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REVIEW ARTICLE

Biochemistry of the glycosyl-phosphatidylinositol membrane protein anchors

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1. Introduction

(a) Anchoring of membrane proteins. It is generally accepted that the predominant structural feature of most biological membranes is a lipid bilayer with proteins either bound to the polar surface or penetrating the bilayer interior to some extent. The net force which holds the latter group of proteins (i.e., integral membrane proteins) in the membrane is the balance of interactions between relatively hydrophobic and polar polypeptide domains with the hydrophobic core of the lipid bilayer and the surrounding medium, respectively (Capaldi, 1982; Singer & Nicolson, 1972). These forces are not only important for attaching these proteins to the membrane, but also permit the protein to adopt its correct conformation and transmembrane orientation so that its function (i.e. solute transport, signal transduction, adhesion, etc.) can be performed correctly.

Membrane proteins vary markedly in the nature and extent of the interactions with the lipid bilayer, and these are summarized in Fig. 1. Many proteins are clearly embedded to a great extent in the lipid bilayer and it is probable that this reflects their functional properties in some way, e.g. transport of ions and other polar molecules across the membrane, etc. (Fig. 1*a*). However, for many other membrane proteins this intimate association with the bilayer is less apparent (Figs. 1*b*–1*f*). Some of these proteins contain a short, hydrophobic stretch of amino acids (commonly referred to as a 'transmembrane domain'), which is believed to cross the bilayer only once, and larger, relatively hydrophilic domains on one or both sides of the cell membrane. The orientation of these proteins and the relative sizes of the two hydrophilic domains can vary substantially. Thus, either the *N*- or the *C*-terminus can be directed towards the cytoplasmic surface of the membrane (Fig. 1*b*). In some proteins, there is only a single hydrophilic region in the polypeptide, and in such

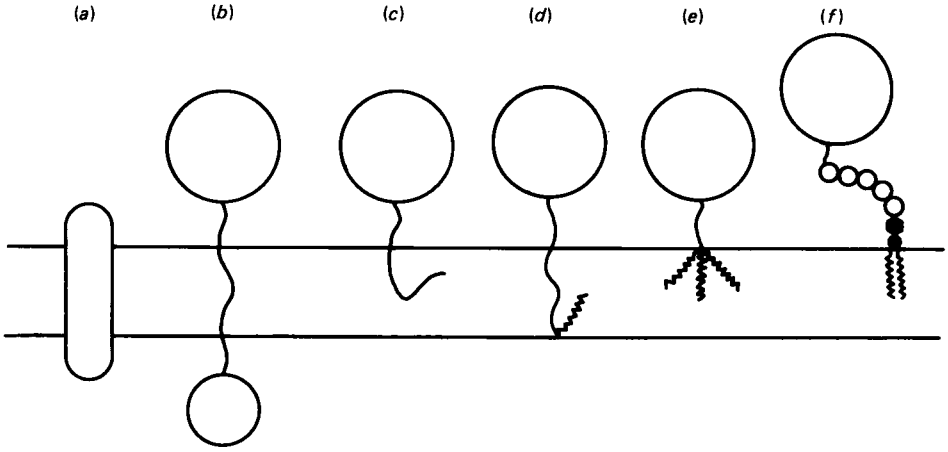

Fig. 1. Anchoring of proteins to membranes

In this Figure the various mechanisms of membrane protein anchoring are illustrated. In (*a*) a substantial proportion of the protein is buried within the bilayer (e.g. rhodopsin; Capaldi, 1982), whereas in (*b*)–(*f*) a small proportion or none of the protein interacts with the bilayer. In (*b*) two hydrophilic domains are connected by a hydrophobic transmembrane domain (e.g. low-density lipoprotein receptor; Sudhof *et al.*, 1985). In (*c*) only one hydrophilic domain is present and consequently the orientation within the bilayer of the hydrophobic domain is less certain (e.g. cytochrome b_5; Gogol & Engelman, 1984; Markello *et al.*, 1985). (*d*)–(*f*) represent proteins with covalently attached lipid; in (*d*) a myristic acid is amide-linked to the α-amino group on the *N*-terminal glycine residue (e.g. NADH:cytochrome b_5 reductase; Ozols *et al.*, 1984); in (*e*) an amide-linked fatty acid and a thioether-linked diacylglycerol are attached to the α-amino and thiol groups of the *N*-terminal cysteine, respectively (e.g. *E. coli* lipoprotein and *B. licheniformis* penicillinase; Hantke & Braun, 1973; Wu & Tokunaga, 1986); in (*f*) a phosphatidylinositol molecule is covalently attached through an intervening glycan structure to the *C*-terminal amino acid of the protein. In this Figure the relative sizes and orientation of the domains are not intended to be accurately represented.

Abbreviations used: AChE, acetylcholinesterase; APase, alkaline phosphatase; VSG, variant surface glycoprotein; DAF, decay accelerating factor; TAP, T-cell activating protein; CRD, cross-reacting determinant; PNH, paroxysmal nocturnal haemoglobinuria; PI-PLC, phosphatidylinositol-specific phospholipase C; N-CAM, neural cell adhesion molecule.

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cases the hydrophobic domain would not necessarily cross the membrane and could assume a relatively variable orientation within the core of the bilayer (Fig. 1*c*).

Until recently, it was believed that the important interactions between proteins and lipids which give the membrane its structural integrity were all non-covalent in nature (Singer & Nicolson, 1972). However, it has been discovered in the last decade that many proteins in both prokaryotic and eukaryotic cell membranes contain covalently attached lipid. Fatty acid has been shown to be covalently attached to many proteins by amide, thioester or *O*-acyl bonds. The attached lipid may be responsible for anchoring of the protein, as is the case with some prokaryotic membrane proteins (Fig. 1*e*), but in most cases the attached lipid does not seem to serve this function (Fig. 1*d*; for reviews see Magee & Schlesinger, 1982; Schlesinger, 1981; Schmidt, 1983). In the final group of proteins (Fig. 1*f*), i.e. those containing a covalently attached glycosylated phosphatidylinositol moiety, there is considerable evidence that the attached lipid is responsible for membrane anchoring. However, the observation that this relatively complex lipid is attached to an extremely diverse group of proteins has suggested that it may serve other functions in addition to attachment to the membrane (Low *et al.*, 1986*a*). The purpose of this Review is to discuss the biochemistry and the functional significance of these novel biological structures.

(*b*) Proteins utilizing glycosyl-phosphatidylinositol as a membrane anchor. The proteins which are believed to have a covalently attached glycosylated phosphatidylinositol anchor are listed in Table 1. Examination of this Table reveals several interesting features. (i) This type of membrane anchor is widely distributed between different organisms. Thus, it has been demonstrated in organisms as diverse as protozoan parasites, the electric ray *Torpedo* and several mammalian species. Although this type of membrane anchor has not yet been observed in higher plants, it seems very likely that it is widely distributed in eukaryotic cells. There are currently no

Table 1. Proteins believed to be anchored to the membrane by covalent attachment to glycosyl-phosphatidylinositol

Protein	Source and function*	References
Alkaline phosphatase	Mammalian tissues; enzyme	Ikezawa *et al.* (1976), Low & Finean (1977*b*), Low & Zilversmit (1980), Low *et al.* (1987), Taguchi & Ikezawa (1978), Taguchi *et al.* (1980)
5′-Nucleotidase	Mammalian tissues; enzyme	Low & Finean (1978), Low *et al.* (1980), Panagia *et al.* (1981*a*), Shukla *et al.* (1980)
Acetylcholinesterase	*Torpedo* electric organ and mammalian blood cells; enzyme	Futerman *et al.* (1983, 1985*a*,*b*,*c*), Low & Finean (1977*a*) Low *et al.* (1987), Roberts & Rosenberry (1986*b*), Taguchi *et al.* (1984), Shukla (1986)
Alkaline phosphodiesterase I	Rat tissues; enzyme	Nakabayashi & Ikezawa (1984, 1986)
Variant surface glycoprotein	*Trypanosoma brucei*; protective coat	Ferguson *et al.* (1985*a*,*b*, 1987)
Thy-1	Mammalian brain and T lymphocytes; antigen	Low & Kincade (1985), Tse *et al.* (1985), Tung *et al.* (1987), Fatemi & Tartakoff (1986)
Trehalase	Rabbit tissues; enzyme	Takesue *et al.* (1986)
Decay accelerating factor	Human blood and HeLa cells; complement regulatory protein	Davitz *et al.* (1986, 1987), Medof *et al.* (1986)
p63 proteinase	*Leishmania major*; enzyme	Bordier *et al.* (1986), Etges *et al.* (1986*a*,*b*)
RT-6	Rat lymphocytes; antigen	Koch *et al.* (1986)
Qa	Mouse T lymphocytes; antigen	Stiernberg *et al.* (1987)
ThB	Mouse lymphocytes antigen	Stiernberg *et al.* (1987)
T-cell activating protein	Mouse T lymphocytes; antigen	Reiser *et al.* (1986)
N-CAM$_{120}$	Rat, mouse and chicken brain; cell–cell interactions	He *et al.* (1986), Sadoul *et al.* (1986), Hemperly *et al.* (1986)
Heparan sulphate proteoglycan	Rat liver; cell–cell and cell–matrix interactions	M. Ishihara *et al.* (1987)

* Although the biochemical nature (i.e. enzymes, antigens, etc.) of these proteins allows them to be identified and characterized, the biological functions of many of them are unknown.

reports of its occurrence in prokaryotic cells. (ii) Evidence for membrane protein anchoring by phosphatidylinositol has been found in every mammalian tissue so far examined (see Shukla, 1982, for examples). (iii) These proteins are obviously functionally diverse even though in many cases their precise roles are unknown. Thus, they range from several different types of enzyme (e.g. phosphomonoesterase, glycosidase, proteinase, etc.) to the variant surface glycoprotein (VSG) whose main function appears to be to form a relatively inert protective coat on a protozoal parasite.

The great diversity in distribution and function of these proteins is in sharp contrast with the fact that they are all mainly located on the cell surface. However, this observation may simply reflect the fact that anchoring of proteins at the cell surface is, in general, easier to study than at intracellular locations. Consequently, the involvement of phosphatidylinositol in the anchoring of, for instance, cytoplasmically oriented proteins has received little attention. Two proteins located inside the cell have been reported to have covalently attached phosphoinositide, i.e., myelin basic protein (Yang *et al.*, 1986) and styrene oxide hydrolase (Griffin & Palakotedy, 1985), but these appear to involve direct linkage of a phosphorylated phosphatidylinositol to serine residues. Preliminary studies suggest that the 195 kDa merozoite surface protein and the transferrin receptor of *Plasmodium falciparum* (Haldar *et al.*, 1985, 1986), VSG in *Trypanosoma equiperdum* (Duvillier *et al.*, 1983), opsin in frog retina (Fliesler & Anderson, 1986), an adenylate cyclase stimulating protein in rat heart (Panagia *et al.*, 1981*b*), and a chicken liver lysosomal ATPase (Maeda *et al.*, 1986) may also contain covalently attached phosphatidylinositol.

