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Biosynthesis, Remodelling and Functions of Mammalian GPI-anchored Proteins: Recent Progress

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More than 100 mammalian proteins are post-translationally modified by glycosylphosphatidylinositol (GPI) at their C-termini and are anchored to the cell surface membrane via the lipid portion. GPI-anchored proteins (GPI-APs) have various functions, such as hydrolytic enzymes, receptors, adhesion molecules, complement regulatory proteins and other immunologically important proteins. GPI-anchored proteins are mainly associated with membrane microdomains or membrane rafts enriched in sphingolipids and cholesterol. It is thought that association with membrane rafts is important for GPI-APs in signal transduction and other functions. Here, we review recent progress in studies on biosynthesis, remodelling and functions of mammalian GPI-APs.

Key words: endoplasmic reticulum, fatty acid, glycosyltransferases, Golgi apparatus, post-translational modification.

Abbreviations: CHO, Chinese hamster ovary; Dol-P-Man, dolichol-phosphate-mannose; ER, endoplasmic reticulum; EtNP, ethanolamine phosphate; GalNAc, β-N-acetylgalactosamine; GlcN, glucosamine; GPI, glycosylphosphatidylinositol; GPI-AP, GPI-anchored protein; GPIET, GPI ethanolaminephosphate transferase; GPI-GnT, GPI-N-acetylglucosamine transferase; GPI-MT, GPI mannosyltransferase; HexNAc, N-acetyl hexosamine; Man, mannose; PE, phosphatidylethanolamine; PI, phosphatidylinositol; TA, transamidase.

STRUCTURE OF MAMMALIAN GPI-APS

Glycan and lipid structures of a number of mammalian glycosylphosphatidylinositol-anchored proteins (GPI-APs) have been determined (*1*, *2*). The core backbone structure, EtNP-6Manα1-2Manα1-6Manα1-4GlcNα1-6*myo*Inositol-phospholipid (where EtNP is ethanolamine phosphate; Man is mannose and GlcN is glucosamine), is common to various eukaryotic GPI-APs, such as yeast (*Saccharomyces cerevisiae*), protozoan parasites (*Trypanosoma brucei* and *Plasmodium falciparum*) and plant (*Pyrus communis*) (*1–5*) (Fig. 1A). All mammalian GPI-APs additionally have an EtNP side branch linked to the 2-position of the first, α1-4 linked Man (Man-1) (*2*). Occasionally, the third EtNP is on the 6-position of the second Man (Man-2) (*2*). In some mammalian GPI-APs, the fourth Man is linked via an α1-2 bond to the third Man (Man-3) (*6*). These modifications occur during biosynthesis of the GPI precursor before attachment to proteins (Fig. 1B) (*2*).

Heterogeneity in the glycan side chain is found even in one GPI-AP. *N*-acetyl hexosamine (HexNAc) is linked to Man-1 at the 4-position in all rat brain Thy-1, and in a fraction of human erythrocyte CD59 and bovine liver CD73 (*7–9*). These HexNAc were determined to be β-*N*-acetylgalactosamine (GalNAc) in a number of examples, such as rat brain Thy-1 and CD59 (*7*, *8*). The GalNAc is occasionally modified by galactose and/or sialic acid (*10*). This HexNAc modification has never been found in GPI precursors, suggesting that it is added after attachment to proteins. There is a report that GPI-APs including mammalian proteins contain βGlcNAc phosphate linked to Man-2 (Fig. 1A) (*11*).

Regarding the lipid structures, mammalian GPI-APs have two characteristics. First, most mammalian GPI-APs on nucleated cells have two saturated fatty chains in their phosphatidylinositol (PI) moiety (*12*, *13*), whereas free PI in the same cells have unsaturated fatty acid at the sn2 position (Fig. 1A) (*14*). This unique fatty chain structure of GPI-APs is elaborated by fatty acid remodelling (see below for more detail) (*15*). The lack of an unsaturated fatty acid chain in GPI-APs is critical for the association with membrane microdomains (see below) (*15*). Second, in many GPI-APs, PI is a mixture of diacyl and 1-alkyl, 2-acyl PI, the latter being usually a major form (*12*, *13*). The biological significance of the alkyl chain has yet to be determined.

