Lipid Linked Sugars in Glycoprotein Synthesis

The oligosaccharide chain of certain glycoproteins is preassembled on a polyprenol carrier.

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The discovery of sugar nucleotides by Leloir and co-workers in 1950 (*1*) initiated a new era of research on the role of these activated compounds as donors of sugar residues in the synthesis of complex glycans and glycoproteins. Fifteen years later a new class of activated sugar derivatives was discovered in bacteria, in which the sugar is glycosidically linked via either a phosphate or a pyrophosphate bridge to the polyisoprenoid alcohol, undecaprenol (*2*). In contrast to the hydrophilic sugar nucleotides, these activated sugar derivatives are hydrophobic, and their synthesis from sugar nucleotide and undecaprenol phosphate is catalyzed by enzymes associated with the cell membrane. Within a few years, it was clear that these hydrophobic compounds played a key role in the synthesis of a great variety of complex glycans of the bacterial cell envelope (*3*). The sequence of reactions involved in synthesis of one such glycan, the lipopolysaccharide of *Salmonella typhimurium* (*4*), is shown in Fig. 1. As soon as the importance of the lipid linked intermediates in the synthesis of bacterial glycans became obvious, investigations were undertaken on the possible existence of similar reactions for the synthesis of the oligosaccharide chains of mammalian glycoproteins.

The mechanism of assembly of the oligosaccharide chains of glycoproteins of eukaryotic cells has been the subject of numerous investigations during the past 20 years. At the present time there is great interest in the biosynthesis and the function of membrane glycoproteins, particularly those associated with the cell surface, because evidence is accumulating that these compounds may be involved in cell-cell interactions. It is thought that enzymatic modifications of the surface glycoproteins, or their interaction with other molecules, may be instrumental in the regulation and differentiation of cell growth (see below).

The earliest biosynthetic studies were consistent with the possibility that polyprenol-linked sugars are formed in mammalian systems and that they participate in the assembly of glycoproteins, but the evidence was by no means compelling. However, in the last 2 or 3 years the participation of lipid linked sugars in the glycosylation of certain membrane-associated proteins has been clearly established. In general terms the process appears to involve three types of reactions:

1) Transfer of a glycose (that is, a monosaccharide) or glycose-1-phosphate residue from sugar nucleotide to a polyprenol phosphate.

2) Assembly of an oligosaccharide linked to lipid (probably a polyprenol) by a phosphodiester or pyrophosphoryl bridge. Glycosyl phosphoryl polyprenols serve as glycosyl donors in this process, but the possibility that sugar nucleotides are also glycosyl donors is not excluded.

3) Transfer of the preassembled oligosaccharide chain from the oligosaccharide-lipid to protein acceptors.

My aim in this article is to review the current state of knowledge of the synthesis and function of these compounds, and to present some ideas and speculations on the possible physiological role of the complex enzyme system involved in their synthesis. Earlier work has been reviewed in detail (*3, 5, 6*).

Monosaccharide Derivatives of Dolichol Phosphate

Twenty years after Leloir's discovery of sugar nucleotides, Behrens and Leloir (*7*) presented firm evidence for formation of a polyprenol-linked derivative of glucose-1-phosphate from uridine diphosphate (UDP)–glucose and an endogenous lipid. The lipid moiety was tentatively identified to be dolichol, and this conclusion was subsequently confirmed by others (see below). The structures of dolichol and glucosyl phosphoryl dolichol are shown in Fig. 2.

The dolichols were first discovered in mammalian tissues more than 10 years ago (*8*). They consist of a family of polyisoprenols ranging in chain length from C_{90} to C_{100}, and thus have some of the largest molecular weights among the aliphatic compounds found in nature [see (*6*) for a review of these compounds]. They differ from the bacterial polyisoprenols (C_{50} to C_{60}) not only in chain length, but because in the dolichols the terminal isoprene unit bearing the hydroxyl group is saturated (Fig. 2). Consequently, the linkage of phosphate to dolichol is stable, whereas its linkage to undecaprenol, an allylic alcohol, is labile to mild acid. Dolichols are widely distributed in mammalian systems (*9*) and are found in largest quantities in the form of an ester of long chain fatty acids, although the free alcohol also is present (*10*). Apparently the level of dolichol phosphate in tissue is extremely low, and it has not yet been possible to assess its cellular concentration directly. Dallner *et al.* (*11*) have attempted to estimate the concentration of dolichol phosphate in different subcellular fractions of liver by measuring the stimulation of synthesis of glucosyl phosphoryl dolichol upon addition of dolichol phosphate isolated from that subcellular fraction. Their results suggest that it is widely but unevenly distributed in all subcellular fractions. However, the limitations of this approach are obvious, and other means of assessing subcellular distribution of dolichol phosphate must be developed.

