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REVIEW

The role of the lipid matrix in the biosynthesis of dolichyl-linked oligosaccharides

John S. Schutzbach*

The University of Alabama at Birmingham, Departments of Microbiology and of Biochemistry and Molecular Genetics, University Station, Birmingham, AL 35294-0019, USA

The enzymes in the dolichol pathway are membrane-proteins that utilize a combination of hydrophilic and extremely hydrophobic substrates. The enzymes in this pathway that have been purified and characterized to any extent have either been shown to be stabilized by mixed phospholipid/detergent micelles, or else require a lipid matrix for catalytic activity. Further understanding of the mechanisms of these essential enzymes may require developing methods for the reconstitution of the glycosyltransferases and their hydrophobic substrates in appropriate lipid matrices.

***Keywords*: dolichol, glycosyltransferases, hexagonal phase, membranes, phospholipids**

***Abbreviations*: CHO, Chinese hamster ovary; Dol, dolichol; DAG, diacylglycerol; DOPC, dioleolylphosphatidylcholine; DOPE, dioleolyphosphatidylethanolamine; ER, endoplasmic reticulum; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol**

The biosynthesis of dolichyl-linked oligosaccharides[‡]

The pathway for biosynthesis of the N-linked oligosaccharides found on eucaryotic glycoproteins is initiated by a concerted series of reactions catalysed by glycosyltransferases found in membranes of the ER [1–3]. Salient features of this pathway include the biosynthesis of dolichyl-linked mannose and glucose intermediates, as well as the formation of a dolichyl-linked oligosaccharide having the composition $Glc_3Man_9GlcNAc_2$ (Figure 1). The GlcNAc residues and five of the mannosyl[‡] residues in this oligosaccharide are derived by glycosyl transfer from nucleotide sugars on the cytoplasmic side of the ER membrane (Figure 2). Following translocation of this heptasaccharide across the ER membrane, four mannosyl and three glucosyl residues are transferred from Dol-linked intermediates on the lumenal side of the ER membrane [4]. The oligosaccharide is then transferred to appropriate asparagine residues in nascent polypeptide acceptors on the lumenal side of the microsomal membrane. The presence of completed oligosaccharide chains on the lumenal side of the ER membrane requires the translocation of hydrophilic carbohydrates across the hydrophobic barrier of the membrane. The lipophilic dolichyl moiety functions to anchor the activated sugar residues, as well as the growing oligosaccharide chains, in membranes of the ER, and the long chain polyisoprene is probably also involved in the translocation of the activated carbohydrates across membranes.

Biosynthesis of complete oligosaccharide-lipid has been demonstrated in crude extracts of many tissues. It has generally been assumed that at least 16 different glycosyltransferases are involved in the synthesis of the tetradecasaccharide-lipid intermediate, but it is important to note that only a few enzymes in the dolichol pathway have been purified to any extent. Genes encoding eight of the enzymes in the dolichol cycle have been identified [3, 5], and five of these have been cloned and sequenced in recent years. It is conceivable, however, that some enzymes in this pathway catalyze the formation of more than one glycosidic-linkage. Proposing such a model would have been untenable as recently as several years ago because of the generally accepted dogma that the formation of each glycosidic linkage in a polysaccharide was catalysed by a single enzyme, 'the one linkage-one enzyme hypothesis' [6]. Inference from biosynthetic pathways for other complex carbohydrates now makes such a proposal less precarious. Enzymes involved in both eucaryotic and procaryotic polysaccharide biosynthesis, translocation, and/or modification have recently been

* Tel: 205 934 5054; Fax: 205 975 7088.

‡ At the present time there are no trivial or convenient names to apply to the enzymes in the dolichol cycle, and for convenience, the mannosyltransferases will be numbered according to the relative order by which mannosyl units are added to the growing oligosaccharide chain *in vivo* [76].