GPI-APs on human erythrocytes, such as CD55 (decay-accelerating factor), CD59 and acetylcholine esterase have an exceptional lipid structure (*8*, *16*). They maintain unsaturated fatty acids at the sn2 position and a palmitoyl chain linked to the 2-position of the inositol ring that is usually removed soon after attachment of GPI to proteins (Fig. 1A) (*8*, *16*). Therefore, GPI-APs on human erythrocytes have three fatty chains, one of which is unsaturated. It is speculated that the three chains form a more stable association with the plasma membrane, being important for stable expression of GPI-APs during long-life of erythrocytes in the blood.

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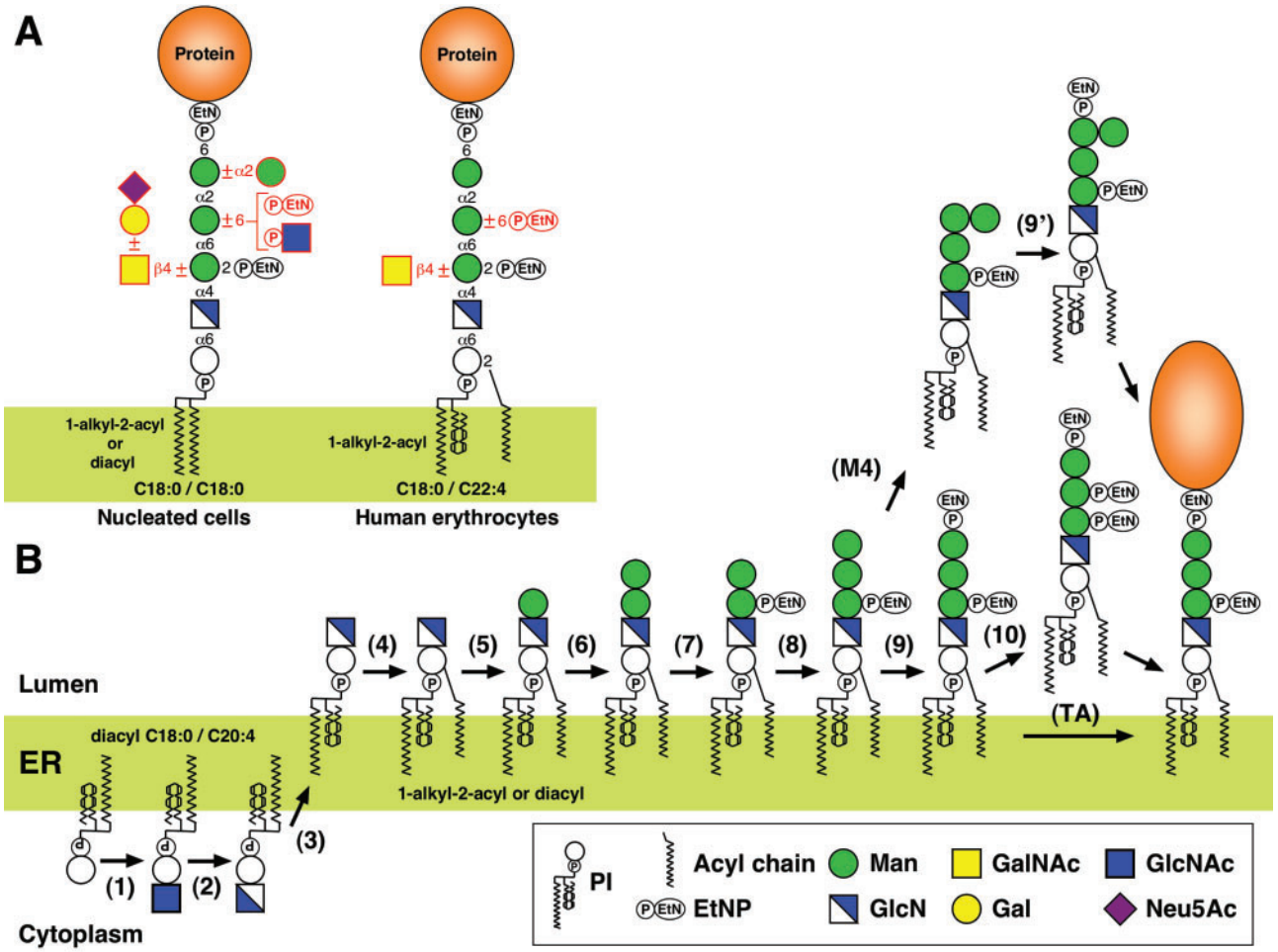

