Genome-wide CRISPR screen reveals CLPTM1L as a lipid scramblase required for efficient glycosylphosphatidylinositol biosynthesis.

Glycosylphosphatidylinositols (GPIs) are complex glycolipids that act as membrane anchors of many eukaryotic cell surface proteins. Biosynthesis of GPIs is initiated at the cytosolic face of the endoplasmic reticulum (ER) by generation of N-acetylglucosaminyl phosphatidylinositol (GlcNAc-PI). The second intermediate, glucosaminyl-phosphatidylinositol (GlcN-PI), is translocated across the membrane to the luminal face for later biosynthetic steps and attachment to proteins (Figure A). The mechanism of the luminal translocation of GlcN-PI is unclear.

The research group of Taroh Kinoshita reported a genome-wide CRISPR knockout screen of genes required for rescuing GPI-anchored protein expression after addition of chemically synthesized GlcNAc-PI to PIGA-knockout cells that cannot synthesize GlcNAc-PI (Figure B).

They identified CLPTM1L (Cleft lip and palate transmembrane protein 1-like), an ER-resident multi-pass membrane protein, as a GlcN-PI scramblase required for efficient biosynthesis of GPIs. Knockout of CLPTM1L in PIGA-knockout cells impaired the efficient utilization of chemically synthesized GlcNAc-PI and GlcN-PI for GPI biosynthesis. Purified CLPTM1L scrambled GlcN-PI, GlcNAc-PI, PI, and several other phospholipids in vitro. CLPTM1L, a member of the PQ-loop family of proteins, represents a new type of lipid scramblase having no structural similarity to known lipid scramblases. Knockout of CLPTM1L in various wild-type mammalian cultured cells partially decreased the level of GPI-anchored proteins. These results suggest that CLPTM1L is the major lipid scramblase involved in cytosol-to-lumen translocation of GlcN-PI across the ER membrane for efficient GPI biosynthesis.

Journal: *Proc. Natl. Acad. Sci. USA*, 119 (14) e2115083119.
Title: Genome-wide CRISPR screen reveals CLPTM1L as a lipid scramblase required for efficient glycosylphosphatidylinositol biosynthesis.
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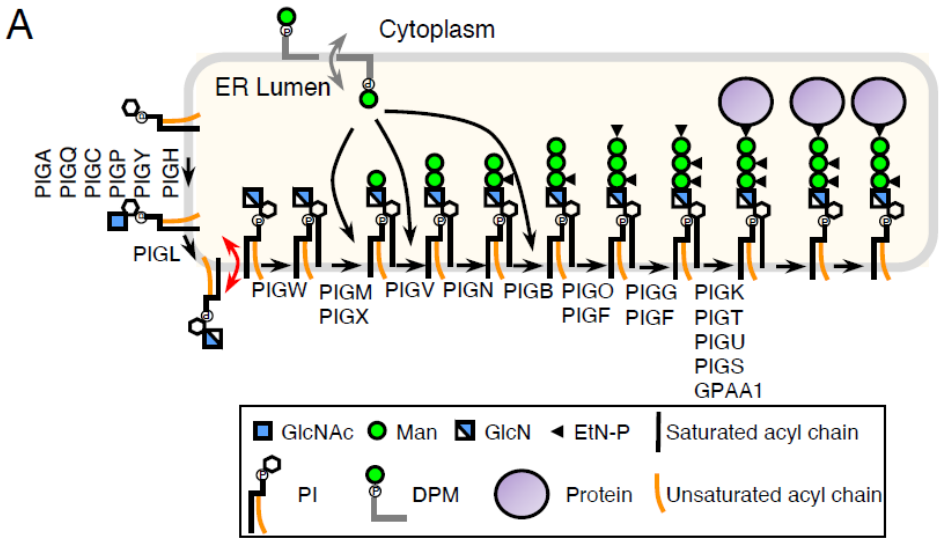
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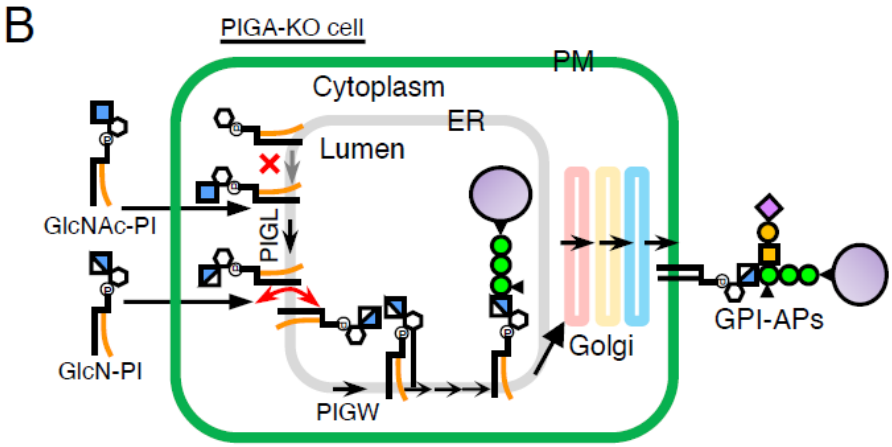
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