Structural Biology of Muscular Dystrophy by X-Ray Crystallography

The POMGnT1 is associated with congenital muscular dystrophy and encodes a glycotransferase which forms the core M1 glycan structure on α-dystroglycan. Another glycan structure, core M3, is essential to connect α-dystroglycan to the extracellular matrix. POMGnT1 consists of a stem and a catalytic domain, as observed in its X-ray crystal structure. The catalytic domain contributes to the formation of core M1 glycan, while the stem domain binds to premature core M3 glycan. Previously, it has been shown that POMGnT1 interacts with other enzymes that are required for further modification of core M3. Thus, POMGnT1 is essential for maturation of the core M3, and the loss of function of this enzyme causes muscular dystrophy.

Congenital muscular dystrophy (CMD) consists of a group of rarely inherited neuromuscular disorders in human. The genes responsible for its occurrence have been identified, and are related to addition of glycans to the proteins. The dystrophin glycoprotein complex physically connects the actin cytoskeleton to the extracellular matrix (Fig. 1). The complex is formed by β-dystroglycan (β-DG) and α-dystroglycan (α-DG), which is heavily glycosylated. The *O*-mannosyl glycans on α-DG are essential for its ability to bind to the extracellular matrix components. Presently, 13 CMD genes are thought to be involved in the glycan synthesis on α-DG [1] Mutations in the gene that encodes the protein *O*-linked mannose β1,2-N-acetylglucosaminyltransferase 1, POMGnT1, were initially linked to a specific type of CMD, muscle-eye-brain disease (MEB). Many CMD-related genes are involved in glycosylation of core M3, a specific glycan structure in α-DG. However, known function of POMGnT1 is synthesis of core M1, another glycan structure on α-DG. Thus, the role of POMGnT1 in glycosylation of core M3 remains unclear.

To elucidate the molecular mechanism by which POMGnT1 influences synthesis of core M1 and core M3, the X-ray crystal structures of POMGnT1 were solved using synchrotron beamlines BL-1A, BL-17A, AR-NE3A (Photon Factory, KEK, Japan), BL-32XU (SPring-8, Japan) and BL-15A (NSRRC, Taiwan) [2]. POMGnT1 consists of three regions: N-terminal transmembrane, stem, and catalytic regions. Since the transmembrane region frequently has bad influence upon protein production and crystallization, the structure without the N-terminal transmembrane region (amino acid residues of 92 ~ 646) was determined. The structure is formed by two domains (stem and catalytic) and a connecting linker region (Fig. 2).

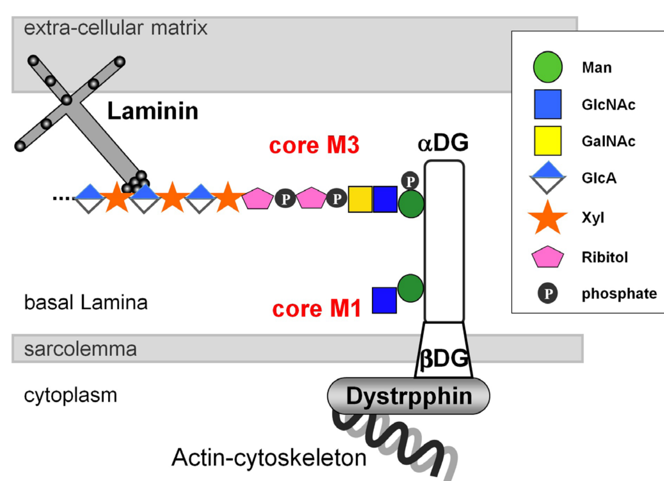

Figure 1: Dystrophin glycoprotein complex.

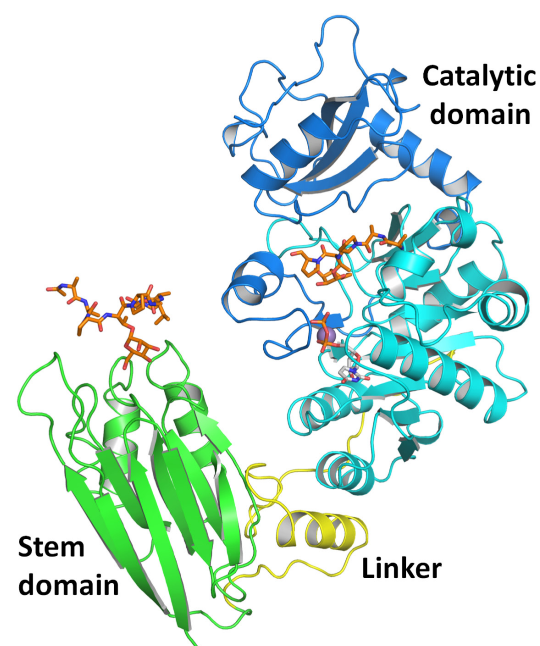

Figure 2: Crystal structure of POMGnT1 (residues 97 ~ 646) with Mn^{2+}-UDP (purple sphere and stick) and mannosylated peptide (orange stick).