Fig. 1. **Structure (A) and biosynthetic pathway (B) of mammalian GPI-APs.** (A) Left, GPI-AP from nucleated cells; right, GPI-AP from human erythrocytes. Mammalian GPI-APs have common core backbone, protein-EtNP-Man-3-Man-2-Man-1-GlcN-PI, and one EtNP side branch linked to Man-1. Occasionally, another EtNP side branch is linked to Man-2; Man-4 is linked to Man-3 as a side branch; HexNAc, which is determined as β-GalNAc in several examples, is linked to Man-1 and is sometimes modified by galactose and/or sialic acid; and there is a report that GlcNAc phosphate is linked to Man-2. GPI-APs from nucleated cells usually have two saturated chains in the PI moiety, whereas those from human erythrocytes have an unsaturated chain in the sn2 position and inositol-linked acyl, usually palmitoyl, chain. (B) Biosynthesis of GPI is initiated by transfer of GlcNAc to PI (step 1). First two steps occur on the cytoplasmic side of the ER, whereas later steps (steps 4-TA) occur on the luminal side. TA, transamidase.

POST-TRANSLATIONAL ATTACHMENT OF GPI ANCHORS TO PROTEINS

Proteins that are to be GPI-anchored have a GPI-attachment signal peptide at the C-terminus. The signal peptide is recognized, cleaved and replaced by pre-assembled GPI by the action of a GPI transamidase residing in the endoplasmic reticulum (ER) (*1*, *2*, *4*). The GPI-attachment signal peptides from various GPI-AP precursors do not contain any consensus sequence but have several common features, i.e. (i) the amino acid residue to which the GPI anchor is amide-bonded is termed the ω-site. The known ω-site amino acids are those with small side-chains, namely G, A, S, N, D and C; (ii) the ω + 2 amino acids are also those with small side-chains, such as G, A and S; (iii) a hydrophilic spacer sequence with six or more residues starting at the ω + 3 site and (iv) a C-terminal hydrophobic sequence long enough to span the ER membrane. The GPI-attachment signal peptides contain all the information necessary and sufficient for GPI-anchoring because they can convert non-GPI-APs to GPI-APs when attached to their C-termini.

The GPI transamidase is a membrane-bound multi-subunit enzyme consisting of PIG-K (also called GPI8), GAA1, PIG-S, PIG-T and PIG-U proteins (Table 1) (*2*). PIG-K is the catalytic subunit that has sequence homology to cysteine protease family members. The other four subunits are also essential or nearly essential for GPI attachment; however, their functions are not clear [see recent review by Orlean and Menon (*2*) for further discussion about GPI transamidase].

BIOSYNTHETIC PATHWAY OF GPI-ANCHOR PRECURSORS

The GPI-anchor precursors are synthesized in the ER from PI through at least nine sequential reaction steps

Table 1. **Proteins involved in biosynthesis of GPI-APs.**

Step[a]	Enzyme	Donor substrate	Protein	Function
1	GPI-GlcNAc transferase (GPI-GnT)	UDP-GlcNAc	PIG-A	Catalytic
			PIG-C	
			PIG-H	
			PIG-P	
			PIG-Q	
			PIG-Y	
			DPM2	
2	GlcNAc-PI de-*N*-acetylase		PIG-L	Catalytic
3	Flippase		Not identified	
4	Inositol acyltransferase	Palmitoyl-CoA	PIG-W	Catalytic
5	α1-4mannosyltransferase I (GPI-MTI)	Dol-P-Man	PIG-M	Catalytic
			PIG-X	Stabilization
6	α1-6mannosyltransferase II (GPI-MTII)	Dol-P-Man	PIG-V	Catalytic
7	EtNP transferase I (GPI-ETI)	PE[b]	PIG-N	Catalytic
8	α1-2mannosyltransferase III (GPI-MTIII)	Dol-P-Man	PIG-B	Catalytic
9	EtNP transferase III (GPI-ETIII)	PE[b]	PIG-O	Catalytic
			PIG-F	Stabilization
10	EtNP transferase II (GPI-ETII)	PE[b]	PIG-G (GPI7)	Catalytic
			PIG-F	Stabilization
M4	α1-2mannosyltransferase IV (GPI-MTIV)	Dol-P-Man	PIG-Z (SMP3)	Catalytic
TA	GPI transamidase		PIG-K	Catalytic
			GAA1	
			PIG-S	
			PIG-T	
			PIG-U	
P1	Inositol deacylase		PGAP1	Catalytic
P2	GPI-AP phospholipase A2		PGAP3	Catalytic?
P3	Lyso-GPI-AP acyltransferase	Stearyl-CoA	PGAP2	Non-catalytic

[a]Steps 1-TA and P1-P3 correspond to those shown in Figs 1 and 2, respectively. [b]Phosphatidylethanolamine.

