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Protein Glycosylation in the Endoplasmic Reticulum: Current Topological Issues†

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The earliest studies on the glycosylation of proteins utilized radioautography and focused on the subcellular sites of protein synthesis and glycosylation [Neutra & Leblond, 1968; summarized by Palade (1975)]. From these studies it appeared that the assembly of the oligosaccharide chains of N-linked glycoproteins occurred in two phases: the initial phase was believed to take place in the endoplasmic reticulum, whereas the second occurred in the Golgi complex. These observations were followed by a fast-paced series of successes: the distinction between membrane-bound and free polysomes (Redman, 1969), the discovery of the signal peptide (Blobel & Sabatini, 1971; Milstein et al., 1972), the demonstration of the cotranslational glycosylation of ovalbumin (Kiely et al., 1976), and the findings that, in accord with the signal hypothesis, newly translated and glycosylated proteins were sequestered within the lumen of the endoplasmic reticulum (Lingappa et al., 1978) and that viral coat proteins were inserted with their carbohydrate chains facing the lumen (Katz et al., 1977). All of these findings set the scene for in-depth biochemical studies on the mechanisms of assembly of the oligosaccharide chains of the glycoproteins. These studies [summarized in Parodi and Leloir (1979), Struck and Lennarz (1980), Hubbard and Ivatt (1981), and Kornfeld and Kornfeld (1985)] have led to the following general conclusions:

(1) All eukaryotic cells are capable of the synthesis of glycoproteins that are destined to become components of the plasma membrane. In addition, many cell types commit a significant portion of their protein biosynthetic activity to the synthesis of secreted and/or lysosome-packaged glycoproteins.

(2) The synthesis of membrane, secretory, or lysosomal glycoproteins is a highly segregated process that occurs within an intracellular membrane system composed of the endoplasmic reticulum, transfer vesicles, Golgi apparatus, and secretory vesicles. During their translation, glycosylation, and processing the glycoproteins are completely isolated from the cytoplasm and travel to the cell surface (in the case of membrane glycoproteins), to the extracellular environment (in the case of secretory glycoproteins), or the lysosomes (in the case of lysosomal enzymes) as part of, or within, these membrane compartments.

(3) The assembly of N-linked oligosaccharide chains occurs in the endoplasmic reticulum and involves the stepwise preassembly of the oligosaccharide chain on dolichyl phosphate followed by en bloc transfer of the oligosaccharyl unit to the growing polypeptide chain.

(4) Subsequent modifications to the oligosaccharide chains of N-linked glycoproteins are initiated in the rough endoplasmic reticulum and completed in the Golgi apparatus.

As a result of the efforts of many laboratories, the individuals steps in the assembly and transfer of the oligosaccharide chain are known to occur as shown in Figure 1. Following elucidation of this assembly pathway and the subsequent steps in processing of the oligosaccharide chain, efforts have come full cycle back to the organelles first studied by radioautography, namely, the rough endoplasmic reticulum and the Golgi complex. The difference, however, is that almost two decades later the focus is on the spatial and topological orientation of the enzymes, substrates, and products rather than on the localization in the organelle per se. Recent studies on the Golgi apparatus with respect to protein glycosylation (Farquhar, 1985) and sugar nucleotide transport (Perez & Hirschberg, 1986a) have been reviewed. Therefore, my review will take up questions of a topological nature that pertain to the rough endoplasmic reticulum and the assembly of the oligosaccharide chains.[1] Related issues concerning transfer of the complete oligosaccharide chain from oligosaccharylpyrophosphoryldolichol to Asn sites in polypeptides have recently been reviewed elsewhere (Kaplan et al., 1987).

Basic Topological Questions

The oligosaccharide chain assembled on dolichyl phosphate and subsequently transferred to nascent polypeptide chains

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[1] Since completion of this paper a review on the same subject has appeared elsewhere (Hirschberg & Snider, 1987).

