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Dolichol Phosphate Mannose Synthase: A Glycosyltransferase with Unity in Molecular Diversities

Dipak K. Banerjee^{1,2}, Zhenbo Zhang¹, Krishna Baksi³, and Jesús E. Serrano-Negrón^{1,4}

¹Department of Biochemistry, School of Medicine, University of Puerto Rico, Medical Sciences Campus, San Juan, PR 00936-5067, USA

²Institute of Functional Nanomaterials, University of Puerto Rico, San Juan, PR 00931-1907, USA

³Department of Anatomy and Cell Biology, School of Medicine, Universidad Central del Caribe, Bayamón, PR 00960-3001, USA

⁴Department of Natural Sciences & Mathematics, Interamerican University of Puerto Rico, Bayamón Campus, Bayamón, PR 00957-6257, USA

Abstract

N-glycans provide structural and functional stability to asparagine-linked (N-linked) glycoprotein, and add flexibility. Glycan biosynthesis is elaborative, multi-compartmental and involves many glycosyltransferases. Failure to assemble N-glycans leads to phenotypic changes developing infection, cancer, congenital disorders of glycosylation (CDGs) among others. Biosynthesis of N-glycans begins at the endoplasmic reticulum (ER) with the assembly of dolichol-linked tetra-decasaccharide (Glc₃Man₉GlcNAc₂-PP-Dol) where dolichol phosphate mannose synthase (DPMS) plays a central role. DPMS is also essential for GPI anchor biosynthesis as well as for O- and C-mannosylation of proteins in yeast and in mammalian cells. DPMS has been purified from several sources and its gene has been cloned from 39 species (e.g., from protozoan parasite to human). It is an inverting GT-A folded enzyme and classified as GT2 by CAZy (carbohydrate active enZyme; <http://www.cazy.org>). The sequence alignment detects the presence of a metal binding DAD signature in DPMS from all 39 species but finds cAMP-dependent protein phosphorylation motif (PKA motif) in only 38 species. DPMS also has hydrophobic region(s). Hydropathy analysis of amino acid sequences from bovine, human, *S. crevisiae* and *A. thaliana* DPMS show PKA motif is present between the hydrophobic domains. The location of PKA motif as well as the hydrophobic domain(s) in the DPMS sequence vary from species to species. For example, the domain(s) could be located at the center or more towards the C-terminus. Irrespective of their catalytic similarity, the DNA sequence, the amino acid identity, and the lack of a stretch of hydrophobic amino acid residues at the C-terminus, DPMS is still classified as Type I and Type II

Correspondence Address: Dipak K. Banerjee, Ph.D. Department of Biochemistry, School of Medicine, University of Puerto Rico, Medical Sciences Campus, San Juan, PR 00936-5067. USA; Tel.: (787) 281-0155; Fax: (787) 274-8724; dipak.banerjee@upr.edu.

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enzyme. Because of an apparent bio-sensing ability, extracellular signaling and microenvironment regulate DPMS catalytic activity. In this review, we highlight some important features and the molecular diversities of DPMS.

Keywords

asparagine-linked; glycoprotein/dolichol; phosphate; mannose; synthase/glycosyltransferase/phosphoprotein/carbohydrate active enzyme

Introduction

Over the years, efforts have been directed towards understanding the catalytic transfer of sugars from sugar nucleotides to glycoproteins, proteoglycans, and glycosyl phosphatidylinositol (GPI) anchors as well as to glycosphingolipids. The oligosaccharide side chains of proteoglycans assemble in Golgi through a direct transfer of sugar residues from sugar nucleotide donors. On the other hand, the synthesis of the glycan chain for N-linked glycoproteins originates in the endoplasmic reticulum (ER), extended and finally terminated in Golgi. Differences therefore exist in the synthesis of core glycan for N-linked glycoproteins, the GPI, and O- and C-mannosylation of proteins. One common aspect however is that they all use dol-p-man, a lipid-linked sugar derivative as mannose donor. The assembly of core oligosaccharide (Glc₃Man₉GlcNAc₂) for N-glycans takes place on dolichol a isoprenoid lipid prior to its *en block* transfer to the asparagine residue in a *sequon* (Asn-X-Ser/Thr) when the protein is in its nascent state [1,2]. This co-translational event is important for protein folding and securing the fidelity of protein production [3].

Dolichylphosphate β-D mannosyl transferase (systemic name: GDP-mannose: dolichylphosphate mannosyl transferase) is commonly known as dolichol phosphate mannose synthase (DPMS; EC 2.4.1.83). Its gene is located in human chromosome 20q13.13. The enzyme was first discovered in *Saccharomyces cerevisiae* in 1971 for catalyzing the transfer reaction
$$\text{GDP-mannose} + \text{Dol-P} \xrightleftharpoons{\text{Mn}^{2+}/\text{Mg}^{2+}} \text{Dol-P-Man} + \text{GDP}$$
 [4]. Since then, DPMS activity has been documented in vertebrates, non-vertebrates Dol-P-Man + GDP [4]. Since then, DPMS activity has been documented in vertebrates, non-vertebrates, fruit fly, plants, fungi, slime molds, protozoan parasites as well as in archaea [5,6]. Immunofluorescence microscopy supports co-localization of both Dol-P and DPMS in the ER (Figure 1a–c). Immunogold electron microscopy of thin sections of capillary endothelial cells also confirm the localization of Dol-P in the ER (Figure 1d). These studies have used amphomycin's affinity for Dol-P [7] as well as antibodies against amphomycin (mouse monoclonal) and DPMS (rabbit polyclonal) as tools [8,9].

In addition to transferring mannose to dolichol (α-saturated C₈₀–C₁₀₀ polyprenol), DPMS also transfers mannose to undecaprenol (α-unsaturated C₅₅ polyprenol), S-citronellol (a water-soluble α-saturated C₁₀ dolichol) as well as to retinol *in vitro* [10–12]. Natural dolichols, a family of long-chain polyisoprene alcohols are composed of one dihydroisoprene unit at the α-end of the polyisoprene chain and two isoprene units in the E- (*trans*-) configuration at the ω-end, with remainder of the chain in the Z- (*cis*-) isoprene units

being species-specific mixtures of polyisoprenes of different chain-length [13]. Dolichols from mammalian cells/tissues contain 8–21 isoprene units (C₉₀–C₁₀₅), whereas yeast dolichols are composed of 14–17 isoprene units (C₇₀–C₈₅), dolichol-15 (C₇₅) and dolichol-16 (C₈₀) being main components.

The structures of other dolichyl-like compounds are comparable to that of dolichol because they share similar kinetic interactions. Shortening the poly-*cis* fragment by five or eight C-isoprene units increase the K_m values approximately 2-fold. Modification of the γ -isoprene unit is less favorable because it increases the K_m 3 to 7-fold higher than that for Dol-P. Similarly, the water-soluble PyNH(CH₂)₂-CS-P (CCS-P) [14] the shortest fragment that occupies the active site is inefficient in binding to the enzyme (K_i ~200 times higher than the K_m for the natural substrate, Dol-P). This suggests CCS-P fragment is a minimum requirement for binding to the enzyme.

DPMS in Glycosylation

The N-linked glycoproteins are evolutionary conserved [15], and the availability of Glc₃Man₉GlcNAc₂-PP-Dol (LLO) is critical for the N-glycosylation reaction [1]. Dol-p-man elongates Man₅GlcNAc₂-PP-dolichol to Man₉GlcNAc₂-PP-dolichol in the ER lumen [16,17] and helps securing the mannose residues to arrange in a tri-antennary structure. Dol-p-man is also a mannose donor for GPI anchor biosynthesis [18], for O-mannosylation of proteins in yeast [19] as well as for O-mannosylation of α -dystroglycan and C-mannosylation of proteins in mammalian cells [20,21]. RFT1 in yeast, an ATP-independent bi-directional polytopic membrane protein with 10 transmembrane spans has claimed to be active in flipping Man₅GlcNAc₂-PP-dolichol from cytosol to the ER lumen [22]. Unfortunately, no flippase has yet been detected in other eukaryotes irrespective of the presence of the gene for a homologous protein in their genome. Similarly, it also remains an open question about the translocation of dol-p-man to the ER lumen to participate in the catalytic process. Uptake of a water-soluble cit-p-man (an analog of dol-p-man) in sealed microsomal vesicles indicates a membrane protein may mediate the transport [11]. The specificity of such translocation prefers saturated α -isoprene to unsaturated *trans*- or *cis* α -isoprene units of Dol-P [23].

DPMS, an M_r31kDa protein has been purified from rat liver, porcine aorta, *Acanthamoeba castellanii*, murine mitochondria, Archae (formerly Archaeobacteria) as well as from bovine liver (Martinez, J.A. and Banerjee, D.K. unpublished observation). The recombinant protein has also been purified from *E. coli* transformed with *S. cerevisiae* DPMS gene as well as the gene from bovine capillary endothelial cells [24,25]. The enzyme from higher eukaryotes is most active in the presence of Mn²⁺ but that from yeast and archaea prefer Mg²⁺. The K_m for GDP-mannose for DPMS ranges from 10⁻⁷M to 10⁻⁶M.