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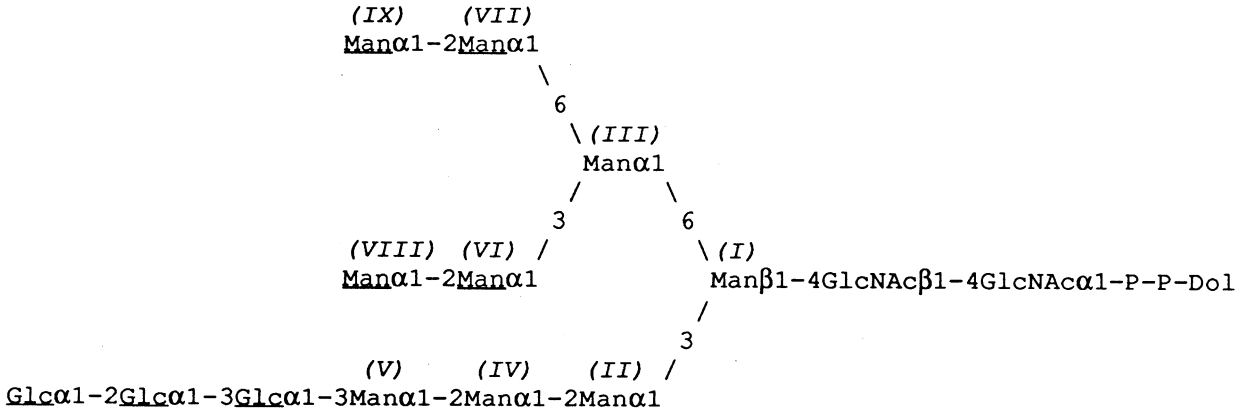

Figure 1. Structure of the oligosaccharide-lipid intermediate. At the present time there are no trivial or convenient names to apply to the enzymes involved in synthesis of this oligosaccharide, and for convenience, the mannosyltransferases are numbered according to the relative order by which mannosyl units are added to the growing oligosaccharide chain *in vivo* [76]. The underlined residues are derived from Dol-P-Man or Dol-P-Glc.

shown to have dual catalytic activities [7–12]. Current information suggests that some of the reactions in the dolichol cycle are closely linked, and it is also possible that the enzymes exist as a multi-enzyme system or complex, although the structural organization of enzymes in this biosynthetic system has not been determined. Understanding the interactions of the individual proteins, or the multi-enzyme system with membranes, as well as mechanisms involved in the translocation of the sugars and oligosaccharides across the ER membrane present formidable scientific challenges, but the resolution of these problems will provide significant new insight about glycosyltransferase function, as well as membrane structure and function.

Struck and Lennarz [13] stated in their 1980 review, "In fact, to fully document the many enzymatic reactions involved in the overall synthesis of the oligosaccharide-lipid . . . , it will be necessary to isolated solubilized, purified forms of each of the enzymes." Most, if not all, of the enzymes in the dolichol cycle are integral membrane proteins. The relatively low abundance and the reported 'apparent' instability of these membrane-bound enzymes during purification, as well as the lack of substrate quantities of appropriate glycosyl-acceptors, has for the most part impeded the purification and detailed characterization of these proteins. Since most proteins are not inherently unstable, the reported lability of purified glycosyltransferase preparations was likely due to the removal of critical protein subunits and/or non-protein cofactors. It is probable that lipids are stripped from the proteins during purification in the presence of detergents, accounting for the apparent instability of some of the enzymes. Thus further characterization of catalytically active glycosyltransferases may require developing methods for the reconstitution of the enzymes in appropriate lipid matrices. The objectives stated in the 1980 review are still relevant, and the tools of molecular biology are now available to facilitate characterization of the enzymes and their properties. This review will primarily focus on studies related to those enzymes in the pathway that have been at least partially purified and characterized.

UDP-GlcNAc: dolichyl phosphate GlcNAc-1-P transferase [EC 2.7.8.15]

The first committed enzyme in the dolichol cycle is the GlcNAc-1-P transferase which catalyses the reversible formation of Dol-P-P-GlcNAc by glycosyl-P transfer from the water soluble donor UDP-GlcNAc to the hydrophobic acceptor Dol-P (Figure 2, Reaction 1). Studies on the topography[§] of the enzyme suggest that the active site faces the cytoplasmic side of the ER membrane [14, 15]. The enzyme has been purified from mammary gland [16] and also partially purified from pig aorta microsomes [17]. Crude microsomal and purified preparations of the GlcNAc-1-P transferase have been shown to be specifically activated by Dol-P-Man. This activation has been proposed as a possible allosteric regulatory mechanism for the enzyme [15] and suggests the presence of a second binding site for a Dol-linked sugar.

The acceptor specificity of the enzyme for the polyprenol-P substrate has been examined in both crude microsomal preparations of the transferase and in mixed phospholipid/detergent micelles. In summary: the enzyme has a preference for longer chain dolichyl-phosphates rather than shorter chain dolichyl-phosphates, a preference for dolichyl-phosphates over the same length totally unsaturated polyprenol-phosphates, and a preference for the S-configuration of dolichol over the R-form [18–21]. These

[§] The word topography will be used in the context as defined by Jennings [77] to include the number and orientation of the membrane-spanning segments relative to the sides of the bilayer as well as the shape of the protein surfaces.

