Transport of Secretory and Membrane Glycoproteins from the Rough Endoplasmic Reticulum to the Golgi

A RATE-LIMITING STEP IN PROTEIN MATURATION AND SECRETION*

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Recent work indicates that transport of newly made secretory and membrane proteins from the rough endoplasmic reticulum (ER)† to the Golgi is a specific and highly regulated event. Different membrane and secreted proteins move from the ER to the Golgi at very different rates, and resident ER proteins do not move at all. Most integral membrane proteins and many secretory proteins undergo extensive covalent modifications and folding while still in the ER; recent studies suggest that proteins will not mature from the ER to the Golgi unless they have achieved a proper conformation. Finally, the nature of some of the signals on proteins that control their export from the ER is being elucidated by a combination of molecular, genetic, and biochemical dissection. However, the complexity and specificity of ER-to-Golgi transport is still not totally understood.

Resident ER Proteins

The membranes of the rough and smooth ER are thought to be continuous, and many predominant polypeptides are found in both. Certain proteins, such as ribophorins, are associated mainly with the rough ER (Kreibich et al., 1978); enzymes involved in lipid metabolism, such as hydroxy-methylglutaryl-CoA reductase, are localized to the smooth ER (Rodwell et al., 1976; Orci et al., 1984). These proteins are cotranslationally inserted into the ER membrane (Brown and Simoni, 1984; Rosenfeld et al., 1984). The evidence that resident ER proteins remain in the ER after synthesis rests, in part, on immunoelectron microscopy. Glucosidase II, an enzyme that catalyzes one of the first steps in processing of asparagine-linked oligosaccharides, is concentrated in rough and smooth ER, but is not detectable in Golgi cisternae (Lucocq et al., 1986).

Additional evidence for selective retention comes from analysis of asparagine-linked oligosaccharides on ER proteins. The same precursor oligosaccharide, of composition \( \text{Glc}_3\text{Man}_n\text{GlcNAc}_m \), is transferred from a dolichol carrier to asparagine residues on secretory and membrane glycoproteins. While the proteins are still in the ER, the 3 glucose residues and 0, 1, or 3 mannose residues are cleaved off by ER-specific glycosidases (reviewed by Kornfeld and Kornfeld, 1988). A detailed analysis of the total population of ER Asn-linked oligosaccharides or of oligosaccharides on specific resident ER proteins revealed mainly \( \text{Man}_0\text{GlcNAc}_0 \) and \( \text{Man}_0\text{GlcNAc}_1 \) (Rosenfeld et al., 1984; Lewis et al., 1985; Brands et al., 1985; Mutsaers et al., 1985). These are structures that could be generated solely from the action of the rough ER enzymes glucosidase I, glucosidase II, and \( \alpha \)-mannosidase. This suggests that the proteins have not moved to the Golgi, wherein are localized enzymes that could process the oligosaccharides further. However, certain secreted and plasma membrane glycoproteins retain high-mannose oligosaccharides (Kornfeld and Kornfeld, 1985). Thus, the lack of processing of oligosaccharides by Golgi \( \alpha \)-mannosidase I or \( N \)-acytethylglucosaminyltransferase I is not solid evidence that the protein has never passed through the Golgi.

Munro and Pelham (1987) showed that three proteins that permanently reside in the lumen of the rough ER, Bip (heavy chain binding protein, also called grp 78 or glucose-regulated 78,000 protein), grp 94, and protein disulfide isomerase, all have the same carboxyl-terminal sequence Lys-Asp-Glu-Leu, and that this sequence appears essential for retention of these proteins within the rough ER. When expressed in transfected fibroblasts, wild-type grp 78 is retained in the rough ER, as might be expected. But, when mutant proteins that lack this carboxyl-terminal sequence or have this sequence extended by random amino acids are expressed in transfected cells, the proteins are secreted. And when these four residues are placed (by \textit{in vitro} mutagenesis) at the carboxyl terminus of lysozyme, a normally secreted protein, the elongated lysozyme is selectively retained in the ER. It is thought that these luminal proteins are anchored to an ER membrane protein by means of the carboxyl-terminal Lys-Asp-Glu-Leu sequence. This, in itself, would explain why these proteins are not transported to the Golgi, but one would also have to explain why the membrane "receptor" would remain in the ER and not be transported to the Golgi along with plasma membrane proteins.

ER-to-Golgi Transport Vesicles

A number of electron microscopic studies suggested that small vesicles transport proteins from the rough ER to the Golgi. Regions of rough ER free of ribosomes, called transitional elements, are thought to be the nascent transport vesicles, but they could also represent vesicles that are fusing with the rough ER (Farquhar and Palade, 1981). At 15 °C, transport of membrane and secreted proteins to the cell surface is blocked, and newly made semiliki forest virus glycoprotein accumulates in smooth-surfaced vesicles near the cis face of the Golgi (Sarasate and Kuimann, 1984). It is difficult to determine, however, whether such vesicles are actually part of the ER, are transport vesicles that have not yet fused with the Golgi, or are part of the cis Golgi cisternae.

