Chapter 2
GPI-Anchored Proteins in Health and Disease

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2.1 Introduction

Many proteins are associated with cell membranes via stretches of transmembrane spanning hydrophobic amino acids; however, other forms of membrane anchorage exist. Of these, one of the best characterised examples is a glycolipid structure which is covalently attached to the C-terminus of many proteins, known as a glycosylphosphatidylinositol (GPI) anchor. This moiety allows the attached protein to anchor to the outer leaflet of the cell membrane. GPI-anchored proteins are produced by most eukaryotic cells, ranging in complexity from protozoa through to vertebrates, and perform a diverse set of functions including roles in signal transduction, cell adhesion and antigen presentation (McConville and Ferguson 1993; Paulick and Bertozzi 2008). The importance of GPI-anchored proteins is evidenced by the embryonic lethality of GPI-deficient mice (Nozaki et al. 1999).

In addition to their normal physiological functions, GPI-anchored proteins are also associated with a range of diseases. For example, variant surface glycoprotein (VSG), a GPI-anchored protein from *Trypanosoma brucei*, is involved in the pathobiology of the parasite by forming a protective coating around it (Ferguson 1999). Moreover, reduced expression of GPI-anchored proteins in hematopoietic stem cells is associated with the human disease paroxysmal nocturnal hemoglobinuria (Brodsky 2006). Perhaps the most notorious of the GPI-anchored proteins is the prion protein (PrP), which is the causative agent of the prion diseases including Creutzfeldt-Jakob disease in humans and bovine spongiform encephalopathy in cattle (Prusiner 1998).

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2.2 Historical Origins and Structure of the GPI Anchor

The discovery of the GPI anchor can be traced back over 30 years ago to 1976 with the discovery that a newly purified enzyme from bacteria, phosphatidylinositol-specific phospholipase C (PI-PLC), was capable of releasing alkaline phosphatase from tissue samples (reviewed in Paulick and Bertozzi 2008). Subsequently, it was observed that other proteins could be released in such a manner leading to the hypothesis that these proteins were covalently attached to the cell membrane via a phosphatidylinositol moiety. In separate studies, structural information gleaned from the C-terminus of a mammalian protein, Thy-1, as well as from *T. brucei* VSG revealed the attachment of ethanolamine, various sugars and certain lipid species (Ferguson et al. 1988; Homans et al. 1988). The original PI-PLC data was rationalised with respect to the latter structural data to create a general structure for a GPI anchor (Fig. 2.1).

Detailed analysis of the structure of GPI anchors from a variety of organisms has revealed a common core motif. Through a phosphoethanolamine bridge, the α-carboxyl group at the C-terminus of the mature protein is linked to a highly conserved core glycan: -6mannose(α1-2)mannose(α1-6)mannose(α1-4)glucosamine(α1-6)*myo*-inositol. In turn, a lipid moiety is linked by a phosphodiester bridge to the inositol ring. The lipid moiety attached to inositol ranges from ceramide (slime mold proteins) to diacylglycerol (protozoa) to 1-alkyl-2-acylglycerol (most mammalian proteins; McConville and Ferguson 1993). The core glycan can be extensively modified by the addition of both ethanolamine and sugar side chains e.g. *N*-acetylglucosamine, mannose and galactose. The types of GPI anchor modification can be protein, tissue

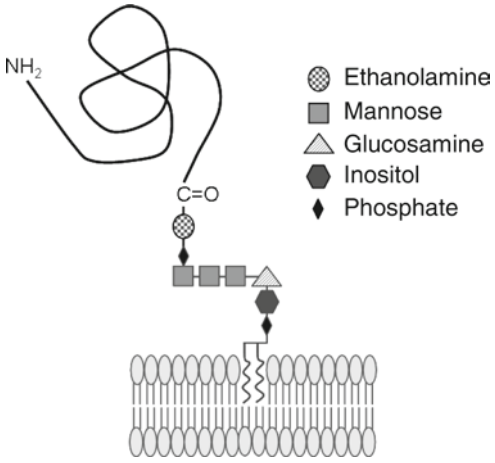

Fig. 2.1 Schematic representation of a GPI anchor. The polypeptide chain is covalently linked at the C-terminus to the core GPI anchor structure ethanolamine-phosphate-$(mannose)_3$-glucosamine-phosphatidyinositol. The lipid tail is attached to the inositol ring and in turn incorporates into the outer leaflet of the lipid bilayer. In this case the lipid is alkyl-acyl-glycerol as found on mammalian proteins

