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Review

Hypoglycosylation due to dolichol metabolism defects

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ABSTRACT

Dolichol phosphate is a lipid carrier embedded in the endoplasmic reticulum (ER) membrane essential for the synthesis of N-glycans, GPI-anchors and protein C- and O-mannosylation. The availability of dolichol phosphate on the cytosolic site of the ER is rate-limiting for N-glycosylation. The abundance of dolichol phosphate is influenced by its de novo synthesis and the recycling of dolichol phosphate from the luminal leaflet to the cytosolic leaflet of the ER. Enzymatic defects affecting the de novo synthesis and the recycling of dolichol phosphate result in glycosylation defects in yeast or cell culture models, and are expected to cause glycosylation disorders in humans termed congenital disorders of glycosylation (CDG). Currently only one disorder affecting the dolichol phosphate metabolism has been described. In CDG-Im, the final step of the de novo synthesis of dolichol phosphate catalyzed by the enzyme dolichol kinase is affected. The defect causes a severe phenotype with death in early infancy. The present review summarizes the biosynthesis of dolichol-phosphate and the recycling pathway with respect to possible defects of the dolichol phosphate metabolism causing glycosylation defects in humans.

1. The de novo synthesis of dolichol

Dolichol was first isolated from animal tissues in 1960 [1]. In 1970 it was demonstrated that many essential biochemical pathways in eukaryotic cells rely on a sufficient supply with dolichol-monophosphate as a carrier of lipid activated glycans [2,3].

Dolichol is the most prevalent polyisoprenyl glycosyl carrier in eukaryotes involved in C- [4] and O-mannosylation of proteins, the formation of glycosylphosphatidylinositol (GPI) anchors [5] and the N-glycosylation of proteins [6].

The first steps of the biosynthetic pathway leading to the biosynthesis of dolichol are common to the majority of neutral lipids. Dolichol, ubiquinone and cholesterol are all products of the mevalonate pathway [7].

The first step towards the biosynthesis of dolichol, cholesterol and ubiquinone is the condensation of two molecules of acetyl-CoA. Two additional carbon atoms descending from acetyl-CoA are added to the resulting acetoacetyl-CoA generating 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) [8]. Subsequently mevalonate is produced by reduction via HMG-CoA reductase. Two phosphorylation and one decarboxylation steps later isopentenylpyrophosphate, an essential building block for all following products, is generated. The condensation of three isopentenylpyrophosphates results in farnesylpyrophosphate [9]. All of the above mentioned biosynthetic reactions are common to the pathways leading to dolichol, cholesterol and ubiquinone, respectively. This review will concentrate on deficiencies that are solely affecting the biosynthesis of dolichol and dolichol-phosphate or their recycling, thus affecting reactions described below.

The first step unique to the biosynthesis of dolichol is the condensation of farnesylpyrophosphate with isopentenylpyrophosphate (Fig. 1). This reaction is carried out by specific cis-prenyltransferases, which use the product polyprenylpyrophosphate and additional isopentenylpyrophosphates to successively elongate the polyprenyl to its final length. Cis-isopentenyltransferases appear to be closely bound to microsomes in mammalian tissues [10]. Later results suggest that dolichol, at least in part, might be produced in the peroxisomes [7].

After the final isopentenylpyrophosphate has been added to the growing polyisoprene pyrophosphate chain, both phosphate residues are released by mono- or pyrophosphatases (Fig. 1). Relevant mono and pyrophosphatase activities could be demonstrated in microsomal fractions [11–13].

The α-isoprene unit of the polyprenol is subsequently reduced by an NADPH dependent microsomal reductase (Fig. 1) [14]. It has been speculated that the activity of the polyprenol reductase might be rate-limiting for the synthesis of dolichol [15]. Finally a dolichol-specific kinase transfers a phosphate from CTP to dolichol [16,17].

It could be shown at least in rats that the contribution of dietary dolichol is negligible for the availability of dolichol in the ER compared to dolichol descending from de novo synthesis [18]. The newly

Abbreviations: Dol, Dolichol; Man, Mannose; Glc, Glucose; GlcNac, N-acetylglucosamine; ER, Endoplamic reticulum; GPI, Glycosylphosphatidylinositol; CTP, Cytidine triphosphate

