INTRODUCTION

Studies of the African trypanosome have played an interesting part in the development of several areas of research, including the structure, biosynthesis and function of glycosylphosphatidylinositol (GPI) membrane anchors (Fig. 1). Martin Low (Low and Finean, 1977, 1978; Low and Zilversmit, 1980) and Hiro Ikezawa (Ikezawa et al., 1976; Taguchi et al., 1980) independently developed the concept of anchorage of proteins to membranes via covalent linkage to phosphatidylinositol in the late 1970s. However, this original work did not receive the attention it deserved until 1985, when a body of compositional data on *Torpedo* electric-organ acetylcholinesterase (AChE) (Futerman et al., 1985), human erythrocyte AChE (Roberts and Rosenberry, 1985) and rat brain and thymocyte THY-1 (Tse et al., 1985), and compositional and structural data on *Trypanosoma brucei* variant surface glycoprotein (VSG) (Ferguson et al., 1985a,b) became available. Since then, researchers from many disciplines have documented the presence of GPI anchors on more than 200 eukaryote cell-surface proteins, and studies of GPI structure, biosynthesis and function have expanded significantly.

The role of the African trypanosome in this saga began with the discovery of the cell surface coat by electron microscopy by Keith Vickerman. The tsetse-fly-transmitted African trypanosomes are extracellular protozoan parasites that live in the blood, lymph and interstitial fluids of the infected mammalian host, where they divide by binary fission. The ability of the trypanosome population to evade the host immune response was evident as long ago as 1910, when Ross and Thomson demonstrated that infected cattle display cyclical waves of parasitaemia (Ross and Thomson, 1910). Vickerman's work established the mechanism of parasite survival: trypanosomes possess a dense and uniform protective coat that undergoes antigenic variation (Vickerman and Luckins, 1969). In the mid-1970s George Cross produced antigenically pure trypanosome clones (variants) and showed that their surface coats comprise ~10^7 copies/cell of 55 kDa variant-specific surface glycoproteins (VSGs) (Cross, 1975). Mervyn Turner and colleagues showed that the VSGs are homodimers and, together with Don Wiley, established the crystal structures of two VSG variants (Blum et al., 1993). These remarkable studies showed that VSGs whose components share only 16% amino acid sequence similarity can adopt almost identical tertiary structures, explaining how antigenically distinct molecules can assemble into functionally identical protective-coat arrays (Fig. 2).

SUMMARY

The discovery of glycosylphosphatidylinositol (GPI) membrane anchors has had a significant impact on several areas of eukaryote cell biology. Studies of the African trypanosome, which expresses a dense surface coat of GPI-anchored variant surface glycoprotein, have played important roles in establishing the general structure of GPI membrane anchors and in delineating the pathway of GPI biosynthesis. The major cell-surface molecules of related parasites are also rich in GPI-anchored glycoproteins and/or GPI-related glycophosholipids, and differences in substrate specificity between enzymes of trypanosomal and mammalian GPI biosynthesis may have potential for the development of anti-parasite therapies. Apart from providing stable membrane anchorage, GPI anchors have been implicated in the sequestration of GPI-anchored proteins into specialised membrane microdomains, known as lipid rafts, and in signal transduction events.

Key words: Glycosylphosphatidylinositol, Trypanosome, Lipid raft

COMMENTARY

The structure, biosynthesis and functions of glycosylphosphatidylinositol anchors, and the contributions of trypanosome research

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INTRODUCTION

Studies of the African trypanosome have played an interesting part in the development of several areas of research, including the structure, biosynthesis and function of glycosylphosphatidylinositol (GPI) membrane anchors (Fig. 1). Martin Low (Low and Finean, 1977, 1978; Low and Zilversmit, 1980) and Hiro Ikezawa (Ikezawa et al., 1976; Taguchi et al., 1980) independently developed the concept of anchorage of proteins to membranes via covalent linkage to phosphatidylinositol in the late 1970s. However, this original work did not receive the attention it deserved until 1985, when a body of compositional data on *Torpedo* electric-organ acetylcholinesterase (AChE) (Futerman et al., 1985), human erythrocyte AChE (Roberts and Rosenberry, 1985) and rat brain and thymocyte THY-1 (Tse et al., 1985), and compositional and structural data on *Trypanosoma brucei* variant surface glycoprotein (VSG) (Ferguson et al., 1985a,b) became available. Since then, researchers from many disciplines have documented the presence of GPI anchors on more than 200 eukaryote cell-surface proteins, and studies of GPI structure, biosynthesis and function have expanded significantly.