After the discovery of glucosyl phosphoryl dolichol, the enzymatic synthesis of a number of other dolichol-linked sugars was reported. These include mannosyl phosphoryl dolichol (*12*), xylosyl phosphoryl dolichol (*13*), *N,N'*-diacetylchitobiosyl pyrophosphoryl dolichol (*14*), and *N*-acetylglucosaminyl pyrophosphoryl dolichol (*15*). It should be emphasized that in some cases the enzymatically synthesized glycosyl lipid has only been partially characterized. Often the basis for calling it a dolichol derivative has been its chromatographic properties and the fact that exogenous dolichol phosphate stimulates its synthesis. In contrast, considerably more evidence is available on the structure and biosynthesis of mannosyl phosphoryl dolichol. Richards and Hemming (*16*), using [^{3}H]dolichol phosphate and guanosine

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diphosphate (GDP)-[^{14}C]mannose, have directly demonstrated its enzymatic synthesis. Also, two groups of investigators (*17, 18*) have analyzed the structure of mannosyl phosphoryl dolichol by chemical and physical methods, and its synthesis by chemical methods has been achieved (*19*). It should also be mentioned that the enzymatic synthesis of a mannosyl derivative of retinol phosphate has been reported (*20*). A description of this compound and its possible role in glycoprotein synthesis have been reviewed (*6*).

Glucose-Containing Oligosaccharide Derivatives of Dolichol Phosphate

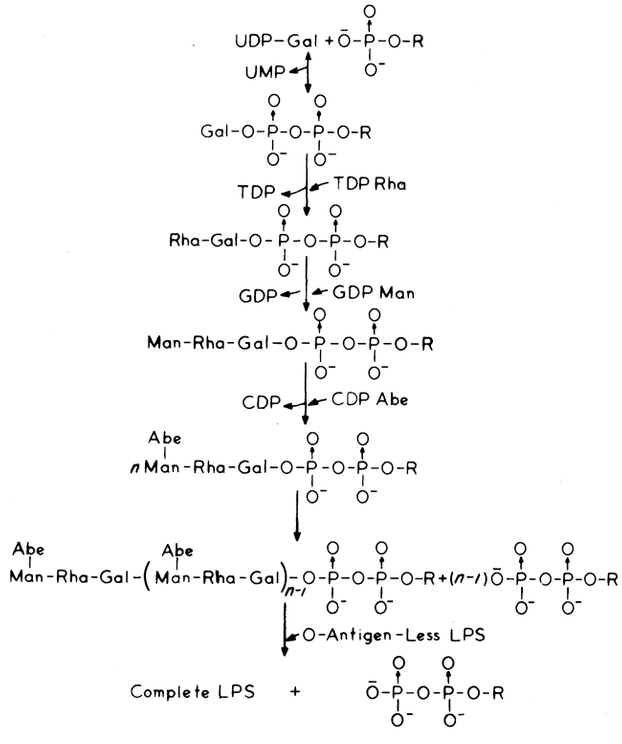
Studying the synthesis of glycosyl phosphoryl dolichol, Behrens and Leloir (*7*) reported that it could serve as a glucosyl donor for synthesis of glucose-containing proteins. Subsequent studies by these workers (*21*) revealed that, in addition to glucoprotein, another glucose-labeled compound was formed. This compound, which had the unusual properties of being insoluble in a mixture of chloroform and methanol and in tricholoroacetic acid, but soluble in a mixture of chloroform, methanol, and water (10 : 10 : 3), was shown to be a glucose-containing oligosaccharide containing approximately 20 hexose residues linked to a lipid (presumably dolichol) by a phosphate or a pyrophosphate bridge. More recent studies indicate that, in addition to glycosyl residues, the oligosaccharide probably contains *N*-acetylhexosamine units (*22*). A survey of microsomal preparations from a variety of tissues revealed that enzymes for synthesis of this oligosaccharide-lipid are widely distributed in animal tissues. The enzymatically synthesized oligosaccharide-lipid isolated from liver microsomes has been shown to serve as a donor of the oligosaccharide chain to endogenous microsomal acceptors that, by a number of criteria, have been shown to be proteins (*23*). Glucose-containing glycoproteins are rare, and it will be of great interest to characterize the glucose-labeled protein (or proteins) in more detail.

