Minireview

Biosynthesis of Glycosyl Phosphatidylinositol Membrane Anchors*

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Faced with the problem of membrane attachment, proteins resort to several solutions. For example, many bond to biological membranes via some type of lipid modification. One kind of lipid anchor, described only in the last few years, is a glycosyl phosphatidylinositol (GPI) (1-3) (see Refs. 1-3 for reviews). GPIs anchor an extensive array of cell surface hydrolases, surface antigens, and adhesion molecules to plasma membranes; they are found in organisms ranging from prokaryotes, yeast, and slime mold to Drosophila and man. None have been reported in prokaryotes or in plants. Recently, detailed structural analyses of several GPI anchors have been performed (see below), and the GPI biosynthetic pathway in one organism has been elucidated (see below).

GPI Structure

The anchor of the Trypanosoma brucei variant surface glycoprotein (VSG) exemplifies the GPI solution to membrane attachment. Its structure (Fig. 1) consists of phosphatidylinositol linked glycosidically to a linear tetrasaccharide composed of one glucosaminyl and three mannosyl residues. The terminal mannosyl, at the nonreducing end of the glycan, is linked to phosphoethanolamine, and the ethanolamine is in amide linkage to the α-carboxyl group of the protein’s C-terminal amino acid residue (4). This structure contains several novel linkages, as well as a non-acetylated glucosamine; the latter compound is rare in eukaryotic oligosaccharides, although common to all GPI structures described (3). Remarkably, the core portion of the VSG anchor (consisting of phosphatidylinositol, the linear tetrasaccharide, and phosphoethanolamine) is also present in the GPI of rat brain Thy-1 (5) and in the GPI of human erythrocyte acetylcholinesterase (6, 7). Where linkages have been determined they are also conserved across a spectrum of organisms. Although this review concentrates on the membrane anchors of proteins, the biosynthetic processes described may also apply to GPI anchors of carbohydrate antigens (9, 10), or to free GPI structures, like the one implicated as an insulin second messenger (11).

How Do GPIs Attach to Proteins?

cDNA sequences for GPI-anchored proteins exhibit a characteristic pattern. They encode a typical N-terminal signal sequence, which directs the protein to the endoplasmic reticulum, and a C-terminal sequence, which predicts 20-30 predominantly hydrophobic amino acids. The predicted C-terminal sequences are absent in mature (GPI-anchored) polypeptides (for examples, see Refs. 12 and 13); presumably they are removed during glycolipid addition.

In the case of the trypanosome VSG, replacement of the C-terminal peptide with an anchor occurs within 1 min of polypeptide synthesis (14, 15). This rapid processing suggested that the GPI may be preconstructed, as shown in the model in Fig. 3. According to this model, the C-terminal peptide serves transiently to tether the newly synthesized polypeptide to the endoplasmic reticulum membrane. The ethanolamine amino group of the GPI precursor may make a nucleophilic attack on the appropriate peptide bond of the protein to be anchored, resulting in transfer of the protein to the GPI.

Several experiments indicate that the transfer of protein to a preformed GPI occurs in the endoplasmic reticulum. These include the rapidity of GPI addition after protein synthesis (mentioned above), as well as study of a yeast mutant (sec18). In sec18 cells vesicular transport of glycoproteins from the rough endoplasmic reticulum to the Golgi apparatus is blocked at 37 °C, but GPI anchors are still added correctly to a 125-kDa plasma membrane glycoprotein; this finding provides direct evidence that GPI addition occurs in the former compartment (16).

GPI addition to a protein has not yet been demonstrated in vitro. However, in the case of placental alkaline phosphatase, cleavage of the C-terminus of the primary translation product occurs in the presence of a microsomal extract (17). This cleavage could be due to an abortive reaction catalyzed by the transferase which usually, in the presence of the appropriate GPI precursor, attaches the protein to its anchor.

Signals for GPI Addition

One focus of recent study has been the identification of the sequence determinants, within a given polypeptide, that direct addition of GPI anchors. Such signals probably reside in or near the C-terminal sequences removed during anchor addition. However, these sequences for various GPI-anchored

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2 The abbreviations used are: GPI, glycosyl phosphatidylinositol; PI, phosphatidylinositol (used to include species with fatty acyl or alkyl groups linked to glycerol); VSG, variant surface glycoprotein; GlcNAc-PI, N-acetylg glucosaminyl phosphatidylinositol; GlcN-PI, glucosaminyl phosphatidylinositol.
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The GPI Biosynthetic Precursor: Glycolipid A

The model for GPI addition in Fig. 3 postulates a preformed glycolipid anchor to which protein is transferred. T. brucei contains a glycolipid with many properties consistent with a role as an anchor precursor (23, 24). This glycolipid, termed glycolipid A, can be radiolabeled with glucosamine, mannose, ethanolamine, phosphate, and myristate (as predicted from the known anchor structure); the kinetics of labeling are those expected of a precursor for the VSG anchor. Glycolipid A shares with the VSG anchor susceptibility to several GPI-specific phospholipases and specific chemical cleavage reactions. The two molecules are also antigenically related. Like the VSG anchor, the only fatty acid in glycolipid A is myristic acid. Unlike the VSG anchor, it contains no galactose (see below). So, although the structure of glycolipid A has not been rigorously determined, all of its known properties (23,
Biosynthesis of Glycolipid A

To address the biosynthesis of GPIs in *T. brucei*, a cell-free system for the biosynthesis of glycolipid A was developed. This system, consisting of washed trypanosome membranes, allows incorporation of radiolabeled sugars or fatty acids into glycolipid A, and into a series of less polar biosynthetic intermediates (25).* Structural characterization of the intermediates, and examination of their precursor-product relationships through pulse-chase experiments, led to the proposed pathway of glycolipid A biosynthesis (shown in Fig. 4 (25, 26)).

