Biogenesis and Expression at PMs of GPI-APs

Subjects: Biochemistry & Molecular Biology
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Glycosylphosphatidylinositol (GPI)-anchored proteins (APs) are produced by coupling of the completed GPI anchor, prefabricated by stepwise transfer from activated precursors of the corresponding carbohydrate and phosphoethanolamine (EtN-P) residues to PI at the luminal face of the ER membranes, to the carboxy-terminus of the polypeptide precursor moiety upon its translation and transient arrest at the endoplasmic reticulum (ER) membranes.

Keywords: adipose cells ; extracellular vesicles ; glycosylphosphatidylinositol (GPI)-anchored proteins (GPI-APs)

1. Introduction

In eukaryotic cells, from yeast to mammalian cells, a specific class of surface proteins is anchored at the outer phospholipid layer of plasma membranes (PMs) via a glycosylphosphatidylinositol (GPI) glycolipid moiety. In mammalian organisms, GPI-APs encompass about 3–5% of the total PM proteins, i.e., about 150 representatives [1][2][3]. The protozoal GPI-APs variant surface glycoprotein (VSG), merozoite surface antigen and promastigote surface protease from the parasites *Trypanosoma brucei* [4], *Plasmodium falciparum* [5] and *Leishmania major* [6], respectively, were the first to have their structure of their anchor elucidated. They are made of a highly conserved hydrophilic glycan core consisting of a non-acetylated glucosamine (GlcN) and three mannose (Man) residues connected via specific glycosidic linkages, which at one end is glycosidically coupled to amphiphilic phosphatidylinositol (PI) (typically diacyl-PI, exclusively, or a mixture of diacyl and 1-alkyl-2-acyl-PI) and at the other non-reducing end, the third mannose, or in rare cases the second mannose, is invariably amide-linked to the carboxy-terminus of the protein moiety via a phosphoethanolamine (EtN-P) bridge [7][8]. This GPI glycan core structure is highly conserved from yeast to protozoa to humans. It can be modified in both mammalian cells, protozoa and fungi by addition of various carbohydrate or acyl moieties (for a review, see [9][10][11]) or by remodeling of the fatty acids (for a review, see [12]), which has meanwhile become amenable to detection by sophisticated analytical methods [13][14].

In particular, in yeast the GPI glycan precursor has the structure Man-(EtN-P)Man-(EtN-P)Man-(EtN-P)Man-GlcN, which after its fabrication at the endoplasmic reticulum (ER) undergoes remodeling in the course of trafficking of the GPI-AP to the PMs. This involves the coupling of a fifth Man residue to the fourth Man residue by certain mannosyltransferases, which have escaped identification so far, and the elimination of the side chain EtN-P of the first and second Man residues by the phosphodiesterases Cdc1 and Ted1, respectively [11][13][15][16]. Furthermore, during infections by protozoa, such as in toxoplasmosis, malaria, and trypanosomiasis, GPI glycolipids actively modulate the host immune system [17]. The PMs of the parasites causing those diseases are known to harbor multiple copies of a particular GPI-AP (and free GPI lipid) which cooperate in the formation of a coat for the protection from infection or as immunomodulators. In vitro and in vivo studies have demonstrated that various modifications of protozoal and fungal GPI glycans elicit a variety of distinct and specific immune responses. For instance, galectin-3-dependent interaction of parasitic GPI glycans with macrophages leads to stimulation of TNF-α synthesis [18]. Similarly, immunization with galactose-harboring GPI glycan of VSG triggers TNF-α production in mice, resulting in their extended life span upon infection with trypanosomes [19]. Elucidation of the structure of the GPI glycan of *Toxoplasma gondii* and *Trypanosoma congolense* has revealed the glycosidic linkage of *N*-acetyl-galactosaminyl as well as glucosyl-*N*-acetyl-galactosaminyl and galactosyl-*N*-acetyl-glucosaminyl side branches, respectively, to the first mannose residue of the glycan core [20]. In trypanosomes, an unusual ß1-6 *N*-acetyl-glucosaminyl-transferase has been suggested to catalyze the modification of the glycan core of their GPI anchors [21].

