THE ENZYMES

Glycosylphosphatidylinositol (GPI) Anchoring of Proteins

Edited by
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VOLUME XXVI

THE ENZYMES

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GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) ANCHORING OF PROTEINS

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The Enzymes

VOLUME XXVI

GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) ANCHORING OF PROTEINS

Preface

This volume is the third publication in this series dealing with posttranslational modification of proteins. Volume 21 dealt with protein lipidation, specifically protein prenylation, *S*-acylation, and *N*-myristoylation, while the topic of volume 24 was protein methyltransferases. The topic of this volume is "Glycosylphosphatidylinositol (GPI) anchoring of cell surface proteins." GPI anchors are glycolipids composed of a phosphatidylinositol whose headgroup is extended by a glucosamine and mannose-containing glycan bearing one or more phosphoethanolamine moieties. The distal phosphoethanolamine links the GPI structure to the C-terminal amino acid of a mature protein via an amide bond. The GPI's hydrophobic lipid moiety serves to anchor the protein to the membrane. GPI-anchored proteins exhibit a variety of functions. They play critical roles in receptor-mediated signal transduction pathways. They are markers of specialized plasma membrane domains and are important in apical protein positioning and in cell wall construction in fungi.

We start with an overview of GPI biosynthesis in Chapter 1. Chapters 2–8 discuss details of the steps involved in this series of biosynthesis reactions. Enzymes, substrates, and enzymatic mechanisms involved in these reactions are discussed. Chapter 9 has special emphasis on the GPIs of protozoa. Chapters 10 and 11 discuss chemical synthesis of GPI anchors and their use in vaccine development. Chapter 12 deals with chemical inhibitors of GPI biosynthesis. Chapters 13–15 discuss aspects of the cell biology of GPI-anchored proteins, including their transport, polarized sorting, and their involvement in cell wall synthesis. Finally, Chapter 16 discusses hemostatic and neurological problems due to GPI deficiency.

The idea for this volume was conceived at the 2006 FASEB meeting on Protein Lipidation, Signaling, and Membrane Domains in Indian Wells, California, where we had a long discussion about the content. We would like to thank the contributors for preparing their chapters in a timely

fashion. We also thank Lisa Tickner for her expert help in organizing the publication. We are also grateful to Gloria Lee who helped edit chapters at UCLA.

Fuyuhiko Tamanoi
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May 11, 2009

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1

Overview of GPI Biosynthesis

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I. Abstract

Glycosylphosphatidylinositol (GPI) anchor is a form of posttranslational modification of many cell surface proteins common to all phyla of eukaryotes. GPI acts to anchor proteins on the outer leaflet of the plasma membrane. The lipid part is either phosphatidylinositol (PI) or inositol phosphoceramide. Due to saturated fatty chains in the lipid part of GPI, GPI-anchored proteins (GPI-APs) are mainly present in membrane microdomains of mammalian and yeast plasma membranes. The glycan part consists of a conserved core backbone and variable side branches. Some of the functions of the glycan part have been characterized in yeast and protozoan parasite trypanosome, while they are largely unclear in mammalian cells. The core backbone structure, EtNP-6Manα1–2Manα1–6Manα1–4GlcNα1–6*myo*Inositol-phospholipid (where EtNP is ethanolamine phosphate; Man is mannose; and GlcN is glucosamine), is common to all of them. The terminal EtNP is linked to the C-terminus of protein via an amide bond. Precursors of GPI-anchor are synthesized in the endoplasmic reticulum (ER) from PI through at least nine sequential reaction steps. The complete GPI precursor is attached to proteins bearing a C-terminal GPI attachment signal peptide by GPI transamidase (GPI-TA). Nascent GPI-APs are then transported via the secretory pathway through the Golgi to

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the plasma membrane. On the way of transportation, the lipid part of GPI is remodeled and the glycan part is modified. At least 23 gene products are involved in biosynthesis and attachment to proteins of GPI-anchor precursors. Several genes involved in lipid remodeling have been identified.

