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# Petri Nets Representation and Analysis of the Synthesis of Dolichol-Linked Precursor of N-Glycans

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Synthesis of dolichol-linked precursor of N-glycans is a complex biological system with numerous interdependent processes. Modeling this process is indispensable if we want to analyze this system for potential problems. We used Petri nets mathematical formalism to construct the synthesis of dolichol-linked precursor of N-glycans. Our analysis show that Dol-P is a critical point, but reduced levels of this substrate can be compensated by the oxidative pathway involving dolichol [9], from Dol pool by phosphorylation (EC 2.7.1.108) or PP-Dol pool by dephosphorylation (EC 3.6.1.4). Reactions of dolichol pathway can be controlled at least at three places by availability of substrates Man-GDP [6], Man (β) – P – Dol [5] and Glc(β) – P – Dol. Reduced levels of proteins with consensus Asn-X-Ser or Asn-X-Thr sites, through lack of essential amino acids, can also be a bottleneck in the synthesis.

*Key words:* petri net, glycosylation.

## Introduction

The process of N-linked glycosylation starts with the formation of dolichol-linked sugar precursor [3]. Sugar molecules are attached to the dolichol through a pyrophosphate linkage and extended through the addition of various sugar molecules to form a precursor oligosaccharide. The assembly of this precursor oligosaccharide occurs in two stages: first stage, which takes place on the cytoplasmic side of the ER, and second stage, which takes place on the luminal side of the ER [2]. Steps involved in stage 1 include addition of two UDP-GlcNAc residues to the dolichol molecule followed by addition of five GDP-Man residues. After stage 1 the lipid-linked glycan is translocated across the membrane into the ER lumen. On the luminal side of the ER membrane 4 mannose and 3 glucose monosaccharides are added. The final product is dolichol - GlcNAc2 - Man9-Glc3. Once the precursor oligosaccharide is formed, the completed glycan is transferred to the newly formed polypeptides in the lumen of the ER membrane [10]. For protein glycosylation completed glycan is attached to asparagine located in a specific consensus sequence in the primary structure (Asn-X-Ser or Asn-X-Thr) [1]. Oligosaccharyltransferase is responsible for the recognition of the consensus sequence and the transfer of the precursor glycan to a polypeptide acceptor.

## Materials and Methods

The main source of pathway information was N-Glycan biosynthesis pathway represented in KEGG/ENZYME database. Bipartite Petri graphs $G = (V_1, V_2; E)$ were constructed from two disjoint sets of nodes, called places ($V = P$) and transitions ($V = T$), $V_1 \cup V_2 = V$, which are connected by edges $e \in E \subseteq V$. The input range $I(x)$ of an element $x \in P \cup T$ of a Petri net is given by $I(x) = \{y|(y, x) \in E\}$, the output range as $O(x) = \{y|(x, y) \in E\}$ – [7] and [8].

## Results

Dolichol is converted to dolichyl phosphate (Dol-P) by CTP:dolichol *O*-phosphotransferase (EC 2.7.1.108) as shown in **Fig. 1**. The next step is synthesis of *N,N*'-diacetylchitobiosyl-diphosphodolichol catalysed by *N*-acetylglucosaminyldiphosphodolichol *N*-acetylglucosaminyltransferase (EC 2.4.1.141). chitobiosyldiphosphodolichol β-mannosyltransferase (EC 2.4.1.142 ) catalyse synthesis of β-(1->4)-D - mannosylchitobiosyldiphosphodolichol. D-Man-α-(1->3)-D-Man-β-(1->4)-D-GlcNAc-β-(1->4)-D-GlcNAc-diphosphodolichol is obtained from GDP-D-mannose and D-Man-β-(1->4)-D-GlcNAc-β-(1->4)-D-GlcNAc-diphosphodolichol by EC 2.4.1.132. Next in the chain of synthesis is D-Man-α-(1->3)-[D-Man-α-(1->6)]-D-Man-β-(1->4)-D-GlcNAc-β-(1->4)-D-GlcNAc-diphosphodolichol, which is product of GDP-Man:Man2GlcNAc2-*PP*-dolichol α-1,6-mannosyltransferase (EC 2.4.1.257). Finally D-Man-α-(1->2)-D-Man-α-(1->2)-D-Man-α-(1->3)-[D-Man-α-(1->6)]-D-Man-β-(1->4)-D-GlcNAc-β-(1->4)-D-GlcNAc-diphosphodolichol is synthesised from 2 GDP-D-mannose and D-Man--α-(1->3)-[D-Man-α-(1->6)]-D-Man-β-(1->4)-D-GlcNAc-β-(1->4)-D-GlcNAc-diphosphodolichol by sucrose synthase (EC 2.4.1.13).