Mannose-Containing Oligosaccharide Derivatives of Dolichol Phosphate

More information is available on the synthesis and structure of a mannose-containing oligosaccharide-lipid and its role in glycoprotein synthesis. Both Richards and Hemming (*16*), using pig liver microsomes, and Baynes *et al.* (*17*), using mouse myeloma microsomes, showed that isolated mannosyl phosphoryl dolichol can serve as a mannosyl donor in the formation of mannose-containing glycoproteins, and several lines of evidence indicated that it is an obligatory intermediate in the process. Similar findings were reported from our laboratory by Waechter *et al.* (*24*), who used a membrane preparation from hen oviducts. Moreover, evidence was presented suggesting that an oligosaccharide-lipid formed from mannosyl phosphoryl dolichol serves as a donor for the introduction of oligosaccharide chains into protein. Direct proof for the participation of the oligosaccharide-lipid in this process was first obtained in the liver system by Behrens *et al.* (*25*), who isolated a mannose-containing oligosaccharide-lipid and showed that when it was added to a microsome preparation the oligosaccharide was incorporated into protein. Indirect evidence that the oligosaccharide of the oligosaccharide-lipid contained not only mannose, but hexosamine residues, was also reported.

On the basis of their earlier studies with a glucose-containing oligosaccharide, Behrens *et al.* (*25*) tentatively suggested that the oligosaccharide might contain not only mannose and hexosamine, but also glucose. However, recent studies by Hsu *et al.* (*26*) indicate this is not the case with the oligosaccharide formed by mouse myeloma. These workers showed that the oligosaccharide moiety isolated from the oligosaccharide-lipid contains only two sugars, mannose and *N*-acetylglucosamine, in a molar ratio of 5 : 2. The proposed structure for the oligosaccharide-lipid is that shown in Fig. 3.

The [^{14}C]mannose-labeled oligosaccharide moiety derived from the oligosaccharide-lipid synthesized by oviduct membranes has been partially characterized by Lucas *et al.* (*27*), and it seems possible that it is identical in structure to that synthe-



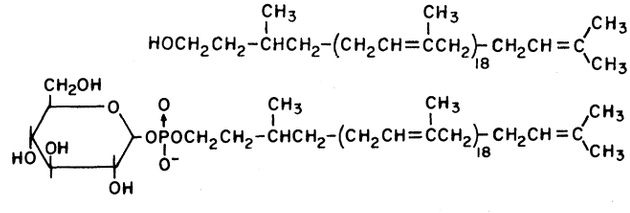


Fig. 1 (left). Sequence of reaction involved in synthesis of the O-antigen chain of the lipopolysaccharide of *Salmonella typhimurium* (*4*). Fig. 2 (above). Structure of dolichol and glucosyl phosphoryl dolichol (*7*).

sized in membrane preparations of mouse myeloma cells. On the basis of paper chromatography and gel filtration studies it was concluded that the oligosaccharide chain contains seven to nine glycose units. Chemical and enzymatic studies revealed that the glycose at the reducing terminus is *N*-acetylglucosamine, whereas mannose is found at the nonreducing end. When UDP-*N*-acetyl[^{14}C]glucosamine was incubated with oviduct membranes in the absence of GDP-mannose, the only hydrophobic product was a disaccharide-lipid containing two *N*-acetylglucosamine residues; no oligosaccharide-lipid was synthesized. When GDP-mannose was also present in the incubation mixture an oligosaccharide-lipid was formed that contained both mannose and *N*-acetyl[^{14}C]glucosaminyl residues. Treatment of this *N*-acetyl[^{14}C]glucosamine-labeled oligosaccharide with partially purified α-mannosidase releases the glucosamine-containing disaccharide [^{14}C]chitobiose. Recent studies with highly purified α-mannosidase on the oligosaccharide indicate that both α- and β-mannosyl units are present.

