Protein Trafficking along the Exocytotic Pathway

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Summary

Proteins of the exocytotic (secretory) pathway are initially targeted to the endoplasmic reticulum (ER) and then translocated across and/or inserted into the membrane of the ER. During their anterograde transport with the bulk of the membrane flow along the exocytotic pathway, some proteins are selectively retained in various intracellular compartments, while others are sorted to different branches of the pathway. The signals or structural motifs that are involved in these selective targeting processes are being revealed and investigations into the mechanistic nature of these processes are actively underway.

Introduction

A schematic outline of the exocytotic pathway in mammalian cells is presented in Fig. 1. It comprises the ER† which is in continuity with the nuclear envelope, the Golgi apparatus, the endosomal/lysosomal system, the secretory granules in cells that possess the regulated secretory pathway, the plasma membrane, and various types of vesicular and/or tubular intermediates that mediate the dynamic connections between these membrane compartments[1-4]. The ER is the entry site of the pathway and targeting to the ER is mediated by various types of ER targeting sequences[5-6]. Proteins destined for secretion and luminal proteins of intracellular compartments are cotranslationally translocated across the ER membrane, while integral membrane proteins are partially translocated across the membrane and are anchored in the lipid bilayer in various ways[5-6]. In the ER, proteins undergo various co- and post-translational modifications, including proteolytic cleavage, topogenesis, glycosylation, formation of disulfide bonds, polypeptide folding, assembly of homo- or hetero-oligomeric structures, lipidation and others[7]. Only properly folded and assembled proteins can exit the ER[8]. Misfolded proteins are retained and degraded probably within the ER[8]. The Golgi apparatus is a complex and dynamic organelle and plays a pivotal role in controlling membrane traffic and sorting of proteins to various branched routes in the exocytotic pathway[1-2,9]. The Golgi apparatus is functionally organized into three major subcompartments: the cis-Golgi network (CGN); the Golgi stack (or medial Golgi); and the trans-Golgi network (TGN). The CGN is located on the cis side of the Golgi apparatus and includes the cisternae and tubular/vacuolar elements morphologically and/or functionally linked to the cis side of the Golgi[1-2,10-13]. The CGN not only receives vesicular traffic from the ER and forwards it to the Golgi stack but is also a major site for the branched route of retrograde transport from the Golgi back to the ER[1-2,10-13]. The Golgi stack most probably includes the medial and trans cisternae of the Golgi and is involved in carbohydrate modification of both proteins and lipids, as well as in forwarding the anterograde traffic to the TGN[1-2]. Whether the Golgi stack is composed of several distinct subcompartments connected to each other by vesicular/tubular transport, or exists as one physically integrated membrane structure remains to be resolved[2]. The TGN, composed of an extensive tubular/vesicular network on the trans side of the Golgi apparatus, is the exit site of membrane traffic from the Golgi apparatus into several branched routes. These include transport to secretory granules, the endosomal/lysosomal system and different domains of the plasma membrane[1-2,9,14-16]. It is believed that upon targeting to the ER, proteins will be transported along the anterograde path-

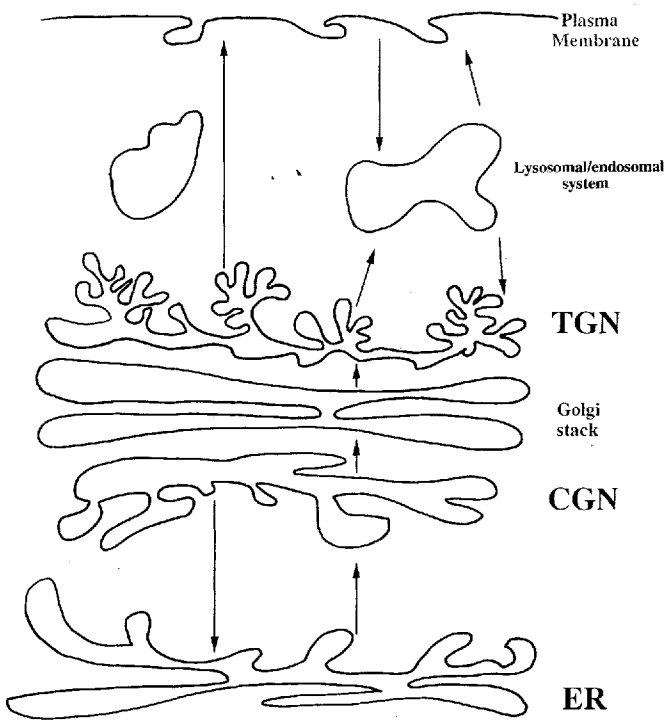

Fig. 1. The organization of the exocytotic pathway: the exocytotic (secretory) pathway is composed mainly of the endoplasmic reticulum (ER), the cis-Golgi network (CGN), the Golgi stack, the trans-Golgi network (TGN), the lysosomal/endosomal system, the plasma membrane, and various vesicular and/or tubular intermediates.