(Fig. 1B) (*2*, *17*). The biosynthetic pathway begins on the cytoplasmic side of the ER membrane by the transfer of GlcNAc to PI from UDP-GlcNAc, generating GlcNAc-PI (Step 1). GlcNAc-PI is next de-*N*-acetylated to generate GlcN-PI (Step 2). It is most likely that GlcN-PI is flipped across the ER membrane into the luminal side (Step 3). An acyl chain, usually palmitic acid, is added to the inositol ring from acyl-CoA to form GlcN-(acyl)PI (Step 4). There is still an argument whether GlcN-(acyl)PI or GlcN-PI is flipped into the luminal side (*18*, *19*). The membrane orientations of GlcN-PI and the catalytic site of the acyltransferase should be definitively determined to resolve this point.

Endogenous cellular PI is predominantly 1-stearoyl, 2-arachidonoyl PI, whereas the PI moiety in mammalian GPI-APs is more heterogeneous and 1-alkyl, 2-acyl PI is dominant. Recently, the fatty chain compositions of GlcNAc-PI, GlcN-PI and GlcN-(acyl)PI each accumulated in mutant Chinese hamster ovary (CHO) cells defective in PIG-L, PIG-W and DPM2, respectively (see below and Table 1 for biosynthetic genes), were determined (*20*). GlcNAc-PI and GlcN-PI had predominantly 1-stearoyl, 2-arachidonoyl PI like free PI, whereas the PI moiety in GlcN-(acyl)PI was a mixture of diacyl and 1-alkyl, 2-acyl PIs, the latter being a major form. The alkyl chain in the sn1 position was palmityl, stearyl or an 18:1 chain very likely an alkenyl form, whereas the acyl chain in the sn2 position was oleic, arachidonic or docosatetraenoic acid. Therefore, the diacyl to 1-alkyl, 2-acyl change occurs in GlcN-(acyl)PI (*20*).

Man-1 and Man-2 are sequentially transferred to GlcN-(acyl)PI from dolichol-phosphate-mannose (Dol-P-Man) to generate Man-Man-GlcN-(acyl)PI (Steps 5 and 6). The EtNP side branch is added to the 2-position of Man-1 from phosphatidylethanolamine (PE) generating Man-(EtNP)Man-GlcN-(acyl)PI (Step 7). Man-3 is then transferred from Dol-P-Man (Step 8). Finally, the so-called 'bridging' EtNP that makes an amide bond with the protein's C-terminus is added to Man-3 from PE (Step 9), generating a form of mature GPI-anchor precursor, EtNP-Man-Man-(EtNP)Man-GlcN-(acyl)PI (*2*, *17*).

The third EtNP can be added to Man-2 as a side branch from PE to generate another form of mature GPI-anchor precursor (Step 10). After step 8, the fourth Man (Man-4) can be transferred from Dol-P-Man (Step M4) to generate an intermediate bearing four mannoses, which is then converted by EtNP addition to Man-3 (Step 9') into the third form of mature GPI-anchor precursor.

It was previously proposed that the immediate intermediate to Man-(EtNP)Man-GlcN-(acyl)PI is (EtNP)Man-GlcN-(acyl)PI, which is found in mutant cells defective in step 8 or 9. However, in mutant cells defective in transfer of Man-2, Man-GlcN-(acyl)PI is predominantly accumulated, whereas only a small amount of (EtNP)Man-GlcN-(acyl)PI is found, indicating that Man-GlcN-(acyl)PI is the major acceptor of Man-2.

