thematic review

Thematic review series: Lipid Posttranslational Modifications

GPI anchoring of protein in yeast and mammalian cells, or: how we learned to stop worrying and love glycophospholipids

Peter Orlean[1,*] and Anant K. Menon[1,†]

Department of Microbiology,* University of Illinois at Urbana-Champaign, Urbana, IL 61801; and Department of Biochemistry,† Weill Cornell Medical College, New York, NY 10021

Abstract Glycosylphosphatidylinositol (GPI) anchoring of cell surface proteins is the most complex and metabolically expensive of the lipid posttranslational modifications described to date. The GPI anchor is synthesized via a membrane-bound multistep pathway in the endoplasmic reticulum (ER) requiring >20 gene products. The pathway is initiated on the cytoplasmic side of the ER and completed in the ER lumen, necessitating flipping of a glycolipid intermediate across the membrane. The completed GPI anchor is attached to proteins that have been translocated across the ER membrane and that display a GPI signal anchor sequence at the C terminus. GPI proteins transit the secretory pathway to the cell surface; in yeast, many become covalently attached to the cell wall. Genes encoding proteins involved in all but one of the predicted steps in the assembly of the GPI precursor glycolipid and its transfer to protein in mammals and yeast have now been identified. Most of these genes encode polytopic membrane proteins, some of which are organized in complexes. The steps in GPI assembly, and the enzymes that carry them out, are highly conserved. GPI biosynthesis is essential for viability in yeast and for embryonic development in mammals. In this review, we describe the biosynthesis of mammalian and yeast GPIs, their transfer to protein, and their subsequent processing.—Orlean, P., and A. K. Menon. **GPI anchoring of protein in yeast and mammalian cells, or: how we learned to stop worrying and love glycophospholipids. *J. Lipid Res.* 2007.** 48: **993–1011.**

Supplementary key words glycosyltransferase • flippase • dolichol • phosphatidylethanolamine • phosphatidylinositol • endoplasmic reticulum • cell wall • glycosylphosphatidylinositol

Roughly 1% of all proteins encoded by eukaryotic genomes, or ~10–20% of all membrane proteins that enter the secretory pathway after being targeted to the endoplasmic reticulum (ER), are posttranslationally modified at their C terminus by glycosylphosphatidylinositol (GPI), a complex glycophospholipid that serves to anchor proteins to the cell surface. Although GPI proteins are functionally diverse, many have, or are predicted to have, hydrolytic activity, or serve as receptors or adhesive proteins. The importance of GPI anchoring in mammals is underscored by the facts that abrogation of GPI biosynthesis results in embryonic lethality (1) and that an inherited hypomorphic promoter mutation that decreases the expression of the mannosyltransferase PIG-M and impairs GPI mannosylation leads to thromboses of the portal and hepatic veins as well as seizures (2). Furthermore, a somatic defect in the first step of GPI biosynthesis in multipotent hematopoietic human stem cells results in paroxysmal nocturnal hemoglobinuria, an acquired hemolytic disease (3). GPIs are essential for viability in yeast and critical for the maintenance of normal cellular morphology (4, 5). They are also important for the cell surface display of a variety of proteins and glycoconjugates in parasitic protozoa, such as the African sleeping sickness parasite *Trypanosoma brucei*, and essential in certain life cycle stages of these parasites (6–8).

Examples of GPI proteins include cell surface receptors (e.g., folate receptor, CD14), cell adhesion molecules (e.g., neural cell adhesion molecule (NCAM) isoforms, carcinoembryonic antigen variants, fasciclin I), cell surface hydrolases (e.g., 5′-nucleotidase, acetylcholinesterase, alkaline phosphatase), complement regulatory proteins [e.g., decay-accelerating factor (CD55)], the scrapie prion, and protozoal coat proteins (e.g., *T. brucei* variant surface glycoprotein). All of these molecules require GPI to be expressed at the cell surface and to function. Unlike conventional hydrophobic transmembrane protein domains that span the membrane bilayer, GPI anchors penetrate

Manuscript received 2 February 2007 and in revised form 12 March 2007.

Published, JLR Papers in Press, March 14, 2007.
DOI 10.1194/jlr.R700002-JLR200

[1] To whom correspondence should be addressed.
e-mail: p-orlean@uiuc.edu (P.O.); akm2003@med.cornell.edu (A.K.M.)

only into a single membrane leaflet. This feature, as well as the saturated fatty acyl chains typically found in the GPI lipid portion, ensures the association of GPI-anchored proteins with lipid rafts, the nanoscale cholesterol and sphingolipid-rich membrane domains that are postulated to play an important role in membrane trafficking and cell signaling (9–11).

Unlike GPI, most other forms of protein lipidation are found on cytoplasmic proteins. Like myristoylation and prenylation, GPI attachment is irreversible. Unlike myristoyl, palmitoyl, and prenyl groups, GPIs can be modified by the addition of side-branching groups to their glycan core, and their lipid moieties can be remodeled as well. These modifications can be species-specific. GPIs represent the sole means of membrane anchoring for GPI proteins; in contrast, single myristoyl, palmitoyl, and prenyl groups are insufficient as membrane anchors, and their anchoring ability must be augmented by additional lipid modifications of the protein, or protein-protein or protein-membrane interactions, to provide stable membrane association (12, 13).

Because GPIs are preassembled stepwise by a series of enzymes located in the membrane of the ER, then transferred to protein in the lumen of that organelle, the GPI-anchoring pathway is analogous to the dolichol pathway for asparagine-linked (*N*-linked) protein glycosylation (14). Both pathways are initiated on the cytoplasmic face of the ER and completed in the ER lumen, necessitating transbilayer flipping of a glycolipid intermediate (15). The pathways share certain activated sugars (UDPGlcNAc, Dol-P-Man), and the same secretory protein can receive both types of posttranslational modification; it is likely that all GPI proteins are modified by *N*-linked glycans. Unlike the dolichol pathway, however, GPI assembly is also dependent on phosphatidylethanolamine (PtdEtn) supply.

Genes for proteins involved in all but one of the predicted steps in the assembly of the GPI precursor glycolipid and its subsequent transfer to protein in mammals and yeast have now been identified. Most of these genes encode polytopic membrane proteins, some of which are organized as protein complexes. The steps in the GPI assembly pathway, and the enzymes that carry them out, are highly conserved. In this review, we describe the biosynthesis of mammalian and yeast GPIs, their transfer to protein, and their subsequent processing. For the most part, we do not discuss the vast literature on protozoal GPI-anchored proteins and glycoconjugates; the reader is referred to other articles for information in this area (7, 16).

STRUCTURE OF GPIs AND SIGNALS FOR THEIR ATTACHMENT

Overview of GPI structure

Detailed analyses of the structures of protein-bound GPIs from mammals, protozoa, and yeast (17–22) reveal that they have a conserved core structure: protein-CO-NH_2-CH_2-CH_2-PO_4-6-Manα1,2Manα1,6Manα1,4GlcNα1,6-*myo*-inositol phospholipid (**Fig. 1A**). The GPI core in yeast and mammals is modified during its biosynthesis by the addition of a palmitoyl group to the 2-OH of the inositol moiety, which renders the GPI resistant to cleavage by PtdIns-specific phospholipase C (PI-PLC), and by the addition of up to three ethanolamine phosphate (Etn-P) moieties to specific positions on the first three mannoses (see below). Addition of a fourth, α1,2-linked mannose (Man) to the third Man (Man-3) is obligatory in yeast but not in mammalian cells, although a fourth Man may be added in a tissue-specific manner (see below). Various side-branching sugars can be added, most likely in the Golgi. The inositol acyl group is normally removed in the ER after transfer of the GPI to protein, and remodeling of the diacylglycerol moiety present on the GPI precursor that is transferred to protein likewise is initiated in the ER and continued in the Golgi (see below). In yeast, the lipid moiety of many GPIs is remodeled to ceramide (23). Protein-bound GPIs in both yeast and mammals can bear Etn-P on Man-1 (20, 24), but it is not clear whether this substituent is invariably retained or whether it can be removed from certain GPIs after anchor transfer to protein. Likewise, the fate of Etn-P moieties added to Man-2 of GPI precursors is unknown.

GPI attachment signals

Direct biochemical demonstration that a protein bears a GPI anchor is not straightforward. Preliminary evidence for GPI anchorage can be obtained if a membrane-associated protein can be made water-soluble by treatment by PI-PLC (25, 26). However, although most, if not all, protein-bound GPIs in *Saccharomyces cerevisiae* are PI-PLC-sensitive, the released proteins tend to remain hydrophobic and partition into the detergent phase in the commonly used Triton X-114 fractionation procedure (27). Moreover, many yeast GPI proteins become covalently cross-linked to cell wall polysaccharide and therefore are difficult to recover for analysis. Proteomics approaches, however, are now permitting increasing numbers of GPI proteins to be identified directly in fractions of yeast and mammalian cells (28, 29).

Biochemical verification of potential GPI modification is now largely eschewed for in silico analyses using predictive algorithms, which reliably identify GPI proteins (30–35). GPI attachment is inferred if a protein's amino acid sequence contains *i*) a hydrophobic N-terminal secretion signal that targets the protein to the ER and *ii*) a C-terminal GPI signal anchor sequence. Yeast GPI proteins additionally often contain stretches of amino acids rich in serine and threonine (30, 31). The features of mammalian and *S. cerevisiae* signal anchor sequences were originally defined experimentally in site-directed mutagenesis studies using model proteins (36, 37; reviewed in Ref. 38) and are shown schematically in Fig. 1B. The amino acid to which the GPI becomes linked is referred to as the "ω" residue, with amino acids N-terminal to it designated ω-minus and those C-terminal to it designated ω-plus. The general features of a signal anchor sequence are as follows: *i*) a stretch of ~10 polar amino acids (ω-10 to ω-1) that

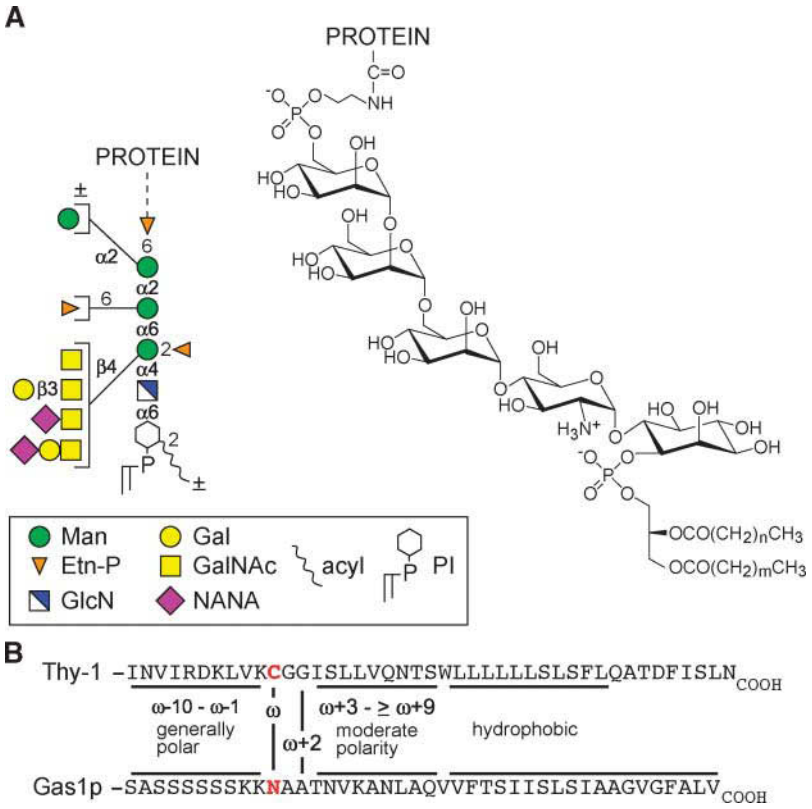

Fig. 1. A: Structure of yeast and mammalian glycosylphosphatidylinositol (GPI)-anchored proteins. The core structure of the GPI anchor is shown in chemical detail on the right and in schematic form on the left. The schematic illustration, used in other figures in this review, makes use of symbology recommended by the Consortium of Glycobiology Editors (La Jolla, CA) in *Essentials in Glycobiology* (Cold Spring Harbor Laboratory Press). GPI consists of a trimannosyl core, glucosamine (GlcN), and PtdIns (PI). The mannose residues are labeled Man-1, Man-2, and Man-3, starting with the residue linked to GlcN. The lipid moiety is typically diacylglycerol with saturated fatty acyl chains, although alkylacylglycerol and ceramide structures are possible as well. The 6-OH of the third mannose residue is modified with ethanolamine phosphate (Etn-P): the amino group of this Etn residue forms an amide bond with the α-carboxyl of the C-terminal amino acid of the protein; this amino acid, termed ω, is an internal amino acid that is exposed during the GPI-anchoring reaction. The schematic illustration shows the core structure of GPI as well as modifications that may or may not be found in the different GPI anchors that have been characterized in yeast and mammals. The 2-OH of inositol in GPI biosynthetic intermediates and the GPI anchor precursor is acylated, usually with palmitic acid. This acyl chain is typically removed before the GPI protein leaves the endoplasmic reticulum (ER) en route to the cell surface. The mannose residues are decorated with Etn-P side chains during GPI biosynthesis; carbohydrate modifications of the mannose residues, presumably acquired in the Golgi, are also seen in some GPI-anchored proteins. Man-3 can be modified by an α1,2-linked mannose to yield a Man_4-GPI; this mannosylation is an essential biosynthetic step in the assembly of yeast GPIs. B: General features of the GPI signal anchor sequences of yeast and mammalian proteins. The C-terminal amino acid sequences of the well-studied rat Thy-1 (18) and yeast Gas1 (36) proteins are presented to illustrate the features of the amino acid sequences surrounding sites of GPI attachment. The GPI is transferred to the ω amino acid with concomitant cleavage of the peptide bond between the ω and ω+1 amino acids. The sequence of 10 amino acids N-terminal to the ω site contains generally polar residues and forms a flexible linker region. Typical ω amino acids are G, A, S, N, D, or C. The ω+1 position is relatively permissive, whereas ω+2 amino acids are predominantly G, A, and S. A moderately polar spacer region of six or more amino acids starts at ω+3 and is followed by a hydrophobic segment amino acid (32–35, 38). The alanine in the ω+1 position of the Gas1p sequence could serve as an alternative, although less optimal, GPI attachment site.

form a flexible linker region; *ii*) the ω amino acid, typically G, A, S, N, D, or C; *iii*) the ω+2 amino acid, the most restrictive position, with predominantly G, A, or S; *iv*) a spacer region of moderately polar amino acids (ω+3 to ω+9 or more); and *v*) a stretch of hydrophobic amino acids variable in length but capable of spanning the membrane (32–35, 38). A given C-terminal sequence may contain more than one set of candidates for the ω and ω+2 amino acids. Slight variations have been noted in the relative lengths of the spacer regions and hydrophobic segments of metazoan and protozoan signal anchor sequences (33).

In yeast, in silico predictions that a GPI attachment sequence is functional have been verified by fusing the sequence to the C terminus of a reporter protein and testing whether it directs cell surface expression of the protein (which is assumed to be GPI-dependent), either in the plasma membrane via a lipid or in a glycosidic link to cell wall β-1,6-glucan (31) (see below).

Biosynthesis of the GPI precursor

The biosynthetic pathway for the glycan core of the GPI precursor was initially charted via in vivo and in vitro radiolabeling experiments in trypanosomes (39–42). The steps in the more complex GPI assembly pathway in yeast and mammals were identified via analyses of GPI biosynthesis, transfer, or processing defects in mutant cell lines (43, 44) or by in vitro radiolabeling experiments (45). Because GPI biosynthesis is dispensable for the growth of mammalian cells in culture, many GPI-deficient mutant cell lines were available early on, facilitating analyses of the pathway. Others were established more recently using innovative selection procedures such as resistance to aerolysin, a GPI binding, pore-forming toxin (46). In contrast to cultured mammalian cells, most genes required for assembly of the GPI precursor in yeast are essential, the exceptions being genes encoding subunits of an enzyme complex that includes essential proteins and genes responsible for two of the known modifications to the GPI core. In these cases, however, viable disruptants are temperature-sensitive or have severe growth defects. Conditional yeast mutants as well as GPI-defective mammalian cell lines were instrumental in defining proteins required for GPI biosynthesis.

The products of >20 genes are directly involved in the assembly of the GPI precursor and its transfer to protein. These proteins are localized in the membrane of the ER, and the vast majority are polytopic membrane proteins. None of the proteins has been structurally characterized at the atomic level, nor is there information on the detailed enzymology of most of the individual steps. Here, we summarize what is known about the individual reaction steps of GPI biosynthesis in yeast and mammals. The proteins associated with each step are listed in **Table 1**, and the biosynthetic pathway is illustrated in **Fig. 2**.

