Synthesis of glycosylphosphatidylinositol (GPI)

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This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the Reactome Textbook.

06/12/2022
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references


Glycosylphosphatidyl inositol (GPI) acts as a membrane anchor for many cell surface proteins. GPI is synthesized in the endoplasmic reticulum. In humans, a single pathway consisting of nine reactions appears to be responsible for the synthesis of the major GPI species involved in membrane protein anchoring. This pathway is shown in the figure. Two additional reactions, not shown, allow the synthesis of variant forms of GPI, one with an additional mannose residue and one with an additional ethanolamine (Tauron et al. 2004; Shishioh et al. 2005). These variant GPI molecules may be used for tissue-specific protein modifications, or may function independently.

The steps of GPI synthesis were first identified by isolating large numbers of mutant cell lines that had lost the ability to express GPI anchored proteins on their surfaces. Somatic cell hybrid analyses of these lines allowed the definition of complementation groups corresponding to distinct mutated genes, and cDNAs corresponding to normal forms of these genes were identified on the basis of their abilities to restore normal cell surface protein expression in mutant cells. Co-precipitation experiments with tagged cloned proteins have allowed the identification of additional proteins involved in GPI anchor biosynthesis.

**Literature references**


**Editions**

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The first step of GPI synthesis is the transfer of N-acetylglucosamine from cytosolic UDP-N-acetylglucosamine to phosphatidylinositol (PI) in the endoplasmic reticulum membrane. The reaction is catalyzed by a multimeric enzyme, also localized to the endoplasmic reticulum membrane, seven components of which have been identified to date by mutagenesis studies in cultured cells and by co-precipitation and reconstitution studies in vitro (Murakami et al. 2005; Watanabe et al. 1996, 1998, 2000).

Followed by: N-acetylglucosaminyl-PI + H2O -> glucosaminyl-PI + acetate

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https://reactome.org
In the second step of GPI synthesis, N-acetylglucosaminyl-PI is hydrolyzed to yield glucosaminyl-PI and acetate. The phosphatidylinositol (PI) derivatives involved in this reaction are located in the endoplasmic reticulum membrane, as is the PIG-L enzyme that catalyzes it (Sharma et al. 1999; Pottekat and Menon 2004).

**Preceded by:** phosphatidylinositol + UDP-N-acetyl-D-glucosamine -> N-acetylglucosaminyl-PI + UDP

**Followed by:** glucosaminyl-PI + fatty acyl-CoA -> glucosaminyl-acyl-PI + CoA-SH

**Literature references**


glucosaminyl-PI + fatty acyl-CoA → glucosaminyl-acyl-PI + CoA-SH

**Location:** Synthesis of glycosylphosphatidylinositol (GPI)

**Stable identifier:** R-HSA-162683

**Type:** transition

**Compartments:** endoplasmic reticulum membrane, endoplasmic reticulum lumen

In the fourth step of GPI synthesis, an acyl group (typically palmitate) is transferred from acyl CoA to glucosaminyl-PI. Mutagenesis and cloning studies suggest that a single protein, PIG-W, catalyzes this reaction (Murakami et al. 2003).

**Preceded by:** N-acetylglucosaminyl-PI + H2O → glucosaminyl-PI + acetate

**Followed by:** Reorientation of glucosaminyl-acyl-PI in the endoplasmic reticulum membrane, glucosaminyl-acyl-PI + dolichol phosphate D-mannose → mannose(α1-4)glucosaminyl-acyl-PI + dolichol phosphate

**Literature references**


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Reorientation of glucosaminyl-acyl-PI in the endoplasmic reticulum membrane

Location: Synthesis of glycosylphosphatidylinositol (GPI)

Stable identifier: R-HSA-162840

Type: transition

Compartments: endoplasmic reticulum membrane

GPI moieties are synthesized anchored to dolichol phosphate in the membrane of the endoplasmic reticulum. The first two steps of the synthetic pathway, leading to the production of glucosaminyl-PI, occur on the cytosolic face of the membrane, while addition of an acyl group (step 4) and all subsequent steps occur on the lumenal face (Murakami et al. 2003). No mutant cell lines defective in the reorientation step have been identified, and the mechanism by which it occurs is unknown.

