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In vitro attachment of glycosyl-inositolphospholipid anchor structures to mouse Thy-1 antigen and human decay-accelerating factor

("glypiation"/protein processing)

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ABSTRACT **Glycosyl-inositolphospholipid (GPL) anchoring structures are incorporated into GPL-anchored proteins immediately posttranslationally in the rough endoplasmic reticulum, but the biochemical and cellular constituents involved in this "glypiation" process are unknown. To establish whether glypiation could be achieved *in vitro*, mRNAs generated by transcription of cDNAs encoding two GPL-anchored proteins, murine Thy-1 antigen and human decay-accelerating factor (DAF), and a conventionally anchored control protein, polymeric-immunoglobulin receptor (IgR), were translated in a rabbit reticulocyte lysate. Upon addition of dog pancreatic rough microsomes, nascent polypeptides generated from the three mRNAs translocated into vesicles. Dispersal of the vesicles with Triton X-114 detergent and incubation of the hydrophobic phase with phosphatidylinositol-specific phospholipases C and D, enzymes specific for GPL-anchor structures, released Thy-1 and DAF but not IgR protein into the aqueous phase. The selective incorporation of phospholipase-sensitive anchoring moieties into Thy-1 and DAF but not IgR translation products during *in vitro* translocation indicates that rough microsomes are able to support and regulate glypiation.**

A number of cell surface proteins are anchored via COOH-terminal glycosyl-inositolphospholipid (GPL) structures that can be cleaved by phosphatidylinositol-specific phospholipase C or D (PI-PLC or -PLD) (reviewed in refs. 1–4). These proteins are initially synthesized with COOH-terminal polypeptide extensions resembling conventional membrane-spanning domains and the GPL moieties are substituted for these extensions immediately posttranslationally via a transamidation reaction termed "glypiation." The cellular machinery responsible for the assembly of GPL-anchor structures and their transfer to nascent polypeptides is unknown.

Two such proteins are Thy-1 antigen, a surface constituent of unknown function on rodent thymocytes and neurons, and decay-accelerating factor (DAF), a surface regulatory protein that restricts autologous complement activation on human blood cells (5–9). The primary translation product of Thy-1 mRNA is a 162-amino acid polypeptide beginning in an NH_2-terminal leader sequence of 19 amino acids and terminating in a COOH-terminal hydrophobic extension 31 amino acids in length (10). In mature Thy-1, the GPL anchoring moiety is attached to Cys-112 (5, 11). DAF mRNA encodes a 347-amino acid protein beginning in an NH_2-terminal leader of 34 amino acids and ending in a COOH-terminal hydrophobic stretch of 23 amino acids (12, 13). The site of GPL-anchor attachment in DAF has not been identified. The importance of the COOH-terminal hydrophobic segment in signaling glypiation has been established, however, by fusing DAF's 3′ cDNA end sequence to upstream cDNA sequences of conventionally anchored CD8 (14) and of herpes simplex virus 1 glycoprotein D (15) and demonstrating that chimeric proteins expressed on transfectants are released by PI-PLC cleavage.

In the present study, GPL-anchor incorporation into Thy-1 and DAF translation products was attempted using a cell-free rabbit reticulocyte lysate system. It was found that upon addition of dog pancreas microsomal membranes, Thy-1 and DAF nascent polypeptides were glypiated. The results establish that all of the components for GPL-anchor processing are contained in this cell fraction.

EXPERIMENTAL PROCEDURES

Reagents. Plasmid pG-TM827, containing the Thy-1 coding region, was prepared by inserting the *Hpa* II–*Hpa* II segment of Thy-1 cDNA from pTM8 (16) into the *Sal* I site of pGEM-2 (Promega Biotec) (17). pB-DF13:2, containing the DAF coding region, was derived by joining DF13 and DF2 cDNAs (14) at their overlapping *Hin*dIII site in pBluescript (Stratagene). pG-IgR, containing the coding region for low molecular weight rabbit polymeric-immunoglobulin receptor (IgR) (ref. 18; E.S., unpublished work), was prepared in pGEM-2 similarly to the Thy-1 plasmid. Salt-washed and nuclease-treated dog pancreatic rough microsomes (DPM) isolated as described (19) were provided by B. Dobberstein (European Molecular Biology Laboratory, Heidelberg).

