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Review

Biosynthesis and function of GPI proteins in the yeast *Saccharomyces cerevisiae*

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Abstract

Like most other eukaryotes, *Saccharomyces cerevisiae* harbors a GPI anchoring machinery and uses it to attach proteins to membranes. While a few GPI proteins reside permanently at the plasma membrane, a majority of them gets further processed and is integrated into the cell wall by a covalent attachment to cell wall glucans. The GPI biosynthetic pathway is necessary for growth and survival of yeast cells. The GPI lipids are synthesized in the ER and added onto proteins by a pathway comprising 12 steps, carried out by 23 gene products, 19 of which are essential. Some of the estimated 60 GPI proteins predicted from the genome sequence serve enzymatic functions required for the biosynthesis and the continuous shape adaptations of the cell wall, others seem to be structural elements of the cell wall and yet others mediate cell adhesion. Because of its genetic tractability *S. cerevisiae* is an attractive model organism not only for studying GPI biosynthesis in general, but equally for investigating the intracellular transport of GPI proteins and the peculiar role of GPI anchoring in the elaboration of fungal cell walls.

Keywords: Glycosylphosphatidylinositol; *Saccharomyces cerevisiae*; Biosynthesis; Cell wall; Ceramide; Lipid remodeling

1. Introduction

Glycosylphosphatidylinositol (GPI) anchors are structurally complex glycophospholipids, which are added posttranslationally to the C-terminal end of secretory proteins after they have been translocated into the ER. GPI anchoring is utilized by most eukaryotes to express proteins at the cell surface. The structural components of some GPI anchors began to be identified in the 1980s and complete structures for the variant surface glycoproteins of *Trypanosoma brucei* and the mammalian Thy-1 glycoprotein had been worked out by Mike Ferguson, Steve Homans and their coworkers in 1988 [1,2]. This pioneering structural work opened the door to studies concerning the biosynthesis of GPI lipids and their attachment to proteins as well as the identification of the genes involved in these processes. Presently, after 20 years of intensive work, genes required for the addition of about everyone of the different structural elements of the GPI anchor have been identified, but new subunits and regulatory elements of the identified enzymes continue to be discovered.

Precursors of GPI anchored proteins have a classical signal sequence for import into the ER at their N-terminus and a GPI anchoring signal at their C-terminus; the C-terminal signal is necessary and sufficient to direct GPI addition [3]. The C-terminal GPI anchoring signal is recognized and removed by a GPI transamidase, which replaces it by the preformed GPI. GPI anchoring signals are composed of a C-terminal hydrophobic domain, which is separated by a short hydrophilic spacer from the cleavage/attachment site (ω site) [4–6]. While several amino acids can serve as anchor attachment sites in other organisms (Ser, Asp, Ala, Asn, Gly, Cys), only Asn and Gly have been found so far in yeast. Nevertheless, the yeast transamidase also can add very efficiently to Ser, and with lower efficiency to Asp, Ala, and Cys [6]. A bioinformatics predictor specially designed for fungal genomes has recently become available and allows to predict GPI proteins and ω sites from protein sequence data more accurately than using the analogous predictors optimized for animal or plant

Abbreviations: aa, amino acids; CPY, carboxypeptidase Y; CWP, cell wall protein; Dol-P-Man, dolicholphosphomannose; EtN-P, phosphorylethanolamine; GlcNAc, N-Acetyl-Glucosamine; $GlcNH_2$, GlcN, Glucosamine; GPI, glycosylphosphatidylinositol; Man, mannose; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PI-PLC, PI-specific phospholipase C; PMP, plasma membrane protein; TM, transmembrane domain

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sequences (http://mendel.imp.univie.ac.at/gpi/fungi_server.html) [7].

Fig. 1A shows the average structure of the yeast GPI anchor as elaborated from the total pool of GPI anchors extracted from yeast cells without the prior purification of any particular GPI protein [8,9]. The structure of the GPI core linking the protein to the lipid moiety is the same as in other organisms. Indeed, this core structure is very conserved among all eukaryotes, and the same is true for the enzymes that elaborate that structure: Almost all the genes identified as being essential for a given enzymatic step in mammalian or yeast cells have found their homologues in other eukaryotes. Amazingly, even certain side chains such as the forth mannose (Man4) or the phosphorylethanolamine (EtN-P) side chains added on Man1 and Man2 (Fig. 1A) are present both, in man and in yeast and thus have been conserved across long evolutionary distances. Thus, the GPI biosynthesis pathway is a very ancient and highly conserved pathway.

Excellent recent reviews summarize current knowledge about the biosynthesis of GPI anchors and the enzymes that are necessary for their elaboration [10–15].

The present review focuses on new data that emerged recently and have extended our understanding of the biosynthesis and role of GPI anchored protein in *S. cerevisiae*.