The next eight reactions of synthesys of dolichol-linked precursor are catalyzed by following enzymes: dolichyl-*P*-Man:Man5GlcNAc2-*PP*-dolichol α-1,3-mannosyltransferase (E.C. 2.4.1.258), dolichyl-*P*-Man:Man6GlcNAc2-*PP*-dolichol α-1,2-mannosyltransferase (E.C. 2.1.4.259), dolichyl-*P*-Man:Man7GlcNAc2-*PP*-dolichol α-1,6-mannosyltransferase (E.C. 2.4.1.260) and dolichyl-*P*-Man:Man8GlcNAc2-*PP*-dolichol α-1,2-mannosyltransferase (E.C. 2.4.1.261), which add dolichyl β-D-mannosyl phosphate to various intermediate products.

In the next three reactions dolichyl-*P*-Glc:Man9GlcNAc2-*PP*-dolichol α-1,3-glucosyltransferase (E.C. 2.4.1.267), dolichyl-*P*-Glc:Glc1Man9GlcNAc2-*PP*-dolichol α-1,3-glucosyltransferase (E.C. 2.4.1.265) and dolichyl-*P*-Glc:Glc2Man9GlcNAc2-*PP*-dolichol α-1,2-glucosyltransferase (E.C. 2.4.1.256) add dolichyl β-D-glucosyl phosphate to obtain the dolichol-linked precursor of glycosylation. This precursor is transferred to protein L-asparagine with the help of dolichyl-diphosphooligosaccharide:protein-L-asparagine oligopolysaccharidotransferase (E.C. 2.4.1.119) to obtain glycoprotein with an oligosaccharide chain attached by *N*-glycosyl linkage to protein L-asparagine.

## Discussion

Analysis of **Fig. 1** shows that Dol-P is a critical point of synthesis of dolichol-linked precursor of N-glycans. Reduced levels of dolichol and its derivative Dol-P affect N-glycosylation of proteins and results in a congenital disorders of glycosylation type I (CDG I)[4]. SRD5A3 is required for converting polyprenol to dolichol and is mutated