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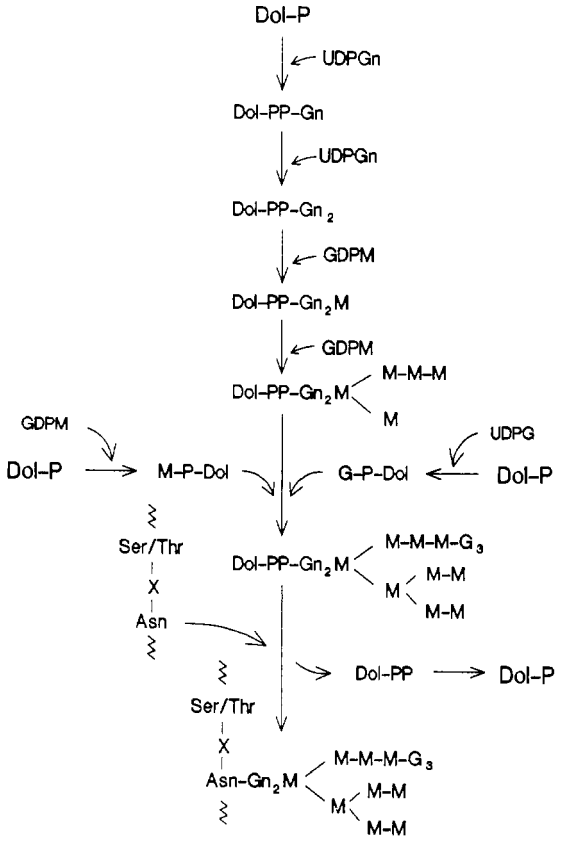

FIGURE 1: Steps in the assembly of the oligosaccharide chain that becomes N-linked to proteins.

contains three sugars: *N*-acetylglucosamine, mannose, and glucose. All three are synthesized in their activated form as sugar nucleotides in the cytoplasm (Coates et al., 1980). A variety of studies (Rothman & Lodish, 1975; Bergman & Kuehl, 1977; Katz et al., 1977; Lingappa et al., 1978; Hanover & Lennarz, 1980) utilizing in vivo pulse-chase techniques as well as in vitro translation in the presence of microsomes revealed that these three sugars, in the form of an oligosaccharide chain, were linked to the polypeptide chains in the lumen of the rough endoplasmic reticulum. Consistent with these observations, it was determined that the Asn residue in ovalbumin destined to be glycosylated did not acquire its carbohydrate chain until at least another 32 amino acid residues beyond this Asn had been added to the growing polypeptide chain (Glabe et al., 1980). Because this number of residues was more than sufficient to span the lipid bilayer, it was concluded that the most likely site of glycosylation of the growing polypeptide chain was at the lumenal face of the rough endoplasmic reticulum.

Three observations indicated that the precursor to the protein-linked oligosaccharide chain, namely, oligosaccharylpyrophosphoryldolichol, was also lumenally oriented:

(1) Studies discussed below using the impermeable lectin concanavalin A to study the topology of the completed oligosaccharylpyrophosphoryldolichol in microsomes indicated that the oligosaccharide moiety was not exposed at the cytoplasmic face of the bilayer (Snider & Robbins, 1982).

(2) Oligosaccharylpyrophosphoryldolichol in "aged" preparations of oviduct microsomes was shown to undergo hydrolytic cleavage, yielding free oligosaccharide that remained entrapped in the lumen (Hanover & Lennarz, 1982). Studies by Anumula and Spiro (1983) subsequently revealed that an analogous process occurred in thyroid microsomes. They suggested that hydrolytic release of the oligosaccharide might be the result of oligosaccharyltransferase's catalyzing transfer

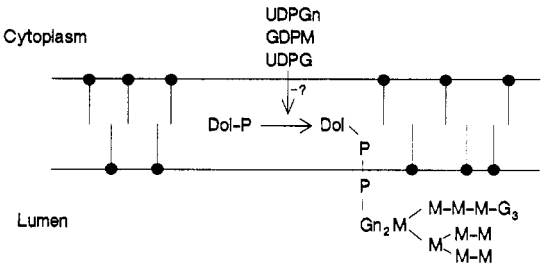

FIGURE 2: Model schematically posing the question: How are cytoplasmic sugars converted to an oligosaccharide in the lumen of the endoplasmic reticulum?

of the oligosaccharide chain to water molecules rather than to Asn residues in nascent polypeptide chains.