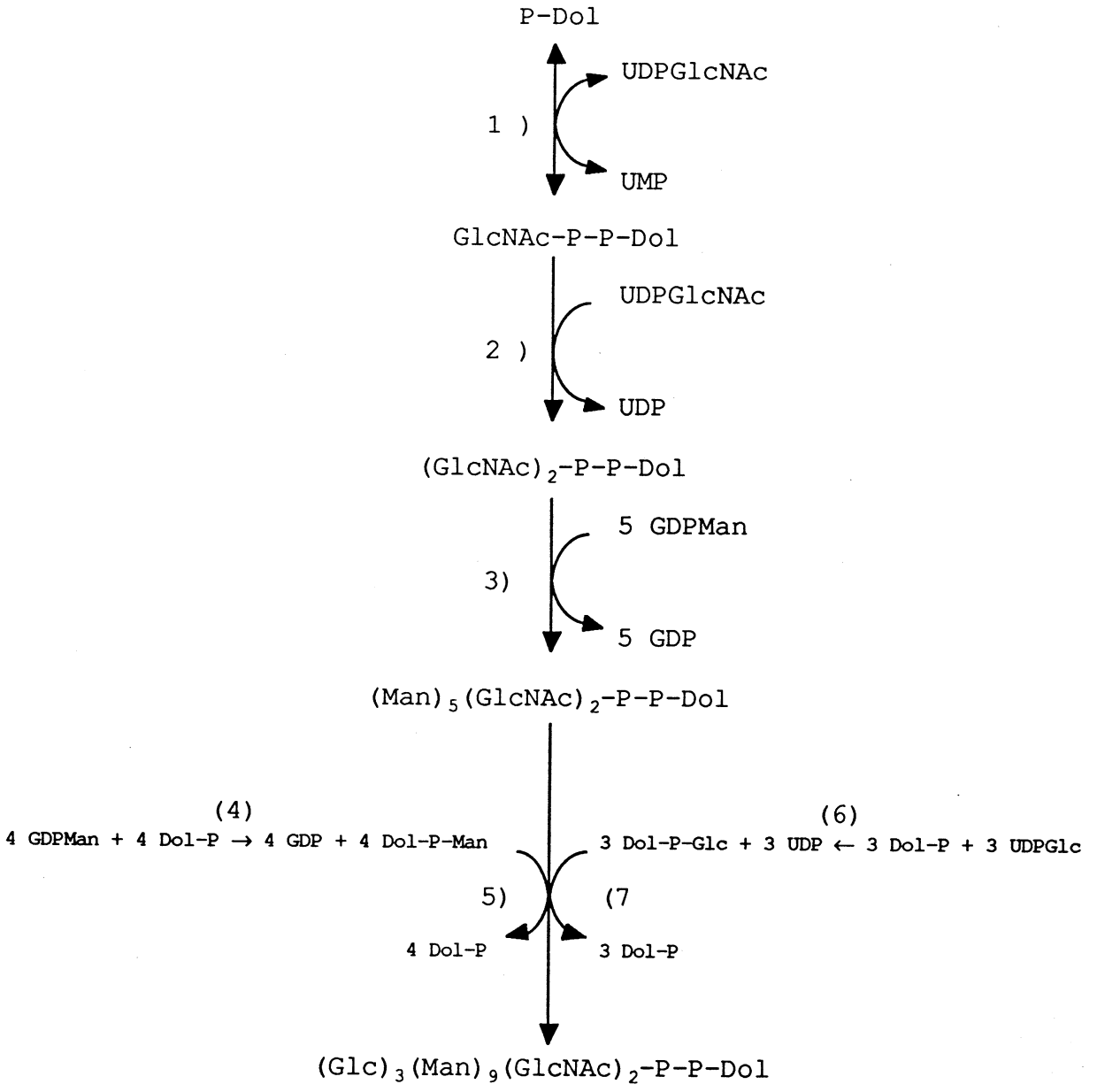

Figure 2. The pathway for the biosynthesis if the dolichyl-linked oligosacchaaride intermediate of N-linked glycoprotein synthesis. Steps 1 to 3 and reaction 4 occur on the cytoplasmic side of the ER membrane. Steps 5 and 7, and probably 6, occur on the lumenal side of the ER membrane. Some of these steps involve the action of more than one enzyme as indicated by the number of sugar residues transferred.

results indicate that the specificity of the enzyme for the polyprenol-P substrates is high, not absolute.