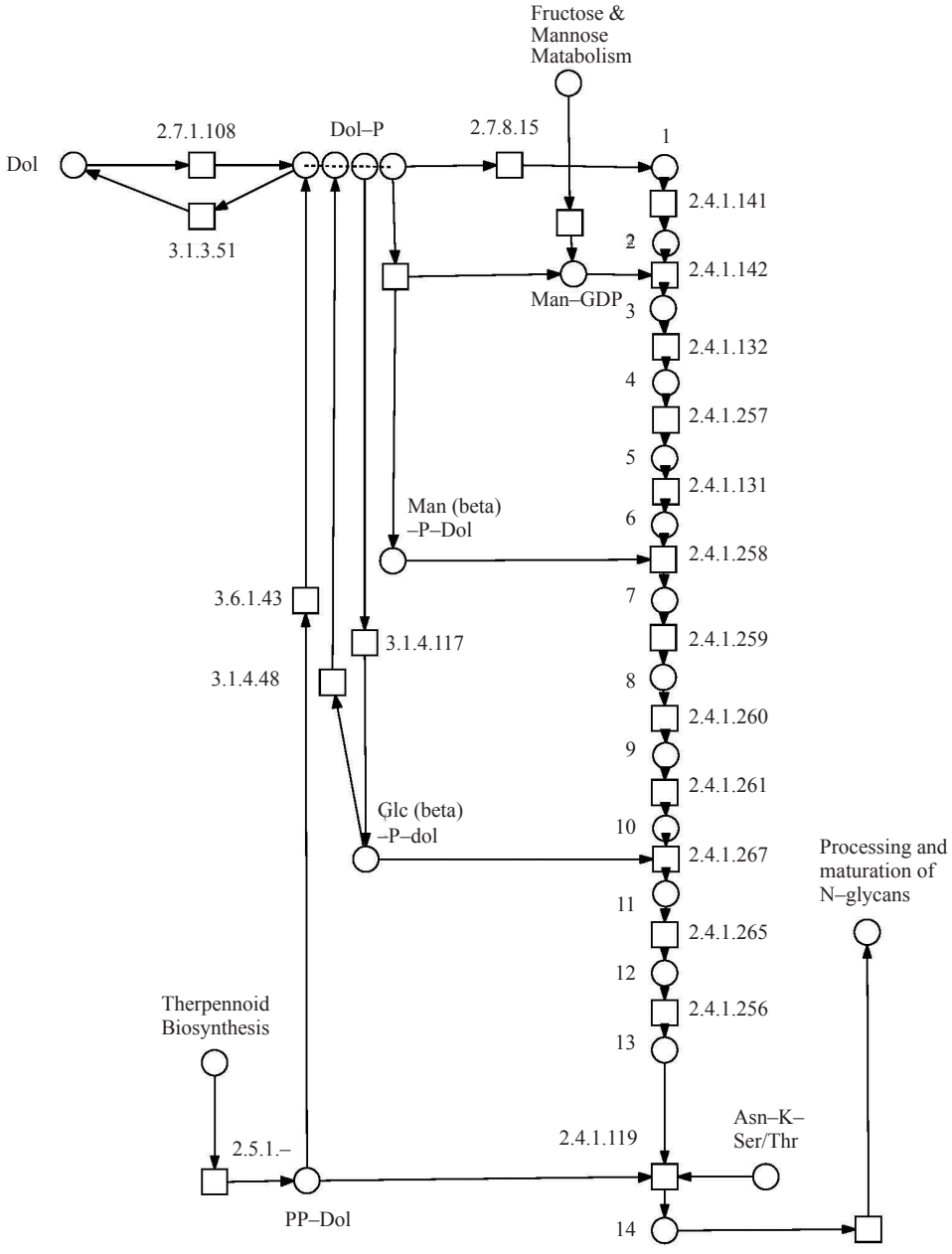


**Fig. 1.** Petri Net presentation of synthesis of dolichol-linked precursor of N-glycans

Legend: *circles represent metabolites and squares represent transitions according to accepted convention. Enzymes are represented according to their number in IUBMB Enzyme Nomenclature.*

in a congenital glycosylation disorder [7]. An alternative pathway for dolichol synthesis must be present, to avoid such disorders. For example, the oxidative pathway involving dolichol could play such a role [9]. Dol-P is precursor for *N*-acetyl-D-glucosaminyl-di-

phosphodolichol, Man (beta)-P-Dol and Glc(beta)-P-Dol. Dol-P can also be consumed by dolichyl-phosphate phosphohydrolase.

Dol-P is replenished from Dol pool by phosphorylation (EC 2.7.1.108) or PP-Dol pool by dephosphorylation (EC 3.6.1.4). PP-Dol comes either from therpenoid biosynthesis or as byproduct after transfer of dolichyldiphosphooligosaccharide to protein L-asparagine. Another source of Dol-P may be Gcl(beta)-P-Dol through the action of dolichyl-β-D-glucosyl-phosphate dolichylphosphohydrolase (EC 3.1.4.48).

These series of reactions can be controlled at least at three places by availability of substrates Man-GDP [6], Man (β) – P – Dol [5] and Glc(β) – P – Dol. Man-GDP is substrate for 2.4.1.142 and lack or low quantities of this substrate can block or impede all subsequent 10 enzymatic reactions and lead to different disorders.

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