(3) Experiments with acceptor peptide substrates for oligosaccharyltransferase indicated that these peptides freely entered and could be washed from the rough endoplasmic reticulum but that after their glycosylation most were entrapped in the lumen (Welply et al., 1983). This implied that both the substrate oligosaccharylpyrophosphoryldolichol and the enzyme oligosaccharyltransferase were lumenally oriented. The observation that synthetic peptides inhibit the cotranslational glycosylation (but not the insertion) of nascent polypeptide chains of both ovalbumin and VSV G protein (Lau et al., 1983) was consistent with this idea.

These observations lead to the following question, which is illustrated schematically in Figure 2: *How are sugar nucleotides in the cytoplasm converted to an oligosaccharide chain facing the lumen of the rough endoplasmic reticulum*? Perhaps it is more useful to rephrase this general question in terms of four more sharply defined questions:

(*1*) *Are sugar nucleotides transported into the lumen of the rough endoplasmic reticulum*? If all three sugar nucleotides are transported into the lumen, the topological aspects of the enzymes and their products become simple: After their transport, the sugar nucleotides in the lumen interact with enzymes whose active sites are exposed at the lumenal face of the endoplasmic reticulum. Under these circumstances the dolichol-linked intermediates, the dolichol-linked end product oligosaccharylpyrophosphoryldolichol, and the glycosylated polypeptide are all lumenally disposed.

However, it appears that this relatively simple situation does not obtain. Initial in vivo and in vitro evidence showed that oviduct microsomes lacked the ability to transport GDP-M[2] (Hanover & Lennarz, 1982). Subsequent studies in liver microsomes supported the idea that GDP-M transport does not occur (Perez & Hirschberg, 1986b). However, positive evidence was reported for the transport of both UDP-Gn (Perez & Hirschberg, 1985) and UDP-G (Perez & Hirschberg, 1986b). In the case of UDP-Gn, the translocation process was temperature dependent, saturable, and inactivated by protease treatment of the endoplasmic reticulum (Perez & Hirschberg, 1985). Preliminary studies suggested that the import of UDP-Gn was coupled to the export of nucleotide monophosphate. Similar observations were made for UDP-G (Perez & Hirschberg, 1986b). Thus, on the basis of the available evidence, the simplest model, involving transport of all three sugar nucleotides into the lumen, followed by assembly and transfer of the oligosaccharide chain at the lumenal face, does not appear to be tenable. However, it is important to remember that the available evidence on transport of GDP-M is negative and that it is always possible that the

[2] Abbreviations: M, mannose; Gn, *N*-acetylglucosamine; G, glucose; Dol-P, dolichyl phosphate.

kinetics of utilization subsequent to transport might be so rapid that it makes detection of this sugar nucleotide in the lumen impossible.