or species specific. A second lipid group, such as palmitic acid, can also be attached to the inositol ring and this modification leaves the GPI anchor resistant to cleavage by PI-PLC (McConville and Ferguson 1993). Another common modification is the remodelling of the fatty acids within the GPI anchor, which must take place to allow the protein to associate with lipid rafts (Maeda et al. 2007).

2.3 Cellular Synthesis of GPI Anchors

Synthesis of the GPI anchor shares similarities to the pathway leading to N-linked protein glycosylation. Both pathways utilise preassembled complexes that are subsequently added to the targeted protein. Furthermore, both pathways are initiated on the cytoplasmic face of the endoplasmic reticulum (ER) and require lipid flippases to translocate a glycolipid intermediate to the luminal face of the ER membrane where the processes are continued (Pomorski and Menon 2006). During synthesis both pathways share certain activated sugars (Dol-P-Man and UDP-GlcNAc) although the synthesis of the GPI anchor differs in its requirement for phosphatidylethanolamine. The core of the GPI anchor is built on the cytoplasmic face of the ER by the sequential addition of sugar moieties and phosphoethanolamine to a phosphatidylinositol molecule (Ferguson 1999; McConville and Menon 2000). Most of the enzymes carrying out the steps involved are well characterised and reviewed elsewhere (McConville and Menon 2000). Initially, UDP-GlcNAc transfers *N*-acetylglucosamine to phosphatidylinositol, and the *N*-acetylglucosamine subsequently undergoes de-*N*-acetylation. Prior to the addition of further sugar molecules inositol acylation must take place in yeast and mammalian cells. Subsequently, the Glc-PI is mannosylated by the addition of three mannose residues that are donated by dolichol-phosphomannose. The GPI anchor precursor is completed by the addition of a phosphoethanolamine molecule derived from phosphatidylethanolamine, and the whole GPI core is flipped across the ER membrane to the lumen where the transamidase enzyme facilitates addition to a protein containing the necessary GPI anchor addition signal.

All GPI-anchored proteins studied to date are resident on either the outer leaflet of the plasma membrane or contained within the lumen of intracellular organelles or vesicles. Therefore, to achieve this orientation GPI-anchored proteins possess two signal sequences. The first is a cleavable N-terminal signal sequence of hydrophobic amino acids that directs the cotranslational entry of the protein into the lumen of the ER through the Sec61 translocon complex (Rapoport 2007). The second sequence directs the addition of the GPI anchor and lies at the very C-terminus of the nascent chain. The sequence comprises 15–25 amino acid residues with a stretch of hydrophobic residues at the C-terminal end. The C-terminal hydrophobic sequence is preceded by a consensus sequence (ω, ω+1 and ω+2) that directs GPI anchor addition. Studies have shown that the ω amino acid residue may only possess a small side chain (Ala, Asn, Asp, Cys, Gly or Ser), with ω+1 being any residue except Pro or Trp and ω+2 being Gly or Ala (or occasionally Ser or

Thr) (Udenfriend and Kodukula 1995). The preassembled GPI anchor is added on the C-terminal side of the ω residue by the transamidase enzyme after the cleavage of the polypeptide chain at this location. The transamidase itself is a complex of several proteins. The protein Gpi8p contains the enzyme's active site, with other components including Gaa1p, Gpi16p (PIG-T) and Gpi17p (PIG-S) (Fraering et al. 2001). Modifications to the GPI anchor of the newly formed GPI-anchored protein occur as the protein is trafficked through the ER and Golgi.

