J. Biochem. **122**, 251-257 (1997)

GPI-Anchor Synthesis in Mammalian Cells: Genes, Their Products, and a Deficiency[1]

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Received for publication, May 16, 1997

Protein GPI anchors are ubiquitous in eukaryotic cells. More than 50 mammalian proteins are anchored to the membrane *via* GPI. GPI anchoring is a posttranslational modification occurring in the endoplasmic reticulum where preassembled GPI anchor precursors are transferred to proteins bearing a C-terminal GPI signal sequence. The GPI anchor precursors are synthesized in the endoplasmic reticulum by sequential addition of sugar and other components to phosphatidylinositol. More than ten genes participate in this biosynthetic pathway, eleven of the mammalian genes having been cloned by means of complementation of mutant cells that are defective in this pathway or based on sequence homology to previously cloned yeast counterparts. A somatic mutation in one of those genes, *PIG-A*, involved in the first reaction step, is responsible for the hemolytic disease, paroxysmal nocturnal hemoglobinuria.

Key words: biosynthesis, endoplasmic reticulum, GPI anchor, paroxysmal nocturnal hemoglobinuria, posttranslational modification.

Many eukaryotic cell surface proteins are anchored to the membrane *via* a glycolipid termed glycosylphosphatidylinositol (GPI) which is posttranslationally linked to the carboxy-terminus *via* an amide bond (*1*). A backbone of GPI consists of ethanolaminephosphate whose amino group forms the amide bond with protein, three mannoses, glucosamine, and inositol phospholipid (*2, 3*). The terminal inositolphospholipid anchors the whole protein to the membrane. This backbone is common to all the GPI-anchored proteins found in eukaryotes, but is variously modified by side structures in different organisms and cell types (*4*). The structure of the inositol phospholipid also varies in different organisms (*4*): mammalian proteins usually have 1-alkyl, 2-acyl phosphatidylinositol, yeast *Saccharomyces cerevisiae* uses inositol phosphoceramide or diacylphosphatidylinositol, whereas *Trypanosoma brucei* uses dimyristoylphosphatidylinositol at the blood stage and lysophosphatidylinositol at the insect stage.

The GPI-anchored proteins are abundant in eukaryotic microorganisms. The blood stage *T. brucei* is coated with variant surface glycoprotein which is GPI-anchored. Sporozoites of malaria parasites are coated with GPI-anchored circumsporozoite proteins. It is likely that the GPI-anchor is essential to these microorganisms because without it, the coat proteins may not be expressed on the cell surface. In yeast *S. cerevisiae,* GPI-anchor is used in a number of cytoplasmic membrane proteins and required for transportation of major cell wall proteins to the cell membrane and their incorporation into the cell wall (*5*). The GPI-anchor is, therefore, essential for growth of yeast. Several temperature sensitive mutants of GPI-anchor synthesis have been isolated (*6-8*). In mammalian cells, more than sixty proteins of various functions are GPI-anchored (*9*). At the cellular level, the GPI-anchor is not essential and many mutant cell lines are defective in GPI-anchor biosynthesis (*3*). This suggests that GPI-anchored proteins play a role in cell to cell and cell to environment interactions. Indeed, GPI-anchor biosynthesis is essential in embryogenesis because GPI-anchor-deficient mice generated by disrupting one of the GPI synthesis genes were embryonic lethal (*10*). An essential role of the GPI-anchor in development and maintainance of skin was recently demonstrated by knocking out one of the GPI synthesis genes in keratinocytes by means of tissue-specific gene targeting (*11*).

The core backbone of the GPI-anchor is assembled in the endoplasmic reticulum (ER) and is transferred to the protein. In this article, we review the biosynthesis of the GPI-anchor in mammalian cells, focusing on the genes and their products involved.

Biosynthesis of GPI anchor precursors

First step. The first intermediate of GPI synthesis is *N*-acetylglucosaminyl phosphatidylinositol (GlcNAc-PI), which is made by a transfer of GlcNAc from UDP-GlcNAc to PI (Fig. 1). A genetic approach revealed that this simple reaction step is regulated by at least three genes; there were three complementation groups of mutants in both mammalian cells (classes A, H, and C) (*12, 13*) and yeast *S. cerevisiae* (*gpi1, 2,* and *3*) (*6, 7*). However, the three mammalian genes, *PIG-A* (*14*), *PIG-H* (*15*), and *PIG-C* (*16*), are not all homologous to the three yeast genes, *GPI1* (*17*), *GPI2* (*7*), and *GPI3* (*18*), *i.e.*, *PIG-A* is homologous to *GPI3* (*7, 18, 19*) as is *PIG-C* to *GPI2* (*16*), but *PIG-H* is not homologous to *GPI1* (Table I). A human homologue of

[1] This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan.