Transcription/Translation. pG-TM827, pB-DF13:2, and pG-IgR were linearized at their respective *Xho* I sites localized in vector sequence and T7 RNA polymerase transcription was carried out in the presence of 0.5 mM m^7GpppG (Pharmacia) (20). Translation was performed using a commercial rabbit reticulocyte lysate kit (New England Nuclear). Reaction mixtures (25 μl) were prepared as suggested by the manufacturer except for the substitution of 80 mM potassium acetate and the omission of magnesium acetate. One hundred to 200 ng of Thy-1, DAF, or IgR mRNA was translated for 60 min at 37°C with 100 μCi (3.7 MBq) of L-[^{35}S]methionine or L-[^{35}S]cysteine without or with 1 μl of DPM (40–60 A_{260} units/ml). After the 60-min incubation, 1 μl of 0.1 M unlabeled L-methionine or L-cysteine was added to provide a "chase," and the mixtures were incubated for 10 min to allow

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Abbreviations: GPL, glycosyl-inositolphospholipid; DAF, decay-accelerating factor; DPM, dog pancreatic rough microsome(s); IgR, polymeric-immunoglobulin receptor; Endo-F, endo-β-*N*-acetylglucosaminidase F; PI-PLC and PI-PLD, phosphatidylinositol-specific phospholipases C and D.

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completion of nascent radioactive polypeptides. Reactions were stopped by addition of 1 μl of RNase A (5 mg/ml) and further incubation for 10 min.

Immunoprecipitation and Sodium Dodecyl Sulfate/Polyacrylamide Gel Electrophoresis (SDS/PAGE)–Fluorography. Thy-1 and DAF translation products were immunoprecipitated with rabbit anti-mouse Thy-1 antiserum and pooled anti-DAF monoclonal antibodies, respectively, followed by protein A-Sepharose beads (21). Immunoprecipitated proteins were eluted from beads by boiling in 1% SDS and eluates were electrophoresed in SDS/polyacrylamide (10 or 12.5%) gels. Gels were dehydrated with dimethyl sulfoxide, impregnated with 2,5-diphenyloxazole and, after drying, exposed at −70°C on X-Omat AR film (Kodak).

Enzyme Digestions and Cell Surface Labeling. Proteinase K (Boehringer Mannheim) digestion of translation products was performed at 4°C for 90 min with enzyme at 0.6 mg/ml in the absence or presence of 0.6% Triton X-100. Proteolysis was stopped by addition of 1 mM phenylmethylsulfonyl fluoride.

Lactoperoxidase-catalyzed cell surface iodination was carried out as described by Hubbard and Cohn (22). Endo-β-*N*-acetylglucosaminidase F (Endo-F; Boehringer Mannheim) digestion of labeled cell surface proteins and of immunoprecipitated translation products was performed for 1 hr at 37°C with 0.4 units of Endo-F (100-μl final volume) mixed with lysates prepared from 10^7 surface-iodinated EL4-6.1 murine thymoma cells and with the products of four *in vitro* translations, respectively.

PI-PLC and -PLD Cleavage Analyses. The equivalent of four *in vitro* translations (100 μl) of Thy-1, DAF, or IgR mRNA performed in the presence of DPM was diluted to 1 ml with 0.1 M Tris·HCl, pH 7.4/0.5 M NaCl/10 mM EDTA. After centrifugation, pelleted membranes were extracted twice with 1% Triton X-114 in a final volume of 1 ml and the last detergent phase (≈80 μl) was adjusted to 400 μl with 0.1 M Tris·HCl, pH 7.4/0.25 M α-methyl D-mannoside/1 mM EDTA containing antipain (5 μg/ml), leupeptin (100 μg/ml), and pepstatin (50 μg/ml).