The glycosyl-phosphatidylinositol anchor structure that has been proposed for the proteins listed in Table 1 is unlikely to be detected unless a particular protein is rigorously examined for its presence. Therefore, it seems probable that many other proteins use this anchoring mechanism also. The foregoing discussion indicates that it is difficult to predict on functional grounds what proteins these might be. However, the possibility of phosphatidylinositol-anchoring should be seriously considered for any cell surface protein having an anchoring domain at the *C*-terminus (see below), that has not directly been shown to include a hydrophobic transmembrane polypeptide sequence.

2. Release of membrane proteins by phosphatidylinositol-specific phospholipases C

(*a*) Background. The first report of the release of a membrane protein by phosphatidylinositol-specific phospholipase C (PI-PLC) was made during investigations of the action of anthrax toxin. A soluble factor in crude toxin preparations from *Bacillus anthracis* caused a rapid and pronounced elevation of serum APase when injected into experimental animals (Slein & Logan, 1960). This effect could also be produced by culture filtrates derived from various non-pathogenic *Bacillus* species and thus, this factor seemed unlikely to be the lethal toxin of anthrax (Slein & Logan, 1962). However, it was observed that a similar effect could be produced *in vitro* by incubating tissue slices with the culture filtrates and assaying the suspending media for released APase. The factor responsible for these effects was partially purified from *B. cereus* and suggested to be a phospholipase C with a relative specificity for phosphatidylinositol (Slein & Logan, 1963, 1965). This striking and unusual phenomenon was essentially ignored for about 10 years. It was subsequently confirmed when highly purified PI-PLC from *B. cereus* (Ikezawa *et al.*, 1976), *Staphylococcus aureus* (Low & Finean, 1977*b*), *Clostridium novyi* (Taguchi & Ikezawa, 1978) and *B. thuringiensis* (Taguchi *et al.*, 1980) became available and were shown to release APase. Since that time, many other membrane proteins have been shown to be releasable (or sensitive to hydrolysis) by bacterial PI-PLC (or the phospholipase C from *Trypanosoma brucei*; see section 5*a*) and these are listed in Table 1.

(*b*) Characteristics of the release process. Extensive studies with a wide range of proteins has revealed several important features of the release process. The phenomenon is quite specific, in that many other membrane components are not released by PI-PLC. This has been determined by fairly general techniques such as measurement of membrane phospholipid release (Low & Finean, 1978) or the number of plant lectin binding sites on the cell surface (J. Stiernberg, M. G. Low, L. Flaherty & P. W. Kincade, unpublished work) but also by looking at the amount of release of particular proteins (see the references in Table 1 for details). This indicates that the releasing effects of PI-PLC are unlikely to be due to a disruptive effect on membrane structure such as microvesiculation, which is often produced by treatment with nonspecific phospholipases C. The molecular masses of APase, 5′-nucleotidase and AChE released from membranes by PI-PLC, when analysed by gel filtration in the absence of detergents, were observed to be relatively low (dimers of approx. 150 kDa) and very similar to that of the native protein solubilized by more conventional techniques (Low & Finean, 1977*a*, 1978; Low & Zilversmit, 1980). Furthermore, determinations of the hydrophobicity of APase (Low & Zilversmit, 1980; Malik & Low, 1986), AChE (Futerman *et al.*, 1985*c*), 5′-nucleotidase (Prasad & Low, 1987), p63 protease (Bordier *et al.*, 1986; Etges *et al.*, 1986*a*,*b*) and RT-6.2 (Koch *et al.*, 1986) by their ability to bind to phospholipid liposomes or partition into Triton X-114 indicated that treatment with PI-PLC had removed the hydrophobic anchoring domain from these proteins. This hydrophobic domain was strongly associated with the protein, since sensitivity to PI-PLC was retained during solubilization by butanol or detergents and, in the case of AChE and p63 protease, through extensive purification procedures. These data support the concept that the protein molecules are directly associated with the polar head group of a phosphatidylinositol or related molecule on an individual basis and not indirectly as part of a complex containing several different proteins. APase and AChE will not reassociate with phosphatidylinositol after they are released from the membrane by PI-PLC (Low & Zilversmit, 1980; Futerman *et al.*, 1985*c*). This observation, in combination with the fact that these proteins cannot be released from the membrane by manipulation of the ionic environment, indicates that the interaction between phosphatidylinositol and the protein is irreversible and non-ionic in character.

Such evidence led to the conclusion that APase and AChE are anchored in the membrane by a covalent interaction with the polar head group of phosphatidyl-

inositol or a related molecule (Low & Zilversmit, 1980; Futerman *et al.*, 1985*c*). Although it could be argued that other interpretations of these data were (and still are) possible, this proposition has been vindicated by subsequent chemical analyses of these and other proteins. This point is of some importance since the conclusion that many of the proteins listed in Table 1 are anchored in the membrane by this mechanism is based on release by PI-PLC.

(*c*) Resistance to release. Although release of a protein from the membrane by PI-PLC is now regarded as reasonably convincing evidence for the involvement of phosphatidylinositol in its membrane anchoring, the converse is not true. It is becoming increasingly evident that some proteins thought to be anchored by this mechanism can, in particular species or cell types, be relatively resistant to PI-PLC release. Thus, 5′-nucleotidase requires approximately 100 times more PI-PLC for release than does APase (Low & Finean, 1978; Shukla *et al.*, 1980; Prasad & Low, 1987). Brain AChE from several mammalian species is not released by PI-PLC (Futerman *et al.*, 1985*b*). Only 5–10% of mouse and human erythrocyte AChE is released by *S. aureus* PI-PLC, whereas 90–100% of this enzyme in pig, ox and rat erythrocytes is releasable (Futerman *et al.*, 1985*b*; Low & Finean, 1977*a*). Similarly, 10–15% of DAF is releasable from human erythrocytes by *S. aureus* PI-PLC compared with 60–80% from leukocytes (Davitz *et al.*, 1986; Medof *et al.*, 1986). Resistant fractions of APase, Qa-2 and Thy-1 have also been observed which seem to vary markedly depending on tissue source or cell type (M. G. Low, J. Stiernberg & P. W. Kincade, unpublished work).

The basis for PI-PLC resistance is unknown. It could be due to masking or interaction with other membrane components, effects of membrane lipid composition of different membranes on PI-PLC activity, or utilization by some of these proteins of an alternative method of anchoring such as a transmembrane polypeptide (see section 4*a*) or binding of a released protein by a cell surface glycosyl-inositol phosphate 'receptor' (see section 6*b*). In the case of 5′-nucleotidase (and some other phosphatidylinositol-anchored proteins), a transmembrane disposition and interaction with the cytoskeleton has been proposed, but detailed studies on the membrane topography of 5′-nucleotidase have not supported this proposition (Baron *et al.*, 1986; Luzio *et al.*, 1987; Low, 1987). By contrast, recent evidence demonstrates that human erythrocyte AChE and DAF are anchored by glycosyl-phosphatidylinositol structures similar to those found in other proteins but modified in some way that renders them resistant to PI-PLC (Medof *et al.*, 1986; W. L. Roberts, B. H. Kim & T. L. Rosenberry, personal communication). It is possible that related modifications of the phosphatidylinositol anchor affect the sensitivity of other proteins to PI-PLC.

(*d*) Specificity. The exact specificities of the PI-PLCs are not known. So far, the *S. aureus* enzyme has been shown to hydrolyse phosphatidylinositol, lysophosphatidylinositol and various glycosylated phosphoinositides (Shukla, 1982; Saltiel & Cuatrecasas, 1986; Saltiel *et al.*, 1986; Turco *et al.*, 1987) but not under comparable assay conditions the major membrane phospholipids or phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate (Ferguson *et al.*, 1985*b*; Shukla, 1986). However, the substrate preference of PI-PLC from mammalian sources is known to be markedly sensitive to assay conditions (see Low *et al.*, 1986*b*, for references) and it is possible that bacterial PI-PLC catalyses hydrolysis of other lipids under conditions not yet tested. Furthermore, since synthetic phosphatidylinositols with particular fatty acid compositions or substituents on the inositol ring are not available, all specificity studies have necessarily been done with a strictly limited, and possibly inappropriate, set of naturally occurring substrates. It is not yet known whether the enzymes produced by the other bacteria have different specificities, since comparative studies have not been reported. The PI-PLC from *Trypanosoma brucei*, however, does seem to be different in its specificity compared with the bacterial enzymes, since it has relatively low activity against phosphatidylinositol compared with its activity against the lipid anchor of VSG (see section 5*a*). Until the specificity of these enzymes is further defined, structural assignments based on the action of PI-PLC must be regarded as tentative.

3. Structural studies of the membrane anchoring domain

The structures of the membrane anchoring domains of most of the proteins listed in Table 1 have not been directly studied. However, direct chemical evidence for much of the anchoring structure for VSG is available and supported by less complete structural studies with several other proteins. It now seems likely that all the other proteins anchored by this type of structure share in common with VSG a similar anchoring structure (Fig. 2). The purpose of this section is to outline the extent of our knowledge of these structures and describe in some detail the various approaches that have been utilized.

(*a*) Location of the membrane anchoring domain. The location of the membrane anchoring domain was first demonstrated for APase in studies where the hydrophobic anchor was removed from the intestinal enzyme by papain (Colbeau & Maroux, 1978). This form of the protein had the same *N*-terminal dipeptide as the intact protein solubilized by detergents, indicating a *C*-terminal location of the anchoring domain. Since then, similar solubilization studies have been done on human placental APase with bromelain (Jemmerson *et al.*, 1984) and subtilisin (Abu-Hasan & Sutcliffe, 1984), on human erythrocyte AChE with papain (Dutta-Choudhury & Rosenberry, 1984) and on *Torpedo* AChE with proteinase K (Futerman *et al.*, 1985*c*; Stieger & Brodbeck, 1985). Cleavage by the proteinase seems to be close to the *C*-terminus, since it has little or no effect on enzyme activity and the change in M_r is so small (less than 2 kDa) that it can barely be distinguished by SDS/polyacrylamide-gel electrophoresis. In the case of the human erythrocyte AChE, this ability to remove the *C*-terminus by selective proteolysis has facilitated the isolation and analysis of the anchoring domain (see below) and it is likely that such an approach will be successfully applied to the membrane anchors of other proteins.