TABLE 1. Protein machinery for GPI anchoring in yeast and mammals

Step[a]	Reaction	Mammalian Protein	Yeast Protein
1	GlcNAc-PI synthesis	PIG-A[b]	Gpi3p[b]
		PIG-C	Gpi2p
		PIG-H	Gpi15p
		PIG-P	Gpi19p
		PIG-Q	Gpi1p
		PIG-Y	Eri1p
		DPM2	—
2	GlcNAc-PI de-*N*-acetylation	PIG-L[b]	Gpi12p[b]
3	GPI flipping	Not identified	Not identified
4	Inositol acylation	PIG-W	Gwt1p
5	α1,4 mannosyltransfer	PIG-M	Gpi14p
		PIG-X	Pbn1p?
6	Etn-P transfer to Man-1	PIG-N	Mcd4p
7	α1,6 mannosyltransfer	PIG-V	Gpi18p
8	α1,2 mannosyltransfer	PIG-B	Gpi10p
9	Etn-P transfer to Man-3	PIG-O	Gpi13p
		PIG-F	Gpi11p?
y	α1,2 mannosyltransfer	hSmp3	Smp3p
10	Etn-P transfer to Man-2	hGpi7	Gpi7p
		PIG-F	Gpi11p?
11	GPI transamidase	PIG-K[b]	Gpi8p[b]
		GAA1	Gaa1p
		PIG-S	Gpi17p
		PIG-T	Gpi16p[b]
		PIG-U	Gab1p
p	Inositol deacylation	PGAP1	Bst1p
p	*sn*-2 deacylation	PERLD1/PGAP3	Per1p
p	*sn*-2 C_{26} acylation		Gup1p
p	*sn*-2 acylation	PGAP2	Cwh43p?

GPI, glycosylphosphatidylinositol.

[a] Steps 1–11 correspond to those shown in Fig. 2A; steps labeled *y* are shown in Fig. 2B; steps labeled *p* concern modification of the GPI structure after it is attached to protein.

[b] Proteins with a single transmembrane span; all other proteins listed here are predicted to be bitopic or polytopic membrane proteins.

The GlcNAc-PI synthetic complex and its regulation

GPI assembly is initiated with the transfer of GlcNAc from UDPGlcNAc to PtdIns (Fig. 2A, step 1). The acyl chains of acceptor PtdIns appear to be the same length as those in bulk membrane phospholipids (47, 48), although in vitro experiments with mammalian cell membranes suggest that there may be some preference for certain acceptor PtdIns molecular species over others (49, 50). The GlcNAc transfer reaction is unexpectedly complex in that many proteins are involved. The mammalian proteins and their yeast orthologs are PIG-A/Gpi3p, PIG-C/Gpi2p, PIG-H/Gpi15p, PIG-P/Gpi19p, PIG-Q(hGpi1p)/Gpi1p, and PIG-Y/Eri1p (50–65). In mammalian cells, a seventh protein, Dpm2p, a noncatalytic subunit of Dol-P-Man synthase, physically associates with the PIG-A, -C, and -Q proteins and enhances GlcNAc-PI synthetic activity (58). *S. cerevisiae* has no Dpm2p ortholog. A defect in PIG-A causes paroxysmal nocturnal hemoglobinuria and overexpression, of PIG-P has been noted in fetal Down syndrome brain (2, 66).

Four of the six *S. cerevisiae* proteins involved in GlcNAc-PI synthesis are essential, but strains lacking the nonessential Gpi1p and Eri1p proteins are temperature-sensitive for growth and lack detectable in vitro GlcNAc-PI synthetic activity (60, 63, 64). PIG-A/Gpi3p is the catalytic subunit because Gpi3p binds a photoactivatable UDPGlcNAc analog (67) and is a member of Glycosyltransferase Family 4 of retaining glycosyltransferases (68, 69). Because the bulk of PIG-A is oriented toward the cytoplasm and anchored in the ER membrane by a single transmembrane domain, synthesis of GlcNAc-PI is likely to take place at the cytoplasmic face of the membrane (62, 70). Consistent with this, newly synthesized GlcNAc-PI is accessible to membrane topological probes on the cytoplasmic face of the ER (71). The mammalian proteins involved in GlcNAc-PI synthesis form a multiprotein complex (GPI-GnT) (50, 58, 65) that has been modeled (62, 72). Genetic interactions between *GPI1*, *GPI2*, and *GPI3* are also consistent with the notion that their products function in a complex

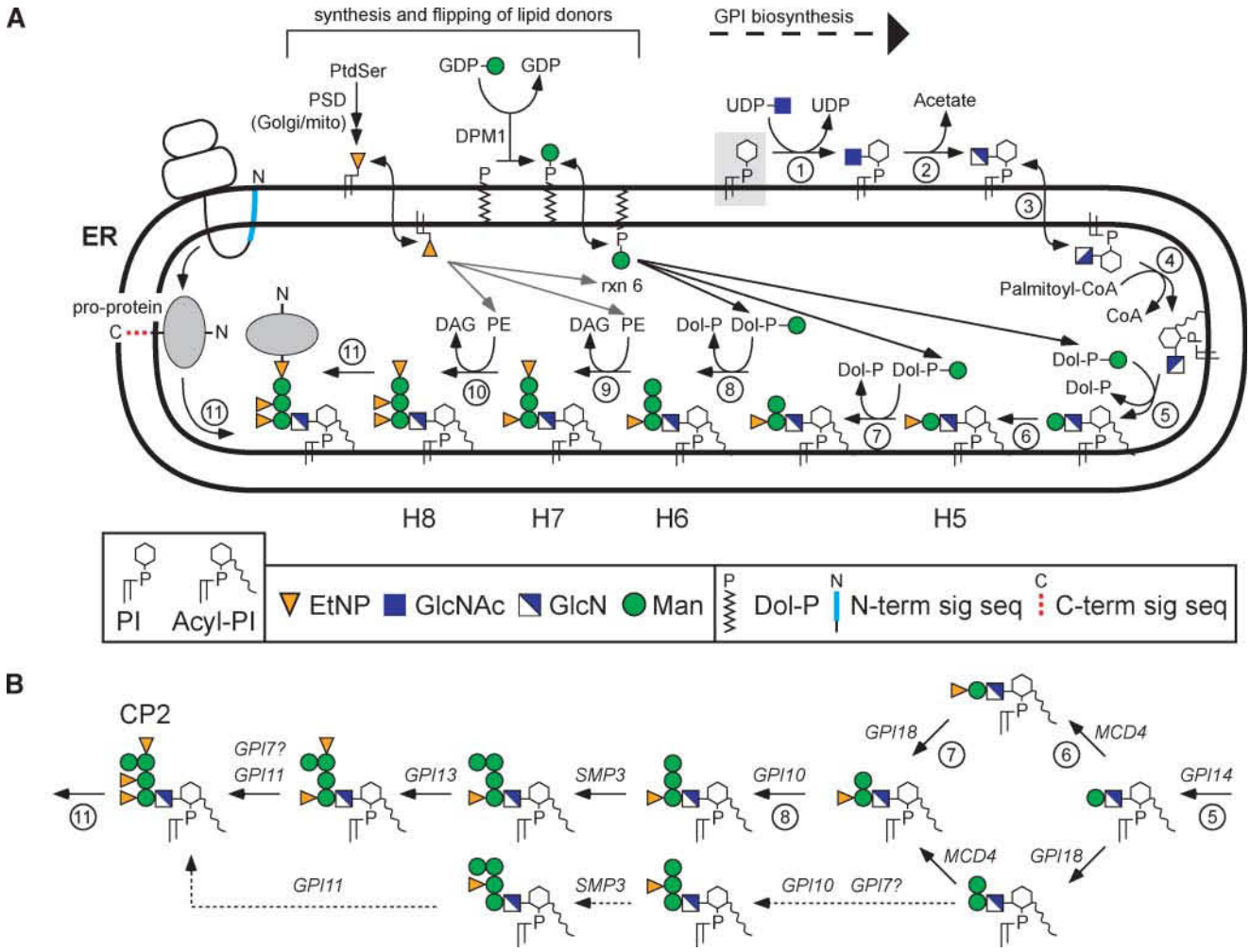

Fig. 2. A: General scheme for GPI biosynthesis in the ER of yeast and mammals. The ER is depicted as a topologically defined compartment. Biosynthesis begins at the top of the figure with a PtdIns acceptor (gray box). In step 1, PtdIns is glycosylated to generate GlcNAc-PI on the cytoplasmic face of the ER. GlcNAc-PI is then de-*N*-acetylated (step 2) to yield GlcN-PI. GlcN-PI is flipped (step 3) into the lumenal leaflet of the ER, where it is inositol-acylated (step 4), inositol-mannosylated, and modified by Etn-P (steps 5–10). Man is derived from Dol-P-Man (synthesized from Dol-P and GDP-Man in a reaction catalyzed by DPM1 on the cytoplasmic face of the ER), and Etn-P is derived from PtdEtn [synthesized typically by decarboxylation of PtdSer via the action of PSD (PtdSer decarboxylase); synthesis from CDP-Etn via the Kennedy pathway is also possible]. Both Dol-P-Man and PtdEtn must be flipped into the lumenal leaflet of the ER to participate in GPI biosynthesis. The Etn-P-capped GPIs that are synthesized by this pathway [H7 and H8 in mammals; CP2 in yeast (23)] (see panel B) are attached (step 11) to ER-translocated proteins displaying a C-terminal GPI signal sequence. Step 11 is catalyzed by GPI transamidase. Man_4-versions of the lipids H6, H7, and H8 may also be synthesized (110). B: Additional steps in the yeast GPI assembly pathway. This panel shows that a fourth Man residue must be added before Etn-P modification of Man-3 can occur (109) and illustrates the possibility that GPI-Man-T-II (Gpi18p) and GPI-Etn-P-T-I (Mcd4p) need not act in a defined order. Also depicted are the different possible orders of addition of Etn-P and Man substituents inferred from the structures of the GPI structural isoforms that accumulate in yeast mutants (109, 130, 133). Analyses of the GPIs that can be radiolabeled in mammalian cells have led to proposals for analogous pathways with branches defined by the presence or absence of Etn-P on Man-1 (108, 110, 113). It is not clear, however, whether the GPI isomers lacking Etn-P on Man-1 represent an alternative route for the assembly of the final Etn-capped yeast GPI anchor precursor CP2.

(52). The roles of the other subunits in the GPI-GnT complex are as yet unclear, but they may mediate regulatory interactions. Yeast GPI-GnT can be joined by another subunit, Ras2p, which, in its GTP-bound form, physically associates with the complex (64). Membranes from *RAS2*-deleted *S. cerevisiae* strains had 8–10 times the activity of membranes from wild-type cells, whereas membranes from cells expressing the activating RAS2-Val-19 mutation made virtually undetectable amounts of GlcNAc-PI (64), indicating that Ras2p-GTP functions as a negative regulator of GlcNAc-PI synthesis. Indeed, depending on the degree of its activation, yeast Ras2p could permit an ~200-fold range in GPI-GnT activity (64). The significance of Ras2-regulation of GPI-GnT is not fully understood. However, because GPI-GnT mutants display the hyperactive Ras phenotypes of filamentous growth and invasion of agar when grown at a semipermissive temperature, it was suggested that Ras2-mediated downregulation of GPI-GnT may contribute to the changes in cell wall architecture that accompany the dimorphic transition to filamentous

growth (59, 63, 64). To date, Ras GTPases have not been found to regulate the activity of mammalian GPI-GnT (65).

GlcNAc-PI de-*N*-acetylation

After its transfer to PI, GlcNAc-PI is de-*N*-acetylated to GlcN-PI by PIG-L/Gpi12p (Fig. 2A, step 2). These orthologs are catalytic because bacterially expressed PIG-L has GlcNAc-PI de-*N*-acetylase activity (73). The GlcNAc-PI de-*N*-acetylation reaction occurs on the cytoplasmic side of the ER membrane, because PIG-L is a type I membrane protein with a large cytoplasmic domain and very few amino acids disposed toward the ER lumen (73–75). Consistent with this, newly synthesized GlcN-PI can be hydrolyzed by PI-PLC on the cytoplasmic face of intact ER vesicles (71).

Flipping

As depicted in Fig. 2A, the GPI biosynthetic pathway is topologically split. The first two reactions occur on the cytoplasmic face of the ER, whereas inositol acylation and mannosylation reactions likely occur in the ER lumen, as described below. For GlcN-PI to be inositol-acylated and -mannosylated, it must be flipped across the ER membrane (Fig. 2A, step 3). Flipping of glycerophospholipids is energetically expensive and does not occur spontaneously: ~20–50 kcal/mol must be expended to transfer the polar head group of an amphipathic phospholipid such as PtdCho through the hydrophobic interior of the bilayer (15). Because the weight of the available evidence suggests that a lipid translocator, or flippase, is needed to flip PtdCho and other glycerophospholipids across the ER membrane in the process of membrane biogenesis, it is likely that a flippase would also be needed to flip GlcN-PI from the cytoplasmic leaflet of the ER to the lumenal side during GPI biosynthesis. Although a GPI flippase has yet to be identified, a recent report describes the successful biochemical reconstitution of GPI flipping in proteoliposomes generated from a detergent extract of rat liver ER vesicles (76). Flipping was demonstrated to be bidirectional and independent of ATP; both GlcNAc-PI and GlcN-PI were flipped. Curiously, both the GPI flippase and the ER phospholipid flippase appeared to be similarly abundant in the detergent extract used for reconstitution, raising the possibility that they could be the same protein (76). This result would explain why, despite extensive efforts, flipping is the only step of GPI assembly for which a mammalian cell mutant has not been identified; this could be because the GPI flippase also operates in a process, such as flipping of bulk phospholipids, that is essential for mammalian cell viability in culture.

Inositol acylation

S. cerevisiae and mammalian mutant cell lines defective in the formation of Dol-P-Man, the donor of the mannoses in the GPI precursor, accumulate GlcN-PI bearing an acyl group on the inositol's hydroxyl, which makes the lipid resistant to PI-PLC (77–79). All mannose-containing GPI precursors characterized in *S. cerevisiae* and mammalian cells are inositol-acylated. Therefore, inositol acylation most likely occurs at the level of GlcN-PI in vivo (Fig. 2A, step 4). Both yeast and mammalian membranes have an acyl-CoA-dependent GlcN-PI:inositol acyltransferase activity (78, 80), although CoA-independent acyltransferase activity has also been reported (81, 82).

The orthologous mammalian PIG-W and yeast Gwt1 proteins are responsible for inositol acylation. *GWT1* was identified because its high level expression confers resistance to the antifungal compound 1-[4-butylbenzyl] isoquinoline, which blocks surface expression of GPI proteins (83). Immunoprecipitated, epitope-tagged PIG-W catalyzes palmitoyl-CoA-dependent inositol acyl transfer (84), and membranes from *pig-w* and *gwt1* cells lack in vitro palmitoyl-CoA-dependent GlcN-PI:inositol acyltransferase activity (84, 85). These results indicate that the major, and perhaps only, in vivo inositol-acylating activity uses acyl-CoA as donor. The acyl group transferred in vivo in *S. cerevisiae* appears to be palmitate, although acyl chains of a range of different lengths can be transferred from the corresponding CoA derivatives by *S. cerevisiae* and *Cryptococcus neoformans* membranes in vitro (78, 86).

The catalytic site of PIG-W is predicted to be located on the lumenal side of the ER (84). Although it has been reported that the ER presents a barrier to the transport of fatty acyl-CoAs (87) that are required for the PIG-W-catalyzed acyl transfer reaction, it is known that a number of secretory proteins, such as Hedgehog and Wnt, are acylated in the ER lumen by members of the acyl-CoA-dependent membrane-bound *O*-acyltransferase (MBOAT) family of proteins (12, 88). Similarly (see below), fatty acid remodeling of the GPI anchor in the ER lumen requires the acyl-CoA-utilizing MBOAT family members Per1p and Gup1p. These points suggest that acyl-CoAs must somehow be available in the lumen of organelles of the secretory pathway, so it seems likely that the inositol acylation reaction occurs at the lumenal face of the ER (Fig. 2A, step 4).

What is the role of the inositol acyl group? Deletion of yeast *GWT1* is either lethal or leads to very slow, temperature-sensitive growth depending on the strain (83), indicating the importance of inositol acylation in yeast. PIG-W is critical for the expression of GPI proteins at the surface of mammalian cells. In vitro, the presence of the inositol acyl group is obligatory for the transfer of α1,4-linked Man-1 to inositol (80). However, radiolabeling studies with *pig-w* mutants reveal that they can still synthesize mannosylated GPIs lacking the inositol acyl group (84), and it was concluded that inositol acylation is not obligatory for the translocation of GlcN-PI into the ER lumen, or for subsequent mannosylation steps. Because PIG-W mutants accumulate GlcN-PI, and this accumulation is eliminated in cells transfected with PIG-W cDNA, inositol acylation clearly enhances the efficiency of the first mannosylation step. In *pig-w* mutants, the absence of the inositol acyl group did not affect the modification of Man-1 with Etn-P, but GPIs bearing Etn-P on Man-3 were not detected, suggesting that the inositol acyl chain is

also important for addition of the Etn-P through which the GPI becomes linked to protein (84).