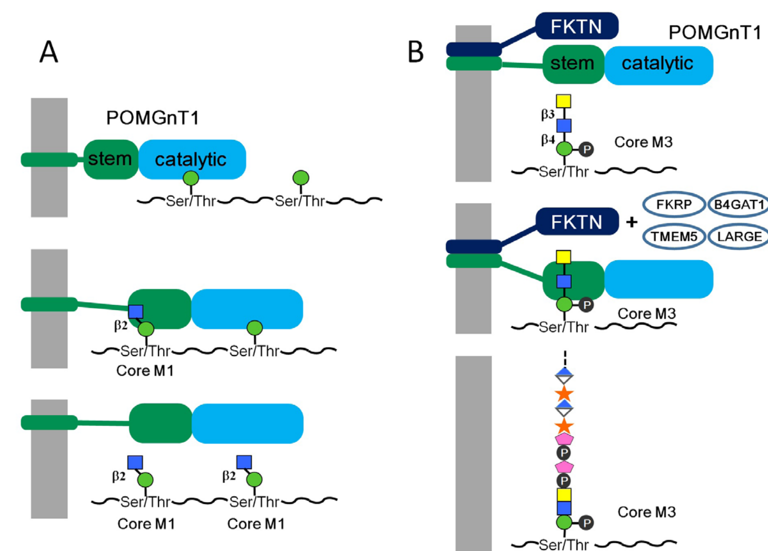

Figure 3: Model of POMGnT1 function in α-DG glycosylation. Gray boxes represent biological lipid membrane. α-DG is shown by wavy line and its mannosylation amino acid residues (Ser/Thr) are indicated.

The stem domain (residues 92 ~ 250) is composed of two stacked β-sheets and two α-helices. The fold resembles that of murine FAM3B/PANDER (PDB ID: 2YOQ), which molecular function remains elusive. The catalytic domain (residues 300 ~ 646) is composed of two structural motifs, a Rossmann-like fold (N-lobe, residues 300 ~ 497), and an open sheet α/β structure (C-lobe, residues 498 ~ 646), which are frequently found in nucleotide-binding proteins, including glycosyltransferases. The overall structure of the catalytic domain of POMGnT1 is similar to that of rabbit GnT I (PDB ID: 1FOA). There are no direct interactions between the stem and catalytic domains; however, both domains are in contact with the linker region (residues 251 ~ 299).

POMGnT1 catalyzes transfer of *N*-acetylglucosamine (GlcNAc) from UDP- GlcNAc to *O*-linked mannosylated protein substrate in the presence of bivalent cation. In addition to the *apo*-form structure, the Mn^{2+}, UDP, and mannosylated peptide-bound complex structure was determined. Since the amino acid residues in GnT I, which interact with UDP-GlcNAc are structurally conserved in POMGnT1, they share a common sugar transfer mechanism, as suggested by a study on GnT I [3]. A mannosylated peptide bound near the UDP-binding site is thought to be an acceptor substrate.

Another mannosylated peptide, found at the stem domain, is absolutely unexpected and its biological function is also unknown. To elucidate whether the stem domain really binds with saccharide in the solution, Frontal Affinity Chromatography and Surface Plasmon Resonance experiments were performed [2]. The stem domain binds with mannosylated peptide (Man-*O*-peptide) and GlcNAc-β1,2-Man peptide, which are the substrate and product of POMGnT1, respectively. In addition, the stem domain also binds with GalNAc-β1,3-GlcNAc-β-pNP, which mimics the core M3 structure.

Next, the structure of the stem domain which forms a complex with GalNAc-β1,3-GlcNAc-β-pNP or GlcNAc-β1,2-Man-peptide was determined. The saccharide derivative and the glycopeptide bind at nearly identical sites on the stem domain, as observed in the case of Mn^{2+}, UDP, and mannosylated peptide-bound complex. Thus, the stem domain recognizes core M3 as well as its enzymatic product.

These results provide a reasonable explanation for two issues that are related to formation of core M1 and modification of core M3 on α-DG. First, the interaction between the stem domain and an enzymatic product of POMGnT1 seems to facilitate clustering of core M1 (Fig. 3A). Second, the stem domain would recognize the core M3 structure. This interaction may recruit other enzymes that interact with POMGnT1, *e.g.*, FKTN, which is required for further modification of core M3 (Fig. 3B).

REFERENCES

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BEAMLINES

BL-1A, BL-17A and AR-NE3A

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