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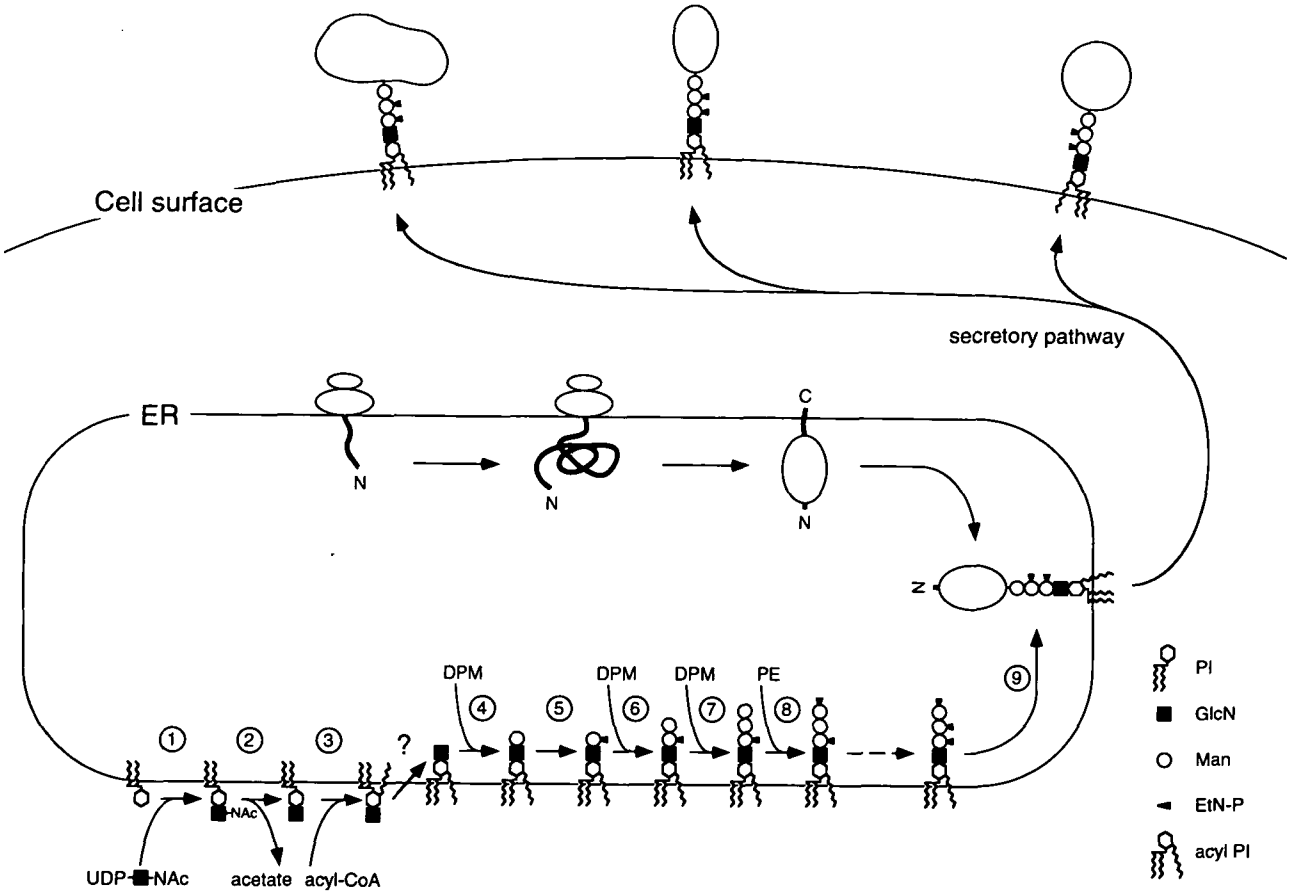

Fig. 1. **Schematic representation of synthesis of GPI-anchored proteins in mammalian cells.** The GPI-anchor is synthesized in the ER and transferred to the newly formed protein bearing a GPI attachment signal. The generated precursor of GPI-anchored protein is transported to the cell surface. Numbering of the reaction steps corresponds to those in the text and Table I.

TABLE I. **Mammalian genes/proteins involved in GPI anchor synthesis.**

Gene	Protein[a]	Yeast homologue[c]	Reaction step	Function	Characteristics	Ref.
PIG-A	484	*GPI3/SPT14/CWH6*	1	GlcNAc transfer	Homology to GlcNAc transferase	*14*
PIG-H (*GPI-H*)	188	?	1	GlcNAc transfer		*15*
PIG-C	269	*GPI2*	1	GlcNAc transfer		*16*
GPI-1	581	*GPI1*	1	GlcNAc transfer		*17*
PIG-L	252[b]	YM8021.07	2	Deacetylation of GlcNAc-PI		*22*
PIG-B	554	YGL142C	7	Third Man transfer	Homology to putative Man transferase ALG9	*31*
PIG-F	219	YDR302W	8	EtN-P transfer		*33*
GAA1	621	*GAA1*	9	GPI attachment		*35*
GPI8	411	*GPI8*	9	GPI attachment	Homology to endopeptidase	*36*
DPM1	260	*DPM1*		Dol-P-Man synthesis	Dol-P-Man synthase	*38*
SL15	248	?		Dol-P-Man usage/synthesis		*40*

[a]Number of amino acids. [b]Rat protein. [c]Gene or ORF.

GPI1 has recently been cloned (Watanabe, R., submitted for publication), therefore, four genes are now known to be involved in this step. The yeast genome does not seem to contain a structural *PIG-H* homologue, suggesting either that the fourth gene of yeast is different from *PIG-H* or that yeast uses only three genes.

PIG-A cDNA was cloned by complementation cloning using class A mutant cells (*14*). It encodes a 484 amino acid, ER transmembrane protein, of which the large amino-terminal portion (400 amino acids) is on the cytoplasmic side and the small carboxy-terminal portion (60 amino acids) is in the lumen (*20*). The amino-terminal portion contains a region homologous to a bacterial GlcNAc transferase for lipopolysaccharide synthesis, suggesting that it bears a catalytic site (*19, 21*). The carboxy-terminal portion is essential for localization of the protein in the ER (*20*).