Chemical and enzymatic studies on the *N*-acetyl[^{14}C]glucosamine-labeled oligosaccharide revealed that the *N*-acetylglucosamine residue at the terminal reducing end of the chain is unlabeled (*27*). The available evidence indicates that the labeled *N*-acetylglucosamine residue is the second, nonreducing unit of the chitobiosyl residue in the oligosaccharide chain. This finding suggests that the enzyme preparation contains endogenous *N*-acetylglucosamine-lipid, which serves as an acceptor of the labeled exogenous *N*-acetylglucosamine and mannose residues according to the sequence shown in Fig. 4.

The above-mentioned studies in the liver (*25*), the mouse myeloma (*26*), and the oviduct systems (*27*) have shown that the isolated, partially purified [^{14}C]mannose-labeled oligosaccharide-lipid serves as a donor of the oligosaccharide chain to glycoprotein. A key question, however, is whether the entire oligosaccharide chain is transferred from the lipid to the protein. We have shown that both labels in doubly labeled oligosaccharide-lipid, containing [^{14}C]mannose and *N*-[^{3}H]acetylglucosamine, are transferred to glycoprotein at a constant ratio (*27*). Both labels were found in a constant ratio in the partially resolved glycopeptide fragments produced by proteolytic digestion of the enzymatically labeled glycoproteins. This finding provides strong evidence that an en bloc transfer of the oligosaccharide chain from oligosaccharide-lipid to protein occurs. Using a different approach, Hsu and co-workers

$$(Man)_5-(GlcNAc)_2-P-P-DoL$$

Fig. 3. Postulated structure of the oligosaccharide-lipid synthesized by mouse myeloma preparations (*26*). Dol-P-P is the abbreviation for dolichol pyrophosphate.

(*26*) have drawn the same conclusion. These workers have cleaved the oligosaccharide chain from the glycoprotein labeled from oligosaccharide-lipid in myeloma preparations. The resulting oligosaccharide contains *N*-acetylglucosamine at the reducing terminus and is chromatographically identical to the oligosaccharide in the oligosaccharide-lipid. Identity of the oligosaccharide of the oligosaccharide-lipid with that found in the glycoprotein has also been established in the oviduct system (*28*).

The structure of the oligosaccharide is of particular interest because of its similarity to the core structure of a variety of soluble secretory glycoproteins. This core oligosaccharide terminates in a chitobiosyl unit, in which the terminal *N*-acetylglucosamine residue is linked to an asparaginyl residue (Fig. 5). In many soluble glycoproteins (*29*), as well as in one membrane glycoprotein (*30*), the first mannosyl residue is linked to the interior *N*-acetylglucosaminyl residue by a β-linkage, whereas the more distal mannoses are linked by α-glycosidic bonds.

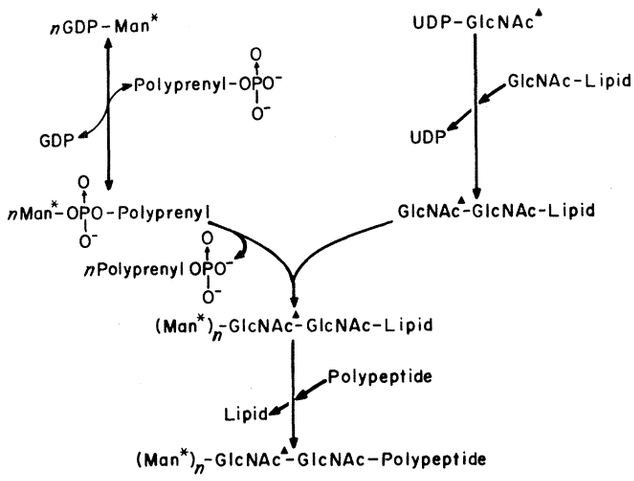
Levy *et al.* (*31*) have recently reported evidence for the formation of a trisaccharide-lipid containing a β-mannosyl unit linked to the chitobiosyl moiety. Moreover, very recent studies (*28*) utilizing highly purified glycosidases, and periodate degradation procedures, provide direct evidence that the oligosaccharide isolated from the oligosaccharide-lipid formed in oviduct has the typical glycoprotein core structure shown in Fig. 5.

