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Chapter Title	Dolichyl-Phosphate (UDP-*N*-Acetylglucosamine) *N*-Acetylglucosaminephospho transferase 1 (GlcNAc-1-P Transferase) (DPAGT1)	
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Abstract	In eukaryotes, *N*-linked protein glycosylation starts with the synthesis of a highly conserved lipid-linked oligosaccharide (LLO) on the endoplasmic reticulum (ER) membrane. As the committed process of *N*-glycosylation, 14 monosaccharide residues are sequentially transferred onto dolichyl pyrophosphate (dol-P) carrier molecule by a series of glycosyltransferases (GTase) to form the core oligosaccharide precursor $Glc_3Man_9GlcNAc_2$-P-P-dol. The first half of GTase reactions in LLO synthesis takes place on the cytoplasmic face of ER (Fig. 124.1), which produces $Man_5GlcNAc_2$-P-P-dol intermediate from the soluble nucleotide sugar substrates uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc) and guanosine diphosphate D-mannose (GDP-man). Once this intermediate is flipped into the lumen of the ER, the next seven sugars are added from dolichol-sugar substrates Man-P-dol and Glc-P-dol to complete the assembly. Another half of GTase catalyzes these reactions (reviewed in Helenius et al. 2004; Kelleher and Gilmore 2006; Weerapana and Imperiali 2006). *DPAGT1* encodes dolichyl-phosphate (UDP-*N*-acetylglucosamine) *N*-acetylglucosaminephosphotransferase 1 that catalyzes the first reaction of LLO synthesis, by adding GlcNAc-1-P from cytoplasmic UDP-GlcNAc to dol-P. *DPAGT1* is essential for *N*-linked protein glycosylation and considered to be a key regulator among the metabolic pathway of protein *N*-glycosylation (Lehrman 1991).

124 Dolichyl-Phosphate (UDP-*N*-Acetylglucosamine) *N*-Acetylglucosaminephospho-transferase 1 (GlcNAc-1-P Transferase) (DPAGT1)

Neta Dean and Xiao-Dong Gao

Contents

Introduction

In eukaryotes, *N*-linked protein glycosylation starts with the synthesis of a highly conserved lipid-linked oligosaccharide (LLO) on the endoplasmic reticulum (ER) membrane. As the committed process of *N*-glycosylation, 14 monosaccharide residues are sequentially transferred onto dolichyl pyrophosphate (dol-P) carrier molecule by a series of glycosyltransferases (GTase) to form the core

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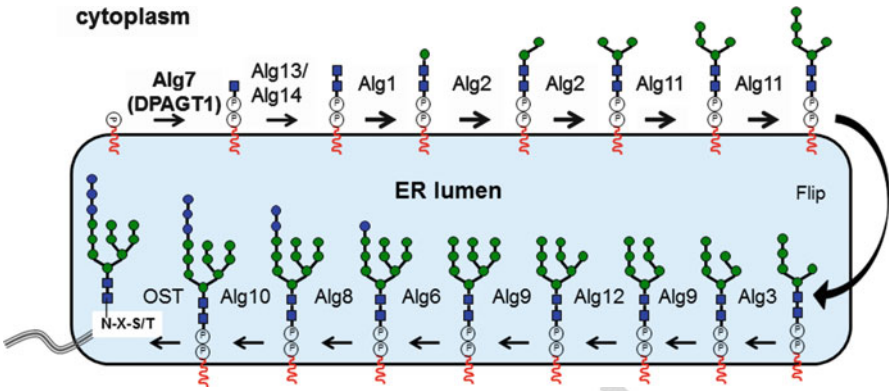

Fig. 124.1 Biosynthetic pathway of lipid-linked oligosaccharides on ER membrane. Shown is a schematic depiction of the sequential assembly of the 14 sugars $Glc_3Man_9GlcNAc_2$ on dolichol pyrophosphate by the Alg (asparagine-linked glycosylation) glycosyltransferases. Seven sugars are added on the cytoplasmic face, and then another seven in the lumen. DPAGT1 (also known as Alg7 glycosyltransferase) catalyzes the first reaction of the assembly, in which GlcNAc-1-P-Dol from UDP-GlcNAc is added to dolichol phosphate (P-Dol) to generate GlcNAc-PP-Dol. The Alg13/Alg14 heterodimeric UDP-GlcNAc transferase then adds the second GlcNAc from UDP-GlcNAc to generate $GlcNAc_2$-PP-Dol. The next five mannoses are added sequentially using GDP-man by the Alg1, Alg2, and Alg11 mannosyltransferases, respectively, to generate Man_5Glc_2-PP-Dol. This intermediate flips into the lumen, where it is further extended by an additional four mannose and three glucose residues from dolichol-linked sugars. Oligosaccharyltransferase transfers this "core" oligosaccharide from dolichol to nascent polypeptides