(*2*) *What is the topological distribution of Dol-P in the bilayer of the rough endoplasmic reticulum*? The arrangement of the Dol-P depicted in Figure 2 begs this question. As noted a number of years ago (Struck & Lennarz, 1980), the hydrocarbon chain of dolichol in its extended form is longer than the width of a typical biological membrane. Thus, the polyisoprenoid chain of dolichyl phosphate is almost certainly in some type of nonextended conformation. The precise nature of this conformation and the location of the polar phosphate head group in what has been called a "superlipid" (McCloskey & Troy, 1980) are important issues that remain to be resolved. Model studies using NMR to examine polyprenoids site specifically labeled with ^2H in phospholipid liposomes suggest that the acetyl esters of geraniol (C_{10}), farnesol (C_{15}), and solanesol (C_{45}) are not arranged in the lipid bilayer with their polar head group exposed at the bilayer interface (deRopp & Troy, 1984; deRopp & Troy, 1985; deRopp et al., 1987). In contrast, the spin-label studies of McCloskey and Troy (1980) and the studies of Valtersson (see below) indicate that dolichyl phosphate does assume a conformation such that the polar portion of the molecule bearing the phosphoryl group is exposed at the bilayer interface. These studies also reveal that spin-labeled analogues of phosphorylated dolichol remain in monomolecular form in phosphatidylcholine vesicles.

In the context of the conformation of dolichyl phosphate, its effect on the physical properties of bilayers is of interest because of the idea that these molecules translocate sugars attached to them. Studies using ^{31}P NMR, small-angle X-ray scattering, differential scanning calorimetry, and freeze–fracture electron microscopy (Valtersson et al., 1985), later complemented by fluorescence resonance energy transfer (van Duijn et al., 1986), led to the following conclusions: First, dolichyl phosphate destabilizes bilayer structures containing unsaturated phosphatidylethanolamine. Second, it abolishes the transition from gel to liquid phase in dimyristoylphosphatidylcholine and increases the fluidity of the fatty acyl chains in phosphatidylethanolamine mixtures. On the basis of these observations, it was postulated that dolichyl phosphate induces disorder and formation of the hexagonal II phase in artificial bilayers. Further, it was suggested that such formation might facilitate transmembrane movement of the dolichol-linked intermediates in natural membranes. Additional evidence that dolichol and its derivatives can affect membrane structure has been derived from fluorescence depolarization of diphenylhexatriene in phosphatidylcholine vesicles (Vigo et al., 1984). However, in contrast to the studies of Valtersson et al. (1985), these studies showed that dolichyl phosphate decreased bilayer fluidity while dolichol increased fluidity; the reason for these conflicting conclusions remains to be established.

With respect to the possible role of dolichyl phosphate in the translocation of saccharides across the membrane, knowledge of the dynamic properties of these molecules in bilayers is very important. However, the available evidence indicates that the $T_{1/2}$ for "flip-flop" of polyprenyl phosphates in artificial bilayers containing phosphatidylcholine is exceedingly long (>5 h at 25 °C) (McCloskey & Troy, 1980; deRopp et al., 1987). The disparity betwen the observed ratio of $T_{1/2}$ >5 h and the rate calculated for dolichol transposition if it is involved in saccharide translocation ($T_{1/2}$ <1 s) led McCloskey and Troy (1980) to conclude that neither the unusual length nor poly cis unsaturation of isoprenoid carriers affords them a uniquely rapid rate of unassisted flip-flop. On this basis, they proposed that incipient oligosaccharide chains are not transported unassisted across the bilayer while covalently bound to lipid; if they do transit the membrane, the process is probably mediated by a specific protein. There is also evidence that an early intermediate in the assembly pathway, Gn_2-PP-Dol, that is formed from dolichyl phosphate does not undergo "flip-flop" in phosphatidylcholine-containing liposomes (see below). If dolichyl phosphate and its glycosylated derivatives in the rough endoplasmic reticulum also do not undergo transverse movements, severe constraints are placed on models for the mechanism of assembly of oligosaccharylpyrophosphoryldolichol (see below).