Purified DPMS from rat liver is inactive in non-ionic detergents. On the other hand, the detergent-free enzyme is active in the presence of phosphatidylethanolamine (PtdEtn) as well as in the presence of phospholipid mixtures of PtdEtn and phosphatidylcholine (PtdCho) when the molar ratio of PtdEtn to PtdCho is 70% or less. The enzyme however is inactive in the presence of PtdCho alone. Unsaturated species of PtdEtn destabilize the

membrane bilayers, but dolichol exhibits the destabilizing effect of PtdEtn on membranes composed of PtdCho and PtdEtn. Thus, DPMS is optimally active in a phospholipid matrix that prefers non-bilayer structural organization [26].

Genetic and metabolic alterations inhibit DPMS activity *in vivo*. DPMS activity is (i) absent in skin fibroblasts from patient with Type Ie variant of congenital disorder of glycosylation (CDG) [27]; (ii) lost in tunicamycin (N-acetylglucosaminyl 1-phosphate transferase inhibitor)-treated capillary endothelial cells [28]; (iii) down regulated in *sh*RNA knockdown capillary endothelial cells [29] or after mutating the PKA site [30]. Amphomycin (a naturally occurring M_r 1,290 Dalton undecapeptide with a fatty acid substitution at the N-terminus) inhibits DPMS activity *in vitro* [5, 31]. Kinetic studies with calf brain DPMS indicate no significant change in the apparent K_m for GDP-mannose but the V_{max} is reduced by >90%. The inhibition is highest in the presence of $Ca^{2+} > Co^{2+} > Mn^{2+} > Ni^{2+} > Mg^{2+}$. Monovalent cations are ineffective. Mechanistically, amphomycin forms a complex with Dol-P in the presence of Ca^{2+} and blocks the availability of Dol-P as an acceptor for mannose. Space-filling model also suggests that Ca^{2+} acts as a bridge between the phosphate head group of Dol-P and the N-terminal aspartic acid residue of amphomycin [32]. The interaction however is lost upon removal of the fatty-acylated aspartic acid residue by mild-acid hydrolysis [17]. Titration of DPMS activity as a function of Dol-P in the presence of amphomycin indicates a co-operative interaction. The substrate-velocity plot is sigmoidal, and the Hill coefficient (n) establishes a positive co-operative interaction between Dol-P and amphomycin [5].

Structure of DPMS

DPMS gene has been cloned and sequenced from 39 different species. Their amino acid sequences indicate a considerable diversity. Natural selection to adapt the changing environment is during evolution is believed to be a contributing factor [33–35]. Phylogenetic comparison and homology of DNA sequences have identified *bDPMS* being closer to *hDPMS* (Figure 2). The accession numbers and the databases used to collect the amino acid/DNA sequences are listed in Table 1.

Amino acid sequences divide DPMS into two classes of enzymes (Table 2). Type I enzymes (*Saccharomyce cerevisiae*, *Ustilago maydis*, *Trypanosoma brucei*, *Leishmania mexicana*, etc.; [36]) share 60–70% amino acid identities and a stretch of hydrophobic amino acid residues near the C-terminus. Type II enzymes (bovine, human, *Saccharomyce pombe*, *Caenorhabditis briggsae*, *Trichoderma reesei*, etc.) however exhibit only 30–40% amino acid identity with Type I enzymes and lack the hydrophobic domain [36]. cDNA encoding *Schistosoma mansoni* DPMS also displays a high homology with *Cricetulus griseus* (Chinese hamster) and *Schizosaccharomyces pombe* [37].

DPMS from *S. cerevisiae* has three cysteine residues, Cys93, Cys172 and Cys259 [38]. Treating with thiol-specific reagents cause inhibition of the synthase activity suggesting sulfhydryl groups play a role in the catalytic mechanism of the enzyme. However, replacing the cysteine residues with serine by site-directed mutagenesis exhibits no change in the specific activities of the mutants indicating none of the cysteine residues is in fact essential

for the catalytic activity. It is suggested that weakly nucleophilic hydroxyl side chain of serine residue might have substituted for cysteine in a molecular reaction mechanism, but the side chain of serine and the sulfhydryl group of cysteine have the potential for hydrogen bonding. Replacing Cys93 with glycine however does not change the outcome. Thus, a direct role of the sulfhydryl residue of cysteine in the catalytic mechanism is highly unlikely. Thiol-blocking reagents may indicate that Cys93 could be located near the catalytic site. GDP-mannose, dolichyl phosphate and substrate analogs have been found to protect against Nbs2 inactivation suggesting Cys93 is physically near, or within, the substrate-binding site of the enzyme [38]. In fact, Cys93 is very close to Ser141 whose phosphorylation enhances the DPMS catalytic activity. DPMS sequence from chimpanzee, Western clawed frog, African clawed frog, Rainbow smelt and *Candida parapsilosis* each contains two cysteine residues but other members of the species have either one or none. Alanine154 in *S. cerevisiae* may also be located in or near the catalytic site because its replacement with threonine (i.e., A154T) causes a total loss of catalytic activity. Furthermore, S141A/A154T double mutant also exhibits a total loss of enzyme activity when subjected to phosphorylation by PKA [39].

A hydrophobic region of 13-amino acids of DPMS (aa245–258) from *Saccharomyces cerevisiae* has been proposed to be a dolichol recognition site [40]. But, modifying the highly conserved amino acid residues of the domain by site-directed mutagenesis or deleting the sequence altogether (i) does not change the catalytic activity of the enzyme; and (ii) all modified enzymes have retained the same affinity for Dol-P as in the wild type when assayed in a phospholipid matrix. Thus, neither the composition nor the sequence of the conserved domain is critical for recognition and binding of Dol-P in a lipid matrix.

The 3-D structure of membrane protein and its topology play an indispensable role for its feature and function. Unfortunately, all attempts to crystallize the recombinant DPMS from yeast have failed because of the hydrophobic nature of the protein [41]. Computing the hydropathy index (hydrophobicity) under ExpASY Bioinformatics Resource Portal (<http://web.expasy.org/protscale/>) (42) using the algorithm of Kyte and Doolittle [43] allows examination of the hydrophobic interior of the membrane-spanning region of DPMS (Figure 3). The positive indices around the amino acid residues aa104–124 and aa166–167 separated by a hydrophilic region of aa125–165 support bDPMS with one membrane-spanning region. The hydropathy plot of bDPMS (Figures 3a) is similar, if not identical to that of the DPMS from human, *S. cerevisiae* and *A. thaliana* (Figures 3b–d). The PKA motif (solid lines in Figure 3) is present between the two hydrophobic domains (i.e., one preceding and the other succeeding the motif) (Figure 3a–d).

Hydropathy plot of DPMS from human, bovine and plant (Figures 3a, b & d) shows three major hydrophobic (positive on the y-axis) regions at aa1–30, aa60–110, aa125–170 and aa180–200, respectively and represents pockets (or clefts) under the shield of hydrophilic domains (negative on the x-axis) of the polypeptide. Given the polarity index window of 19, (18–21 nonpolar amino acid residues form α -helix) one of these lipophilic regions could be an ER membrane-spanning domain, as well as a binding site for Dol-P. The yeast DPMS (Figure 3c) displays a highly lipophilic profile and opens the possibility for more than one membrane-spanning domain for Type 1 enzymes.

Mutation or deletion of a fragment to adapt environmental change could trigger a frame shift. This may lead to a different membrane spanning region and different location of the PKA motif in DPMS. The PKA motifs at aa141 for *S. cerevisiae*; aa165 for bovine; aa158 for human and aa151 for *Arabidopsis thaliana* (Figure 3d) are perfect examples. Furthermore, constructing in-frame insertion, deletion, and nonsense mutation were shown that the distance of a functional domain to N or C-terminus is important to affect function [44].

Unique features of DPMS also allow the use of Fluorescence Resonance Energy Transfer (FRET) technique to elucidate its structure. For example, triangulation of distances between Trp133, modified Cys93, and the fluorescent probes of the dolichyl-derived compounds delineate the selected structural properties of the enzyme's active site and its dolichol-binding pocket.

DPMS is an inverting GT-A fold enzyme [45, 46] and classified as GT2 in Carbohydrate Active enZYme database (CAZy; <http://www.cazy.org>). Like other GT-A fold enzymes, DPMS from all sources also carry a DXD sequence signature for metal binding (Figure 4d). In recent years, the 3-D structures of a couple of glycosyltransferases with GT-A fold have been elucidated. These are DNA-modifying β -glucosyltransferase from bacteriophage T4 [47], SpsA from *B. subtilis* [48], polyprenyl-phosphate glycosyltransferase GtrB from *Synechocystis* sp. PCC6803 [49], oligosaccharyl transferase (STT3) from a thermophilic archaeon, *Pyrococcus furiosus* [50], and yeast MNN1 α -1,3-mannosyltransferase [51]. DPMS structure when studied with sensitive fold recognition analyses using the META server (<http://bioinfo.pl/Meta>) [52] indicates that they are similar to one another at the primary, secondary, and tertiary structure levels. Also, all DPMS are similar in fold to the structure of the spore coat polysaccharide biosynthesis protein A (SpsA) glycosyltransferase (GT) from *Bacillus subtilis* for which a 3D X-ray structure has been elucidated (protein data bank [pdb code: 1h7l]) [48]. A 3-D model of DPMS analogous to that of SpsA agrees nearly perfectly with the available experimental data allowing identification of the active site cleft, its properties, and a putative functional mechanism [53].