2.4 Identification of a GPI Anchor

The presence of a GPI anchor on a protein can be investigated experimentally by a number of means. As mentioned, these include the common approach of assessing the protein's susceptibility to release from the cell membrane using bacterial PI-PLC. Other strategies include antibody detection of the cross-reacting determinant, inositol-1,2-cyclic monophosphate, exposed after cleavage by PLC (Broomfield and Hooper 1993). Alternatively, metabolic labelling using radiolabelled GPI anchor constituents or detergent insolubility of the protein can be used to assess whether it possesses a GPI anchor (Hooper 2001). Latterly, the potential for GPI anchor addition to a protein is often assessed using bioinformatics approaches such as the Web-based 'big Π predictor' algorithm (Eisenhaber and Eisenhaber 2007; Eisenhaber et al. 2004).

2.5 Properties Conveyed on a Protein by a GPI Anchor

In addition to its primary function in membrane anchorage of its attached protein, the GPI anchor can convey additional properties to the protein which may regulate its function.

2.5.1 Phospholipase Cleavage

In addition to their release by bacterial PI-PLCs, endogenous phospholipases exist that can cleave the GPI anchor in vivo and release the attached protein, perhaps in response to a stimulus (Fig. 2.2a). The secreted protein may possess the same or slightly different functions to the membrane anchored protein. Several secreted mammalian GPI-anchored proteins have been identified which possess the cross-reacting determinant suggesting cleavage by a PLC, though the identity of this enzyme remains unknown (Movahedi and Hooper 1997). To date, three other enzymes have been suggested to cleave GPI anchors. A GPI-specific phospholipase D, which is present in blood plasma, has been shown to cleave GPI-anchored proteins