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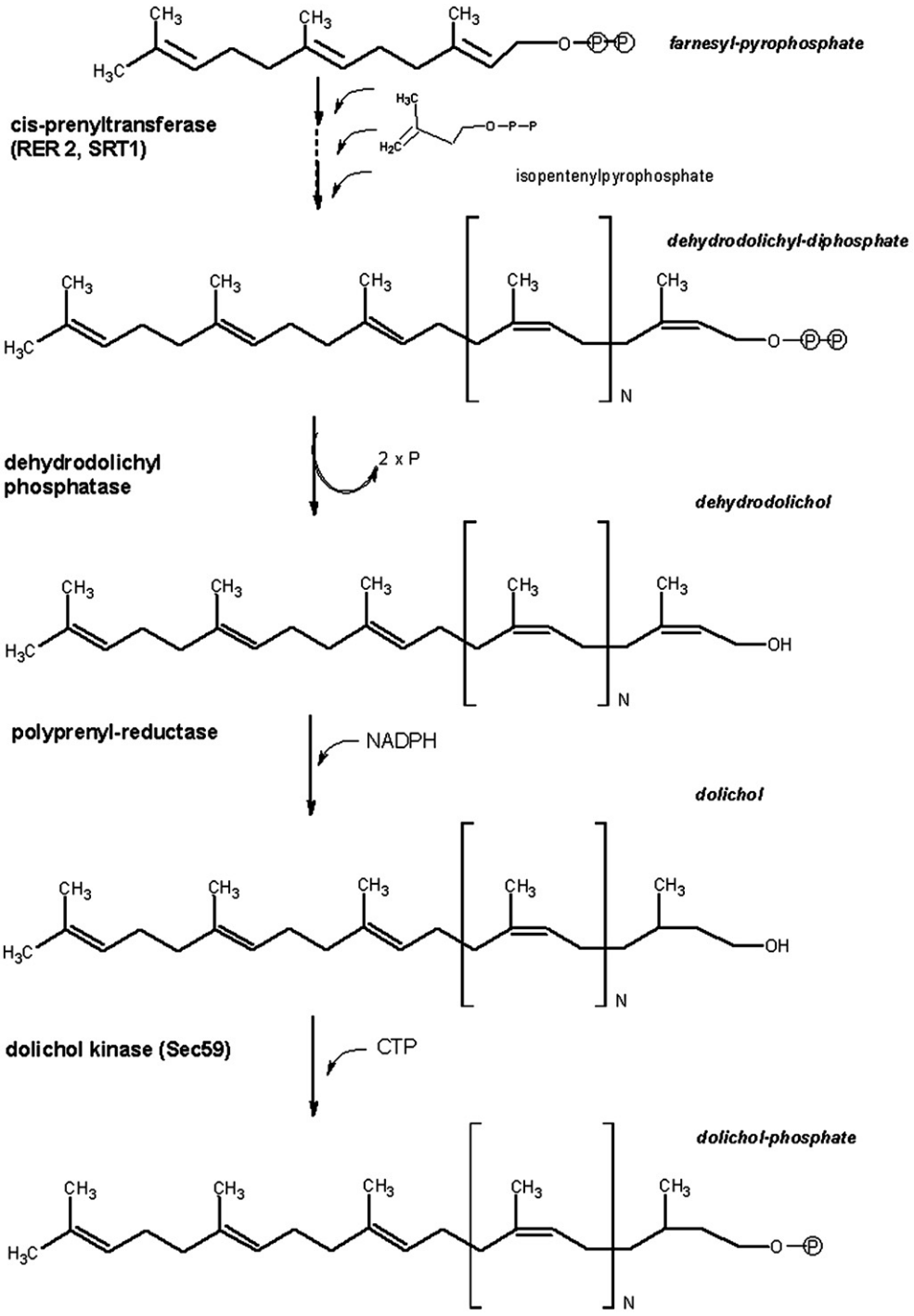

Fig. 1. De novo biosynthesis of dolichol-phosphate. Cis-prenyltransferases promote the condensation of farnesylpyrophosphate with several molecules of isopentenylpyrophosphate. The arising polyprenylpyrophosphate is dephosphorylated and reduced. Dolichol is re-phosphorylated by the dolichol kinase.

synthesized dolichol-phosphate residing on the cytoplasmatic leaflet of the ER might then serve as a glycan carrier in the ER membrane.

The chain length of eukaryotic dolichol molecules is species specific and differs from 14 to 17 isoprene units in unicellular organisms like the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* [19]. Mammalian cells produce longer dolichol molecules with 18–21 isoprene units [20].

Even bacterial cells that prefer undecaprenyl phosphate (also known as bactoprenol phosphate) which is fully unsaturated, sometimes use dolichol as a glycosyl carrier [21,22].

Due to the overlap between the cholesterol, the farnesyl and the dolichol pathways, the effect of conditions affecting the availability and metabolism of cholesterol on the Dol-P pool and thereby on glycosylation is of special interest. It could be shown that the availability and metabolism of cholesterol does not directly correlate with the availability and metabolism of Dol-P [23]. While cholesterol metabolism varied 2200-fold under experimental conditions, the Dol-P metabolism only varied six-fold. In further experiments on rat liver slices, Keller et al. did not find a significant variation in Dol-P while cholesterol synthesis varied about 40-fold, indicating that the rate of dolichol phosphate synthesis in the liver can be maintained on almost the same level under conditions in which the rate of cholesterol synthesis varies dramatically [24].