Most striking of DPMS is that DPMS from every known source except *C. parapsilosis* has a PKA motif [54, 55] equivalent to that found in *S. cerevisiae* (RRVIS¹⁴¹S). DPMS from *Saccharomyces cerevisiae* and *Ustilago maydis* also has a serine residue immediately following the phosphorylation consensus site, i.e., RRVISS¹⁴²/RRIISS¹⁷¹ [54, 56], the significance of which is currently unknown. However, when Ser141 in yeast DPMS is mutated, Ser142 partially fulfils the need for Ser141 [30]. The sequence alignment of DPMS from all 39 species except *Candida parapsilosis* highlights this conserved motif and is presented in Figure 4d. The rest of the figure is included in a supplemental file sf4.

The defect in dol-p-man synthesis also results in defective GPI and consequently the surface expression of defective Thy-1, a GPI-anchored protein [57]. Irrespective of a defect in dol-p-man synthesis, Thy-1-negative lymphoma of complementation class E [58] and CHO-derived Lec15 mutant [59] cells are identified with different complementation groups [60], and suggest the participation of more than one protein. Yeast DPMS DNA complements both mouse Thy-1 negative lymphoma mutant cells of complementation class E and the Lec

15 mutant of CHO cells [61,62] but not human or the mouse homolog of DPMS, i.e., hDPMS or mDPMS. This observation suggests that mammalian DPMS might be a multi-component enzyme [63,64]. A similar claim has also been made for the DPMS from *Candida albicans* and *Arabidopsis thaliana* [65,66]. DPM2, an 84 amino acid membrane protein whose gene located on chromosome 9q33 has been proposed to form a complex with DPM1 [67]. Two putative transmembrane domains and a double lysine sequence near the C-terminus act as ER localization signal for DPM2. DPM1–DPM2 interaction however is lost upon substitution of two amino acids in the first DPM2 transmembrane domain.

Affinity isolation of DPMS complex from human cells identifies an M_r8kDa protein with 92 amino acid residues called DPM3 whose gene is on chromosome 1q12–21. DPM1, DPM2 and DPM3 when incorporated into liposomes, show a full DPMS activity with a K_m of GDP-mannose comparable to that of the DPMS purified from rat liver. DPMS as a heteromeric enzyme also reported for *Schizosaccharomyces pombe* [36], *Arabidopsis thaliana* [65], *Trichoderma reesei* [68], and for a pathogenic yeast *Candida albicans* [69]. Schematic representation of a structural model proposed for human DPMS suggests DPM2 associates with the N-terminal hydrophobic portion of DPM3, and DPM3, *via*, its C-terminal hydrophilic portion association with DPM1 [63]. This idea needs to be tested following co-crystallization of DPM1 with DPM2 and DPM3. This is important, because the model ignores the presence of the transmembrane domain in DPMS as well as dephosphorylation rendering DPMS catalytically inactive [Figure 3].

Regulation of DPMS Catalytic Activity

DPMS plays a critical role during extracellular signaling and/or cellular microenvironment regulating internal events leading to increased protein N-glycosylation [70,71]. For example, capillary endothelial cells when cultured in the absence of 5% CO₂ exhibit (i) a distinctive morphology; (ii) enhanced protein N-glycosylation; and (iii) increased catalytic activity of DPMS by lowering the K_m for GDP-mannose by ~35% without compromising the V_{max} [71]. This may perhaps explain the “Warburg effect” in tumor angiogenesis [72]. On the other hand, isoproterenol (a β-agonist) enhances protein N-glycosylation in rat parotid acinar cells by increasing the DPMS activity. There is no significant alteration in the apparent K_m for GDP-mannose, but the V_{max} is enhanced ~2-fold [70]. This is comparable to the DPMS activation in microsomal membrane phosphorylated *in vitro* with PKA, and reproduces in phosphorylated bovine brain as well as hen oviduct microsomal membranes. Increased DPMS activity in rat parotid acinar cells correlates with increased mannosylated oligosaccharide-PP-Dol synthesis, and its turnover as well as protein glycosylation [73].

Phosphorylation activation of ER glycosyltransferases is relatively unknown but somatic cell genetics and molecular cell genetics support the concept for DPMS:

- a. *Somatic cell genetics*: The model is wild type Chinese hamster ovary (CHO) cells and its PKA deficient mutants. CHO cells do not express β-adrenergic receptor but exogenous addition of 8Br-cAMP enhances protein glycosylation in wild type cells 120%. However, it is only 7%–23% in PKA deficient mutants. The mutants also undergo morphological changes in the presence of 8Br-cAMP.

These mutants express quantitatively low level of LLO, and the $t_{1/2}$ for LLO turnover is twice as high as in the wild type [74]. Both wild type and the mutants express $\text{Glc}_3\text{Man}_9\text{GlcNAc}_2\text{-PP-Dol}$ as the most predominating species, and there is no accumulation of $\text{Man}_5\text{GlcNAc}_2\text{-PP-Dol}$ in mutants [74]. The K_m for GDP-mannose for DPMS is 160%–400% higher in mutants than with the wild type cell. The k_{cat} is also reduced 2–4 fold in mutants. Exogenous addition of Dol-P does not rescue the k_{cat} for DPMS in mutants but *in vitro* phosphorylation restores the catalytic activity to the level of the wild type [74]. Similarly, in MRI, a revertant (i.e., a pseudo wild type), in which the deficiency of the regulatory subunit (RI subunit) for PKA type I is corrected, restores the level of LLO biosynthesis, turnover and protein glycosylation [75].

- b. *Molecular cell genetics:* Recombinant DPMS from bovine adrenal medullary capillary endothelial cells and *Saccharomyces cerevisiae* express active enzymes in *E. coli* [38,39,54]. Kinetics of recombinant DPMS from *Saccharomyces cerevisiae* indicate no change in the K_m for GDP-mannose but ~ 6-fold increase in the V_{max} after *in vitro* phosphorylation along with higher enzyme turnover (k_{cat}) and catalytic efficiency (k_{cat}/K_m). Autoradiography of ^{32}P -labeled DPMS detects an M_r 31-kDa phosphoprotein on SDS-PAGE. Immunoblotting with anti-phosphoserine antibody also establishes the presence of a phosphorylated serine residue in DPMS. To further evaluate the phosphorylation regulation of DPMS catalytic activity, a S141A DPMS mutant is isolated by replacing serine-141 of the consensus sequence (RRVIS¹⁴¹S) with alanine by site-directed mutagenesis. When phosphorylated, the DPMS mutant exhibits 50% less catalytic activity [30]. Nevertheless, replacing serine-142 with alanine has no bearing on the enzyme activity before or after phosphorylation. However, the DPMS activity is undetectable even after phosphorylation in a mutant in which both serine-141 and serine-142 are replaced with alanine (i.e., S141A/S142A) [39].

Impact of DPMS in Biological Functions and in Human Health

(i) Biological Functions

- a. *Viability of yeast:* DPMS is a structural gene in *Saccharomyces cerevisiae* and is essential for its viability. Disruption of DPM1 is lethal and does not allow the organism to grow at non-permissive temperature [62]. It is not only the disruption of DPMS gene but disruption of the phosphorylation site also has serious consequences on cellular proliferation. Mutation at serine-141 (S141A) or both at serine-141 and serine-142 (S141A/S142A) or in combination of serine-141 and alanine-154 (S141A/A154T) makes yeast cells grow slower both at 22°C and at 37°C [39].
- b. *Development of Arabidopsis Plant:* Comparative genomic analysis has identified dol-p-man synthesis in *Arabidopsis* plant (*A. thaliana*) mediated by DPM1, DPM2 and DPM3 assembled into a functional DPMS unit. *Arabidopsis* accumulates dolichols from Dol-14 to Dol-23 [76], and detects dol-(14)p-man in an *in vitro* assay with Dol-14. Loss of function and overexpression studies show

DPM1, in contrast to DPM2 and DPM3 exerts a broad influence on various aspects of *Arabidopsis* development. Root growth, seed architecture, vascular bundle, organization, activation of *unfolded protein response* (*UPR*), induction of chlorosis, and sensitivity to ammonium ion [77] is consistent with the role played by GPI-anchored proteins in yeast cell wall formation [78]. *Arabidopsis*, like other members of the Brassicaceae is sensitive to ammonium ion [77], and dol-p-man is linked to such sensitivity. The loss-of-function mutants of DPM2 and DPM3 retain a wild-type phenotype, but the loss-of-function mutant of DPM1 and DPM1-RNAi exacerbates the hypersensitivity of *Arabidopsis* to ammonium ion. The leaves become strongly chlorotic and the root growth is drastically reduced [77].