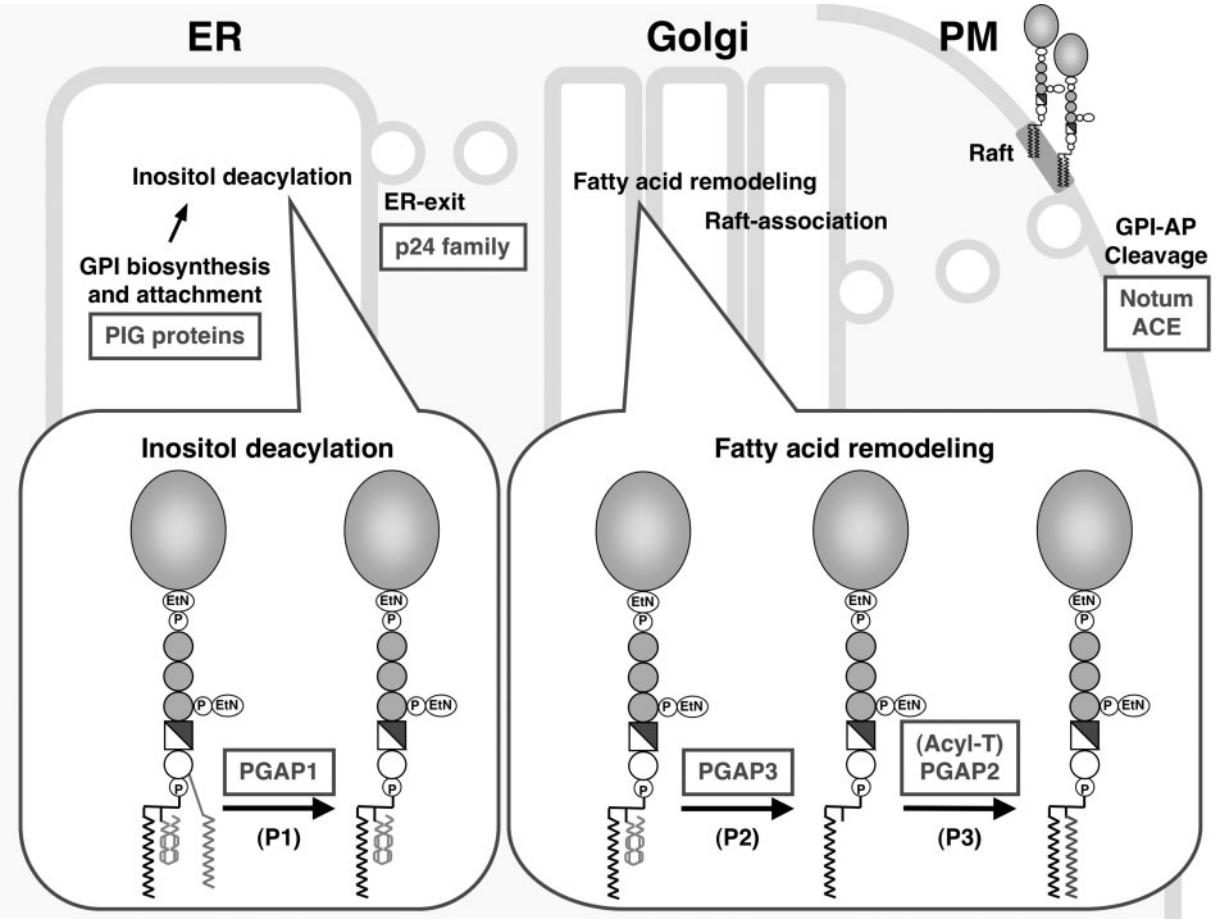

Fig. 2. **Inositol-deacylation and fatty acid remodelling of mammalian GPI-APs.** In the ER, soon after attachment of GPI-anchor to proteins and before departure for the Golgi, the acyl chain linked to inositol is removed by PGAP1, a deacylase. Inositol-deacylated GPI-APs are transported via secretory pathway. p24 family proteins are involved in efficient ER-to-Golgi transport of GPI-APs. In the Golgi, an unsaturated fatty acid at the sn2-position of PI moiety is removed, generating lyso-GPI-AP intermediate, which is then reacylated with a saturated fatty acid, predominantly stearic acid. Fatty acid remodelled GPI-APs bearing two saturated fatty chains are associated with membrane rafts.

The pathway is, therefore, modified as described above (Fig. 2) (*17*). This reaction sequence is consistent with that of *S. cerevisiae* [see recent review by Pittet and Conzelmann (*4*)].