Recently ER-to-Golgi transport vesicles have been detected biochemically in homogenates of Hep G2 human hepatoma cells (Lodish et al., 1987). On equilibrium sucrose-D-\( \Omega \) gradients these vesicles are very light in density, lighter than even Golgi vesicles and much lighter than the ribosome-

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† The abbreviations used are: ER, endoplasmic reticulum; VSV, vesicular stomatitis virus; HA, hemagglutinin; H and L chains, heavy and light chains, respectively.
studded rough ER. They contain newly made secretory proteins with oligosaccharides mainly of structure Man,GlcNAc and Man,GlcNAc, characteristic of pre-Golgi processing. Different secretory proteins enter these vesicles at rates commensurate with their rates of exit from the ER and eventual secretion from the cell.

Cell-free Systems

An exciting development is that of cell-free systems that reconstitute ER-to-Golgi transport of newly made glycoproteins. One system utilizes as "donor" membranes yeast ER vesicles from a ts mutant cell that accumulates (at the non-permissive temperature) radiolabeled, newly made invertase and other secretory proteins in the ER. The donor cells are also deficient in a Golgi enzyme that in normal (wild-type) cells adds mannose residues to the outer chains of Asn-linked oligosaccharides. The deficiency in the Golgi enzyme would not affect eventual secretion of the protein from the cell. The "acceptor" extract is prepared from unlabeled cells and contains Golgi vesicles that contain the "missing" enzyme. In vitro transfer of the radiolabeled glycoprotein from the donor ER to the acceptor Golgi is monitored by addition of outer-chain mannose residues to invertase catalyzed by the enzyme in the acceptor Golgi. Additional experiments establish that the labeled protein is initially in, and remains in, membrane-limited vesicles (Haselbeck and Schekman, 1986).

The other and more efficient system (Balch et al., 1987) uses as donor ts045 VSV G protein in ER membrane vesicles. The donor cells are lacking in functional Golgi α-mannosidase I and thus would be unable to process the oligosaccharides on the G protein if the G reached the Golgi fraction. The acceptor Golgi contains this α-mannosidase. The assay for movement is removal by Golgi α-mannosidase I of the mannose residues from the G protein, forming the characteristic Golgi processing intermediate Man,GlcNAc. Both systems require energy in the form of ATP and cytosol and membrane proteins. No vesicular intermediates have yet been detected in these reactions. Since movement to the Golgi is monitored solely by action of a Golgi oligosaccharide processing enzyme on the protein from the donor ER vesicles, it is not excluded that in these systems ER and Golgi membrane vesicles fuse promiscuously, allowing Golgi enzymes access to ER proteins. Such fusion reactions are probably not physiological.

Specificity in Transport of Proteins to the Golgi

The rates of transport of normal, native secretory and membrane glycoproteins from the rough ER to the Golgi complex can vary by almost an order of magnitude. For example, in human hepatoma Hep G2 cells, half of the newly made albumin and α1-antitrypsin mature to the Golgi rapidly, about 25 min at 32 °C; pro-C3 and α1-antichymotrypsin take 70 min, and half of the newly made transferrin requires over 180 min for movement to the Golgi (Lodish et al., 1983). Other studies on hepatomas have yielded similar results (Lefkowitz and Davis, 1983; Yeo et al., 1985). All these proteins require about the same period of time, 20 min, to be secreted from the Golgi, though minor differences in the rates of secretion from the Golgi could exist (Yeo et al., 1985). In the exocrine pancreas, several newly made secretory proteins (among them trypsinogen and chymotrypsin) move to the Golgi twice as rapidly as do others, such as procarboxypeptidase B (Scheele and Tartakoff, 1985). In a murine lymphoma line newly synthesized H-2Kk cell-surface glycoprotein reaches the Golgi over four times as rapidly as does the very similar (80% sequence identity) protein H-2Dk; the half-lives are 1 and 4.5 h, respectively (Williams et al., 1985). And two membrane glycoproteins encoded by a murine retrovirus leave the ER, and reach the cell surface, at different rates (Pitting and Kabat, 1982).