(ii) Human Health

- a. *Aging*: Reduced N-linked protein glycosylation correlates with nearly 2-fold reduction of DPMS activity in parotid acinar cells from 22–24 months old rats. There is no change in the K_m for GDP-mannose between young and old rats but the V_{max} is reduced by nearly 2-fold in older animals. Failure to restore the enzymatic activity after supplementation with Dol-P supports reduction of DPMS level during aging [79].
- b. *Thy-1 Lymphoma*: Absence of DPMS activity in class E thy-1 lymphoma cells correlates with the accumulation of $\text{Man}_5\text{GlcNAc}_2\text{-PP-Dol}$ in the ER. Terminal glucosylation of $\text{Man}_5\text{GlcNAc}_2\text{-PP-Dol}$ transfers it to the asparagine residue but the rate is much slower than that of the full length LLO [16]. There is nothing known about processing of a truncated N-glycan in the ER nor could the clinical information in the medical record of the patient confirm if DPMS deficiency is a cause or manifestation of the disease. Similarly, complete absence of DPMS causes a systemic deficiency of GPI anchor biosynthesis as well as O- and C-mannosylation of proteins. In fact, blocking of GPI biosynthesis in DPM2 knockout mice is embryonically lethal [80].
- c. *Congenital Disorder of Glycosylation*: CDG Type 1e patients with the absence of DPMS activity exhibit developmental delay, seizures, hypotonia and dysmorphic function [27, 80–82]. Fibroblasts from these patients also have a shorter life span. Detailed analyses reveal dol-p-man deficiency in humans gradually affects two sub-pathways of protein glycosylation. Mild deficiency due to mutation in DPM3 affects mainly O-mannosylation pathways and leads to α -dystroglycanopathies. On the other hand, severe dol-p-man deficiency perturbs N-glycosylation and GPI pathways and provokes abnormal clotting and epilepsy [82,83]. A single missense mutation in mammalian DPM3 also reduces drastically the catalytic activity of DPM1 and leads to muscular dystrophy [79].
- d. *Angiogenesis*: Angiogenesis is an essential physiological process for growth and development, and a ‘key’ element for tumor progression [84]. Increased bDPMS activity in 8Br-cAMP-treated capillary endothelial cells correlates with the synthesis and turnover of LLO. As a result, N-glycosylation of the endothelial cell marker glycoprotein Factor VIIIc as well as the cell surface N-glycans are

also enhanced following 8Br-cAMP treatment. The consequence is activation of “angiogenic switch” and increased cellular proliferation. It is important to note that bDPMS has a PKA motif (RKIIS¹⁶⁵R) [25] (Figure 4d). The underline mechanism includes: (i) no changes in the expression of Bcl-2 (a pro-angiogenic protein) as well as caspase-3, and -9 (pro-apoptotic proteins) activities in 8Br-cAMP treated cells; and (ii) 4.5–4.8 fold and 1.5–1.6 fold reduced expression of ER chaperones GRP-78/Bip and GRP-94 whereas 1.4–1.6 fold increased expression of cytoplasmic chaperones HSP-70 and HSP-90 in 8Br-cAMP treated cells. HSP-70 binds to an unfolded region of the protein that is rich in hydrophobic residues and blocks protein folding until it has translocated across the membrane [85]. HSP-90, on the other hand is involved in protein folding that is associated with signal transduction [86]. The proposed model is HSP-70 activates the hypoxia-inducing factor 1 (HIF1) and controls expression of eight out of ten enzymes of the glycolytic pathway, whereas HSP-90 binds to activated p53 (i.e., p53p) and translocates the transcription factor to the nucleus to activate gene expression and angiogenesis [87,88].

The relationship between DPMS and angiogenesis strengthens further in capillary endothelial cells overexpressing DPMS. These cells express nearly four times more DPMS protein and ~108% higher DPMS activity. As a result there are increased expression of surface N-glycans with GlcNAc- β -(1,4)-GlcNAc)1,4- β -GlcNAc-NeuAc epitope and increased cellular proliferation [89]. Overexpression of DPMS also accelerates wound healing [89]. DPMS activity however is lost when tunicamycin-treated capillary endothelial cells undergo cell cycle arrest and induction of apoptosis [28]. Silencing of *DPMS* with *shRNA* also reduces the angiogenic potential [29].

Future Direction

Current understanding on the role of glycobiology in growth and development, infection diseases, cancer and CDGs has recognized the central role for DPMS. Partitioning dol-p-man in more than one pathway has raised additional question about the dynamicity of its distribution. Therefore, it is worth addressing the extent of extra- or intra-cellular signaling responsible for such dynamicity. In addition, it is important to quantify how much dol-p-man is withdrawn per unit time from each pool (in case there are multiple-pools) to understand this complex behavior. Successful answering of these questions is expected to help developing next generation therapeutics, i.e., glycotherapy for diseases such as breast cancer.

Our knowledge on DPMS and GDP-mannose (i.e., the substrate) interaction or between DPMS and Dol-P (i.e., the acceptor) interaction is based on theoretical considerations. Therefore, evaluation of DPMS protein structure is of utmost importance. The presence of DAD motif supports metal binding (Mn^{2+} or Mg^{2+}), but elucidation of the protein structure is expected to highlight why the requirement for metal ion changes (i.e., Mn^{2+} v/s Mg^{2+}) with the source of the enzyme such as mammalian v/s yeast. Similarly, X-ray crystallographic analyses of S141A, S142A, and S141A/S142A DPMS mutants from *S. cerevisiae* are equally important to explain how serine-142 partially fulfills the need of serine-141.

The role of two accessory proteins, DPM2 and DPM3 for stabilizing the catalytic component DPM1 in mammalian species and in *C. albicans* and *Arabidopsis* needs clarification. A stretch of 27 hydrophobic amino acids is missing at the C-terminus from DPMS in these species. Also, *E. coli* cannot express recombinant protein when transformed with *DPMS* gene from these species. Fortunately, recombinant bDPMS is fully active when (a) the *E. coli* transformed with the *bDPMS* gene [25]; and (b) the native enzyme is purified from bovine liver by isoelectric focusing (Martinez and Banerjee, unpublished observation).

Hydrophobic residues are important in controlling protein activity by regulating phosphorylation as indicated by dephosphorylation of tyrosine residue in ERK [90,91]. It is therefore reasonable to assume that the presence of highly conserved hydrophobic regions in DPMS may play an intricate role for its function [92,93]. Also, reports indicate that hydrophobic regions of the N- and C-terminus impact protein function [94,95]. bDPMS has hydrophobic regions close to its N- and C-terminus. It would be highly significant to study their roles in ER membrane insertion.

Kinetic behavior of phosphorylated DPMS or after its overexpression in capillary endothelial cells supports enhanced protein N-glycosylation. It is due to increased rate of LLO synthesis and turnover [74,85]. This in fact, has raised a fundamental question about the processing of N-glycans. The number of N-glycosylation site(s) are fixed in an N-linked glycoprotein. Could increased LLO synthesis and turnover dictate enhanced glycoprotein gene transcription and translation, or hold the protein unfolded for a period until all putative N-glycosylation sites are populated?

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DPMS	dolichol phosphate mannose synthase
GPT	N-acetylglucosaminyl 1-phosphate transferase
LLO	lipid-linked oligosaccharide
GPI	glycophosphatidyl anchor
ER	endoplasmic reticulum
PKA	cAMP-dependent protein kinase
PtdEtn	phosphatidylethanolamine

PtdCho	phosphatidylcholine
cAMP	3',5'-cyclic adenosine monophosphate
CDG	congenital disorders of glycosylation