ENZYMES INVOLVED IN BIOSYNTHESIS OF GPI-ANCHOR PRECURSORS

At least 18 gene products are involved in biosynthesis of GPI-anchor precursors (Table 1) (*2*, *17*). Step 1, generation of GlcNAc-PI, is mediated by GPI-GlcNAc transferase (GPI-GnT) that is an unusually complex glycosyltransferase, consisting of six core (PIG-A, PIG-C, PIG-H, PIG-P, PIG-Q and PIG-Y) and one extra (DPM2) subunits (*2*, *21*). PIG-A is the catalytic subunit belonging to glycosyltransferase family 4 (GT4) in the CAZy classification (*22*, *23*). The functions of the five other core subunits are not clear yet but all of them are essential or nearly essential. Functionally important sites in some of these subunits, including the catalytic site in PIG-A are on the cytoplasmic side of the ER. DPM2, one of the three subunits of Dol-P-Man synthase, is associated with a fraction of GPI-GnT and enhances GPI-GnT activity 3-fold (*2*). GPI biosynthesis and Dol-P-Man biosynthesis may be co-regulated by DPM2.

Step 2 is mediated by ER-membrane protein PIG-L having GlcNAc-de-*N*-acetylase activity. Functionally important sites in PIG-L face the cytoplasm, indicating that GlcN-PI is generated on the cytoplasmic side (*2*). A putative enzyme that mediates step 3, flipping of GlcN-PI into the luminal side, has not been identified (*2*). Step 4, transfer of the acyl chain to inositol, is mediated by multi-transmembrane protein PIG-W having acyltransferase activity (*2*). It was reported recently that yeast Arv1p is required for efficient delivery of GlcN-(acyl)PI to the first GPI-mannosyltransferase (GPI-MTI) (*18*).

Step 5 is mediated by α1-4 mannosyltransferase, GPI-MTI, consisting of PIG-M and PIG-X (*2*). PIG-M is the catalytic subunit belonging to the GT50 family (*23*), and has 10 transmembrane domains and a functionally important DXD motif within the first luminal domain. PIG-X, an ER transmembrane protein with a large luminal domain, is associated with PIG-M and is required for the stable expression of PIG-M. Whether PIG-X has any role in mannose transfer is not clear.

Step 6, transfer of Man-2 requires PIG-V. Although its catalytic activity has not been demonstrated, PIG-V is most likely the GPI-MTII, α1-6 mannosyltransferase, because PIG-V has multiple (eight) transmembrane domains and a functionally important acidic amino acid (Asp) in the first luminal domain like other ER-resident, Dol-P-monosaccharide-utilizing glycosyltransferases. PIG-V is classified into GT76 (*2*). Gpi18p, *S. cerevisiae* PIG-V homologue, requires another gene product Pga1p (*24*). Mammalian genomes have no homologue of Pga1, suggesting either that PIG-V alone is sufficient or that a co-factor protein not homologous to Pga1p is involved.

Step 7, EtNP-transfer to Man-1, requires PIG-N, which is most likely the GPI-ethanolamine phosphate transferase I (GPI-ETI) itself. PIG-N has three motifs conserved in phosphatases (*2*). Man-3 is then added by PIG-B, GPI-MTIII, an α 1–2 mannosyltransferase (step 8) (*2*). In step 9, the bridging EtNP is transferred by GPI-ETIII, a complex of PIG-O and PIG-F. PIG-O must be the catalytic subunit because it has three conserved motifs similar to PIG-N and forms a protein family with PIG-N and PIG-G (also called GPI7) (*2*). PIG-F, a hydrophobic protein with two transmembrane domains, binds to and stabilizes PIG-O (*2*). The addition of the EtNP side branch to Man-2 (step 10) is mediated by GPI-ETII consisting of PIG-G and PIG-F. Similarly to GPI-ETIII, PIG-G is stabilized by PIG-F (*2*). Transfer of Man-4 is mediated by PIG-Z (also called SMP3), an α 1-2 mannosyltransferase (*2*). PIG-B, PIG-Z and ALG9 and ALG12 (α mannosyltransferases involved in *N*-glycan synthesis) form a family that is classified as GT22 (*23*).

EVENTS OCCURRING AFTER ATTACHMENT OF GPI-ANCHORS TO PROTEINS

Soon after attachment of GPI to proteins and before the exit from the ER, the inositol-linked acyl chain is removed by the action of PGAP1 (*2*, *4*). PGAP1, post-GPI attachment to proteins 1 (*25*), is a deacylase bearing a lipase motif with a catalytic serine. The inositol-deacylation occurs in most cell types with the exception of human erythrocytic lineage cells.