For PI-PLC digestions, aliquots (100 μl) of samples were incubated at 30°C for 60 min alternatively with 0.5 unit (1 unit = 1 μmol/min) of purified *Bacillus thuringiensis* PI-PLC (provided by M. Low, Columbia University, New York) (23), with 10 units of *Bacillus cereus* PLC (Sigma) (23), or with buffer alone, each in the presence of 1 mM EDTA to inhibit phosphatidylcholine-specific PLC activity (24). Following incubation, reaction mixtures were extracted with Triton X-114 and separated into aqueous and detergent phases. After three further extractions of each phase, the separated phases were precipitated with 30% (wt/vol) trichloroacetic acid, and precipitates were washed in ice-cold acetone and analyzed by SDS/PAGE–fluorography.

For PI-PLD digestions, the detergent phase of Triton X-114 extractions, adjusted to 400 μl as described above, was supplemented with 10 mM $CaCl_2$. Duplicate aliquots (100 μl) of samples were placed at 37°C, and one aliquot was treated with human serum (1 μl) (25) twice over a 1-hr period. Treated and untreated aliquots were subjected to SDS/PAGE–fluorography as above.

RESULTS

DPM Processing of Nascent Thy-1 Polypeptide. Upon *in vitro* translation in rabbit reticulocyte lysate, mRNA generated by transcription of Thy-1 cDNA (in pG-TM827) directed the synthesis of a single polypeptide (Fig. 1*A*, lane c). This polypeptide corresponded in size (≈16 kDa) to nonglycosylated Thy-1 precursor (17, 21). When DPM membranes were added to the translation mix, a 25-kDa product (Fig. 1*A*, lane a) was observed in addition to the 16-kDa species. This 25-kDa product migrated on SDS/PAGE identically to mature endogenous Thy-1 antigen immunoprecipitated from surface-^{125}I-labeled EL4-6.1 thymoma cells (Fig. 1*C*, compare lanes a and c). Neither the 16- nor the 25-kDa translation product was observed when Thy-1 mRNA was omitted from the translation reaction (Fig. 1*A*, lanes b and d). The amount of 25-kDa product synthesized in the system varied directly with the amounts of Thy-1 mRNA and of DPM added to the reaction mix (data not shown). To further verify the specificity of the 16- and 25-kDa Thy-1 products, control studies were performed in which mRNA generated by transcription of IgR cDNA was translated and the translation mix was treated with DPM in an identical manner. In contrast to the Thy-1 products, ≈50- and 78-kDa bands (see Fig. 2, lanes e and f) corresponding in size to nascent and translocated IgR polypeptides were generated.

Characterization of DPM-Processed Thy-1 Protein. To establish whether the 25-kDA Thy-1 product observed upon addition of DPM was translocated across microsomal membranes, its accessibility to degradation by proteases was assessed. Upon incubation with proteinase K (Fig. 1*B*, lanes