References

1. Kornfeld R, Kornfeld S. Assembly of asparagine-linked oligosaccharides. *Annu Rev Biochem.* 1985; 54:6631–6.
2. Helenius A, Aebi M. Roles of N-linked glycans in the endoplasmic reticulum. *Annu Rev Biochem.* 2004; 73:1019–49. [PubMed: 15189166]
3. Trombetta ES. The contribution of N-glycans and their processing in the endoplasmic reticulum to glycoprotein biosynthesis. *Glycobiology.* 2005; 13:77R–91R.
4. Tanner W, Jung P, Behrens NH. Dolicholmonophosphates: Mannosyl acceptors in a particulate in vitro system of *S. cerevisiae*. *FEBS Lett.* 1971; 16:245–48. [PubMed: 11945952]
5. Waechter CJ, Lennarz WJ. The role of polyprenol-linked sugars in glycoprotein synthesis. *Annu Rev Biochem.* 1976; 45:95–112. [PubMed: 786163]
6. Richards JB, Evans PJ, Hemming FW. Dolichol phosphates as acceptors of mannose from guanosine diphosphate mannose in liver systems. *Biochem J.* 1971; 124:957–59. [PubMed: 5131017]
7. Banerjee DK. Amphomycin inhibits mannosylphosphoryldolichol synthesis by forming a complex with dolichylmonophosphate. *J Biol Chem.* 1989; 264:2024–28. [PubMed: 2464586]
8. Banerjee DK, Diaz AM, Campos TM, Grande C, Kozek WJ, Baksi K. Monoclonal antibody to amphomycin. A tool to study the topography of dolichol monophosphate in the membrane. *Carbohydr Res.* 1971; 236:301–13.
9. Banerjee DK, DaSilva JJ, Bigio B. Mannosylphosphodolichol synthase activity is associated with 32kDa phosphoprotein. *Biosci Rep.* 1999; 19:169–77. [PubMed: 10513894]
10. Lahav M, Chiu TH, Lennarz WJ. Studies on the Biosynthesis of Mannan in *Micrococcus lysodeikticus* II. The enzymatic synthesis of mannosyl-1-phosphoryl-undecaprenol. *J Biol Chem.* 1969; 244:5890–98. [PubMed: 5350943]
11. Rush JS, Waechter CJ. Transmembrane movement of a water-soluble analogue of mannosylphosphoryl dolichol is mediated by an endoplasmic reticulum protein. *J Cell Biol.* 1995; 130:529–36. [PubMed: 7622555]
12. Shidoji Y, De Luca LM. Rat liver microsomes catalyse mannosyl transfer from GDP-D-mannose to retinyl phosphate with high efficiency in the absence of detergents. *Biochem J.* 1981; 200:529–38. [PubMed: 6177313]
13. Vladimir, N., Shibaev, VN., Danilov, LL. Synthesis of intermediates in the dolichol pathway of protein glycosylation. In: Large, DG., Warren, CD., editors. *Glycopeptides and Related Compounds: Synthesis, Analysis, and Applications.* Marcel Dekker Inc; NY, Basel, Hong Kong: 1997. p. 427-504.
14. Xing J, Forsee WT, Lamani E, Maltsev SD, Danilov LL, Shibaev VN, Schutzbach JS, Cheung HC, Jedrzejewski MJ. Investigations of the active site of *Saccharomyces cerevisiae* dolichylphosphate-mannose synthase using fluorescent labeled dolichylphosphate derivatives. *Biochemistry.* 2000; 39:7886–94. [PubMed: 10891068]
15. Sinhoara H, Maruyama T. Evolution of glycoproteins as judged by the frequency of occurrence of the tripeptides Asn-X-Ser and Asn-X-Thr in proteins. *J Molec Evolution.* 1973; 2:117–22.
16. Chapman A, Trowbridge IS, Hyman R, Kornfeld S. Structure of the lipid-linked oligosaccharides that accumulate in class E Thy-1-negative mutant lymphomas. *Cell.* 1979; 17:509–15. [PubMed: 476829]
17. Banerjee DK, Scher MG, Waechter CJ. Amphomycin: effect of the lipopeptide antibiotic on the glycosylation and extraction of dolichyl monophosphate in calf brain membranes. *Biochemistry.* 1981; 20:1561–68. [PubMed: 7225346]
18. Englund PT. The structure and biosynthesis of glycosyl phosphatidylinositol protein anchors. *Annu Rev Biochem.* 1993; 62:121–38. [PubMed: 8352586]

19. Tanner W, Lehle L. Proteins glycosylation in yeast. *Biochim Biophys Acta*. 1987; 906:81–99. [PubMed: 3552049]
20. Many H, Chiba A, Yoshida A, Wang X, Chiba Y, Jigami Y, Margolis RU, Endo T. Demonstration of mammalian protein O-mannosyltransferase activity: coexpression of POMT1 and POMT2 required for enzymatic activity. *Proc Natl Acad Sci USA*. 2004; 101:500–05. [PubMed: 14699049]
21. Doucey M-A, Hess D, Cacan R, Hofsteenge J. Protein C-Mannosylation is enzyme-catalyzed and uses Dolichyl-Phosphate-Mannose as a Precursors. *Molec Biol Cell*. 1998; 9:291–300. [PubMed: 9450955]
22. Helenius J, Ng DT, Marolda CL, Walter P, Valvano MA, Aeby M. Translocation of lipid-linked oligosaccharides across the ER membrane requires Rft1 protein. *Nature*. 2002; 415:447–50. [PubMed: 11807558]
23. Sanyal S, Menon AK. Stereoselective transbilayer translocation of mannosyl phosphoryl dolichol by an endoplasmic reticulum flippase. *Proc Natl Acad Sci USA*. 2010; 107:11289–94. [PubMed: 20534553]
24. Schutzbach JS, Zimmerman JW, Forsee WT. The purification and characterization of recombinant yeast dolichyl-phosphate-mannose synthase. *J Biol Chem*. 1993; 268:24190–96. [PubMed: 8226966]
25. Baksi K, Zhang Z, Banerjee A, Banerjee DK. Cloning and expression of mannosylphospho dolichol synthase from bovine adrenal medullary capillary endothelial cells. *Glycoconj J*. 2009; 26:635–45. [PubMed: 19214747]
26. Jensen JW, Schutzbach JS. Activation of dolichyl-phospho-mannose synthase by phospholipids. *Eur J Biochem*. 1985; 153:41–48. [PubMed: 4065149]
27. Marquardt T, Denecke J. Congenital disorders of glycosylation: review of their molecular basics, clinical presentations and specific therapies. *Eur J Pediatr*. 2003; 162:359–70. [PubMed: 12756558]
28. Banerjee DK. N-glycans in cell survival and death: cross-talk between glycosyltransferases. *Biochim Biophys Acta*. 2012; 1820:1338–46. [PubMed: 22326428]
29. Baksi K, Zhang Z, Banerjee A, Serrano JE, Perez LE, Linares L, Seijo A, Sanchez N, Katiyar U, Banerjee DK. Silencing Mannosylphospho Dolichol Synthase with shRNA Impacts Differentiation of Capillary Endothelial Cells. *FASEB J*. 2016; 30:844.1.
30. Banerjee DK, Carrasquillo EA, Hughey P, Schutzbach JS, Martinez JA, Baksi K. In vitro phosphorylation by cAMP-dependent protein kinase up-regulates recombinant *Saccharomyces cerevisiae* mannosylphospho dolichol synthase. *J Biol Chem*. 2005; 280:4174–81. [PubMed: 15548536]
31. Elbein AD. Inhibitors of the biosynthesis and processing of N-linked oligosaccharide chains. *Annu Rev Biochem*. 1987; 56:497–534. [PubMed: 3304143]
32. Banerjee DK. A recent approach to the study of dolichyl monophosphate topology in the rough endoplasmic reticulum. *Acta Biochim Pol*. 1994; 41:275–80. [PubMed: 7856398]
33. Rokas A, Carroll SB. Frequent and widespread parallel evolution of protein sequences. *Mol Biol Evol*. 2008; 25:1943–53. [PubMed: 18583353]
34. Edrees BM, Athar M, Abduljaleel Z, Al-Allaf FA, Taher MM, Khan W, Bouazzaoui A, Al-Harbi N, Safar R, Al-Edressi H, Alansary K, Anazi A, Altayeb N, Ahmed MA. Functional alterations due to amino acid changes and evolutionary comparative analysis of ARPKD and ADPKD genes. *Genomics Data*. 2016; 10:127–34. (complete). [PubMed: 27843768]
35. Jacquemyn H, Roldán-Ruiz I, Honnay O. Evidence for demographic bottlenecks and limited gene flow leading to low genetic diversity in a rare thistle. *Conserv Genet*. 2010; 11:1979–87.
36. Colussi FA, Taron CH, Mack JC, Orlean P. Human and *Saccharomyces cerevisiae* dolichol phosphate mannose synthases represent two classes of the enzyme, but both function in *Schizosaccharomyces pombe*. *Proc Natl Acad Sci USA*. 1997; 94:7873–78. [PubMed: 9223280]
37. Tempone AJ, Furtado DR, Gimba ER, Oliveira FM, Rumjanek FD. Dolichol phosphate mannose synthase is differentially expressed in male and female worms of *Schistosoma mansoni*. *Comp Biochem Physiol B Biochem Mol Biol*. 2002; 131:465–74. [PubMed: 11959028]