†Abbreviations used in this review: BFA, brefeldin A; CGN, cis-Golgi network; ER, endoplasmic reticulum; ERES, ER exit sites; GPI, glycosylphosphatidylinositol; LDL, low density lipoprotein; MDCK, Madin-Darby canine kidney; M6P, mannose-6-phosphate; TGN, trans-Golgi network.

way up to the plasma membrane with the bulk membrane flow by default unless they are retained in specific compartments or sorted to various branched routes[17]. Although several sorting signals (summarized in Table 1) and the main features of the exocytotic pathway have now been defined, many fundamental questions remain to be answered and understanding of how sorting signals function is still lacking. Recent work done in this and other laboratories is now beginning to shed some light on several key grey areas. These include ER retention, ER-Golgi transport, Golgi retention and Golgi-cell surface transport. This review will deal mainly with these issues. Other important aspects of protein trafficking such as protein translocation across the ER, the biochemistry of vesicular transport and other aspects of protein transport have been reviewed extensively by other authors[1-3,5-6,14-16].

ER Localization

The signal for ER localization for a family of type I integral membrane proteins in mammalian cells has recently been identified as the carboxyl terminal KKXX/KXKXX (K=Lys and X=any amino acid) or related sequences facing the cytosol[18-19]. This signal is present in several cellular and viral ER membrane proteins[18-19] as the type I membrane orientation (single transmembrane domain with N-terminus in the lumen of intracellular organelles or extracellular space and the C-terminus in the cytosol). Interestingly, a type I membrane protein that is located in the yeast nuclear envelope and the ER also contains this consensus sequence, indicating that a similar signal may operate in yeast[20]. Furthermore, this consensus sequence is also found at the C-terminal cytoplasmic domain of two ER membrane proteins with type III membrane orientation (the protein traverses the membrane several times): the human HMG CoA reductase[18] and a recently identified ER integral membrane protein that is involved in the early process of polypeptide translocation[21]. Whether the consensus sequence is necessary or sufficient for ER localization of type III membrane proteins remains to be investigated. Recently, a different cytoplasmic ER retention signal has been identified in the CD3ε chain of the T-cell receptor[22].

The mechanism for the retention of proteins bearing this signal is not known, although several possible models have been proposed[18]. The addition of N-acetylgalactosamine (the first sugar residue of O-linked glycosylation) to ER-localized polypeptides bearing this signal indicates that the signal may function at a post-ER/early Golgi compartment[18], as the initiation of O-linked glycosylation is believed to occur in the CGN[2]. One model, similar to that proposed for the ER localization of luminal proteins (see below), is that membrane proteins bearing this signal actually exit the ER and are then selectively retrieved from a post-ER compartment (e.g. the CGN) by binding to cytosolic, and/or membrane receptor (s), which triggers routing of the complex back to the ER. The potential components that interact with this signal have not yet been described. Identification, purification, characterization and molecular cloning of these putative interacting components will provide greater understanding of ER localization mediated by this type of signal. Several approaches may be employed, and the first will be to screen for mutants in yeast that are defective in ER localization mediated by this signal, followed by cloning and characterization of the mutant genes. The yeast system can only be employed if the KKXX/KXKXX or related sequence could mediate ER localization in yeast, which remains to be established. Alternatively, the anti-idiotypic antibody approach could be employed once monospecific antibodies to this signal are available. Finally, biochemical purification of proteins that can specifically bind this targeting signal sequence may prove to be a viable alternative.

The signal responsible for ER localization of luminal proteins has been revealed to be a carboxyl terminal KDEL or closely-related sequences in both mammalian and yeast cells[12,23]. The mechanism of ER localization mediated by this signal involves selective retrieval of signal-bearing luminal proteins at a post-ER compartment. Yeast ER luminal proteins bear a C-terminal HDEL sequence and are recognized by the HDEL receptor, encoded by the ERD2 gene[12,24-25]. Two related human homologues of the yeast ERD2 gene have been cloned[13,26-27]. The protein encoded by one of them has been shown to interact with the corresponding KDEL sequence-bearing ligands in mammalian cells[28]. Moreover, the specificity of the receptor molecule could be altered by changing the stretch of residues between amino acids 51 to 57, which may be the ligand binding domain. Antibodies against the C-terminal sequence of the mammalian homologue (named p23) of the yeast ERD2 gene