Identification of the anomeric configurations of the linkages between the glycosyl residues in the oligosaccharide-lipid may provide a clue about the exact sequence of addition of the five or more mannosyl residues that are attached to the chitobiosyl unit. Behrens and co-workers (*25*) have speculated on the anomeric configuration of the glycosidic bonds in mannosyl phosphoryl dolichol, chitobiosyl pyrophosphoryl dolichol, and the oligosaccharide-lipid. They suggested that each transfer, starting with the original sugar nucleotides, both of which contain α-anomeric glycosidic bonds, might proceed with inversion of the anomeric configuration. If this proves to be the case, the sequence might be as shown in Fig. 6. This sequence would explain the presence of both α- and β-anomers linked to mannose unit. The recent demonstration (*32*) that conversion of *N*,*N*′-diacetylchitobiosyl pyrophosphoryl dolichol to the oligosaccharide-lipid is dependent on GDP-mannose, and does not proceed with mannosyl phosphoryl dolichol alone, is consistent with this scheme.

Nature of the Glycosylated Proteins

Little is known about the mannose-containing glycoproteins that are glycosylated via the lipid intermediate pathway. As would be expected, the proteins are insoluble in trichloroacetic acid and in organic solvents, and are susceptible to digestion by proteolytic enzymes. It is likely that the linkage of the oligosaccharide chain to the polypeptide is via an *N*-glycosidic linkage between C-1 of the terminal *N*-acetylglucosamine and an asparaginyl residue, but this point has not been proved. It is clear that the glycoprotein products found in liver (*25*), myeloma cells (*26*), and oviduct systems (*27*) are membrane bound. Thus, although both myeloma cells and oviduct tissues are responsible for synthesis of relatively large amounts of soluble, secretory glycoproteins (ovalbumin in oviduct and kappa-type immunoglobulin light chain in myeloma), the labeled glycoproteins are membrane associated. Moreover, immunological studies with appropriate antiserums and the solubilized membrane proteins indicate that no more than 10 to 20 percent of the myeloma product is antigenically reactive with antibody to kappa chains (*26*), and less than 10 percent of the oviduct product reacts with antibody to ovalbumin (*27*).

Sodium dodecyl sulfate–polyacrylamide disc gel electrophoresis of the oviduct membrane glycoproteins labeled with GDP-mannose has revealed that a number of polypeptide chains, differing in molecular weight, are labeled (*27*). Less than 10 percent of the label is found in the 45,000 molecular weight region of the gel, where ovalbumin migrates. Three proteins (apparent molecular weights of 75,000, 55,000, and 25,000) account for nearly two-thirds of the mannose-labeled glycoprotein products when GDP-mannose and either the crude membrane fraction or a total oviduct homogenate were used (*33*). Thus, all of the mannose acceptor proteins in the oviduct homogenate appear to be membrane bound. Recently it has been found that when oligosaccharide-lipid, rather than GDP-mannose, is used as substrate a polypeptide chain with an apparent molecular weight of 25,000 is the principal acceptor