2. Biogenesis and Expression at PMs of GPI-APs

GPI-APs are produced by coupling of the completed GPI anchor, prefabricated by stepwise transfer from activated precursors of the corresponding carbohydrate and EtN-P residues to PI at the luminal face of the ER membranes, to the carboxy-terminus of the polypeptide precursor moiety upon its translation and transient arrest at the ER membranes (for a review, see [22][23]). Total synthesis of the GPI anchor in mammalian cells requires 13 reactions catalyzed by more than 23

gene products, among them for transfer and amide coupling of the GPI anchor to the polypeptide moiety the membrane-bound GPI transamidase (GPI-T), including the catalytic subunits PIG-K and GPAA1 and the regulatory subunits PIG-S, PIG-T and PIG-U in mammals [24], including humans [25], and their homologues in Drosophila [26] and yeast (for instance GPI8 as PIG-K homolog) (for a review, see [27][28][29][30]). Consequently, the polypeptide precursor is equipped with two signals, a GPI attachment signal sequence at their carboxy-terminus recognized by the GPI-T [31][32][33] and a typical secretory pathway signal sequence at their amino-terminus for translocation into and quality control (and degradation) at the ER membranes [34][35][36][37] and subsequent transport to the PMs [38]. Both signals consist of stretches of hydrophobic amino acids [32] and are subsequently clipped off by signal peptidase and GPI-T, respectively. Unexpectedly, the canonical signal recognition particle-dependent pathway engaged by the majority of membrane and secretory proteins is not involved in the translocation of GPI-APs across the ER membranes, which instead depends on a network of cytosolic proteins and factors [39]. For the prediction of GPI anchorage of cell surface proteins, algorithms such as Big-PI [1], GPI SOM [33] and FragAnchor [3], as well as web-based prediction tools [40][41][42][43], are available. Importantly, the entire information required and adequate for “glypiation”, i.e., the post-translational addition of GPI anchors, seems to be contained in the GPI attachment signal sequence and is decoded by GPI-T, since it manages to transform soluble proteins into the corresponding GPI-AP upon recombinant addition to the carboxy-terminus, as has been exemplified with numerous secretory protein-GPI fusion constructs (e.g., ref. [44]). Interestingly, subunits of the GPI-T, such as PIG-U in mammals, as well as certain GPI-APs, have been recognized to be involved in the initial stages of tumor development and tumor progression and may be used as putative biomarkers for specific diagnosis, therapy and prognosis [45].

The data available demonstrate that soon after coupling of the GPI anchor to the protein moiety and in the course of transport of the assembled GPI-AP along the secretory pathway to the cell surface, the lipid portion of the GPI anchor is subjected to structural remodeling which is critical for proper functioning and intracellular trafficking (for a review see [12][22][28]). For instance, in the yeast *Saccharomyces cerevisiae*, lipid remodeling has been shown to happen at the ER, starting with deacylation of the GPI inositol moiety by Bst1 (orthologue to mammalian PGAP1) [46], a process thought to confer quality control for correct GPI assembly, continued by removal of an unsaturated fatty acid at the sn-2 position by Per1 phospholipase A_2 (orthologue to mammalian PGAP3) and subsequent coupling of a very-long-chain saturated fatty acid (C26) by Gup1 reacylase (not orthologue to mammalian PGAP2, but belonging to the mammalian MBOAT family of acyltransferases) [47] and then in the majority of yeast GPI-APs finished by replacement of the C26 diacylglycerol with ceramide, which also harbors a very-long-chain saturated fatty acid by Cwh43 (the amino- and carboxy-terminal parts corresponding to mammalian PGAP2 and PGAP2-interacting protein p24, respectively; for a review, see [22]) [48][49].

Interestingly, yeast and mammalian cells differ in the sites where GPI-APs become sorted from typical secretory proteins, with ER operating as the major site of sorting for the former and the Golgi apparatus for the latter (for a review see [22]). Importantly, in yeast, typical ceramide-harboring GPI-APs undergo clustering into discrete ER zones which are equipped with specific ER exit sites (ERES) [50]. Very recent studies indicate that in yeast ceramide remodeling is a prerequisite for clustering at the ER of GPI-APs and their resulting subsequent sorting, trafficking to PMs and insertion into lipid rafts. In greater detail, it has become apparent that a GPI-AP upon its remodeling by Cwh43 becomes recognized by the GPI-glycan remodelase Ted1 in order to facilitate its interaction with the p24 complex and in consequence its export from the ER, as part of a quality control mechanism of GPI-AP cargo sorting at the level of the ER [50]. It is tempting to speculate that GPI anchors harboring C26 ceramide associate with free C26 ceramide present in the ER membranes to produce ceramide-enriched lipid rafts, which possibly constitute interdigitated phases resulting in segregation of the GPI-APs from transmembrane proteins [51].