Table 1. *Summary of the known sorting signals along the exocytotic pathway*

Subcellular localization	Type of protein	Sorting signal
ER	Luminal protein and type II membrane proteins	C-terminal -Lys-Asp-Glu-Leu (KDEL) facing the lumen
ER	Type I membrane proteins	C-terminal -Lys-Lys-X-X-(KKXX) or KXKXX exposed to the cytosol (X=any amino acid)
Golgi	Type II (and possibly type I) membrane proteins	The transmembrane domain (the signal/anchor of type II membrane proteins)
TGN	A type I membrane protein	The Tyr-containing C-terminal sequence facing the cytosol
Lysosome	Luminal enzymes	Mannose-6-phosphate (M-6-P) attached to the N-linked glycan
Lysosome	Type I membrane proteins	Tyr-containing C-terminal sequences exposed to the cytosol
Apical surface	Glycosylphosphatidylinositol (GPI)-linked proteins	GPI-linker
Basolateral surface	Type I membrane proteins	A short cytoplasmic sequence that is either distinct or resembles the endocytotic signal
Rapid endocytosis	Type I and type II membrane proteins	A cytoplasmic reverse-turn with an essential Tyr residue

product show that the protein is present in all cell types examined and is predominantly confined to the cis side of the Golgi (the CGN), thus providing independent morphological evidence in support of the retrieval model for the retention of luminal ER proteins and establishing that the site of retrieval is in the CGN[13]. The details of the retrieval process, including the possibility of ligand-induced recycling of the receptor-ligand complex, is unknown. Recently, the transmembrane topology of p23 has been established (our unpublished results). Several regions, including the carboxy-terminal 21-residue sequence of p23 are exposed to the cytosol. These cytoplasmic regions, especially the C-terminal cytoplasmic tail, may potentially interact with components of a more elaborate recycling machinery upon binding the KDEL ligand (which occurs in the luminal domains). This interaction may lead to selective retrieval back to the ER. In support of this hypothesis, a mutation in the yeast ERD2 gene that resulted in the deletion of the last 12 residues of the C-terminus led to a loss of the ERD2 gene function[24], establishing the importance of the C-terminal cytoplasmic tail in the function of this protein. By following the subcellular dynamics of p23 when the cells were subjected to a low temperature treatment, we demonstrated that the mammalian homologue of the yeast ERD2 gene product could indeed cycle between the Golgi and the ER, as has been described for some other proteins[10-11,29]. The retrograde route from the Golgi back to the ER has been demonstrated and investigated by employing the fungal metabolite, brefeldin A, which inhibits the anterograde transport of the exocytotic pathway and causes extensive recycling of Golgi components back to the ER[3]. Interestingly, overexpression of the mammalian homologues of the yeast ERD2 gene in mammalian cells caused brefeldin A-like effects, indicating that this protein may also be involved in regulating the balance between the retrograde and the anterograde traffic, and/or other important aspects of the exocytotic pathway[27].

Two integral membrane proteins of type II membrane orientation (traversing the membrane once with the N-terminus in the cytosol and the C-terminus in the lumen of intracellular organelles or extracellular space), the products of SEC20 and SED4 genes, have been shown to have the HDEL sequence[30-31]. As a type II membrane protein with its C-terminus exposed to the lumen, it could be envisaged that the protein is recognized and retrieved in the same way as luminal proteins bearing the same signal. Indeed, when the KDEL sequence is appended to the C-terminus of a type II surface membrane protein, the protein is retained in the ER[32]. Endogenous mammalian membrane proteins bearing the KDEL signal remain to be identified. The C-terminal KDEL or related sequence is thus capable of conferring ER localization for luminal as well as type II integral membrane proteins, and this sequence functions as a retrieval signal at the CGN for channeling proteins back to the ER by a retrograde transport route.

ER to Golgi Transport

Transport between the ER and the Golgi apparatus has long been thought to be mediated by carrier vesicles budding from the ER and fusing with the Golgi. Vesicular intermediates of ER-Golgi transport have been isolated and characterized[1-2]. By *in vitro* reconstitution using membrane fractions of viral-infected cells, Rothman and co-workers have characterized intermembrane vesicular transport in great detail[1]. Recent studies suggest that there exists an intermediate compartment between the ER and the Golgi that may mediate ER-Golgi transport[29]. Whether the intermediate compartment is an independent compartment or a sub-region of the ER or the Golgi has not been clearly resolved[2,29]. Although there are several explanations for the nature of the intermediate compartment[2,29], the simplest one would be that the intermediate compartment is equivalent to the transitional elements of the ER observed in pancreatic cells[33-34] and represents specialized regions of the ER that are actively involved in vesicle budding. The intermediate compartment could thus be ER exit sites (ERES)[2,35].