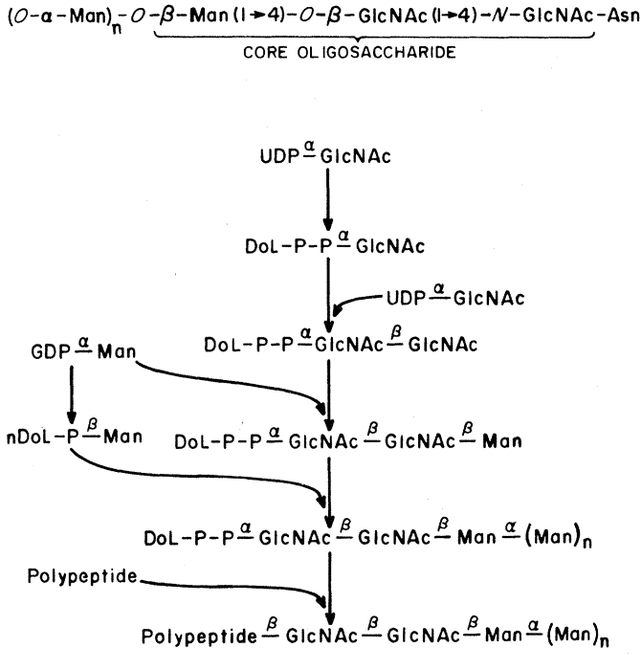


Fig. 4 (left). Reactions in the synthesis of the oligosaccharide chain of oviduct membrane glycoproteins. This scheme is similar to that proposed for the assembly of oligosaccharide-lipid and glycoproteins in liver (*25*) and mouse myeloma preparations (*26*). The lipid to which *N*-acetylglucosamine is linked has not yet been identified. Fig. 5 (top right). The structure of the core oligosaccharide of some secretory glycoproteins showing α-mannosyl residues attached to the β-linked mannose unit. Fig. 6 (bottom right). A biosynthetic sequence for synthesis of the "core" oligosaccharide showing possible inversions of the anomeric configuration of the various intermediates. No inversion is shown in the first reaction since the moiety transferred is the glycose-1-phosphate, not the glycose.

for the oligosaccharide moiety (*33*). Thus the product labeled by oligosaccharide-lipid appears to be identical to one of the three glycoproteins formed when GDP-mannose was incubated with the oviduct membrane preparation. It is of particular interest that analyses of the glycoproteins labeled from GDP-mannose in membrane fractions from hen kidney, liver, brain, and oviduct indicate that a labeled polypeptide of apparent molecular weight 25,000 is the only major protein product common to the four preparations.

Possible Function of the Lipid-Mediated Assembly Process

There seems to be little doubt now that there are two distinct types of reactions involved in the assembly of glycoproteins. The process whereby single sugars are sequentially transferred from their respective nucleotide derivatives to the distal portion of incomplete oligosaccharide chains of glycoproteins has been studied by many workers (*34*). In some cases the individual glycosyl transferases have been highly purified and shown to catalyze transfer of single sugar residues to purified protein acceptors. Under these circumstances there is little possibility that lipid intermediates are involved.

What, then, is the reason for the existence of a second process involving lipid linked intermediates in the assembly of the core oligosaccharide chains? A study of the subcellular distribution of the enzymes involved in assembly of the oligosaccharide-lipid and in transfer of the oligosaccharide chain to protein may provide some clues. The available information suggests that highest activities are found in the endoplasmic reticulum (*11, 16, 26*) and possibly in the outer mitochondrial membrane fractions (*11*). However, in no case has a thorough study establishing the purity of the subcellular fractions and the true specific activities in each fraction been reported. Thus, at present the only points that are certain are that the enzymes involved in assembly and transfer of the oligosaccharide chain are associated with a membrane fraction and, in cell-free preparations, the proteins that are glycosylated remain tightly associated with the membranes.

On the basis of this very limited information, one working hypothesis is that the lipid-mediated assembly process functions in a step in which nascent proteins already associated with the different membranous components of the cell are converted to glycoproteins by addition of the core oligosaccharide (Fig. 7). Evidence that membrane glycoproteins are asymmetrically distributed, with the majority of the oligosaccharide chains near or beyond the periphery of the membrane has been summarized (*35*). Perhaps proteins destined to be membrane glycoproteins are introduced into that particular membrane without their bulky, hydrophilic oligosaccharide chains. The oligosaccharide chains linked to the hydrophobic polyprenol could be transferred to amino acid residues of the polypeptide chain that are located near the surface of the membrane. Alternatively, the oligosaccharide chains could be transferred to amino acid residues located in regions of the polypeptide chain that are in the interior of the membrane. The presence of the bulky, hydrophilic oligosaccharide groups in the hydrophobic interior of the membrane might provide sufficient driving force for a conformational rearrangement of the protein, resulting in placement of the oligosaccharide residues at the surface of the membrane.

Although this proposed mechanism could be operative in a variety of membranes, such as those of the Golgi and the endoplasmic reticulum, it is illustrated (Fig. 7) in the more specific context of the glycoproteins of the plasma membrane of animal cells. A number of studies have shown that intact cells possess surface-associated glycosyltransferase activities that catalyze transfer of sugars from exogenous sugar nucleotides to glycoproteins (*36*). Recent evidence with the use of GDP-mannose (which presumably cannot enter the cell) as the glycosyl donor suggests that mannosylated intermediates, probably linked to lipid, are formed in chick embryo liver cells (*37*), normal and transformed mouse fibroblasts (*38*), and in hen oviduct slices and cell suspensions (*39*). Thus, if indeed GDP-mannose cannot permeate the