The structural gene for the transferase (ALG7) has been cloned from yeast [22], from CHO cells [23, 24], and from the mouse [25]. ALG7 encodes a protein with multiple potential membrane-spanning domains and two potential glycosylation sites [5]. The cDNA encoding the CHO transferase predicts a protein of 408 amino acids including 10 potential membrane spanning hydrophobic segments. The mammalian enzyme is 41–43% identical in amino acid sequence to the protein encoded by ALG7, and several of the hydrophobic domains of the mammalian enzyme have 60% or greater identity with the yeast enzyme. The predicted molecular mass of the enzyme is 46 kDa which corresponds quite well with the calculated mass of the purified protein(s). The gene for mouse GlcNAc-1-P transferase was shown to reside on mouse chromosome 17 and encodes a protein 96% identical at the amino acid level to the CHO enzyme [25].

During purification, the pig aorta enzyme was separated from an unidentified heat-stable factor that stimulated transferase activity about five-fold when added back to

reaction mixtures containing partially purified enzyme. The activating factor was found to be non-dialysable, resistant to mild acid hydrolysis, not extractable into lipid solvents, and was resistant to hydrolysis with Pronase or ribonuclease. The presence of a similar heat-stable stimulatory factor was not reported for the mammary gland enzyme. Phospholipase A_2 treatment of rat lung microsomal preparations was shown to result in a time-dependent loss of 65 to 75% of the GlcNAc-1-P transferase activity [26] and deoxycholate delipidated protein fractions were also shown to be devoid of transferase activity [27]. In both studies, activity could be restored by the addition of PG, but maximal activity with detergent delipidated membranes was obtained with PC/PG (1 : 1) mixtures. GlcNAc-1-P transferase preparations solubilized in nonionic detergents were reported to be 'apparently' quite unstable losing most activity in 12 h. The solubilized preparations could be stabilized by the inclusion of 20% glycerol, 20 mg PG ml^{-1}, 5 mM Dol-P and 2.5 mM UDP-GlcNAc in the purification buffers. The purified enzyme preparations, however, were still reported to be unstable losing 80% activity in 24 h [16]. This multiple membrane spanning protein must therefore retain the ability to recognize the two disparate substrates, one hydrophilic the other lipophilic, while sheathed in a mixed micelle, but the results also suggest that maximal catalytic activity and stability of this integral membrane glycosyltransferase may require reconstitution with appropriate phospholipids.

A highly conserved sequence of 11 to 13 amino acids was identified in a number of yeast enzymes that utilize dolichols as substrates, and this sequence was originally proposed as a potential dolichol recognition domain [28]. The mammalian GlcNAc-1-P transferase was found to contain two copies of this conserved domain, possibly accounting for the recognition of both the Dol-P acceptor and the allosteric activator Dol-P-Man. Disruption of the potential Dol recognition sequences in the transferase, either by scramble mutation or by conservative (triple alanine) amino acid replacements, was shown to result in an inactive enzyme [29]. Although this result was initially interpreted to support a role for the conserved sequences as dolichol recognition domains, recent studies that will be described later do not support this conclusion [30, 31]. Since all of the enzymes shown to contain this sequence are ER proteins, it is possible that this conserved sequence is a recognition signal, or retention marker for these proteins. The *N*-acetylglucosaminyltransferase [EC 2.4.1.141] catalysing reaction 2 (Fig. 2) has not been purified or characterized.

Mannosyltransferase I [EC 2.4.1.142]

Mannosyltransferase I catalyses mannosyltransfer from GDP-Man to $(GlcNAc)_2$-P-P-Dol with the formation of βMan-4-$(GlcNAc)_2$-P-P-Dol. The enzyme was solubilized with Nonidet P-40 from pig aorta [32], but properties of the enzyme were not reported. Mannosyltransferase I has also been solubilized from cultured soybean cell microsomes and purified approximately 700-fold [33]. The plant enzyme was shown to require either detergent or phospholipid for maximum activity, with PC giving maximal activity. The gene encoding this essential enzyme activity (*ALG1*) was cloned by complementation of a temperature sensitive yeast mutant that had been isolated by a tritium suicide procedure [34, 35]. The predicted protein sequence contains 449 amino acids with a mass of 51.9 kDa. The amino terminus includes a domain of 27 hydrophobic residues, residues 8–34, predicted to form a signal sequence and membrane-spanning domain. This domain also contains the conserved 13-amino acid peptide proposed as the putative dolichol recognition domain. Mutant constructs of *ALG1* have been prepared that contain a 34 amino acid deletion from residues 2–35 [36]. Both the wild type and mutant genes were expressed in *E. coli* as active enzymes that were shown to catalyse transfer of mannose to an alternative glycosyl-acceptor, phytanyl-pyrophosphoryl-α-*N*,*N*′-diacetylchitobioside. The apparent K_m values for the aceptor were 25 and 17 μM, respectively, for the wild type and truncated enzymes. These results demonstrated that the hydrophobic amino terminal domain was not required either for catalytic activity or for binding hydrophobic substrate. The mammalian gene for this enzyme has not yet been cloned.