oligosaccharide precursor $Glc_3Man_9GlcNAc_2$-P-P-dol. The first half of GTase reactions in LLO synthesis takes place on the cytoplasmic face of ER (Fig. 124.1), which produces $Man_5GlcNAc_2$-P-P-dol intermediate from the soluble nucleotide sugar substrates uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc) and guanosine diphosphate D-mannose (GDP-man). Once this intermediate is flipped into the lumen of the ER, the next seven sugars are added from dolichol-sugar substrates Man-P-dol and Glc-P-dol to complete the assembly. Another half of GTase catalyzes these reactions (reviewed in Helenius et al. 2004; Kelleher and Gilmore 2006; Weerapana and Imperiali 2006). *DPAGT1* encodes dolichyl-phosphate (UDP-*N*-acetylglucosamine) *N*-acetylglucosaminephosphotransferase 1 that catalyzes the first reaction of LLO synthesis, by adding GlcNAc-1-P from cytoplasmic UDP-GlcNAc to dol-P. *DPAGT1* is essential for *N*-linked protein glycosylation and considered to be a key regulator among the metabolic pathway of protein *N*-glycosylation (Lehrman 1991).

Databanks

IUBMB enzyme nomenclature: EC 2.7.8.15

Dolichyl-phosphate (UDP-*N*-acetylglucosamine) *N*-acetylglucosaminephosphotransferase 1 (GlcNAc-1-P transferase) (DPAGT1)

Species	Gene symbol	GenBank accession number	UniProt ID	PDB accession number
Homo sapiens	*DPAGT1*	Z82022	9QH3H5	N/A
Saccharomyces cerevisiae	*ALG7*	Z36112	P07286	N/A

Name and History

Dolichyl-phosphate (UDP-*N*-acetylglucosamine) *N*-acetylglucosaminephosphotransferase 1 transfers GlcNAc-P from cytosolic UDP-GlcNac to dol-P on the ER membrane to produce GlcNAc-P-P-dol. As shown in Fig. 124.1, this enzyme is responsible for the first step of LLO biosynthesis. The enzyme activity was first demonstrated from a solubilized membrane fraction from pig aorta (Heifetz et al. 1977). Tunicamycin, an antibiotic isolated from *Streptomyces lysosuperficus*, is a potent inhibitor of DPAGT1 and has been used for identification of *DPAGT1* genes. The first tunicamycin-resistance gene was identified in yeast (Rine et al. 1983) and found to correspond to *ALG7* (Barnes et al. 1984). The *DPAGT1* gene (commonly referred to as *ALG7*) has been also cloned from *Schizosaccharomyces pombe* (Zou et al. 1995), *Leishmania amazonensis* (Liu and Chang 1992), mouse (Rajput et al. 1992), CHO cells (Zhu and Lehrman 1990), and humans (Eckert et al. 1998). The first purification of DPAGT1 has been done from the lactating mammary gland of bovine (Shailubhai et al. 1988).

Structure

Hamster DPAGT1 consists of 408 amino acids and is predicted to have ten membrane-spanning domains (Zhu and Lehrman 1990). Its topological features on ER membrane have been further elucidated (Dan et al. 1996). The largest hydrophilic loop between the two C-terminal transmembrane spans, which contains a number of conserved residues essential for DPAGT1 activity, is identified to the cytosolic face. Results demonstrated a cytoplasmic orientation of DPAGT1 consisting with the idea that assembly of GlcNAc-P-P-dol happens on the cytosolic side of ER membrane. DPAGT1 in vivo exists as a dimer and/or a higher-order structure. In addition to chemical cross-linking studies that reveal the dimeric form of DPAGT1 (Dan and Lehrman 1997) in mouse cell lysates, biochemical and genetic analyses demonstrate that in yeast, Alg7 interacts with the heterodimeric Alg13/14 UDP-GlcNAc transferase that catalyzes the second step of LLO biosynthesis (Noffz et al. 2009).

Enzyme Activity Assay and Substrate Specificity

This enzyme catalyzes the following reaction: UDP-*N*-acetyl-D-glucosamine + phosphodolichol <=> *N*-acetyl-D-glucosaminyl-diphosphodolichol + UMP.

This reaction requires divalent metals such as Mg^{2+} or Mn^{2+} (Kean 1983; Kaushal and Elbein 1985) and is activated by dol-P-Man and phosphatidylglycerol (Kean 1985; Kaushal and Elbein 1985). Tunicamycin is a well-known inhibitor of DPAGT1 and has been a useful reagent for the study of this enzyme. In addition, the reaction product (i.e., GlcNAc-P-P-dol) as well as the product of the following reaction (i.e., GlcNAc-GlcNAc-P-P-dol) inhibits enzyme activity, suggesting feedback regulation by its product (Kean et al. 1999). UDP-GlcNAc-1-P transferase activity is assayed by using dol-P as acceptor and UDP-[^{14}C]GlcNAc as the donor. The reaction contained in a final volume of 0.06 ml: 28 mM *tris*–HCl, pH 7.4, 10 mM $MgCl_2$, 0.7 M NaCl, 0.7 mM DTT, 0.3 % Nonidet P40, 3.5 mM diheptanoyl phosphatidylcholine, 23 % glycerol, 0.05 mCi UDP-[^{14}C]GlcNAc, 2 mg dol-P, and solubilized enzyme (equivalent to 0.2 mg membrane protein). Lipid-linked products are extracted with chloroform/methanol and analyzed by thin layer chromatography (Würde et al. 2012).