In investigating the subcellular dynamics of the mammalian KDEL receptor, p23[13], we observed that p23 could be distributed from its main perinuclear Golgi location to spotty structures upon 15°C incubation. These spotty structures were identified to be the ERES described above by the colocalization of p23 with the G protein of a temperature sensitive VSV strain (ts045), which is known to accumulate in the ERES at 15 °C. p23 seemed to recycle from the perinuclear Golgi region to the ER and then accumulate in the ERES. Upon warming up to 37°C, tubular extensions were observed to emanate from the ERES, and this tubular network apparently connects the ERES to the perinuclear Golgi region. Within a short period of time, p23 could be chased exclusively into the perinuclear Golgi. There are several ways to account for the observation of the tubules and the tubular network emanating from the ERES. One hypothesis is that there may exist physical connections between the ERES and the entry sites of the Golgi and that vesicles derived from the ERES are transported along these physical connections to the Golgi. We speculate that these physical connections could consist of several central 'highways' leading to the Golgi and each ERES is connected to these central highways by its local 'driveway'. The tubular structures observed upon release from the low temperature block[13] could be the result of a continuous stream of transport vesicles moving along these 'driveways' and 'highways". These physical connections may be specialized structures of the cellular cytoskeletal network and movement of vesicles along these connections may be facilitated by the common cellular motor proteins dynein, kinesin[36], or a host of motor proteins yet to be identified. A schematic depiction of this hypothesis is presented in Fig 2.

The concentration of p23, ts045 VSV G-protein and other proteins in the ERES at 15°C could be the result of an accumulation of the budded vesicles around the ERES, which is most likely due to an inhibition of movement of the budded vesicles along the transport 'driveway' and 'highway'. The accumulation of vesicles in the ERES at 15 °C results in the formation of ultrastructurally identifiable structures composed mainly of clustered vesicles and short tubules[37], the structure of which is morphologically similar to the transitional elements of the ER[33-34]. These structures may also be

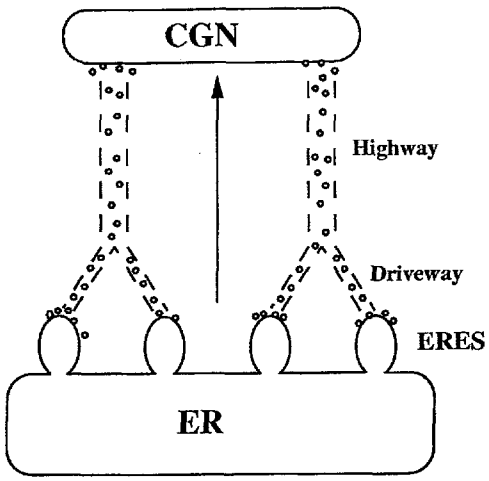

Fig. 2. A hypothetical model for ER-Golgi transport. Proteins in the ER are packaged at the ER exit sites (ERES) into transport vesicles. The ERES might be equivalent to the recently identified intermediate compartment involved in ER-Golgi transport. Transport vesicles bud from the ERES and are transported to the CGN via structural connections ('driveways' and 'highways') between the ERES and the CGN

detected at 37°C, especially in cells which possess massive secretion such as the pancreatic cells[33-34]. The detailed molecular composition and the nature of the ERES awaits further investigation. The existence of 'driveways' and 'highways' between the ERES and the Golgi is presently speculative and will be resolved in future experiments.