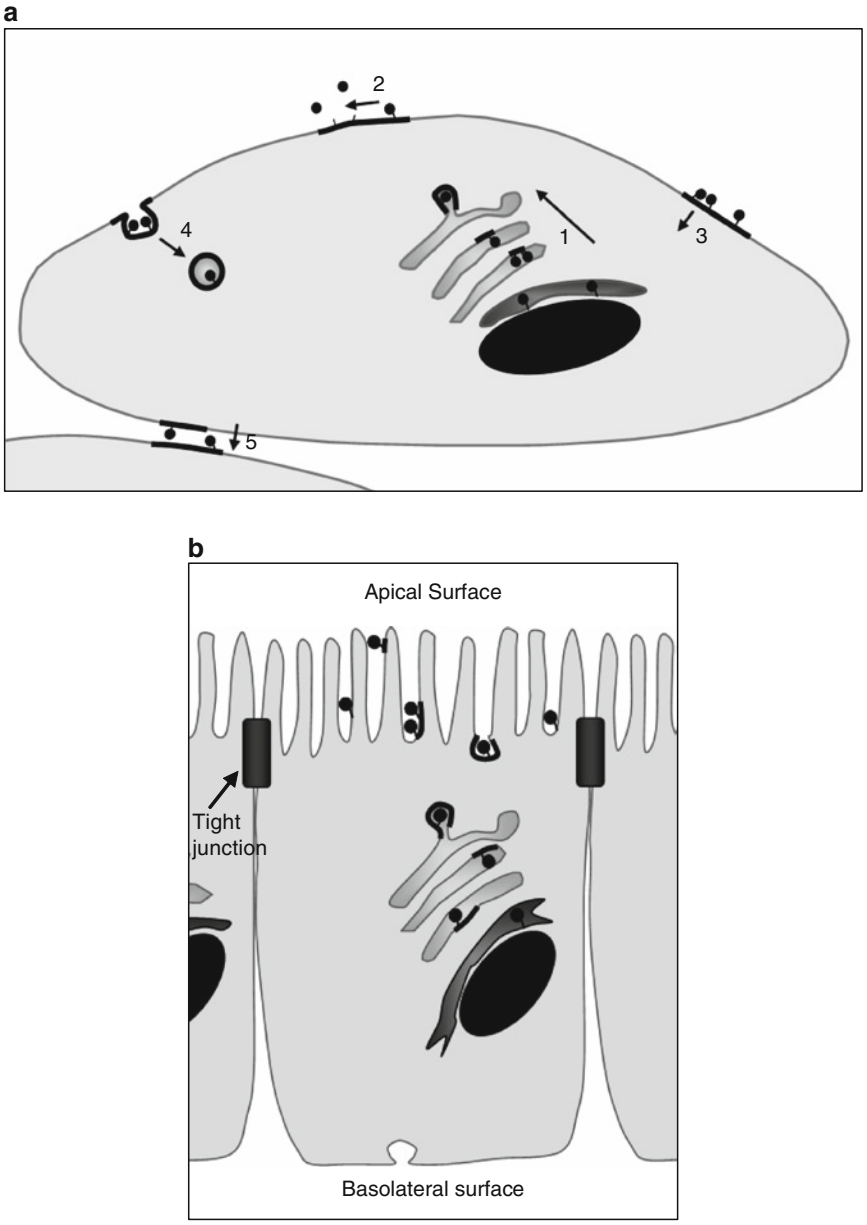

Fig. 2.2 Functions conveyed on a protein by the GPI anchor. (**a**) The GPI anchor is rapidly added to proteins possessing the necessary signal peptides within the ER. The GPI anchor conveys the following properties on its attached protein. (1) Association of the protein with lipid rafts in the Golgi on route to the cell surface. (2) Regulated release of the attached protein by enzymatic cleavage within the GPI anchor. (3) Transduction of signals across cell membranes. (4) Targeting of the attached protein for endocytosis. (5) Intercellular transfer of the protein from one cell to another in the process of cell painting. (**b**) In polarised cells the plasma membrane is spatially separated into apical and basolateral domains by tight junctions. The GPI-anchor is proposed to act as an apical targeting determinant owing to the association of GPI-anchored proteins with lipid rafts in the secretory pathway

in vitro (Low and Huang 1991). Additionally, phospholipase D was able to cleave GPI anchor synthesis intermediates and therefore may provide a mechanism by which the levels of such intermediates can be regulated (Mann et al. 2004). A protein first identified in a screen of Wnt signalling inhibitors, Notum, has also been shown to be capable of cleaving the GPI anchor of several proteins including members of the glypican family which regulate Wnt signalling (Traister et al. 2008). In contrast to phospholipase D, Notum was able to cleave its substrates extracellularly (Traister et al. 2008). Angiotensin-converting enzyme (ACE) has also recently been reported to be capable of cleaving GPI anchors (Kondoh et al. 2005), although the validity of this report remains controversial (Kondoh et al. 2005; Leisle et al. 2005).

2.5.2 Lipid Raft Association

There is substantial evidence for the compartmental organisation of the plasma membrane into specialised microdomains termed lipid rafts (Simons and Vaz 2004). Lipid rafts are enriched in sphingomyelin, glycosphingolipids and cholesterol and are believed to serve as locations for diverse cellular functions such as signalling and endocytosis (Simons and Ikonen 1997; Simons and Toomre 2000). Owing to favourable hydrophobic interactions between the saturated acyl chains of the GPI anchor and lipid raft-resident membrane lipids, GPI-anchored proteins are enriched within lipid rafts (Fig. 2.2a). The long saturated chains of sphingolipids self-associate within rafts to form a more tightly packed gel-like phase than the unsaturated kinked side chains of the surrounding phospholipid bilayer (Simons and Vaz 2004). Cholesterol preferentially integrates into the sphingolipid-rich phase hence lipid raft integrity is sensitive to cholesterol depletion (Rajendran and Simons 2005).

The close packing of raft lipids renders them partially resistant to solubilisation with certain non-ionic detergents. Indeed, Triton X-100 insolubility at 4°C has become the most widely used assessment of lipid raft association for a protein (Munro 2003). GPI-anchored proteins in particular are enriched in the isolated detergent resistant membranes (DRMs) owing to favourable interactions between the saturated acyl chains of the GPI anchor and the surrounding lipids (Mayor and Riezman 2004). However, whether DRMs equate to physiologically-relevant lipid rafts is a subject of much debate in the field (Lichtenberg et al. 2005).