(*b*) Inositol content of the purified proteins. Although in most cases the membrane anchoring domain probably comprises less than 5% of the total mass of the protein,

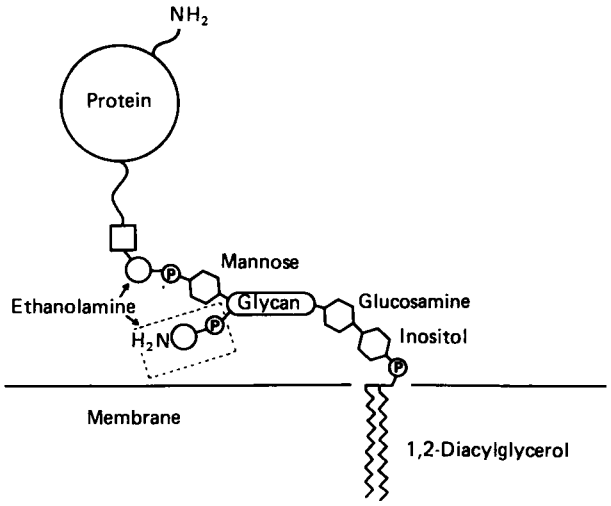

Fig. 2. Glycosyl-phosphatidylinositol structure proposed to anchor proteins to the membrane

The arrangement of the components in this pictorial representation of the anchoring structure is based on work with VSG, although composition and specific degradation studies of the anchors in other proteins are consistent with this model (see section 3*f* for details). The structure shown in the box is proposed to be present in Thy-1, human erythrocyte AChE and possibly other proteins (excepting VSG). The structure has three regions. (i) A phosphatidylinositol molecule whose 1,2-diacylglycerol moiety is embedded in the bilayer and is responsible for anchoring; removal of this diacylglycerol by PI-PLC results in release of the protein from the membrane. (ii) A glycan of varied structure and composition. The linkage between the glycan and the membrane phosphatidylinositol molecule is via a glycosidic linkage with a glucosamine that has a free amino group. (iii) An ethanolamine is amide-linked via its amino group to the α-carboxyl of the *C*-terminal amino acid (□). The non-reducing end of the glycan contains a mannose 6-phosphate which is phosphodiester-linked to the hydroxyl of this ethanolamine residue. Phosphodiester linkages are represented as (P). The purpose of this Figure is to indicate the likely arrangement of components in the anchoring structure. The size and orientation of those components and of the protein and lipid domains are therefore not necessarily portrayed accurately. For example, the *C*-terminal cysteine in Thy-1 is disulphide-linked to Cys-9 in the mature protein and AChE, APase and 5′-nucleotide probably exist as dimers in the membrane.

it is possible to derive considerable information from studies on the purified protein itself without prior isolation of the membrane anchoring region. This particularly applies to the inositol content of these proteins, since *myo*-inositol is not a normal constituent of proteins and can be quantified by specific and sensitive g.l.c.–m.s. techniques. In this fashion the first direct evidence for covalent attachment between a protein and *myo*-inositol was obtained for purified detergent-solubilized AChE from *Torpedo* (Futerman *et al.*, 1985*a*). The location of the *myo*-inositol in the membrane anchoring domain was confirmed by inositol analysis of AChE solubilized by proteinase K (which removes the entire membrane anchoring domain) or by PI-PLC (which removes only 1,2-diacylglycerol). More recently, *myo*-inositol has been detected in human placental APase solubilized by PI-PLC (Low *et al.*, 1987) and in human erythrocyte AChE solubilized by detergent (Roberts & Rosenberry, 1986*b*) in amounts consistent with a stoichiometry of 1 mol of inositol/mol of polypeptide.

(*c*) Composition of the purified membrane anchoring domains. Direct chemical composition data on the *C*-terminal structure containing the membrane anchoring domain has been determined for three proteins. (i) A hydrophobic 'peptide' was prepared by tryptic cleavage of Thy-1 and contained the anchoring structure plus the *C*-terminal amino acid cysteine (Campbell *et al.*, 1981; Tse *et al.*, 1985). The argument for this being the membrane anchor is somewhat indirect, i.e. it is the only hydrophobic structure in the whole molecule. (ii) A similar hydrophobic structure prepared by papain treatment of human erythrocyte AChE contained the *C*-terminal dipeptide His-Gly (Haas *et al.*, 1986; Roberts & Rosenberry, 1985, 1986*a*,*b*; W. L. Roberts, B. H. Kim & T. L. Rosenberry, personal communication). Here, the evidence for involvement of membrane anchoring was very clear, since the papain treatment releases the protein from the membrane (see section 3*a*). (iii) A *C*-terminal glycopeptide was produced by Pronase digestion of VSG, and contained aspartate or serine as the only amino acid (Ferguson *et al*, 1985*b*; Holder, 1985; Holder & Cross, 1981; Strang *et al.*, 1986). The Pronase glycopeptide prepared from the soluble form of VSG (which results from the action of an endogenous phospholipase C; see section 5*a*) did not contain glycerol and fatty acid, in contrast with the Pronase glycopeptide prepared from the intact membrane form of VSG (Ferguson *et al.*, 1985*a*). It is therefore probable that the *C*-terminal Pronase glycopeptide is responsible for membrane anchoring.

A comparison of the analytical data (see above for references) for these structures from diverse origins suggests that they are similar. They all contain ethanolamine (1 mol/mol in VSG and 2 mol/mol in Thy-1 and AChE), glucosamine (1 mol/mol), *myo*-inositol (1 mol/mol) and, where analysed, glycerol, phosphate and fatty acid. In VSG and AChE, the glucosamine has a free amino group. In contrast with these similarities, certain features of the analyses do indicate that structural differences are likely. Thus, additional sugars may be present as well as glucosamine, i.e. mannose (2 mol/mol) and galactose (0–8 mol/mol) in VSG and mannose (2–3 mol/mol) and galactosamine (0–1 mol/mol) in Thy-1; comparable data for AChE have not yet been reported. The fatty acid compositions are also quite different (Ferguson *et al.*, 1985*a*; Roberts & Rosenberry, 1985; Tse *et al.*, 1985). VSG contains exclusively myristate (14:0) and AChE contains a mixture of fatty acids with the predominant species being palmitate (16:0). Thy-1 contains principally stearate (18:0) and two unidentified residues behaving like C_{20} and C_{22} fatty acids on g.l.c.

(*d*) Biosynthetic labelling studies of the membrane anchors. Incorporation of radioactive precursors into the phosphatidylinositol anchors has tended to confirm the structural information described above. Fatty acid labelling (i.e. with [^{3}H]-myristate and -palmitate) of phosphatidylinositol-anchored proteins has been achieved with VSG in *T. brucei*, p63 protease in

Leishmania major, Thy-1 in murine T lymphoma cells, and the human placental type of APase expressed in HeLa cells (Etges *et al.*, 1986*a*; Fatemi & Tartakoff, 1986; Ferguson & Cross, 1984; R. Jemmerson & M. G. Low, unpublished work). A substantial proportion of the label could be removed by nitrous acid or PI-PLC and, in the case of VSG, the released ^3H has been shown to be associated with phosphatidylinositol and 1,2-diacylglycerol, respectively (Ferguson *et al.*, 1985*a*). Since many other proteins in eukaryotic cells have been shown to be labelled with fatty acids by similar procedures (i.e. autoradiography of SDS/polyacrylamide gels; see Magee & Courtneidge, 1985, for references), it is likely that some of these contain the fatty acid as part of a phosphatidylinositol moiety. Certainly, those in which an *O*-acyl linkage is suspected (i.e. sensitive to strong alkali or alkaline hydroxylamine) should be examined in this regard.

[^3H]Ethanolamine has been selectively incorporated into VSG, as well as into HeLa cell DAF and APase; proteolytic cleavage and kinetic studies indicate that the incorporated radioactivity is localized at the *C*-terminus (Medof *et al.*, 1986; Rifkin & Fairlamb, 1985; R. Jemmerson & M. G. Low, unpublished work). [^3H]Ethanolamine has also been incorporated into Thy-1 in murine T lymphoma cells (Fatemi *et al.*, 1987). This was shown to be localized to a hydrophobic fragment after Pronase digestion. Only half of the incorporated ethanolamine was sensitive to methylation (Fatemi *et al.*, 1987), which supports the concept that one of the two ethanolamines in this protein (Tse *et al.*, 1985) is amide-linked to the *C*-terminal amino acid (see section 3*f*).

***(e)* Immunological studies.** It has been known for some time that the soluble form of *T. brucei* VSG contains a common determinant that is expressed on most of the different variant forms of this protein (Cross, 1979). This determinant (the cross-reacting determinant or CRD) is cryptic in the membrane form of VSG and is only exposed when this is converted to the soluble form by the action of the endogenous phospholipase C (Cardoso de Almeida & Turner, 1983). This suggested that the epitope (or epitopes) recognized by the antibodies was localized in the membrane anchoring domain and the observation that the CRD was present in the *C*-terminal Pronase glycopeptide supported this conclusion (Holder, 1985; Holder & Cross, 1981). Subsequently, the anti-CRD antibodies were shown to cross-react with *Torpedo* and human erythrocyte AChE and with p63 protease after hydrolysis by *T. brucei* phospholipase C (see section 5*a*) as well as with DAF and human placental APase hydrolysed by *S. aureus* PI-PLC (M. G. Low, unpublished work; Bordier *et al.*, 1986; Davitz *et al.*, 1987; Stieger *et al.*, 1986). A CRD has also been demonstrated recently in *Paramecium* surface antigens (Capceville *et al.*, 1986). In all cases, the determinant was masked in the membrane form of the protein before removal of the diacylglycerol by phospholipase C. The observation that this determinant is shared by other phosphatidylinositol-anchored proteins suggests that it could be useful in determining the structure of the anchoring domain. The precise location and extent of the CRD epitope within the anchoring domain is currently unknown, although it is believed to include part of the glycan, since VSG 118, which has no galactose in this part of the anchor, is only weakly cross-reactive (Cross, 1979; Holder, 1985). In spite of this, the anti-CRD antibodies should be useful as an adjunct to PI-PLC in the identification (by immunoassay or immunoblotting) and isolation (using immobilized antibodies) of novel phosphatidylinositol-anchored proteins and for studying the biosynthetic attachment of anchor components (see section 4*a*).