A related issue is the possible asymmetric distribution of dolichyl phosphate in the lipid bilayer. This is the case because three reactions in the pathway involve dolichyl phosphate:

$$\text{Dol-P + UDP-Gn} \rightleftarrows \text{Dol-PP-Gn + UMP}$$

$$\text{Dol-P + GDP-M} \rightleftarrows \text{Dol-P-M + GDP}$$

$$\text{Dol-P + UDP-G} \rightleftarrows \text{Dol-P-G + UDP}$$

As indicated in Figure 1, the first of these reactions is the first step in the assembly pathway, whereas the second and third provide the glycosyl donors utilized at the terminal stages of the biosynthetic process. If flip-flop does not occur and if the head group of dolichyl phosphate is localized to one face of the bilayer, even more rigid constraints are imposed on models for assembly of oligosaccharylpyrophosphoryldolichol. Despite the importance of this issue, no experiments to test the possible asymmetric distribution of dolichyl phosphate in the rough endoplasmic reticulum have been reported. Experiments on a related question, the topological distribution of dolichol in microsomes, suggest that the active site of dolichol kinase and the hydroxyl group of the polyprenoid are exposed to the cytoplasm (Adair & Cafmeyer, 1983). This idea is based on the following findings: Treatment with the impermeable probe mercury dextran or with trypsin resulted in a loss of dolichol kinase activity. This observation, coupled with the finding that exogenous CDP inhibited dolichol kinase activity, suggested that the active site of the enzyme was exposed on the cytoplasmic face of the microsomes. Further, the observation that incubation with [^{32}P]CTP, which is presumed to be inaccessible to the lumen of microsomes, resulted in formation of ^{32}P-labeled dolichyl phosphate indicated that at least some of the dolichol was exposed at the cytoplasmic face. However, the issue of the possible asymmetric distribution of dolichyl phosphate remains to be resolved.

(*3*) *What is the topological distribution of the saccharide chains of the various intermediates involved in assembly of oligosaccharylpyrophosphoryldolichol*? Of the various questions pertaining to the overall assembly process, the most effort has been devoted to the issue of the sidedness of the saccharide chains. Initially, it was established that galactosyltransferase could serve as a probe of the disaccharide chain of Gn_2-PP-Dol, because in the presence of detergent and UDP-Gal it catalyzed its quantitative conversion to Gal-Gn_2-PP-Dol (Hanover & Lennarz, 1979). Furthermore, when Gn_2-PP-Dol was incorporated into unilamellar liposomes, galactosyltransferase derivativized only approximately 50% of the disaccharide chains. This indicated that (a) isolated Gn_2-PP-Dol was incorporated into the artificial bilayer in an essentially symmetric fashion and that (b) galactosyltransferase could act on this glycolipid in a phospholipid bilayer.

When this same probe was applied to hen oviduct rough endoplasmic reticulum that had been preincubated with labeled UDP-Gn in order to generate labeled Gn_2-PP-Dol, virtually

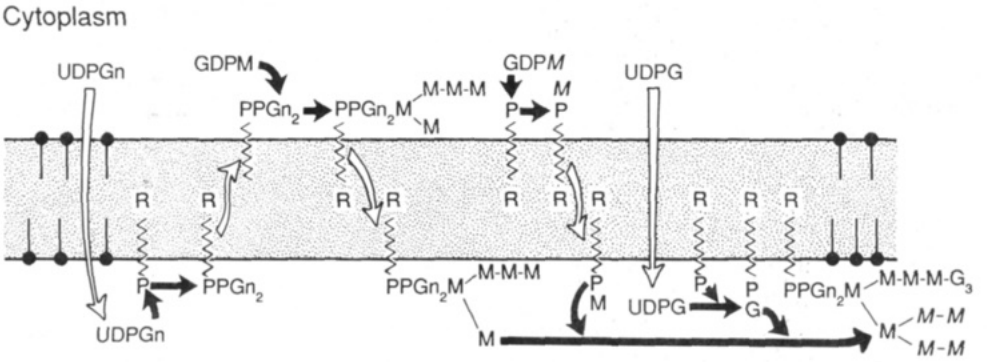

FIGURE 3: "Consensus" model derived from the studies discussed in the text depicting assembly of the oligosaccharide chain of oligosaccharylpyrophosphoryldolichol. R〰P represents dolichyl phosphate. The solid, bold arrows represent the progression of reaction sequences from left to right. The open, tapered arrows represent translocation events. The italic M indicates the mannose units that arise from M-P-Dol.