38. Forsee WT, McPherson D, Schutzbach JS. Characterization of recombinant yeast dolichyl mannosyl phosphate synthase and site-directed mutagenesis of its cysteine residues. *Eur J Biochem.* 1997; 244:953–58. [PubMed: 9108271]
39. Carrasquillo, EA. PhD Thesis. The University of Puerto Rico Medical Sciences Campus; San Juan, PR, USA: 2002. Role of cAMP-dependent phosphorylation domain on the functions (in vitro/in vivo) of mannosylphosphodolichol (Dol-P-Man) synthase in *Saccharomyces cerevisiae*.
40. Albright CF, Orlean P, Robbins PW. A 13-amino acid peptide in three yeast glycosyltransferases may be involved in dolichol recognition. *Proc Natl Acad Sci USA.* 1989; 86:7366–69. [PubMed: 2678101]
41. Zimmerman JW, Robbins PW. The hydrophobic domain of dolichyl-phosphate-mannose synthase is not essential for enzyme activity or growth in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 1993; 268:16746–53. [PubMed: 8344954]
42. Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, MR., Appel, RD., Bairoch, A. Protein identification and analysis tools on the ExPASy server. In: Walker, JM., editor. *The Proteomics Protocols Handbook*. Humana Press; Totowa, NJ: 2005. p. 571-607.
43. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *J Mol Biol.* 1982; 157:105–32. [PubMed: 7108955]
44. Werstuck G, Capone JP. Mutational analysis of the herpes simplex virus trans-inducing factor Vmw65. *Gene.* 1989; 75:213–24. [PubMed: 2541050]
45. Coutinho PM, Deleury E, Davies GJ, Henrissat B. An Evolving Hierarchical Family Classification for Glycosyltransferases. *J Mol Biol.* 2003; 328:307–17. [PubMed: 12691742]
46. Lairson LL, Henrissat B, Davies GJ, Withers SG. Glycosyltransferases: Structures, Functions, and Mechanisms. *Annu Rev Biochem.* 2008; 77:521–55. [PubMed: 18518825]
47. Liu J, Mushegian A. Three monophyletic superfamilies account for the majority of the known glycosyltransferases. *Protein Sci.* 2003; 12:1418–31. [PubMed: 12824488]
48. Tarbouriech N, Charnock SJ, Davies GJ. Three-dimensional structures of the Mn and Mg dTDP complexes of the family GT-2 glycosyltransferase SpsA: a comparison with related NDP-sugar glycosyltransferases. *J Mol Biol.* 2001; 314:655–61. [PubMed: 11733986]
49. Ardiccioni C, Clarke OB, Tomasek D, Issa HA, von Alpen DC, Pond HL, Banerjee S, Rajashankar KR, Liu Q, Guan Z, Li C, Kloss B, Bruni R, Kloppmann E, Rost B, Manzini MC, Shapiro L, Mancia F. Structure of the polyisoprenyl-phosphate glycosyltransferase GtrB and insights into the mechanism of catalysis. *Nat Commun.* 2016; 7:10175. [PubMed: 26729507]
50. Igura M, Maita N, Kamishikiryo J, Yamada M, Obita T, Maenaka K, Kohda D. Structure-guided identification of a new catalytic motif of oligosaccharyltransferase. *EMBO J.* 2008; 27:234–43. [PubMed: 18046457]
51. Wiggins CA, Munro S. Activity of the yeast MNN1 alpha-1,3-mannosyltransferase requires a motif conserved in many other families of glycosyltransferases. *Proc Natl Acad Sci USA.* 1998; 95:7945–50. [PubMed: 9653120]
52. Bujnicki JM, Elofsson A, Fischer D, Rychlewski L. Structure prediction meta server. *Bioinformatics.* 2001; 17:750–51. [PubMed: 11524381]
53. Lamani E, Mewbourne RB, Fletcher DS, Maltsev SD, Danilov LL, Veselovsky VV, Lozanova AV, Grigorieva NY, Pinsker OA, Xing J, Forsee WT, Cheung HC, Schutzbach JS, Shibaev VN, Jedrzejewski MJ. Structural studies and mechanism of *Saccharomyces cerevisiae* dolichyl-phosphate-mannose synthase: insights into the initial step of synthesis of dolichyl-phosphate-linked oligosaccharide chains in membranes of endoplasmic reticulum. *Glycobiology.* 2006; 16:666–78. [PubMed: 16549409]
54. Orlean P, Albright C, Robbins PW. Cloning and sequencing of the yeast gene for dolichol phosphate mannose synthase, an essential protein. *J Biol Chem.* 1988; 263:17499–507. [PubMed: 3053713]
55. Taylor SS, Buechler JA, Yonemoto W. cAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. *Annu Rev Biochem.* 1990; 59:971–1005. [PubMed: 2165385]
56. Zimmerman JW, Specht CA, Cazares BX, Robbins PW. The isolation of Dol-P-man synthase from *Ustilago maydis* that functions in *Saccharomyces cerevisiae*. *Yeast.* 1996; 12:765–71. [PubMed: 8813763]

57. Sugiyama E, DeGasperi R, Urakaze M, Chang HM, Thomas LJ, Hyman R, Warren CD, Yeh ETH. Identification of defects in glycosylphosphatidylinositol anchor biosynthesis in the Thy-1 expression mutants. *J Biol Chem.* 1991; 266:12119–22. [PubMed: 1829456]
58. Chapman A, Fujimoto K, Kornfeld S. The primary glycosylation defect in class E Thy-1-negative mutant mouse lymphoma cells is an inability to synthesize dolichol-P-mannose. *J Biol Chem.* 1980; 255:4441–46. [PubMed: 7372584]
59. Camp LA, Chauhan P, Farrar JD, Lehrman MA. Defective mannosylation of glycosylphosphatidylinositol in Lec35 Chinese hamster ovary cells. *J Biol Chem.* 1993; 268:6721–28. [PubMed: 8454644]
60. Singh N, Tartakoff AM. Two different mutants blocked in synthesis of dolichol-phosphoryl-mannose do not add glycopospholipid anchors to membrane proteins: quantitative correction of the phenotype of a CHO cell mutant with tunicamycin. *Mol Cell Biol.* 1991; 11:391–400. [PubMed: 1986234]
61. DeGasperi R, Thomas LJ, Sugiyama E, Chang HM, Beck PJ, Orlean P, Albright C, Waneck G, Sambrook JF, Warren CD, Yeh ETH. Correction of a defect in mammalian GPI anchor biosynthesis by a transfected yeast gene. *Science.* 1990; 250:988–91. [PubMed: 1978413]
62. Beck PJ, Orlean P, Albright C, Robbins PW, Gething MJ, Sambrook JF. The *Saccharomyces cerevisiae* DPM1 gene encoding dolichol-phosphate-mannose synthase is able to complement a glycosylation-defective mammalian cell line. *Mol Cell Biol.* 1990; 10:4612–22. [PubMed: 2201896]
63. Maeda Y, Tanaka S, Hino J, Kangawa K, Kinoshita T. Human dolichol-phosphate-mannose synthase consists of three subunits, DPM1, DPM2 and DPM3. *EMBO J.* 2000; 19:2475–82. [PubMed: 10835346]
64. Tomita S, Inoue N, Maeda Y, Ohishi K, Takeda J, Kinoshita T. A Homologue of *Saccharomyces cerevisiae* Dpm1p is not sufficient for synthesis of dolichol-phosphate-mannose in mammalian cells. *J Biol Chem.* 1998; 273:9249–54. [PubMed: 9535917]
65. Jadid N, Mialoundama AS, Heintz D, Ayoub D, Erhardt M, Mutterer J, Meyer D, Alioua A, Dorselaer AV, Rahier A, Camara B, Bouvier F. Dolichol phosphate mannose synthase1 mediates the biogenesis of isoprenyl-linked glycans and influences development, stress response, and ammonium hypersensitivity in *Arabidopsis*. *The Plant Cell.* 2011; 23:1985–2005. [PubMed: 21558543]
66. Mora-Montes HM, Ponce-Noyola P, Villagómez-Castro JC, Gow NA, Flores-Carreón A, López-Romero E. Protein glycosylation in *Candida*. *Future Microbiol.* 2009; 4:1167–83. [PubMed: 19895219]
67. Maeda Y, Tomita S, Watanabe R, Ohishi K, Kinoshita T. DPM2 regulates biosynthesis of dolichol phosphate-mannose in mammalian cells: correct subcellular localization and stabilization of DPM1, and binding of dolichol phosphate. *EMBO J.* 1998; 17:4920–29. [PubMed: 9724629]
68. Kruszewska JS, Saloheimo M, Migdalski A, Orlean P, Penttilä M, Palamarczyk G. Dolichol phosphate mannose synthase from the filamentous fungus *Trichoderma reesei* belongs to the human and *Schizosaccharomyces pombe* class of the enzyme. *Glycobiology.* 2000; 10:983–91. [PubMed: 11030744]
69. Juchimiuk M, Kruszewska J, Palamarczyk G. Dolichol phosphate mannose synthase from the pathogenic yeast *Candida albicans* is a multimeric enzyme. *Biochim Biophys Acta.* 2015; 1850:2265–75. [PubMed: 26299246]
70. Banerjee DK, Kousvelari EE, Baum BJ. cAMP-mediated protein phosphorylation of microsomal membranes increases mannosylphospho dolichol synthase activity. *Proc Natl Acad Sci USA.* 1987; 84:6389–93. [PubMed: 2819874]
71. Banerjee DK. Microenvironment of endothelial cell growth and regulation of protein N-glycosylation. *Indian J Biochem Biophys.* 1988; 25:8–13. [PubMed: 3141265]
72. Vander Heiden MG, Cantly LC, Thompson CB. Undersatnding the Warburg effect: the metabolic requirements of cell proliferation. *Science.* 2009; 324:1029–33. [PubMed: 19460998]
73. Grant SR, Kousvelari EE, Banerjee DK, Baum BJ. β -Adrenergic stimulation alters oligosaccharide pyrophosphoryl dolichol metabolism in rat parotid acinar cells. *Biochem J.* 1985; 231:431–38. [PubMed: 4062905]