Mannosyltransferase II [EC 2.4.1.132]

Mannosyltransferase II catalyses the next sequential reaction in the dolichol cycle involving transfer from GDP-Man to βMan-4-$(GlcNAc)_2$-P-P-Dol resulting in the formation of αMan-3-βMan-4-$(GlcNAc)_2$-P-P-Dol. Mannosyltransferase II can also utilize the trisaccharide-lipid αMan-6-βMan-4-$(GlcNAc)_2$-P-P-Dol as an acceptor with the addition of α-1,3-linked mannose and the formation of a tetrasaccharide-P-P-lipid [37]. The enzyme was purified 660-fold from rabbit liver microsomes and completely separated from mannosyltransferases IV and V, which synthesize α-1,2-mannosyl-mannose linkages, but the most highly purified preparations still contained mannosyltransferase III activity, which synthesizes the α-1, 6-mannosyl-linkage (Jensen, J. W. and Schutzbach, J. S., unpublished results).

Purified mannosyltransferase II was active in the presence of non-ionic detergents, but enzyme activity was sensitive to detergent concentration with optimal activity at 0.0225% Nonidet P-40. The purified enzyme could be readily reconstituted with PE dispersions but not with other naturally occurring phospholipids [38, 39]. Unsaturated species of PE, unlike PC, do not readily form bilayers in an aqueous environment but instead form a nonbilayer or hexagonal phase. The results suggested that mannosyltransferase II was optimally active in the presence of this nonbilayer phase. Evidence for the formation of an enzyme-phospholipid complex was obain-18ed from kinetic

studies, by the stabilization of the enzyme by PE, and by co-sedimentation of the enzyme with phospholipids.

A temperature sensitive mutation in yeast (*Alg2*) has been identified that results in the accumulation of a mixture of lipid-linked $Man_1GlcNAc_2$ and $Man_2GlcNAc_2$ when cells were grown at the non-permissive temperature [40]. At the permissive temperature growth was normal with the formation of the expected $Glc_3Man_9GlcNAc_2$ structure. The *ALG2* gene was cloned from multicopy plasmids based on the ability to rescue growth at the non-permissive temperature [41]. *ALG2* encodes a protein of 529 amino acids with a mass of 60 kDa. The predicted sequence contains one or two potential membrane-spanning domains, and the N-terminal region contains a typical leader sequence. The predicted protein sequence also contains the putative dolichol recognition sequence in a hydrophobic potential membrane-spanning domain near the C-terminus. Although the gene product of *ALG2* has not been confirmed as mannosyltransferase II, the gene product does restore normal oligosaccharide synthesis in the yeast mutant. It is interesting to note that the temperature sensitive mutants contain a mixture of tri- ($Man_1GlcNAc_2$) and tetra- ($Man_2GlcNAc_2$) saccharide-lipids. In light of the inability to physically separate mannosyltransferase II from mannosyltransferase III, this result may suggest that the gene product of *ALG2* catalyses the synthesis of both the α-1,3- and the α-1,6 mannosyl-mannose linkages. Resolution of this paradox must await the demonstration of both reactions in a single enzyme, or else separation of the two mannosyltransferasse activities as separate proteins.

Mannosyltransferase IV and mannosyltransferase V [EC 2.4.1.131]

These two enzymes catalyze sequential transfer from GDP-Man to the α-1,3-linked mannosyl arm of the growing oligosaccharide with the formation of α-1,2-linkages. The two transferase activities were completely separated from each other, and from mannosyltransferase II, by column chromatography on hydroxylapatite [37]. Mannosyltransferase V was purified 40-fold from rabbit liver microsomes. In contrast to other enzymes of the dolichol cycle, mannosyltransferase V does not require divalent cations for activity. The effects of a lipid environment on these enzymes has not been studied, although lability of enzyme activity in detergent solutions may indicate a requirement for phospholipid for stability.