2. The role of dolichol-phosphate for protein glycosylation

Fourteen distinct oligosaccharide-pyrophosphoryl dolichol derivates are synthesized during the assembly of the final N-glycan precursor in the ER [6]. During N-glycosylation of proteins, dolichol phosphate is used by three different enzymes. The GlcNAc-1-phosphotransferase transfers a GlcNAc-1-P residue descending from UDP-GlcNAc to Dol-P. The resulting GlcNAc-P-P-Dol is subsequently elongated to become $Man_5GlcNAc_2$-P-P-Dol. After this structure has been flipped to the luminal side of the ER, it is elongated by the action of different enzymes to the complete precursor oligosaccharide $Glc_3Man_9GlcNAc_2$-P-P-Dol [6,25].

Incubation of cells with radioactive mannose showed that 1 min after the addition of the labeled sugar, a significant amount of radioactivity is incorporated in $Glc_3Man_9GlcNAc_2$-P-P-dolichol and after 5 min $Man_9GlcNAc_2$-P-P-dolichol and larger LLOs start to accumulate implicating that the process of N-glycan formation on dolichol can be performed within minutes [26].

This preassembled oligosaccharide structure is transferred to specific asparagine residues of the respective protein by a multisubunit enzyme complex termed oligosaccharyltransferase [6] (Fig. 2A). As a consequence, Dol-P-P is released on the luminal leaflet of the ER.

All mannose and glucose residues attached to the $Man_5GlcNAc_2$-P-P-Dol structure inside the ER descend from Man-P-Dol or Glc-P-Dol, respectively. Both substrates are generated on the outer ER membrane by the action of Dol-P-Mannose synthase (dpm1) [27] and Dol-P-glucose synthase (Alg5) [28], and subsequently flip on the luminal leaflet of ER membrane by mechanisms that are not yet fully understood. The biosynthesis of both Man-P-Dol and Glc-P-Dol requires sufficient amounts of dolichol-phosphate.

Eight molecules of Dol-P are required for the synthesis of one N-glycan. However, C- and O-mannosylation and GPI anchor biosynthesis also depend on a sufficient supply of Dol-P [4–6].

All three mannose residues that are part of the core structure common to all GPI-anchors descend from Man-P-Dol (Fig. 2B) which