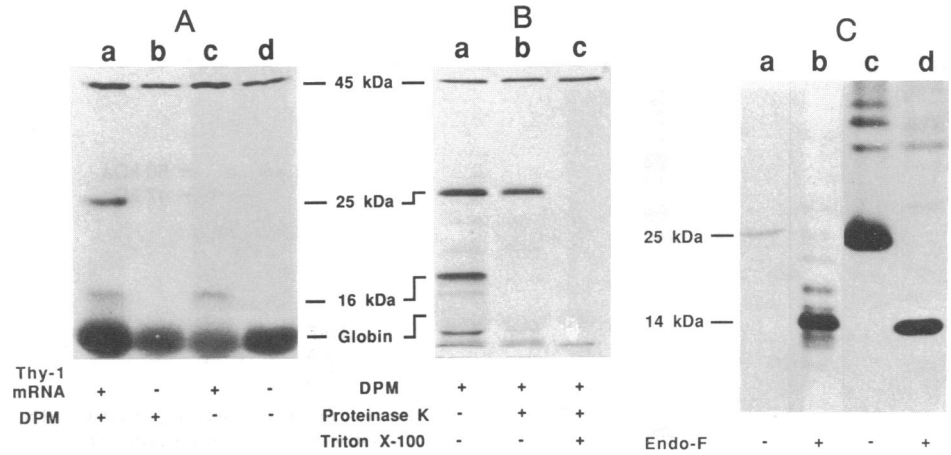

FIG. 1. *In vitro* translation of murine Thy-1 mRNA in rabbit reticulocyte lysate. (*A*) Translation in the presence (lane a) or absence (lane c) of DPM. Control mixtures lacked Thy-1 mRNA (lanes b and d). (*B*) Proteinase K protection of DPM-processed Thy-1 translation products. Lanes: a, untreated control; b, enzyme-treated; c, enzyme-treated in the presence of Triton X-100. (*C*) Endo-F treatment of immunoprecipitated DPM-processed Thy-1 translation products. Lanes: a, untreated control; b, Endo-F-treated; c, untreated surface-iodinated endogenous EL4-6.1 Thy-1; d, Endo-F-treated EL4-6.1 Thy-1. The apparent molecular masses were determined by comparison with the following standards: β-galactosidase (116 kDa); phosphorylase *b* (94 kDa); transferrin (78 kDa); albumin (66 kDa); ovalbumin (46 kDa); glyceraldehyde-3-phosphate dehydrogenase (34 kDa); α-chymotrypsinogen (25 kDa); cytochrome *c* (12.5 kDa).

a and b), >90% of the protein remained intact. Resistance to proteolysis was abolished, however, when the membranes were dispersed in 0.6% Triton X-100 prior to addition of the enzyme (Fig. 1*B*, lane c). Moreover, the nontranslocated 16-kDa precursor was entirely degraded even in the absence of detergent (Fig. 1*B*, lane b). To ascertain whether the 25-kDa translocated Thy-1 species was glycosylated, the two Thy-1 products were immunoprecipitated with anti-Thy-1 antibodies and the precipitated proteins were treated with Endo-F. This treatment converted the 25-kDa component into a 14-kDa species (Fig. 1*C*, lanes a and b) comparable to the product of Endo-F treatment of iodinated mature, cell surface Thy-1 (Fig. 1*C*, lanes c and d) (cf. also ref. 21). Moreover, similar (partially deglycosylated) intermediates of ≈21 and ≈17 kDa were observed in Endo-F digests of both Thy-1 preparations, arguing that the *in vitro* synthesized and endogenous proteins were identical.

PI-PLC Cleavage of Translocated Thy-1 Protein. Since partitioning of Thy-1 protein into the hydrophobic phase of Triton X-114 detergent depends upon the presence of an anchoring domain in the Thy-1 molecule (26, 27), Triton X-114 extraction in conjunction with PI-PLC digestion analyses was exploited to determine whether the *in vitro* synthesized 25-kDa Thy-1 product was glypiated. Upon Triton X-114 extraction of Thy-1 translation products generated in the presence of DPM, both the 25-kDa translocated product and the 16-kDa precursor partitioned into the detergent phase (Fig. 2, lanes c and d). After incubation of the mixture with purified *B. thuringiensis* PI-PLC, the 16-kDa Thy-1 precursor remained unaffected. In contrast, a portion of the 25-kDa Thy-1 product moved into the aqueous phase (Fig. 2, lanes a and b). Densitometry showed that 10% of the detergent-binding Thy-1 molecules were released. No change in SDS/PAGE mobility was associated with this transition. As a control, Triton X-114 extracts of *in vitro* synthesized, DPM-processed IgR were prepared. Similar to the findings with Thy-1, both the 50-kDa precursor and the 78-kDa translocated protein partitioned in the detergent phase. In contrast, however, PI-PLC treatment of the detergent phase induced no release of translocated protein into the aqueous phase (Fig. 2, lanes e and f), excluding an indirect effect of the enzyme on Triton X-114 partitioning of the translocated 25-kDa Thy-1 product.

PI-PLD Cleavage of Translocated Thy-1 Protein. To confirm that the 25-kDa Thy-1 protein was glypiated, a further set of digestion analyses were carried out with PI-PLD, a second