Preceded by: glucosaminyl-PI + fatty acyl-CoA -> glucosaminyl-acyl-PI + CoA-SH

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glucosaminyl-acyl-PI + dolichol phosphate $\rightarrow$ mannose(al1-4)glucosaminyl-acyl-PI + dolichol phosphate

**Location:** Synthesis of glycosylphosphatidylinositol (GPI)

**Stable identifier:** R-HSA-162830

**Type:** transition

**Compartments:** endoplasmic reticulum membrane

In the fifth step of GPI synthesis, a mannose residue is added to glucosaminyl-acyl-PI. The reaction takes place at the luminal surface of the endoplasmic reticulum membrane. It is catalyzed by a complex of at least two components, PIG-M and PIG-X (Maeda et al. 2001; Ashida et al. 2005).

**Preceded by:** glucosaminyl-PI + fatty acyl-CoA $\rightarrow$ glucosaminyl-acyl-PI + CoA-SH

**Followed by:** mannose(al1-4)glucosaminyl-acyl-PI + phosphatidylethanolamine $\rightarrow$ (ethanolamineP) mannose(al1-4)glucosaminyl-acyl-PI + diacylglycerol

**Literature references**


Kinoshita, T., Watanabe, R., Ohishi, K., Hong, Y., Kinoshita, K., Harris, CL. et al. (2001). PIG-M transfers the first mannose to glycosylphosphatidylinositol on the luminal side of the ER. *EMBO J*, 20, 250-61.

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mannose(a1-4)glucosaminyl-acyl-PI + phosphatidylethanolamine -> (ethanolamineP) mannose(a1-4)glucosaminyl-acyl-PI + diacylglycerol

Location: Synthesis of glycosylphosphatidylinositol (GPI)

Stable identifier: R-HSA-162798

Type: transition

Compartments: endoplasmic reticulum membrane

In the sixth step of GPI synthesis, a phosphoethanolamine group is transferred from phosphatidylethanolamine onto the first mannose of the GPI precursor. The human protein that catalyzes this reaction was first identified because it could complement a yeast mutant strain defective for GPI synthesis (Gaynor et al. 1999); its specific function in phosphoethanolamine transfer is inferred from functional studies of the homologous mouse protein (Hong et al. 1999). The reaction is annotated here with phosphatidylethanolamine as the donor of the phosphoethanolamine group on the basis of studies in yeast (Imhof et al. 2000).

Preceded by: glucosaminyl-acyl-PI + dolichol phosphate D-mannose -> mannose(a1-4)glucosaminyl-acyl-PI + dolichol phosphate

Followed by: (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate D-mannose -> mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate

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(ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate D-mannose -> mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate

**Location:** Synthesis of glycosylphosphatidylinositol (GPI)

**Stable identifier:** R-HSA-162873

**Type:** transition

**Compartments:** endoplasmic reticulum membrane

In the seventh reaction of GPI synthesis, a second mannose residue is added to the glycolipid on the luminal face of the endoplasmic reticulum membrane. PIG-V was identified as the catalyst, or a component of the catalyst, of this reaction, on the basis of its ability to correct the metabolic defects of yeast and mammalian mutant cells arrested at this stage of the GPI synthetic process (Fabre et al. 2005; Kang et al. 2005).

**Preceded by:** mannose(a1-4)glucosaminyl-acyl-PI + phosphatidylethanolamine -> (ethanolamineP) mannose(a1-4)glucosaminyl-acyl-PI + diacylglycerol

**Followed by:** mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate D-mannose -> mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate

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In the eighth reaction of GPI synthesis, a third mannose residue is added, catalyzed by PIG-B (Takahashi et al. 1996).