The first step in the biosynthesis of glycolipid A is the transfer of GlcNAc from UDP-GlcNAc to phosphatidylinositol (PI; Fig. 4, a and b). Whether this glycosylation step is specific for a certain class of PIs is not known. The resulting N-acetylglucosaminyl PI (GlcNAc-PI) is then deacetylated to form glucosaminyl PI (GlcN-PI; Fig. 4c (26)). The two activities involved in GlcN-PI formation, the sugar transfer from UDP-GlcNAc to endogenous PI and the deacetylation of GlcNAc-PI, have been demonstrated in the cell-free system (26), and both have been detergent-solubilized.3

Once GlcN-PI has been formed, it is sequentially mannosylated in the presence of GDP-mannose (Fig. 4, d-f (25)). The immediate sugar donor of these steps has not been determined. GDP-mannose may be the direct donor of one or more residues, and a species related to mannosyl phosphoryldolichol appears to be involved. Evidence for the latter includes failure of a cell line deficient in mannosyl phosphoryldolichol synthase activity to form GPI structures (27, 28). Also, inhibitor studies indicate the involvement of mannosyl phosphoryldolichol in the biosynthesis of trypanosome GPI species (29).

The triply mannosylated GPI intermediate is next modified by the incorporation of phosphoethanolamine from an as yet unknown donor (Fig. 4g (25)). Based on studies with the cell-free system, this phosphoethanolamine donor, present in trypanosome membranes, is probably not CDP-ethanolamine (25); it may be an ethanolamine containing lipid, perhaps phosphatidylethanolamine (a phosphoethanolamine donor to oligosaccharides in other systems (30)). Phosphoethanolamine incorporation yields a compound designated glycolipid A' (Fig. 4g) with the same glycan structure as glycolipid A (25). However, the lipid portions of glycolipids A and A' are not the same, as discussed below.

Fatty Acid Remodeling

*T. brucei* VSG is unusual in that it contains only myristate in its GPI anchor; other anchors contain mixtures of fatty acyl or fatty alkyl groups (3, 7). Because of this strict requirement of trypanosome anchors for myristate, it was surprising that no early biosynthetic precursors of glycolipid A could be labeled with [3H]myristate. It is now clear that glycolipid A' and all earlier GPI intermediates contain fatty acids more hydrophobic than myristate (25). These fatty acids are replaced with myristate late in biosynthesis through a series of remodeling steps (Fig. 4, g-j). Studies in the cell-free system indicate that the first steps are the removal of a fatty acid from glycerol and its replacement, at position sn-2, with myristate from a myristoyl-coenzyme A donor. Subsequently the other fatty acid is replaced by myristate to form glycolipid A, but the details of this reaction are not yet worked out.2

Modifications of the Gycan Core

Up to fatty acid remodeling, the biosynthetic pathway (Fig. 4) yields the core structure common to the several GPIs (from different species) that have been studied in detail. Therefore, these steps could be common to GPI biosynthetic pathways in all cells. Little information is available about when non-core components of GPI structures (see Fig. 2) are added. In the trypanosome case, galactosylation of the core is a late modification of the completed anchor; these residues are added roughly 15 min after the anchor is attached to protein (31, 32), possibly in the Golgi apparatus. In general, anchor modifications may be governed by the structure of the anchored protein, the cell type, or the metabolic state of the cell.

Defective GPI Biosynthesis Is Implicated in a Human Disease

Patients with paroxysmal nocturnal hemoglobinuria experience periodic hemolysis due to increased sensitivity of blood cells to autologous complement-mediated lysis (for review see Refs. 33, 34). This is due to the absence of two proteins, decay-accelerating factor and C8 binding protein, from the cell surface of erythrocytes, platelets, and leukocytes. Both are GPI-anchored proteins (35-37). On normal cells, these molecules act to prevent such incidental host cell lysis during complement activation. Since the defective cells also lack acetylcholinesterase, alkaline phosphatase, and other GPI-anchored proteins, it is likely that the molecular basis of

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* W. J. Masterson, unpublished data.
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paroxysmal nocturnal hemoglobinuria is a defect of GPI biosynthesis or of anchor attachment.

What Now?

Despite the rapid accumulation of information about GPI-anchored proteins, there remains a myriad of fascinating questions and exciting prospects for obtaining answers. Structural analysis of other anchors should reveal the extent of shared features and of novel modifications. Rerational pathways, with their enzymology and regulation, must be explored. Yet to be unscrambled are the peptide sequence or conformational features that signal the addition of glycolipid anchors. In addition, the enzymology of anchor attachment remains largely uncharted; the transfer of protein to a preformed GPI has never been demonstrated in vitro.

It is likely that many of these questions will be addressed, at least in the immediate future, in trypanosomes. These unicellular parasites produce exceptionally large quantities of GPI anchored proteins, there remains a myriad of fascinating questions and exciting prospects for obtaining answers. Rerational pathways, with their enzymology and regulation, must be explored. Yet to be unscrambled are the peptide sequence or conformational features that signal the addition of glycolipid anchors. In addition, the enzymology of anchor attachment remains largely uncharted; the transfer of protein to a preformed GPI has never been demonstrated in vitro.

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