Mannosylation

Dol-P-Man is the donor of the core mannosyl residues (Man-1, Man-2, and Man-3) of the GPI precursor (89) and is presumed also to contribute the fourth mannose residue that is essential in the yeast GPI biosynthetic pathway (Fig. 2B). Mammalian Dol-P-Man synthesis-defective mutants (termed Class E) and the temperature-sensitive yeast *dpm1* mutants are blocked in GPI anchoring (77, 90, 91), as is a mammalian cell mutant with a putative defect in Dol-P-Man usage (92). GPI mannosylation can be blocked in mammalian cells by treatment with mannosamine, a compound that may act as a chain terminator (93). Four Dol-P-Man-utilizing, inverting GPI-mannosyltransferases (GPI-Man-Ts) have been identified. These multiple-membrane-spanning proteins are highly conserved functionally, for the mammalian genes encoding GPI-Man-T-II, -III, and -IV all rescue lethal null mutations in their *S. cerevisiae* orthologs. By analogy with the Dol-P-Man-dependent steps in the dolichol-linked pathway for protein *N*-glycosylation (94), mannosyltransfer to GPIs is expected to occur on the lumenal face of the ER. This means that Dol-P-Man, a lipid that is synthesized from Dol-P and GDP-Man on the cytoplasmic face of the ER (Fig. 2A), must flip across the ER membrane. Although a transporter/flippase for Dol-P-Man remains to be found, ATP-independent Dol-P-Man transport activity was recently reconstituted, raising the possibility that this transporter could be identified biochemically in the near future (95).

GPI-Man-T-I. PIG-M/Gpi14p is responsible for the addition of Man-1-linked α1,4 to GlcN (Fig. 2A, step 5) (96). These proteins have been assigned to CAZy Glycosyltransferase Family 50 (68) and are members of a large family of proteins with 12 predicted transmembrane domains (97) in a subgroup with Dol-P-Man-utilizing α1,3 Man-T Alg3p, which acts in the dolichol pathway for *N*-glycosylation. A lumenally oriented loop between transmembrane domains I and II contains a "DXD motif" common to many glycosyltransferases (98) and necessary for PIG-M function. The lumenal location of the functionally important DXD motif is one of the pieces of evidence in support of the lumenal orientation of GPI mannosyltransfer reactions. However, whether the DXD motif serves a ligand binding function in PIG-M/Gpi14p remains to be determined, because it was previously demonstrated to play a role in coordinating nucleotide sugars rather than lipid-linked sugars such as Dol-P-Man.

A second protein, PIG-X, is also required for GPI-Man-T-I function in mammalian cells. This membrane protein, which physically associates with and stabilizes PIG-M, does not appear to be involved in Dol-P-Man synthesis or its utilization for *N*-glycan synthesis (99). The essential 416 amino acid Pbn1 protein is the functional homolog of PIG-X in yeast. Demonstration that *PBN1* complements the GPI-anchoring defect in *pig-x* cells required that *GPI14* be cotransfected with *PBN1*, suggesting that the functional interactions between PIG-X/PIG-M and Pbn1p/Gpi14p are specific (99). Analyses of Pbn1p-deficient yeast strains, however, indicate that this protein is involved in processing diverse proteins that pass though the ER, including a protease destined for the vacuole, a vacuolar membrane protein, and the GPI protein Gas1p, attachment of whose anchor was not apparently affected (100). Therefore, Pbn1p has additional function in yeast, and its role in GPI biosynthesis may be a minor one.

GPI-Man-T-II. PIG-V/Gpi18p is required for the addition of α1,6-linked Man-2 (Fig. 2A, step 7) (101, 102). Mammalian *pig-v* mutants accumulate a GPI intermediate with a single mannose, as well as a GPI with a single mannose additionally modified with Etn-P (Fig. 2A, lipid H5). Likewise, yeast *gpi18* mutants accumulate a Man_1-GPI bearing Etn-P on its mannose, but an unmodified Man_1-GPI was not resolved unambiguously (101). Thus, PIG-V/Gpi18p could, in principle, use Man_1-GPIs, with or without Etn-P on their mannose, as acceptor, and this possibility is illustrated in Fig. 2B.

The PIG-V/Gpi18p proteins have been assigned to single-member CAZy Glycosyltransferase Family GT-76. In contrast to GPI-Man-T-I, -II, and -IV, PIG-V/Gpi18p proteins are predicted to have eight transmembrane domains and no obvious sequence similarity to Family 22 or 50 glycosyltransferases or obvious homologs in the mammalian or yeast proteomes. However, sequences of protein: *O*-mannosyltransferases and the Stt3p subunit of oligosaccharyltransferases are among the many proteins identified after multiple reiterations of PSI-BLAST using the PIG-V sequence as a probe (102), raising the possibility that these glycan-transferring proteins are distantly related.

GPI-Man-T-III. The PIG-B/Gpi10p mannosyltransferase adds the third α1,2-linked Man to GPIs (Fig. 2A, step 8) (103–105). Mammalian *pig-b* and yeast *gpi10* mutants accumulate a Man_2-GPI with an Etn-P substituent on Man-1 (104–108). The preferred acceptor for Gpi10p appears to be a Man_2-GPI with Etn-P on Man-1, but, as discussed below, the requirement for Etn-P on Man-1 is not absolute. PIG-B/Gpi10p is a member of CAZy Glycosyltransferase Family 22, along with Smp3p (as well as Alg9p and Alg12p, respectively, α1,2- and α1,6-Man-T that function in the assembly of the dolichol-linked precursor in *N*-glycosylation). No Family 22 protein has a DXD motif. Protease protection experiments on PIG-B suggested that the bulk of the protein is lumenally oriented and membrane-anchored by a single transmembrane domain near its N terminus (103). However, in silico analyses predict 12 transmembrane domains (97).

GPI-Man-T-IV. The human and yeast Smp3 proteins, also members of Glycosyltransferase Family 22, add a fourth α1,2-linked Man to Man-3 of the GPI (Fig. 2B) (109, 110). Smp3p function is essential in yeast (109), because addition of this side-branching fourth Man to the 2-OH of Man-3 obligatorily precedes the addition of Etn-P to the

6-OH of Man-3 (109). Mammalian cells do not have this stringent requirement for a fourth mannose, for unlike yeast, they transfer Man_3-GPIs to protein (18) and make trimannosyl GPI precursors bearing Etn-P on Man-3 (108, 111–114). Furthermore, the major GPI precursors that accumulate in GPI transamidase-defective mammalian cell lines are trimannosyl structures (112, 115). However, a fourth mannose can be present on the GPIs of some mammalian proteins, notably in brain (18, 116, 117), and traces of putative tetramannosyl GPIs have been detected in radiolabeling experiments with cultured mammalian cells (108, 113, 118). An mRNA for an Smp3p ortholog is expressed in human tissues, the highest levels being in the brain and colon, and expression of *hSMP3* cDNA in human cells results in the formation of Man_4-GPIs (110). Therefore, hSmp3p may be expressed in a tissue-specific manner.

Phosphoethanolamine addition

Proteins involved in Etn-P addition. In addition to the Etn-P moiety at the 6-position of Man-3, through which the GPI becomes linked to protein, Etn-P moieties can be added to the 2-OH of Man-1 and the 6-OH of Man-2 as well (Fig. 1). These side branches are added during precursor biosynthesis. Protein-bound GPIs of both mammalian and yeast proteins can bear side-branching Etn-Ps on their first mannosyl residue (18, 20, 24). There is no evidence, however, that side-branching Etn-Ps are involved in linkages to protein (105). The Etn-P moieties on Man-1 and Man-3 are transferred from Ptd-Etn, not CDP-Etn (119, 120). Because all Etn-P additions are likely to occur in the ER lumen, this requires flipping of PtdEtn from the cytoplasmic face of the ER to the lumenal face (Fig. 2A). Three related proteins, PIG-N/Mcd4p, hGpi7p/Gpi7p, and PIG-O/Gpi13p (GPI-Etn-P-T-I, -II, and -III), appear to be responsible for the addition of an Etn-P moiety to each of the three mannoses. These large proteins are predicted to have 10–14 transmembrane domains and a lumenal hydrophilic loop between transmembrane segments 1 and 2 containing sequences characteristic of members of the alkaline phosphatase superfamily (113, 114, 121–123). The alkaline phosphatase domain is critical for protein function, because the G227E mutation encoded by the *mcd4-174* allele that leads to temperature sensitivity and a conditional block in GPI anchoring (121) is in one of the two metal binding sites in alkaline phosphatase family proteins (123). The presence of the alkaline phosphatase domain is consistent with a role for these proteins in the addition or removal of a phosphodiester-liked substituent, and indeed, deficiencies in these proteins prevent the addition of Etn-P moieties to mannose 1, 2, or 3 of GPI precursors. The small, hydrophobic PIG-F/Gpi11 protein functions as an auxiliary subunit of at least two of the GPI-Etn-P-Ts, but its relative importance varies between yeast and mammalian cells. The phenotypes of yeast mutants defective in Etn-P addition are complex, suggesting that the substituents they add are important for diverse processes involving the GPI; there is also evidence that the GPI-Etn-P-Ts themselves may have additional functions in yeast.

GPI-Etn-P-I. The PIG-N/Mcd4 proteins are involved in the addition of Etn-P to Man-1 (Fig. 2A, B, step 6), but this modification is of different relative importance in mammalian and yeast cells. Mammalian cells lacking PIG-N synthesize GPIs lacking Etn-P on Man-1 and are significantly affected in surface expression of GPI proteins (113), consistent with their inability to synthesize a full complement of mature GPIs. In contrast, a conditionally lethal yeast *mcd4* allele blocks GPI anchoring (121) and leads to the accumulation of a Man_2-GPI lacking Etn-P (124). In vitro GPI synthesis by *mcd4* membranes also does not proceed beyond Man_2-GPI (24). The finding that Mcd4p defects lead to the accumulation of Man_2-GPI lacking Etn-P on Man-1 suggests that Mcd4p normally transfers Etn-P to a dimannosyl GPI, but the accumulation of a Man_1-GPI with Etn-P on its lone mannose in both yeast and mammalian GPI-Man-T-II mutants (see above) indicates that singly mannosylated GPIs can serve as acceptors for PIG-N/Mcd4p as well. The accumulation of a Man_2-GPI lacking Etn-P on Man-1 in Mcd4p-deficient cells further suggests that an Etn-P on Man-1 is required for the GPI-Man-T-III-dependent addition of the third mannose. However, *mcd4* disruptants can be partially bypassed by high-level expression of *GPI10*, and a Man_4-GPI probably bearing one Etn-P on Man-2 is still formed in an *mcd4/gpi11* double mutant (124). Therefore, Gpi10p's requirement for Etn-P on Man-1 is not absolute.

A screen for inhibitors of GPI-dependent processing of the model yeast protein Gas1p identified the terpenoid lactone YW3548. Treatment of yeast cells with this compound led to the accumulation of an unsubstituted Man_2-GPI, consistent with the idea that YW3548 blocks the Mcd4p-dependent addition of Etn-P to Man-1 (104, 125). YW3548 was shown to elicit a similar block in GPI biosynthesis in mammalian cells (109).

Yeast *mcd4* disruptants partially bypassed by native or heterologous GPI-Man-T-III genes are slow-growing (124, 126), indicating that in addition to possibly enhancing the efficiency of Man-3 addition by Gpi10p, this side branch may be required for later functions of the GPI. Indeed, *mcd4Δ* cells partially rescued by *T. brucei GPI10* transfer GPIs to protein less efficiently, exhibit retarded export of GPI proteins from the ER, no longer remodel the lipid moiety of GPI anchors to ceramide, and are defective in axial bud site selection, suggesting that recognition of Etn-P on Man-1 is important in diverse contexts (126). An Etn-P moiety on Man-1 is important for the recognition of GPIs by the human GPI transamidase (118) (see below).

Mcd4p may have additional functions in yeast. The *mcd4*-P301L allele, but not the G227E mutant, exhibits a defect in PtdSer transport to the Golgi and vacuole for subsequent decarboxylation, yet it is not obviously defective in GPI anchoring, suggesting that Mcd4p may have a role in transport-dependent metabolism of PtdSer (127). Furthermore, high-level expression of *MCD4* results in extracellular ATP release and enrichment of both Mcd4p and ATP uptake activity in the Golgi, leading to the suggestion that Mcd4p may normally mediate

the symport of ATP and PtdEtn into the lumen of the ER (128).

GPI-Etn-P-T-II. The human and yeast Gpi7 proteins are involved in Etn-P addition to Man-2 (Fig. 2A, step 10). Viable, although temperature-sensitive, yeast *gpi7* disruptants accumulate a Man_4-GPI with Etn-P on Man-1 and Man-3 but unmodified on Man-2 (122), and analogously, hGpi7p-depleted human cells accumulate a Man_3-GPI bearing Etn-P on Man-1 and Man-3 but not on Man-2 (129).

The hydrophobic PIG-F protein binds to and stabilizes hGpi7p (129) (as well as PIG-O; see below). Although an interaction between Gpi11p and Gpi7p in yeast has not been demonstrated, a role for PIG-F/Gpi11 in Gpi7p function was proposed, because the profile of Man_4-GPI precursors that accumulated in *gpi7* disruptants was similar to that of precursors that accumulated in *gpi11* mutants (130).

In yeast, the *gpi7* deletion leads to a range of defects. These include impaired ER-to-Golgi transport of GPI proteins and ceramide remodeling (122) and inefficient cross-linking of GPI proteins into the cell wall (131). In addition, *gpi7* disruptants have a cell-separation defect attributable to mistargeting of Egt2p, a daughter cell-specific protein that normally is involved in degradation of the division septum (132). These phenotypes suggest that the Etn-P moiety on Man-2 of protein-bound GPIs may be a signal recognized by components of the intracellular transport machinery, by lipid-remodeling enzymes, or by proteins involved in cross-linking GPI to the cell wall.

GPI-Etn-P-III. Human PIG-O and yeast Gpi13p are required for the addition of Etn-P to α1,2-linked Man-3 (Fig. 2A, step 9) (114, 130, 133). This Etn-P is the one that becomes amide-linked to the C terminus of a GPI protein, and as would be expected, *GPI13* is essential in yeast. The Zn^{2+} chelator 1,10-phenanthroline inhibits GPI anchor synthesis in mammalian cells by blocking the PIG-O-catalyzed Etn-P transfer reaction (134); metabolic labeling experiments with phenanthroline-treated cells show an accumulation of H6 (Fig. 2A), a triply mannosylated GPI intermediate with Etn-P on Man-1. As is the case for hGpi7p, PIG-O is also bound and stabilized by the PIG-F protein (114), and indeed, the GPI accumulation phenotype of *pig-f* mutants indicates that they are blocked in Etn-P addition to Man-3 (107, 108, 135, 136). In yeast, however, the PIG-F ortholog Gpi11p does not have a major role in Etn-P addition to Man-3, because the GPIs accumulated in strains lacking Gpi11 or PIG-F function include a Man_4 species bearing Etn-P on its third mannose (130). Therefore, yeast and human cells may differ in the extent to which their GPI-Etn-P-II and -III proteins depend on a partnership with PIG-F/Gpi11p. Yeast Gpi11p's role, however, is not solely to partner Gpi7p, for unlike *GPI7*, *GPI11* is an essential gene. Indeed, both Gpi11p and PIG-F may also act earlier in GPI assembly, because in addition to the two Man_4-GPIs that have been characterized, *gpi11* mutants accumulate two more nonpolar mannolipids (130) and *pig-f* mutants also accumulate multiple mannolipids (108, 135–137). PIG-F, however, does not appear to have a role in PIG-N function, because these two proteins do not interact physically (114).

What is the normal GPI assembly pathway?

Structural characterization of the head groups of GPIs that accumulate in mammalian and yeast GPI mutants has identified a series of potential intermediates that can be arranged in a linear pathway for the assembly of the complete GPI precursor, a Man_3- or possibly a Man_4-GPI with Etn-Ps on Man-1, -2, and -3 in mammals and a Man_4-GPI with Etn-Ps on Man-1, -2, and -3 in yeast (Fig. 2A).