***(f)* Structure of the anchoring domain.** Some information is available on the arrangement of the various components in the *C*-terminal anchoring domain. (i) Manual Edman sequencing revealed the *C*-terminal amino acid to be amide-linked to an ethanolamine in both VSG and AChE, and partial acid hydrolysis studies suggest a similar arrangement in Thy-1 (Haas *et al.*, 1986; Holder, 1983; Williams & Tse, 1985). Methylation studies indicate that there is another ethanolamine residue in Thy-1, AChE and DAF which has a free amino group (Haas *et al.*, 1986; Medof *et al.*, 1986; Fatemi *et al.*, 1987). (ii) The single ethanolamine in VSG is believed to be linked by a phosphate to mannose, since ethanolamine phosphate and mannose 6-phosphate have been identified by partial acid hydrolysis of the *C*-terminal glycopeptide (Ferguson *et al.*, 1987). Phosphodiester linkages for the two ethanolamines seem likely in Thy-1 also since they, in combination with the phosphatidylinositol phosphodiester, would account for the three phosphates detected in analyses of the anchoring region (Tse *et al.*, 1985). (iii) The inositol in VSG, human erythrocyte and *Torpedo* AChE and human placental APase can be removed by nitrous acid deamination (Ferguson *et al.*, 1985*b*; Low *et al.*, 1987; Roberts & Rosenberry, 1986*b*). This indicates that the inositol in the membrane anchoring domain is attached through a glycosidic linkage to the glucosamine residue, which has a free amino group. (iv) The arrangement of other sugars within the anchoring structure is uncertain but, since the known sugar compositions (i.e. for several different VSGs and Thy-1) differ considerably, a common structure in this central region is not possible (Holder, 1985; Tse *et al.*, 1985). Furthermore, high resolution gel filtration analysis of the glycan of VSG 117 indicates considerable size microheterogeneity (Ferguson *et al.*, 1987). However, it has been suggested that the different VSGs may have a common 'core' structure of Man_2GlcN, which is modified with 0–8 residues of galactose (Ferguson *et al.*, 1987). A similar core structure for Thy-1 would be consistent with the amounts of mannose and glucosamine detected in the anchoring domain (Tse *et al.*, 1985). (v) Periodate oxidation of the membrane form of VSG labelled with [^3H]myristate released the radioactivity from the protein, suggesting that it is the 4-hydroxyl on the inositol ring which is glycosidically linked to glucosamine (Ferguson *et al.*, 1987).

4. Biosynthesis of the membrane anchors

***(a)* The polypeptide precursor.** Even before the structure of the VSG anchor was determined, it had been shown that attachment of the *C*-terminal structure was very rapid and could be detected within 1 min of translation of the protein (Bangs *et al.*, 1985, 1986; Ferguson *et al.*, 1986). Furthermore, this post-translational processing not only involved attachment of the

glycophospholipid structure (as detected by the anti-CRD antibodies) but also removal from the *C*-terminus of a hydrophobic peptide sequence of 17 or 23 amino acid residues that was predicted from cDNA sequences but not present in the mature protein (Fig. 3). Although the processing of VSG is very rapid, it seems unlikely to be a late cotranslational event since the length of the *C*-terminal peptide removed is not long enough to cross the bilayer in addition to the ribosomal subunits (Fig. 4). A similar situation exists in Thy-1, where comparison of the predicted and actual amino acid sequences indicates that 31 residues are removed during processing (Moriuchi & Silver, 1985; Seki *et al.*, 1985*a*,*b*).

Complete amino acid sequences for the other phosphatidylinositol-anchored proteins are not available, but it seems likely that a similar process is involved in lipid attachment. This is based on the observation that cDNA sequences for other proteins believed to be anchored by phosphatidylinositol predict short (approx. 15–20 residues) hydrophobic amino acid sequences at the *C*-terminus, with insignificant or nonexistent 'cytoplasmic' domains (Fig. 3), similar to those found in VSG and Thy-1. Since it is not clear whether these would effectively anchor the protein to the cell surface, the implication is that they are replaced with the glycosylated phosphatidylinositol anchor immediately after translation, as observed with VSG. In fact, it could be argued that the function of these *C*-terminal hydrophobic peptide sequences is to provide a means for transiently retaining the newly synthesized peptide at the lumenal surface of the endoplasmic reticulum in order to facilitate such a process.

Protein	*C*-Terminal sequence (Hydrophobic region)
VSG 117A	WENNACKD*SSILVTKKFALTVVSAAFVALLF
VSG 221A	NINITGSS*NSFVISKTPLWLAVLLF
Thy-1	KLVKC*GGISLLVQNTSWLLLLLLSLSFLQATDFISL
APase P	LAPPAGTTDAAHPGRSVVPALLPLLAGTLLLLETATAP
APase I	LAPPACTTDAAHP VAASLPLLAGTLLLLGASAAP
APase L	IGANLGHCAPASSAGSLAAGPLLVALALYPLSVLF
Ly-6E.1	CQEDLCNAAVPNGGSTWTMAGVLLFSLSSVLLQTLL
N-CAM$_{120}$	PTVIPATLGSPSTSSSFVSLLLSAVTLLLLC

Fig. 3. Predicted *C*-terminal amino acid sequences for precursor polypeptides of phosphatidylinositol-anchored proteins

These sequences have been tentatively aligned at the *N*-terminal end of the hydrophobic sequence (in some cases this is difficult to distinguish precisely). Underlined sequences represent those shown to be present in the mature protein by amino acid sequencing. In VSG and Thy-1 the *C*-terminal amino acid residue in the mature protein is indicated (*) and, since peptide sequences near the *C*-terminus are available for some of the alkaline phosphatases, potential sites for the *C*-termini in these proteins are also indicated (●). The amino acid sequences are predicted from cDNA sequences determined for VSG (Cross, 1984), rat Thy-1 (Moriuchi & Silver, 1985; Seki *et al.*, 1985*a*,*b*), Ly-6E.1 (LeClair *et al.*, 1986), N-CAM$_{120}$ (Hemperly *et al.*, 1986) and the major types of alkaline phosphatase: human liver (L; Weiss *et al.*, 1986), human intestine (I; Berger *et al.*, 1987) and human placenta (P; two allelic variants that have identical *C*-terminal sequences; Henthorn *et al.*, 1986; Kam *et al.*, 1985; Millan, 1986; Ovitt *et al.*, 1986). The gap is inserted in the intestinal APase sequence to indicate homology with the placental sequence. The Ly-6E.1 sequence is included since two antigens encoded or regulated by the *Ly*-6 locus are known to be phosphatidylinositol-anchored, i.e. ThB and TAP (see Table 1). Although genomic sequences for several genes in the *Qa*-2,3 locus are known (Devlin *et al.*, 1985), they are not presented since they do not accurately predict the correct *N*-terminal sequence of the mature *Qa*-2 (Soloski *et al.*, 1982).

The observation that a relatively complex structure, such as that shown in Fig. 2, is attached to a diverse but limited set of membrane proteins implies a fairly high degree of specificity in the processing mechanism. Some information or 'signal' must be present in the polypeptide which directs the processing enzymes to cleave the polypeptide and attach the glycosylated phosphatidylinositol. However, this 'signal' is unlikely to be a segment of defined length and sequence, such as that found near the *N*-terminus of lipid-modified proteins in *E. coli* (i.e. Leu-Ala-Gly-Cys; Wu & Tokunaga, 1986). Inspection of the sequences shown in Fig. 3 reveals neither conservation nor consensus in the hydrophobic region or in the vicinity of actual (i.e. VSG and Thy-1) or likely (i.e. APase and Ly-6) processing sites. The lack of sequence conservation in this region is particularly apparent in the case of the mammalian APases; the placental type contains an extra four residues not present in the intestinal type from which it is believed to have evolved (see Fig. 3). A more likely possibility is that the signal sequences are relatively diverse, as has been found for *N*-terminal cotranslational insertion signals as well as stop-transfer signals (Kreil, 1981; Sabatini *et al.*, 1982). It has been suggested that the information contained in the *N*-terminal insertion signal is conformational in character (Kreil, 1981) and such a situation may also exist at the processing site in the phosphatidylinositol-anchored proteins. Thus, the polypeptide chain may adopt a relatively unstructured conformation, in the region between the *N*-terminal (presumably globular) domain and the *C*-terminal hydrophobic segment embedded in the bilayer, that is especially susceptible to cleavage. To answer these questions, more precursor polypeptide sequences and the precise location of the processing sites within them will have to be determined.

Support for the concept that the *C*-terminal sequence has some role in directing lipid attachment comes from the recent identification of a cDNA clone that appears to encode for chicken brain N-CAM$_{120}$ (Hemperly *et al.*, 1986). This clone predicts a sequence which is very similar to that found for the larger PI-PLC resistant forms of N-CAM (i.e. N-CAM$_{130}$ and N-CAM$_{160}$) that were already known to contain hydrophobic transmembrane and hydrophilic cytoplasmic domains and consequently unlikely to be anchored by phosphatidylinositol. The first 574 residues of this sequence are identical with the larger N-CAM polypeptide sequences, but the remaining 25 residues are completely different. This unique sequence contains a moderately hydrophobic stretch of 15 residues at the *C*-terminus (Fig. 3) and presumably arises as a result of an alternative mRNA splicing event of a single initial gene transcript. Since the amount of this type of mRNA increases during embryonic development in parallel with increased expression of N-CAM$_{120}$ (Hemperly *et al.*, 1986), it is likely that the presence of this unique sequence in some