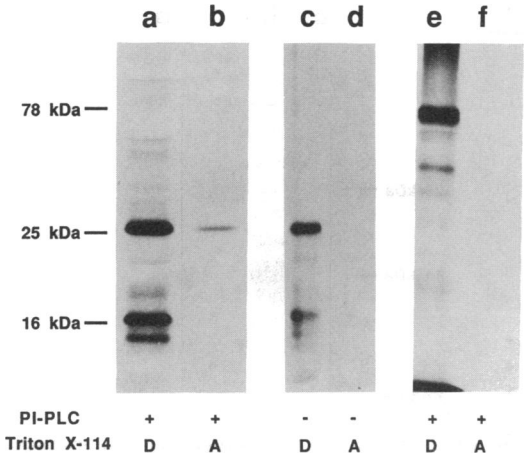

FIG. 2. PI-PLC digestion of *in vitro* DPM-processed Thy-1 and IgR translation products. Detergent (D) and aqueous (A) phases of Thy-1 were treated with enzyme (lanes a and b) or with buffer control (lanes c and d). Detergent (D) and aqueous (A) phases of IgR were treated with PI-PLC (lanes e and f).

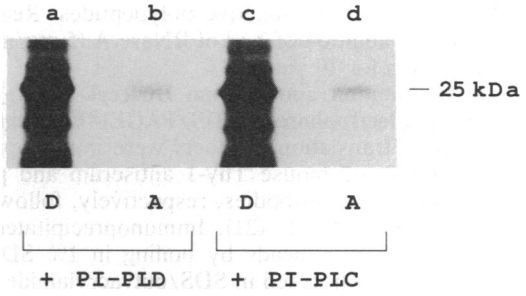

FIG. 3. PI-PLD and PI-PLC digestions of DPM-processed Thy-1 translation products. Detergent (D) and aqueous (A) phases of samples treated with PI-PLD (lanes a and b) or PI-PLC (lanes c and d) were analyzed.

enzyme specific for GPL-anchor structures. Triton X-114 extracts of Thy-1 products generated in the presence of DPM were incubated with PI-PLD, and the hydrophobic and aqueous phases were examined by SDS/PAGE. As shown in Fig. 3, similar to findings with PI-PLC, serum PLD activity converted an identical proportion of the 25-kDa protein into a water-soluble Thy-1 form. This PI-PLD-digested derivative was indistinguishable on SDS/PAGE from the product of PI-PLC digestion.

DPM Processing of Nascent DAF Polypeptide and PI-PLC Cleavage of the Translocated Protein. To ascertain whether *in vitro* glypiation is applicable to other GPL-anchored proteins and whether the extent of PI-PLC release is a function of the mRNA used or is a property of the translation system, a similar set of experiments was conducted with DAF. Upon incubation of DAF cDNA (pB-DF13:2) in the transcription/translation system in the absence of DPM membranes (data not shown), a 46-kDa polypeptide was synthesized. Addition of DPM to the reaction mix converted most of this species into a 47-kDa component (Fig. 4*A*, lane b). This component was immunoprecipitated by anti-DAF monoclonal antibody IA10 (Fig. 4*A*, lane c) and its apparent molecular mass closely approximated that of the HeLa cell DAF precursor observed after 10 min of pulse-labeling with [^{35}S]cysteine (data not shown). Some of the increased size of this component was attributable to N-glycosylation, since Endo-F treatment reduced its apparent molecular mass back to 45 kDa (Fig. 4*B*, lanes a and b). Upon Triton X-114 extraction of the mem-

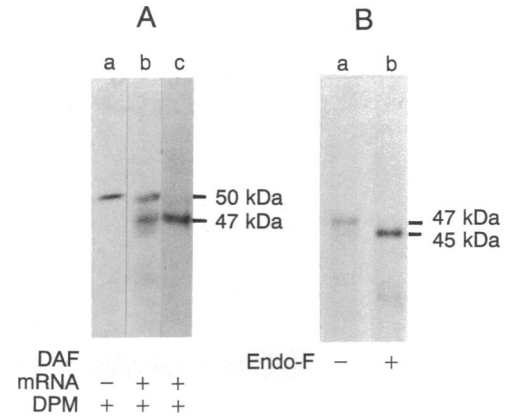

FIG. 4. *In vitro* translation of DAF mRNA and characterization of the DPM-processed translation product. (*A*) Translation in the presence of DPM without (lane a) or with (lane b) DAF mRNA. One-fifth of each translation mixture was loaded directly on the gel. Four-fifths of the DAF mRNA translation mix was taken for immunoprecipitation with anti-DAF monoclonal antibody IA10 (lane c). Autoradiographic exposure time was 2 days (lanes a and b) or ≈2 weeks (lane c). (*B*) Buffer control (lane a) and Endo-F (lane b) treatment of immunoprecipitated DPM-processed DAF translation products.