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STRUCTURAL STUDIES

The Cross and Turner groups were intrigued by the enigma that the VSG coats are stably associated with the parasite plasma...
membrane but can be isolated as freely water-soluble glycoproteins (sVSGs) after cell lysis. This latter observation also appeared to be at variance with VSG cDNA sequences that were predicted to encode C-terminal hydrophobic peptide sequences reminiscent of transmembrane domains (Boothroyd et al., 1980). Analyses of mature sVSG C-termini by Tony Holder showed that these C-terminal hydrophobic peptides are removed and replaced by an unusual ethanolamine-carbohydrate moiety (Holder, 1983). However, the hydrophilic nature of the C-terminal moiety isolated from sVSGs did not explain VSG-membrane association. Cardoso de Almeida and Turner (1983) provided the missing link. They showed that, when they isolated VSG under denaturing conditions (i.e. in boiling detergent buffers), it retained the amphiphilic properties expected of an integral membrane glycoprotein. This membrane form of VSG (mfVSG) was readily converted to sVSG during cell lysis by the action of an endogenous heat-labile enzyme that was later identified as a GPI-specific phospholipase C. Thus, the combined observations of Holder and Cross and Cardoso de Almeida and Turner provided evidence for modification of VSG by some sort of complex glycolipid that serves as the sole membrane anchor.

The sheer abundance of VSG, and the ease of cultivation of batches of $10^{11}$ T. brucei bloodstream forms, allowed purification of 40-60 mg amounts of sVSG and mfVSG, and this greatly assisted the structural analyses that, over a period of about five years, defined the first partial GPI-anchor structure (Ferguson et al., 1985b) and, subsequently, the complete structure (Ferguson et al., 1988). Central to the solution of the complete structure, and shortly afterwards that of the rat brain THY-1 GPI anchor (Homans et al., 1988), was two-dimensional proton nuclear magnetic resonance (NMR) spectroscopy. The structures of >20 GPI-anchor structures have since been partially or completely solved, and are reviewed elsewhere (McConville and Ferguson, 1993; Treumann et al., 1998). These studies have revealed a conserved core structure.
(Fig. 1) and tissue- and species-specific features. In recent years, methodologies for analysis of subnanomole amounts of GPI anchors have been established (Schneider and Ferguson, 1995; Treumann et al., 1998; Zitzmann and Ferguson, 1999).

The innate complexity of GPI anchors, which contain carbohydrate, lipid, phosphate and amines, has attracted several high-powered synthetic-organic-chemistry groups, and partial and complete (Murkata and Ogawa, 1992; Campbell and Fraser-Reid, 1995; Baeschlin et al., 1998; Tailler et al., 1999) GPI structures have been synthesised; these are reviewed elsewhere (Gigg and Gigg, 1997). Some of these compounds, and their analogues, have been used in biosynthesis studies (see below).

GPI-RELATED MOLECULES OF TRYPANOSOMATIDS

By defining GPI structures as those that contain the structural motif Man\(\alpha_1-4\)GlcN\(\alpha_1-6\)PI, we can exclude the mannosylated PI glycolipids (note: PI is used throughout this article as a global abbreviation for diradylphosphatidylinositol and inositol phosphoceramide) of plants, yeast, fungi and mycobacteria and identify three families of eukaryote GPI structure. The type-1 GPs, which include all of the protein-linked GPI anchors, are based on the Man\(\alpha_1-6\)Man\(\alpha_1-4\)GlcN\(\alpha_1-6\)PI motif, type-2 GPs are based on the Man\(\alpha_1-3\)Man\(\alpha_1-4\)GlcN\(\alpha_1-6\)PI motif, and the hybrid GPs are based on the Man\(\alpha_1-6\)Man\(\alpha_1-3\)Man\(\alpha_1-4\)GlcN\(\alpha_1-6\)PI motif (McConville and Ferguson, 1993). The type-2 and hybrid GPs are much less widespread than the type-1 GPs, but are particularly abundant in certain protozoa such as *Tetrahymena* and trypanosomatid parasites (i.e. parasites of the order Kinetoplastida and belonging to the same family as trypanosomes) such as the *Leishmania*, *Endotrypanum*, *Leptomonas* and *Crithidia* (McConville and Ferguson, 1993; Routier et al., 1995).