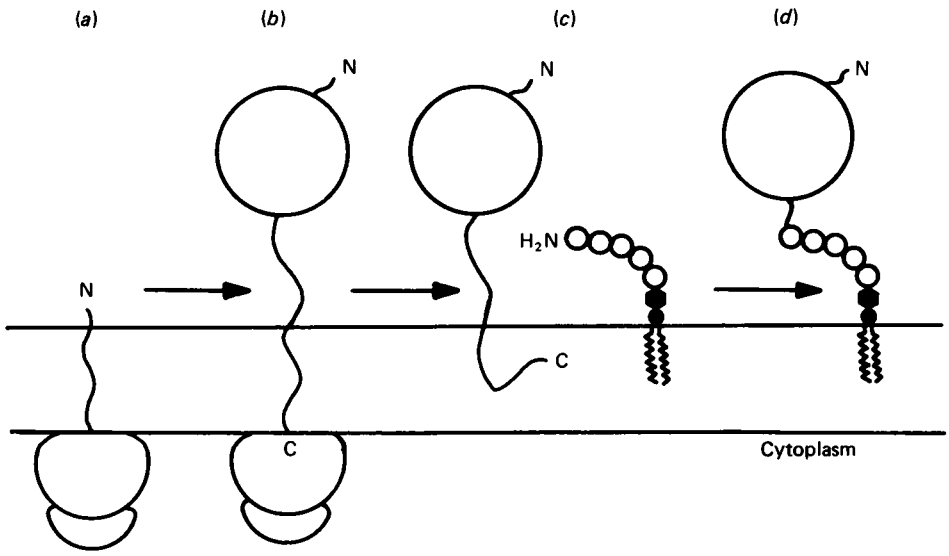

Fig. 4. Biosynthesis and processing of phosphatidylinositol-anchored proteins

This simple scheme illustrates the major steps that have been inferred from studies of VSG, but similar events also seem likely for proteins in higher eukaryotes (see section 4 for details). (*a*), (*b*) Membrane-bound ribosomes transfer the newly synthesized polypeptide into the lumen of the endoplasmic reticulum by conventional mechanisms (not illustrated) involving *N*-terminal proteolytic processing and glycosylation of the polypeptide. After termination of protein synthesis the protein is transiently anchored by a *C*-terminal hydrophobic sequence of approx. 15–20 residues. (*c*) The *N*-terminal domain of this precursor polypeptide is then attached to a preassembled glycosyl-phosphatidylinositol anchor precursor. Such a reaction, catalysed by a transamidase, presumably involves simultaneous peptide bond cleavage and amide bond formation with an amino group on the anchor precursor. (*d*) The mature phosphatidylinositol-anchored protein is then transferred to the cell surface via the Golgi apparatus by conventional mechanisms, which may also involve addition of extra sugars to the anchoring region (not illustrated). Abbreviations: N, *N*-terminus; C, *C*-terminus.

way identifies the polypeptide for lipid attachment. Although these data indicate that mRNA processing could be involved in regulation of lipid attachment, there is at present no evidence that this applies to other phosphatidylinositol-anchored proteins. Multiple mRNA species coding for *Torpedo* AChE or human placental APase have been identified by RNA blot analyses or inferred by comparison of 3′ nucleotide sequences of cDNA clones (Schumacher *et al.*, 1986; Ovitt *et al.*, 1986; Henthorn *et al.*, 1986). However, none of these has been correlated with differences in protein sequence or sensitivity to PI-PLC. *Torpedo* AChE is known to be retained at the cell surface by two quite distinct mechanisms involving covalent attachment to collagen or phosphatidylinositol, but cDNA clones corresponding to the latter type of AChE are not yet available for comparison (Schumacher *et al.*, 1986). Resistance of other proteins to release by PI-PLC has also indicated a degree of heterogeneity in the mechanism of membrane attachment (see section 2*c*) and even though other possibilities have been suggested the involvement of different mRNA processing events is an attractive alternative that should not be discounted.

(*b*) The glycosylated phosphatidylinositol precursor. In the preceding section, it has been tacitly assumed that the event occurring simultaneous with removal of the *C*-terminal hydrophobic peptide was the attachment of a preformed lipid located at the lumenal surface of the endoplasmic reticulum (Fig. 4*c*). The enzyme(s) carrying out such a transformation is completely unknown, but it is probably a transamidase which catalyses simultaneous cleavage of a peptide bond by the ethanolamine amino group and formation of the amide bond. There are several lines of evidence supporting this conclusion. (i) The rapidity with which the anchor is attached and the similar kinetics of CRD and myristate attachment to VSG (see section 4*a*) appear to rule out a step-wise addition of components to the *C*-terminal amino acid (Bangs *et al.*, 1985; Ferguson *et al.*, 1986). These data, and the observation of [^{3}H]ethanolamine incorporation into a 48 kDa pro-DAF (Medof *et al.*, 1986), also seem to exclude the occurrence of anchor attachment in the Golgi apparatus or on the plasma membranes. (ii) A lipid which is rapidly labelled with myristate, which is nitrous acid- and PI-PLC sensitive and which contains the CRD has been identified in *T. brucei* (Krakow *et al.*, 1986). However, the incorporation of ethanolamine, mannose or galactose and other components of the anchor were not determined. (iii) In mammalian liver and muscle, a lipid sensitive to nitrous acid and PI-PLC and which can be labelled with glucosamine and inositol has been identified and is believed to be the precursor for intracellular mediators of insulin action (Saltiel & Cuatrecasas, 1986; Saltiel *et al.*, 1986, 1987). These data indicate that higher eukaryotic cells also have the biosynthetic capability of producing glycosylated phosphatidylinositols with at least some of the characteristics expected of the protein anchors. (iv) Studies with Thy-1$^-$ lymphoma cells indicate a tight coupling between removal of the hydrophobic peptide and lipid attachment (see section 4*c*). (v) As indicated in the previous section, the most efficient mechanism seems to be one in which the precursor polypeptide retains its hydrophobic peptide 'anchor' until it can be replaced by the lipid. This could only occur if the glycosylated phosphatidylinositol

anchor was added essentially simultaneously (Fig. 4*c*), since attachment of the ethanolamine to the protein by amide linkage with an α-carboxyl group necessarily implies that a peptide bond is cleaved by either a protease or transamidase with release of the hydrophobic peptide. Although attachment of a relatively intact lipid anchor to the protein in the endoplasmic reticulum seems the most likely, this does not exclude the possibility of extensive processing of the anchoring domain after attachment. It has been suggested for VSG that the anchor contains a 'core' glycan structure of Man_2GlcN (Ferguson *et al.*, 1987). An anchor precursor with this sugar composition might therefore be attached to the protein and subsequently be modified during its transit through the Golgi apparatus by the addition of galactose or other sugars (see section 3*c*).

The close structural relationship of the glycophospholipid anchor and the insulin-sensitive glycophospholipid suggests that they share early biosynthetic steps in common (see above). This immediately raises a topological problem, since degradation of the glycophospholipid in response to insulin stimulation is presumed to occur at the cytoplasmic surface of the plasma membrane, in contrast with the cell surface location of phosphatidylinositol-anchored proteins. Diffusion of the anchor precursor across the endoplasmic reticulum membrane to an appropriate location either before or after attachment to protein seems inherently unlikely but cannot be excluded. A more radical alternative would restrict these glycophospholipids, the enzymes that make them and the phospholipase C that degrades them to the endoplasmic reticulum lumen, the cell surface or other extra-cytoplasmic compartments, a novel concept for which there is some preliminary experimental support (see section 6*b*).

(*c*) Defects in biosynthesis of the anchors. Although the details of anchor biosynthesis are unknown, there are two situations which suggest that absence of surface expression of phosphatidylinositol-anchored proteins is due to defects in biosynthesis or attachment of the glycosylated phosphatidylinositol anchor. Thus, in the class A and E mutants of the murine T lymphoma BW-5147, Thy-1 and two antigens encoded or regulated by genes in the *Ly-6* locus are not expressed on the cell surface (Horton & Hyman, 1983; Hyman, 1985). Since Thy-1 and proteins encoded or regulated by the *Ly-6* locus (i.e. ThB and TAP) are phosphatidylinositol-anchored (Table 1), it is likely that these mutations affect some cellular process to which anchor biosynthesis or attachment is particularly sensitive. It was suggested (Chapman *et al.*, 1980) that the class E phenotype is due to a deficiency in the enzyme that transfers mannose from GDP-mannose to dolichol phosphate (and other lipid mannosyl acceptors such as phosphatidic acid and retinyl phosphate; Creek *et al.*, 1986) resulting in abnormal *N*-linked glycosylation of proteins, and subsequent intracellular degradation. However, since Ly-6E.1 does not contain *N*-linked glycans, this seems improbable as a general explanation (Palfree & Hammerling, 1986). The biochemical event affected in the class A mutant is not known. It has recently been observed that E mutant cells secrete a large proportion of the abnormal Thy-1 into the extracellular medium in a hydrophilic form (Fatemi & Tartakoff, 1986). Furthermore, the E mutant Thy-1, unlike wild-type Thy-1, does not contain biosynthetically incorporated [^{3}H]palmitate or [^{3}H]ethanolamine (Fatemi & Tartakoff, 1986; Fatemi *et al.*, 1987). It has therefore been proposed that the defect in dolichol-phosphate-mannose synthesis prevents either assembly of the mannose-containing anchor precursor itself or attachment of the anchor to the incorrectly glycosylated Thy-1 (Fatemi & Tartakoff, 1986; Fatemi *et al.*, 1987). In a different study it was shown that Thy-1 extracted from A and E mutant cells does have a hydrophobic anchor but, unlike the wild type, it was not sensitive to PI-PLC (Conzelmann *et al.*, 1986). This is presumably due to the retention by A and E mutant Thy-1 of the *C*-terminal hydrophobic peptide; the observation that [^{3}H]tryptophan is incorporated into mutant Thy-1 supports this conclusion, since the single tryptophan residue in Thy-1 is located in this region. The implication of this study is that inability to make the glycophospholipid precursor (i.e. in the E mutant) not only prevents attachment of the anchor but also removal of the *C*-terminal peptide. This is consistent with a single reaction catalysed by a transamidase being involved in attachment of the glycosyl-phosphatidylinositol anchor (Fig. 4). Whether the class A phenotype is due to a deficiency in such an enzyme is not known.

The expression of several phosphatidylinositol-anchored proteins appears to be decreased in a rare acquired haemolytic disorder, paroxysmal nocturnal haemoglobinuria (PNH). In this disorder many of the erythrocytes are abnormally sensitive to autologous complement-mediated haemolysis. This is in part due to a deficiency in a subpopulation of cells of a complement regulatory component, decay accelerating factor (DAF). This protein is now known to be anchored to membranes by phosphatidylinositol on the basis both of its ability to be released from membranes by PI-PLC and biosynthetic labelling studies (Davitz *et al.*, 1986, 1987; Medof *et al.*, 1986). Deficiencies of erythrocyte AChE and leukocyte APase in PNH (Chow *et al.*, 1985; Craddock *et al.*, 1976; Tanaka *et al.*, 1960) seem to point to a common biosynthetic defect being responsible for their inability to be expressed at the cell surface. Since defects in other components of PNH cells, not anchored by phosphatidylinositol (e.g. glycophorin; Parker *et al.*, 1984), have been reported, it seems likely that the inability to express DAF, AChE and APase relates specifically to some general biochemical defect, similar to the one identified for the class E Thy-1 mutant, to which these proteins are especially sensitive.

