



GPI Membrane Anchors

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GPI Membrane Anchors

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Glycosylphosphatidylinositol (GPI) membrane anchors are complex glycolipids found covalently attached to a wide variety of externally disposed plasma membrane proteins in eukaryotes⁽¹⁻³⁾. Some lumenally disposed secretory granule proteins are also GPI-anchored, but there are no examples of cytoplasmically oriented GPI-anchored proteins. The primary function of GPI anchors is to afford a stable association of protein with the membrane lipid bilayer. They should be thought of as an alternative anchoring mechanism to the use of a hydrophobic peptide transmembrane domain by class 1 membrane proteins (Figure 1).

All GPI anchors have a conserved basic core structure: ethanolamine—PO₄—6Man α 1—2Man α 1—6Man α 1—4GlcNH₂ α 1—6myo-inositol-1-PO₄—lipid with species- and tissue-specific variations in substituents and lipid structure^(4,5) (Figure 2). The myo-inositol—1—PO₄—lipid component is loosely described as a ‘phosphatidylinositol’ (PI). The lipid moiety is quite variable; most commonly it is *sn*-1-alkyl-2-acyl-glycerol, followed by *sn*-1,2-diacyl-glycerol, *sn*-1-acyl-2-lysoglycerol and ceramide. In many instances the inositol ring contains an additional lipid modification in the form of an ester-linked palmitic acid (C16:0). This inositol-palmitoylation renders the anchor resistant to the action of bacterial phosphatidylinositol-specific phospholipase C (PI-PLC), an enzyme widely used to release GPI-anchored proteins from the surface of cells. It should be noted that the PI-PLC enzymes that cleave phosphatidylinositol 4,5-bisphosphate (PIP₂), as part of the inositol 1,4,5-trisphosphate (IP₃) second messenger system, and those that cleave GPI structures are mutually exclusive. The GPI is attached to the protein through an amide bond between the C-terminal amino acid α -carboxyl group and the amino group of the ethanolamine phosphate bridge. The presence of extra ethanolamine phosphate substituents does not occur in protozoa or in yeast GPIs, but is a ubiquitous modification in the GPIs of higher eukaryotes. In some anchors there can be two of these extra units.

GPI ANCHOR BIOSYNTHESIS

GPI anchors are synthesized as precursors in the endoplasmic reticulum. This process involves the sequential transfer of monosaccharides to a phosphatidylinositol acceptor and the transfer of ethanolamine phosphate from phosphatidylethanolamine to the terminal mannose.

A protein destined to receive a GPI anchor must contain an N-terminal signal sequence for entry into the lumen of the endoplasmic reticulum via the signal recognition particle and a GPI signal sequence at the C-terminus. The GPI-signal sequence is extremely degenerate (reviewed in references 1,3,6-8). The most common feature is a run of 12-20 hydrophobic residues at the very C-terminus of the

primary translation product, although there are exceptions. The GPI-signal sequence is cleaved and replaced by a pre-assembled GPI precursor⁽⁹⁻¹¹⁾. From kinetic and genetic data the addition of GPI to proteins is believed to occur in the endoplasmic reticulum. For reviews on GPI biosynthesis, see references 5 and 12-15.

IDENTIFICATION OF GPI ANCHORS

The presence of a GPI membrane anchor on a protein can be inferred indirectly from the analysis of cDNA sequences, or directly by structural analysis for the components ethanolamine and myo-inositol using amino-acid analysis and gas chromatography-mass spectrometry, respectively. As an alternative to chemical analysis, biosynthetic radiolabeling of proteins with [³H]-ethanolamine and [³H]myo-inositol may be used for cells in tissue culture. Another approach is the use of *Bacillus thuringiensis* PI-PLC. This enzyme is not cytotoxic and releases GPI-anchored proteins from the surface of living cells. The release of a particular protein can be monitored either by following the appearance of solubilized protein in the medium, or by following its loss from the cell surface. The latter criterion is often conveniently measured by fluorescence flow cytometry using a fluorescently labeled antibody to the protein of interest. The PI-PLC approach can also be used on cell lysates and purified proteins. For example, the release of the lipid moiety by PI-PLC changes the phase-partition behavior of GPI-anchored proteins. In a Triton X-114 (TX-114) phase-separation experiment, GPI-anchored proteins partition into the detergent phase before PI-PLC treatment, and into the aqueous phase after PI-PLC treatment. In addition, PI-PLC-cleaved GPI-anchored proteins express an epitope called ‘the cross-reacting determinant’ (CRD) which can be recognized by anti-CRD antibodies on Western blots.