**Preceded by:** (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate \(\rightarrow\) mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate

**Followed by:** mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI \(\rightarrow\) mannose (a1) mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + phosphatidylethanolamine \(\rightarrow\) (ethanolamineP) mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI (acyl-GPI) + diacylglycerol

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The final step in the main pathway for the synthesis of GPI moieties in human cells is the addition of an ethanolamine phosphate to the third mannose residue of the glycolipid, donated by phosphatidylethanolamine. This reaction has been experimentally characterized in the mouse, where studies with mutated cell lines defective in GPI biosynthesis have established the role of two proteins, PIG-F and PIG-O, in this reaction (Hong et al. 2000). While a human PIG-F protein has been identified and shown to be involved in this event (Inoue et al. 1993), the human event has not been fully characterized and is therefore annotated here as inferred from studies of the mouse event.

**Preceded by:** mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate D-mannose -> mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate

**Followed by:** (ethanolamineP) mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI - (ethanolamineP) mannose (a1-2) (ethanolamineP) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI

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mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI -> mannose (a1) mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI

Location: Synthesis of glycosylphosphatidylinositol (GPI)

Stable identifier: R-HSA-162797

Type: transition

Compartments: endoplasmic reticulum membrane

Most human GPI anchors are thought to contain three mannose residues, while most yeast GPI anchors contain four. Recently, a human homologue of the yeast enzyme responsible for addition of the fourth mannose residue to GPI molecules was identified and shown to mediate synthesis of human GPI molecules with four mannose residues. While the mannose donor and the nature of the bond linking the third and fourth mannose residues have not been established directly in studies with the human enzyme, these features are known for yeast and the normal human gene restores GPI synthesis in mutant yeast. This observation, together with the sequence similarities between human PIGZ and and Saccharomyces cerevisiae smp3, supports the inference that the human enzyme uses dolichol-P-mannose as a donor. The functional distinction between GPI anchors with three and four mannose residues is unknown, although the latter appear to be abundant in many human tissues (Taron et al. 2004).

Preceded by: mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate D-mannose -> mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate

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Most human GPI anchors have ethanolamine phosphate groups attached to their first and third mannose residues, but GPI anchors with ethanolamine phosphates attached to all three mannose residues have also been identified. Addition of the third ethanolamine phosphate can be catalyzed by a complex of PIG-F and a newly described human protein, GPI7. The donor of the ethanolamine phosphate for this reaction is unknown (Shishioh et al. 2005).

**Preceded by:** mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + phosphatidylethanolamine -> (ethanolamineP) mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI (acyl-GPI) + diacylglycerol

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</table>
Table of Contents

Introduction 1

Synthesis of glycosylphosphatidylinositol (GPI) 2

phosphatidylinositol + UDP-N-acetyl-D-glucosamine -> N-acetylglucosaminyl-PI + UDP 3

N-acetylglucosaminyl-PI + H2O -> glucosaminyl-PI + acetate 4

glucosaminyl-PI + fatty acyl-CoA -> glucosaminyl-acyl-PI + CoA-SH 5

Reorientation of glucosaminyl-acyl-PI in the endoplasmic reticulum membrane 6

glucosaminyl-acyl-PI + dolichol phosphate D-mannose -> mannose(a1-4)glucosaminyl-acyl-PI + dolichol phosphate 7

mannose(a1-4)glucosaminyl-acyl-PI + phosphatidylethanolamine -> (ethanolamineP) mannose(a1-4)glucosaminyl-acyl-PI + diacylglycerol 8

(ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate D-mannose -> mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate 9

mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate D-mannose -> mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + dolichol phosphate 10

mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI + phosphatidylethanolamine -> (ethanolamineP) mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI (acyl-GPI) + diacylglycerol 11

mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI -> mannose (a1) mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI 12

(ethanolamineP) mannose (a1-2) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI -> (ethanolamineP) mannose (a1-2) (ethanolamineP) mannose (a1-6) (ethanolamineP) mannose (a1-4) glucosaminyl-acyl-PI 13

Table of Contents 14