none of the labeled disaccharyl lipid was found to be accessible to the enzyme. In contrast, when detergent was added to the microsomes at a concentration just sufficient to expose latent, lumenal marker enzymes, the majority of disaccharyl lipid was accessible to the enzyme probe. This result suggested that the disaccharyl unit of endogenously synthesized Gn_2-PP-Dol was not exposed on the cytoplasmic face of the rough endoplasmic reticulum.

The above experiment measuring the topology of labeled Gn_2-PP-Dol after it had been synthesized in a preincubation did not exclude the possibility that assembly occurred on the cytoplasmic face but that a subsequent translocation process moved the Gn_2-containing molecule to the lumen where it was inaccessible to the probe. Therefore, an experiment was performed in which the assembly reaction with labeled UDP-Gn and the probing reaction with galactosyltransferase and UDP-Gal were carried out concurrently rather than sequentially (Hanover & Lennarz, 1981). Under these conditions Gn_2-PP-Dol exposed even transiently on the outer leaflet of the bilayer should be accessible to the transferase and hence converted to the trisaccharide. No such conversion was observed. On the basis of these results it was concluded that synthesis of Gn_2-PP-Dol did not occur at the cytoplasmic face. While the obvious interpretation of these essentially negative experiments was that assembly occurred at the lumenal face, the alternative possibility, that the disaccharide moiety (perhaps associated with an enzyme complex) was buried in the bilayer, could not be excluded.

In an extension of the earlier mentioned studies on the topology of completed oligosaccharide chains, concanavalin A was used to probe the α-linked mannosyl units of the growing oligosaccharide chain (Snider & Rogers, 1984). Experiments with liver microsomes labeled with GDP-M and then probed with concanavalin A revealed that the short-chain oligosaccharylpyrophosphoryldolichol, containing from two to five mannosyl units, was accessible to the lectin. This indicated that these intermediates are located at the cytoplasmic face of the bilayer. The longer intermediates and the completed oligosaccharylpyrophosphoryldolichol could not be detected with concanavalin A in intact microsomes, implying that, unlike the shorter intermediates, they were not present at the cytoplasmic face of the membrane and therefore could not interact with the lectin. One limitation of these studies was that the putative oligosaccharyl lipids that bound to concanavalin A were never identified as such. In any event, on the basis of these observations it was postulated that short- and intermediate-length oligosaccharide chains that are formed directly from GDP-M are assembled at the cytoplasmic face of the membrane; after all of these mannose units are added, the remaining units, which arise from M-P-Dol, are added in the bilayer or at the lumenal face of the endoplasmic reticulum.

A model that incorporates all of the experimental observations discussed in this section is shown in Figure 3. As depicted, the assembly of oligosaccharylpyrophosphoryldolichol is initiated by the transport of cytoplasmic UDP-Gn into the lumen. In the lumen the nucleotide reacts with dolichyl phosphate; after addition of a second Gn unit the resulting disaccharyl lipid is translocated back to the cytoplasm where it reacts with cytoplasmic GDP-M, which cannot enter the lumen. The product of this reaction is M_5Gn_2-PP-Dol at the cytoplasmic face of the membrane. Further elongation involves M-P-Dol as a donor, which is formed from cytoplasmic GDP-M. Both M-P-Dol and M_5Gn_2-PP-Dol are either translocated, and then react to form the longer oligosaccharide chain, or react while they are undergoing translocation. Finally, UDP-G is translocated and converted to G-P-Dol at the lumenal face, where it reacts with the growing oligosaccharyl lipid to produce the mature oligosaccharide chain. This model has the attractive feature of having individual sugar transfer reactions compartmentalized. Thus, as indicated in the model, Gn transfer to initiate the synthesis of oligosaccharyl lipid occurs in the lumen, initial M transfer reactions occur at the cytosolic face, and G addition occurs in the lumen. Such compartmentalization could provide multiple sites for control of assembly of oligosaccharide chains.

