Heterodimeric Alg13/Alg14 UDP-GlcNAc transferase

Introduction

As one of the most frequent and common posttranslational modifications in eukaryotic cell, protein *N*-glycosylation begins with the multistep biosynthesis of an evolutionarily conserved lipid-linked oligosaccharide (LLO), which is extended by a series of membrane-associated Alg (asparagine-linked glycosylation) glycosyltransferases to produce $Glc_3Man_9GlcNAc_2$-PP-dolichol (dol) in the endoplasmic reticulum (ER). As shown in figure 1, assembly of the first seven sugars in the LLO synthetic pathway takes place on the cytoplasmic face of the ER membrane, and is catalyzed by cytosolic glycosyltransferases that produce $Man_5GlcNAc_2$-PP-dol from the soluble nucleotide sugar substrates UDP-GlcNAc and GDP-man. This dol-linked intermediate then flips into the lumen of the ER where the next seven sugars are added from dol-linked sugar substrates to complete the assembly of the tetradecasaccharide on dolichol diphosphate. An UDP-*N*-acetylglucosamine transferase (accepted name: *N*-acetylglucosaminyldiphosphodolichol *N*-acetylglucosaminyltransferase) catalyzes the second step of LLO synthesis that adds *N*-acetylglucosamine (GlcNAc) to GlcNAc-pp-dol to produce $GlcNAc_2$-PP-dol. Unlike any other Alg glycosyltransferases, this UDP-GlcNAc transferase is unique because it comprised of two subunits, Alg13 and Alg14 proteins (Figure 1). Alg13 and Alg14 homologous can be found in virtually all eukaryotic cells including man, mammals, insects, worms, plants and fungi. In yeast, both Alg13 and Alg14 subunits are essential for cell viability, suggesting the importance of heterodimeric Alg13/Alg14 UDP-GlcNAc trasferase (reviewed in: Helenius et al. 2004; Kelleher and Gilmore 2006; Weerapana and Imperiali 2006).

Databanks

IUBMB enzyme nomenclature: EC 2.4.1.141

Species	Gene symbol	Genbank accession number	Uniprot ID	PDB accession number
Saccharomyces cerevisiae	ALG13	Z72569	P53178	N/A
Saccharomyces cerevisiae	ALG14	Z35939	P38242	N/A
Homo sapiens	ALG13	BC005336	Q9NP73	2jzc
Homo sapiens	ALG14	BC011706	Q96F25	N/A

Name and History

In LLO synthetic pathway, *N*-acetylglucosaminyldiphosphodolichol *N*-acetylglucosaminyltransferase catalyzes the transfer of a β1, 4-linked GlcNAc from UDP-GlcNAc to GlcNAc-pp-dol to produce $GlcNAc_2$-PP-dol. Early purification and biochemical studies of this enzyme have been performed by using yeast (Sharma et al. 1982) and mung bean seedling (Kaushal and Elbein. 1986), and lately, its substrate specificity was tested against membrane fraction of CHO cells (McLachlan and Krag. 1994) and crude microsomal membranes prepared from pig liver (Vincent et al. 2001). Because of the unusual heterodimeric structure, it has been taken quite long time to identify the *ALG* genes, which encored this UDP-GlcNAc transferase. Five years after all other 12 *ALG* genes have been cloned, two *S. cerevisiae* ORFs (*YGL047w* and *YBR070c*) were eventually found responsible for the activity (Chantret et al. 2005; Gao et al. 2005; Bickel et al. 2005). Unlike any other eukaryotic ER glycosyltransferases, Ygl047w, which was designated as Alg13, is predicted to contain a consensus glycosyltransferase catalytic domain but lucks any predicted membrane-spanning domains. The other polypeptide, Ybr070c, which was designated as Alg14, contains at least one predicted membrane-spanning domain but lacks any sequences that are predicted to participate in sugar transfer. Bioinformatics approach demonstrated that Alg13 and Alg14 shear their structural homology with the bacterial MurG protein (Chantret et al. 2005). MurG is an UDP-GlcNAc undecaprenyl-PP-MurNAc pentapeptide: *N*-acetylglucosaminyl transferase that plays an essential role in peptidoglycan biosynthesis of *Escherichia coli* (Mengin-Lecreulx et al. 1991), and has a distinct two-domain structure (Ha et al. 2000). Alg13 is homologous to the C-terminal catalytic domain of MurG, while Alg14 is homologous to its N-terminal domain (Gao et al. 2008; Wang et al. 2008). Remarkably, homologous of X-linked genes *ALG13* and *ALG14* have been confirmed in virtually all eukaryotic cells.