PIG-A is homologous to yeast *GPI3*, also termed *SPT14* or *CWH6*, bearing about 50% amino acid identity (*18, 19*).

PIG-H cDNA was cloned by complementation cloning using class H mutant cells (*15*). It encodes a 188 amino acid, ER protein. PIG-H protein has a hydrophobic portion in the middle of the molecule which may not be a transmembrane domain and both its amino- and carboxy-termini reside on the cytoplasmic side of the ER, suggesting that it is oriented to the cytoplasm (*20*). There is no protein in data bases that has homology to PIG-H. So, it is not possible to predict the function of PIG-H from its primary sequence.

PIG-C was identified as a homologue of yeast *GPI2* because a cDNA of the human homologue of *GPI2* complemented class C mutant cells (*16*). The predicted PIG-C protein consists of 269 amino acids and has six or seven putative transmembrane domains. It is expressed in the ER. Amino acid identity between PIG-C and Gpi2p is about 20% (*16*).

A human homologue of yeast *GPI1* was found in a data base of Expressed Sequence Tags (EST) and its full-length cDNA was recently cloned. It encodes a 581 amino acid protein having 24% amino acid identity with Gpi1p. Human GPI1 has at least one putative transmembrane domain (Watanabe, R., submitted for publication).

Biochemical studies with epitope-tagged proteins demonstrated that all four gene products form a protein complex in the ER and that the protein complex affinity-purified from digitonin extract had GPI GlcNAc transferase activity *in vitro*, *i.e.*, it generated GlcNAc-PI from UDP-GlcNAc and PI (Watanabe, R., submitted for publication). Therefore, the first step is mediated by the unusually complex GlcNAc transferase. Among the four proteins, PIG-A may have a catalytic site because it has homology to a bacterial GlcNAc transferase as described above. GPI1 may be important in stabilizing the protein complex because an amount of complex of PIG-A, -H, and -C increased with an increased expression of human GPI1 and because human GPI1 directly associates with all three proteins. This notion is consistent with a report that *GPI1*- disrupted yeasts can synthesize the GPI-anchor at 25°C but not 37°C (*17*). The functions of PIG-C and PIG-H are unclear but the complex structure of the GPI GlcNAc transferase may be relevant to the regulation of the enzyme.

Second step. The second step is deacetylation of GlcNAc-PI to form glucosaminyl-PI (GlcN-PI) (Fig. 1). The isolated GPI GlcNAc transferase complex did not support the second step, suggesting that the deacetylase is not contained in the complex (Watanabe, R., submitted for publication). A rat cDNA that complements deacetylase-deficient mutant CHO cells of class L (*22*) has recently been cloned and the gene was termed *PIG-L* (*22*). It encodes a protein of 252 amino acids with no homology to other known proteins. PIG-L protein is an ER membrane protein, most of it being on the cytoplasmic side (*22*). Whether PIG-L is GlcNAc-PI deacetylase itself is not known, however, since overexpression of PIG-L in CHO cells caused overexpression of the deacetylase activity and since it is expressed in the ER where deacetylation is taking place, PIG-L may be closely involved in deacetylation (*22*).

Third step. GlcN-PI is then acylated on inositol to form GlcN-(acyl)PI (Fig. 1) (*23, 24*). Palmitoylation is a major form. An enzyme that mediates this acylation has not been cloned or isolated. Acylation of PI is an obligatory step in mammalian cells because a synthetic dioctanoyl GlcN-acylPI analogue was further processed upon incubation with microsomes much better than dioctanoyl GlcN-PI (*25*). Consistent with this, GlcN-(acyl)PI is accumulated in class E mouse thymoma mutant cells and Lec15 CHO mutant cells which do not synthesize dolichol-phosphate-mannose (Dol-P-Man) (*23, 26*), and in Lec35 CHO mutant cells which do not use Dol-P-Man efficiently (*27*). In contrast, acylation does not seem necessary in trypanosomes because GlcNAc-PI was efficiently processed in an *in vitro* system (*28*).

Fourth and fifth steps. Mannose is transferred from Dol-P-Man to GlcN-acylPI, generating Man-GlcN-(acyl)PI (Fig. 1). A mutant in this step has yet to be established, and the gene(s) involved has not been cloned. As described above, the specificities of mammalian and trypanosomal transferases for this mannose differ.

Ethanolaminephosphate (EtN-P) is added to position 3 of mannose, generating (EtN-P)Man-GlcN-(acyl)PI (Fig. 1) (*26, 29, 30*). It is not known whether this is an obligatory step. The donor of EtN-P has yet to be elucidated.

Sixth and seventh steps. Two more mannoses are transferred from Dol-P-Man to generate the seventh intermediate, Man-Man-(EtN-P)Man-GlcN-(acyl)PI (Fig. 1). A mutant that is defective in the second mannose transfer has yet to be established, and the gene(s) responsible has not been cloned.