II. Introduction of GPI-Anchored Proteins

Glycosylphosphatidylinositol (GPI) anchor is a form of posttranslational modification of many cell surface proteins common to all phyla of eukaryotes [1–5]. GPI acts to anchor proteins on the outer leaflet of the plasma membrane. Whereas two other major posttranslational modifications for membrane anchoring of proteins, namely acylation and prenylation, include only lipids, GPI consists of lipid and glycan parts. The lipid part is either phosphatidylinositol (PI) or inositol phosphoceramide. Due to saturated fatty chains in the lipid part of GPI, GPI-anchored proteins (GPI-APs) are mainly present in membrane microdomains (membrane rafts or lipid rafts) in mammalian and yeast plasma membranes [6]. The glycan part consists of the conserved core backbone and the variable side branches. Some of the functions of the glycan part have been characterized in yeast and protozoan parasite trypanosome, while they are largely unclear in mammalian cells.

About 150 human proteins with various functions are GPI anchored. GPI-APs include hydrolytic and other enzymes (alkaline phosphatase, 5′-nucleotidase/CD73, erythrocyte acetylcholinesterase, renal dipeptidase, and mono-ADP-ribosyltransferase ART), adhesion molecules (neural cell adhesion molecule 120, TAG1, and isoform of CD58), receptors (folate receptor, CD14, CD16b, uPA receptor/CD87, ciliary neurotrophic factor receptor α subunit, and glial-cell-derived neurotrophic factor receptor α subunit), complement regulatory proteins (CD55 and CD59), immunologically important proteins (CD24, CD48, CD52, and CD90/Thy-1), and other proteins (prion protein and glypicans). Complete deficiency of GPI causes early embryonic lethality due to malformation of the brain as shown by knockout mice with defective GPI biosynthesis [7]. Inherited partial deficiency causes inherited GPI deficiency, a disease characterized by hepatic and/or portal vein thromboses and seizures [8]. Acquired GPI deficiency due to a somatic mutation in the hematopoietic stem cell causes paroxysmal nocturnal hemoglobinuria characterized by complement-mediated hemolytic anemia, venous thrombosis, and bone marrow failure [9, 10].

It is estimated that about 60 out of 6000 proteins of yeast *Saccharomyces cerevisiae* are GPI anchored, based on the presence of GPI-attachment

signal peptide at the C-terminus of the protein sequence predicted from genome data [2, 11]. Many, perhaps majority, of the GPI-APs are cell wall proteins rather than plasma membrane proteins. Cell wall localization of GPI-APs is achieved by a transglycosidation reaction between the glycan part of GPI and cell wall β-1,6 glucan [12]. GPI biosynthesis is essential for the growth of *S. cerevisiae* [2].

The plant Arabidopsis may have 248 GPI-APs as predicted in a similar way [4]. They include various enzymes and receptors. GPI-APs are required for root development, cell wall synthesis, pollen germination, and tube growth [13, 14].

GPI-APs are by far the most popular type of the cell surface proteins in protozoa, such as trypanosomes and malaria parasites [15, 16]. African trypanosome, *Trypanosoma brucei*, has two proliferative stages, a bloodstream form that grows in the blood plasma of mammalian hosts and a procyclic form that grows in the midgut of tsetse fly vector. Bloodstream form parasites have a dense cell surface coat consisting of 10 million molecules of a single GPI-AP, variant surface glycoprotein (VSG) [15]. GPI biosynthesis is essential for growth of the bloodstream form, being exploited as a target of antitrypanosomal drug development [17–20]. The surface of procyclic form parasites is coated by one million molecules of procyclins, GPI-AP with a large side branch of GPI-containing terminal sialic acids. GPI biosynthesis is not essential for growth of the procyclic form in *in vitro* culture [17], whereas it is critical for survival in tsetse fly [21, 22].

Major cell surface proteins of sporozoites and merozoites of malaria parasites are also GPI anchored. Sporozoites that invade hepatocytes after injection by mosquitos are covered by GPI-anchored circumsporozoite proteins [23]. Merozoites that invade erythrocytes have major GPI-APs, such as MSP1 and MSP2 [24].