Structure

Alg13 and Alg14 were first identified *in silico* by their structural homology to MurG protein. MurG belongs to the GT-B glycosyltransferases (GTases) superfamily (Bourne and Henrissat. 2001). Crystal structure of MurG reveals two distinct domains, each of which contains several Rossmann folds that are postulated to be involved nucleotide sugar binding and transfer, as well as lipid acceptor recognition (Ha et al. 2000, Hu et al. 2003). Alg13 protein contains the conserved catalytic domain, found in the C-terminal domain of MurG. NMR structure of yeast Alg13 (2jzc) retains typical GT-B structural characteristics in its C-terminal half but the N-terminal half of the protein contains a Rossmann-like fold with a mixed parallel and antiparallel β sheet rather than the conventional Rossmann fold found in all GT-B enzymes, indicating a unique topology among glycosyltransferases (Wang et al. 2008). Alg14

contains the predicted lipid acceptor and a membrane-associating domain, found in the N-terminal domain of MurG (Chantret et al. 2005). However, eukaryotic Alg14 possesses an evolved N-terminal region that is missing in bacterial orthologs (Lu et al. 2012).

Alg14 is a membrane protein that recruits the cytosolic Alg13 protein to the ER to form a hetero-oligomeric complex that catalyzes the biosynthesis of $GlcNAc_2$-PP-Dol (Gao et al. 2005). A transmembrane domain involved in the evolved N-terminal region of Alg14 contributes to its localization to the ER membrane. In addition, there is a second membrane association site contains a conserved amphiphilic-like α-helix located in the central part of Alg14, which mediates an integral interaction with the ER (Lu et al. 2012). Alg13 contains the catalytic domain of the UDP-GlcNAc transferase, but cytosolic soluble Alg13 is not active unless bound to Alg14 at the ER membrane, suggesting the formation of the Alg13/14 complex is crucial for UDP-GlcNAc transferase activity (Bickel et al. 2005). Complex formation is mediated by a short C-terminal α-helix of Alg13 in cooperation with the last three amino acids of Alg14 (Gao et al. 2008).

Enzyme Activity Assay and Substrate Specificity

This enzyme catalyzes the following reaction: UDP-*N*-acetyl-D-glucosamine + *N*-acetyl-D-glucosaminyl -diphosphodolichol <=> UDP + *N*,*N*'-diacetylchitobiosyl-diphosphodolichol. The reaction was stimulated by the addition of divalent metals such as Mg^{2+} and Ca^{2+} (Sharma et al. 1982), but uridine nucleotides, especially UDP and UDP-Glc, were considered to be inhibiters (Kaushal and Elbein. 1986). Substrate specificity of the enzyme was tested by using four unnatural dolichol diphosphate monosaccharides with the C-2 acetamido group in the natural substrate Dol-PP-GlcNAc replaced by fluoro, ethoxy, trifluoroacetamido, and amino functionalities. Results demonstrated that enzyme fraction prepared from pig liver was found highly specific for its glycosyl acceptor and the acetamido group shown to be a key functional determinant for UDP-GlcNAc transferase reaction (Vincent et al. 2001).

The method for assaying UDP-GlcNAc transferase activity in solubilized extracts obtained from a microsomal membrane fraction of fibroblasts was recently developed using [^{14}C]$GlcNAc_1$-PP-Dol as acceptor and UDP-GlcNAc as donor. The standard reaction mixture contains the following in a final volume of 0.06 ml: 27 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 0.8 mM dithiothreitol, 27% glycerol, 0.45% NP-40, 0.2 mM UDP-GlcNAc and [^{14}C]$GlcNAc_1$-PP-Dol (3000 cpm), and solubilized membrane fractions (equivalent to 0.1 mg membrane protein). After incubation at 24 °C for the indicated time, the reaction was stopped by chloroform/methanol with a ratio of chloroform/methanol/water of 3/2/1. Then, $GlcNAc_{1/2}$-PP-Dol was extracted and analyzed by TLC as described above. Radioactivity was detected by phosphorimaging or x-ray film (Timal et al. 2012).

Preparation

UDP-GlcNAc transferase activity has been documented in *in vitro* experiments from different sources such as yeast cells (Sharma et al. 1982), CHO cells (McLachlan and Krag. 1994), mung bean seedling (Kaushal and Elbein. 1986), porcine aorta (Heifetz and Elbein. 1977), hen oviduct (Chen and Lennarz. 1977), rat liver (Leloir and Behrens. 1973) and pig liver (Vincent et al. 2001). Like other membrane-binding glycosyltransferases, UDP-GlcNAc transferase activity is found in the microsomal membrane fraction and solubilized by extraction with neutral detergent such as Triton X-100 and Nodidet P-40.