Class B mutant cells accumulate an intermediate bearing two mannoses, indicating that the third mannosylation is defective (*13, 29*). *PIG-B* gene has been cloned by means of complementation cloning using class B cells (*31*). *PIG-B* encodes an ER transmembrane protein of 554 amino acid residues. Although the mannosyltransferase activity of PIG-B is not shown directly, it is probably the enzyme itself because it has homology to yeast Alg9p, a putative mannosyltransferase for the seventh mannose in the *N*-glycan precursor (*32*). A 30-amino-acid amino-terminal portion of PIG-B is on the cytoplasmic side of the ER while the large carboxy-terminal portion is in the lumen (*31*). The amino-terminal portion was not necessary for complementation, indicating that the functional sites are on the lumenal side (*31*).

Eighth step. EtN-P is added to position 6 of the third mannose from phosphatidylethanolamine, generating EtN-P-Man-Man-(EtN-P)Man-GlcN-(acyl)PI (Fig. 1). Class F mutant is defective in this step (*13, 26*). *PIG-F* which complements this defect was cloned by expression cloning (*33*). *PIG-F* encodes a very hydrophobic ER protein of 219 amino acids (*33*). Whether PIG-F is EtN-P transferase has yet to be established.

Another EtN-P may be added to the second mannose to generate EtN-P-Man-(EtN-P)Man-(EtN-P)Man-GlcN-(acyl)PI (Fig. 1). Both of these molecules bearing three mannoses may be transferred to proteins, *i.e.*, they are mature GPI anchor precursors.

Transfer of GPI anchor precursor to protein

The mature GPI anchor precursor is added to the carboxy-terminus of protein *via* an amide bond formed between the carboxy-terminus and EtN. Proteins that are to be GPI-anchored have two signal sequences. One is an amino-terminal signal sequence for translocation across the ER membrane that is common to secreted and cell-surface

membrane proteins. The other is a carboxy-terminal sequence that signals its own cleavage and replacement with the GPI-anchor (*1*). This replacement reaction is thought to be a transamidation (*1*). In a cell-free system in which a model protein miniPLAP made by *in vitro* transcription/translation was processed and modified by the GPI anchor in the presence of microsomes, an addition of hydrazine (or hydroxylamine) resulted in the linkage of hydrazine (or hydroxylamine) in place of the GPI-anchor forming a hydrazide (or a hydroxamate) of miniPLAP (*34*).

There are two yeast mutants, *gaa1* (*35*) and *gpi8* (*8*), that are defective in this step and are synthetic lethal (*8*), indicating the involvement of at least two genes. The transamidation may be mediated by a protein complex because it is a rather complex reaction involving recognitions of the GPI signal sequence and the GPI anchor precursor and substitution of the former with the latter.

Yeast *GAA1* gene encodes an ER membrane protein of 614 amino acids with a large lumenal domain (*35*). It is unclear whether Gaa1p recognizes a GPI signal sequence or a GPI anchor precursor, but the presence of a large lumenal domain is compatible with the notion that transamidation occurs on the lumenal side of the ER. Human and mouse homologues of *GAA1* have recently been cloned (Ohishi, K. and Inoue, N., unpublished). They encode proteins of 621 amino acids that have 22% amino acid identity with yeast Gaa1p. Mouse F9 embryonic carcinoma cells in which *GAA1* was disrupted by gene targeting were defective in the surface expression of GPI-anchored proteins and accumulated mature GPI-anchor precursors, indicating that this gene is indeed involved in the transfer of the GPI-anchor (Ohishi, K., unpublished).

Yeast *GPI8* gene encodes a 411-amino-acid membrane protein residing on the lumenal side of the ER that has homology to a family of plant endopeptidases one of which has transamidase activity (*36*). So, GPI8 may be a catalytic component of transamidase. Human *GPI8* has also been cloned and shown to complement *gpi8* mutant (*36*).

Class K mutant of human K562 cells synthesizes mature GPI anchor precursors but is unable to transfer them to proteins due to a defective transamidation (*34*). Transfection of human *GPI8* but not *GAA1* cDNA complemented class K mutant (Ohishi, K., unpublished), confirming that two genes are also involved in this step in mammalian cells.

Biosynthesis and usage of a mannose donor, Dol-P-Man

All three mannoses in the GPI-anchor are donated by Dol-P-Man. So, its defective synthesis and/or usage would result in GPI anchor deficiency. Class E and Lec15 mutants are defective in the synthesis of Dol-P-Man. Somatic cell hybridization experiments suggested that they represent different mutant classes (*37*), hence, two genes are involved in Dol-P-Man synthesis in mammalian cells. This is in contrast to the synthesis of Dol-P-Man in yeast *S. cerevisiae* for which one gene is thought to be sufficient.

A *dpm1* mutant of *S. cerevisiae* is defective in Dol-P-Man synthesis, its gene *DPM1* having been cloned (*38*). *DPM1* encodes a protein of 267 amino acids with a carboxy-terminal hydrophobic segment, a putative transmembrane domain. Dpm1p expressed in *E. coli* exhibited Dol-P-Man synthase activity, suggesting that it is the synthase itself (*38*). Defective Dol-P-Man synthesis of both class E and Lec15 mutants were complemented by transfection with *DPM1* DNA (*39*).

A human homologue of *DPM1* has been identified. Human *DPM1* encodes a protein of 260 amino acids that has 30% amino acid identity with yeast Dpm1p (Tomita, S., unpublished). However, it lacked a transmembrane domain. Human *DPM1* cDNA restored synthesis of Dol-P-Man in class E cells, suggesting that it is a catalytic component of human Dol-P-Man synthase. In contrast to yeast *DPM1*, human *DPM1* did not complement Lec15, indicating that the gene mutated in Lec15 is also essential for synthesis of Dol-P-Man in mammalian cells (Tomita, S., unpublished). A major difference between yeast and human DPM1 proteins is the lack of a transmembrane domain in the latter. Since both yeast and mammalian Dol-P-Man synthases are membrane-bound, the gene mutated in Lec15 may be necessary for membrane association of mammalian DPM1.