2.5.3 Signal Transduction

Antibody cross-linking of GPI-anchored proteins has long been known to evoke signalling responses, including rises in intracellular Ca^{2+} or tyrosine phosphorylation (Fig. 2.2a) (Kasahara and Sanai 2000). The importance of the GPI anchor is

evidenced by an absence of signalling when the same proteins are anchored by a transmembrane domain (Jones and Varela-Nieto 1998). The targeting of GPI-anchored proteins to lipid rafts appears to be critical for their signalling functions, and this is not surprising given the enrichment of signalling molecules in such domains (Allen et al. 2007; Hugel et al. 2004). The mechanism by which the GPI anchor transduces signals across the membrane is not fully understood, although direct association with signalling transmembrane proteins in lipid rafts is one possibility (Simons and Toomre 2000).

In recent studies it appears that the functional specificity of the signalling mediated by a GPI-anchored protein may reside within the GPI anchor signal sequence. When the GPI anchor signal sequence of differentiation-promoting neural cell adhesion molecule (NCAM) was exchanged for the signal sequence from carcinoembryonic antigen (CEA), a mature protein with NCAM external domains but CEA-like differentiation-blocking activity was produced (Screaton et al. 2000). The altered signalling capabilities of the chimeras appeared to be due to GPI anchor mediated association with specific subpopulations of membrane microdomains (Nicholson and Stanners 2006). Surprisingly, the function of NCAM could be changed by the insertion of the CEA GPI signal sequence residues GLSAG 6–10 amino acids downstream of the GPI attachment site in NCAM (Nicholson and Stanners 2007).

2.5.4 GPI Anchor Dependent Endocytic Targeting

Like all proteins, once at the cell surface GPI-anchored proteins are targeted for down-regulation and degradation through endocytosis. The best characterised endocytic mechanism is clathrin-dependent endocytosis. Clathrin-dependent endocytosis involves the recognition of endocytic motifs in the cytoplasmic tail of transmembrane proteins by endocytic adaptor proteins which act as a bridge between the transmembrane protein and clathrin. Indeed, there is evidence that despite lacking a cytoplasmic tail, GPI-anchored proteins can be targeted for clathrin-dependent endocytosis. Clathrin-dependent endocytosis of PrP and the GPI-anchored urokinase plasminogen activator receptor (uPAR) is facilitated by interaction with the transmembrane low density lipoprotein receptor-related protein-1 (Conese et al. 1995; Parkyn et al. 2008; Taylor and Hooper 2007). However, in the absence of lateral association with transmembrane proteins, other mechanisms of endocytosis for GPI-anchored proteins exist, which are dependent on the GPI anchor itself (Fig. 2.2a).

Initial studies suggested that GPI anchored proteins were internalised through caveolae, a subpopulation of lipid rafts coated with caveolin (Anderson 1998). Nonetheless, it is now established that GPI-anchored proteins are not normally enriched in caveolae (Mayor et al. 1994). Many endocytic pathways, including clathrin- and caveolin-dependent endocytosis require scission of the endocytic vesicle from the cell membrane by a GTPase, dynamin. However, it is now known

that many GPI-anchored proteins are capable of undergoing endocytosis in a dynamin-independent manner (Ricci et al. 2000; Sabharanjak et al. 2002). It is proposed that GPI-anchored proteins are internalised through a pathway known as the GPI-anchored-protein-enriched early endosomal compartments (GEEC) pathway (Mayor and Riezman 2004). Uptake of GPI-anchored proteins via this pathway is dependent on the GPI anchor and is susceptible to inhibition of the Rho-family GTPase cdc42 (Sabharanjak et al. 2002). It appears that recruitment of GPI-anchored proteins into the GEEC pathway is sensitive to cholesterol depletion and requires the recruitment of the actin polymerisation machinery by cdc42 (Chadda et al. 2007). The protein GRAF1 appears to be a specific non-cargo marker for the GEEC pathway and appears to coordinate small G protein signalling and membrane remodelling to enable internalisation of GPI-anchored proteins into the pathway (Lundmark et al. 2008). Recently, it has been shown that GPI-anchored proteins are enriched in another novel clathrin and dynamin-independent pathway mediated by the raft protein, flotillin-1 (Glebov et al. 2006). Whether the GPI anchor directly interacts with flotillin-1 remains to be determined.