The cell surfaces of the trypanosomatids are dominated by GPI-anchored or GPI-related molecules at all stages of their life cycles (Fig. 3). Many of these organisms express abundant glycoinositol phospholipids (GIPLs). These GPI structures are

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Fig. 3. The cell surface molecules of trypanosomatid parasites. The general dimensions and copy numbers of the major cell-surface molecules of (A) *T. brucei* bloodstream forms, (B) *Leishmania* promastigote forms and (C) *T. cruzi* epimastigote forms are shown in cartoon form (top panels) together with models of 20 nm×20 nm sections of their plasma membranes (Ferguson, 1997, and references therein). All of the molecules shown are GPI-anchored proteins (*T. brucei* VSG, *Leishmania* PSP and *T. cruzi* mucus), or GPI-anchored phosphosaccharide (*Leishmania* LPG) or GPI-related GIPLs. Detailed descriptions of these structures can be found elsewhere (McConville and Ferguson, 1993; Ferguson, 1997). The shapes shaded red are protein components, and those shaded blue are carbohydrate components. The small yellow ovals on the *T. cruzi* mucus represent sialic acid residues.
The presence of an evolutionarily conserved core region in protein-linked GPI membrane anchors (Fig. 2) suggests that the biosynthetic machinery that assembles GPI anchors is also conserved. Studies of the African trypanosome played a key role in the development of methodologies for studying GPI biosynthesis. Thus, intact GPI-anchor precursors were first identified and characterised in T. brucei (Krakow et al., 1986; Mayor et al., 1990a,b), and Masterson et al. (1989) used this organism to develop the first cell-free system, which they used to delineate the pathway for GPI biosynthesis.

The cell-free system is based on washed membranes primed with UDP-GlcNAc and GDP-[3H]mannose in the presence of tunicamycin, which suppresses the synthesis of dolichol-cycle intermediates. The radiolabelled GPI intermediates formed in the membranes from endogenous PI are extracted in solvent and analysed by TLC. This system was adapted for study of GPI biosynthesis in mammalian cells (Hirose et al., 1992; Puoti and Conzelmann, 1993) and yeast (Costello and Orlean, 1992; Canivenc-Gansel et al., 1998; Sütterlin et al., 1998), as well as other protozoa, such as Plasmodium (Gerold et al., 1994), Toxoplasma (Tomavo et al., 1992), Paramecium (Azzouz et al., 1995) and Leishmania (Smith et al., 1997a; Balaton and McConville, 1998). These studies, together with in vivo labelling experiments, established the essential features of GPI-anchor biosynthesis (see Fig. 4A,B).

The T. brucei studies not only gave the first detailed descriptions of the assembly of the conserved GPI core but also revealed the process of fatty acid remodelling, a mechanism whereby the longer fatty acids of the PI moiety are sequentially removed and replaced by myristate (Masterson et al., 1990). GPI-lipid remodelling was originally thought to be unique to African trypanosomes, but we now know that it also occurs in Leishmania (Ratlon and McConville, 1998) and in yeast, in which diacylglycerol is exchanged for ceramide (Reggiori et al., 1997). Furthermore, the unusual composition of the PI moieties of some mammalian GPI anchors (e.g. CD52; Teumann et al., 1995) suggests that lipid remodelling of some sort may also occur in mammalian cells.

One area in which studies of trypanosomes have not contributed significantly to our understanding of GPI biosynthesis (owing to our inability to generate GPI-minus mutants in this organism) is the cloning of genes that encode enzymes, and associated proteins, involved in GPI biosynthesis. Kinoshita et al. (1997) have cloned many of the mammalian genes using an expression-cloning approach. Several groups have cloned other genes in yeast by making and rescuing temperature-sensitive GPI-minus mutants (Hamburger et al., 1995; Leidich et al., 1995; Benghezal et al., 1996) (Fig. 4C). The full extent to which the components of the GPI pathway exist in multicomponent complexes remains to be determined; however, not all the components are associated. For example, there is no evidence for an association between the mammalian GlcNAc-PI de-N-acetylase (the PIG-L gene product, which catalyses the second step of the pathway; Nakamura et al., 1997) and the GlcNAc-
transfase complex. Furthermore, there may be differences between different species, given that there is biochemical evidence for the association of the de-N-acetylase and the first mannosyltransferase in T. brucei and L. major but not in human cells (Smith et al., 1997a;b; Sharma et al., 1997).