A hamster cDNA that complemented Lec15 mutant has been cloned and termed *SL15* for suppressor of Lec15 (*40*). *SL15* encodes a protein of 248 amino acids which has two putative transmembrane domains and a double-lysine ER-retention signal near the carboxy-terminus, suggesting that it is an ER membrane protein (*40*). SL15 has no homology to human DPM1. Although these characteristics are consistent with the notion that SL15 is necessary for membrane association of DPM1, the story is not that simple. Lec35 mutant cells synthesize Dol-P-Man but are defective in its usage (*27*). This does not involve a defect in the first mannosyltransferase because the cells are also defective in the usage of Dol-P-Man for *N*-glycan (*27*). Although somatic cell hybridization indicated that Lec35 and Lec15 are of different complementation classes (*27*), SL15 cDNA also complemented Lec35 (*40*). At present, whether *SL15* is mutated in Lec15 and/or Lec35 is not known. It is necessary to determine this to further understand the synthesis and usage of Dol-P-Man in mammalian cells.

Membrane topology of GPI-anchor synthesis

The first and second intermediates face the cytoplasmic side of the ER because they were cleaved by PI-PLC when intact microsomes were incubated with the enzyme (*41*). A large domain of PIG-A having homology to a GlcNAc transferase was located on the cytoplasmic side of the ER, and PIG-H and PIG-L are oriented to the cytoplasmic side (*20, 22*). Taken together, the first two reaction steps would occur on the cytoplasmic side of the ER. On the other hand, the transfer of the mature GPI-anchor precursors to proteins is thought to occur on the lumenal side of the ER. Therefore, a post-deacetylation intermediate or the mature GPI-anchor precursors should flip into the lumen.

The step at which this flip occurs is unclear at the moment because analyses of membrane orientation of biosynthesis proteins and GPI intermediates gave different results. PIG-B, which is involved in the transfer of the third mannose was oriented to the lumenal side, suggesting that the third mannose is transferred from Dol-P-Man to a GPI intermediate on the lumenal side (*31*). In Lec35 mutant CHO cells, Dol-P-Man is synthesized but not used in the GPI-anchor or *N*-glycan precursor (*27*). Dol-P-Man acts as a mannosyl donor for the last four mannoses of *N*-glycan precursor on the lumenal side of the ER. Since Dol-P-Man

is synthesized on the cytoplasmic side, it must be translocated into the lumen to be competent as a mannosyl donor for *N*-glycan. It is, therefore, likely that Lec35 cells are defective in the translocation of Dol-P-Man into the lumen. The fact that Lec35 mutation affects usage of Dol-P-Man in both *N*-glycan and GPI-anchor suggests that translocation of Dol-P-Man is also essential for GPI-anchor synthesis. This together with the lumenal orientation of PIG-B supports the idea that Dol-P-Man is used for GPI-anchor synthesis on the lumenal side. If this is correct, GlcN-(acyl)PI would flip into the lumen in mammalian and yeast systems and GlcN-PI would flip in trypanosomes. In contrast, analysis of the membrane orientation of mannose-bearing GPI intermediates in trypanosomes and mammalian cells suggests that they are cytoplasmically oriented (*42*). If this is correct, the mature GPI-anchor precursors would flip into the lumen.

Paroxysmal nocturnal hemoglobinuria: a GPI anchor deficiency

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematopoietic stem cell disorder. Patients with PNH have abnormal cell populations in various hematopoietic cell lineages that are defective in the surface expression of GPI-anchored proteins (*9*). A major clinical symptom is intravascular hemolysis caused by own complement. A lack of the GPI-anchored complement regulatory proteins, CD59 and decay accelerating factor (CD55), on the surface of abnormal erythrocytes is causally related to the abnormally high susceptibility to complement, leading to intravascular hemolysis upon activation of complement during infections and other events (*9*).

The first step of GPI-anchor synthesis is defective in affected hematopoietic cells from patients with PNH (*43, 44*). This is due to a somatic mutation in the *PIG-A* gene (*45-47*). *PIG-A* is an X-linked gene mapped at Xp22.1 (*45*). So, one somatic mutation in male cells is sufficient to cause a GPI-anchor deficient state. In affected female cells, normal and mutated *PIG-A* alleles were found but the allele bearing a somatic mutation was selectively expressed, indicating that the somatic mutation occurred in the active X-chromosome (*45*). Since affected neutrophils and B-lymphocytes in the same patient had the same mutation, it should have occurred in the multipotential hematopoietic stem cell (*45*).

In more than a hundred patients with PNH characterized, *PIG-A* abnormalities were found (*48*). There is no example of PNH caused by mutation in other GPI-synthesis genes. The most likely explanation for this would be that all other genes involved in the synthesis of the GPI-anchor or its transfer to proteins are autosomal and hence two mutations are needed in the same cell to cause a GPI-anchor deficient state. Since this condition would be extremely rare, patients with PNH caused by a somatic mutation in an autosomal gene would be difficult to find. In fact, seven other genes have been mapped to various autosomes (Table II) (*16, 31, 49, 50*).

