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Enzymatic mechanism of GPI anchor attachment clarified

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A group of eukaryotic cell surface proteins are anchored to the outer leaflet of the plasma membrane by glycosylphosphatidylinositol (GPI). In human cells, about 150 GPI-anchored proteins of various functions, such as hydrolytic enzymes, receptors, protease inhibitors, adhesion molecules, and complement regulatory proteins, have been known, and more will be added to the list. GPI is a glycolipid that is attached to the protein's C terminus as a post-translational modification.[1] GPI acts not only as a membrane anchor, but also confers unique functional properties to the proteins, such as association with lipid microdomains or rafts, apical sorting in polarized cells, and release of intact proteins after a cleavage of GPI moiety by angiotensin-converting enzyme and other GPIases.

Proteins that are modified by GPI have signal peptides at both the N and C termini in the preproproteins (**Fig. 1**, step 1). The N-terminal signal is for translocation across the ER membrane into the lumen and is cleaved by signal peptidase after the lumenal translocation (step 2). The C-terminal peptide directs attachment of GPI to the ω site amino acid.[2] The GPI attachment signal peptide has 4 elements: an unstructured linker of about 10 amino acids from ~ω-11 to ω-1 sites; amino acids with short side chains at ω and ω+2 sites; a hydrophilic stretch of 5–10 amino acids from ω+3 site; and a hydrophobic segment of 15–20 amino acids at the C-terminal end. The GPI attachment signal peptide is cleaved between ω and ω+1 amino acids and replaced by the preassembled GPI by transamidation (steps 3–5).[2] This reaction is mediated by GPI transamidase, which is a complex of 5 proteins. The subunits of human enzyme are PIG-K, GPAA1, PIG-S, PIG-T, and PIG-U, and yeast counterparts are GPI8, GAA1, GPI17, GPI16, and GAB1/CDC91, respectively. All of them are ER resident membrane proteins bearing 1–7 transmembrane domains. PIG-K/GPI8 is a member of C13-clade cysteine proteinases and is disulfide-linked to PIG-T/GPI16 in the complex.[3] GPAA1/GAA1 is the first component of GPI transamidase to be identified and has been shown to associate with GPI.[4,5] Roles of PIG-S/GPI17 and PIG-U/GAB1 are unclear at the moment.

The first step of transamidation is generation of a carbonyl intermediate between the substrate protein and the cysteine in the catalytic site of PIG-K (**Fig. 1**, step 3). GPI, which consists of phosphatidylinositol (PI), inositol-linked palmitic acid, glucosamine (GlcN), 3 mannoses (Man), and 3 ethanolamine phosphate (EtNP) attached to each of 3 mannoses (see an enlarged box in step 4), is preassembled in the ER through a pathway involving 11 biosynthetic steps.[1] In the second step in transamidation, the thioester is attacked by an amino group of terminal ethanolamine phosphate of the preassembled GPI (steps 4 and 5). Until recently, how the GPI transamidase catalyzes the second step has been totally unknown.

In this issue of *Cell Cycle*, Eisenhaber et al. report compelling bioinformatics evidence that GPAA1/GAA1 is the enzyme that catalyzes the second step in transamidation to complete GPI–anchor attachment.[6] GPAA1 has an N-terminal transmembrane domain, a lumenal region of about 320 amino acids, and the hydrophobic C-terminal region containing 6 transmembrane domains. Eisenhaber et al. took an elegant bioinformatics approach and demonstrated that the luminal region of about 300 amino acids of GPAA1/GAA1 orthologs from a wide variety of organisms has similarity to M28-type peptidases. Authors proposed that the region in GPAA1/GAA1 makes an α/β-hydrolase fold with a central β-sheet consisting of 8 strands surrounded by 7 α-helices. Authors further proposed that GPAA1/GAA1 may have one Zn-biding site like some of the M28 family peptidases. It was suggested that GPAA1/GAA1 catalyzes formation of an amide bond between the C terminus of

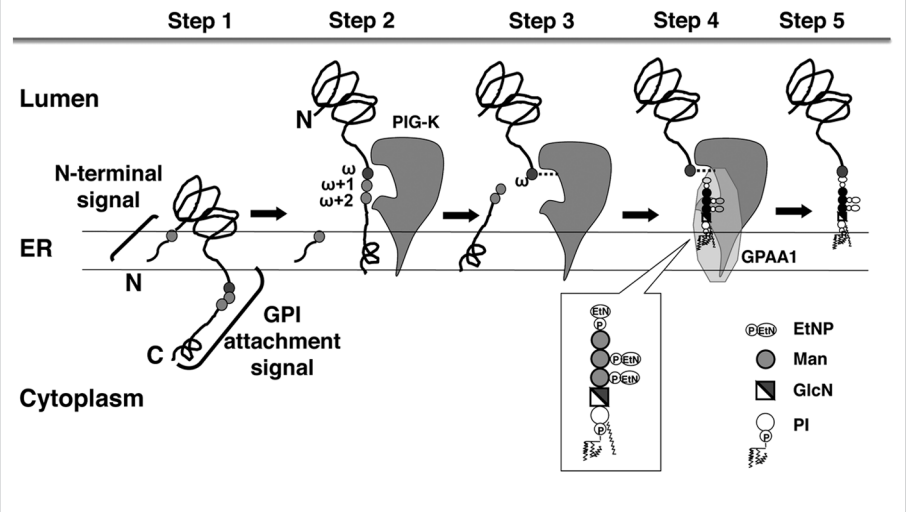

Figure 1. Action of GPI transamidase. Step 1: Preproprotein bearing N- and C-terminal signals. Step 2: Cleavage of N-terminal signal and recognition of GPI attachment signal by GPI transamidase. Only PIG-K is shown, for simplicity. Step 3: Cleavage between ω and ω+1 sites by PIG-K, resulting in carbonyl intermediate linked by a thioester bond. Step 4: GPAA1-mediated attack of the thioester. Step 5: Completion of transamidation.

ω site amino acid and terminal ethanolamine phosphate of GPI by a reverse reaction of the peptidase.

Now that the structural basis of transamidation during GPI–anchor attachment is much more clearly understood than before, development of ways to inhibit or to activate GPI transamidase will be facilitated in view of upregulation of GPI transamidase in various cancers[7] and inherited partial deficiency of GPI transamidase.

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