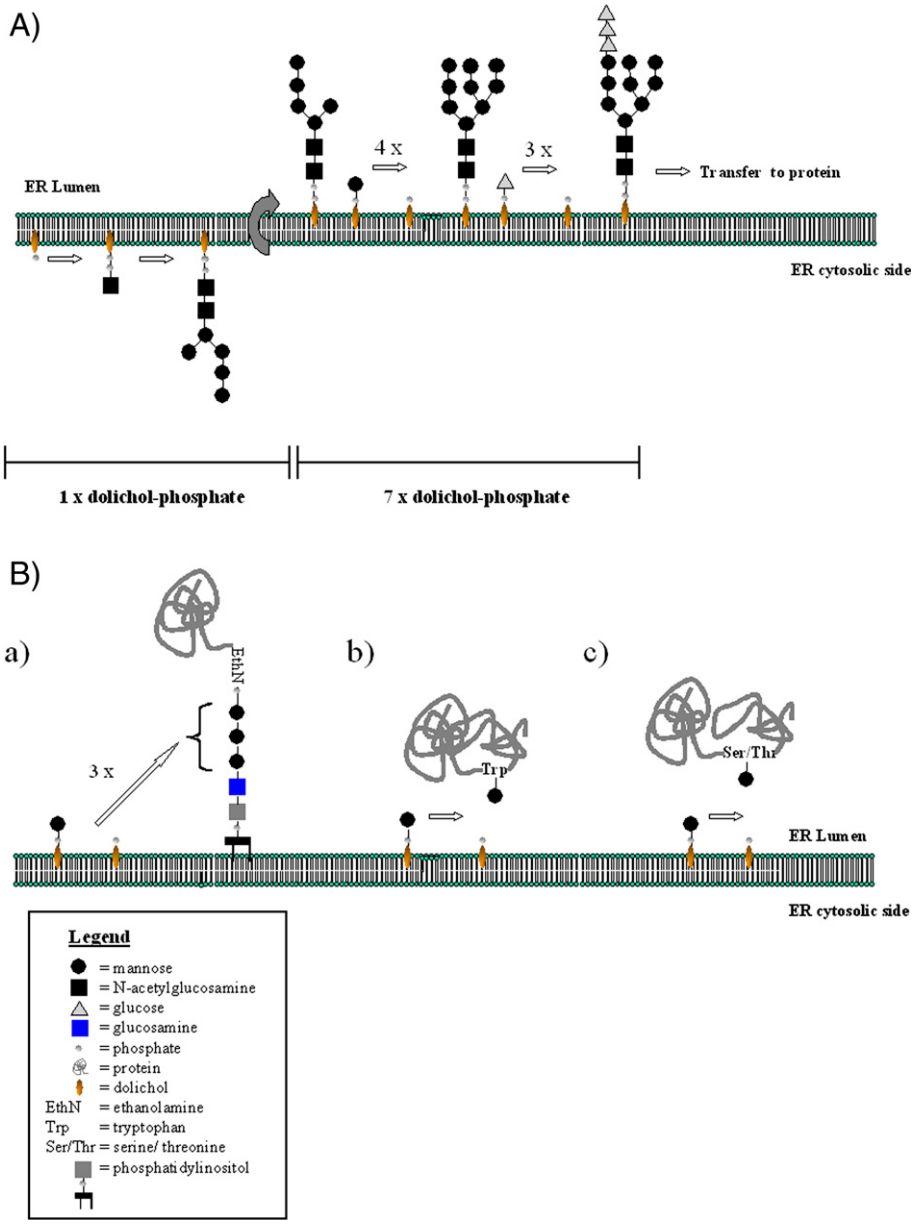

Fig. 2. Schematic representation of the N-glycosylation, C- and O-mannosylation pathway. (A) The oligosaccharide precursor structure $Glc_3Man_9GlcNAc_2$ is assembled on the outer and inner leaflet of the ER. The preassembled oligosaccharide is transferred to the nascent protein en bloc via oligosaccharyltransferase. The number of required dolichol-P molecules is given under the bar. (B) Proteins will be modified by GPI-anchors (a) and C- (b) or O-mannosylation (c) on the inner leaflet of the ER.

also applies to C- and O-mannosylation, both equally depending on Man-P-Dol as a required substrate (Fig. 2B) [4–6].

Dolichol-phosphate is essential for a variety of different glycosylation processes implicating glycosylation disorders as a result of defects affecting the synthesis of Dol-P. Disorders mainly affecting protein glycosylation are comprised in the rapidly growing group of congenital disorders of glycosylation (CDG), thus Dol-P synthesis and recycling defects are suggested to be members of these metabolic disorders [25].

While a relevant decrease in Dol-P causes impaired N-glycosylation, additionally supplied Dol-P results in increased N-glycosylation, suggesting that the availability of Dol-P is rate limiting for the N-glycosylation pathway [29–31].

Moreover, some enzymes involved in the N-glycosylation pathway are closely regulated by the abundance of certain dolichol linked glycans. Man-P-dolichol, for example, activates the N-acetylglucosamine-1-phosphate transferase (GPT-1) that initiates the synthesis of the precursor N-glycan $Glc_3Man_9GlcNAc_2$ on dolichol by allosteric activation [32]. Glc-P-dolichol, on the other hand is a competitive inhibitor of the activation of GPT-1 by Man-P-dolichol [32]. Both GlcNAc-P-P-dolichol and GlcNAc-GlcNAc-P-P-dolichol inhibit the de novo synthesis of GlcNAc-P-P-dolichol by feedback inhibition [26].

GlcNAc-P-P-dolichol also stimulates Man-P-dolichol synthesis up to tenfold [33]. Thus N-glycosylation is not only regulated by the availability of Dol-P but also by the presence of primary Dol-P-P-glycans. Rosenwald et al. showed that Dol-P accounts for approximately 10% of total dolichol metabolites, and that the level of Dol-P is constant even when the activity of enzymes utilizing this substrate is considerably varied [34]. It is well known that a total disruption of N-glycosylation is not compatible with life [35]. Therefore, no defects in dolichol metabolism completely abolishing glycosylation are expected to occur in humans.

3. Recycling of dolichol phosphate

During the synthesis of one N-glycan, seven Dol-P and one Dol-P-P with luminally-oriented phosphate residues are released at the inner ER membrane. Similarly, three molecules of Dol-P are released during the assembly of one GPI anchor and one is released during C- and O-mannosylation processes, respectively [36]. In addition to the de novo synthesis of dolichol phosphate, a high-throughput recycling pathway for Dol-P exists to supply enough Dol-P sufficient for the different glycosylation pathways [36]. Due to the compartmentalization of the N-glycan synthesis beginning on the cytosolic side and ending on the luminal side of the ER membrane, a recycling pathway of Dol-P is necessary (Fig. 3).

