). During normal brain development, the top of the cerebral cortex is defined by a basement membrane – the glia limitans – at the end of the radial glia fibers that guide neurons on their way to the cortex. This prevents neurons from migrating out of the brain into the subarachnoid space. Cobblestone lissencephaly induced by diminished *O*-mannosylation of α-DG is associated with gaps in the glia limitans, as well as the failure of neurons to organize themselves within the cortex region and their migration into the subarachnoid space (Ross 2002). Similar defects are induced in the conditional knockout of murine dystroglycan in glia cells and brain neurons that result in brain malformations resembling those found in WWS and MEB patients (Moore et al. 2002). There are indications that dystroglycan is also involved in the organization of synapses and of the basal membrane of the retina (Moukhles et al. 2000; Sato et al. 2008). Thus, on the

whole, brain and ocular malformations of dystroglycanopathy patients might be explained by diminished function of α-DG due to reduced *O*-mannosylation.

In addition, emerging evidence suggests that changes in *O*-mannosyl glycan composition are modulating not only laminin- but also integrin-dependent adhesion and migration of human neuronal cells (Abbott et al. 2006). In this respect, it was recently suggested that the developmentally regulated and neuron-specific receptor protein-tyrosine phosphatase beta (RPTPβ) is carrying the *O*-mannosyl-linked HNK-1 modification. Changes in *O*-mannosylation might thus regulate RPTPβ signalling thereby influencing cell–cell and cell–matrix interactions in the developing nervous system (Abbott et al. 2008).

Protein *O*-mannosylation in prokaryotes

Prokaryotes, including bacteria and archaea, are able to glycosylate proteins (Abu-Qarn et al. 2008). More than 70 glycoproteins have been reported (Upreti et al. 2003). Among these, some glycoproteins containing *O*-mannosyl glycans have been reported including β1,4-glucanase Cex of *Cellumonas fimi*, and *Streptomyces lividans* (Ong et al. 1994) and the phosphate binding protein PstS of *Streptomyces coelicolor* (Wehmeier et al. 2009). Further, *O*-mannosyl glycans containing rhamnose, 2-*O*-methyl mannose, glucose, and glucuronic acid, attached at consensus sites corresponding to Asp-Ser or Asp-Thr have been isolated from protease P40 and the endoglycosidases Endo F2 and Endo F3 of the Gram-negative bacterium *Flavobacterium menigosopticum* (Plummer et al. 1995). The alanin- and proline-rich antigen (Apa) and superoxide dismutase (SodC) glycoproteins of the Gram-positive bacterium *Mycobacterium tuberculosis* (Dobos et al. 1996; Sartain and Belisle 2008) and the secreted antigen MPB83 of *Mycobacterium bovis* (Michell et al. 2003) are *O*-mannosylated at Thr residues in Thr/Pro-rich sequences. Characterization of Apa and SodC revealed short linear α1,2-linked oligomannose chains of up to three mannosyl residues (Dobos et al. 1996; Sartain and Belisle 2008), whereas MBP83 contains mannose and α1,3-linked mannobiose (Michell et al. 2003). The structural similarities of mycobacterial and fungal *O*-mannosyl glycans already suggest a conserved biosynthetic pathway.

Biosynthesis of bacterial *O*-mannosyl glycans

The biosynthetic pathway of *O*-mannosyl glycans in bacteria was initially characterized using a cell-free peptide glycosylation assay. Incubation of a membrane fraction from *Mycobacterium smegmatis* with GDP-[^{14}C]mannose and synthetic peptides derived from the Apa protein, led to the synthesis of polyprenol monophosphate [^{14}C]mannose and then to [^{14}C]mannose-peptide (Cooper et al. 2002).

Consequently, polyprenyl monophosphomannose synthases (Ppm1) with significant homology to the *S. cerevisiae* Dol-P-Man synthase Dpm1p have been identified in mycobacteria and corynebacteria. Their function in polyprenol monophosphate mannose (PPM) synthesis has been demonstrated (Baulard et al. 2003; Cooper et al. 2002; Gibson et al. 2003; Gurcha et al. 2002). Recently, direct involvement of *S. coelicolor* Ppm1 in the protein *O*-mannosylation pathway could be shown (Wehmeier et al. 2009). Ppm1 catalyzed the transfer of mannose to endogenous polyprenol phosphate containing nine repeated isoprenyl units in membrane preparations of *S. coelicolor*. Further, mutation in *ppm1* abolished the transfer of mannose to acceptor peptides/protein.