In addition to these complete precursors, it is possible that counterparts of these GPIs lacking Etn-P on Man-2, but probably not Man-1, are also transferred to protein. However, transfer of these structures may be suboptimal, because the double mutant between *gpi7* and transamidase mutant *gpi8* has a synthetic growth defect (122). This synthetic phenotype, though, could also reflect a need for some threshold level of protein-bound GPIs with Etn-P on Man-2.

A feature of the linear pathway is the early addition of Etn-P to Man-1. However, although the (Etn-P)Man-1-GPI pathway is a direct route to a transfer-competent precursor, both yeast and mammalian mutants also synthesize mannosylated GPIs that cannot be placed in it. Some of these GPIs have no Etn-Ps, whereas others lack Etn-P on Man-1 but receive this moiety on Man-2 instead (109, 110, 113, 129, 130, 133) (Fig. 2B). These GPIs can arise in both yeast and mammals, because neither PIG-N nor Mcd4p is obligatory for the addition of Man-3 (113, 124), although GPI-Man-T-III likely has a strong preference for acceptors bearing Etn-P on Man-1.

It is not known, however, whether the GPI isoforms lacking Etn-P on Man-1 are physiologically relevant and represent alternative routes to GPI transamidase substrates. These glycolipids may remain as free GPI and have additional functions of their own, but their accumulation could also be toxic to cells.

TRANSFER OF GPIs TO PROTEIN

As discussed above, genes encoding GPI-anchored proteins specify two signal sequences in the primary translation product: an N-terminal signal sequence for ER targeting and a C-terminal sequence that directs the attachment of a GPI anchor (Fig. 1B). Both sequences are removed during processing of the preproprotein to its mature GPI-anchored form. The removal of the C-terminal GPI signal sequence and its replacement with GPI on the lumenal face of the ER (Fig. 2A, step 11) are catalyzed by GPI transamidase (GPIT) (**Fig. 3A**), a multisubunit membrane-bound enzyme.

Protein translation and translocation must be complete for the C-terminal GPI signal sequence of the proprotein to emerge on the lumenal side of the ER, where it is

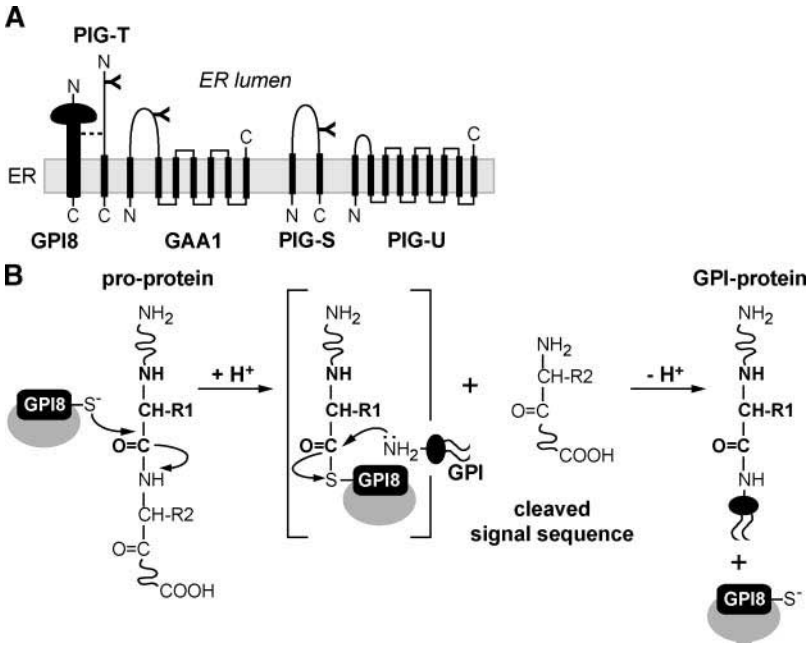

Fig. 3. A: GPI transamidase. The enzyme consists of the catalytic center, GPI8/Gpi8p, and four other subunits (PIG-T/Gpi16p, GAA1/Gaa1p, PIG-S/Gpi17p, and PIG-U/Gab1p) that are all essential for function. The yeast subunits can be divided into two subcomplexes, one containing Gpi8p, Gpi16p, and Gaa1p and the other containing Gab1p and Gpi17p (149, 151, 160). GPI8 and PIG-T are disulfide-linked. PIG-T stabilizes the complex. GAA1 has a role in GPI recognition, because GPIT containing a mutant GAA1 subunit lacking the last transmembrane domain span binds proproteins but not GPIs. PIG-U has a sequence motif that suggests that it may also participate in GPI recognition. PIG-S has no known function. Although none of the subunits bears a recognizable ER retention-retrieval motif, the transmembrane domain of PIG-T may function to localize the complex to the ER (201). This panel shows predicted or tested topological models of the five mammalian subunits; the grouping of the subunits GPI8/PIG-T/GAA1 and PIG-S/PIG-U reflects the subcomplex architecture suggested for the yeast enzyme. B: GPI anchoring of proteins. ER-translocated proteins with a C-terminal GPI signal sequence are recognized by GPIT on the lumenal face of the ER. The C-terminal signal sequence is cleaved between residues ω and ω+1 and replaced with GPI. GPI is attached to the ω residue by an amide bond between the α-COOH of ω and the NH_2 of the capping Etn-P residue in GPI.

recognized by GPIT. GPIT activates the carbonyl group of the ω amino acid in the proprotein by displacing the GPI signal sequence [residues ω+1 through the C terminus; the metabolic fate of the cleaved signal sequence is unknown, but it may be processed in a manner analogous to that described for cleaved ER-targeting N-terminal signals (138)]. Nucleophilic attack on the activated carbonyl by the amino group of an Etn-P-capped GPI substrate (such as mammalian H7 or H8 or yeast CP2, a Man_4-GPI with Etn-P on Man-1, -2, and -3; Fig. 2A) yields a GPI-anchored protein and regenerates GPIT (139–141) (Fig. 3B). Small nucleophiles such as hydrazine and hydroxylamine can replace GPI in microsome-based assays of GPI anchoring (140, 141), providing a simple test of carbonyl activation. Nucleophilic attack by water is also seen in these assays (142), raising the possibility that a small percentage of proprotein products is simply secreted without receiving a GPI anchor. The proportion of secreted proteins may increase when GPI supply is short (93).

GPIT is a complex of five membrane proteins; in mammals and yeast, these are PIG-K (or GPI8)/Gpi8p, GAA1/Gaa1p, PIG-S/Gpi17p, PIG-T/Gpi16p, and PIG-U/Gab1p (Fig. 3A). Gaa1p and Gpi8p were the first to be identified through genetic approaches (143–146) and were subsequently shown to form a complex (147); the other subunits were identified mainly because they specifically coimmunoprecipitated with the GPI8/Gpi8p-GAA1/Gaa1p complex (148–151). GPITs from *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Arabidopsis thaliana* are similar to the mammalian/yeast enzyme. In contrast, GPITs from trypanosomatids such as *T. brucei* share three subunits with mammalian/yeast GPIT (homologs of GPI8, GAA1, and PIG-T termed *Tb*GPI8, *Tb*GAA1, and *Tb*GPI16/PIG-T, respectively) but have two novel subunits (TTA1 and TTA2) in lieu of PIG-S and PIG-U (152, 153). All five subunits are essential in both human and trypanosomatid-type GPIT, and all are needed for the nucleophilic attack on the ω residue that produces the activated carbonyl intermediate to initiate transamidation (147, 148, 150).

GPI8/Gpi8p is presumed to be the catalytic center of the enzyme, because *i*) it shares sequence homology with a family of cysteine proteases, one member of which has transamidase activity in vitro (154); *ii*) mutagenesis of a putative cysteine-histidine catalytic dyad in PIG-K/GPI8

inactivates GPIT (147, 154); and *iii*) it is in physical proximity to the proprotein substrate (155, 156). Mammalian/yeast GPI8/Gpi8p is a type I membrane protein with the majority of its sequence located in the ER lumen; GPI8 in many lower eukaryotes such as nematodes and trypanosomes is a soluble protein of the ER lumen, suggesting that the transmembrane domain is functionally dispensable (152, 157). Consistent with this, a human GPI8 construct lacking the transmembrane domain can rescue GPI anchoring in GPI8$^-$ K562 cells (147). GPI8 is disulfide-linked to PIG-T (158); although this association is not essential, it promotes GPIT activity in vivo. *Escherichia coli*-expressed *T. brucei* GPI8 cleaves a tetrapeptide substrate (159), indicating that *Tb*GPI8, in the absence of other GPIT subunits, is catalytically active, at least as a protease. Genetic and biochemical analyses of the interactions among the subunits of the yeast GPT transamidase complex suggest that the enzyme may consist of two subcomplexes, one containing Gpi8p, Gpi16p, and Gaa1p and the other containing Gab1p and Gpi17p (149, 151, 160) (Fig. 3A).

It is unclear why GPIT consists of five essential subunits when, in principle, the GPI8/Gpi8p subunit should suffice. With the possible exception of the PIG-U/Gab1p subunit that shares a minor resemblance to fatty acid elongase proteins and may therefore have a lipid binding role within GPIT (150), the function of the other "noncatalytic" subunits of GPIT cannot be predicted from their primary sequence. These subunits resemble only their counterparts in other eukaryotes and share no sequence similarity with proteins of known function. A similar conundrum exists for the ER-localized oligosaccharyltransferase (OST) complex that has eight subunits, many of which are essential (161). OST, like GPIT, engages protein (ER-translocated nascent chains with an -N-X-S/T- sequon) and lipid (dolichol-PP-oligosaccharide) to generate an *N*-glycosylated protein. Because a single OST subunit, STT3, is able to carry out oligosaccharyl transfer alone in a bacterial system (162), the question of the variety and essentiality of the other OST subunits in eukaryotes remains. For both GPIT and OST, it seems likely that the noncatalytic subunits select and recruit the two very different substrates that are handled by the enzymes, or that they regulate substrate access to the catalytic site. Recent data support this idea.

Site-specific photo-cross-linking experiments indicate that GAA1 is physically close to proproteins that are bound to GPIT (155), and coimmunoprecipitation analyses show that it is required for GPI8 to recognize its protein substrate (163). Capitalizing on the observation that interactions between GPIT and its proprotein and GPI substrates can be preserved under suitable detergent extraction conditions (164, 165), recent work established that human GPIT complexes containing a defective GAA1 subunit (either lacking the last transmembrane span or with a proline residue in this span mutated to another amino acid) could bind proproteins but not GPI (164, 166). These results indicate that GAA1 plays a key role in substrate recognition by GPIT.

The coimmunoprecipitation methodology used to identify a role for GAA1 in GPI binding was also used to identify the molecular features of GPI that are recognized by human GPIT. Surprisingly, the Etn-P cap linked to Man-3 was shown not to be a prerequisite for GPI recognition, but the Etn-P residue linked to Man-1 was critical (165). Thus, in an in vitro assay, human GPIT was able to pull down the minimal GPI (Etn-P)2Manα1-4GlcN-acylPI (165) and its more elaborate variants. However, Etn-P-capped GPIs lacking the Etn-P modification on Man-1 were poorly recognized. This is a point of some interest for future work, because trypanosomatids do not modify GPIs with Etn-P on Man-1 and, as described above, trypanosomatid GPIT complexes are compositionally distinct from the mammalian/yeast family of GPITs.

Components of the GPI transamidase complex have additional, novel functions. Depletion of PIG-U/Gab1p or Gpi8p in yeast, but not of Gaa1p, PIG-T/Gpi16p, or PIG-S/Gpi17p, results in the accumulation of bar-like structures of actin that are closely associated with the perinuclear ER and decorated with the actin binding protein cofilin (151). Because formation of actin bars is not a characteristic of yeast GPI assembly mutants, and therefore not a general consequence of a defect in GPI anchoring, it was suggested that this phenotype reflects perturbation of a functional interaction between resident proteins of the ER membrane and the actin cytoskeleton, leading to collapse of the ER network around the nucleus (151). It can be speculated that Gab1p and Gpi8p are involved in this interaction, although it is not clear whether the interaction is a direct one. Curiously, recent results indicate that the gene for PIG-U, the human Gab1 ortholog, is amplified in human bladder cancer, and overexpression of PIG-T and GAA1 has been linked to breast cancer (167, 168). In the case of PIG-U, it is not yet known whether this gene amplification affects the actin cytoskeleton in human cells.

LIPID MODIFICATIONS OF PROTEIN-BOUND GPIs AND CELL WALL CROSS-LINKING

Both the glycan and lipid portions of protein-bound GPIs are modified as the GPI protein transits the secretory pathway. In yeast, an α1,2- or α1,3-linked mannose can be added to Man-4 in the Golgi (169) by as yet unknown transferases. In the case of mammals, however, neither the subcellular sites at which Man-1 of GPIs receive additional sugars (Fig. 1A) nor the transferases involved have been identified. It is plausible, though, that Golgi-localized transferases are involved. Our focus is on the modifications to the lipid portion of protein-bound GPIs that are initiated even before the GPI protein exits the ER and continue as the protein travels to the cell surface. These modifications are removal of the inositol acyl chain and introduction of longer chain lipids and are important for the transport of GPI proteins and for modulating the association of GPI proteins with membranes. A further processing event in yeast involves the glycan portion of the GPI, which participates in a transglycosylation reaction in which

the entire GPI protein becomes cross-linked into cell wall β1,6-glucan.

Inositol deacylation

In what appears to be the first posttransfer GPI processing event, the inositol group introduced before mannosylation of the GPI precursor is removed by an ER-localized deacylase (170). The orthologous PGAP1/Bst1 proteins, which contain a lipase consensus motif, are responsible (171, 172).

The phenotypes of yeast and mammalian mutants suggest roles for inositol deacylation in the incorporation of GPI proteins into transport vesicles and in the quality control of GPI proteins. In mammalian cells, loss of PGAP1 function delays the transport of GPI proteins from the ER to the Golgi (171). *S. cerevisiae bst1* mutants were isolated as bypass suppressors of the lethality of a mutation in *SEC13*, which encodes a component of the coat protein complex of COPII-coated vesicles (173). *bst1* mutants are defective in a quality control mechanism in which misfolded proteins are transported to the Golgi by COPII vesicles, then retrieved to the ER (174). Maturation of the GPI protein Gas1p, which normally exits the ER in a class of vesicles distinct from those used by non-GPI proteins (175, 176; reviewed in Ref. 177), is slowed as well. These findings suggest a role for Bst1p in sorting cargo proteins (173, 174); interference in this process presumably allows proteins to exit the ER some other way and bypass the *sec13* defect. It has been speculated that the lethality of *sec13* may be attributable to the accumulation of inositol-deacylated GPI proteins in association with Emp24p and Erp1p cargo receptors that normally facilitate the incorporation of GPI proteins into transport vesicles (178, 179); this interaction is prevented in *bst1* (171). Loss of Bst1p function not only affects the forward transport of normal GPI proteins but also their degradation. *BST1* disruptants are viable but are delayed in ER-associated degradation of a mutant form of Gas1p that is normally misfolded and rapidly degraded in the ER, indicating a role for inositol deacylation in quality control of GPI proteins (172).

Lipid remodeling

In yeast, the remodeling of a GPI's lipid moiety is initiated in the ER, whereas the homologous remodeling activities in mammalian cells seem to start in the Golgi. Analyses of the lipid moieties of the anchors of Gas1p, as well as bulk GPI proteins, indicate the existence in yeast of pathways for remodeling the shorter acyl chains of the diacylglycerol of freshly transferred GPIs to either base-labile $C_{26:0}/C_{26:0}$ diacylglycerols or to a base-stable ceramide consisting of $C_{18:0}$ phytosphingosine and a hydroxy-$C_{26:0}$ fatty acid (23). The remodeling events start in the ER with the removal of the acyl chain at the *sn*-2 position of the diacylglycerol to form a lyso-GPI. Per1, an ER membrane protein, is required for this activity in yeast (180). Next, a $C_{26:0}$ acyl chain is introduced at *sn*-2 by the *O*-acyltransferase Gup1p, an MBOAT family member and resident of the ER membrane (181). The Per1p- and Gup1p-dependent remodeling events are required for optimal ER-to-Golgi transport of GPI proteins and for their association with lipid rafts (180, 181). The GPI remodeled by Gup1p at *sn*-2 is a plausible intermediate in the formation of $C_{26:0}/C_{26:0}$ diacylglycerol-containing GPIs as well as ceramide-based anchors, because the *gup1* deletion severely affects, if not altogether blocks, the formation of base-stable, ceramide-containing GPIs (181). Formation of the latter by replacement of the diacylglycerol with ceramide is also an ER event (48, 182), but the phytosphingosine $C_{26:0}$ ceramide that is initially attached in the ER can in turn be replaced in the Golgi with a phytosphingosine hydroxy-$C_{26:0}$ ceramide (182) to generate the major ceramide-based GPI species found in yeast. The proteins involved in ceramide remodeling are as yet unknown. The Etn-P side branches introduced by Mcd4p and Gpi7p appear to be important for optimal remodeling (122, 126). It is not yet clear, however, whether the diacylglycerol on any given GPI protein can be remodeled to ceramide; if only specific proteins are targeted for this modification, then it remains to be determined what features of the GPI or protein dictate remodeling.