An early model of dolichol recycling proposed a complete dephosphorylation of Dol-P and Dol-P-P with a subsequent flipping or diffusion of the free α saturated polyisoprenol from the luminal to the cytosolic leaflet [36]. Subsequently, dolichol would have to be re-phosphorylated by a dolichol kinase in order to be reused as glycan carrier. However, Rush et al. could demonstrate that the known dolichol kinase (DK1) does not contribute to the recycling pathway of Dol-P and that CTP is not necessary for the recycling process. This suggests that Dol-P flips through the ER membrane either by diffusion or by a flippase and is directly reused for the synthesis of dolichol linked oligosaccharides (Fig. 3A–B) [37]. Nevertheless, since flipping of Dol-P is an energetically unfavorable event due to the hydrophilic head group that has to traverse the hydrophobic ER membrane, it should be considered that Dol-OH flips through the ER membrane and is re-phosphorylated on the cytosolic leaflet of the ER by a currently unknown enzyme with an alternative phosphorylation mechanism independent of CTP (Fig. 3C). Dol-P-P released by the oligosaccharyltransferase must be converted to Dol-P before translocation to the cytosolic site of the ER making the action of a pyrophosphatase converting Dol-P-P to Dol-P an essential feature of the recycling process [36]. In yeast, a pyrophosphatase (CWH8) with an active site oriented towards the ER lumen has been characterized catalyzing the hydrolysis of the beta phosphate from Dol-P-P. As a result of the CWH8 deficiency, cells accumulate Dol-P-P [38]. Additionally, a hypoglycosylation phenotype caused by a diminished abundance of the full-length dolichol linked N-glycan precursor ($Glc_3Man_9GlcNAc_2$-P-P-Dol) could be demonstrated [38,39], underlining the conversion of Dol-P-P to Dol-P as an important step in the recycling of Dol-P. The mammalian homologue to the *CWH8* gene, which has been termed DolPP1 was cloned and could be shown to correct the phenotype of CWH8-deficient yeasts [40].

It is still unknown how Dol-P might flip through the ER membrane. In an experimental model, the diffusion rate of Dol-P in vesicles was far too slow to meet the physiological demand [41] implicating another model than diffusion for Dol-P flipping. However, no potential Dol-P-flippase has been characterized to date.

Given that the de novo synthesis of Dol-P is not sufficient to fully cover the demand of the N-glycosylation pathway, that the deficiency of a pyrophosphatase affecting the conversion of Dol-P-P to Dol-P causes a deficiency of N-glycosylation, that there is a rapid appearance of Dol-P on the cytoplasmic face of the ER derived from luminally oriented Dol-P-P, and that no catabolic pathway for

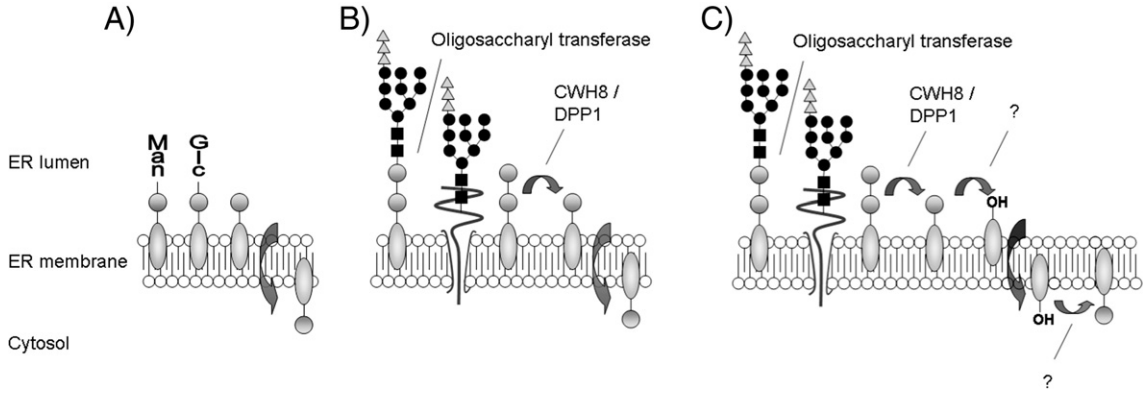

Fig. 3. Recycling of dolichol-phosphate. Recycling of Dol-P and Dol-P-P from the luminal leaflet of the ER membrane to the cytosolic leaflet. (A) After the transfer of mannose or glucose from Man-P-Dol and Glc-P-Dol into the lumen of the ER by either mannosyl- or glycosyltransferases Dol-P with luminal orientation is released and flips on the cytosolic leaflet of the ER membrane. (B) The oligosaccharyltransferase transfers the dolichol linked N-glycan to the nascent protein releasing Dol-P-P on the luminal leaflet of the ER. Subsequently Dolichol-pyrophosphatase (CWH8/DPP1) clears the terminal phosphate enabling Dol-P to flip through the ER-membrane either by diffusion or by a yet unknown flippase. (C) Due to the energetically unfavorable transfer of Dol-P through the ER membrane another model of Dol-P-P and Dol-P recycling might be possible with a de-phosphorylation of dolichol in the ER by CWH8/DPP1 or another not identified enzyme consecutively flipping through the ER membrane and with a subsequent cytosolic re-phosphorylation by another yet unidentified enzyme distinct from dolichol kinase. Symbols are the same as in Fig. 2.

dolichol is known to enable a high-throughput turnover of dolichol, Dol-P is thus subject to an efficient recycling pathway allowing multiple rounds of dolichol-linked oligosaccharide synthesis on the basis of an existing Dol-P pool.