GPI-APs are transported from the ER to the Golgi via secretory vesicles. In *S. cerevisiae*, p24 family proteins Emp24p and Erv25p are involved in efficient exit of GPI-APs from the ER (*26*). In mammalian cells, knock-down of p23, one of the p24 family members, resulted in delayed transport of GPI-APs, but not transmembrane proteins, from the ER to the Golgi, suggesting a similar role of p24 family proteins in yeast and mammalian cells (*27*). In yeast, physical association of Emp24p and Gas1p, a GPI-AP, was reported. Whether the association involves direct recognition of the GPI structure by p24 proteins has not been determined. Association of mammalian p24 with GPI-APs has been studied but has not been demonstrated (*27*).

Recently, fatty acid remodelling of the PI moiety in the Golgi has been clarified (*28*). As described earlier, GPI precursors and GPI-APs have different PI moieties, i.e. GPI precursors have an unsaturated fatty acid at the sn2 position, whereas PI in GPI-AP has a saturated fatty acid, usually stearic acid, at the sn2 position (*15*). The sn1 chain is almost exclusively saturated in both GPI precursors and GPI-APs. Recent studies demonstrated that unsaturated sn2 chains in both 1-alkyl, 2-acyl and diacyl types of GPI are replaced with stearic acid in the Golgi apparatus (*15*). The first step of the fatty acid remodelling is removal of the unsaturated sn2 chain, generating a lyso-GPI-AP intermediate. PGAP3, a 35-kDa membrane protein mainly expressed in the Golgi, is required for this step. PGAP3 is the homolog of yeast Per1p, which is involved in similar fatty acid remodelling (*29*) and has no homology to phospholipases. Whether Per1p is the phospholipase A2 itself is yet to be determined. Lyso-GPI-APs are then reacylated with stearic acid. PGAP2, a 27 kDa membrane protein mainly expressed in the Golgi, is required for reacylation (*30*). PGAP2 is perhaps not an acyltransferase itself because it has no homology to acyltransferases and because Gup1p, a membrane-bound *O*-acyltransferase (MBOAT) family member, is required for transfer of C26 : 0 fatty acid in similar fatty acid remodelling reactions in *S. cerevisiae* (*31*). The trypanosome Gup1p homolog, TbGup1, is involved in transfer of myristic acid (C14:0) in GPI fatty acid remodelling in *Trypanosoma brucei* (*32*). It is likely that one of the mammalian MBOAT family members is involved in reacylation with stearic acid (C18:0). The *S. cerevisiae* homolog of PGAP2, Cwh43p, is involved in another type of remodeling of GPI-AP known to occur in *S. cerevisiae*; a diacylglycerol to ceramide change (*33*, *34*). The exact reaction involved in this type of remodelling has not been determined but it may be an exchange reaction between the diacylglycerol form of GPI and a donor of ceramide by the action of either a phospholipase C or D type enzyme. Cwh43p consists of an N-terminal part homologous to mammalian PGAP2 and the C-terminal part having a metal hydrolase motif (*33*, *34*). The possible role of PGAP2 in fatty acid remodelling, therefore, does not seem to be that of a catalytic component but seems to be a substrate-recruiting or -recognition component.

IMPORTANCE OF LIPID STRUCTURE IN FUNCTIONS OF GPI-APS

On the surface of PGAP1-defective CHO cells, inositol-acylated GPI-APs are expressed (*25*). Transport of inositol-acylated GPI-APs from the ER to the Golgi apparatus was at a 4-fold reduced rate compared to that of normal GPI-APs in the wild-type CHO cells, indicating that lipid structure is important for intracellular transport of GPI-APs (*25*). The steady-state levels of the cell surface expression of GPI-APs on the PGAP1-defective CHO cells and blood cells from PGAP1-knockout mice are normal (*35*). Nevertheless, the majority of the PGAP1-knockout mice died quickly after birth often with the phenotype of otocephaly due to malformation of the jaw, indicating the importance of GPI lipid structure in functions of GPI-APs involved in development (*35*). Survivors grew with significantly reduced body weight. Females were fertile, whereas all males were nearly completely sterile due to the sperms' inability to ascend from the uterus to the oviduct and to attach to the zona

pellucida of the egg. A prominent feature of the sperm from the PGAP1-knockout mice is the elevated levels of sperm-associated GPI-APs, such as CD52 and CD55 (*35*). CD52 is synthesized in the epididymis epithelial cells and is transferred to sperm possibly via vesicles called epididymosomes (*36*). Inositol-acylated CD52 may be more efficiently transferred or more stably associated with the sperm surface. For sperm maturation, inhibitory components called decapacitation factors should be removed from the surface of sperm and there is a report that a receptor for one of the decapacitation factors is a sperm-associated GPI-AP (*37*). Inositol-acylated GPI-anchored decapacitation factor receptor might be associated with sperm at a higher level and hence more decapacitation factor would be associated, inhibiting sperm maturation. This may be a basis for the male infertility of PGAP1-knockout mice.

