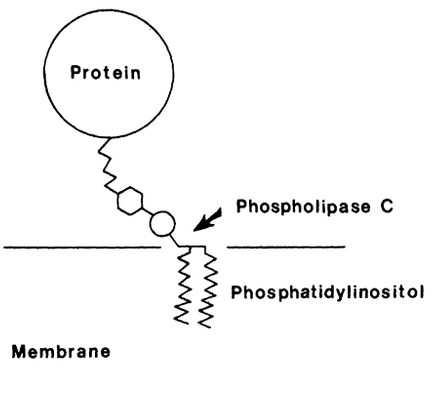
Novel Protein/Membrane Attachment

Several investigators, working independently, have discovered an unsuspected method by which proteins attach to cell surface membranes. The linkage, which may turn out to be ubiquitous, may also be important in the specific release of proteins from membranes.

Among the first to come upon this membrane attachment phenomenon were Martin Low and his colleagues at the University of Birmingham (Low is now at the Oklahoma Medical Research Foundation in Oklahoma City). Low started out in the fall of 1976, working with the enzyme phosphatidylinositol-specific phospholipase C, which, when added to cells, causes them to release alkaline phosphatase into the medium. The enzyme works by cleaving phosphatidylinositol, the membrane phospholipid. The break occurs between the glycerol backbone and the phosphate. Although Low and his colleagues were investigating other properties of the enzyme, they found that the enzyme clipped several proteins from cell surfaces, indicating that these proteins might be anchored to the membrane in some unusual way.

It is widely known that many proteins are attached to membranes by hydrophobic sections that seek out the fatty acids of the membrane and are repelled by the aqueous surrounding medium. But Low's enzyme cleaved phosphatidylinositol and released three unrelated membrane proteins—acetylcholinesterase, 5′nucleotidase, and alkaline phosphatase. Perhaps, Low reasoned, these proteins are attached to the membrane because they are covalently linked to lipid, specifically to the inositol ring of phosphatidylinositol.



Protein/membrane linkage

Low's hypothesis was widely disregarded. "People found our evidence difficult to accept," Low remarks. "There were no examples of covalent links between phospholipids and proteins and it was generally accepted that hydrophobic peptides hold membrane proteins in membranes."

But corroborating evidence did turn up—although from an entirely unexpected source. About 2 years ago, Michael Ferguson and George Cross at Rockefeller University managed to isolate the membrane anchor of the variable surface glycoprotein, or VSG, from African trypanosomes, which cause sleeping sickness. This protein coats the surface of the organisms and changes repeatedly, enabling trypanosomes to avoid attacks by the immune system. They knew from previous work by Tony Holder and M. Lucia Cardosa de Almeida of Wellcome Laboratories and Mervin Turner of Cambridge University that the membrane anchor of VSG was not a peptide. Now Ferguson and Cross could set out to find what it was.

Upon further investigation, they learned that VSG is attached to a glycophospholipid in the membrane, the lipid portion of which is phosphatidylinositol. This attachment occurs almost immediately upon synthesis of the protein. When VSG proteins are made, they have a hydrophobic sequence at their carboxy terminus, but, within 1 minute after synthesis, this hydrophobic section is cut off and is replaced by the glycophospholipid.

Next, Ferguson and Kasturi Haldar identified the endogenous enzyme in the trypanosomes that can release the VSG protein from the membrane. The enzyme is a phospholipase C. "We don't know what role this enzyme plays," Ferguson says, "but it is possible that trypanosomes have to shed their VSG coats during their life cycles." They lose their coats entirely when they are in the gut of the tsetse fly and this may be when the enzyme is used, Ferguson speculates.

While Ferguson and his colleagues were accumulating evidence that VSG is anchored to membranes with a glycophospholipid, Low and Israel Silman of the Weizmann Institute in Rehovot found that acetylcholinesterase from torpedo is covalently linked to phosphatidylinositol. In the meantime, Alan Williams of Oxford University discovered that the brain and lymphocyte protein thy-1 is also attached to membranes in this unusual way. Williams first noticed that thy-1 has no hydrophobic sequences. Then he found that it is attached in membranes to a glycophospholipid. Moreover, when thy-1 is synthesized, it originally has a hydrophobic region at its carboxy terminus but, like the VSG protein, this region is quickly cleaved and is replaced by the glycophospholipid. Finally, Low and his colleague Paul Kincade found that the enzyme phophatidylinositol-specific phospholipase C releases thy-1 from cell surfaces.

The leading question, of course, is why would proteins be attached to membranes in this unexpected way? "Martin [Low] and my pet theory is that it allows the cells to release the proteins in response to stimuli," Ferguson says. "The advantage of attaching proteins to a glycolipid is that it allows much greater specificity. You can hit a particular protein rapidly without damaging other membrane proteins." Such a system would clearly endow a cell with a very useful dimension of metabolic flexibility.

Although the linkage method is not unique to trypanosomes, Ferguson hopes it might be exploited in attacking them. "Trypanosomes are absolutely dependent on the integrity of their coats," he says. "If the attachment of VSG proteins to the membrane is disrupted, trypanosomes are not viable. You don't have to affect this attachment for long and it may be that trypanosomes are affected more by drugs [that break the protein-phospholipid bond] than are their hosts."—**GINA KOLATA**

Additional Readings

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4. M. A. J. Ferguson *et al.*, *ibid.*, in press.
5. M. A. J. Ferguson and G. A. N. Cross, *ibid.*, **259**, 3011 (1984).