Mannosyltransferase VI [EC 2.4.1.130]

Mannosyltransferase VI catalyses transfer from Dol-P-Man to the α-1,6-linked mannosse of the $Man_5GlcNAc_2$ heptasaccharde with the formation of an α-1,3-linkage. The mannosyltransferase was purified almost 1000-fold from pig aorta [42], contained two protein bands on SDS-PAGE of 55 and 65 kDa, and is the only enzyme catalysing mannosyltransfer from Dol-P-Man in the dolichol cycle to be purified. Glycerol, but not phospholipid, was found to partially stabilize the enzyme, although the purified enzyme preparation was reported as being very unstable. The enzyme was specific for heptasaccharide lipid acceptor, but could utilize unsaturated polyprenol-P-Man as a glycosyl-donor although with a K_m seven-fold higher and a 40% lower V_m [43]. Even though this enzyme was not purified to homogeneity, the results obtained in this work are quite impressive. The investigators had to prepare not only the $Man_5GlcNAc_2$-P-P-Dol acceptor in substrate quantities but also the radioactive mannosyl donor Dol-P-[^{14}C]Man. Enzyme assays involved incorporating Dol-P-Man, $Man_5GlcNAc_2$-P-P-Dol, along with purified enzyme in detergent micelles, and required separation of the radioactive octasaccharide-lipid product from the glycosyl donor, Dol-P-[^{14}C]Man.

A recent investigation [44] of glycosyl-donor specificity using pig brain microsomes and endogenous acceptors suggests that the enzyme is specific for both the reduced α-isoprene unit of dolichol and the β-anomer of Dol-P-Man. Totally unsaturated polyprenyl[11]-P-Man and polyprenyl[19]-P-Man were extremely poor substrates for the enzyme, and the rate of transfer from the α-anomer was only 7% the rate of transfer from the β-anomer. The length of the dolichol chain was also an important variable. The apparent K_m values for C-95-, C-55- and C-10-dolichyl-P-Man were 3, 10, and 200 μM, respectively. The reaction product formed in these studies, however, was the $Man_9GlcNAc_2$ oligosaccharide, and therefore the kinetic constants represent a sum of four different mannosyltransfer reactions. Regardless, models for the mechanism of these mannosyltransferases must account for the ability of the active site to interact with, and recognize, both the sugar and the polyprenyl moieties of the two amphipathic substrates.

Dol-P-Man synthase [EC 2.4.1.83]

Dol-P-Man synthase catalyses the reversible transfer of mannose from GDP-Man to Dol-P (Figure 2. Reaction 4). The enzyme was solubilized from liver microsomes and purified 880-fold (over the microsomal fraction) in the presence of 0.1% NP-40 [45]. Following removal of detergent, the purified enzyme preparation was stable for months when stored at $-20\,^{\circ}C$. Partially purified fractions of liver Dol-P-Man synthase, however, were found to be inactive in the presence of detergents, whereas, the analogous enzyme from yeast is active in detergent solution [46, 47]. Detergent-free mammalian Dol-P-Man synthase was found to be optimally active when reconstituted with PE, or with phospholipid mixtures of PE and PC when the molar proportion of PC was 70% or less. The mode of activation of the synthase by PE suggested that specific structural features of PE, and/or its unusual macroscopic organization, were

involved in the activation. Thus other lipids, including monogalactosydiglyceride, which preferentially adopt the hexagonal H_{II} phase in aqueous solution were also found to optimally activate the transferase. The results support the proposal that the physical state of the lipid/substrate complex, rather than specific lipids, largely determines whether the enzyme forms a productive complex with its hydrophobic substrate [48]. The mammalian enzyme was found to physically associate only with those lipid matrices that provided a suitable matrix for enzyme activity.