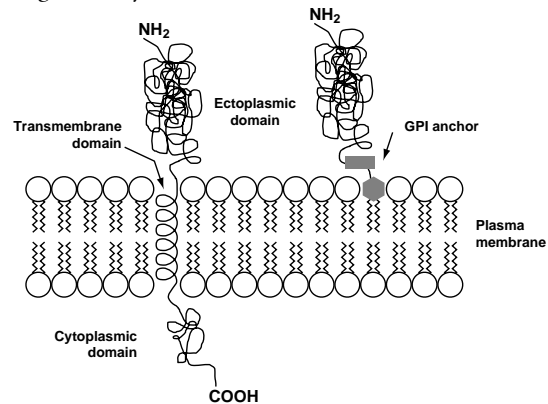


Figure 1. Comparison between transmembrane and GPI-anchored plasma membrane proteins. A class 1 transmembrane protein (left) has an N-terminal ectoplasmic domain, a transmembrane domain consisting of an α -helix of about 20 hydrophobic amino acids, and a C-terminal cytoplasmic domain. A GPI-anchored protein (right) has an N-terminal ectoplasmic domain and is embedded in the outer leaflet of the lipid bilayer solely via the lipid portion of the GPI anchor.

GPI Membrane Anchors

Although positive results with PI-PLC are powerful criteria of GPI anchor identification, negative results are ambiguous as many GPI anchors are inherently resistant to PI-PLC because of inositol-palmitoylation.

FUNCTION AND PROPERTIES

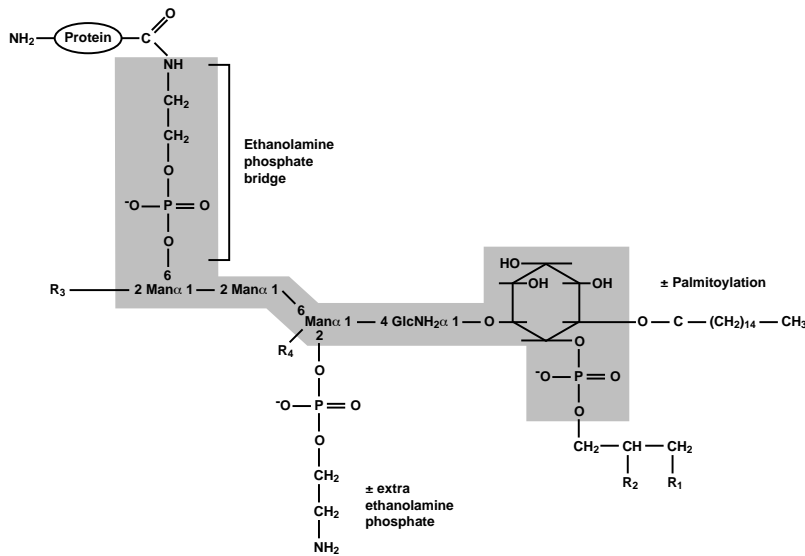
The basic function of a GPI anchor is to afford a stable association of protein with the membrane. The GPI anchor is an efficient and stable anchor comparable with a hydrophobic peptide domain. Additionally, possession of a GPI anchor has many consequences.

1. In mammalian cells GPI-anchored proteins may be found in specialized membrane micro-domains which are insoluble in the neutral detergent Triton X-100.

These specialized micro-domains contain mixtures of many GPI-anchored proteins⁽¹⁶⁻¹⁸⁾ and appear to be rich in sphingolipids, glycosphingolipids and probably cholesterol⁽¹⁹⁾.

2. The presence of a GPI anchor generally causes the targeting of proteins to the apical membrane of polarized cells^(20,21).

3. GPI-anchored proteins are generally excluded from the clathrin-mediated endocytic pathway. Their levels of surface expression are generally very high and their turnover rates are generally low. However, in some cases, e.g. the GPI-anchored folate binding protein, a novel clathrin-independent form of endocytosis (called potocytosis) is thought to occur^(22,23).