Glycan and lipid structures have been determined for various GPI-APs from mammalian cells [5, 25–27], yeast (*S. cerevisiae*) [28], protozoan parasites (*T. brucei* and *Plasmodium falciparum*) [24, 29], and plant (*Pyrus communis*) [30] (Figure 1.1). The core backbone structure, EtNP-6Manα1–2Manα1–6Manα1–4GlcNα1–6*myo*Inositol-phospholipid (where EtNP is ethanolamine phosphate; Man is mannose; and GlcN is glucosamine), is common to all of them [1–3, 5]. The terminal "bridging" EtNP is linked to the C-terminus of the protein via an amide bond. Various side branches decorate the core backbone of GPI [1–3].

Precursors of the GPI anchor are synthesized in the ER from PI through sequential reaction steps, such as addition of monosaccharides, EtNP, and fatty acid, and removal of acetyl group from *N*-acetylGlcN (GlcNAc) and fatty acid [1–3, 5] (Figure 1.2). The complete GPI precursor is then attached

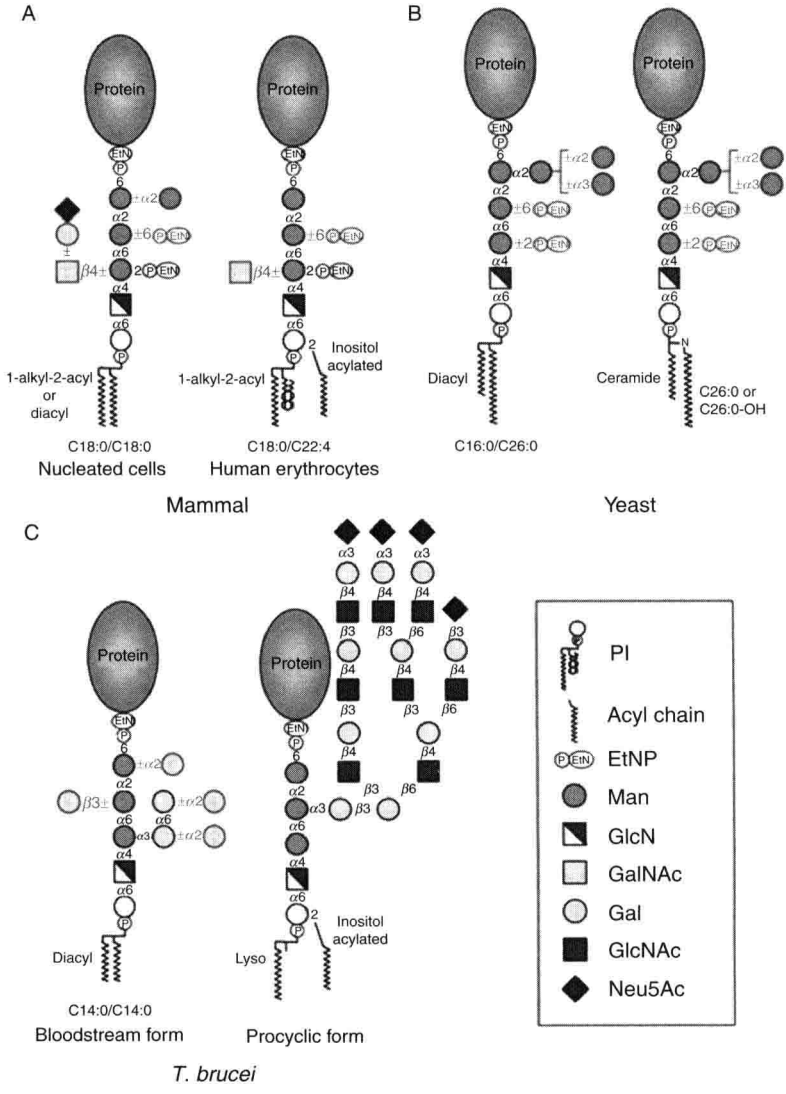

FIG. 1.1. Structures of GPI-APs in mammalian cells (A), yeast (B), and trypanosome (C). (A) Left, GPI-AP from nucleated cells; right, GPI-AP from human erythrocytes. (B) Left, diacylglycerol-type GPI-AP; right, ceramide-type GPI-AP in budding yeast, *S. cerevisiae*. (C) Left, GPI-AP from blood stream form of *T. brucei*; right, GPI-AP from procyclic form of *T. brucei* [5]. Symbol representations of monosaccharides are according to Ref. [148].