The effects of Dol-P recycling on the availability of Dol-P for N-glycosylation have yet to be determined. Since CWH8 is not known to be involved in the de novo synthesis of Dol-P-P and a CWH8 deficiency causes a reduction of dolichol linked oligosaccharides to about 20% in yeast [39], one must conclude, that a relevant amount of Dol-P available for N-glycosylation results from recycling of luminally-oriented Dol-P and Dol-P-P.

4. Enzymes involved in the de novo synthesis and the recycling of dolichol phosphate

4.1. Cis-prenyltransferases

The cis-prenyltransferases are thought to be the first enzymes committed to the biosynthesis of dolichol alone [42]. They are considered to catalyze the head-to-tail condensation of farnesyl-diphosphate with several isopentenyl diphosphate molecules to generate polyprenyl diphosphate also known as dehydro-dolichol diphosphate.

The mechanism which leads to the length determination of the polyprenylphosphate has yet to be determined [43]; but two mechanisms have been suggested. It has been proposed that the addition of isopentenol-P-P instead of isopentenyl-P-P leads to the determination of the chain length [44]. Alternatively, Crick et al. [10] suggest that chain elongation may simply be terminated by the number of isoprene units themselves. According to the latter hypothesis, the polyprenyl chain will not be a substrate for cis-isoprenyltransferase once a specific length is reached [44].

Cis-prenyltransferases have been cloned and characterized from both prokayotic and eukaryotic organisms such as *Micrococcus luteus* [45], *Escherichia coli* [46], *Haemophilus influenzae* [46], *Streptococcus pneumoniae* [46], *Arabidopsis thaliana* [47] and *Saccharomyces cerevisiae* [48].

Two proteins with known cis-prenyltransferase activities have been discovered in yeast, RER2 and SRT1. RER2 mutants exhibit a very severe growth restriction even at 23 °C. A complete growth inhibition occurs at 37 °C [49].

N-glycosylation and GPI-anchor biosynthesis are severely affected. Several ER membrane proteins like Sec12 are mislocalized and abnormal membrane structures occur within the cells [50].

With SRT1, another cis-prenyltransferase, probably accounting for the biosynthesis of dolichol in *Saccharomyces cerevisiae* has been described. Although SRT1 deficient cells do not show any of the above mentioned deficiencies, cells that are harbouring simultaneous disruptions of the RER2 and *SRT1* gene show a lethal phenotype [50].

Interestingly, spontaneous revertants of RER2 deleted cells (Δrer2) have been found to overexpress the *SRT1* gene product. Dolichol molecules descending from these revertants are approximately five isoprene units longer than dolichol of wild type cells. Wild type yeast cells synthesize dolichol molecules of 14-17 isoprene units whereas SRT1 over expressing Δrer2 cells produce dolichol with lengths of 19-22 isoprene units [51].

4.2. Polyprenylpyrophosphate phosphatase

The enzyme activity needed to dephosphorylate polyprenyl pyrophosphate has been demonstrated several times [11,12,52,53].

The described activities would be in accordance with a role of CWH8 in the de novo synthesis of dolichol-phosphate, where polyprenyl pyrophosphate has to be converted to polyprenyl before the reduction of the α-isoprene unit can occur [14]. Since polyprenyl pyrophosphate is produced by cis-prenyltransferases whose active sites are exposed to the cytoplasmic surface of the ER [10,54], the active site of CWH8 has the wrong topology, since it is oriented to the luminal side of the ER [38].

Yet, no mutant or enzyme with polyprenylpyrophosphate phosphatase activity and the right topological orientation or impact on glycosylation could be identified.

4.3. Dolichol pyrophosphate phosphatase

Previous studies described the capability of crude microsomal fractions to hydrolyze exogenous Dol-P-P [55–58]. In *Saccharomyces cerevisiae*, LPP1 and DPP1 have been shown to dephosphorylate dolichol pyrophosphate and dolichol phosphate [59,60] *in vitro*. However, since no growth or glycosylation deficiencies occur in cells deficient in these enzymes, it is questionable if dolichol phosphates are their primary substrates *in vivo*. One enzyme that was shown to convert Dol-P-P to Dol-P and to a slower rate Dol-P to Dol in yeast was found to be CWH8 [38]. CWH8 is an integral membrane protein with four transmembrane domains and a luminally oriented active site [38].

As described in the chapter dealing with the recycling of dolichol phosphate CWH8, deficient cells have a severe hypoglycosylation phenotype. Although the authors already proposed a pyrophosphatase and phosphatase activity and an ER localization of CWH8, they did not prove the function of the coded protein [39]. In a follow-up study, a mouse homologue of the *CWH8* gene was described (DOLPP1) [40]. The authors could show a marked preference of the coded enzyme for Dol-P-P and demonstrate an ER localization of the protein with its active domain oriented to the ER lumen. Impaired N-glycosylation in cells deficient in dolichol pyrophosphate phosphatase is due to a relative lack of Dol-P on the outer leaflet of the ER; but might be additionally influenced by a product inhibition of the oligosaccharyltransferase complex by the accumulation of Dol-P-P on the inner leaflet of the ER. The latter aspect is underscored by the fact that the lack of CWH8 mainly affects the N-glycosylation pathway. O- and C-mannosylation and GPI anchoring do not release Dol-P-P but Dol-P and are thus unlikely to be inhibited by an accumulation of Dol-P-P. Accordingly, these pathways are only moderately affected [61].