5. Degradation of membrane anchors

(*a*) Anchor-removing enzymes. Several activities which remove the anchors from the phosphatidylinositol-anchored proteins have been reported; however, the only one to be purified and characterized in any detail is that from *Trypanosoma brucei*. This is a membrane bound phospholipase C which is responsible for the rapid conversion of the hydrophobic membrane form of VSG to a soluble form during osmotic or cold detergent lysis (Cardoso de Almeida & Turner, 1983; Ferguson & Cross, 1984; Ferguson *et al.*, 1985*a*). It has a molecular mass of approx. 37–40 kDa, does not require Ca^{2+}, and is inhibited by thiol blockers (Bulow & Overath, 1986; Fox *et al.*, 1986; Hereld *et al.*, 1986). An unexpected finding was that phosphatidylinositol and the polyphosphoinositides were not good substrates for this

enzyme (Bulow & Overath, 1986; Fox *et al.*, 1986; Hereld *et al.*, 1986). The only substrates that seemed to be hydrolysed at rates approaching that of the membrane form of VSG were the *C*-terminal fragment of this protein (prepared by Pronase treatment) and a lipid (see section 4*b*) isolated from *T. brucei* that is believed to be a precursor of the lipid anchor (Fox *et al.*, 1986; Hereld *et al.*, 1986; Krakow *et al.*, 1986). This enzyme will also remove the glycophospholipid anchors from other proteins, i.e. the p63 protease and AChE (Bordier *et al.*, 1986; Etges *et al.*, 1986*b*; Stieger *et al.*, 1986). Since the insulin-sensitive glycophospholipid (believed to be the source of water soluble glycosylinositol phosphate insulin mediators) is also hydrolysed by this enzyme, it seems likely that the head group specificity is for a glucosaminyl-phosphatidylinositol structure (Fox *et al.*, 1986; Saltiel & Cuatrecasas, 1986). A recent report indicates that a 52 kDa insulin-sensitive phospholipase C purified from liver plasma membranes has very similar properties (Fox *et al.*, 1987). These novel phospholipases C share some properties with the bacterial PI-PLC enzymes (see section 2*d*) in that they are inhibited by thiol blockers and do not require Ca^{2+} for activity, but are clearly more specific than the bacterial enzymes which have relatively high activity against phosphatidylinositol. Furthermore, these three types of phospholipase C are distinct from the mammalian cytosolic phospholipases C which have high molecular mass (70 kDa and greater), are Ca^{2+} dependent and are capable of hydrolysing polyphosphoinositides in addition to phosphatidylinositol (see Low & Weglicki, 1983; Low *et al.*, 1986*b*, for references). The ability of these latter enzymes to hydrolyse protein anchors or other glycosylated forms of phosphatidylinositol has yet to be established.

Several activities which seem to be capable of removing the phosphatidylinositol anchors from proteins have been reported in mammalian tissues, but not purified. It is not clear whether they are one of the forms of PI-PLC found in these tissues or different types of enzyme also capable of hydrolysing the anchoring domain. Since the anchoring domains are quite complex in their structures, it is not difficult to conceive of other enzymes that would be capable of cleaving them in a highly specific fashion (e.g. phospholipase D, endoglycosidase, phosphodiesterase and proteinase). A limited amount of information is available on the properties of these anchor-removing activities. The butanol-activated enzyme that converts APase from a hydrophobic aggregated form to a hydrophilic form is most active at acid–neutral pH and is thiol- and Ca^{2+} dependent, but its bond specificity and subcellular location are unknown (Kominami *et al.*, 1985; Low & Zilversmit, 1980; Malik & Low, 1986; Miki *et al.*, 1985). Thiol-dependent, deoxycholate-stimulated, soluble and membrane-associated activities capable of releasing APase or AChE from membranes have been reported in rat heart and kidney, sheep platelets and sheep basal ganglia (Low & Weglicki, 1981; Majumdar & Balasubramanian, 1982, 1985; Low & Prasad, 1987). However, conflicting information is available on their Ca^{2+} sensitivities.

***(b)* Release of phosphatidylinositol-anchored proteins *in vivo*.** There are a number of reports indicating that phosphatidylinositol-anchored proteins can be released *in vivo* or from intact cells in culture. This suggests that the enzyme activities described above may have a role in releasing some proteins as part of a physiologically significant process. Thus, APase is released into the serum after fat ingestion, during pregnancy and in a variety of diseases (McComb *et al.*, 1979). AChE is released from certain areas of the brain and adrenal chromaffin cells in response to a variety of stimuli (Greenfield, 1984; Mizobe *et al.*, 1984) and Qa-2 is secreted from concanavalin A-stimulated T lymphocytes (Soloski *et al.*, 1986). N-CAM_{120} is found in both membrane-bound and soluble forms (e.g. Gennarini *et al.*, 1984). The VSG coat of the African trypanosome is exchanged during antigenic variation in the blood stream of the mammalian host, or lost during differentiation in the insect host (Bulow & Overath, 1985; Overath *et al.*, 1983). However, even though *T. brucei* contains a very active and specific anchor-cleaving phospholipase C (see section 5*a*) capable of carrying out such changes, there is no definitive evidence showing that it is involved in either of these processes or how its activity is regulated. Thus, soluble VSG can be detected in the blood of infected animals, but this could simply be a consequence of organism death, since the phospholipase C is known to become active upon cell lysis (see Turner, 1984, for references). Release of VSG from intact trypanosomes has been observed *in vitro* in response to Ca^{2+} and the ionophore A23187 (Bowles & Voorheis, 1982) but, since the purified phospholipase C is known to be Ca^{2+}-independent, it is not clear whether the phospholipase C is directly involved in this process.

6. Function of the glycosyl-phosphatidylinositol anchors

The evidence cited above has demonstrated the involvement of phosphatidylinositol in the anchoring of a diverse group of membrane proteins and, although there are still many gaps in our knowledge of the structure, biosynthesis and metabolism of the anchoring domains, a consistent picture is emerging. By contrast, information on the functional aspects of the phosphatidylinositol anchor is completely lacking. Although these structures serve to anchor the proteins to the membrane, many other proteins are anchored by mechanisms not involving covalently attached lipid (see Fig. 1). Why are these more conventional mechanisms not used for the proteins listed in Table 1? Presumably, the phosphatidylinositol anchor allows the protein to carry out its physiological function more efficiently than if it were anchored by other mechanisms. Even though the true physiological function of many of these proteins is unknown, their biochemical properties indicate that they are likely to be functionally diverse and it is difficult to see how the anchor is functionally relevant in all of them. Perhaps a more instructive approach would be to consider what novel properties a phosphatidylinositol anchor might confer on a protein. Two possibilities have emerged that warrant further discussion. As more phosphatidylinositol-anchored proteins are identified, it is likely that other functions for the phosphatidylinositol-anchoring domain will become apparent.

***(a)* Mobility.** Most cell surface proteins with a large extracellular domain have in addition a cytoplasmic domain, the two being connected by a relatively short, hydrophobic transmembrane domain (Fig. 1*b*). By contrast, a phosphatidylinositol-anchored cell surface protein would most likely have its anchor entirely

located within the outer leaflet of the bilayer. Since interactions between the cytoskeleton and cytoplasmic domains have been reported to reduce the lateral mobility of proteins (Jacobson *et al.*, 1987), it is likely that phosphatidylinositol-anchored proteins would be relatively more mobile. Fluorescence photobleaching studies have in fact shown that the mobile fraction of Thy-1, which constitutes about one-half of the total, has a relatively high diffusion coefficient ($D \sim 10^{-9}$ cm^2/s) compared with many cell surface proteins (Dragsten *et al.*, 1979; Ishihara *et al.*, 1987; Jacobson *et al.*, 1987; Woda & Gilman, 1983), but it is not known if there is any relationship between the immobile fraction and PI-PLC resistant forms of Thy-1 (section 2*c*). Thy-1 has been reported to interact indirectly with the cytoskeleton, and this may also contribute to the immobile fraction (Bourgignon *et al.*, 1986). However, until the diffusion coefficients of a larger number of phosphatidylinositol-anchored proteins (especially dimeric ones such as AChE and APase) have been determined, the functional significance of high mobility will remain uncertain.

(*b*) Release and uptake of proteins. The existence of enzymes which readily release proteins from membranes by hydrolysing the phosphatidylinositol anchor (see section 5) has quite naturally led to the suggestion that this might be one function of the anchor (Low *et al.*, 1986*a*). It is not difficult to envisage the potential utility to the cell of a selective release mechanism for certain proteins. This might be particularly valuable for proteins which are involved in adhesion or homing of cells (e.g. N-CAM$_{120}$, heparan sulphate proteoglycan and possibly also Thy-1) and protective coat proteins in parasites.

An additional possibility is that cleavage of the phosphatidylinositol anchor would expose an unusual glycan on the soluble protein. This might be a site for specific recognition and binding by a surface receptor on the same or another cell. Evidence for such a suggestion has recently been presented for hepatocyte heparan sulphate proteoglycan, which is partially releasable by either inositol phosphates or PI-PLC (M. Ishihara *et al.*, 1987). The relative proportion of these two pools of releasable heparan sulphate can be modulated by inclusion of insulin in the culture media. Since insulin has been reported to stimulate a phospholipase C-mediated hydrolysis of a glycophospholipid with structural homology to the glycosyl-phosphatidylinositol anchors (Saltiel & Cuatrecasas, 1986; Saltiel *et al.*, 1986; Fox *et al.*, 1987), it is probable that insulin also stimulates release of the phosphatidylinositol-anchored form of heparan sulphate, which then binds to a glycosyl-inositol phosphate 'receptor' on the cell surface prior to endocytosis (M. Ishihara *et al.*, 1987). Indirect evidence suggests that a similar phenomenon may be occurring with other phosphatidylinositol-anchored proteins. APase levels in rat osteosarcoma cells are markedly decreased after culturing in the presence of insulin (Levy *et al.*, 1986). AChE, which is known to be released from cells in response to a variety of stimuli (Greenfield, 1984; Mizobe *et al.*, 1984), is found in rat brain bound to a cell surface lectin or receptor from which it can be dissociated by sugar phosphates (Gaston *et al.*, 1982). It is noteworthy that AChE in that tissue is resistant to release by PI-PLC (Futerman *et al.*, 1985*b*).