A mammalian counterpart of Gup1p has not yet been identified, but Per1p's ortholog is the PERLD1/PGAP3 protein (180, 183). PGAP3-dependent removal of unsaturated fatty acyl chains at the *sn*-2 position, however, occurs predominantly in the Golgi. The PGAP2 protein, likewise Golgi-localized, is involved in the subsequent introduction of a saturated ($C_{18:0}$) fatty acid at *sn*-2 (47). Mutations in the yeast gene that encodes a homolog of PGAP2, albeit a much larger protein, cause cell wall abnormalities consistent with defects in cell surface anchorage of GPI proteins (47, 184). In mammals, remodeling at *sn*-2 requires prior inositol deacylation by PGAP1 (183). The PGAP3- and PGAP2-dependent remodeling activities, in turn, are necessary for the GPI-anchored proteins to associate with lipid rafts (183).

Cell wall cross-linking

For many fungal GPI proteins, the outer face of the plasma membrane is not the final destination. Rather, an additional processing step occurs in which some, perhaps the majority, of GPI proteins becomes cross-linked to the cell wall via a remnant of their GPI anchor. This post-secretory pathway processing event seems to be characteristic of fungi and has no counterpart in mammals. Pulse-chase and cell fractionation experiments with the *S. cerevisiae* Agα1p mating agglutinin led to the proposal that the GPI of a membrane-anchored protein is cleaved within its glycan, with loss of the inositol-containing lipid moiety and transient formation of a soluble intermediate whose GPI remnant is then transferred to cell wall β1,6-glucan, yielding a GPI-Cell Wall Protein (GPI-CWP) (185, 186). The glucan portion of GPI-CWP-β1,6-glucan is in turn covalently cross-linked to β1,3-glucan and chitin (187–189).

Analyses of the linkage region between GPI-CWPs and β1,6-glucan suggest that the GPI is cleaved between its

GlcN moiety and the first mannose and that the latter's reducing end is glycosidically linked to the nonreducing end of a β1,6-glucan chain or to an internal β1,6-linked glucose (189, 190) (**Fig. 4**). The exact nature of the GPI-Glc linkage is not yet known, nor have the enzymatic activities involved been characterized.

Many yeast GPI proteins are predominantly cell wall-localized, a few are mainly localized in the plasma membrane, and yet others can be detected in both locations (31, 191–195). Although GPI could serve as a generic cell wall-targeting signal whose efficiency varies widely between proteins (193), the ability of a protein to be directed to one or the other location can be critical for it to fulfill its function (194, 196). The importance of protein localization, together with the differential distribution of GPI proteins, suggests the existence of signals in the GPI protein that promote or prevent the cross-linking reaction at the cell surface.

Studies in which the C-terminal sequences of putative GPI proteins, including amino acids immediately N-terminal to the protein's predicted GPI attachment site (the ω-minus region), are exchanged between GPI proteins or fused to heterologous proteins indicate that the ω-minus residues are key determinants of plasma membrane or cell wall localization (191–193, 195). These analyses, together with in silico analyses of the sequences of predicted *S. cerevisiae* GPI proteins (30), indicate that, in general, if the ω-minus acids include two basic amino acids, the protein will be retained in the plasma membrane, and if basic residues are absent or replaced with hydrophobic amino acids, the protein is shifted to the cell wall (191, 192, 194). However, the dibasic motif alone is not the sole determinant of plasma membrane or cell wall localization (193, 195), for it can be overridden, for example, if a long serine- and threonine-rich stretch of amino acids is also present, which results in cell wall anchorage. Whether any structural feature of the GPI anchor itself also plays a role in determining cross-linkage is unknown.

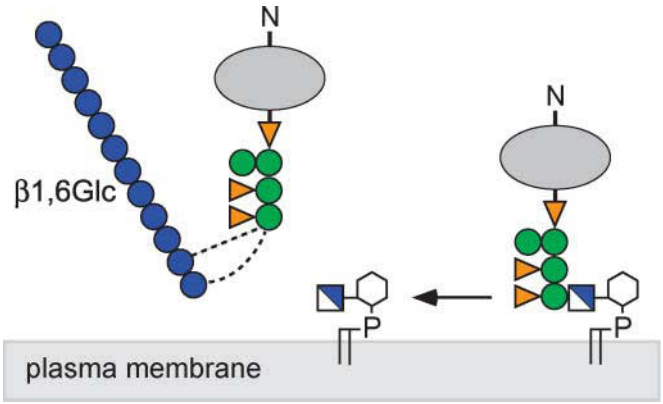

Fig. 4. Postulated transglycosylation reaction in which a GPI-Cell Wall Protein becomes cross-linked to cell wall β1,6-glucan via its GPI glycan. The GPI is likely to be cleaved between Man-1 and GlcN (189, 190) and transferred to a terminal or an internal β1,6-linked Glc in cell wall β1,6-glucan. Features of a GPI glycoprotein that correlate with cell wall anchorage include the presence of serine- and threonine-rich regions in the protein and the absence of two basic amino acids from the ω-minus region (30, 31, 191–195). It is not known whether the presence of Etn-P side branches, or of long acyl chains or a ceramide on the GPI, is important for transglycosylation to occur, or whether Etn-P side branches are retained after cross-linking.

CONCLUDING REMARKS: WHITHER GPI PATHWAY RESEARCH?

In this review, we describe the almost exponential progress that has been made recently in providing genetic and biochemical definitions of the pathway for GPI anchoring, a highly conserved glycosylation pathway dependent on aminophospholipid supply and critical for modification, transport, and surface localization of a subset of proteins. Efforts to chart the GPI pathway have not only identified genes and proteins associated with most of the committed biochemical steps but have also highlighted fascinating issues for future exploration, such as the importance of specific modifications to the protein-bound GPI, the mechanism of GPI flipping, and the enzymology and structure of the membrane proteins involved.

Most of the proteins of the GPI biosynthetic pathway are polytopic membrane proteins, and some function in membrane-bound complexes, making purification a challenge and three-dimensional structural analysis a distant prospect. It is also possible that there is a higher order organization of some or all of the biosynthetic enzymes, enabling substrate channeling. This possibility has been suggested for the dolichol pathway of protein *N*-glycosylation (197). The structural complexity of substrate GPIs has restricted enzymological studies. However, advances in synthetic chemistry have allowed the synthesis of selected GPIs, with which GPI-Man-T-I specificity and GPI flipping have already been investigated, and a chemical-biological analysis of the entire pathway, including the acceptor specificity of pathway enzymes, is likely to follow (76, 80, 198). The availability of defined GPI structures may also aid in the detection of elusive enzyme activities, such as lipid remodelases, the glycosyltransferases that modify protein-bound GPIs, and cell wall cross-linkers.

The pathway for the assembly of GPIs and subsequent remodeling of their lipid moieties has the potential to generate considerable diversity in anchor forms, a diversity already apparent in vivo from the occurrence of species- and tissue-specific variations in GPI structure. Analyses of mutants defective in Etn-P addition and in lipid remodeling are revealing the functional importance of GPI modification at the level of bulk GPI protein in yeast and cultured mammalian cells. What remains to be learned is whether individual proteins receive GPIs with specific head group variations, whether GPI head group structure influences lipid remodeling, and whether the structure of the head group and the lipid moiety of the GPI on an individual protein affects transport, localization, and function in a protein-specific way. Alternatively, are tissue-specific structural variations a consequence of

the altered expression of specific GPI biosynthetic genes? For example, does upregulation of *SMP3* expression cause a wholesale increase in the abundance of Man_4-GPIs on brain glycoprotein? Answers to these questions will require the determination of detailed structures of the GPIs of individual proteins in different tissues and cell types as well as during development and in disease. Advances in proteomic analyses focusing on posttranslational modifications may soon make these studies possible (29).

The biological significance of GPI structural diversity in multicellular organisms remains to be explored. For example, what developmental defects might mice exhibit if they are unable to attach a fourth mannose or add Etn-P to Man-2? Might any human genetic diseases be associated with subtle alterations in GPI structure or diminished ability to transfer GPIs to protein, in the same way that perturbations in the assembly of the dolichol-linked precursor oligosaccharide lead to the range of developmental and neurological defects seen in congenital disorders of glycosylation (199)?

Not only may structural features of GPIs be important, but individual proteins in the GPI-anchoring pathway may themselves contribute to normal cell growth in novel ways. The findings that the genes for the GPI transamidase subunits GAA1 and PIG-U are amplified in human cancers, and that the gene for the PIG-P component of GPI-GnT is overexpressed in fetal Down syndrome brain (66), raise questions of whether and how increased expression of these noncatalytic subunits affects GPI anchoring and, ultimately, gives rise to clinical symptoms. Whether the actin bar phenotype seen when the PIG-U ortholog, Gab1p, and GPI transamidase subunit Gpi8p are depleted in yeast cells (151) will give insights into PIG-U's novel role in mammalian cells is not yet clear, but the striking actin phenotype in yeast highlights the possibility that individual components of the GPI-anchoring machinery have functions beyond their known roles in a glycosylation pathway.

From the outset of work on GPIs in parasitic protozoa, the notion has been pursued that there are species-specific steps in GPI assembly that could be exploited as targets for new antiparasitic drugs (7, 8). The identification of the genes involved in so many of the steps in GPI anchoring in humans and pathogenic eukaryotic microbes is highlighting new potential drug targets, which are beginning to be validated by gene disruption in pathogenic protozoa and fungi (6, 200).

Further studies of GPIs and the proteins that make them are clearly needed to reveal all of the functions of this complex lipid modification in normal cells and in normal development and to determine what goes awry when GPI anchoring is perturbed. There is much to be done, and we hope that others, like us, will learn to stop worrying and love glycophospholipids.

Work in the authors' laboratories was supported by National Institutes of Health Grants GM-55427 (A.K.M.) and GM-46620 (P.O.). The authors thank S. Kubrick and B. Dylan for the usual reasons, and Sam Canis for his unique insights.

REFERENCES

1. Nozaki, M., K. Ohishi, N. Yamada, T. Kinoshita, A. Nagy, and J. Takeda. 1999. Developmental abnormalities of glycosylphosphatidylinositol-anchor-deficient embryos revealed by Cre/loxP system. *Lab. Invest.* **79:** 293–299.
2. Almeida, A. M., Y. Murakami, D. M. Layton, P. Hillmen, G. S. Sellick, Y. Maeda, S. Richards, S. Patterson, I. Kotsianidis, L. Mollica, et al. 2006. Hypomorphic promoter mutation in PIGM causes inherited glycosylphosphatidylinositol deficiency. *Nat. Med.* **12:** 846–851.
3. Inoue, N., Y. Murakami, and T. Kinoshita. 2003. Molecular genetics of paroxysmal nocturnal hemoglobinuria. *Int. J. Hematol.* **77:** 107–112.
4. Leidich, S. D., D. A. Drapp, and P. Orlean. 1994. A conditionally lethal yeast mutant blocked at the first step in glycosyl phosphatidylinositol anchor synthesis. *J. Biol. Chem.* **269:** 10193–10196.
5. Pittet, M., and A. Conzelmann. 2006. Biosynthesis and function of GPI proteins in the yeast *Saccharomyces cerevisiae. Biochim. Biophys. Acta.* In press.
6. Nagamune, K., T. Nozaki, Y. Maeda, K. Ohishi, T. Fukuma, T. Hara, R. T. Schwarz, C. Sütterlin, R. Brun, H. Riezman, et al. 2000. Critical roles of glycosylphosphatidylinositol for *Trypanosoma brucei. Proc. Natl. Acad. Sci. USA.* **97:** 10336–10341.
7. Ferguson, M. A. J. 1999. The structure, biosynthesis and functions of glycosylphosphatidylinositol anchors, and the contributions of trypanosome research. *J. Cell Sci.* **112:** 2799–2809.
8. Ferguson, M. A. J. 2000. Glycosylphosphatidylinositol biosynthesis validated as a drug target for African sleeping sickness. *Proc. Natl. Acad. Sci. USA.* **97:** 10673–10675.
9. Brown, D. A., and E. London. 1998. Functions of lipid rafts in biological membranes. *Annu. Rev. Cell Dev. Biol.* **14:** 111–136.
10. Simons, K., and D. Toomre. 2000. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **1:** 31–39.
11. Helms, J. B., and C. Zurzolo. 2004. Lipids as targeting signals: lipid rafts and intracellular trafficking. *Traffic.* **5:** 247–254.
12. Resh, M. D. 2006. Trafficking and signaling by fatty acylated and prenylated proteins. *Nature Chem. Biol.* **2:** 584–590.
13. Linder, M. E., and R. J. Deschenes. 2007. Palmitoylation: policing protein stability and traffic. *Nat. Rev. Mol. Cell Biol.* **8:** 74–84.
14. Helenius, J., and M. Aebi. 2002. Transmembrane movement of dolichol linked carbohydrates during N-glycoprotein biosynthesis in the endoplasmic reticulum. *Semin. Cell Dev. Biol.* **13:** 171–178.
15. Pomorski, T., and A. K. Menon. 2006. Lipid flippases and their biological functions. *Cell. Mol. Life Sci.* **63:** 2908–2921.
16. McConville, M. J., and A. K. Menon. 2000. Recent developments in the cell biology and biochemistry of glycosylphosphatidylinositol lipids. *Mol. Membr. Biol.* **17:** 1–16.
17. Ferguson, M. A. J., S. W. Homans, R. A. Dwek, and T. W. Rademacher. 1988. Glycosylphosphatidylinositol moiety that anchors *Trypanosoma brucei* variant surface glycoprotein to the membrane. *Science.* **239:** 753–759.
18. Homans, S. W., M. A. Ferguson, R. A. Dwek, T. W. Rademacher, R. Anand, and A. F. Williams. 1998. Complete structure of the glycosyl phosphatidylinositol membrane anchor of rat brain Thy-1 glycoprotein. *Nature.* **333:** 269–272.
19. Roberts, W. L., J. J. Myher, A. Kuksis, M. G. Low, and T. L. Rosenberry. 1988. Lipid analysis of the glycoinositol phospholipid membrane anchor of human erythrocyte acetylcholinesterase. Palmitoylation of inositol results in resistance to phosphatidylinositol-specific phospholipase C. *J. Biol. Chem.* **263:** 18766–18775.
20. Roberts, W. L., S. Santikarn, V. N. Reinhold, and T. L. Rosenberry. 1988. Structural characterization of the glycoinositol phospholipid membrane anchor of human erythrocyte acetylcholinesterase by fast atom bombardment mass spectrometry. *J. Biol. Chem.* **263:** 18776–18784.
21. Brewis, I. A., M. A. Ferguson, A. Mehlert, A. J. Turner, and N. M. Hooper. 1995. Structures of the glycosyl-phosphatidylinositol anchors of porcine and human renal membrane dipeptidase. Comprehensive structural studies on the porcine anchor and interspecies comparison of the glycan core structures. *J. Biol. Chem.* **270:** 22946–22956.
22. Fankhauser, C., S. W. Homans, J. E. Thomas-Oates, M. J. McConville, C. Desponds, A. Conzelmann, and M. A. J. Ferguson.