4.4. Polyprenylreductase

A general hypoglycosylation of proteins has been found in Lec9 Chinese hamster ovary cells (CHO). This cell line shows hypoglycosylation of a variety of proteins. Besides glucuronidase and vesicular stomatitis virus glycoprotein (G protein), the bulk of [^{3}H]mannose-labeled proteins revealed an aberrant glycosylation phenotype. Moreover, the compartmentalization of β-3-hexosaminidase and β-3-glucuronidase is impaired in Lec9 cells and the function of the mannose 6-phosphate receptor is altered [62]. This cell line has been shown to exhibit a dramatic reduction in tumorigenicity if injected in nude mice [63]. It could be shown that the amount of the fully glycosylated lipid-linked precursor structure $Glc_3Man_9GlcNAc_2$-P-P-Dol was markedly decreased [64]. Lec9 cells incorporate approximately 40-fold less [^{3}H]-mannose into the fully glycosylated LLO structure. The incorporation of radioactivity into the minor LLO intermediate $Man_5GlcNAc_2$-P-P-Dol was also decreased, but to a much lower extent and this truncated structure was more frequently transferred to protein compared to parental cells. The reduction and size variation of the lipid-linked oligosaccharides ultimately led to a fivefold decrease in [^{3}H]-mannose incorporation into protein [64]. It has been found that Lec9 cells are defective in the synthesis of dolichol. Lec9 cells synthesize polyprenols of the same chain lengths as parental cells, but do not efficiently reduce the double bond of the terminal isoprene unit of long-chain prenols. Although cis-α-unsaturated polyprenol is used as a substrate for oligosaccharide lipid intermediate biosynthesis, many enzymes have a preference for the usage of dolichol over its unsaturated polyprenol [62]. It is noteworthy that Lec9 cells are not totally defective for dolichol synthesis, as a certain amount of alpha-saturated dolichol could be detected.

All mutant CHO cell lines belonging to the Lec9 complementation group share very similar phenotypes. CHB11-1-3 [65], B211 [66] Lec9.4A [67] and F2A8 [68,69] share the temperature sensitive growth phenotype and the hypoglycosylation of dolichol and proteins. A revertant of CHB11-1-3 exhibits a higher specific cis-prenyltransferase activity [65]. It has been speculated that CHB11-1-3 is a Km mutant, since a higher amount of substrate reverses the defect.

Another mutant defective for the reduction of polyprenol to dolichol has been found in *Trypanosoma brucei* [70]. The insect form of the protozoan parasite causing the sleeping sickness in Sub-Saharan Africa displays mutants that are resistant to ConcanavalinA treatment, since its major surface glycoprotein procyclin is underglycosylated, and carries abnormally glycosylated glycostructures. Dolichol linked oligosaccharides have been shown to be truncated.

Defective ConA 1–1 mutants produce equal amounts of polyprenol and dolichol, whereas wild type cells almost exclusively produce dolichol. The relatively high amount of residual dolichol leads to the conclusion that these cells harbour just one defective allele, or that the mutated enzyme has a considerable rest activity [70].

Ohkura et al. [71] could show that even in some CDG fibroblasts that were not further characterized but derived from patients with a type one isoelectric focusing pattern of serum transferrin, polyprenol rather than dolichol accumulated. These findings imply that in these patients, the lack of dolichol-phosphate might be in part responsible for the described phenotypes, or that the dolichol synthesis might be affected by defects in the subsequent metabolism of dolichol linked oligosaccharides.

So far, no polyprenol reductase gene has been identified in any eukaryotic organism. Thus, no mutations leading to the loss of polyprenol reductase activity have been detected. Moreover, all putative mutants producing polyprenols instead of dolichol produced at least a small amount of the fully saturated dolichol, implicating that null mutants are lethal.

Mutant cells use the polyprenol compounds to synthesize glycosylated polyprenols of the same structure; and nearly the same amount as wild type cells produce the dolichol equivalents. As $Man_5GlcNAc_2$-P-P-Dol accumulates in mutant cells, at least Man-P-Polyprenol and Glc-P-Polyprenol seem to be utilized at much slower rates than their dolichol counterparts [68,69].

4.5. Dolichol kinase

Dolichol kinase activity was firstly described in various cells by Allen et al. [16]. Dolichol kinase (DK) catalyzes the CTP-dependent phosphorylation of dolichol and hence the final step of the de novo synthesis of Dol-P [72]. It could be shown that DK activity varies with the cell cycle and tissue differentiation [29,73,74]. Previously it has been discussed, that DK also plays a key role in the recycling of Dol-P [36], but currently it could be shown, that the known enzyme coded by the *DK1* gene is not involved [37]. However, DK activity would be important to recruit Dol-P from a further pool, the free dolichol pool, which is located in the ER membrane. The relevance of this pool for a stable Dol-P level is yet not fully understood.