brane fraction, both the 47-kDa (processed) and 46-kDa (unprocessed) DAF components were recovered in the hydrophobic phase (Fig. 5, lane a). In a fashion parallel to that with Thy-1 and IgR, the hydrophobic phase was incubated with *B. thuringiensis* PI-PLC. A 44-kDa polypeptide appeared in the aqueous phase (Fig. 5, lane d), indicating that the 47-kDa translocated DAF molecule contained a GPL anchor. Similar to findings with Thy-1, partial conversion was observed.

DISCUSSION

The results of the present study demonstrate that, in the presence of DPM, *in vitro* synthesized Thy-1 and DAF translation products not only are N-glycosylated and released from their NH_2-terminal signal sequences but, additionally, are glypiated. This processing is specific to sequences in nascent polypeptides of GPL-anchored proteins since, under identical conditions, nascent polypeptides of a conventionally anchored protein, IgR, are not glypiated.

The 16-kDa Thy-1 polypeptide generated upon initial translation of *in vitro* transcribed Thy-1 mRNA corresponds in size to the Thy-1 precursor predicted from the 486-nucleotide open reading frame in Thy-1 cDNA. The 25-kDa size of the Thy-1 product that emerged upon addition of DPM is consistent with that expected for processed Thy-1 protein devoid of its NH_2-terminal signal and COOH-terminal extension sequences and containing high-mannose N-linked oligosaccharides. The inaccessibility of this product to proteinase K digestion indicated that it was positioned intraluminally. In agreement with Thy-1 cDNA sequence, which predicts no downstream charged residues (as found in conventionally anchored proteins), the lack of enzyme effect on the apparent size of the molecule indicated that no cytoplasmic region was exposed extraluminally. Endo-F digestion decreased the apparent molecular mass of the 25-kDa product to 14 kDa. The difference in mass (2 kDa) between this 14-kDa deglycosylated derivative and the 16-kDa *in vitro* synthesized Thy-1 precursor corresponds to the predicted size of the NH_2-terminal signal and COOH-terminal extension sequences expected in precursor but not mature Thy-1 protein.

The lack of change in apparent molecular mass of the 25-kDa Thy-1 product following PI-PLC cleavage is consistent with previous observations concerning endogenous cell-associated Thy-1 protein (21, 27). The finding that, in contrast to the 25-kDa Thy-1 species, the 16-kDa precursor was not cleaved by PI-PLC and remained in the detergent phase is consistent with the presence in the 16-kDa molecule of both

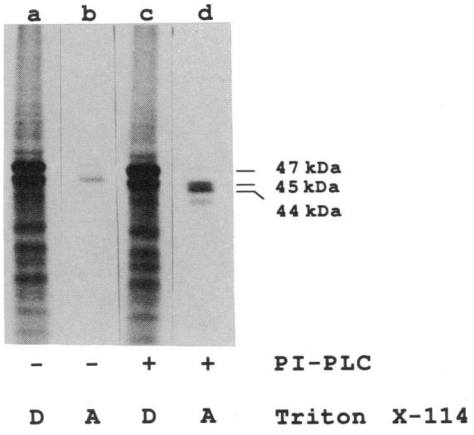

FIG. 5. PI-PLC digestion of DPM-processed DAF translation products. Detergent (D) and aqueous (A) phases of buffer-treated control sample (lanes a and b) and of PI-PLC-treated sample (lanes c and d) were analyzed.

NH_2-terminal leader and COOH-terminal hydrophobic detergent-binding sequences (and the absence in the PLC preparation of contaminating protease activity). Since the PI-PLD derivative of glypiated Thy-1 should differ from the PI-PLC derivative only by the absence of phosphate on the inositol ring, the finding that the two derivatives exhibited identical SDS/PAGE mobility provides supporting evidence for the presence of a GPL anchoring structure in the *in vitro* synthesized 25-kDa translocated Thy-1 product.