to proteins bearing a C-terminal GPI attachment signal peptide on the luminal side of the ER membrane [1]. The GPI attachment is mediated by a transamidase that cleaves the C-terminal signal peptide and replaces it

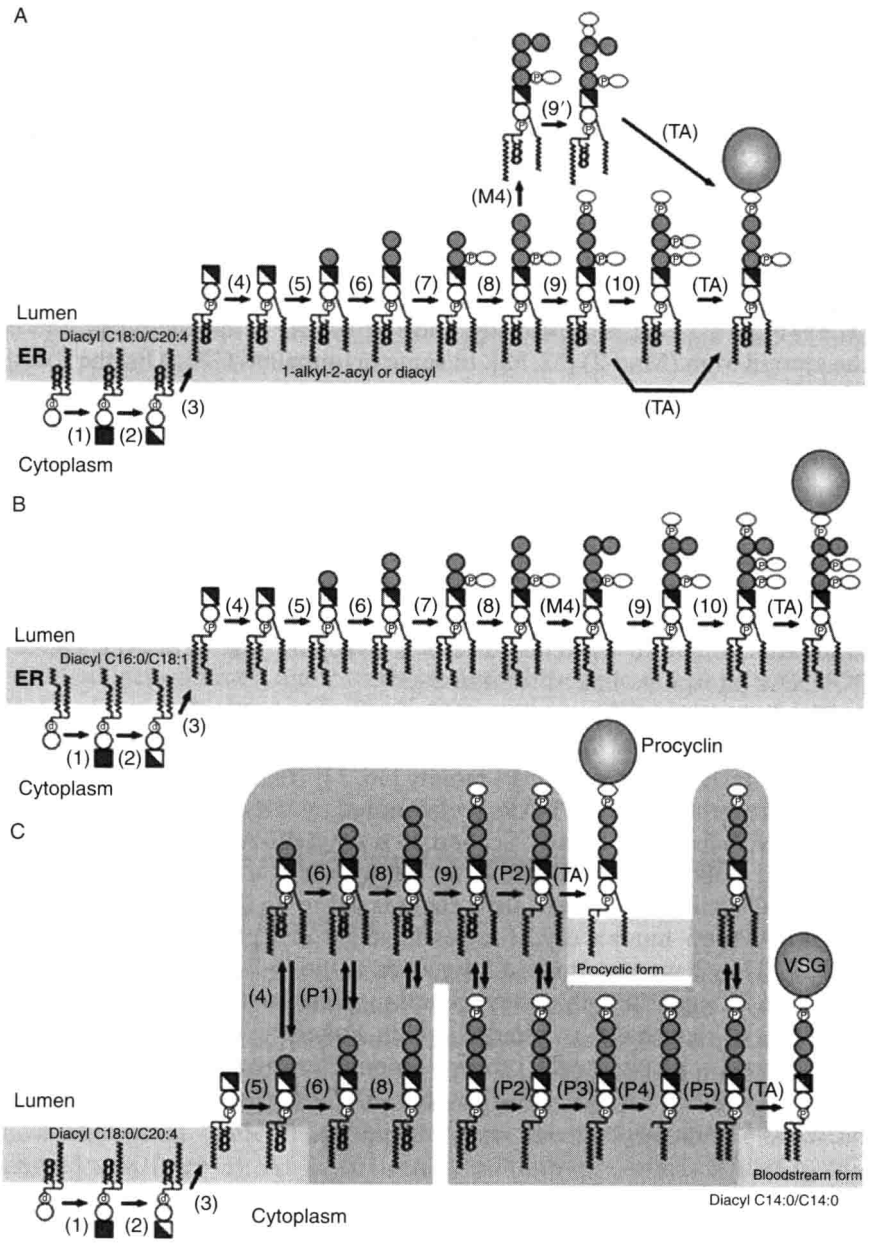

FIG. 1.2. Biosynthesis of GPI on the ER membrane in mammalian cells (A), yeast (B), and trypanosome (C). Steps 1-TA correspond to those in Tables 1.1 and 1.2. In C, reactions are not numbered in order, but those equivalent to mammalian steps (A) are given with the same numbers.