74. Banerjee DK, Aponte E, DaSilva JJ. Low expression of lipid-linked oligosaccharide due to a functionally altered Dol-P-Man synthase reduces protein glycosylation in cAMP-dependent protein kinase deficient Chinese hamster ovary cells. *Glycoconj J*. 2004; 21:479–86. [PubMed: 15750789]
75. Banerjee DK. Requirement of protein kinase type I for cAMP-mediated up-regulation of lipid-linked oligosaccharide for asparagine-linked protein glycosylation. *Cell Mol Biol (Noisy-le-grand)*. 2007; 53:55–63. [PubMed: 17531150]
76. Jozwiak AP, Skorupinska-Tudek K, Maluszynska J, Kania M, Danikiewicz W, Swiezewska E. Polyisoprenoid alcohols are accumulated in Arabidopsis roots. *Chem Phys Lip*. 2009; 160:S47.
77. Britto DT, Kronzucker HJ. NH_4^+ toxicity in higher plants: A critical review. *J Plant Physiol*. 2002; 159:567–84.
78. Gillmore CS, Lukowitz W, Brininostool G, Sedbrook JC, Hamann T, Poindexter P, Soerville C. Glycophosphatidyl-inositol-anchored proteins are required for cell wall synthesis and morphogenesis in Arabidopsis. *Plant Cell*. 2005; 17:1128–40. [PubMed: 15772281]
79. Kousvelari EE, Banerjee DK, Murty L, Baum BJ. N-Linked protein glycosylation in the rat parotid gland during aging. *Mech Aging and Dev*. 1988; 42:173–81.
80. Nozaki M, Ohishi K, Yamada N, Kinoshita T, Nagy A, Takeda J. Developmental abnormalities of glycophosphatidylinositol-anchor deficient embryos revealed by Crc/loxP system. *Lab Invest*. 1999; 79:293–99. [PubMed: 10092065]
81. Kim S, Westphal V, Srikrishna G, Mehta DP, Peterson S, Filano J, Karnes PS, Patterson MC, Freeze HH. Dolichol phosphate mannose synthase (DPM1) mutations define congenital disorder of glycosylation 1c (CDG-1c). *J Clin Invest*. 2000; 105:191–98. [PubMed: 10642597]
82. Haeuptle MA, Hennot T. Congenital disorders of glycosylation: an update on defects affecting the biosynthesis of dolichol-linked oligosaccharides. *Hum Mutat*. 2009; 30:1628–41. [PubMed: 19862844]
83. Lefeber DJ, Schönberger J, Morava E, Guillard M, Huyben KM, Verrijp K, Grafakou O, Evangelio A, Preijers FW, Manta P, Yildiz J, Grünewald S, Spilioti M, van den Elzen C, Klein D, Hess D, Ashida H, Hofsteenge J, Maeda Y, van den Heuvel L, Lammens M, Lehle L, Wevers RA. Deficiency of Dol-P-Man synthase subunit DPM3 bridges the congenital disorders of glycosylation with the dystroglycanopathies. *Am J Hum Genet*. 2009; 85:76–86. [PubMed: 19576565]
84. Endo T. Glycobiology of α -dystroglycan and muscular dystrophy. *J Biochem*. 2015; 157:1–12. [PubMed: 25381372]
85. Banerjee, DK., Martinez, JA., Baksi, K. Significance of protein N-glycosylation in breast tumor angiogenesis. In: Maragoudakis, ME., Papadimitriou, E., editors. *Angiogenesis: Basic Science and Clinical Applications*. Transworld Research Network; Trivandrum, Kerala: 2007. p. 281-302.
86. Martinez JA, Tavarez JJ, Oliveira CM, Banerjee DK. Potentiation of angiogenic switch in capillary endothelial cells by cAMP: A cross-talk between up-regulated LLO biosynthesis and the HSP-70 expression. *Glycoconj J*. 2006; 23:209–20. [PubMed: 16691504]
87. Pearl LH, Prodromon C. Structure and in vivo function of Hsp90. *Curr Opin Struct Biol*. 2000; 10:46–51. [PubMed: 10679459]
88. Bukau B, Horwich AL. The Hsp70 and Hsp60 chaperone machines. *Cell*. 1998; 92:351–66. [PubMed: 9476895]
89. Zhang Z, Banerjee A, Baksi K, Banerjee DK. Mannosylphospho dolichol synthase overexpression supports angiogenesis. *Biocatal Biotransformation*. 2010; 28:90–98. [PubMed: 20640223]
90. Li R, Xie DD, Dong JH, Li H, Li KS, Su J, Chen LZ, Xu YF, Wang HM, Gong Z, Cui GY, Yu X, Wang K, Yao W, Xin T, Li MY, Xiao KH, An X, Huo Y, Xu Z, Sun JP, Pang Q. Molecular mechanism of ERK dephosphorylation by striatal-enriched protein tyrosine phosphatase. *J Neurochem*. 2014; 128:315–29. [PubMed: 24117863]
91. Samaddar S, Dutta A, Sinharoy S, Paul A, Bhattacharya A, Saha S, Chien KY, Goshe MB, DasGupta M. Autophosphorylation of gatekeeper tyrosine by symbiosis receptor kinase. *FEBS Lett*. 2013; 587:2972–79. [PubMed: 23962520]

92. Woe PC, Blount P, Kung C. Functional and structural conservation in the mechanosensitive channel MscL implicates elements crucial for mechanosensation. *Mol Microbiol.* 1998; 28:583–92. [PubMed: 9632260]
93. Yang Y, Igumenova TI. The C-Terminal V5 Domain of Protein Kinase Ca Is Intrinsically Disordered, with Propensity to Associate with a Membrane Mimetic. *PLoS One.* 2013; 8:e65699. [PubMed: 23762412]
94. García-Alai MM, Alonso LG, De Prat-Gay G. The N-terminal module of HPV16 E7 is an Intrinsically disordered domain that confers conformational and recognition plasticity to the oncoprotein. *Biochemistry.* 2007; 46:10405–12. [PubMed: 17715947]
95. Higgins DG, Thompson JD, Gibson TJ. Using CLUSTAL for multiple sequence alignments. *Methods Enzymol.* 1996; 266:383–402. [PubMed: 8743695]

Figure 1. ER Localization of Dol-P and DPMS

(a) *Detection of Dol-P*: Fixed capillary endothelial cells were incubated with amphomycin in a buffer containing Ca^{2+} and stained with anti-amphomycin mouse monoclonal antibody [8] followed by FITC-conjugated rabbit anti-mouse IgG secondary antibody before collecting images in a fluorescence microscope. (b) *Detection of DPMS*: Fixed capillary endothelial cells were incubated with anti-DPMS rabbit polyclonal antibody [9] followed by Rhodamin-conjugated goat anti-rabbit IgG before collecting images in a fluorescence microscope. (c) *Detection of Dol-P and DPMS*: Fixed capillary endothelial cells were incubated with amphomycin in a buffer containing Ca^{2+} followed by anti-amphomycin antibody (mouse monoclonal) and then with anti-DPMS antibody (rabbit polyclonal) as above. This followed staining with FITC-conjugated rabbit anti-mouse IgG and Rhodamin-conjugated goat anti-rabbit IgG antibodies prior to collecting images in a fluorescence microscope. (d) *Detection of Dol-P by electron microscopy*: Thin (60nm–100nm) sections of capillary endothelial cells on copper grids treated with amphomycin in a buffer containing Ca^{2+} followed by anti-amphomycin antibody. The immune complexes then treated with 20nm gold conjugated-rabbit anti-mouse IgG before collecting images in a Phillips –200 electron microscope at a magnification of 20,000.

Figure 2. Unrooted phylogenetic tree with branch lengths for DPM1 sequences from 39 species
 The sequence sources of DPM1 from different species are from Table 1. After alignment the dendrogram was generated using the CLUSTAL W program [43] on the GenomeNet server (<http://www.genome.jp/tools/clustalw/>). GenomeNet is a Japanese network of database and computational services for genome research operated by the Kyoto University is Bioinformatics Center.

Figure 3. Hydropathy profile of DPMS from four different species

For scanning the polypeptide sequences of DPMS, 19 amino acids faulted as one window and hydropathy index was calculated based on one window. (a) Bovine capillary endothelial cells; (b) human; (c) *S. cerevisiae*; and (d) *A. thaliana*. The hydropathic character of DPMS is computed under ExpASY Bioinformatics Resource Portal (<http://web.expasy.org/portscale/>) [42] on the logarithm established [43] with a window of 19.