The observation that DAF and Thy-1 precursor polypeptides were glypiated in an identical fashion upon addition of DPM indicates that glypiation *in vitro* is not unique to a particular protein but rather has general applicability. The 46-kDa DAF precursor that appeared upon initial translation of *in vitro* transcribed DAF mRNA corresponds in size (347 amino acids) to the nonglycosylated DAF precursor predicted from sequence analyses of DAF cDNA (13). The increase in molecular mass (1 kDa) associated with processing of this precursor by DPM is consistent with removal of the 17-amino acid signal peptide and addition of the single high-mannose N-linked glycan, which is expected at amino acid 63 (assuming that COOH-terminal extension cleavage and GPL-anchor addition have equal but opposite effects). The DPM product is comparable in apparent size to the DAF precursor identified in biosynthetic labeling studies of HeLa (see *Results*) and HL-60 cells, although its correspondence to the 43- or 46-kDa precursor identified in the latter cell type has not been established. The detection, following Triton X-114 extraction, of some 46-kDa polypeptide in the hydrophobic phase along with the 47-kDa DPM product presumably resulted from the presence of NH_2-terminal leader and COOH-terminal extension in 46-kDa DAF precursor, as observed with Thy-1 and IgR translation products. Unlike findings with Thy-1, PI-PLC digestion of the 47-kDa translocated DAF product resulted in a significant decrease in apparent molecular mass. This decrease (3 kDa) is consistent with that reported (9) to occur following PI-PLC cleavage of endogenous human erythrocyte DAF. Similar to findings in the present study with Thy-1, however, a nearly identical proportion of the 47-kDa DAF protein remained uncleaved.

The observation that portions of the translocated 25-kDa Thy-1 and 47-kDa DAF translation products resisted PI-PLC (and PI-PLD) cleavage and remained in the detergent phase is unexplained. The same results with Thy-1 were obtained in 20 different experiments using different batches of *B. thuringiensis* PI-PLC. No greater release was observed with *B. cereus* PI-PLC. One possibility is that the residual uncleaved materials could represent nonglypiated Thy-1 and DAF precursors. This could occur if glypiation efficiency *in vitro* were diminished, leaving some molecules unprocessed. It has been reported that in Thy-1-deficient lymphoma mutants, aberrant Thy-1 molecules that are synthesized retain their COOH-terminal hydrophobic polypeptide extensions and partition in the detergent phase of Triton X-114 (27). Alternatively, incomplete glypiation could occur if the amounts of preformed GPL-anchor units or of GPL-anchor precursors in the DPM preparation employed were limiting (28–31). However, our attempts to augment glypiation by adding potential precursor substrates to incubation mixtures were unsuccessful. A second possibility is that GPL-anchor structures in some of the 25-kDa Thy-1 and 47-kDa DAF glypiated molecules could be chemically modified—e.g., palmitoylated, as described for human erythrocyte acetylcholinesterase (32) and DAF (33)—rendering them resistant to PI-PLC cleavage. This notion is supported by reports (6, 27, 28) that similar to these erythrocyte proteins, only 30–50% of the endogenous Thy-1 molecules in cells are released following PI-PLC digestion *in situ*.

The *in vitro* incorporation of GPL anchors into murine and human membrane proteins through the use of DPM mem-

branes supports the notion that this processing occurs in the rough endoplasmic reticulum, as suggested initially by biosynthetic labeling studies of variant surface glycoproteins (30) and subsequently by studies of DAF (9) and Thy-1 (28), and establishes that glypiation of proteins occurs via a posttranslational pathway that is conserved across species. The availability of a cell-free translation system containing all the ingredients necessary for *in vitro* glypiation should provide an experimental model to define sequences in precursor proteins that regulate glypiation as well as to identify and characterize the enzyme activities involved in the glypiation pathway.