cell, it seems possible that the lipid-mediated oligosaccharide assembly process first detected in cell-free membrane preparations is operative at the cell surface. These findings with exogenous sugar nucleotides are somewhat puzzling since it seems highly unlikely that under normal conditions the extracellular space in tissues contains significant concentrations of sugar nucleotides. One possibility is that the enzymes that catalyze the initial transfer of glycose units from the sugar nucleotide to dolichol derivatives are located within the plasma membrane in such a way that they can act on sugar nucleotide substrate on either side of the membrane, although normally they only encounter sugar nucleotide on the cytoplasmic side of the membrane.

The hypothesis of Roseman (*40*) that *trans* glycosylation between adjacent cells is involved in the cell adhesion process should be mentioned in the context of the lipid linked activated sugars. This hypothesis holds that an enzyme on the surface of one cell catalyzes the glycosylation of a protein on the surface of another cell. However, it is unclear how the necessary sugar nucleotide can pass through the permeability barrier of the cell membrane into the extracellular space. However, if the scheme outlined in Fig. 7 is correct, this problem is obviated because the activated sugar that must penetrate the membrane is linked to a lipid and can readily diffuse to the outer surface of the membrane. The activated sugar (or oligosaccharide) derivative would thus be equally available to participate as a substrate in *trans* glycosylation, or in *cis* glycosylation on the same cell, as shown in Fig. 7. It should be emphasized, however, that the recent findings on membrane-associated, lipid linked sugars provide no direct evidence for or against the basic features of Roseman's hypothesis. They merely provide a possible explanation for one aspect of the hypothesis that was not readily explicable.

Soluble Glycoproteins

In this article I have focused by attention on membrane glycoproteins, and have not considered the important question of whether or not the soluble, secretory glycoproteins are glycosylated via lipid linked intermediates. The fact is that there is not yet enough information to answer this question. Clearly, there is similarity, if not identity, in the structure of the mannose- and *N*-acetylglucosamine-containing oligosaccharide linked to lipid and the structure of the oligosaccharide core of many soluble glycoproteins. Perhaps the lipid-mediated reactions are involved only in assembly of this core region of the secretory glycoproteins. The finding that only a small fraction of the glycoprotein labeled in cell-free preparations of myeloma and oviduct tissue appears to be the secretory glycoproteins that are made in large quantities by these cells does not preclude this possibility. Perhaps components essential for the completion of these secretory glycoproteins are absent from the membrane preparations used in these studies. Or possibly the level of nascent, unglycosylated polypeptide that serves as glycosyl acceptor is extremely low in the membrane preparations. Although there is a great deal of published experimental evidence (*34*) in favor of the "classical" process for secretory glycoprotein synthesis, involving the sequential addition of single sugar residues, there is relatively little information on transfer of the sugars directly from sugar nucleotides to the core region of the oligosaccharide chain. Possibly it is only this core that is assembled via a lipid linked process. In any case, considering the intense research activity in this field, it should not be long before we have the answer to this important question.

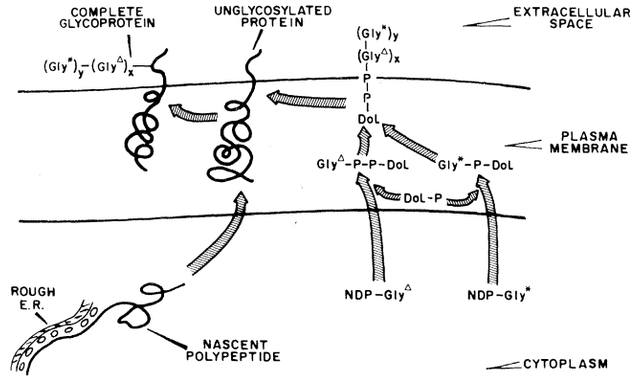


Fig. 7. A model for the glycosylation of plasma membrane glycoproteins via the lipid intermediate pathway. Gly^{Δ} and Gly* represent two different glycosyl residues.