Another noteworthy feature is that the PIG-B gene product, which is believed to be the third mannosyltransferase, is predicted to have a polytopic topology in the endoplasmic reticulum (ER) membrane that is similar to other ER-resident glycosyltransferases (Dan and Lehrman, 1997) and different from the type-2 membrane-protein structure that is typical of Golgi glycosyltransferases.

The abundance of GPI-anchored virulence-factor molecules on the surface of trypanosomatid parasites (Fig. 3) suggests that inhibitors of GPI biosynthesis might make useful drugs. Other pathogens that also have essential GPI-anchored proteins include the apicomplexan parasites Plasmodium (Gerold et al., 1996) and Toxoplasma (Manger et al., 1998). Furthermore, the essential nature of GPI biosynthesis to Saccharomyces cerevisiae (Leidich et al., 1994) and the role of GPI anchors in mannanprotein crosslinking to the cell-wall glucan (Lu et al., 1995; Kollár et al., 1997) suggest that the GPI pathway also represents a good antifungal target. However, although GPI minus mammalian cells survive well in culture, GPI mutations are lethal in mice (Tarutani et al., 1997). With this in mind, one must compare the enzymes of mammalian GPI biosynthesis with their counterparts in the protozoal and fungal pathogens to select appropriate targets, that is, those that have a good chance of producing pathogen-specific effects. Fundamental differences between the parasite and human pathways have been revealed by use of phenylmethylsulphonyl fluoride (Güther et al., 1994) and synthetic substrate analogues (Doerrer et al., 1996; Smith et al., 1997a;b; Sharma et al., 1999) in parasite and human cell-free systems (Fig. 4A,B). Furthermore, parasite-specific substrates (Smith et al., 1997b) and inhibitors (unpublished data) have been synthesised (Crossman et al., 1997). Differences in the GPI pathways of different species are also evident from the selection of a natural compound that inhibits GPI biosynthesis in yeast and human cells but not in Plasmodium or T. brucei (Sütterlin et al., 1997). Taken together, the observations described above suggest that species-specific anti-GPI therapeutic agents should be attainable.

The study of GPI biosynthesis in mammalian cells has also provided an explanation of the disease paroxysmal nocturnal haemoglobinuria at the molecular level. In this disease, erythrocytes are destroyed by complement because they lack complement-regulating GPI-anchored molecules, such as CD59 and decay-accelerating factor (DAF). The metabolic lesion in the progenitor cells is an inability to form the first intermediate of the GPI pathway, namely GlcN-PI, due to mutations in the pig-A gene that encodes the catalytic subunit of the GlcNac-transferase complex (Armstrong et al., 1992; Takahashi et al., 1993; Bessler et al., 1994).

**THE IMPACT OF GPI ANCHORS ON GENERAL EUKARYOTE CELL BIOLOGY**

**Distribution of GPI-anchored proteins**

The realisation that many classical plasma-membrane markers, such as 5′-nucleotidase and alkaline phosphatase, are GPI-anchored rather than transmembrane proteins stimulated researchers from many fields to reassess the mode of anchorage of numerous cell-surface proteins. Low and Ikezawa had previously developed an approach for the treatment of whole cells, or membrane preparations, with bacterial phosphoinositide-specific phospholipases Cs (PI-PLCs) that became a standard test for the presence of GPI-anchored proteins (Taguchi et al., 1985; Koch et al., 1986). The search for such proteins was aided by another product of trypanosome research: antibodies to the so-called cross-reacting determinant (CRD). These antibodies recognise epitopes shared by different sVSG variants that are located in the GPI anchor carbohydrate (Zamze et al., 1988). One of the these epitopes contains the cyclic inositol 1,2-phosphate that is produced by GPI anchors after cleavage by PI-PLCs. Thus, the conversion from CRD negative to CRD positive after PI-PLC digestion provides compelling evidence for the presence of a GPI anchor.