2. General properties of GPI proteins of yeast

All known GPI proteins of yeast seem to end up either at the plasma membrane or in the cell wall. A certain number of cell wall proteins (CWPs) of *S. cerevisiae* can be extracted from the glucan mesh simply by boiling in SDS cells in the presence of a reducing agent to break disulfide bridges. A larger part of cell wall proteins however is covalently linked to the insoluble glucan meshwork. Of those, the majority are first made as GPI anchored proteins, which at a certain maturation stage loose the lipid moiety of the GPI anchor and get attached through the remaining mannose residues of the anchor to β1,6-glucans. If these GPI-CWPs are expressed without the C-terminal GPI attachment signal, they are not attached to the glucan mesh and in many cases are secreted [16–18]. Similarly, certain GPI proteins are secreted when the amount of β1,6glucan is greatly diminished [19–21]. GPI-CWPs can be selectively removed from the cell walls by treatment with endo-β1,6-endoglucanase or hydrofluoric acid (HF) [22]. A minor subpopulation of cell wall proteins are not GPI proteins but are nevertheless attached to glucans through a covalent, alkali-sensitive linkage; these proteins have been named ASL-CWPs [23]. Recent reviews summarize current knowledge about the yeast cell wall architecture [23–28].

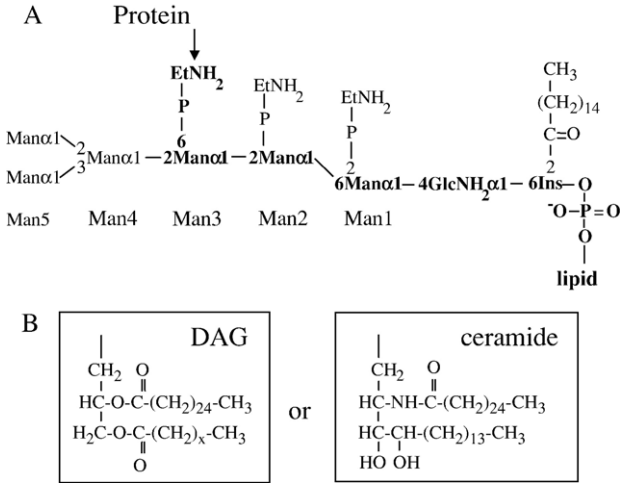

Fig. 1. Structure of GPI anchors of yeast. (A) The average structure of the yeast GPI anchor as elaborated from the total pool of GPI anchors [8,9]. The conserved carbohydrate core structure is in bold characters. The most mature GPI lipid (CP2) accumulating in GPI transamidase mutants contains 4 mannoses (Man1 to Man 4) and phosphorylethanolamine ($EtNH_2$-P) substituents on Man1, Man2 and Man3. A 5th mannose (Man5) can be added to GPI proteins while they transit through the Golgi apparatus, whereby Man5 is added either in α1–2 or in α1–3 linkage [8,111]. Although present on CP2, it is not certain that the $EtNH_2$-P on Man2 is present on GPI anchors of proteins [9]. The palmitic acid on the inositol (Ins) is present on all but the earliest free GPI lipids but it is removed as soon as the GPI lipid is attached to a protein. (B) The two alternative lipid moieties, diacylglycerol (DAG) or ceramide are found on mature GPI proteins. In both anchor lipids the C26 fatty acid can be α-hydroxylated [8]. x probably is 14 or 16.

Comprehensive lists of GPI proteins have been compiled by in silico analysis of the yeast genome [7,29–31] but only for a minority of them the existence of a GPI anchor has been established biochemically. Biochemical evidence can be obtained through indirect methods, e.g., through the demonstration of a loss of hydrophobicity upon treatment with PI-specific phospholipase C (PI-PLC), through metabolic labeling with anchor components such as [^{3}H] inositol, by the loss of surface localization upon site directed mutation of a putative ω site, or by the demonstration of a covalent, alkali-resistant, glucanase-sensitive association with the cell wall, for which the addition of a GPI anchor is a prerequisite. A more direct demonstration of GPI anchoring is obtained through purification of GPI proteins and the analysis of their C-terminal end by chemical and physical methods, e.g., mass spectrometric analysis [32].

3. Biosynthesis of GPI lipids in yeast

3.1. Technical aspects

Contrary to other systems, metabolic labeling of yeast cells with components of the GPI anchor such as mannose or inositol does not allow to detect any free GPI lipids, whereas inositol-labeled GPI proteins can readily be detected. This suggests that the steady state level of free GPI lipids is very low. However, the main reason for the failure to detect free GPI lipids after metabolic labeling may be the fact that the GPI lipids are not made in excess of what is needed for GPI anchoring and that yeast does not contain any free GPIs in its cell membranes as is the case in other organisms. The main pathway generates CP2, which contains 4 mannoses and 3 EtN-P's as depicted in Fig. 2. CP2 is the largest and most polar GPI precursor lipid of yeast and is supposed to be attached to proteins. It is unclear whether CP2 is the only GPI lipid that gets attached to proteins in as much as other GPI lipids lacking EtN-P side chains on Man1 or Man2 equally accumulate, when the transamidase ceases to work and since there presently is no biochemical prove that yeast GPI protein anchors carry an EtN-P on Man2 [9].