It must be emphasized that the model shown in Figure 3 represents an extreme interpretation of the data. Thus, the fact that Gn_2-PP-Dol is not accessible to an impermeable probe does not mean that it *must* be at the lumenal face; it merely means that it is not at the cytoplasmic face. Similarly, the finding that oligosaccharyl lipids containing chains longer than M_5Gn_2 are not accessible to concanavalin A does not mean that they must be oriented toward the lumen. Moreover, the model shown in Figure 3 has two very unattractive features, both of which relate to the postulated wholesale shuttling of saccharide chains back and forth across the membrane as oligosaccharylpyrophosphoryldolichol is assembled. First, as noted above, although there is evidence for translocation of certain sugar nucleotides, there is no evidence for flip-flop of glycosyl lipids. Haselbeck and Tanner (1982) inferred that flip-flop might occur on the basis of studies on the apparent ability of crude mannosylphosphoryldolichol synthase incorporated into liposomes to translocate mannosyl units to entrapped GDP. However, aside from this observation there is no experimental support for such translocation. The second unattractive feature of this multiple translocation model is the apparent energy requirement. F. A. Troy (personal communication) has estimated that the movement of one saccharide unit attached to dolichyl phosphate from an aqueous environment to the hydrophobic environment of the bilayer, with

the concomitant disruption of four hydrogen bonds between the four hydroxyl groups and water, would require the expenditure of 25–50 kcal/mol or the equivalent of 2.5–5 ATP molecules per translocation. Clearly, this expenditure would increase as the number of glycosyl units in the oligosaccharide chain increased; i.e., for M_5Gn_2 linked to dolichyl pyrophosphate it could be as large as 130–260 kcal/mol. Thus, if such translocations occur, either the saccharide chain is not directly exposed to the hydrophobic environment or a great deal of energy is expended in accomplishing the process. On the basis of all of these considerations, it seems reasonable to conclude that this "consensus" model, incorporating the results of all the published studies, may require considerable modification in the future as additional experimental information becomes available.

(*4*) *What is the topological distribution of the enzymes involved in the synthesis of oligosaccharylpyrophosphoryldolichol*? A question obviously related to the sidedness of the saccharyl lipids in the endoplasmic reticulum is the topological orientation of the enzymes that catalyze their synthesis. Recently, the polypeptide chain of the last enzyme in the dolichol-linked pathway, oligosaccharyltransferase, has been identified and a number of the properties of the enzyme elucidated (Kaplan et al., 1987). However, aside from oligosaccharyltransferase, nothing is known about the structural features of the enzymes in this pathway. Consequently, approaches to defining the topological orientation of their active sites have been limited to studying the effect of proteolysis of sealed microsomes on the activity of these enzymes. This experimental approach makes the assumption that if inactivation of enzyme occurs, the active site of the enzyme faces the cytoplasm, and conversely, if no inactivation occurs, it faces the lumen. However, there are several potential circumstances that could confound conclusions on the sidedness of the active site. For example, cleavage of the polypeptide chain of the enzyme at a site other than the active site could lead to inactivation, or cleavage might not occur if the enzyme is largely buried in the membrane. If either of these conditions obtain, it would be impossible to deduce the topological orientation of the active site. At best, one can conclude from the results of these early studies (Snider et al., 1980; Hanover & Lennarz, 1982) that some of the enzymes in the pathway may differ in the sidedness of their active sites. Recently, more detailed comparative studies of the properties of M-P-Dol and G-P-Dol synthases, including their sensitivity to trypsin, suggest that the former enzyme is exposed at the cytoplasmic face, whereas the latter is not (Koro et al., unpublished observations). This would be consistent with the earlier discussed difference in transport of the sugar nucleotides utilized by these two enzymes.