Unfortunately, not all GPI anchors are PI-PLC sensitive. Terry Rosenberry and colleagues first showed that some GPI anchors are PI-PLC resistant because the anchors have an extra fatty acid (usually palmitate) attached to the inositol ring (Roberts et al., 1988a,b) at the 2-position (Treumann et al., 1995). Particular species, tissues or cell types tend to express predominantly PI-PLC-sensitive or PI-PLC-resistant (inositol-acylated) GPI anchors on their cell surfaces (Richier et al., 1992; Wong and Low, 1992). Almost all mammalian GPI-biosynthesis intermediates are inositol acylated, but some or all of the GPI anchors are inositol deacylated after transfer of the anchor to protein (Chen et al., 1998). The timing (and role) of inositol acylation and deacylation is rather different in African trypanosomes (Güther and Ferguson, 1985) (see Fig. 4A,B).

Despite the existence of PI-PLC-resistant anchors, many GPI anchors can be predicted from cDNA analysis. Most GPI-anchored proteins can be identified by the presence of an N-terminal signal peptide, which directs sequestration into the lumen of the ER, and of a C-terminal GPI-addition signal peptide (GPI-sp). The latter has no strict consensus sequence but is easily identified by its general properties (Udenfriend and Kodukula, 1995). Interestingly, although GPI-sp sequences often work across species, T. brucei and Plasmodium GPI-sp sequences are bulkier than most at the site of GPI addition and do not work well in mammalian cells (Moran and Caras, 1994). This might also be an exploitable difference between protozoan parasites and their mammalian hosts.

**Lipid rafts**

The concept of ‘lipid rafts’ (i.e. lipid-dependent membrane microdomains) has gained popularity in recent years (Harder and Simons, 1997; Simons and Inkonen, 1997; Brown and London, 1997, 1998). The original concept was based on the sorting of certain cell-surface glycosphingolipids to the apical surface of MDCK cells (Simons and van Meer, 1988), a process that suggested physical association of particular lipid contents in the trans-Golgi to allow packaging and vectorial delivery. The preferential delivery of GPI-anchored proteins to the apical membrane of polarised epithelial cells, demonstrated by Rodriguez-Boulan and colleagues (Lisanti et al., 1990), further suggested that these proteins co-associate with glycosphingolipid-rich domains in the Golgi. It was known that most GPI-anchored proteins are insoluble in cold neutral detergents (Hooper and Turner, 1988); pulse-chase studies that
recorded the acquisition of detergent insolubility by GPI-anchored proteins as they entered the Golgi (the site of glycosphingolipid synthesis) therefore provided considerable support for the formation of glycosphingolipid- and GPI-protein-containing rafts in the Golgi (Brown and Rose, 1992).

The lipid-raft hypothesis has received further indirect support from a variety of studies, most of which utilise cold neutral detergent extraction to generate so-called detergent-resistant membranes (DRMs), also known as glycolipid-enriched membranes (GEMs) and detergent-insoluble
glycolipid-enriched domains (DIGs). Typically, these fractions are isolated by extraction with Triton X-100 at 0-4°C, followed by flotation in a sucrose density gradient. The fractions are relatively rich in sphingolipids, glycosphingolipids, cholesterol, GPI-anchored proteins and certain non-receptor tyrosine kinases, and relatively poor in phospholipids and transmembrane proteins (Stefanová et al., 1991; Brown and Rose, 1992; Cinek and Horejsí, 1992; Fiedler et al., 1993; Stevens and Tang, 1997). The tyrosine kinases found in these complexes (e.g. LCK and LYN) are acylated with at least two saturated fatty acids (Shenoy-Scaria et al., 1993). The cosequestration of GPI-anchored proteins and non-receptor tyrosine kinases (predicted by the lipid-raft model) provided a possible explanation for the perplexing, but well-characterised, ability of GPI-anchored proteins to transduce signals across the plasma membrane. There are many examples of transmembrane signalling through the crosslinking of GPI-anchored proteins (Horejsi et al., 1998). Cellular responses include rises in intracellular Ca²⁺, tyrosine phosphorylation, proliferation, cytokine induction and oxidative burst (Robinson, 1997). These antibody-induced signalling events clearly depend on the presence of a GPI anchor (Robinson et al., 1997). The physiological receptor-ligand pair that signals in a GPI-dependent way has been identified. Thus, GPI-anchored proteins known to be involved in transmembrane signalling, such as the ciliary-neurotrophic-factor receptor α (CNTFR-α) and the glial-cell-line-derived-factor receptor α (GDNFR-α), need to be associated with transmembrane β co-receptors to transmit their signals (Massagué, 1996). The GPI anchor can be removed from CNTFR-α without loss of α/β receptor function (Stahl and Yancopoulos, 1993). Similarly, GPI-anchored CD14 (the LPS-LPS-binding-protein receptor) functions equally well with a GPI anchor or with a spliced transmembrane domain (Lee et al., 1993); the signal-transducing partner for CD14 has been identified as the transmembrane Toll-like receptor 2 (Yang et al., 1998). The ability of these GPI-anchored receptors to interact laterally with transmembrane (signal-transducing) proteins is inconsistent with the lipid-raft model, in which transmembrane proteins are largely excluded from these membrane microdomains.