If protein release is mediated by cell surface phospholipases C regulated by insulin, or other hormones and growth factors, then an important consequence of such a release event would be generation at the cell surface of 1,2-diacylglycerol (an activator of protein kinase C) and of glycosyl-inositol phosphate mediators either attached to protein or in a free form (Saltiel, 1987; Saltiel *et al.*, 1986, 1987). These second messengers could be delivered to their intracellular target sites by the processes of transbilayer diffusion and receptor-mediated endocytosis, respectively. Alternatively, the anchor might be removed by other enzymes which do not produce 1,2-diacylglycerol or the glycosyl-inositol phosphates (e.g. phospholipase D, proteinases, endoglycosidases, etc.; see section 5*a*). Thus, a glycosyl-phosphatidylinositol anchoring system could offer the cell a relatively economical mechanism for selectively co-ordinating cell surface expression of a particular protein with intracellular metabolism via several independent second messenger pathways. The physiological consequences of such a mechanism cannot at present be predicted, but it is likely that the capability for specific release and uptake of cell surface molecules will be of importance in many areas of cell biology. The mere existence of a novel biological structure, which has been documented in this Review, generally implies the existence of novel functions. Whether this concept is true or not in the present case will no doubt be revealed by further investigation.

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References

Abu-Hasan, N. S. & Sutcliffe, R. G. (1984) in Human Alkaline Phosphatase, pp. 117–126, Alan R. Liss, New York

Bangs, J. D., Hereld, D., Krakow, J. L., Hart, G. W. & Englund, P. T. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 3207–3211

Bangs, J. D., Andrews, N. W., Hart, G. W. & Englund, P. T. (1986) J. Cell Biol. **103**, 255–263

Baron, M. D., Pope, B. & Luzio, J. P. (1986) Biochem. J. **236**, 495–502

Berger, J., Garattini, E., Hua, J.-C. & Udenfriend, S. (1987) Proc. Natl. Acad. Sci. U.S.A., in the press

Bordier, C., Etges, R. J., Ward, J., Turner, M. J. & Cardoso de Almeida, M. L. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 5988–5991

Bourgignon, L. Y. W., Suchard, S. J. & Kalomiris, E. L. (1986) J. Cell Biol. **103**, 2529–2540

Bowles, D. J. & Voorheis, H. P. (1982) FEBS Lett. **139**, 17–21

Bulow, R. & Overath, P. (1985) FEBS Lett. **187**, 105–110

Bulow, R. & Overath, P. (1986) J. Biol. Chem. **261**, 11918–11923

Campbell, D. G., Gagnon, J., Reid, K. B. M. & Williams, A. F. (1981) Biochem. J. **195**, 15–30

Capaldi, R. A. (1982) Trends Biochem. Sci. **7**, 292–295

Capceville, Y., Baltz, T., Deregnaucort, C. & Keller, A. M. (1986) Exp. Cell Res. **167**, 75–86

Cardoso de Almeida, M. L. & Turner, M. L. (1983) Nature (London) **302**, 349–352

Chapman, A., Fujimoto, K. & Kornfeld, S. (1980) J. Biol. Chem. **255**, 4441–4446

Chow, F.-L., Telen, M. J. & Rosse, W. F. (1985) Blood **66**, 940–945

Colbeau, A. & Maroux, S. (1978) Biochim. Biophys. Acta **511**, 39–51

Conzelmann, A., Spiazzi, A., Hyman, R. & Bron, C. (1986) EMBO J. **5**, 3291–3296
Craddock, P. R., Fehr, J. & Jacob, H. S. (1976) Blood **47**, 931–939
Creek, K. E., Rimoldi, D., Glifford, A. J., Silverman-Jones, C. S. & DeLuca, L. M. (1986) J. Biol. Chem. **261**, 3490–3500
Cross, G. A. M. (1979) Nature (London) **277**, 310–312
Cross, G. A. M. (1984) Philos. Trans. R. Soc. London Ser. B **307**, 3–12
Davitz, M. A., Low, M. G. & Nussenzweig, V. (1986) J. Exp. Med. **163**, 1150–1161
Davitz, M. A., Gurnett, A. M., Low, M. G., Turner, M. J. & Nussenzweig, V. (1987) J. Immunol. **138**, 520–523
Devlin, J. J., Weiss, E. H., Paulson, M. & Flavell, R. A. (1985) EMBO J. **4**, 3203–3207
Dragsten, P., Henkart, P., Blumenthal, R., Weinstein, J. & Schlessinger, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 5163–5167
Dutta-Choudhury, T. A. & Rosenberry, T. L. (1984) J. Biol. Chem. **259**, 5653–5660
Duvillier, G., Nouvelot, A., Richet, C., Baltz, T. & Degand, P. (1983) Biochem. Biophys. Res. Commun. **114**, 119–125
Etges, R., Bouvier, J. & Bordier, C. (1986*a*) EMBO J. **5**, 597–601
Etges, R., Bouvier, J. & Bordier, C. (1986*b*) J. Biol. Chem. **261**, 9098–9101
Fatemi, S. H. & Tartakoff, A. M. (1986) Cell **46**, 653–657
Fatemi, S. H., Haas, R., Jentoft, N., Rosenberry, T. L. & Tartakoff, A. M. (1987) J. Biol. Chem., in the press
Ferguson, M. A. J. & Cross, G. A. M. (1984) J. Biol. Chem. **259**, 3011–3015
Ferguson, M. A. J., Haldar, K. & Cross, G. A. M. (1985*a*) J. Biol. Chem. **260**, 4963–4968
Ferguson, M. A. J., Low, M. G. & Cross, G. A. M. (1985*b*) J. Biol. Chem. **260**, 14547–14555
Ferguson, M. A. J., Duszenko, M., Lamont, G. S., Overath, P. & Cross, G. A. M. (1986) J. Biol. Chem. **261**, 356–362
Ferguson, M. A. J., Dwek, R. A., Homans, S. W. & Rademacher, T. W. (1987) in NATO/ASI Volume: Host–Parasite Cellular and Molecular Interactions in Protozoal Infections (Chang, K.-P., ed.), Springer-Verlag, Heidelberg, in the press
Fliesler, S. J. & Anderson, R. E. (1986) Biochem. Biophys. Res. Commun. **136**, 815–821
Fox, J. A., Duszenko, M., Ferguson, M. A. J., Low, M. G. & Cross, G. A. M. (1986) J. Biol. Chem. **261**, 15167–15771
Fox, J. A., Soliz, N. M. & Saltiel, A. R. (1987) Proc. Natl. Acad. Sci. U.S.A., in the press
Futerman, A. H., Low, M. G. & Silman, I. (1983) Neurosci. Lett. **40**, 85–89
Futerman, A. H., Low, M. G., Ackermann, K. E., Sherman, W. L. & Silman, I. (1985*a*) Biochem. Biophys. Res. Commun. **129**, 312–317
Futerman, A. H., Low, M. G., Michaelson, D. M. & Silman, I. (1985*b*) J. Neurochem. **45**, 1487–1494
Futerman, A. H., Fiorini, R.-M., Roth, E., Low, M. G. & Silman, I. (1985*c*) Biochem. J. **226**, 369–377
Gaston, S. M., Marchase, R. B. & Jakoi, E. R. (1982) J. Cell. Biochem. **18**, 447–459
Gennarini, G., Rougon, G., Deagostini-Bazin, H., Hirn, M. & Goridis, C. (1984) Eur. J. Biochem. **142**, 57–64
Gogol, E. P. & Engelman, D. M. (1984) Biophys. J. **46**, 491–495
Greenfield, S. (1984) Trends Neurosci. **7**, 364–368
Griffin, M. J. & Palakotedy, R. B. (1985) in Inositol and Phosphoinositides: Metabolism and Regulation (Bleasdale, J. E., Eichberg, J. & Hauser, G., eds.), p. 669, Humana Press, Clifton, NJ
Haas, R., Brandt, P. T., Knight, J. & Rosenberry, T. L. (1986) Biochemistry **25**, 3098–3105
Haldar, K., Ferguson, M. A. J. & Cross, G. A. M. (1985) J. Biol. Chem. **260**, 4969–4974
Haldar, K., Henderson, C. L. & Cross, G. A. M. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 8565–8569
Hantke, K. & Braun, V. (1973) Eur. J. Biochem. **34**, 284–296
He, H.-T., Barbet, J., Chaix, J.-C. & Goridis, C. (1986) EMBO J. **5**, 2489–2494
Hemperly, J. J., Edelman, G. M. & Cunningham, B. A. (1986) Proc. Natl. Acad. Sci. U.S.A., **83**, 9822–9826
Henthorn, P. S., Knoll, B. J., Raducha, M., Rothblum, K. N., Slaughter, C., Weiss, M., Lafferty, M. A., Fischer, T. & Harris, H. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 5597–5601
Hereld, D., Krakow, J. L., Bangs, J. D., Hart, G. W. & Englund, P. T. (1986) J. Biol. Chem. **261**, 13813–13819
Holder, A. A. (1983) Biochem. J. **209**, 261–262
Holder, A. A. (1985) Curr. Top. Microbiol. Immunol. **117**, 57–74
Holder, A. A. & Cross, G. A. M. (1981) Mol. Biochem. Parasitol. **2**, 135–150
Horton, M. A. & Hyman, R. (1983) Immunogenetics **17**, 261–270
Hyman, R. (1985) Biochem. J. **225**, 27–40
Ikezawa, H., Yamanegi, M., Taguchi, R., Miyashita, T. & Ohyabu, T. (1976) Biochim. Biophys. Acta **450**, 154–164
Ishihara, A., Hou, Y. & Jacobson, K. (1987) Proc. Natl. Acad. Sci. U.S.A., in the press
Ishihara, M., Fedarko, N. S. & Conrad, H. E. (1987) J. Biol. Chem., in the press
Jacobson, K., Ishihara, A. & Inman, R. (1987) Annu. Rev. Physiol., in the press
Jemmerson, R., Shah, N., Takeya, M. & Fishman, W. H. (1984) in Human Alkaline Phosphatases, pp. 105–115, Alan R. Liss, New York
Kam, W., Clauser, E., Kim, Y. S., Kan, Y. W. & Rutter, W. J. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 8715–8719
Koch, F., Thiele, H.-G. & Low, M. G. (1986) J. Exp. Med. **164**, 1338–1343
Kominami, T., Miki, A. & Ikehara, Y. (1985) Biochem. J. **227**, 183–189
Krakow, J. L., Hereld, D., Bangs, J. D., Hart, G. W. & Englund, P. T. (1986) J. Biol. Chem. **261**, 12147–12153
Kreil, G. (1981) Annu. Rev. Biochem. **50**, 317–348
LeClair, K. P., Palfree, R. G. E., Flood, P. M., Hammerling, U. & Bothwell, A. (1986) EMBO J. **5**, 3227–3234
Levy, J. R., Murray, E., Manolagas, S. & Olefsky, J. M. (1986) Endocrinology (Baltimore) **119**, 1786–1792
Low, M. G. (1987) in Thy-1: Immunology, Neurology and Therapeutic Applications (Reif, A. E. & Schlesinger, M., eds.), Marcel Dekker, New York, in the press
Low, M. G. & Finean, J. B. (1977*a*) FEBS Lett. **82**, 143–146
Low, M. G. & Finean, J. B. (1977*b*) Biochem. J. **167**, 281–284
Low, M. G. & Finean, J. B. (1978) Biochim. Biophys. Acta **508**, 565–570
Low, M. G. & Kincade, P. W. (1985) Nature (London) **318**, 62–64
Low, M. G. & Prasad, A. R. S. (1987) Fed. Proc. Fed. Am. Soc. Exp. Biol. **46**, in the press
Low, M. G. & Weglicki, W. B. (1981) Fed. Proc. Fed. Am. Soc. Exp. Biol. **40**, 826
Low, M. G. & Weglicki, W. B. (1983) Biochem. J. **215**, 325–334
Low, M. G. & Zilversmit, D. B. (1980) Biochemistry **19**, 3913–3918
Low, M. G., Owens, K. & Weglicki, W. B. (1980) Fed. Proc. Fed. Am. Soc. Exp. Biol. **39**, 2102
Low, M. G., Ferguson, M. A. J., Futerman, A. H. & Silman, I. (1986*a*) Trends Biochem. Sci. **11**, 212–215
Low, M. G., Carroll, R. C. & Cox, A. C. (1986*b*) Biochem. J. **237**, 139–145
Low, M. G., Futerman, A. H., Ackermann, K. E., Sherman, W. R. & Silman, I. (1987) Biochem. J. **241**, 615–619
Luzio, J. P., Baron, M. D. & Bailyes, E. M. (1987) in Mammalian Ectoenzymes (Kenny, A. J. & Turner, A. J., eds.), Elsevier Science Publishers, Amsterdam, in the press
Maeda, K., Ohta, Y., Nakabayashi, T., Taguchi, R. & Ikezawa, H. (1986) Biochem. Int. **12**, 855–863