2.5.5 Intercellular Transfer

A number of GPI-anchored proteins have been shown to transfer between one cell and another in a process termed ‘cell painting’ (Fig. 2.2a) (Anderson et al. 1996; Kooyman et al. 1995; Liu et al. 2002). The lack of a cytoplasmic tail is a key to this event and it is well established that GPI-anchored proteins can spontaneously insert into lipid bilayers (Milhiet et al. 2002). The physiological significance of this process is poorly understood but may provide a means by which cells that cannot synthesise their own proteins under normal circumstances (e.g. mature erythrocytes) could express GPI-anchored proteins.

2.5.6 Apical Targeting Signal

Within polarised cells there are marked differences in both the lipid and protein composition between the apical and basolateral cell surfaces which are separated by tight junctions (Rodriguez-Boulan et al. 2005). The establishment of polarity allows for the compartmentalisation of specialised functions and the GPI anchor itself has extensively been suggested to impart apical targeting of the attached protein (Fig. 2.2b) (Folsch 2008; Schuck and Simons 2006). In 1992, Brown and Rose demonstrated that GPI-anchored proteins are targeted to the apical surface owing to their association with detergent insoluble lipid rafts in the Golgi (Brown and Rose 1992). A large body of work supports this observation (Schuck and Simons 2006).

Nonetheless there are several caveats to the notion that a GPI anchor confers apical targeting. The majority of GPI-anchored proteins are targeted to the

basolateral membrane of Fisher rat thyroid cells (Zurzolo et al. 1993). Moreover, both N-glycans and protein oligomerisation have been suggested to impart apical targeting on GPI-anchored proteins (Benting et al. 1999; Paladino et al. 2007; Pang et al. 2004). Recently, it has been shown that sorting of GPI-anchored proteins to the apical or basolateral membrane of polarised cells is dependent on the GPI anchor attachment sequence itself (Paladino et al. 2008). In this study, the GPI anchor addition sequences for either the folate receptor or PrP were fused to the C-terminus of green fluorescent protein (GFP) and targeting of the resulting fusion proteins assessed (Paladino et al. 2008). The GFP-folate receptor fusion oligomerised readily and was targeted to the apical surface, however, as is observed with PrP^{C}, the GFP-PrP fusion was targeted to the basolateral surface (Paladino et al. 2008; Sarnataro et al. 2002). It was speculated that differences in the targeting may be a consequence of differences in the structure of the GPI anchor and/or in the surrounding lipid environment.

2.6 GPI-Anchored Proteins and Disease

A body of evidence has demonstrated that GPI-anchored proteins are involved in a variety of disease states. In the following sections some examples are provided with particular emphasis on the role of the GPI anchor itself.

2.6.1 Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal haematopoietic stem cell disorder which can arise either de novo or develop from acquired aplastic anaemia, a bone marrow failure resulting after certain drug treatments (Nagarajan et al. 1995; Young and Maciejewski 1997). PNH leads to complement-system mediated intravascular hemolysis, thrombosis and bone marrow failure (Moyo et al. 2004). The underlying cause of PNH is a somatic mutation in stem cells that means they and all their resultant progeny completely lack GPI-anchored proteins at their cell surface (Brodsky 2006). This defect is the consequence of mutation of the *PIG-A* gene, required for the final step in GPI anchor synthesis (Miyata et al. 1994). A large number of genes are involved in the biosynthesis of the GPI anchor, yet the deficiency in GPI-anchored proteins is due solely to mutations in *PIG-A* (Bessler et al. 1994; Takeda et al. 1993). To date over 100 mutations have been identified that span the whole of *PIG-A*, with most mutations being small insertions or deletions which result in frameshifts or premature termination of transcription (Nafa et al. 1995).