| Example | R1 | R2 | R3 | R4 | Extra ethanolamine phosphate | Palmitoylation |
|-------------------------------------|--|--|---|--------------------------------|------------------------------|----------------|
| <i>Trypanosoma brucei</i> VSG | C _{14:0} fatty acid (exclusively) | C _{14:0} fatty acid (exclusively) | - | [α Gal] _{0,5} | - | - |
| <i>Trypanosoma brucei</i> PARP | C _{18:0} fatty acid (exclusively) | - | [NANA ₅ ,Gal ₉ ,GlcNAc ₉] | - | + | - |
| <i>Trypanosoma cruzi</i> IG7 | C _{16:0} alkyl | C _{18:0} fatty acid | α Man | - | - | - |
| <i>Leishmania major</i> PSP | C _{24:0} alkyl | C _{16:0} fatty acid | - | - | - | - |
| Yeast Proteins | Ceramide | [α Man] _{1,2} | - | - | - | - |
| <i>Dictyostelium discoideum</i> PSA | Ceramide | \pm α Man | - | + | - | - |
| <i>Torpedo</i> AChE | C _{16:0} alkyl | C _{16:0} fatty acid | α Glc | \pm β GalNAc | + | - |
| Human erythrocyte AChE | C _{18:0} alkyl | C _{22:4} fatty acid | - | - | + | + |
| Human erythrocyte DAF | Alkyl | C _{22:4} fatty acid | ? | ? | + | + |
| Rat brain Thy-1 | ? alkyl | C _{18:0} fatty acid | \pm α Man | β GalNAc | + | - |
| Hamster Prp | ? | ? | \pm α Man | [NANA-Hex-HexNAc] | - | + |

Figure 2. The chemical structure of GPI anchors. All GPI anchors contain a conserved core structure (shaded box) with species- and tissue-specific variations in the lipid structure and core substituents, as indicated. AChE, Acetylcholinesterase; VSG, Variant surface glycoprotein; ?, unknown; PARP, Procylic Acidic Repetitive Protein; PSP, Promastigote Surface Protease; DAF, Decay Accelerating Factor; PSA, Pre-sporo Antigen; Prp, Prion protein.

GPI Membrane Anchors

4. Although GPI-anchored proteins have no cytoplasmic domain they can effect transmembrane signalling^(24,25). In T cells, for example, cross-linking of any GPI-anchored protein into patches causes a rise in intracellular calcium, and mitogenesis in the presence of phorbol esters. The mechanism by which the transmembrane signal is mediated is unknown.
5. The mobility of GPI-anchored proteins in the plane of the membrane is dictated by their interactions with other membrane components and can range from extremely fast to essentially immobile.
6. There is evidence that some GPI-anchored proteins are released from membranes by the action of GPI-specific phospholipases. However, the generality and the mechanism of control of this phenomenon is unknown. The normal sera of all mammals are known to contain GPI-specific phospholipase D which is capable of cleaving between the inositol ring and the phosphate of the PI moiety of all GPI anchors in detergent solution. However, the action of this enzyme on the surface of living cells has not been demonstrated⁽²⁶⁾.
7. The expression of alternatively anchored forms of the same protein (GPI versus transmembrane) through mRNA splicing or differential gene expression may be used to modulate the function of some proteins⁽²⁾. For example, ligation of the transmembrane form of the FcγRIII receptor (CD16) with immune complexes directly triggers cytotoxic reaction and phagocytosis in monocytes, whereas ligation of the GPI-anchored form of the same receptor in neutrophils only potentiates these cells to respond to signals via other transmembrane receptors⁽²⁷⁻²⁹⁾.
8. Defects in GPI anchor biosynthesis are associated with the human disease paroxysmal nocturnal hemoglobinuria (PNH). Patients with PNH lack GPI-anchored proteins on a range of cells (erythrocytes, platelets, granulocytes, monocytes, natural killer cells, and some B cells and T cells) owing to the proliferation of mutant hemopoietic stem cells. The lack of complement-regulating proteins, such as the GPI-anchored CD59 and decay accelerating factor (DAF), on the erythrocytes of PNH patients is thought to be responsible for the autohemolysis characteristic of this disease.
9. The use of GPI anchors is more common in lower eukaryotes such as protozoa and yeast than in higher eukaryotes such as mammals. The parasitic kinetoplastid protozoa (e.g. *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* spp.) are known to express extremely high levels of GPI anchors and/or GPI-related molecules which are not linked to protein. One of these GPI-related molecules is the lipophosphoglycan (LPG) of *Leishmania*

parasites which contains a large repeating phosphosaccharide chain linked to a GPI-like glycolipid core. The LPG is known to confer virulence on *Leishmania* both in the mammalian host and the insect (sand-fly) vector⁽⁵⁾. The presence of novel parasite-specific GPI-related molecules may reflect evolutionary elaboration of the conserved GPI anchor biosynthetic pathway.

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