1993. Structures of glycosylphosphatidylinositol membrane anchors from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **268:** 26365–26374.
23. Sipos, G., A. Puoti, and A. Conzelmann. 1994. Glycosylphosphatidylinositol membrane anchors in *Saccharomyces cerevisiae*: absence of ceramides from complete precursor glycolipids. *EMBO J.* **13:** 2789–2796.
24. Imhof, I., I. Flury, C. Vionnet, C. Roubaty, D. Egger, and A. Conzelmann. 2004. Glycosylphosphatidylinositol (GPI) proteins of *Saccharomyces cerevisiae* contain ethanolamine phosphate groups on the α1,4-linked mannose of the GPI anchor. *J. Biol. Chem.* **279:** 19614–19627.
25. Menon, A. K. 1994. Structural analysis of glycosylphosphatidylinositol anchors. *Methods Enzymol.* **230:** 418–442.
26. Hooper, N. M. 2001. Determination of glycosylphosphatidylinositol membrane protein anchorage. *Proteomics.* **1:** 748–755.
27. Conzelmann, A., C. Fankhauser, and C. Desponds. 1990. Myoinositol gets incorporated into numerous membrane glycoproteins of *Saccharomyces cerevisiae*: incorporation is dependent on phosphomannomutase (*SEC53*). *EMBO J.* **9:** 653–661.
28. Yin, Q. Y., P. W. de Groot, H. L. Dekker, L. de Jong, F. M. Klis, and C. G. de Koster. 2005. Comprehensive proteomic analysis of *Saccharomyces cerevisiae* cell walls: identification of proteins covalently attached via glycosylphosphatidylinositol remnants or mild alkali-sensitive linkages. *J. Biol. Chem.* **280:** 20894–20901.
29. Elortza, F., S. Mohammed, J. Bunkenborg, L. J. Foster, T. S. Nuhse, U. Brodbeck, S. C. Peck, and O. N. Jensen. 2006. Modification-specific proteomics of plasma membrane proteins: identification and characterization of glycosylphosphatidylinositol-anchored proteins released upon phospholipase D treatment. *J. Proteome Res.* **5:** 935–943.
30. Caro, L. H., H. Tettelin, J. H. Vossen, A. F. Ram, H. van den Ende, and F. M. Klis. 1997. *In silico* identification of glycosyl-phosphatidylinositol-anchored plasma-membrane and cell wall proteins of *Saccharomyces cerevisiae*. *Yeast.* **13:** 1477–1489.
31. Hamada, K., S. Fukuchi, M. Arisawa, M. Baba, and K. Kitada. 1998. Screening for glycosylphosphatidylinositol (GPI)-dependent cell wall proteins in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **258:** 53–59.
32. De Groot, P. W. J., K. J. Hellingwerf, and F. M. Klis. 2003. Genome-wide identification of fungal GPI proteins. *Yeast.* **20:** 781–796.
33. Eisenhaber, B., P. Bork, and F. Eisenhaber. 1998. Sequence properties of GPI-anchored proteins near the omega-site: constraints for the polypeptide binding site of the putative transamidase. *Protein Eng.* **11:** 1155–1161.
34. Eisenhaber, F., B. Eisenhaber, W. Kubina, S. Maurer-Stroh, G. Neuberger, G. Schneider, and M. Wildpaner. 2003. Prediction of lipid posttranslational modifications and localization signals from protein sequences: big-Pi, NMT and PTS1. *Nucleic Acids Res.* **31:** 3631–3634.
35. Eisenhaber, B., G. Schneider, M. Wildpaner, and F. Eisenhaber. 2004. A sensitive predictor for potential GPI lipid modification sites in fungal protein sequences and its application to genome-wide studies for *Aspergillus nidulans*, *Candida albicans*, *Neurospora crassa*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *J. Mol. Biol.* **337:** 243–253.
36. Nuoffer, C., P. Jeno, A. Conzelmann, and H. Riezman. 1991. Determinants for glycophospholipid anchoring of the *Saccharomyces cerevisiae* GAS1 protein to the plasma membrane. *Mol. Cell. Biol.* **11:** 27–37.
37. Nuoffer, C., A. Horvath, and H. Riezman. 1993. Analysis of the sequence requirements for glycosylphosphatidylinositol anchoring of *Saccharomyces cerevisiae* Gas1 protein. *J. Biol. Chem.* **268:** 10558–10563.
38. Udenfriend, S., and K. Kodukula. 1995. How glycosylphosphatidylinositol-anchored membrane proteins are made. *Annu. Rev. Biochem.* **64:** 563–591.
39. Krakow, J. L., D. Hereld, J. D. Bangs, G. W. Hart, and P. T. Englund. 1986. Identification of a glycolipid precursor of the *Trypanosoma brucei* variant surface glycoprotein. *J. Biol. Chem.* **261:** 12147–12153.
40. Menon, A. K., S. Mayor, M. A. Ferguson, M. Duszenko, and G. A. Cross. 1988. Candidate glycophospholipid precursor for the glycosylphosphatidylinositol membrane anchor of *Trypanosoma brucei* variant surface glycoproteins. *J. Biol. Chem.* **263:** 1970–1977.
41. Masterson, W. J., T. L. Doering, G. W. Hart, and P. T. Englund. 1989. A novel pathway for glycan assembly: biosynthesis of the glycosylphosphatidylinositol anchor of the trypanosome variant surface glycoprotein. *Cell.* **56:** 793–800.
42. Menon, A. K., R. T. Schwarz, S. Mayor, and G. A. M. Cross. 1990. Cell-free synthesis of glycolipid precursors for the glycosylphosphatidylinositol membrane anchor of *Trypanosoma brucei* variant surface glycoproteins: structural characterization of putative biosynthetic intermediates. *J. Biol. Chem.* **265:** 9033–9042.
43. Leidich, S. D., D. A. Drapp, and P. Orlean. 1995. Isolation and characterization of yeast glycosylphosphatidylinositol anchoring mutants. *Methods Enzymol.* **250:** 560–571.
44. Kinoshita, T., T. Miyata, N. Inoue, and J. Takeda. 1995. Expression cloning strategies for glycosylphosphatidylinositol-anchor biosynthesis enzymes and regulators. *Methods Enzymol.* **250:** 547–560.
45. Hirose, S., J. J. Knez, and M. E. Medof. 1995. Mammalian glycosylphosphatidylinositol-anchored proteins and intracellular precursors. *Methods Enzymol.* **250:** 582–614.
46. Maeda, Y., H. Ashida, and T. Kinoshita. 2006. CHO glycosylation mutants: GPI anchor. *Methods Enzymol.* **416:** 182–205.
47. Tashima, Y., R. Taguchi, C. Murata, H. Ashida, T. Kinoshita, and Y. Maeda. 2006. PGAP2 is essential for correct processing and stable expression of GPI-anchored proteins. *Mol. Biol. Cell.* **17:** 1410–1420.
48. Sipos, G., F. Reggiori, C. Vionnet, and A. Conzelmann. 1997. Alternative lipid remodelling pathways for glycosylphosphatidylinositol membrane anchors in *Saccharomyces cerevisiae*. *EMBO J.* **16:** 3494–3505.
49. Stevens, V. L., and C. R. H. Raetz. 1991. Defective glycosylphosphatidylinositol biosynthesis in extracts of three Thy-1 negative lymphoma cell mutants. *J. Biol. Chem.* **266:** 10039–10042.
50. Watanabe, R., N. Inoue, B. Westfall, C. H. Taron, P. Orlean, J. Takeda, and T. Kinoshita. 1998. The first step of glycosylphosphatidylinositol biosynthesis is mediated by a complex of PIG-A, PIG-H, PIG-C and GPI1. *EMBO J.* **17:** 877–885.
51. Miyata, T., J. Takeda, Y. Iida, N. Yamada, N. Inoue, M. Takahashi, K. Maeda, T. Kitani, and T. Kinoshita. 1993. The cloning of PIG-A, a component in the early step of GPI-anchor biosynthesis. *Science.* **259:** 1318–1320.
52. Leidich, S. D., Z. Kostova, R. R. Latek, L. C. Costello, D. A. Drapp, W. Gray, J. S. Fassler, and P. Orlean. 1995. Temperature-sensitive yeast GPI anchoring mutants gpi2 and gpi3 are defective in the synthesis of *N*-acetylglucosaminyl phosphatidylinositol. Cloning of the *GPI2* gene. *J. Biol. Chem.* **270:** 13029–13035.
53. Schönbächler, M., A. Horvath, J. Fassler, and H. Riezman. 1995. The yeast *spt14* gene is homologous to the human *PIG-A* gene and is required for GPI anchor synthesis. *EMBO J.* **14:** 1637–1645.
54. Vossen, J. H., A. F. Ram, and F. M. Klis. 1995. Identification of *SPT14/CWH6* as the yeast homologue of hPIG-A, a gene involved in the biosynthesis of GPI anchors. *Biochim. Biophys. Acta.* **1243:** 549–551.
55. Inoue, N., R. Watanabe, J. Takeda, and T. Kinoshita. 1996. PIG-C, one of the three human genes involved in the first step of glycosylphosphatidylinositol biosynthesis is a homologue of *Saccharomyces cerevisiae GPI2*. *Biochem. Biophys. Res. Commun.* **226:** 193–199.
56. Kamitani, T., H. M. Chang, C. Rollins, G. L. Waneck, and E. T. Yeh. 1993. Correction of the class H defect in glycosylphosphatidylinositol anchor biosynthesis in Ltk- cells by a human cDNA clone. *J. Biol. Chem.* **268:** 20733–20736.
57. Yan, B. C., B. A. Westfall, and P. Orlean. 2001. Ynl038wp (Gpi15p) is the *Saccharomyces cerevisiae* homologue of human Pig-Hp and participates in the first step in glycosylphosphatidylinositol assembly. *Yeast.* **18:** 1383–1389.
58. Watanabe, R., Y. Murakami, M. D. Marmor, N. Inoue, Y. Maeda, J. Hino, K. Kangawa, M. Julius, and T. Kinoshita. 2000. Initial enzyme for glycosylphosphatidylinositol biosynthesis requires PIG-P and is regulated by DPM2. *EMBO J.* **19:** 4402–4411.
59. Newman, H. A., M. J. Romeo, S. E. Lewis, B. C. Yan, P. Orlean, and D. E. Levin. 2005. Gpi19, the *Saccharomyces cerevisiae* homologue of mammalian PIG-P, is a subunit of the initial enzyme for glycosylphosphatidylinositol anchor biosynthesis. *Eukaryot. Cell.* **4:** 1801–1807.
60. Leidich, S. D., and P. Orlean. 1996. Gpi1, a *Saccharomyces cerevisiae* protein that participates in the first step in glycosylphosphatidylinositol anchor synthesis. *J. Biol. Chem.* **271:** 27829–27837.
61. Tiede, A., J. Schubert, C. Nischan, I. Jensen, B. Westfall, C. H. Taron, P. Orlean, and R. E. Schmidt. 1998. Human and mouse Gpi1p homologues restore glycosylphosphatidylinositol membrane anchor biosynthesis in yeast mutants. *Biochem. J.* **334:** 609–616.
62. Tiede, A., C. Nischan, J. Schubert, and R. E. Schmidt. 2000. Characterisation of the enzymatic complex for the first step in

glycosylphosphatidylinositol biosynthesis. *Int. J. Biochem. Cell Biol.* **32:** 339–350.
63. Sobering, A. K., M. J. Romeo, H. A. Vay, and D. E. Levin. 2003. A novel Ras inhibitor, Eri1, engages yeast Ras at the endoplasmic reticulum. *Mol. Cell. Biol.* **23:** 4983–4990.
64. Sobering, A. K., R. Watanabe, M. J. Romeo, B. C. Yan, C. A. Specht, P. Orlean, H. Riezman, and D. E. Levin. 2004. Yeast Ras regulates the complex that catalyzes the first step in GPI-anchor biosynthesis at the ER. *Cell.* **117:** 637–648.
65. Murakami, Y., U. Siripanyaphinyo, Y. Hong, Y. Tashima, Y. Maeda, and T. Kinoshita. 2005. The initial enzyme for glycosylphosphatidylinositol biosynthesis requires PIG-Y, a seventh component. *Mol. Biol. Cell.* **16:** 5236–5246.
66. Ferrando-Miguel, R., M. S. Cheon, and G. Lubec. 2004. Protein levels of genes encoded on chromosome 21 in fetal Down syndrome brain (Part V): overexpression of phosphatidyl-inositol-glycan class P protein (DSCR5). *Amino Acids.* **26:** 255–261.
67. Kostova, Z., D. M. Rancour, A. K. Menon, and P. Orlean. 2000. Photoaffinity labelling with P3-(4-azidoanilido)uridine 5′-triphosphate identifies Gpi3p as the UDP-GlcNAc-binding subunit of the enzyme that catalyses formation of GlcNAc-phosphatidylinositol, the first glycolipid intermediate in glycosylphosphatidylinositol synthesis. *Biochem. J.* **350:** 815–822.
68. Coutinho, P. M., E. Deleury, G. J. Davies, and B. Henrissat. 2003. An evolving hierarchical family classification for glycosyltransferases. *J. Mol. Biol.* **328:** 307–317.
69. Kostova, Z., B. C. Yan, S. Vainauskas, R. Schwartz, A. K. Menon, and P. Orlean. 2003. Comparative importance *in vivo* of conserved glutamates in the EX_7E-motif retaining glycosyltransferase Gpi3p, the UDP-GlcNAc-binding subunit of the first enzyme in glycosylphosphatidylinositol assembly. *Eur. J. Biochem.* **270:** 4507–4514.
70. Watanabe, R., T. Kinoshita, R. Masaki, A. Yamamoto, J. Takeda, and N. Inoue. 1996. PIG-A and PIG-H, which participate in glycosylphosphatidylinositol anchor biosynthesis, form a protein complex in the endoplasmic reticulum. *J. Biol. Chem.* **271:** 26868–26875.
71. Vidugiriene, J., and A. K. Menon. 1993. Early lipid intermediates in glycosyl-phosphatidylinositol anchor assembly are synthesized in the ER and located in the cytoplasmic leaflet of the ER membrane bilayer. *J. Cell Biol.* **121:** 987–996.
72. Eisenhaber, B., S. Maurer-Stroh, M. Novatchkova, G. Schneider, and F. Eisenhaber. 2003. Enzymes and auxiliary factors for GPI lipid anchor biosynthesis and post-translational transfer to proteins. *Bioessays.* **25:** 367–385.
73. Watanabe, R., K. Ohishi, Y. Maeda, N. Nakamura, and T. Kinoshita. 1999. Mammalian PIG-L and its yeast homologue Gpi12p are *N*-acetylglucosaminylphosphatidylinositol de-*N*-acetylases essential in glycosylphosphatidylinositol biosynthesis. *Biochem. J.* **339:** 185–192.
74. Nakamura, N., N. Inoue, R. Watanabe, M. Takahashi, J. Takeda, V. L. Stevens, and T. Kinoshita. 1997. Expression cloning of PIG-L, a candidate *N*-acetylglucosaminyl-phosphatidylinositol deacetylase. *J. Biol. Chem.* **272:** 15834–15840.
75. Pottekat, A., and A. K. Menon. 2004. Subcellular localization and targeting of *N*-acetylglucosaminyl phosphatidylinositol de-*N*-acetylase, the second enzyme in the glycosylphosphatidylinositol biosynthetic pathway. *J. Biol. Chem.* **279:** 15743–15751.
76. Vishwakarma, R. A., and A. K. Menon. 2005. Flip-flop of glycosylphosphatidylinositols (GPI's) across the ER. *Chem. Commun.* **2005:** 453–455.
77. Orlean, P. 1990. Dolichol phosphate mannose synthase is required *in vivo* for glycosyl phosphatidylinositol membrane anchoring, O mannosylation, and N glycosylation of protein in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **10:** 5796–5805.
78. Costello, L. C., and P. Orlean. 1992. Inositol acylation of a potential glycosyl phosphoinositol anchor precursor from yeast requires acyl coenzyme A. *J. Biol. Chem.* **267:** 8599–8603.
79. Urakaze, M., T. Kamitani, R. DeGasperi, E. Sugiyama, H. M. Chang, C. D. Warren, and E. T. Yeh. 1992. Identification of a missing link in glycosylphosphatidylinositol anchor biosynthesis in mammalian cells. *J. Biol. Chem.* **267:** 6459–6462.
80. Doerrler, W. T., J. Ye, J. R. Falck, and M. A. Lehrman. 1996. Acylation of glucosaminyl phosphatidylinositol revisited. Palmitoyl-CoA dependent palmitoylation of the inositol residue of a synthetic dioctanoyl glucosaminyl phosphatidylinositol by hamster membranes permits efficient mannosylation of the glucosamine residue. *J. Biol. Chem.* **271:** 27031–27038.
81. Stevens, V. L., and H. Zhang. 1994. Coenzyme A dependence of glycosylphosphatidylinositol biosynthesis in a mammalian cell-free system. *J. Biol. Chem.* **269:** 31397–31403.
82. Doerrler, W. T., and M. A. Lehrman. 2000. A water-soluble analogue of glucosaminylphosphatidylinositol distinguishes two activities that palmitoylate inositol on GPI anchors. *Biochem. Biophys. Res. Commun.* **267:** 296–299.
83. Tsukahara, K., K. Hata, K. Sagane, N. Watanabe, J. Kuromitsu, J. Kai, M. Tsuchiya, F. Ohba, Y. Jigami, K. Yoshimatsu, et al. 2003. Medicinal genetics approach towards identifying the molecular target of a novel inhibitor of fungal cell wall assembly. *Mol. Microbiol.* **48:** 1029–1042.
84. Murakami, Y., U. Siripanyapinyo, Y. Hong, J. Y. Kang, S. Ishihara, H. Nakakuma, Y. Maeda, and T. Kinoshita. 2003. PIG-W is critical for inositol acylation but not for flipping of glycosylphosphatidylinositol-anchor. *Mol. Biol. Cell.* **14:** 4285–4295.
85. Umemura, M., M. Okamoto, K. Nakayama, K. Sagane, K. Tsukahara, K. Hata, and Y. Jigami. 2003. *GWT1* gene is required for inositol acylation of glycosylphosphatidylinositol anchors in yeast. *J. Biol. Chem.* **278:** 23639–23647.
86. Franzot, S. P., and T. L. Doering. 1999. Inositol acylation of glycosylphosphatidylinositols in the pathogenic fungus *Cryptococcus neoformans* and the model yeast *Saccharomyces cerevisiae*. *Biochem. J.* **340:** 25–32.
87. Polokoff, M. A., and R. M. Bell. 1978. Limited palmitoyl-CoA penetration into microsomal vesicles as evidenced by a highly latent ethanol acyltransferase activity. *J. Biol. Chem.* **253:** 7173–7178.
88. Hofmann, K. 2000. A superfamily of membrane-bound *O*-acyltransferases with implications for Wnt signaling. *Trends Biochem. Sci.* **25:** 111–112.
89. Menon, A. K., S. Mayor, and R. T. Schwarz. 1990. Biosynthesis of glycosyl-phosphatidylinositol lipids in *Trypanosoma brucei*: involvement of mannosylphosphoryldolichol as the mannosyl donor. *EMBO J.* **9:** 4249–4258.
90. Fatemi, S. H., and A. M. Tartakoff. 1986. Hydrophilic anchor-deficient Thy-1 is secreted by a Class E mutant lymphoma. *Cell.* **46:** 653–657.
91. Conzelmann, A., A. Spiazzi, R. Hyman, and C. Bron. 1986. Anchoring of membrane proteins via phosphatidylinositol is deficient in two classes of Thy-1 negative mutant lymphoma cells. *EMBO J.* **5:** 3291–3296.
92. Anand, M., J. S. Rush, S. Ray, M. A. Doucey, J. Weik, F. E. Ware, J. Hofsteenge, C. J. Waechter, and M. A. Lehrman. 2001. Requirement of the Lec35 gene for all known classes of monosaccharide-P-dolichol-dependent glycosyltransferase reactions in mammals. *Mol. Biol. Cell.* **12:** 487–501.
93. Lisanti, M. P., M. C. Field, I. W. Caras, A. K. Menon, and E. Rodriguez-Boulan. 1991. Mannosamine, a novel inhibitor of glycosylphosphatidylinositol incorporation into proteins. *EMBO J.* **10:** 1969–1977.
94. Helenius, A., and M. Aebi. 2004. Roles of N-linked glycans in the endoplasmic reticulum. *Annu. Rev. Biochem.* **73:** 1019–1049.
95. Rush, J. S., and C. J. Waechter. 2004. Functional reconstitution into proteoliposomes and partial purification of a rat liver ER transport system for a water-soluble analogue of mannosylphosphoryldolichol. *Biochemistry.* **43:** 7643–7652.
96. Maeda, Y., R. Watanabe, C. L. Harris, Y. Hong, K. Ohishi, K. Kinoshita, and T. Kinoshita. 2001. PIG-M transfers the first mannose to glycosylphosphatidylinositol on the lumenal side of the ER. *EMBO J.* **20:** 250–261.
97. Oriol, R., I. Martinez-Duncker, I. Chantret, R. Mollicone, and P. Codogno. 2002. Common origin and evolution of glycosyltransferases using Dol-P-monosaccharides as donor substrate. *Mol. Biol. Evol.* **19:** 1451–1463.
98. Wiggins, C. A., and S. Munro. 1998. Activity of the yeast MNN1 alpha-1,3-mannosyltransferase requires a motif conserved in many other families of glycosyltransferases. *Proc. Natl. Acad. Sci. USA.* **95:** 7945–7950.
99. Ashida, H., Y. Hong, Y. Murakami, N. Shishioh, N. Sugimoto, Y. U. Kim, Y. Maeda, and T. Kinoshita. 2005. Mammalian PIG-X and yeast Pbn1p are the essential components of glycosylphosphatidylinositol-mannosyltransferase I. *Mol. Biol. Cell.* **16:** 1439–1448.
100. Subramanian, S., C. A. Woolford, E. Drill, M. Lu, and E. W. Jones. 2006. Pbn1p: an essential endoplasmic reticulum membrane protein required for protein processing in the endoplasmic reticulum of budding yeast. *Proc. Natl. Acad. Sci. USA.* **103:** 939–944.