Regarding PNH pathology, erythrocytes originating from PNH stem cells are particularly vulnerable to lysis mediated by the complement system. This is a consequence of a lack of two GPI-anchored proteins, membrane inhibitor of

reactive lysis (CD59) and decay accelerating factor (CD55), which are both involved in complement system regulation. As a result of increased erythrocyte lysis, large amounts of free hemoglobin are released into the plasma which subsequently mediates scavenging of nitric oxide (Rother et al. 2005). The increased consumption of nitric oxide results in the clinical symptoms associated with PNH such as abdominal pain, fatigue, esophageal spasm and possibly thrombosis.

One unanswered question in the PNH field remains the issue of clonal dominance. Why are PNH stem cells spared from immunological attack (e.g. as seen in aplastic anaemia)? It is speculated that PNH cells are spared from the immunological attack owing to the absence of certain GPI-anchored proteins (Young and Maciejewski 1997). In a recent study it was shown that there is an ongoing immune attack against haematopoietic stem cells in both PNH and aplastic anaemia, however, PNH stem cells were resistant to attack (Savage et al. 2009). Furthermore, *PIG-A* mutations appear to confer a cellular resistance to stresses that would otherwise lead to apoptosis (Savage et al. 2009).

2.6.2 Prion Diseases

The prion diseases are characterised by the post-translational misfolding of the normal cellular form of the prion protein (PrP^{C}) into the infectious isoform (PrP^{Sc}). According to the protein-only hypothesis, interaction between PrP^{Sc} and endogenous PrP^{C} is sufficient to result in the template-driven formation of more PrP^{Sc} (Prusiner 1998). A wealth of literature suggests that lipid rafts may act as a site for the conformational conversion of PrP^{C} to PrP^{Sc} (Campana et al. 2005). Given that PrP's GPI anchor is one of the strongest determinants of its lipid raft targeting, it is not surprising that a body of literature has attempted to assess the role of the GPI anchor in prion disease (Taylor and Hooper 2006).

Like other GPI-anchored proteins, PrP^{C} can be transferred efficiently between cells (Liu et al. 2002). This process requires cell contact, an intact GPI anchor and activation of protein kinase C (Liu et al. 2002). Given that PrP^{Sc} has to be inserted into a contiguous lipid raft membrane with PrP^{C} for misfolding to occur, GPI anchor dependent cell transfer of PrP^{Sc} as hypothesised by Liu et al. from infected to uninfected cells could provide a mechanism for prion spread (Baron et al. 2002; Liu et al. 2002).

Recently, the GPI anchor of PrP has been suggested to play a role in the toxicity associated with prion disease (Chesebro et al. 2005). In this study transgenic mice expressing an anchorless, secreted form of PrP^{C} were shown to accumulate PrP^{Sc} in plaques yet the mice never developed clinical disease although the PrP^{Sc} produced was capable of infecting other transgenic mice expressing GPI-anchored PrP (Chesebro et al. 2005). Therefore, the authors of this study were able to discriminate between prion conversion/infectivity and prion toxicity. One interpretation of these data is that the GPI anchor itself is important in transducing neurotoxic

signals that result in the onset of clinical symptoms (Aguzzi 2005). Removal of the GPI anchor of PrP^{Sc} by the aspartic endoprotease cathepsin D was shown in in vivo and cell-based assays to have little effect on either PrP^{Sc} propagation or infectivity supporting the conclusions of Chesebro et al. (Chesebro et al. 2005; Lewis et al. 2006).