<i>S.cerevisiae</i>	MDAD----	LQHPPETVPKLFESLH--DHAFTLGTRYAPGVGIDKDWPMYRRVVISSTARMMARP	150
<i>C.glabrata</i>	MDAD----	LQHPPEVPSLFDLSR--KHPFVLGTRYAPGVGIDKDWPLHRRVISTARMMARP	150
<i>U.maydis</i>	MDAD----	LQHPPEAVPSLLALNG-QKSFVLGTRYGVGVSMKDWPLHRRRIISSGARMLARP	179
<i>T.brucei</i>	MDAD----	LQHPKTPVCLLRALKPGVEFVCGTRYGAGVEIDKDWPLHRRFISWGARLLARP	154
Bovine	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFITQI	174
Pig	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFITQI	173
<i>G.panda</i>	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFITQI	174
Dog	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFLTQI	174
Horse	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFITQI	174
Human	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFLTQI	174
<i>S.orangutan</i>	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFLTQI	229
Chimpanzee	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFLTQI	174
<i>R.monkey</i>	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFLTQI	229
<i>W.marmoset</i>	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFLTQI	174
<i>N.rat</i>	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFITQI	174
<i>H.mouse</i>	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFITQI	174
<i>C.hamster</i>	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFITQI	180
<i>R.J.fowl</i>	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGDGGVYG-WDLRKLISRANFITQV	156
<i>P.guttata</i>	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKLISRANFLTQV	156
<i>G.opossum</i>	MDAD----	LSHHPKFIPEFIRKQKEGNFDIVSGTRYKGNNGGVYG-WDLRKKIISRANFLTQI	178
<i>W.frog</i>	MDAD----	LSHHPKFIPEFIRKQKEGSYDIVSGTRYSGNGGVYG-WDLRKLISRANFVTQV	161
<i>A.frog</i>	MDAD----	LSHHPKFIPEFIRKQKEGSYDIVSGTRYAGNGGVYG-WDLRKLISRANFITQV	161
Bullfrog	MDAD----	LSHHPKFIPEFIRKQKEGDFDVSGTRYIGNGGVYG-WDLRKLISRANFVLSQV	162
Zebrafish	MDAD----	LSHHPKFIPEFIRKQKEGGYDLVSGTRYRGDGGVYG-WDLRKLISRANFVTQV	164
<i>R.smelt</i>	MDAD----	LSHHPKFIPEFIRKQKEGGYDVVAGTRYRGDGGVYG-WDLRKLISRANFVTQV	164
<i>C.intestinalis</i>	MDAD----	LSHHPKFIPEFIRKQKEELDCDIVTGTTRYEGEGGVYG-WDLRKLISRANFVTQV	155
<i>J.wasp</i>	MDAD----	LSHHPKFIPEFIRKQKEELDCDIVTGTTRYAGNGGVYG-WDLRKLISRANFITQI	165
<i>H.B.louse</i>	MDAD----	LSHHPKFIPEFIRKQKEELDCDIVTGTTRYKGNNGGVYG-WDLRKLISRANFLTQF	167
<i>R.beetle</i>	MDAD----	LSHHPKFIPEFIRKQKSKDYDVVSGTRYVSGGGVYG-WDLRKLISRANFLTQF	159
<i>P.aphid</i>	MDAD----	LSHHPKFIPEFIRKQKAEQDYDVVTSRYIGNGGVYG-WDLRKLISRANFVSQL	152
<i>D.melanogaster</i>	MDAD----	LSHHPKFIPEFIRKQKEGNYDIVSGTRYAGNGGVYG-WDLRKLISRANFVSQL	162
<i>B.manjavacas</i>	MDAD----	LSHHPKFIPEFIRKQKEQDYDIVTGTTRYAGDGGVYG-WDLRKLISRANFVSQL	151
<i>C.Albicans</i>	MDAD----	FSHHPKFIPEFIRKQKSDYDIVTGTTRYAGDGGVYG-WDLRKLISRANFLASV	153
<i>C.dubliniensis</i>	MDAD----	FSHHPKFIPEFIRKQKSDYDIVTGTTRYAGDGGVYG-WDLRKLISRANFLASV	153
<i>L.elongisporus</i>	MDAD----	FSHHPKFIPEFIRKQKTEDYDIVTGTTRYAGNGGVYG-WDLRKLISRANFLATV	153
<i>S.pombe</i>	MDAD----	FSHHPKFIPEFIRKQKEHNYDIVLGTTRYAKDGGVYG-WDLRKLISRANLLAST	151
<i>C.briggsae</i>	MDAD----	LSHHPKFIPEFIRKQKLETNASIVTGTTRYVKGGGVYG-WDLRKLISRANFLAQF	155
<i>A.thaliana</i>	MDAD----	LSHHPKFIPEFIRKQKLETNASIVTGTTRYVKGGGVYG-WDLRKLISRANFLAQF	160
<i>C.parapsilosis</i>	ADADGATQFSDVAKLIDYLGSYNPEPAIAGSRAHMVNTDAVVK-RSLIRNFMVGLHTLVFI	221	

Figure 4d. Alignment of amino acid sequence of DPMS

The alignment of DPMS sequences from all 39 species used the CLUSTAL W program [43] (<http://www.genome.jp/tools/claustalw/>). The PKA-motif is highlighted in blue and is present in all DPMS sequences except in *C. parapsilosis*. In addition, the sequences from *S. cerevisiae*, *G. glabrata* and *U. maydis* each has a second serine residue following the primary phosphorylation site and is absent from the rest. The significance of this discrepancy may be evolution derived. The experimental results suggest that serine-142 in *S. cerevisiae* could partially fulfil the need of serine-141 if mutated [30]. The metal (Mn^{2+} or Mg^{2+}) binding DAD motif is present in DPMS sequence from all 39 species and highlighted in magenta. The complete alignment of all DPMS sequences is in the Supplement Figure sf4.

Table 1

Source of collected amino acid sequences for species

Abbreviations	Full Name	Accession Number	Sources of Database
A. frog	African clawed frog	NM_001095996	NCBI *
<i>A. thaliana</i>	<i>Arabidopsis thaliana</i>	NP_564118	NCBI
Bovine	Bovine (cloned in our lab)	GQ367549	NCBI
Bullfrog	American Bullfrog	BT081832	NCBI
<i>B. manjavacas</i>	<i>Brachionus manjavacas</i>	FJ829247	NCBI
<i>C. briggsae</i>	<i>Caenorhabditis briggsae</i>	AAC98796	NCBI
<i>C. intestinalis</i>	<i>Ciona intestinalis</i>	XM_002131455	NCBI
Chimpanzee	Chimpanzee	XM_001168349	NCBI
C. hamster	Chinese hamster	AF121895	NCBI
<i>C. albicans</i>	<i>Candida albicans</i>	C1_08010W_A	CGD **
<i>C. dubliniensis</i>	<i>Candida dubliniensis</i>	Cd36_07530	CGD
<i>C. glabrata</i>	<i>Candida glabrata</i>	CAGL0G09955g	CGD
<i>C. parapsilosis</i>	<i>Candida parapsilosis</i>	CPAR2_801230	CGD
Dog	Dog	XM_534456	NCBI
<i>D. melanogaster</i>	<i>Drosophila melanogaster</i>	ACX31360	NCBI
Horse	Horse	XM_001488051	NCBI
G. panda	Giant panda	XM_002912977	NCBI
<i>M. domestica</i>	<i>Monodelphis domestica</i>	XM_001378490	NCBI
Human	Human	D86198	NCBI
H. B. louse	Human body louse	XM_002429856	NCBI
H. mouse	House mouse	NM_010072	NCBI
J. wasp	Jewel wasp	XM_001599275	NCBI
<i>L. elongisporus</i>	<i>Lodderomyces elongisporus</i>	XP_001528001	NCBI
N. rat	Norway rat	NM_001106544	NCBI
<i>P. guttata</i>	<i>Poephila guttata</i>	DQ214713	NCBI
P. aphid	Pea aphid	XM_001952186	NCBI
R. J. fowl	Red jungle fowl	XM_417511	NCBI
R. smelt	Rainbow smelt	BT075095	NCBI
R. beetle	Red flour beetle	XM_968905	NsCBI
R. monkey	Rhesus monkey	XM_001094366	NCBI
<i>S. pombe</i>	<i>Schizosaccharomyces pombe</i>	NP_594017	NCBI
<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>	NP_015509	NCBI
Pig	Pig	AB529457	NCBI
S. orangutan	Sumatran orangutan	XM_002830419	NCBI
<i>T. brucei</i>	<i>Trypanosoma brucei</i>	AJ86675	NCBI
<i>U. maydis</i>	<i>Ustilago maydis</i>	U54797	NCBI

Abbreviations	Full Name	Accession Number	Sources of Database
W. frog	Western clawed frog	NM_001011407	NCBI
W. marmoset	White-tufted-ear marmoset	XM_002747659	NCBI
Zebrafish	Zebrafish	NM_001003596	NCBI

* NCBI: The National Center for Biotechnology Information;

** CGD: Candida Genome Database

Table 2

Proposed Classification of Dol-P-Man Synthase based on sequence information

Class I	Class II
<i>Saccharomyces cerevisiae</i> , <i>Ustilago maydis</i> , <i>Trypanosoma brucei</i> , <i>Candida glabrata</i>	<i>Schizosaccharomyces pombe</i> , <i>Caenorhabditis briggsiae</i> , Human, <i>Drosophila melanogaster</i> , <i>Trichoderma reesei</i> , A. Frog, <i>A. Thaliana</i> , Mouse, Bovine, Bullfrog, <i>Brachionus manjavacas</i> , <i>Ciona intestinalis</i> , Chimpanzee, Chinese hamster, <i>Candida albicans</i> , <i>Candida dubliniensis</i> , <i>Candida parapsilosis</i> , Dog, Horse, Giant panda, <i>Monodelphis domestica</i> , Human body louse, House mouse, Jewel wasp, <i>Lodderomyces elongisporus</i> , Norway rat, <i>Poephila guttata</i> , Pea aphid, Red jungle fowl, Rainbow smelt, Red flour beetle, Rhesus monkey, Pig, Sumatran orangutan, Western clawed frog, White-tufted-ear marmoset, Zebrafish

Type I enzymes share 60–70% amino acid identity and the presence of a stretch of hydrophobic amino acids at the C-terminus [36]; Type II enzymes exhibit only 30–40% amino acid identity with Type I enzymes and lack the C-terminus hydrophobic domain [36].