TABLE II. **Chromosomal location of GPI anchor synthesis genes.**

Human		Mouse	
Gene	Location	Gene	Location
PIG-A	Xp22.1	*Piga*	XF3/4
PIG-H (*GPI-H*)	14q[a]	*Pigh*	12
PIG-C	1q23-q25		
PIG-B	15q21-q22		
PIG-F	2p16-p21	*Pigf*	17E4/5
GAA1	8q24.3	*Gaa1*	15E
DPM1	20q13		
SL15	17		

[a]Predicted from the location of mouse *Pigh*.

Concluding remarks

As described above, eleven genes necessary for biosynthesis of the mammalian GPI-anchor precursor and its transfer to proteins have been cloned. Five or more genes are yet to be cloned, indicating that more than fifteen genes are involved in this pathway. For cloning other genes, mutant cell lines defective in those genes should be established or yeast counterparts should be cloned first. Elucidation of mammalian and yeast GPI-anchor synthesis genes would facilitate cloning and characterization of their counterparts in pathogenic protozoas and fungi. This, in turn, would facilitate better understanding of differences and similarities between GPI-anchor synthesis enzymes and regulators in host and parasites.

We thank Drs. Mark A. Lehrman, Peter Orlean, and Howard Riezman for communicating unpublished results.

REFERENCES

(Not all relevant references are cited due to the limited number of references allowed.)

1. Udenfriend, S. and Kodukula, K. (1995) How glycosylphosphatidylinositol-anchored membrane proteins are made. *Annu. Rev. Biochem.* **64**, 563-591
2. Stevens, V.L. (1995) Biosynthesis of glycosylphosphatidylinositol membrane anchors. *Biochem. J.* **310**, 361-370
3. Takeda, J. and Kinoshita, T. (1995) GPI-anchor biosynthesis. *Trends Biochem. Sci.* **20**, 367-371
4. McConville, M.J. and Ferguson, M.A.J. (1993) The structure, biosynthesis and function of glycosylated phosphatidylinositols in the parasitic protozoa and higher eukaryotes. *Biochem. J.* **294**, 305-324
5. Lu, C.F., Montijn, R.C., Brown, J.L., Klis, F., Kurjan, J., Bussey, H., and Lipke, P.N. (1995) Glycosyl phosphatidylinositol-dependent cross-linking of alpha-agglutinin and beta-1,6-glucan in the *Saccharomyces cerevisiae* cell wall. *J. Cell Biol.* **128**, 333-340
6. Leidich, S.D., Drapp, D.A., and Orlean, P. (1994) A conditionally lethal yeast mutant blocked at the first step in glycosyl phosphatidylinositol anchor synthesis. *J. Biol. Chem.* **269**, 10193-10196
7. Leidich, S.D., Kostova, Z., Latek, R.R., Costello, L.C., Drapp, D.A., Gray, W., Fassler, J.S., and Orlean, P. (1995) Temperature-sensitive yeast GPI anchoring mutants gpi2 and gpi3 are defective in the synthesis of *N*-acetylglucosaminyl phosphatidylinositol: cloning of the GPI2 gene. *J. Biol. Chem.* **270**, 13029-13035
8. Benghezal, M., Lipke, P.N., and Conzelmann, A. (1995) Identification of six complementation class involved in the biosynthesis of glycosylphosphatidylinositol anchors in *Saccharomyces cerevisiae*. *J. Cell Biol.* **130**, 1333-1344
9. Kinoshita, T., Inoue, N., and Takeda, J. (1995) Defective glycosyl phosphatidylinositol anchor synthesis and paroxysmal nocturnal hemoglobinuria. *Adv. Immunol.* **60**, 57-103
10. Kawagoe, K., Kitamura, D., Okabe, M., Taniuchi, I., Ikawa, M., Watanabe, T., Kinoshita, T., and Takeda, J. (1996) GPI-anchor