It is not known what elements might regulate the differential rates of maturation of normal proteins. One possibility is selective binding and/or retention of transported proteins in the transport vesicles as they bud from the rough ER; this would be analogous to the selective internalization of receptor proteins and their bound ligands from the plasma membrane during receptor-mediated endocytosis. Such a hypothesis would require a receptor for transported secretory proteins, a receptor that accumulates specifically in the transport vesicles. Despite much effort, we have been unable to detect such a "transport receptor" by binding newly made proteins to ER membranes. One problem could be the intrinsic low affinity of such receptors for their secretory-protein ligands that would make it difficult to detect specific binding. If, for instance, a single protein molecule were in a spherical vesicle 0.05 μm in diameter (volume = 6.5 × 10⁻¹⁰ cm³), this protein would be at a concentration of 2.5 × 10⁻⁶ M; thus the affinity of the putative receptor for this protein ligand could be on the order of 10⁻¹⁰ to 10⁻¹ⁱ M, much less than that of typical cell-surface hormone receptors.

Another possibility, not exclusive of the other, is that selected secretory or membrane proteins are specifically retained in the rough ER, and thus prevented from moving into or accumulating in the transport vesicles. Yet a third possibility is that all ER proteins are transported to the Golgi and that resident ER proteins are immediately recycled back to the ER. There is no evidence supporting or disproving any of these models.

Retention in the ER of Abnormal Proteins

But one conclusion is made clear by abundant recent work: abnormal secretory or plasma membrane proteins, whether generated by mutation or by pharmacological treatment of the secretory cell, often are specifically retained within the rough ER and are not secreted. In at least some cases, it has been shown that the conformation of the protein is altered, and it would appear reasonable to hypothesize that, unless a membrane or secreted glycoprotein folds into a native or near-native conformation within the rough ER, it will not be moved.

One well studied case is the mutant Z allele of the hepatic protein α1-antitrypsin (also called α1-antiprotease inhibitor) (reviewed by Carrell and Travis, 1985). The mutant Z protein is normally cotranslationally inserted into the rough ER, but, unlike the wild-type protein which is secreted rapidly, the mutant does not leave the ER; it accumulates in membrane-limited inclusion bodies. The substitution of lysine for glutamate at residue 342 in the Z variant may affect the conformation of the protein (Loebenberg et al., 1984) but this site is not near regions of oligosaccharide attachment nor of proteolytic processing. When expressed in macrophages or, after microinjection of mRNA, in oocytes, the Z variant is not secreted though the wild-type α1-antitrypsin is secreted normally. This suggests that the absence of ER to Golgi movement of the Z variant is not specific to the liver (Perlmutter et al., 1985; Verbanac and Heath, 1986). Additionally, subtle changes in the oligosaccharides attached to α1-antitrypsin can also prevent its maturation from the rough ER (Lodish and Kong, 1984).

Mutations in secretory proteins that prevent cleavage of the amino-terminal hydrophobic "signal" sequence prevent, or drastically slow, transport of proteins from the rough ER. Possibly this is because the altered protein remains bound to
the ER membrane by the signal peptide. For instance, a single alanine-to-valine mutation at the signal cleavage site of yeast invertase causes a 50-fold reduction in the rate of invertase transport to the Golgi, attributable to defective signal peptide cleavage (Schauer et al., 1985). A deletion in the yeast cell wall enzyme acid phosphatase that spans the signal peptide cleavage site causes most of the newly made acid phosphatase to accumulate in the rough ER. Cells expressing this modified acid phosphatase exhibit an exaggerated ER with a greatly distended lumen (Hagaesu-Tsapis et al., 1986). Importantly, certain mutations in the body of yeast invertase also block its maturation from the rough ER (Schauer et al., 1985).

In general, it is difficult if not impossible to determine whether the small amount of aberrant protein that accumulates in the rough ER is of normal or abnormal conformation. However, the structure of one viral glycoprotein, influenza HA, is known in molecular detail (Wilson et al., 1981) and several recent studies show the importance of HA conformation for maturation from the ER. The HA glycoprotein spike is formed as a trimer of three identical HAo polypeptides; late in maturation (in the Golgi or on the cell surface) each HAo is cleaved by a specific protease to generate an HA1 and HAb peptide. There is a single, hydrophobic segment of residues that spans the phospholipid bilayer as an α-helix and a short hydrophilic carboxyl-terminal segment that faces the cytoplasm. The bulk of the protein is exoplasmic, that is, it faces the lumen of the ER. Folding and assembly of HAo monomers into trimeric structures takes about 7 min and is completed before the protein leaves the rough ER (Gething et al., 1986; Copeland et al., 1986). Importantly, mutants of the HA polypeptide that fail to be transported from the rough ER are blocked at different stages of the folding pathway. Such mutations include HAα8 with alterations in the carboxyl-terminal cytoplasmic tail or at the base of the fibrous stalk of the trimer near its junction with the membrane-spanning segment. These observations indicate that formation of the HAα trimer requires interactions between the cytoplasmic and possibly membrane-spanning segments, as well as between the large exoplasmic regions. They also suggest that formation of a correctly folded quaternary structure within the rough ER constitutes a key event that regulates transport of the protein to the Golgi.