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1. Cross, G. A. M. (1987) *Cell* **48,** 179–181.
2. Low, M. G. (1987) *Biochem. J.* **244,** 1–13.
3. Low, M. G. & Saltiel, A. R. (1988) *Science* **239,** 268–275.
4. Ferguson, M. A. J. & Williams, A. F. (1988) *Annu. Rev. Biochem.* **57,** 285–320.
5. Tse, A. G. D., Barclay, A. N., Watts, A. & Williams, A. F. (1985) *Science* **230,** 1003–1008.
6. Low, M. G. & Kincade, P. W. (1985) *Nature (London)* **318,** 62–64.
7. Nicholson-Weller, A., Burge, J., Fearon, D. T., Weller, P. F. & Austen, K. F. (1982) *J. Immunol.* **129,** 184–189.
8. Davitz, M. A., Low, M. G. & Nussenzweig, V. (1986) *J. Exp. Med.* **163,** 1150–1161.
9. Medof, M. E., Walter, E. I., Roberts, W. L., Haas, R. & Rosenberry, T. L. (1982) *Biochemistry* **25,** 6740–6746.
10. Seki, T., Moriuchi, T., Chang, H.-C., Denome, R. & Silver, J. (1985) *Nature (London)* **313,** 485–487.
11. Homans, S. W., Ferguson, M. A. J., Dwek, R. A., Rademacher, T. W., Anand, R. & Williams, A. F. (1988) *Nature (London)* **333,** 269–272.
12. Caras, I. W., Davitz, M. A., Rhee, L., Weddel, G., Martin, D. W. & Nussenzweig, V. (1987) *Nature (London)* **325,** 545–549.
13. Medof, M. E., Lublin, D. M., Holers, V. M., Ayers, D. J., Getty, R. R., Leykam, J. F., Atkinson, J. P. & Tykocinski, M. L. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2007–2011.
14. Tykocinski, M. L., Shu, H.-K., Ayers, D. J., Walter, E. I., Getty, R. R., Groger, R. K., Hauer, C. A. & Medof, M. E. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3555–3559.
15. Caras, I. W., Weddel, G. N., Davitz, M. A., Nussenzweig, V. & Martin, D. W. (1987) *Science* **238,** 1280–1283.
16. Hedrick, S. M., Cohen, D. I., Nielsen, E. A. & Davis, M. M. (1984) *Nature (London)* **308,** 149–153.
17. Melton, D., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12,** 7035–7056.
18. Kuhn, L. & Kraehenbuhl, J. P. (1981) *J. Biol. Chem.* **256,** 12490–12495.
19. Blobel, G. & Dobberstein, B. J. (1975) *Cell Biol.* **67,** 852–862.
20. Connolly, T. & Gilmore, R. J. (1986) *Cell Biol.* **103,** 2253–2261.
21. Luescher, B. & Bron, C. (1985) *J. Immunol.* **134,** 1084–1089.
22. Hubbard, A. L. & Cohn, A. Z. (1975) *J. Cell Biol.* **64,** 438–460.
23. Ikezawa, H. & Tagnini, R. (1985) *Methods Enzymol.* **7,** 731–741.
24. Ikezawa, J., Yamanegi, M., Taguchi, R., Miyashita, T. & Ohyabu, T. (1976) *Biochim. Biophys. Acta* **450,** 154–164.
25. Davitz, M. A., Hereld, D., Shak, S., Krakow, J., Englund, P. T. & Nussenzweig, V. (1987) *Science* **238,** 81–84.
26. Bordier, C. J. (1981) *Biol. Chem.* **256,** 1604–1607.
27. Conzelmann, A., Spiazzi, A., Hyman, R. & Bron, C. (1986) *EMBO J.* **5,** 3291–3296.
28. Conzelmann, A., Spiazzi, A. & Bron, C. (1986) *Biochem. J.* **246,** 605–610.
29. Davitz, M. A., Herold, D., Shak, S., Krakow, J. L., Englund, P. T. & Nussenzweig, V. (1987) *Science* **238,** 81–84.
30. Bangs, J. D., Herold, D., Krakow, J. L., Hart, G. W. & Englund, P. T. (1985) *Proc. Natl. Acad. Sci. USA* **32,** 3207–3211.
31. Ferguson, M. A. J., Duvzenko, M., Lamont, G. S., Overath, P. & Cross, G. A. M. (1986) *J. Biol. Chem.* **261,** 356–362.
32. Roberts, W. L., Kim, B. H. & Rosenberry, T. L. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7817–7821.
33. Walter, E. I., Roberts, W. L., Rosenberry, T. L. & Medof, M. E. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46,** 772 (abstr.).