101. Fabre, A-L., P. Orlean, and C. H. Taron. 2005. *Saccharomyces cerevisiae* Ybr004c and its human homologue are required for addition of the second mannose during glycosylphosphatidylinositol precursor assembly. *FEBS J.* **272:** 1160–1168.
102. Kang, J. Y., Y. Hong, H. Ashida, N. Shishioh, Y. Murakami, Y. S. Morita, Y. Maeda, and T. Kinoshita. 2005. PIG-V involved in transferring the second mannose in glycosylphosphatidylinositol. *J. Biol. Chem.* **280:** 9489–9497.
103. Takahashi, M., N. Inoue, K. Ohishi, Y. Maeda, N. Nakamura, Y. Endo, T. Fujita, J. Takeda, and T. Kinoshita. 1996. PIG-B, a membrane protein of the endoplasmic reticulum with a large lumenal domain, is involved in transferring the third mannose of the GPI anchor. *EMBO J.* **15:** 4254–4261.
104. Sütterlin, C., M. V. Escribano, P. Gerold, Y. Maeda, M. J. Mazon, T. Kinoshita, R. T. Schwarz, and H. Riezman. 1998. *Saccharomyces cerevisiae GPI10*, the functional homologue of human PIG-B, is required for glycosylphosphatidylinositol-anchor synthesis. *Biochem. J.* **332:** 153–159.
105. Canivenc-Gansel, E., I. Imhof, F. Reggiori, P. Burda, A. Conzelmann, and A. Benachour. 1998. GPI anchor biosynthesis in yeast: phosphoethanolamine is attached to the α1,4-linked mannose of the complete precursor glycophospholipid. *Glycobiology.* **8:** 761–770.
106. Puoti, A., C. Desponds, C. Fankhauser, and A. Conzelmann. 1991. Characterization of glycophospholipid intermediate in the biosynthesis of glycophosphatidylinositol anchors accumulating in the Thy-1-negative lymphoma line SIA-b. *J. Biol. Chem.* **266:** 21051–21059.
107. Sugiyama, E., R. DeGasperi, M. Urakaze, H. M. Chang, L. J. Thomas, R. Hyman, C. D. Warren, and E. T. Yeh. 1991. Identification of defects in glycosylphosphatidylinositol anchor biosynthesis in the Thy-1 expression mutants. *J. Biol. Chem.* **266:** 12119–12122.
108. Hirose, S., G. M. Prince, D. Sevlever, L. Ravi, T. L. Rosenberry, E. Ueda, and M. E. Medof. 1992. Characterization of putative glycoinositol phospholipid anchor precursors in mammalian cells. Localization of phosphoethanolamine. *J. Biol. Chem.* **267:** 16968–16974.
109. Grimme, S. J., B. A. Westfall, J. M. Wiedman, C. H. Taron, and P. Orlean. 2001. The essential Smp3 protein is required for addition of the side-branching fourth mannose during assembly of yeast glycosylphosphatidylinositols. *J. Biol. Chem.* **276:** 27731–27739.
110. Taron, B. W., P. A. Colussi, J. M. Wiedman, P. Orlean, and C. H. Taron. 2004. Human Smp3p adds a fourth mannose to yeast and human glycosylphosphatidylinositol precursors *in vivo*. *J. Biol. Chem.* **279:** 36083–36092.
111. Ueda, E., D. Sevlever, G. M. Prince, T. L. Rosenberry, S. Hirose, and M. E. Medof. 1993. A candidate mammalian glycoinositol phospholipid precursor containing three phosphoethanolamines. *J. Biol. Chem.* **268:** 9998–10002.
112. Mohney, R. P., J. J. Knez, L. Ravi, D. Sevlever, T. L. Rosenberry, S. Hirose, and M. E. Medof. 1994. Glycoinositol phospholipid anchor-defective K562 mutants with biochemical lesions distinct from those in Thy-1- murine lymphoma mutants. *J. Biol. Chem.* **269:** 6536–6542.
113. Hong, Y., Y. Maeda, R. Watanabe, K. Ohishi, M. Mishkind, H. Riezman, and T. Kinoshita. 1999. Pig-n, a mammalian homologue of yeast Mcd4p, is involved in transferring phosphoethanolamine to the first mannose of the glycosylphosphatidylinositol. *J. Biol. Chem.* **274:** 35099–35106.
114. Hong, Y., Y. Maeda, R. Watanabe, N. Inoue, K. Ohishi, and T. Kinoshita. 2000. Requirement of PIG-F and PIG-O for transferring phosphoethanolamine to the third mannose in glycosylphosphatidylinositol. *J. Biol. Chem.* **275:** 20911–20919.
115. Ohishi, K., N. Inoue, and T. Kinoshita. 2001. PIG-S and PIG-T, essential for GPI anchor attachment to proteins, form a complex with GAA1 and GPI8. *EMBO J.* **20:** 4088–4098.
116. Stahl, N., M. A. Baldwin, R. Hecker, K. M. Pan, A. L. Burlingame, and S. B. Prusiner. 1992. Glycosylinositol phospholipid anchors of the scrapie and cellular prion proteins contain sialic acid. *Biochemistry.* **31:** 5043–5053.
117. Nakano, Y., K. Noda, T. Endo, A. Kobata, and M. Tomita. 1994. Structural study on the glycosyl-phosphatidylinositol anchor and the asparagine-linked sugar chain of a soluble form of CD59 in human urine. *Arch. Biochem. Biophys.* **311:** 117–126.
118. Vainauskas, S., and A. K. Menon. 2006. Ethanolamine phosphate linked to the first mannose residue of glycosylphosphatidylinositol (GPI) lipids is a major feature of the GPI structure that is recognized by human GPI transamidase. *J. Biol. Chem.* **281:** 38358–38364.
119. Menon, A. K., and V. L. Stevens. 1992. Phosphatidylethanolamine is the donor of the ethanolamine residue linking a glycosylphosphatidylinositol anchor to protein. *J. Biol. Chem.* **267:** 15277–15280.
120. Imhof, I., E. Canivenc-Gansel, U. Meyer, and A. Conzelmann. 2000. Phosphatidylethanolamine is the donor of the phosphorylethanolamine linked to the α1,4-linked mannose of yeast GPI structures. *Glycobiology.* **10:** 1271–1275.
121. Gaynor, E. C., G. Mondesert, S. J. Grimme, S. I. Reed, P. Orlean, and S. D. Emr. 1999. *MCD4* encodes a conserved endoplasmic reticulum membrane protein essential for glycosylphosphatidylinositol anchor synthesis in yeast. *Mol. Biol. Cell.* **10:** 627–648.
122. Benachour, A., G. Sipos, I. Flury, F. Reggiori, E. Canivenc-Gansel, C. Vionnet, A. Conzelmann, and M. Benghezal. 1999. Deletion of *GPI7*, a yeast gene required for addition of a side chain to the glycosylphosphatidylinositol (GPI) core structure, affects GPI protein transport, remodeling, and cell wall integrity. *J. Biol. Chem.* **274:** 15251–15261.
123. Galperin, M. Y., and M. J. Jedrzejas. 2001. Conserved core structure and active site residues in alkaline phosphatase superfamily enzymes. *Proteins.* **45:** 318–324.
124. Wiedman, J. M., A-L. Fabre, B. W. Taron, C. H. Taron, and P. Orlean. 2007. *In vivo* characterization of the GPI assembly defect in yeast *mcd4-174* mutants and bypass of the Mcd4p-dependent step in *mcd4* null mutants. *FEMS Yeast Res.* **7:** 78–83.
125. Sutterlin, C., A. Horvath, P. Gerold, R. T. Schwarz, Y. Wang, M. Dreyfuss, and H. Riezman. 1997. Identification of a species-specific inhibitor of glycosylphosphatidylinositol synthesis. *EMBO J.* **16:** 6374–6383.
126. Zhu, Y., C. Vionnet, and A. Conzelmann. 2006. Ethanolaminephosphate side chain added to GPI anchor by Mcd4p is required for ceramide remodeling and forward transport of GPI proteins from ER to Golgi. *J. Biol. Chem.* **281:** 19830–19839.
127. Storey, M. K., K. L. Clay, T. Kutateladze, R. C. Murphy, M. Overduin, and D. J. Voelker. 2001. Phosphatidylethanolamine has an essential role in *Saccharomyces cerevisiae* that is independent of its ability to form hexagonal phase structures. *J. Biol. Chem.* **276:** 48539–48548.
128. Zhong, X., R. Malhotra, and G. Guidotti. 2003. ATP uptake in the Golgi and extracellular release require Mcd4 protein and the vacuolar H^+-ATPase. *J. Biol. Chem.* **278:** 33436–33444.
129. Shishioh, N., Y. Hong, K. Ohishi, H. Ashida, Y. Maeda, and T. Kinoshita. 2005. GPI7 is the second partner of PIG-F and involved in modification of glycosylphosphatidylinositol. *J. Biol. Chem.* **280:** 9728–9734.
130. Taron, C. H., J. M. Wiedman, S. J. Grimme, and P. Orlean. 2000. Glycosylphosphatidylinositol biosynthesis defects in Gpi11p- and Gpi13p-deficient yeast suggest a branched pathway and implicate Gpi13p in phosphoethanolamine transfer to the third mannose. *Mol. Biol. Cell.* **11:** 1611–1630.
131. Richard, M., P. De Groot, O. Courtin, D. Poulain, F. Klis, and C. Gaillardin. 2002. *GPI7* affects cell-wall protein anchorage in *Saccharomyces cerevisiae* and *Candida albicans*. *Microbiology.* **148:** 2125–2133.
132. Fujita, M., T. Yoko-o, M. Okamoto, and Y. Jigami. 2004. *GPI7* involved in glycosylphosphatidylinositol biosynthesis is essential for yeast cell separation. *J. Biol. Chem.* **279:** 51869–51879.
133. Flury, I., A. Benachour, and A. Conzelmann. 2000. YLL031c belongs to a novel family of membrane proteins involved in the transfer of ethanolaminephosphate onto the core structure of glycosylphosphatidylinositol anchors in yeast. *J. Biol. Chem.* **275:** 24458–24465.
134. Sevlever, D., K. J. Mann, and M. E. Medof. 2001. Differential effect of 1,10-phenanthroline on mammalian, yeast, and parasite glycosylphosphatidylinositol anchor synthesis. *Biochem. Biophys. Res. Commun.* **288:** 1112–1118.
135. Kamitani, T., A. K. Menon, Y. Hallaq, C. D. Warren, and E. T. Yeh. 1992. Complexity of ethanolamine phosphate addition in the biosynthesis of glycosylphosphatidylinositol anchors in mammalian cells. *J. Biol. Chem.* **267:** 24611–24619.
136. Puoti, A., and A. Conzelmann. 1993. Characterization of abnormal free glycophosphatidylinositols accumulating in mutant lymphoma cells of classes B, E, F, and H. *J. Biol. Chem.* **268:** 7215–7224.