The metabolic regulation of Dol-P-Man synthase is not well understood, but could possibly involve covalent modification of the enzyme [49–51], or changes in the concentrations of the glycosyl donor, the glycosyl acceptor, or the synthase itself [13]. Changes in the lipid microenvironment, however, could also potentially regulate mammalian Dol-P-Man synthase [52]. Diacylglycerols and lysophospholipids are present at low concentrations in biological membranes as the result of phospholipid metabolism [53], and the presence of either DAG or lyso-PC would be expected to disturb lipid packing and alter local membrane architecture. Purified Dol-P-Man synthase reconstituted with phospholipids was found to be modulated by the incorporation of these compounds into the lipid matrix [52]. DAG enhances mannosyl transfer, probably as a consequence of the ability of this conically shaped lipid to physically alter the structure of bilayer membranes [54–56]. DAG lowers the apparent K_m for Dol-P in PC membranes from 9 μM to 0.3 μM but has no effect on the K_m for GDP-Man, suggesting that the incorporation of DAG into PC membranes alters the physical properties of the lipid matrix in a manner that increases the apparent affinity of the enzyme for Dol-P. The increased affinity of the enzyme for Dol-P in PC/DAG mixtures may be the result of DAG-induced phospholipid headgroup spreading, which would allow the synthase increased access to the polar phosphate group of the polyprenol substrate. In contrast, the incorporation of lyso-PC into either PE dispersions or into PE/PC membranes was found to reduce transferase activity. The diminished activity noted in the presence of phospholipid dispersions containing low concentrations of lyso-PC was not the result of micelle formation [57, 58], and the inhibitory effects of lyso-PC on transferase activity can be ascribed to the ability of lysolipids to stabilize the bilayer phase in phospholipid mixtures containing PE.

The fact that synthase activity can be modulated by the incorporation of either DAG or lyso-PC into the lipid matrix, suggests that activity might also be regulated by interacellular phospholipases. Thus when enzyme was reconstituted in PC vesicles containing 2.4% Dol-P, which supports minimal activity, the rate of mannosyl transfer was significantly increased by treatment with phospholipase C [52], and the rate of mannosyl transfer was proportional to the quantity of DAG generated in situ by phospholipase C. DAG concentrations of 0.75 mol % stimulated a five-fold increase in enzyme activity, and maximal levels of enzyme activity were noted at 4 mol % DAG. Activation at a DAG concentration of 0.75 mol % is within the physiological concentration range for this compound [53]. By comparison, when the enzyme was reconstituted in PE/PC membranes which support mannosyl transfer, activity was inversely proportional to the amount of phospholipase A_2 added to the reaction mixture. The modulation of synthase activity observed when phospholipases were added to the reactions mixtures is consistent with the results obtained when either DAG or lyso-PC was directly incorporated into the lipid matrix. Since these compounds arise in vivo as a result of phospholipid metabolism, modulation of transferase activity by these phospholipids may represent a potential regulatory mechanism for the synthesis of oligosaccharide-lipids.

Dol-P-Man synthase has also been purified from chicken liver mitochondria [59] and purified to homogeneity from mouse liver mitochondria [60, 61]. The latter enzyme had a molecular mass of 30 kDa based on SDS-PAGE. The phospholipid specificity of the mitochondrial enzymes was examined and they were shown to be active in the presence of a variety of phospholipids. The amounts of detergent in the enzyme assays was not well controlled, however, and the concentrations of Dol-P would have been sufficient to have disrupted normal phospholipid bilayer structure. Thus it is difficult to assess the optimal lipid environment required for activity of the mitochondrial enzymes.

The structural gene for yeast Dol-P-Man synthase (*DPM1*) has been cloned, and shown to be expressed as an active protein in *E. coli* [62]. *DPM1* codes for a protein of 267 amino acids with an apparent molecular mass of 30.36 kDa having a potential membrane spanning domain of 25 amino acids at its carboxyl terminus. The NH_2 terminus meets existing criteria for yeast signal sequences, but there is no site for cleavage by signal peptidase and the active site of the enzyme appears to have a cytoplasmic orientation. Although the enzyme had been purified to homogeneity from yeast [63], the procedure did not provide useful amounts of enzyme for further characterization. Recently an *E. coli* high expression vector for the synthase was prepared that allows the isolation of mg quantities of a stable, homogeneous protein [30]. The recombinant enzyme was found to be active at 25 °C when assayed in reaction mixtures containing non-ionic detergents with a K_m for GDP-Man of 1.5 μM and a K_m for Dol-P of 10.7 μM. When detergent-free enzyme was reconstituted with PE, the K_m for GDP-Man was unchanged but the apparent K_m for Dol-P decreased four-fold. The phospholipid specificity for optimal reconstitution of the recombinant yeast enzyme is similar to that for the mammalian synthase. Thus dispersions of nonbilayer phospholipid (PE) or phospholipid mixtures that form destabilized bilayers provide the best support for transferase activity [30, 64]. The similarity of the yeast and mammalian enzymes in lipid

requirements may be significant because the yeast enzyme has been shown to be expressed as an active protein in CHO cells where it corrects the genetic defect for this activity [65]. Results obtained with the purified recombinant enzyme [64] do not support the idea that the synthase itself is involved in the translocation of activated mannosyl residues across phospholipid bilayers [47, 66].