Biological Aspects

Alg13 and Alg14 comprise a heterodimeric UDP-*N*-acetylglucosamine transferase (EC 2.4.1.141) that adds the second GlcNAc moiety to the growing lipid-linked oligosaccharide (LLO) on the cytosolic side of the ER. Both A*LG13* and *ALG14* genes are essential for cell growth. Cells in which *ALG13* or *ALG14* expression has been repressed exhibit slow growth and defective protein glycosylation and accumulate $GlcNAc_1$-PP-Dol (Chantret et al. 2005). In heterodimeric complex, Alg13p is the catalytic subunit, which lacks any hydrophobic membrane-spanning domain. Its localization to the ER membrane requires interaction with the integral ER membrane protein Alg14. Consistent with this model, over-expression of *ALG13* or attenuation of *ALG14* causes Alg13 protein to be partitioned into the cytoplasm. Co-expression of the human homologs of *ALG13* and *ALG14* can functionally complement the loss of either *ALG13* or *ALG14*, but neither individual human gene can complement deletion of its yeast homolog because the yeast and human proteins fail to properly pair with each other (Gao et al. 2005). Membrane protein Alg14 also interacts with Alg7, the GlcNAc-1-phosphate transferase that catalyzes the first step of LLO pathway, to form a heterooligomeric glycosyltransferase complex (Noffz et al. 2009). The evolved N-terminal TMD in Alg14 responses for this interaction, while Alg13 does not directly interact with Alg7 (Lu et al. 2012). These findings implicate Alg14 as the core coordinator in recruitment of the catalytic partners Alg7 and Alg13 to ER membrane to initiate *N*-glycosylation.

Knockout and Transgenic mice

There is no report regarding *ALG13* or *ALG14* gene knockout and transgenic mice.

Human disease

The first ALG13-CDG (or CDG-Ir) was recently identified in a group of patients with CDG-I by using whole-exome sequencing (WES) technology (Bamshad et al. 2011) in combination with the knowledge of the protein *N*-glycosylation pathway for gene identification (Timal et al. 2012). A missense mutation (c.280A > G; p.Lys94Glu) was found in *ALG13* gene. Lysine 94 is located in the C-terminal glycosyltransferase domain of Alg13 and is fully conserved among eukaryotic cells down to yeast. The corresponding amino acid in yeast (lysine 153) is positioned at the C-terminal end of the α5 helix, which is thought to be involved in UDP-GlcNAc binding on the basis of structural comparison with the bacterial homolog MurG (Ha et al. 2000; Wang et al. 2008). The UDP-GlcNAc transferase activity in patient fibroblasts was severely reduced when compared with control with a residual activity of 17%. This ALG13-CDG patient represented a diverse clinical spectrum, and died in his infantile (Timal et al. 2012).

There is no report documenting the discovery of any CDG related to *ALG14*.

Future Perspectives

The unique heterodimeric nature of the Alg13p/ Alg14p UDP-GlcNAc transferase has raised the question of why this enzyme has evolved such an unusual configuration compared with other eukaryotic glycosyltransferases. This Alg13p/14 complex is a well-suited target for regulation of LLO assembly because it catalyzes one of the earliest steps in this pathway. One possible explanation is that this "split" subunit arrangement provides a mechanism to regulate its activity. It has been demonstrated that the cytosolic Alg13p catalytic subunit is a target for proteasomal degradation. Failure to degrade cytosolic excess Alg13p causes a glycosylation defect, supporting the model that the proteolytic degradation of unassembled Alg13p contributes to the regulation of *N*-glycosylation (Averbeck et al. 2008). Further experiments that investigate the degradation system of Alg13 are required for understand how an excess of Alg13p can inhibit glycosylation. An important future goal of the studies on this heterodimeric UDP-GlcNAc transferase will be to undercover the molecular mechanism for regulation of *N*-glycosylation.

Cross References

Dolichyl-phosphate (UDP-*N*-acetylglucosamine) *N*-acetylglucosaminephosphotransferase 1 (DPAGT1/Alg7)

Further Reading

Chantret et al. 2005; Gao et al. 2005; Bickel et al. 2005: The first cloning and characterization of Alg13/Alg14 complex

Wang et al. 2008: the first solution structure of Alg13

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