A gene coding for a dolichol kinase was firstly identified in yeast termed SEC59 [75]. Sec59 mutants stop dividing and become enlarged at the restrictive temperature of 37 °C [75,76]. The cells accumulate and express incompletely glycosylated secretory proteins at the restrictive temperature [77]. Additionally O-mannosylation and GPI anchor synthesis are almost completely blocked [75]. Analysis of dolichol-linked oligosaccharides in Sec59 deficient yeast showed not only decreased levels of lipid linked oligosaccharides, but also an altered LLO pattern with accumulation of $Man_{5-6}GlcNAc2$-P-P-Dol [76].

Dol-P levels in SEC59 cells are about 50% of wild type yeasts at the permissive temperature decreasing to less than 10% at the restrictive temperature. A consecutive increase of Dol was not observed [75].

In 2002, a mammalian *DK* gene was cloned from a mouse brain library [40]. At the same time the human *DK* gene was characterized [78]. The dolichol kinase is located in the ER with a CTP binding domain and dolichol kinase activity on the cytoplasmic site of the ER [79]. Using site-directed mutagenesis it could be shown that deleting the putative CTP binding site (AA459-474) or a mutation of selected conserved residues within the cytoplasmic loop significantly impairs or abolishes dolichol kinase activity [79]. Dolichol kinase is a key regulator of the Dol-P synthesis and therefore indirectly regulates the rate of protein glycosylation [80–82].

5. Glycosylation disorders in humans caused by dolichol phosphate synthesis or recycling defects

Currently only one disorder affecting the biosynthetic pathway or recycling of dolichol phosphate has been described.

5.1. Dolichol kinase deficiency

A deficiency of dolichol kinase, catalyzing the final step of dolichol phosphate synthesis was the first defect of the Dol-P pathway known to cause a severe hypoglycosylation phenotype in humans. We could describe four patients from two unrelated families suffering from a dolichol kinase defect named CDG-Im. IEF of serum transferrin showed a severe hypoglycosylation. A decrease in the activity of glycosylated clotting factor, typical for CDG I, was present. All patients died within the first year of life [83].

Both pairs of siblings were of consanguineous ancestry, one of German, the other of Turkish origin. Length, weight and head circumference were normal at birth but secondary microcephaly, developing in the first month of life was a common finding in all patients. Dry, ichthyosiform or parchmentlike skin was present in every child and a lack of hair growth or progressive hair loss was noticed partly involving eyebrows and eyelashes. One child developed seizures with hypsarrhythmia in the EEG. Muscular hypotonia was common and severe. Two children had progressive dilative cardiomyopathy, one child suffered from recidivating hypoglycemia. The children died at a mean age of 6 months.

Two different missense mutations (c.295T>A; p.99Cys>Ser and c.1322A>C; p.441Tyr>Ser) were found in the patients' DK1 gene and dolichol kinase activity in dermal fibroblasts was reduced to fewer than 5% of healthy controls. Mutated alleles were unable to complement a yeast stain mutant in dolichol kinase. Since Dol-P is of special importance for the transport of Man and Glc into the ER, one would expect that fibroblast of CDG-Im show truncated dolichol-linked oligosaccharides between the assembly of $Man_5GlcNAc_2$-P-P-Dol and $Glc_3Man_5GlcNac_2$-P-P-Dol. However, labeling of patients' fibroblasts showed no significant alteration in the dolichol linked oligosaccharide pattern while the amount of dolichol linked oligosaccharides was decreased [83].

6. Conclusions and future perspective

Dolichol phosphate is an essential carrier of oligosaccharides during the synthesis of N-glycans, GPI-anchor synthesis and protein O- and C-mannosylation. Since a total lack of glycosylation is not compatible with life in yeast and mammals, biochemical defects completely disabling Dol-P synthesis are not to be expected in humans. Generally defects affecting the de novo synthesis have to be differentiated from defects affecting the recycling of Dol-P.

Currently only one defect primarily affecting Dol-P synthesis has been described. A deficiency of dolichol kinase, the latest step of the de novo synthesis of Dol-P causes a severe disorder belonging to the group of congenital disorders of glycosylation with death of all four described patients within the first year of life. The de novo synthesis of Dol-P shares several metabolic steps with other important metabolic pathways including cholesterol, ubiquinone, geranyl and farnesyl

synthesis. Thus early defects are expected to cause severe phenotypes or embryonic lethality.

It can be assumed, that in the future, further defects affecting the de novo synthesis and the recycling of Dol-P will be detected revealing more clues about the complex impact on glycosylation and membrane organization due to a lack of dolichol phosphate.

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