137. Lemansky, P., D. K. Gupta, S. Meyale, G. Tucker, and A. M. Tartakoff. 1991. Atypical mannolipids characterize Thy-1-negative lymphoma mutants. *Mol. Cell. Biol.* **11:** 3879–3885.
138. Weihofen, A., M. K. Lemberg, H. L. Ploegh, M. Bogyo, and B. Martoglio. 2000. Release of signal peptide fragments into the cytosol requires cleavage in the transmembrane region by a protease activity that is specifically blocked by a novel cysteine protease inhibitor. *J. Biol. Chem.* **275:** 30951–30956.
139. Mayor, S., A. K. Menon, and G. A. M. Cross. 1991. Transfer of glycosylphosphatidylinositol membrane anchors to polypeptide acceptors in a cell-free system. *J. Cell Biol.* **114:** 61–71.
140. Maxwell, S. E., S. Ramalingam, L. D. Gerber, L. Brink, and S. Udenfriend. 1995. An active carbonyl formed during glycosylphosphatidylinositol addition to a protein is evidence of catalysis by a transamidase. *J. Biol. Chem.* **270:** 19576–19582.
141. Sharma, D. K., J. Vidugiriene, J. D. Bangs, and A. K. Menon. 1999. A cell-free assay for glycosylphosphatidylinositol anchoring in African trypanosomes. Demonstration of a transamidation reaction mechanism. *J. Biol. Chem.* **274:** 16479–16486.
142. Maxwell, S. E., S. Ramalingam, L. D. Gerber, and S. Udenfriend. 1995. Cleavage without anchor addition accompanies the processing of a nascent protein to its glycosylphosphatidylinositol-anchored form. *Proc. Natl. Acad. Sci. USA.* **92:** 1550–1554.
143. Hamburger, D., M. Egerton, and H. Riezman. 1995. Yeast Gaa1p is required for attachment of a completed GPI anchor onto proteins. *J. Cell Biol.* **129:** 629–639.
144. Benghezal, M., A. Benachour, S. Rusconi, M. Aebi, and A. Conzelmann. 1996. Yeast Gpi8p is essential for GPI anchor attachment onto proteins. *EMBO J.* **15:** 6575–6583.
145. Chen, R., S. Udenfriend, G. M. Prince, S. E. Maxwell, S. Ramalingam, L. D. Gerber, J. Knez, and M. E. Medof. 1996. A defect in glycosylphosphatidylinositol (GPI) transamidase activity in mutant K cells is responsible for their inability to display GPI surface proteins. *Proc. Natl. Acad. Sci. USA.* **93:** 2280–2284.
146. Yu, J., S. Nagarajan, J. J. Knez, S. Udenfriend, R. Chen, and M. E. Medof. 1997. The affected gene underlying the class K glycosylphosphatidylinositol (GPI) surface protein defect codes for the GPI transamidase. *Proc. Natl. Acad. Sci. USA.* **94:** 12580–12585.
147. Ohishi, K., N. Inoue, Y. Maeda, J. Takeda, H. Riezman, and T. Kinoshita. 2000. Gaa1p and Gpi8p are components of a glycosylphosphatidylinositol (GPI) transamidase that mediates attachment of GPI to proteins. *Mol. Biol. Cell.* **11:** 1523–1533.
148. Ohishi, K., N. Inoue, and T. Kinoshita. 2001. PIG-S and PIG-T, essential for GPI anchor attachment to proteins, form a complex with GAA1 and GPI8. *EMBO J.* **20:** 4088–4098.
149. Fraering, P., I. Imhof, U. Meyer, J. M. Strub, A. van Dorsselaer, C. Vionnet, and A. Conzelmann. 2001. The GPI transamidase complex of *Saccharomyces cerevisiae* contains Gaa1p, Gpi8p, and Gpi16p. *Mol. Biol. Cell.* **12:** 3295–3306.
150. Hong, Y., K. Ohishi, J. Y. Kang, S. Tanaka, N. Inoue, J. Nishimura, Y. Maeda, and T. Kinoshita. 2003. Human PIG-U and yeast Cdc91p are the fifth subunit of GPI transamidase that attaches GPI-anchors to proteins. *Mol. Biol. Cell.* **14:** 1780–1789.
151. Grimme, S. J., X-D. Gao, P. S. Martin, K. Tu, S. E. Tcheperegine, K. Corrado, A. E. Farewell, P. Orlean, and E. Bi. 2004. Deficiencies in the endoplasmic reticulum (ER)-membrane protein Gab1p perturb transfer of glycosylphosphatidylinositol to proteins and cause perinuclear ER-associated actin bar formation. *Mol. Biol. Cell.* **15:** 2758–2770.
152. Nagamune, K., K. Ohishi, H. Ashida, Y. Hong, J. Hino, K. Kangawa, N. Inoue, Y. Maeda, and T. Kinoshita. 2003. GPI transamidase of *Trypanosoma brucei* has two previously uncharacterized (trypanosomatid transamidase 1 and 2) and three common subunits. *Proc. Natl. Acad. Sci. USA.* **100:** 10682–10687.
153. Hong, Y., K. Nagamune, K. Ohishi, Y. S. Morita, H. Ashida, Y. Maeda, and T. Kinoshita. 2006. TbGPI16 is an essential component of GPI transamidase in *Trypanosoma brucei*. *FEBS Lett.* **580:** 603–606.
154. Meyer, U., M. Benghezal, I. Imhof, and A. Conzelmann. 2000. Active site determination of Gpi8p, a caspase-related enzyme required for glycosylphosphatidylinositol anchor addition to proteins. *Biochemistry.* **39:** 3461–3471.
155. Vidugiriene, J., S. Vainauskas, A. E. Johnson, and A. K. Menon. 2001. Endoplasmic reticulum proteins involved in glycosylphosphatidylinositol-anchor attachment: photocrosslinking studies in a cell-free system. *Eur. J. Biochem.* **268:** 2290–2300.
156. Spurway, T. D., J. A. Dalley, S. High, and N. J. Bulleid. 2001. Early events in glycosylphosphatidylinositol anchor addition: substrate proteins associate with the transamidase subunit Gpi8p. *J. Biol. Chem.* **276:** 15975–15982.
157. Sharma, D. K., J. Hilley, J. D. Bangs, G. H. Coombs, J. Mottram, and A. K. Menon. 2000. Soluble GPI8 restores glycosylphosphatidylinositol anchoring in a trypanosome cell-free system depleted of soluble endoplasmic reticulum proteins. *Biochem. J.* **351:** 717–722.
158. Ohishi, K., K. Nagamune, Y. Maeda, and T. Kinoshita. 2003. Two subunits of glycosylphosphatidylinositol transamidase, GPI8 and PIG-T, form a functionally important intermolecular disulfide bridge. *J. Biol. Chem.* **278:** 13959–13967.
159. Kang, X., M. Szallies, H. Rawer, M. Echner, and M. Duszenko. 2002. GPI anchor transamidase of *Trypanosoma brucei*: in vitro assay of the recombinant protein and VSG anchor exchange. *J. Cell Sci.* **115:** 2529–2539.
160. Zhu, Y., P. Fraering, C. Vionnet, and A. Conzelmann. 2005. Gpi17p does not stably interact with other subunits of glycosylphosphatidylinositol transamidase in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta.* **1735:** 79–88.
161. Kelleher, D. J., and R. Gilmore. 2006. An evolving view of the eukaryotic oligosaccharyltransferase. *Glycobiology.* **16:** 47R–62R.
162. Wacker, M., D. Linton, P. G. Hitchen, M. Nita-Lazar, S. M. Haslam, S. J. North, M. Panico, H. R. Morris, A. Dell, B. W. Wren, et al. 2002. N-linked glycosylation in *Campylobacter jejuni* and its functional transfer into *E. coli*. *Science.* **298:** 1790–1793.
163. Chen, R., V. Anderson, Y. Hiroi, and M. E. Medof. 2003. Proprotein interaction with the GPI transamidase. *J. Cell. Biochem.* **88:** 1025–1037.
164. Vainauskas, S., and A. K. Menon. 2004. A conserved proline in the last transmembrane segment of Gaa1 is required for GPI recognition by GPI transamidase. *J. Biol. Chem.* **279:** 6540–6545.
165. Vainauskas, S., and A. K. Menon. 2006. Ethanolamine phosphate linked to the first mannose residue of glycosylphosphatidylinositol (GPI) lipids is a major feature of the GPI structure that is recognized by human GPI transamidase. *J. Biol. Chem.* **281:** 38358–38364.
166. Vainauskas, S., Y. Maeda, H. Kurniawan, T. Kinoshita, and A. K. Menon. 2002. Structural requirements for the recruitment of Gaa1 into a functional glycosylphosphatidylinositol transamidase complex. *J. Biol. Chem.* **277:** 30535–30542.
167. Guo, Z., J. F. Linn, G. Wu, S. L. Anzick, C. F. Eisenberger, S. Halachmi, Y. Cohen, A. Fomenkov, M. O. Hoque, K. Okami, et al. 2004. CDC91L1 (PIG-U) is a newly discovered oncogene in human bladder cancer. *Nat. Med.* **10:** 374–381.
168. Wu, G., Z. Guo, A. Chatterjee, X. Huang, E. Rubin, F. Wu, E. Mambo, X. Chang, M. Osada, M. S. Kim, et al. 2006. Overexpression of glycosylphosphatidylinositol (GPI) transamidase subunits phosphatidylinositol glycan class T and/or GPI anchor attachment 1 induces tumorigenesis and contributes to invasion in human breast cancer. *Cancer Res.* **66:** 9829–9836.
169. Sipos, G., A. Puoti, and A. Conzelmann. 1995. Biosynthesis of the side chain of yeast glycosylphosphatidylinositol anchors is operated by novel mannosyltransferases located in the endoplasmic reticulum and the Golgi apparatus. *J. Biol. Chem.* **270:** 19709–19715.
170. Chen, R., E. I. Walter, G. Parker, J. P. Lapurga, J. L. Millan, Y. Ikehara, S. Udenfriend, and M. E. Medof. 1998. Mammalian glycophosphatidylinositol anchor transfer to proteins and posttransfer deacylation. *Proc. Natl. Acad. Sci. USA.* **95:** 9512–9517.
171. Tanaka, S., Y. Maeda, Y. Tashima, and T. Kinoshita. 2004. Inositol deacylation of glycosylphosphatidylinositol-anchored proteins is mediated by mammalian PGAP1 and yeast Bst1p. *J. Biol. Chem.* **279:** 14256–14263.
172. Fujita, M., T. Yoko-o, and Y. Jigami. 2006. Inositol deacylation by Bst1p is required for the quality control of glycosylphosphatidylinositol-anchored proteins. *Mol. Biol. Cell.* **17:** 834–850.
173. Elrod-Erickson, M. J., and C. A. Kaiser. 1996. Genes that control the fidelity of endoplasmic reticulum to Golgi transport identified as suppressors of vesicle budding mutations. *Mol. Biol. Cell.* **7:** 1043–1058.
174. Vashist, S., W. Kim, W. J. Belden, E. D. Spear, C. Barlowe, and D. T. Ng. 2001. Distinct retrieval and retention mechanisms are required for quality control of endoplasmic reticulum protein folding. *J. Cell Biol.* **155:** 355–368.
175. Muniz, M., P. Morsomme, and H. Riezman. 2001. Protein sorting upon exit from the endoplasmic reticulum. *Cell.* **104:** 313–320.
176. Morsomme, P., C. Prescianotto-Baschong, and H. Riezman. 2003. The ER v-SNAREs are required for GPI-anchored protein sorting

from other secretory proteins upon exit from the ER. *J. Cell Biol.* **162:** 403–412.
177. Watanabe, R., and H. Riezman. 2004. Differential ER exit in yeast and mammalian cells. *Curr. Opin. Cell Biol.* **16:** 350–355.
178. Marzioch, M., D. C. Henthorn, J. M. Herrmann, R. Wilson, D. Y. Thomas, J. J. Bergeron, R. C. Solari, and A. Rowley. 1999. Erp1p and Erp2p, partners for Emp24p and Erv25p in a yeast p24 complex. *Mol. Biol. Cell.* **10:** 1923–1938.
179. Muniz, M., C. Nuoffer, H. P. Hauri, and H. Riezman. 2000. The Emp24 complex recruits a specific cargo molecule into endoplasmic reticulum-derived vesicles. *J. Cell Biol.* **148:** 925–930.
180. Fujita, M., M. Umemura, T. Yoko-o, and Y. Jigami. 2006. PER1 is required for GPI-phospholipase A2 activity and involved in lipid remodeling of GPI-anchored proteins. *Mol. Biol. Cell.* **17:** 5253–5264.
181. Bosson, R., M. Jaquenoud, and A. Conzelmann. 2006. *GUP1* of *Saccharomyces cerevisiae* encodes an O-acyltransferase involved in remodeling of the GPI anchor. *Mol. Biol. Cell.* **17:** 2636–2645.
182. Reggiori, F., E. Canivenc-Gansel, and A. Conzelmann. 1997. Lipid remodeling leads to the introduction and exchange of defined ceramides on GPI proteins in the ER and Golgi of *Saccharomyces cerevisiae*. *EMBO J.* **16:** 3506–3518.
183. Maeda, Y., Y. Tashima, T. Houjou, M. Fujita, T. Yoko-o, Y. Jigami, R. Taguchi, and T. Kinoshita. 2007. Fatty acid remodeling of GPI-anchored proteins is required for their raft association. *Mol. Biol. Cell.* In press.
184. Martin-Yken, H., A. Dagkessamanskaia, P. De Groot, A. Ram, F. Klis, and J. Francois. 2001. *Saccharomyces cerevisiae YCRO17c/CWH43* encodes a putative sensor/transporter protein upstream of the BCK2 branch of the PKC1-dependent cell wall integrity pathway. *Yeast.* **18:** 827–840.
185. Lu, C. F., J. Kurjan, and P. N. Lipke. 1994. A pathway for cell wall anchorage of *Saccharomyces cerevisiae* α-agglutinin. *Mol. Cell. Biol.* **14:** 4825–4833.
186. Lu, C. F., R. C. Montijn, J. L. Brown, F. Klis, J. Kurjan, H. Bussey, and P. N. Lipke. 1995. Glycosyl phosphatidylinositol-dependent cross-linking of α-agglutinin and β-1,6-glucan in the *Saccharomyces cerevisiae* cell wall. *J. Cell Biol.* **128:** 333–340.
187. Kapteyn, J. C., R. C. Montijn, E. Vink, J. de la Cruz, A. Llobell, J. E. Douwes, H. Shimoi, P. N. Lipke, and F. M. Klis. 1996. Retention of *Saccharomyces cerevisiae* cell wall proteins through a phosphodiester-linked β-1,3-/β-1,6-glucan heteropolymer. *Glycobiology.* **6:** 337–345.
188. Van Der Vaart, J. M., R. te Biesebeke, J. W. Chapman, F. M. Klis, and C. T. Verrips. 1996. The β-1,6-glucan containing side-chain of cell wall proteins of *Saccharomyces cerevisiae* is bound to the glycan core of the GPI moiety. *FEMS Microbiol. Lett.* **145:** 401–407.
189. Kollar, R., B. B. Reinhold, E. Petrakova, H. J. Yeh, G. Ashwell, J. Drgonova, J. C. Kapteyn, F. M. Klis, and E. Cabib. 1997. Architecture of the yeast cell wall. β(1→6)-glucan interconnects mannoprotein, β(1→)3-glucan, and chitin. *J. Biol. Chem.* **272:** 17762–17775.
190. Fujii, T., H. Shimoi, and Y. Iimura. 1999. Structure of the glucan-binding sugar chain of Tip1p, a cell wall protein of *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta.* **1427:** 133–144.
191. Hamada, K., H. Terashima, M. Arisawa, and K. Kitada. 1998. Amino acid sequence requirement for efficient incorporation of glycosylphosphatidylinositol-associated proteins into the cell wall of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **273:** 26946–26953.
192. Hamada, K., H. Terashima, M. Arisawa, N. Yabuki, and K. Kitada. 1999. Amino acid residues in the omega-minus region participate in cellular localization of yeast glycosylphosphatidylinositol-attached proteins. *J. Bacteriol.* **181:** 3886–3889.
193. De Sampaïo, G., J-P. Bourdineaud, and J-M. Laquin. 1999. A constitutive role for GPI anchors in S*accharomyces cerevisiae*: cell wall targeting. *Mol. Microbiol.* **34:** 247–256.
194. Frieman, M. B., and B. P. Cormack. 2003. The omega-site sequence of glycosylphosphatidyl-inositol-anchored proteins in *Saccharomyces cerevisiae* can determine distribution between the membrane and the cell wall. *Mol. Microbiol.* **50:** 883–896.
195. Frieman, M. B., and B. P. Cormack. 2004. Multiple sequence signals determine the distribution of glycosylphosphatidylinositol proteins between the plasma membrane and cell wall in *Saccharomyces cerevisiae*. *Microbiology.* **150:** 3105–3114.
196. Terashima, H., K. Hamada, and K. Kitada. 2003. The localization change of Ybr078w/Ecm33, a yeast GPI-associated protein, from the plasma membrane to the cell wall, affecting the cellular function. *FEMS Microbiol. Lett.* **218:** 175–180.
197. Gao, X. D., A. Nishikawa, and N. Dean. 2004. Physical interactions between the Alg1, Alg2, and Alg11 mannosyltransferases of the endoplasmic reticulum. *Glycobiology.* **14:** 559–570.
198. Schofield, L., M. C. Hewitt, K. Evans, M. A. Siomos, and P. H. Seeberger. 2002. Synthetic GPI as a candidate anti-toxic vaccine in a model of malaria. *Nature.* **418:** 785–789.
199. Aebi, M., and T. Hennet. 2001. Congenital disorders of glycosylation: genetic model systems lead the way. *Trends Cell Biol.* **11:** 136–141.
200. Grimme, S. J., P. A. Colussi, C. H. Taron, and P. Orlean. 2004. Deficiencies in the essential Smp3 mannosyltransferase block glycosylphosphatidylinositol assembly and lead to defects in growth and cell wall biogenesis in *Candida albicans*. *Microbiology.* **150:** 3115–3128.
201. Vainauskas, S., and A. K. Menon. 2005. Endoplasmic reticulum localization of Gaa1 and PIG-T, subunits of the glycosylphosphatidylinositol (GPI) transamidase complex. *J. Biol. Chem.* **280:** 16402–16409.