A bioinformatic approach led to the identification of the first bacterial protein *O*-mannosyltransferase (Rv1002c/*pmt*) from *M. tuberculosis*. The Mt-Pmtp protein displays 22–24% homology to the PMTs of *S. cerevisiae* (VanderVen et al. 2005). Overexpression of Mt-*pmt* in *M. smegmatis* led to increased Pmt activity of membrane fractions in a cell-free peptide glycosylation assay (VanderVen et al. 2005). Pmt proteins that initiate biosynthesis of bacterial *O*-mannosyl glycans have also been characterized from *Corynebacterium glutamicum* and from *S. coelicolor* (Mahne et al. 2006; Wehmeier et al. 2009). Disruption of both, Cg-*pmt* and Sc-*pmt*, completely abolishes *O*-mannosylation of specific glycoproteins in vivo (Mahne et al. 2006; Wehmeier et al. 2009). To date, a whole plethora of bacterial Pmt homologs has been identified and categorized as glycosyltransferases of the GT39 family (http://www.cazy.org). Despite their moderate homology to fungal Pmt proteins, the hydropathy profiles of bacterial and eukaryotic Pmts are substantially similar (Mahne et al. 2006; VanderVen et al. 2005; Figure 4). Yet, MIR motifs of the large hydrophilic loop 5 domain are absent in bacterial Pmts. Interestingly, specific arginine residues in the first and second transmembrane domain are conserved between bacterial and eukaryotic PMTs (Figure 4). In *S. cerevisiae*, these conserved residues are crucial for the formation of PMT complexes, suggesting that bacterial Pmts might also act as complexes. In addition, an Asp-Glu motif present in the putative catalytic loop 1 domain is crucial for mannosyltransferase activity for both, yeast and mycobacterial Pmts (Girrbach et al. 2000; VanderVen et al. 2005). Conservation of the basal mechanisms of *O*-mannosyl transfer between pro- and eukaryotes is further supported by the finding that in mycobacteria protein *O*-mannosylation depends on protein translocation via the secretory pathway (VanderVen et al. 2005).

Although enzymes catalyzing the initial steps of bacterial *O*-mannosylation have been identified, it is not clear to date how *O*-linked mannose is further extended. However, bacterial mannosyltransferases of the GT-C superfamily that add α1,2 branches to the mannan core of lipomannan/lipoarabinomannan have been characterized (Kaur et al. 2006). Whether these or similar enzymes are also involved in the biosynthesis of *O*-mannosyl glycans remains to be elucidated in the future.

Biological significance of protein *O*-mannosylation in prokaryotes

Despite the progress in the eukaryotic field, little is known about the functional role of protein *O*-mannosylation in bacteria. *pmt* mutants of *C. glutamicum* are viable and do not show any obvious phenotype (Mahne et al. 2006). Insights into the biological significance of protein *O*-mannosylation could be obtained from the function of the respective glycoproteins. *M. tuberculosis* causes persistent infection by evading the defense mechanisms of the infected host (Ragas et al. 2007). In this context, mannose-specific C-type lectins have emerged to be the preferential receptor used by mycobacteria to enter their target cells (Nguyen and Pieters 2005). The *M. tuberculosis* cell wall-associated adhesion protein Apa has recently been

demonstrated to bind these mannose-specific lectins which suggests a role of the attached *O*-mannosyl glycans in colonization and invasion of the host cell by the pathogen. On the other hand, Apa is one of the major immunodominant antigens secreted by *M. tuberculosis*. Experiments using nonmannosylated Apa proteins demonstrated that its capacity to stimulate T-lymphocyte responses is highly dependent on the glycosylation. Thus, *O*-mannosyl glycans contribute to the antigenicity of Apa (Horn et al. 1999; Romain et al. 1999). In *S. coelicolor*, mutations in *pmt* and *ppm1* result in increased resistance to the bacteriophage ΦC31 (Cowlishaw and Smith 2001). This phenotype is also observed in *ppm1* mutants (Cowlishaw and Smith 2001). These observations lead to the suggestion that the receptor for ΦC31 is a yet undefined *O*-mannosylated cell surface glycoprotein of *S. coelicolor* (Cowlishaw and Smith 2001). In summary, these initial studies suggest that *O*-mannosyl glycans of specific secretory proteins contribute to virulence of pathogenic mycobacteria and might fulfill other yet unidentified important functions.

Concluding remarks

During the last 35 years, the biosynthetic pathway of *O*-mannosyl glycans was established in bakers' yeast. The identification of PMTs and *pmt* mutants was a major step toward deciphering the biological role of this essential protein modification in fungi. Unexpectedly, *O*-mannosyl glycans and PMTs were also identified in animals and humans in the late 1990s, and their association with neuromuscular diseases has focused the attention of different research areas on protein *O*-mannosylation. Today, the basic principles of *O*-mannosylation in eukaryotes are quite well understood. However, 3D structures of PMTs, their association with other ER components, and signals that define *O*-mannosylation still remain to be explored. Certainly, bakers' yeast will again be an important model organism to solve these issues. Furthermore, the recent identification of PMTs in bacteria has proven that *O*-mannosylation is conserved between pro- and eukaryotes. The future analysis of bacterial Pmts will help to resolve basic principles of *O*-mannosylation and to explain how the PMT family has evolved to satisfy the different demands in the ER of eukaryotes.

Funding

We thank The Deutsche Forschungsgemeinschaft and the European Union for continuing support.

Acknowledgements

We thank all the present and former members of the laboratories of W. Tanner and ours who helped to throw light on protein *O*-mannosylation in yeasts and in mammals. We are grateful to F. Hutzler and J. Hutzler for localization of yeast PMTs shown in Figure 2B. Thanks to M. Büttner for the critical reading of the manuscript. S. Strahl is especially thankful to Mark Lehrman for his immense patience and encouragement while this review was in progress. S. Strahl is member of CellNetworks – Cluster of Excellence (EXC81).

Conflict of interest statement

None declared.

Abbreviations

CMD, congenital muscular dystrophy; CWI, cell wall integrity pathway; α-DG, α-dystroglycan; Dol-P, dolichol-phosphate; HNK-1 epitope, human natural killer-1 epitope; LGMD, limb-girdle muscular dystrophy, MEB, muscle-eye-brain disease; WWS, Walker–Warburg syndrome.

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