In addition to remodeling of the lipid portion of the GPI anchor, its glycan portion is also subjected to another modification process prior to exit from the ER. It has been demonstrated that after elimination of the EtN-P side chain, which had initially been attached to the second mannose residue of the glycan core, by a specific phosphodiesterase, encoded by Ted1 and Cdc1 in yeast [52] and PGA5P/MPPE1 in mammalian cells [53], the yeast transmembrane cargo receptor p24 associates with the remodeled glycan for subsequent specific recruitment of Lst1, which represents a specific isoform of the major COPII cargo interacting subunit Sec24 [53] and mediates the biogenesis of COPII vesicles enriched in GPI-APs for their sorting at ERES in *Saccharomyces cerevisiae* [52][54]. On the basis of the finding that in PGAP5-deficient mammalian cells GPI-APs did not efficiently enter into ERES, it has been suggested that PGAP5- and Ted1-mediated removal of the EtN-P side chain is a prerequisite for productive recruitment of GPI-APs into ERES and subsequent trafficking from the ER to the Golgi apparatus in both mammals [55] and yeast [53][54], respectively.

In mammalian cells, upon arrival of GPI-APs at the Golgi apparatus, the unsaturated fatty acyl chains in 1-alkyl-2-acylglycerols at the sn2 position as well as in diacylglycerols of the GPI anchor are exchanged for stearic acid by the action of the phospholipase A_2 PGAP3, an orthologue to yeast Per1 [56]. During this remodeling, lyso-intermediates of GPI-APs are generated, which become subsequently re-acylated with stearic acid by the acyltransferase PGAP2,

operating like Gup1 in yeast [57]. In both yeast and mammalian cells, GPI-APs are then transported from the *trans*-Golgi network (TGN) to PMs (for a review, see [58]). In addition, mammalian-polarized cells, such as epithelial or endothelial cells, sort and segregate their membrane proteins during their transport from the TGN to the cell surface along either the apical or basolateral cognate routes. One of the first signals for apical sorting postulated was the GPI anchor, since the majority of GPI-APs are, in fact, expressed at the extracellular leaflet of the apical PM domains [59][60][61][62][63]. In particular, in polarized epithelial cells, GPI-APs are predominantly sorted to the apical surface at the TGN under the control of specific factors by clustering into lipid rafts, which may operate as apical sorting platforms. Apical sorting of GPI-APs seems to depend on their accumulation at lipid rafts formed at the TGN membrane as defined by their resistance towards extraction by cold non-ionic detergent in the course of arrival and subsequent remodeling of the GPI anchor fatty acids for the expression of long-chain saturated fatty acids at the Golgi apparatus [64]. However, association of GPI-APs with lipid rafts is assumed to be required but not sufficient for apical sorting [65][66][67][68]. Importantly, the experimental evidence hints to differences in the organization of GPI-APs at PMs between polarized and non-polarized cells. Distinct mechanisms engaged by GPI-APs for their selective transport along the secretory pathway with emphasis on the control of their export from the ER and/or the TGN, and multiple molecules and genes responsible for the polarized trafficking and transport of GPI-APs to distinct PM domains in polarized epithelial cells, have been proposed (for a review, see [69]). Interestingly, some native GPI-APs and GPI-anchored fusion proteins have been found to be transported across cells (transcytosis), such as the folate receptor from the basolateral to apical surface domains of intestinal [70] as well as retinal pigment epithelial cells [71] and tissues, such as LY6A across the blood–brain barrier [72]. The transcellular transport of GPI-APs, which seems to involve transcytosis via caveolae, may be useful for the oral delivery of protein therapeutics, such as insulin, as has previously been speculated [73].

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