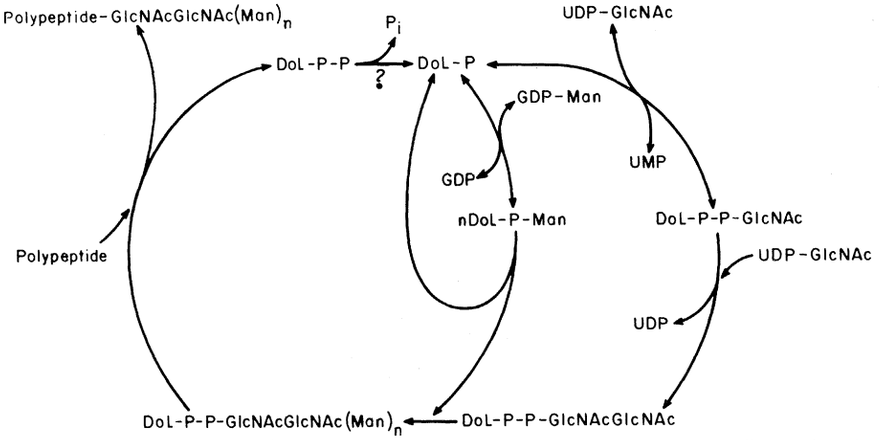


Fig. 8. Postulated pathway for the assembly of oviduct membrane glycoproteins illustrating the function of dolichol phosphate as a coenzyme.

Summary

It is now clear that one of the pathways of synthesis of glycoproteins involves the preassembly of at least a portion of the oligosaccharide chain while it is attached to the lipid dolichol phosphate. A sequence for the overall process similar to that postulated by Hsu and co-workers (*26*) is illustrated in Fig. 8, although it should be emphasized that not all the individual reactions have been documented in detail. The general features of this process are remarkably similar to those involved in synthesis of the complex glycans of bacteria, especially the O-antigen chain of lipopolysaccharide (Fig. 1). In the postulated sequence the dolichol phosphate functions as a coenzyme, either directly or after removal of the second phosphate group of dolichol pyrophosphate. It will be of interest to determine whether the level of dolichol phosphate in membranes plays a role in the cellular regulation of glycoprotein synthesis.

Although rapid progress is being made in elucidation of the reactions involved in

assembly of the oligosaccharide-lipid, little is yet known about the membrane glycoproteins, and possibly secretory glycoproteins, that are formed via this pathway. The elucidation of the structure and the function of these glycoproteins remains as a formidable challenge to biochemists and cell biologists.

Note added in proof: Very recently, experiments with intact oviduct cells in suspension (*41*) showed that the surface of these cells contains enzymes that catalyze synthesis of both mannosyl phosphoryl dolichol and oligosaccharide-lipid from exogenous GDP-mannose. In relation to the question of the participation of lipid linked intermediates in glycosylation of secretory glycoproteins, evidence indicating that this may indeed be so in the case of the kappa-type immunoglobulin light chain has very recently been reported (*42*).

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Asilomar Conference on Recombinant DNA Molecules*

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I. Introduction and General Conclusions

This meeting was organized to review scientific progress in research on recombinant DNA molecules and to discuss appropriate ways to deal with the potential biohazards of this work. Impressive scientific achievements have already been made in this field, and these techniques have a remarkable potential for furthering our understanding of fundamental biochemical processes in pro- and eukaryotic cells. The use of recombinant DNA methodology promises to revolutionize the practice of molecular biology. Although there has as yet been no practical application of the new techniques, there is every reason to believe that they will have significant practical utility in the future.

Of particular concern to the participants at the meeting was the issue of whether the pause in certain aspects of research in this area, called for by the Committee on Recombinant DNA Molecules of the National Academy of Sciences in the letter published in July 1974 (*1*), should end, and, if so, how the scientific work could be undertaken with minimal risks to workers in laboratories, to the public at large, and to the animal and plant species sharing our ecosystems.

The new techniques, which permit combination of genetic information from very different organisms, place us in an area of biology with many unknowns. Even in the present, more limited conduct of research in this field, the evaluation of potential biohazards has proved to be extremely difficult. It is this ignorance that has compelled us to conclude that it would be wise to exercise considerable caution in performing this research. Nevertheless, the participants at the Conference agreed that most of the work on construction of recombinant DNA molecules should proceed, provided that appropriate safeguards, principally biological and physical barriers adequate to contain the newly created organisms, are employed. Moreover, the standards of protection should be

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