2.6.3 Malaria

Malaria is an infectious disease responsible for the deaths of two million people a year, with more than 80% of cases occurring in Africa (Nebl et al. 2005). There are four species of malarial parasites known to infect humans, with the bulk of cases attributed to *Plasmodium falciparum* infection. Disease symptoms include acute respiratory disease, renal failure, pulmonary oedema and seizures (White and Ho 1992). Site specific localisation of parasites coupled with both local and global inflammatory response to cytokine production is thought to underlie disease (Stevenson and Riley 2004). A number of years ago it was suggested that GPI anchors play a key role in *P. falciparum* infection as purified GPI-anchored proteins from this parasite were shown to be potent activators of immune cells capable of producing pro-inflammatory cytokines such as TNF-α (Schofield and Hackett 1993; Vijaykumar et al. 2001). The effect was due to the GPI anchor and not the attached protein as the agent was susceptible to reagents that disrupt the integrity of GPI anchors, but not to proteinase digestion (Schofield and Hackett 1993). Further studies have shown that the GPI anchor of *P. falciparum* alone is sufficient to cause malarial symptoms in various host tissues and cell types (Schofield and Hackett 1993; Schofield et al. 1996; Tachado and Schofield 1994). It is interesting to note that the GPI anchors from *T. brucei*, *Trypanosoma cruzi* and *Toxoplasma gondii* all possess similar properties to the GPI anchor from *P. falciparum*, suggesting that there are shared pathogenic mechanisms between trypanosomiasis, Chagas' disease, toxoplasmosis and malaria.

2.6.4 Pore Forming Toxins and the GPI Anchor

The bacterial toxin aerolysin produced by the human pathogen *Aeromonas hydrophilia* has its cytolytic effect by forming oligomers that insert into the cell membrane and form a channel (van der Goot et al. 1994). A number of GPI-anchored proteins are known to bind the toxin, such as Thy-1, and the determinant for binding was shown to be the GPI anchor itself (Diep et al. 1998). Aerolysin could not bind to all GPI-anchored proteins implying that certain GPI anchor modifications impart binding specificity (Diep et al. 1998). One such motif may be the β-*N*-acetylglucosamine sidechain present on the GPI anchor of placental-like alkaline phosphatase (Fukushima et al. 2003).

2.7 Conclusions and Future Perspectives

The GPI anchor is a complex post-translational modification that is implicated in the regulation of a host of physiological processes by conveying properties to the protein to which it is attached. However, there remain large gaps in our knowledge of GPI anchors, both in health and disease. As the phosphoinositol, glucosamine and mannose moieties within the core of the GPI anchor can be extensively modified by the addition of phosphoethanolamine and sugars, diverse functional properties on the GPI anchor would be expected to be imparted, beyond a simple role in membrane anchorage. However, due precisely to this structural heterogeneity, the roles of different GPI anchor modifications have been difficult to assess (Paulick and Bertozzi 2008).

Attempts to manipulate GPI anchor modifications in a cellular context have failed owing both to incomplete knowledge of the biosynthetic enzymes as well as the fact that their disruption often results in complete loss of GPI anchorage (Bastisch et al. 2000; Kawagoe et al. 1996; Kinoshita and Inoue 2000). Recently, several groups have adopted an alternative approach to understanding GPI anchor function to advance the field by attempting its chemical synthesis (Paulick and Bertozzi 2008). Currently, the results of such experiments are unsatisfactory owing to the complicated nature of the synthesis pathways and the fact that the synthetic product is often not able to be coupled to a protein. Nonetheless, advances are being made; a study showed that a short synthetic peptide of 12 amino acids could be added to a synthetically produced GPI anchor (Shao et al. 2004).

Moreover, the behaviour of GFP fused to GPI anchor analogues by chemical ligation was recently studied in a cell model and provided evidence that individual components of the GPI anchor impart certain properties on the attached protein (Paulick et al. 2007). The lateral mobility of the GFP conjugates in the membrane was shown to be drastically reduced by deletions within the glycan core, whilst endocytosis was unaltered (Paulick et al. 2007). However, although such studies are promising synthetic production of a native GPI-anchored protein has not been reported. Significantly though, semi synthesis of a GPI-anchored prion protein was recently reported (Becker et al. 2008). In this study, a synthetic cysteine-tagged GPI anchor was fused with recombinant PrP containing a C-terminal thioester (Becker et al. 2008). The quest to produce synthetic GPI anchors may have potential in disease therapeutics. This is evidenced by the fact that synthetic GPI anchors have been suggested as a candidate anti-toxic vaccine for malaria (Kamena et al. 2008; Schofield et al. 2002).

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