deficient mice: Implications for clonal dominance of mutant cells in paroxysmal nocturnal hemoglobinuria. *Blood* **87**, 3600-3606
11. Tarutani, M., Itami, S., Okabe, M., Ikawa, M., Tezuka, T., Yoshikawa, K., Kinoshita, T., and Takeda, J. (1997) Tissue specific knock-out of the mouse Pig-a gene reveals important roles for GPI-anchored proteins in skin development. *Proc. Natl. Acad. Sci. USA* **94**, in press
12. Stevens, V.L. and Raetz, C.R. (1991) Defective glycosyl phosphatidylinositol biosynthesis in extracts of three Thy-1 negative lymphoma cell mutants. *J. Biol. Chem.* **266**, 10039-10042
13. Sugiyama, E., DeGasperi, R., Urakaze, M., Chang, H.M., Thomas, L.J., Hyman, R., Warren, C.D., and Yeh, E.T.H. (1991) Identification of defects in glycosylphosphatidylinositol anchor biosynthesis in the Thy-1 expression mutants. *J. Biol. Chem.* **266**, 12119-12122
14. Miyata, T., Takeda, J., Iida, Y., Yamada, N., Inoue, N., Takahashi, M., Maeda, K., Kitani, T., and Kinoshita, T. (1993) Cloning of PIG-A, a component in the early step of GPI-anchor biosynthesis. *Science* **259**, 1318-1320
15. Kamitani, T., Chang, H.M., Rollins, C., Waneck, G.L., and Yeh, E.T.H. (1993) Correction of the class H defect in glycosylphosphatidylinositol anchor biosynthesis in Ltk-cells by a human cDNA clone. *J. Biol. Chem.* **268**, 20733-20736
16. Inoue, N., Watanabe, R., Takeda, J., and Kinoshita. T. (1996) PIG-C, one of the three human genes involved in the first step of glycosylphosphatidylinositol biosynthesis is a homologue of *Saccharomyces cerevisiae* GPI2. *Biochem. Biophys. Res. Commun.* **226**, 193-199
17. Leidich, S.D. and Orlean, P. (1996) Gpi1, a *Saccharomyces cerevisiae* protein that participates in the first step in glycosylphosphatidylinositol anchor synthesis. *J. Biol. Chem.* **271**, 27829-27837
18. Schonbachler, M., Horvath, A., Fassler, J., and Riezman, H. (1995) The yeast spt14 gene is homologous to the human PIG-A gene and is required for GPI anchor synthesis. *EMBO J.* **14**, 1637-1645
19. Vossen, J.H., Ram, A.F., and Klis, F.M. (1995) Identification of SPT14/CWH6 as the yeast homologue of hPIG-A, a gene involved in the biosynthesis of GPI anchors. *Biochim. Biophys. Acta* **1243**, 549-551
20. Watanabe, R., Kinoshita, T., Masaki, R., Yamamoto, A., Takeda, J., and Inoue, N. (1996) PIG-A and PIG-H, which participate in glycosylphosphatidylinositol anchor biosynthesis, form a protein complex in the endoplasmic reticulum. *J. Biol. Chem.* **271**, 26868-26875
21. Kawagoe, K., Takeda, J., Endo, Y., and Kinoshita, T. (1994) Molecular cloning of murine Pig-a, a gene for GPI-anchor biosynthesis, and demonstration of interspecies conservation of its structure, function and gene locus. *Genomics* **23**, 566-574
22. Nakamura, N., Inoue, N., Watanabe, R., Takahashi, M., Takeda, J., Stevens, V.L., and Kinoshita, T. (1997) Expression cloning of PIG-L, a candidate *N*-acetylglucosaminyl-phosphatidylinositol deacetylase. *J. Biol. Chem.* **272**, 15834-15840
23. Urakaze, M., Kamitani, T., DeGasperi, R., Sugiyama, E., Chag, H.M., Warren, C.D., and Yeh, E.T.H. (1992) Identification of a missing link in glycosylphosphatidylinositol anchor biosynthesis in mammalian cells. *J. Biol. Chem.* **267**, 6459-6462
24. Stevens, V.L. and Zhang, H. (1994) Coenzyme A dependence of glycosylphosphatidylinositol biosynthesis in a mammalian cell-free system. *J. Biol. Chem.* **269**, 31397-31403
25. Doerrler, W.T., Ye, J., Falck, J.R., and Lehrman, M.A. (1996) Acylation of glucosaminyl phosphatidylinositol revisited. *J. Biol. Chem.* **271**, 27031-27038
26. Puoti, A. and Conzelmann, A. (1993) Characterization of abnormal free glycophosphatidylinositols accumulating in mutant lymphoma cells of classes B, E, F, and H. *J. Biol. Chem.* **268**, 7215-7224
27. Camp, L.A., Chauhan, P., Farrar, J.D., and Lehrman, M.A. (1993) Defective mannosylation of glycosylphosphatidylinositol in Lec35 Chinese hamster ovary cells. *J. Biol. Chem.* **268**, 6721-6728
28. Smith, T.K., Cottaz, S., Brimacombe, J.S., and Ferguson, M.A. (1996) Substrate specificity of the dolichol phosphate mannose: glucosaminyl phosphatidylinositol alpha1-4-mannosyltransferase of the glycosylphosphatidylinositol biosynthetic pathway of African Trypanosomes. *J. Biol. Chem.* **271**, 6476-6482
29. Puoti, A., Desponds, C., Fankhauser, C., and Conzelmann, A. (1991) Characterization of glycophospholipid intermediate in the biosynthesis of glycophosphatidylinositol anchors accumulating in the Thy-1-negative lymphoma line SIA-b. *J. Biol. Chem.* **266**, 21051-21059
30. Hirose, S., Prince, G.M., Sevlever, D., Ravi, L., Rosenberry, T.L., Ueda, E., and Medof, M.E. (1992) Characterization of putative glycoinositol phospholipid anchor precursors in mammalian cells. Localization of phosphoethanolamine. *J. Biol. Chem.* **267**, 16968-16974
31. Takahashi, M., Inoue, N., Ohishi, K., Maeda, Y., Nakamura, N., Endo, Y., Fujita, T., Takeda, J., and Kinoshita, T. (1996) PIG-B, a membrane protein of the endoplasmic reticulum with a large lumenal domain, is involved in transferring the third mannose of the GPI anchor. *EMBO J.* **15**, 4254-4261
32. Burda, P., te Heesen, S., Brachat, A., Wach, A., Dusterhoft, A., and Aebi, M. (1996) Stepwise assembly of the lipid-linked oligosaccharide in the endoplasmic reticulum of *Saccharomyces cerevisiae*: Identification of the ALG9 gene encoding a putative mannosyl transferase. *Proc. Natl. Acad. Sci. USA* **93**, 7160-7165
33. Inoue, N., Kinoshita, T., Orii, T., and Takeda, J. (1993) Cloning of a human gene, PIG-F, a component of glycosylphosphatidylinositol anchor biosynthesis, by a novel expression cloning strategy. *J. Biol. Chem.* **268**, 6882-6885
34. Chen, R., Udenfriend, S., Prince, G.M., Maxwell, S.E., Ramalingam, S, Gerber, L.D., Knez, J., and Medof, M.E. (1996) A defect in glycosylphosphatidylinositol (GPI) transamidase activity in mutant K cells is responsible for their inability to display GPI surface proteins. *Proc. Natl. Acad. Sci. USA* **93**, 2280-2284
35. Hamburger, D., Egerton, M., and Riezman, H. (1995) Yeast Gaa1p is required for attachment of a completed GPI anchor onto proteins. *J. Cell Biol.* **129**, 629-639
36. Benghezal, M., Benachour, A., Rusconi, S., Aebi, M., and Conzelmann, A. (1996) Yeast Gpi8p is essential for GPI anchor attachment onto proteins. *EMBO J.* **15**, 6575-6583
37. Singh, N. and Tartakoff, A.M. (1991) Two different mutants blocked in synthesis of dolichol-phosphoryl-mannose do not add glycophospholipid anchors to membrane proteins: Quantitative correction of the phenotype of a CHO cell mutant with tunicamycin. *Mol. Cell. Biol.* **11**, 391-400
38. Orlean, P., Albright, C., and Robbins, P.W.(1988) Cloning and sequencing of the yeast gene for dolichol phosphate mannose synthase, an essential protein. *J. Biol. Chem.* **263**, 17499-17507
39. Beck, P.J., Orlean, P., Albright, C., Robbins, P.W., Gething, M.J., and Sambrook, J.F. (1990). The *Saccharomyces cerevisiae* DPM1 gene encoding dolichol-phosphate-mannose synthase is able to complement a glycosylation-defective mammalian cell line. *Mol. Cell. Biol.* **10**, 4612-4622
40. Ware, F.E. and Lehrman, M.A. (1996) Expression cloning of a novel suppressor of the Lec15 and Lec35 glycosylation mutations of Chinese hamster ovary cells. *J. Biol. Chem.* **271**, 13935-13938
41. Vidugiriene, J. and Menon, A.K. (1993) Early lipid intermediates in glycosyl-phosphatidylinositol anchor assembly are synthesized in the ER and located in the cytoplasmic leaflet of the ER membrane bilayer. *J. Cell Biol.* **121**, 987-996
42. Vidugiriene, J. and Menon, A.K. (1994) The GPI anchor of cell-surface proteins is synthesized on the cytoplasmic face of the endoplasmic reticulum. *J. Cell Biol.* **127**, 333-341
43. Takahashi, M., Takeda, J., Hirose, S., Hyman, R., Inoue, N., Miyata, T., Ueda, E., Kitani, T., Medof, M.E., and Kinoshita, T. (1993) Deficient biosynthesis of *N*-acetylglucosaminyl phosphatidylinositol, the first intermediate of glycosyl phosphatidylinositol anchor biosynthesis, in cell lines established from patients with paroxysmal nocturnal hemoglobinuria. *J. Exp. Med.* **177**, 517-521
44. Hillmen, P., Bessler, M., Mason, P.J., Watkins, W.M., and Luzzatto, L. (1993) Specific defect in *N*-acetylglucosamine incorporation in the biosynthesis of the glycosylphosphatidyl-