A similar conclusion was reached from a study of a temperature-sensitive mutation in the gene encoding the VSV G glycoprotein (Kreis and Lodish, 1986). Wild-type G protein, like HA, normally forms an oligomer, probably a trimer, while it is still in the rough ER. The ts045 mutation causes the G protein to remain in the rough ER as long as the cells are maintained at the nonpermissive temperature, 39 °C. When the temperature is lowered to 32 °C the G matures normally and synchronously to the Golgi and thence to the cell surface. At 39 °C the ts045 G protein remains a monomer; when the temperature is lowered the G protein forms an oligomer and does so before it leaves the ER. Thus the single amino acid substitution in the exoplasmic segment of ts045 G (Gallione and Rose, 1985) causes temperature-sensitive oligomerization, and it could be concluded that, like HA, oligomerization is an essential prerequisite for maturation of G from the rough ER.

The behavior of mutant versions of several other transmembrane proteins, whose transport from the rough ER is either blocked or slowed, can be explained satisfactorily in terms of defects in achieving a normal tertiary or quaternary conformation (see Rizzolo et al., 1985 and the discussions in Gething et al., 1986; Copeland et al., 1986; and Kreis and Lodish, 1986). There is no evidence that these proteins bear specific "transport signals" that in themselves direct movement from the rough ER to the Golgi. However, requirements for proper folding and maturation of viral glycoproteins are somewhat flexible. For example, mutant VSV G proteins that lack the cytoplasmic tail are not transported to the Golgi or are transported very slowly. But chimeric proteins that contain the exoplasmic and transmembrane segments of G, linked to the cytoplasmic domain of influenza HA or of the immunoglobulin heavy chain, are transported from the rough ER at a normal or near-normal rate (Puddington et al., 1986). Importantly, yet other mutant G proteins and chimeric membrane proteins accumulate within Golgi vesicles (Gabel and Bergmann, 1985; Guan and Rose, 1984); thus, certain structural or conformational features of a protein may be compatible with transport to the Golgi from the rough ER, but not for subsequent transport to the cell surface.

**Bip and Protein Folding**

Finally, let us consider what might cause the retention of abnormal membrane and secreted proteins within the ER. An important clue comes from recent studies of myeloma cells that make immunoglobulin heavy but not light chains; these heavy chains are retained in the ER. Such H chains are bound tightly but not covalently to the luminal protein termed Bip. As discussed above, Bip is selectively retained in the ER by a carboxyl-terminal sequence of amino acids. Studies of mutant Ig heavy chains show that the CH1 region is critical for binding of the heavy chains to Bip (Kreis et al., 1987).

In hybridosomes that secrete complete HAo immunoglobulins, a small and transient fraction of newly made H chains is bound to Bip (Bole et al., 1986). These authors suggest that the interaction of Bip with H chains facilitates binding of H to L chains, but there is no direct evidence that the small amount of H chains bound to Bip eventually binds L chains and is secreted and not, in contrast, degraded within the cell. Though Bip is found in many cell types, its importance in catalyzing folding of ER proteins and/or retention of abnormal proteins in the ER remains to be tested. Some newly made monomeric HAo is bound to Bip (Gething et al., 1986) but, again, one cannot be certain whether Bip-HAα is an intermediate in HAα folding or in HAα degradation. Using similar immunoprecipitation protocols we have been unable to detect an association of Bip with any monomeric ts04G protein that accumulates at 39 °C. Nor can we detect any interaction of Bip with any Hep G2 secretory proteins; binding to Bip apparently is not a determinant in the differential rate of export of Hep G2 secretory proteins.

It would appear, in conclusion, that the transport of secretory and membrane proteins from the rough ER to the Golgi is far more complex and specific than was envisaged even a few years ago.

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**Note Added in Proof**—Very recently, two groups have reported that very gentle procedures for shearing off pieces of the plasma membrane yield extracts in which ER-to-Golgi transport of newly made proteins occurs at very high efficiency (Beckers, C. J. M., Kessler, D. S., and Belch, W. (1987) Cell 50, 523–534; Simons, K., and Vizueta, H. (1987) EMBO J. 6, 2221–2228). An experiment published by Wieland et al. (Wieland, F. T., Gleason, M. A., Serafin, T. A., and Rothman, J. E. (1987) Cell 50, 289–300) suggests that "bulk flow" of proteins from the ER to the Golgi occurs very rapidly, within 10 min, and thus that selective retention of proteins in the ER is responsible for differential ER-to-Golgi transport rates and efficiencies. In this study, a small radiolabeled peptide is added to cells; it diffuses into the rough ER where it is glycosylated, and the glycosylated peptide is rapidly secreted. However, it is not certain that the glycosylated
peptide follows the normal secretory pathway and, in particular, it may not traverse the Golgi before secretion.

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