Preparation

DPAGT1 is found in all eukaryotes. Like other membrane-associated LLO GTases, UDP-GlcNAc-1-P transferase activity is found in the ER microsomal membrane fraction and can be solubilized by extraction with neutral detergents such as Triton X-100 and Nonidet P-40. Because of its purported instability, the enzyme should be stabilized and activated by phospholipids such as phosphatidylglycerol and dolichol phosphate (Plouhar and Bretthauer 1982). There is a report documented, a purification of DPAGT1 from the lactating mammary gland of bovine (Shailubhai et al. 1988). Bovine DPAGT1 can be purified from solubilized microsome preparations with 0.25 % Nonidet P-40 using $(NH_4)_2S0_4$ precipitation followed by gel filtration, anion exchange chromatography, and hydroxylapatite chromatography.

Biological Aspects

The DPAGT1 initiates the protein *N*-glycosylation by catalyzing the synthesis of GlcNAc-P-P-dol. However, its substrates, UDP-GlcNAc and dolichol phosphate, are also required for protein *O*-glycosylation, *O*-GlcNAcylation, the biosynthesis of glycosylphosphatidylinositol (GPI) anchors and proteoglycans. Because of its unique feature, DPAGT1 can be considered to be the key regulator of cellular glycoprotein biosynthesis. *DPAGT1* gen has been found in all eukaryotes from yeast to human cells. Human *DPAGT1* can complement an *alg7* conditional yeast mutant (Eckert et al. 1998), demonstrating the high functional conservation throughout evolution. Mutations in human *DPAGT1* cause the congenital disorder

of glycosylation CDG-Ij (DPAGT1-CDG) (Freeze 2006). As in yeast, mammalian *ALG7* (*DPAGT1*) is essential since mice deleted for DPAGT1 are embryonic lethal (Rine et al. 1983; Marek et al. 1999).

Knockout and Transgenic Mice

Embryos with knocked out *DPAGT1* gene can complete development through the morula and blastocyst stages but die shortly after uterine implantation, demonstrating the essential role of this gene in early embryogenesis (Marek et al. 1999).

Human Disease

As the first enzyme of LLO synthetic pathway, defects of the human *DPAGT1* cause CDG-Ij (DPAGT1-CDG). There are four clinical reports of five patients suffering this CDG (Wu et al. 2003; Würde et al. 2012; Timal et al. 2012; Carrera et al. 2012). Patients described had very severe clinical manifestations including muscular hypotonia, intractable seizures, developmental delay, mental retardation, and microcephaly. Most of them died within the first years of life.

It has been reported that mutations in *DPAGT1* also cause a Limb-Girdle congenital myasthenic syndrome (CMS) (Belaya et al. 2012), an inherited disorder of neuromuscular transmission characterized by muscle weakness (Engel et al. 2012). Symptoms of the patients are limited to neuromuscular function similar to those of CMSs due to defects in glutamine-fructose-6-phosphate transferase 1 (GFPT1), which is involved in the synthesis of UDP-*N*-acetylglucosamine (see the chapter describing *GFPT1*). It is Au2 not yet clear why mutations in *DPAGT1* lead to the development of CMSs without showing other nonmuscle abnormalities characteristic of DPAGT-CDGs.

DPAGT1 gene is a target of the canonical Wnt/b-catenin signaling pathway (Sengupta et al. 2010). Partial inhibition of *DPAGT1* reduces Wnt signaling, while overexpression leads to aberrant *N*-glycosylation of E-cadherin. Hypoglycosylated E-cadherin affects the stability of cadherin-mediated cell-cell adhesion and inhibits Wnt signaling and *DPAGT1* expression (Nita-Lazar et al. 2009; Jamal et al. 2012). Such cross talk among the *DPAGT1*/*N*-glycosylation, Wnt signaling and E-cadherin adhesion is a key mechanism underlying squamous cell carcinoma (OSCC), suggesting *DPAGT1* may represent an effective target for oral cancer therapy.

Future Perspectives

For the past few decades, enormous work has been done to elucidate the structure, function, and regulation of this DPAGT1. In the post-genomic era, growing evidence has implicated DPAGT1 in various human diseases, such as CDGs, CMSs, and oral cancer. Knowing the secondary effects of protein *N*-glycosylation in other

regulatory pathways of the cell represents a big challenge. It will be of interest to understand the molecular details of how DPAGT1 regulates the cross talk between protein *N*-glycosylation and other cell essential pathways.

Cross-References

► Heterodimeric Alg13/Alg14 UDP-GlcNAc Transferase (ALG13,14)

Further Reading

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Sengupta et al. (2010): First report describing the interactions among canonil Wnt signaling, DPAGT1 and E-cadherin.
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