A well-known characteristic of GPI-APs is association with membrane microdomains or membrane rafts. Membrane rafts are small (10–200 nm) and dynamic membrane domains enriched in selective lipids, such as sphingolipids and cholesterol. Lipid-modified proteins, such as GPI-APs are found in the outer leaflet and src family tyrosine kinases are found in the inner leaflet of membrane rafts. GPI-APs usually have two saturated fatty chains in the PI moiety. It has been thought that two saturated straight fatty chains make associations with two saturated chains in the ceramide moiety of sphingolipids and with cholesterol, the basis of raft-association of GPI-APs. Another typical characteristic of GPI-APs is that they are efficiently recovered into the detergent-resistant membrane (DRM) fraction when cells are extracted with cold non-ionic detergent, such as Triton X-100 (*26*, *38*). Glycosphingolipids are also efficiently recovered into the DRM, suggesting that associations among components of lipid microdomains contribute to resistance against extraction by cold non-ionic detergent. As described above, the GPI-APs' PI moiety bearing two saturated chains is generated by fatty acid remodelling and two Golgi-membrane proteins PGAP2 (*30*) and PGAP3 (*15*) are required for it. On the surface of PGAP2- and PGAP3-double mutant CHO cells, unremodelled GPI-APs bearing an unsaturated fatty acyl chain, such as oleic and arachidonic acid, are expressed (*15*). These GPI-APs are not efficiently recovered into the DRM when the cells are extracted with cold Triton X-100, whereas normally remodelled GPI-APs on the wild-type CHO cells are (*15*). It seems that GPI with an unsaturated acyl chain loses its association with sphingolipids and cholesterol, becoming extractable with cold Triton X-100. It will be interesting to determine the effects of loss of raft-association of GPI-APs on various biological functions of GPI-APs. We are in progress of generating PGAP3-knockout mice and these will be available soon for such studies (Y. Wang, Y. Maeda, Y. Murakami and T. Kinoshita, unpublished data).

CLEAVAGE OF GPI-APS IN RELATION TO THEIR FUNCTIONS

Glypicans that are GPI-anchored heparan sulphate proteoglycans, are involved in regulation of a number of morphogenic factors, such as Wnts and Hedgehogs. In *Drosophila*, Notum was identified by genetic screen for inhibitors of wingless signalling and was shown to inhibit wingless by releasing Dlp, a glypican, by cleaving GPI-anchor (*39*). Notum is a member of a hydrolase family with catalytic serine. Mammalian Notum has recently been shown to have an ability to release glypicans and other GPI-APs from the cell surface by cleaving GPI (*40*). Notum's GPI cleaving ability has been proposed to be due to its GPI-specific phospholipase C activity (*39*, *40*). It was also shown that mammalian Notum can act as a negative regulator of Wnt by releasing glypican-3 from the cell surface (*40*), suggesting that released glypican acts as competitive inhibitor of Wnt and Wnt receptor interaction.

In transgenic mice expressing GPI-anchored form of GFP body widely, release of GFP into extracellular compartments was observed in several organs, such as pancreas and testis, suggesting the presence of GPI-cleaving enzyme(s) (*41*). An enzyme that cleaves GPI-anchored alkaline phosphatase has been purified from testis germ cells and identified to be testicular isoform of angiotensin converting enzyme (tACE) (*42*). Both testis-specific tACE and widely expressed somatic ACE had GPI-cleaving activity apart from their classic activities to cleave angiotensin I and bradykinin (*42*). ACE knock-out male mice are infertile due to defective sperm–egg binding at the zona pellucida (*43*). It was speculated that some GPI-AP must be released from sperm by the action of tACE (known to be associated with sperm), in order for sperm to acquire competence to bind with the zona pellucida. ACE cleavage site within GPI has been reported to be between mannoses (*42*). Others, however, reported inability to show ACE-mediated cleavage of GPI-AP (*44*, *45*). Further study is required to solve the controversy regarding this important point.

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