Magee, A. I. & Courtneidge, S. A. (1985) EMBO J. **4**, 1137–1144
Magee, A. I. & Schlesinger, M. J. (1982) Biochim. Biophys. Acta **694**, 279–289
Majumdar, R. & Balasubramanian, A. S. (1982) FEBS Lett. **146**, 335–338
Majumdar, R. & Balasubramanian, A. S. (1985) Biochem. Pharmacol. **34**, 4109–4115
Malik, A. S. & Low, M. G. (1986) Biochem. J. **240**, 519–527
Markello, T., Zlotnick, A., Everett, J., Tennyson, J. & Holloway, P. W. (1985) Biochemistry **24**, 2895–2901
McComb, R. B., Bowers, G. N. & Rosen, S. (1979) in Alkaline Phosphatases, pp. 525–786, Plenum Press, New York and London
Medof, M. E., Walter, E. I., Roberts, W. L., Haas, R. & Rosenberry, T. L. (1986) Biochemistry **25**, 6740–6747
Miki, A., Kominami, T. & Ikehara, Y. (1985) Biochem. Biophys. Res. Commun. **126**, 89–95
Millan, J. L. (1986) J. Biol. Chem. **261**, 3112–3115
Mizobe, F., Iwamoto, M. & Livett, B. G. (1984) J. Neurochem. **42**, 1433–1438
Moriuchi, T. & Silver, J. (1985) FEBS Lett. **178**, 105–108
Nakabayashi, T. & Ikezawa, H. (1984) Cell Struct. Funct. **9**, 247–263
Nakabayashi, T. & Ikezawa, H. (1986) J. Biochem. (Tokyo) **99**, 703–712
Overath, P., Czichos, J., Stock, U. & Nonnengaesser, C. (1983) EMBO J. **2**, 1721–1728
Ovitt, C. E., Strauss, A. W., Alpers, D. H., Chou, J. Y. & Boime, I. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 3781–3785
Ozols, J., Carr, S. A. & Strittmatter, P. (1984) J. Biol. Chem. **259**, 13349–13354
Palfree, R. G. E. & Hammerling, U. (1986) J. Immunol. **136**, 594–600
Panagia, V., Heyliger, C. E., Choy, P. C. & Dhalla, N. S. (1981*a*) Biochim. Biophys. Acta **640**, 802–806
Panagia, V., Michiel, D. F., Dhalla, K. S., Nijjai, M. S., & Dhalla, N. S. (1981*b*) Biochim. Biophys. Acta **676**, 395–400
Parker, C. J., Soldato, C. M. & Rosse, W. F. (1984) J. Clin. Invest. **73**, 1130–1143
Prasad, A. R. S. & Low, M. G. (1987) Fed. Proc. Fed. Am. Soc. Exp. Biol. **46**, in the press
Reiser, H., Oettgen, H., Yeh, E. T. H., Terhorst, C., Low, M. G., Benacerraf, B. & Rock, K. L. (1986) Cell **47**, 365–370
Rifkin, M. R. & Fairlamb, A. H. (1985) Mol. Biochem. Parasitol. **15**, 245–256
Roberts, W. L. & Rosenberry, T. L. (1985) Biochem. Biophys. Res. Commun. **133**, 621–627
Roberts, W. L. & Rosenberry, T. L. (1986*a*) Biochemistry **25**, 3091–3098
Roberts, W. L. & Rosenberry, T. L. (1986*b*) Fed. Proc. Fed. Am. Soc. Exp. Biol. **45**, 1816
Sabatini, D. D., Kreibich, G., Morimoto, T. & Adesnik, H. (1982) J. Cell Biol. **92**, 1–22
Sadoul, K., Meyer, A., Low, M. G. & Schachner, M. (1986) Neurosci. Lett. **72**, 341–346
Saltiel, A. R. (1987) Endocrinology (Baltimore), in the press
Saltiel, A. R. & Cuatrecasas, P. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 5793–5797
Saltiel, A. R., Fox, J. A., Sherline, P. & Cuatrecasas, P. (1986) Science **233**, 967–972
Saltiel, A. R., Sherline, P. & Fox, J. A. (1987) J. Biol. Chem., in the press
Schlesinger, M. J. (1981) Annu. Rev. Biochem. **50**, 193–206
Schmidt, M. F. G. (1983) Curr. Top. Microbiol. Immunol. **102**, 101–129
Schumacher, M., Camp, S., Maulet, Y., Newton, M., MacPhee-Quigley, K., Taylor, S. S., Friedmann, T. & Taylor, P. (1986) Nature (London) **319**, 407–409
Seki, T., Chang, H.-C., Moriuchi, T., Denome, R., Ploegh, H. & Silver, J. (1985*a*) Science **227**, 649–652
Seki, T., Moriuchi, T., Chang, H.-C., Denome, R. & Silver, J. (1985*b*) Nature (London) **313**, 485–487
Shukla, S. D. (1982) Life Sci. **30**, 1323–1335
Shukla, S. D. (1986) Life Sci. **38**, 751–755
Shukla, S. D., Coleman, R., Finean, J. B. & Michell, R. H. (1980) Biochem. J. **187**, 277–280
Singer, S. J. & Nicholson, G. L. (1972) Science **175**, 720–731
Slein, M. W. & Logan, G. F. (1960) J. Bacteriol. **80**, 77–85
Slein, M. W. & Logan, G. F. (1962) J. Bacteriol. **83**, 359–369
Slein, M. W. & Logan, G. F. (1963) J. Bacteriol. **85**, 369–381
Slein, M. W. & Logan, G. F. (1965) J. Bacteriol. **90**, 69–81
Soloski, M. J., Uhr, J. W. & Vitetta, E. S. (1982) Nature (London) **296**, 759–761
Soloski, M. J., Vernachio, J., Einhorn, G. & Lattimore, A. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 2949–2953
Stieger, A. & Brodbeck, U. (1985) J. Neurochem. **44**, 48–56
Stieger, A., Cardoso de Almeida, M.-L., Blatter, M.-C., Brodbeck, U. & Bordier, C. (1986) FEBS Lett. **199**, 182–186
Stiernberg, J., Low, M. G., Flaherty, L. & Kincade, P. W. (1987) J. Immunol., in the press
Strang, A.-M., Williams, J. M., Ferguson, M. A. J., Holder, A. A. & Allen, A. K. (1986) Biochem. J. **234**, 481–484
Sudhof, T. C., Goldstein, J. L., Brown, M. S. & Russell, D. W. (1985) Science **228**, 815–822
Taguchi, T. & Ikezawa, H. (1978) Arch. Biochem. Biophys. **186**, 196–201
Taguchi, R., Asahi, Y. & Ikezawa, H. (1980) Biochim. Biophys. Acta **619**, 48–57
Taguchi, R., Suzuki, K., Nakabayashi, T. & Ikezawa, H. (1984) J. Biochem. (Tokyo) **96**, 437–446
Takesue, Y., Yokota, K., Nishi, Y., Taguchi, R. & Ikezawa, H. (1986) FEBS Lett. **201**, 5–8
Tanaka, K. R., Valentine, W. N. & Fredricks, R. E. (1960) N. Engl. J. Med. **262**, 912–918
Tse, A. G. D., Barclay, A. N., Watts, A. & Williams, A. F. (1985) Science **230**, 1003–1008
Tung, E., Wang, I. Y., Xu, C. S. & Wang, A. C. (1987) Immunol. Invest., in the press
Turco, S. J., Johnson, C. L., King, D. L., Orlandi, P. & Wright, B. L. (1987) in NATO/ASI Volume: Host–Parasite Cellular and Molecular Interactions in Protozoal Infections (Chang, K.-P., ed.), Springer-Verlag, Heidelberg, in the press
Turner, M. J. (1984) Philos. Trans. R. Soc. London Ser. B **307**, 27–40
Weiss, M. J., Henthorn, P. S., Lafferty, M. A., Slaughter, C., Raducha, M. & Harris, H. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 7182–7186
Williams, A. F. & Tse, A. G. D. (1985) Biosci. Rep. **5**, 999–1005
Woda, B. A. & Gilman, S. C. (1983) Cell Biol. Int. Rep. **7**, 203–209
Wu, H. C. & Tokunaga, M. L. (1986) Curr. Top. Microbiol. Immunol. **125**, 127–157
Yang, J. C., Chang, P. C., Fujitaki, J. M., Chiu, K. C. & Smith, R. A. (1986) Biochemistry **25**, 2677–2681