Notwithstanding the wealth of publications based on DRM, GEMs and DIGs, and the fact that the lipid-raft hypothesis could explain certain membrane-sorting and signal-transduction phenomena, it should be noted that the existence of lipid rafts in biological membranes is controversial; see recent reviews (Edidin, 1997; Brown, 1998; Jacobson and Dietrich, 1999). Indeed, a recent fluorescence-energy-transfer study found no evidence for the association of two ‘raft components’ (the glycosphingolipid GM1 and GPI-anchored 5'-nucleotidase) on the apical membrane of MDCK cells (Kenworthy and Edidin, 1998). However, studies using a novel fluorescence-depolarisation technique with GPI-anchored DAF (Varma and Mayor, 1998) and chemical crosslinking of GPI-anchored THY-1 (Freidrichson and Kurzhalia, 1998) provided strong evidence for the cholesterol-dependent clustering of GPI-anchored proteins into extremely small (<70 nm diameter) membrane microdomains. In further support of lipid rafts, Brown and colleagues have shown that artificial lipid mixtures rich in cholesterol and sphingolipids can form detergent-resistant liquid-ordered domains within a liquid-crystalline membrane environment (Brown and London, 1997, 1998; Schroeder et al., 1998). Such domains would tend to sequester GPI-anchored proteins because of the generally saturated nature of their PI moieties.

In summary, the physical nature and significance of lipid rafts is beginning to emerge, but this is still a complicated and confusing area and no real consensus view is yet available. Certainly the use of cold detergent extracts (i.e. DRM/GEM/DIG preparations) continues to provide a useful experimental tool, but it is far from clear what these fractions are. One quantitative study showed that the detergent-insoluble membranes from Caco-2 cells contain more than 50% of the total cell-lipid content (Stevens and Tang, 1997). Therefore, it would be wrong to assume that detergent-insoluble membranes are a defined subtraction of the plasma membrane. Indeed, one could argue that some or all of the detergent-resistant complexes represent the artificial coalescence of the mosthydrophobic components within a given lipid bilayer upon detergent extraction of the less-hydrophobic and lower-melting-temperature phospholipid species. In this regard, the fact that detergent-insoluble GPI-anchored proteins contain PI moieties that are significantly more hydrophobic than is the average cellular phospholipid might be relevant.

**GPI anchors as tools**

The replacement of C-terminal transmembrane domains of proteins by GPI-addition signal peptides allows the expression of the proteins on the plasma membrane of transfected mammalian cells in GPI-anchored form. This can be useful way to produce soluble forms of membrane proteins. For example, the T-cell receptor could not be expressed in a soluble form simply by deletion of the transmembrane domain, but it could be expressed in GPI-anchored form and then rendered soluble by the action of bacterial PI-PLC (Lin et al., 1990). In addition, purified GPI-anchored proteins can be used to coat hydrophobic surface-plasmon-resonance chips, thus providing a convenient way of orienting and presenting proteins for binding studies (Harrison et al., 1997).

There are several examples of the exchange of GPI-anchored proteins between cell surfaces (Ilagumaran et al., 1996; Medof et al., 1996, and references therein). Although the precise mechanism of exchange is unknown, it is clear that purified GPI-anchored proteins will spontaneously insert into lipid bilayers. The key difference between GPI-anchored proteins and transmembrane proteins, in this regard, is the lack of any cytoplasmic domain. The physiological significance of GPI-protein exchange is still uncertain, particularly given that all mammals express potent GPI-PLD activity in serum that can remove the lipid (phosphatidic acid) component of the GPI anchor and, therefore, prevent GPI-protein reinsertion. However, the transfer property of GPI-anchored proteins is being exploited experimentally to ‘paint’ exogenous proteins...
onto cell surfaces (Medof et al., 1996), and two groups are using this approach to generate potential cellular cancer vaccines and cellular immunotolerisation therapies (Brunschiwig et al., 1995; McHugh et al., 1995).

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