inositol anchor in cloned cell lines from patients with paroxysmal nocturnal hemoglobinuria. *Proc. Natl. Acad. Sci. USA* **90**, 5272-5276

45. Takeda, J., Miyata, T., Kawagoe, K., Iida, Y., Endo, Y., Fujita, T., Takahashi, M., Kitani, T., and Kinoshita, T. (1993) Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria. *Cell* **73**, 703-711
46. Bessler, M., Mason, P.J., Hillmen, P., Miyata, T., Yamada, N., Takeda, J., Luzzatto, L., and Kinoshita, T. (1994) Paroxysmal nocturnal haemoglobinuria (PNH) is caused by somatic mutations in the PIG-A gene. *EMBO J.* **13**, 110-117
47. Miyata, T., Yamada, N., Iida, Y., Nishimura, J., Takeda, J., Kitani, T., and Kinoshita, T. (1994) Abnormalities of PIG-A transcripts in granulocytes from patients with paroxysmal nocturnal hemoglobinuria. *N. Engl. J. Med.* **330**, 249-255
48. Kinoshita, T., Inoue, N., and Takeda, J. (1996) Role of phosphatidylinositol-linked proteins in paroxysmal nocturnal hemoglobinuria pathogenesis. *Annu. Rev. Med.* **47**, 1-10
49. Ware, R.E., Howard, T.A., Kamitani, T., Chang, H.M., Yeh, E.T.H., and Seldin, M.F. (1994) Chromosomal assignment of genes involved in glycosylphosphatidylinositol anchor biosynthesis: Implications for the pathogenesis of paroxysmal nocturnal hemoglobinuria. *Blood* **83**, 3753-3757
50. Ohishi, K., Inoue, N., Endo, Y., Fujita, T., Takeda, J., and Kinoshita, T. (1995) Structure and chromosomal localization of the GPI-anchor synthesis gene PIG-F and its pseudogene ΨPIG-F. *Genomics* **29**, 804-807