Conclusions

The uncertainties and equivocations in the answers to topological questions about the assembly process in the endoplasmic reticulum reflect the difficulties in dealing with the structural organization of lipids and proteins in membranes. Clearly, "solid-state" biochemistry has a number of inherent limitations that are not present when one is dealing with enzymes and substrates that are soluble in an aqueous environment. Before a clear picture of the topological features of the glycosylation process emerges, it will be necessary to know more about three aspects: (1) the physical organization of dolichyl phosphate in the membrane (at present the best way to study this appears to be by use of physical–chemical techniques in model membrane systems, but ultimately one will have to devise ways to study this in biological membranes); (2) the sidedness of the saccharyl lipid intermediates (new impermeable probes must be developed that exhibit high specificity for the molecule of interest); (3) the enzymes in the pathway (these proteins must be isolated and sequenced by using either state-of-the-art biochemical techniques, the methods of recombinant DNA, or molecular genetics). Such sequence information, coupled with proteolysis and inhibitor studies on these enzymes in the endoplasmic reticulum, should allow one to deduce their arrangement in the bilayer. However, only by combining the information obtained by all three approaches will we be able to understand fully the topological features of this complex assembly process.

Acknowledgments

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Accelerated Publications

fd Gene 5 Protein Binds to Double-Stranded Polydeoxyribonucleotides Poly(dA·dT) and Poly[d(A-T)·d(A-T)]†

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ABSTRACT: Circular dichroism (CD) data indicated that fd gene 5 protein (G5P) formed complexes with double-stranded poly(dA·dT) and poly[d(A-T)·d(A-T)]. CD spectra of both polymers at wavelengths above 255 nm were altered upon protein binding. These spectral changes differed from those caused by strand separation. In addition, the tyrosyl 228-nm CD band of G5P decreased more than 65% upon binding of the protein to these double-stranded polymers. This reduction was significantly greater than that observed for binding to single-stranded poly(dA), poly(dT), and poly[d(A-T)] but was similar to that observed for binding of the protein to double-stranded RNA [Gray, C. W., Page, G. A., & Gray, D. M. (1984) *J. Mol. Biol. 175*, 553–559]. The decrease in melting temperature caused by the protein was twice as great for poly[d(A-T)·d(A-T)] as for poly(dA·dT) in 5 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), pH 7. Upon heat denaturation of the poly(dA·dT)–G5P complex, CD spectra showed that single-stranded poly(dA) and poly(dT) formed complexes with the protein. The binding of gene 5 protein lowered the melting temperature of poly(dA·dT) by 10 °C in 5 mM Tris-HCl, pH 7, but after reducing the binding to the double-stranded form of the polymer by the addition of 0.1 M Na^+, the melting temperature was lowered by approximately 30 °C. Since increasing the salt concentration decreases the affinity of G5P for the poly(dA) and poly(dT) single strands and increases the stability of the double-stranded polymer, the ability of the gene 5 protein to destabilize poly(dA·dT) appeared to be significantly affected by its binding to the double-stranded form of the polymer.

The fd gene 5 protein (G5P)[1] is one of the most extensively studied single-strand DNA binding proteins (Ray, 1978; Kowalczykowski et al., 1981). This model protein is encoded by the fd filamentous phage (Marvin & Hohn, 1969). A known biological function of this protein in the phage life cycle is to control the switch from the replication of double-stranded RF DNA to the synthesis of single-stranded viral DNA

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[1] Abbreviations: CD, circular dichroism; G5P, gene 5 protein; RF, replicative form; T_m, melting temperature; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; R, ratio of nucleotide to protein molar concentrations (R = [nucleotide]/[gene 5 protein monomer]); SDS, sodium dodecyl sulfate; dsDNA, double-stranded DNA; dsRNA, double-stranded RNA; EDTA, ethylenediaminetetraacetic acid.

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