Yeast Dol-P-Man synthase also contains the putative dolichol recognition sequence within the hydrophobic domain near the carboxyl terminus [28]. A series of site directed mutations and deletions were made in this domain of the enzyme [30, 31]. All of the mutant enzymes, as well as enzyme containing a deletion of the entire hydrophobic domain, were catalytically active with no change in apparent affinity for Dol-P when the enzymes were reconstituted with phospholipids. Therefore, this domain does not appear to be involved in either the recognition or binding of Dol-P. Since all of the enzymes that contain this conserved sequence are localized in the ER, perhaps it is more likely that this sequence represents an ER specific label.

Dol-P-Glc synthase [EC 2.4.1.117]

This enzyme catalyses the reversible formation of Dol-P-Glc (Figure 2, Reaction 6) and has been highly purified from MOPC-315 plasmacytoma tissue [67], and from human liver microsomes to near homogeneity [68]. The latter enzyme has a molecular mass of 36 kDa based on SDS-PAGE. The molecular mass of the plasmacytoma enzyme (37 kDa) was determined by sedimentation velocity on a sucrose gradient in the presence of 0.35% Triton X-100. Surprisingly, this membrane enzyme did not appear to associate with either phospholipids or detergents because such an interaction would probably have resulted in aberrant migration through the gradient. Optimal activity of the plasmacytoma enzyme was obtained in the presence of PC, whereas the liver enzyme was maximally active in the presence of PE. A protein of similar mass (35 kDa) has been identified as Dol-P-Glc synthase by photoaffinity labeling in rat mammary gland [69] and rat liver microsomes [70]. Photoaffinity labeling was also used to identify a 35 kDa protein in yeast membranes [71] that was decreased or absent in membranes from two yeast mutants (*Alg5* and *Dpg1*) known to be deficient in the synthesis of Dol-P-Glc, lending support to the idea that the 35 kDa protein is the synthase.

The structural gene for Dol-P-Glc synthase (*ALG5*) was recently cloned from yeast by complementation of *alg5-1 wbpl-1* double mutants that do not grow at 30 °C [72]. *ALG5* was expressed as an active protein in *E. coli* confirming its identity as the structural gene for this enzyme. The open reading frame encodes for a protein of 38.3 kDa with two potential N-linked glycosylation sites and has a region of 26 hydrophobic and uncharged amino acids near the amino terminus. The glucosyltransferase was identical in 58 out of 262 amino acids with Dol-P-Man synthase. Histidinol dehydrogenase fusions with N-terminal portions of the *ALG5* protein were used to provide evidence that the glucosyltransferase is a transmembrane protein with several membrane spanning domains.

Oligosaccharyltransferase [2.4.1.119]

The action of this enzyme represents the exit from the dolichol cycle. The enzyme catalyses transfer of completed oligosaccharide chains from the activated lipid-intermediate to the asparagine residue of Asn-X-Ser/Thr sequons in nascent polypeptide chains on the lumenal surface of the ER membrane. A protein complex containing protein subunits of 66, 63 and 48 kDa was purified from dog pancreas microsomes [73] and identified as the transferase. The 66 and 63 kDa subunits were also identified as ribophorins I and II by protein immunoblotting. The oligosaccharyltransferase was shown to be stabilized and greatly activated when reconstituted with phospholipids [74]. The enzyme was highly specific for PC over other naturally occurring phospholipids, and activity was dependent on both the length and degree of unsaturation of the acyl chains.

Summary

As discussed throughout this review, the membrane-bound proteins of the dolichol cycle that have been at least partially purified were usually found to be stabilized by phospholipids, or to require reconstitution with lipids for enzyme activity. Other enzymes in the pathway have not yet been purified or characterized as to the effects of lipids. Based on known results, however, it is tempting to speculate that purification of the other enzymes in the dolichol pathway will also require stabilization and reconstitution with phospholipids. As genes encoding enzymes in the pathway are cloned and sequenced, the deduced amino acid sequences will allow identification of homologies between the enzymes, and characterization of the protein properties based on the sequence data. Also, it is possible that the dolichol pathway is under some type of physiological control, and that this control may be important in the regulation on *N*-linked glycoprotein synthesis [75]. Recent studies are focusing on the mechanism of control of the pathway through regulation of gene transcription and post-translational changes, but review of this latter topic is beyond the scope of the present article.

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