JCB Journal of Cell Biology

SPOTLIGHT

GPI anchors: Regulated as needed

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GPI anchoring is an essential post-translational modification in eukaryotes that links proteins to the plasma membrane. In this issue, Liu et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202208159) suggest, for the first time, a regulation on demand of the GPI glycolipid precursor biosynthesis.

Glycosylphosphatidylinositol-anchored proteins (GPI-APs) are an important family of cell surface proteins linked to the outer leaflet of the plasma membrane by an extremely complex glycolipid, the GPI anchor. This conserved post-translational modification defines the spatiotemporal distribution of proteins and, in turn, their correct functioning (1). At least 0.5% of the eukaryotic proteins are GPI-APs (2). In mammals, 150 GPI-APs have been described and the GPI anchor is essential for embryogenesis, neurogenesis, immune response, and fertilization (3).

The GPI is transferred en bloc to newly synthesized precursor proteins through a transamidation reaction in the lumen of the ER. The GPI backbone includes a phospholipid tail, a glycan core, and a phosphoethanolamine linker, by which the protein is attached. In addition, once the GPI is linked to the protein, its glycan and lipid portions are further modified, with consequences in transport and protein localization. Since its discovery in 1976 (4), huge efforts have been made to uncover the biosynthetic pathway of the GPI and its subsequent remodeling. The synthesis of GPI is a sophisticated stepwise pathway that starts in the cytoplasmic leaflet of the ER and ends up in its luminal leaflet. In mammals, 22 genes are involved in the synthesis and protein attachment of the GPI anchor (5). Previous work from the Kinoshita lab has greatly contributed to the identification and characterization of genes involved in this intricate biosynthetic pathway (3). However, whether and how this is regulated in response to cellular needs was unknown. The study by Liu et al. (6) addressed this question and provided an exciting hint: the synthesis of GPI responds to the amount of specific GPI-AP precursors.

Previously, the authors developed an original free GPI cell surface expression system to search for new players in the GPI biosynthetic pathway using genome-wide CRISPR/Cas9 screening (7). One of the main discoveries obtained with this powerful tool was that under defective transfer of GPI anchors to precursor proteins, deficiencies in the ER-associated degradation pathway that handles luminal proteins (ERAD-L) lead to stimulation of GPI biosynthesis. In the present work, the authors implemented a modified version of the free GPI cell surface expression system with genome-wide CRISPR screening to question the underlying mechanism of this upregulation. The screening revealed that the unanchored precursor of the ubiquitously expressed complement decay-accelerating factor, CD55, was key to increased GPI biosynthesis. Upon impairment of GPI anchor transamidation, the lack of a functional ERAD-L pathway produces an accumulation of CD55 precursor proteins that triggers upregulation of GPI biosynthesis. Furthermore, the GPI attachment signal peptide of CD55 was identified as the crucial active element in the upregulation of GPI synthesis. Consistently, the increase in GPI synthesis is dose-dependent on the specific GPI attachment signal peptide. One of the most intriguing findings of this article is that these characteristics do not extend to all GPI-APs. Only the GPI attachment signal peptides of CD55, CD48, and PLET1 were determined as potential signals for the regulation of GPI synthesis. It remains unclear why only some GPI-AP precursors and not others activate the GPI synthesis.

To identify effectors that could bridge the signal of the specific precursor GPI proteins and GPI synthesis, the authors took advantage of biotin-ligase-based proximity labeling and bimolecular fluorescence complementation assays to detect that the ER-resident protein ARV1 is spatially close to the unanchored CD55 precursor. Interestingly, ARV1, which had previously been linked to GPI-AP synthesis in other organisms (8), was also found to be a positive regulator of GPI synthesis in the genome-wide CRISPR screening of this study. That means that ARV1 is functionally required for CD55-dependent GPI upregulation. Furthermore, the authors also show that ARV1 is associated with GPI-GlcNAc transferase, the first enzyme in the pathway, as previously observed in the trypanosome and yeast (9, 10). This result provided evidence that upregulation of GPI synthesis occurs at an early step of the pathway, as confirmed through the study of the metabolic flux of GPI synthesis using radioactive precursors (9, 10). Together, these pieces of evidence led the authors to propose that ARV1 may balance the rate of GPI synthesis to be needed in an early step of the pathway by somehow sensing the accumulation of the specific GPI anchor signal peptide CD55 in the ER. Nevertheless, further

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characterization of the relationship between the CD55 precursor and ARV1 will be necessary to support the proposed model.

Overall, this is an important and impressive work that addresses the regulation of the GPI biosynthetic pathway, providing further evidence that GPI biosynthesis is regulated on demand at an early stage by the presence of specific unanchored GPI precursor proteins through their GPI attachment signal. One appealing possibility is that this regulatory system might function to optimize the GPI anchoring acting as a quantity control mechanism. However, interestingly, the picture seems to be more complex as free GPIs are also produced and delivered to the cell surface by some specific tissues and cell types in mammals. Since this study has shown that GPI biosynthesis is upregulated at early stages, it would be important to test whether this mechanism could differentially balance free GPI biosynthesis and the formation of GPI-APs in the ER in response to physiological requirements.

To conclude, the work of Liu et al. (6) provides a new first step toward the understanding of GPI anchor biology, and further research will be required to understand the precise underlying mechanism of this regulatory system and its physiological significance.

Acknowledgments

Funding provided by Ministerio de Ciencia e Innovación/Agencia Estatal de Investigación (Award ID: PID2020-119505GB-I00/AEI/10.13039/501100011033).

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