Golgi Localization

The majority of resident Golgi proteins that have been cloned belongs to a family of type II membrane proteins that are involved in the modification of carbohydrate chains of glycoproteins and glycolipids[38]. Recent studies performed in this and other laboratories have demonstrated that the transmembrane domain (the type II signal/anchor) of three Golgi glycosyltransferases, α2,6-sialylytransferase[39-41], β1,4-galactosyltransferase[42-45] and N-acetylglucosaminyltransferase I[46], is sufficient to confer Golgi localization of reporter molecules. In one case, it was also shown that the transmembrane domain of α2,6-sialyltransferase functions intracellularly[39]. In some cases, the efficiency of Golgi localization is enhanced by the inclusion of sequences flanking the transmembrane domain. The mechanism for this membrane-anchor mediated retention is not known, but two possible working models are currently being considered. One envisages that the transmembrane domain of these Golgi proteins is recognized by a receptor-like protein(s), which mediate their Golgi localization via either a retrieval mechanism or by selectively incorporating them into a Golgi membrane region which is excluded from further trafficking. The other suggests that the Golgi environment causes resident Golgi proteins to aggregate into large complexes that is similarly excluded from transport. These two models are, in fact, not mutually exclusive because a protein component(s) may facilitate or mediate the aggregation of the Golgi protein (functioning as a chaperone), in conjunction with either the ionic and/or lipid environment of the Golgi. Interestingly, when the rat α2,6-sialyltransferase was expressed in yeast, the protein was mainly confined to the yeast Golgi, suggesting that a highly conserved process is involved in Golgi localization (unpublished observation). Golgi localization has also been investigated extensively using viral proteins. The first of the three transmembrane domains of the E1 protein of the avian coronavirus infectious bronchitis virus is sufficient for Golgi localization[47]. Golgi localization was abolished by point mutations in the transmembrane region, resulting in their surface expression. Furthermore, the Golgi-localized protein but not the surface protein was in a large complex that was SDS insoluble and sedimented to the bottom of a sucrose gradient, suggesting a selective aggregation of Golgi-localized proteins[47]. This suggests that aggregate formation could be important for Golgi localization mediated by this transmembrane domain. What triggers the aggregation is yet unknown and remains one of the immediate questions to be answered. Whether similar mechanisms are involved in the retention of endogenous Golgi proteins also needs to be investigated. However, no significant amount of SDS- resistant aggregates have been observed for Golgi-localized fusion proteins mediated by the transmembrane domain of α2,6-sialylytransferase[39] and N-acetylglucosaminyltransferase I[46]. In contrast to the E1 protein of the infectious bronchitis virus, the first transmembrane domain of the E1 protein of another coronavirus (mouse hepatitis virus A59) was not sufficient for Golgi localization[48].

The three Golgi sugar transferases discussed above are most likely confined to the Golgi stack[2, 49]. No Golgi localization signal has been examined for cellular proteins that are preferentially localized to the CGN. Whether the transmembrane domain of the E1 protein of avian coronavirus infectious bronchitis virus mediates the Golgi stack and/or CGN localization is not clear, although the viral protein is confined to the cis side of the Golgi[50]. The TGN localization signal has been investigated with a type I membrane protein known as TGN38[51]. TGN38 was localized to the Golgi in transfected cells. A mutant form in which the cytoplasmic tail has been deleted was delivered to the cell surface, suggesting that the cytoplasmic tail of TGN38 is necessary for Golgi localization. When the cytoplasmic domain of TGN38 is transfered to a cell surface protein, the chimeric protein is indeed localized to the TGN, demonstrating that the cytoplasmic tail of TGN 38 is sufficient for TGN localization. Detailed dissection of this region revealed that a critical Tyr residue and some of its flanking residues are essential for the TGN localization (our unpublished observations). The nature of this cytoplasmic signal and the mechanism governing TGN localization are currently being investigated.

Targeting to the Lysosomal/Endosomal System

The mannose 6-phosphate moiety (M6P) on N-linked glycans of soluble lysosomal enzymes is a well-known signal for targeting these proteins to lysosomes[52]. M6P is recog-

nized by two homologous but distinct receptors in the TGN, which deliver the lysosomal proteins to a prelysosomal (late endosomal) compartment for subsequent transport to the lysosome. The M6P receptors shuttle between the TGN and the prelysosomal compartment. A Golgi localized adaptin protein (γ-adaptin) has been proposed to participate in the targeting of lysosomal enzymes from the TGN to the prelysosomal compartment, possibly by binding to the cytoplasmic tail of the M6P receptor[52-53]. Recently, it has been shown that the cytoplasmic region of one of the receptors contains two signals for lysosomal enzyme sorting in the Golgi[54]. Integral membrane proteins of the lysosome do not contain the M6P signal and a different mechanism may be employed for their lysosomal targeting[55]. Experiments with several lysosomal membrane proteins have shown that the cytoplasmic tail of these proteins is important for lysosomal localization and the sequence is transferable to reporter molecules[55]. Interestingly, there is a tyrosine residue within the defined sequence which is essential for targeting. Recently, a di-leucine motif was identified to be involved in Golgi sorting to the lysosome. This di-leucine motif is present in the same cytoplasmic domain harboring the Tyr-containing lysosomal signal, and these two signals can together enhance the sorting efficiency in the Golgi[54,56]. Two possible routes may be involved in delivery of lysosomal membrane proteins. One utilizes direct targeting from the TGN to the endosomal/lysosomal system. Alternatively, lysosomal membrane proteins may be initially delivered to the plasma membrane followed by selective retrieval from the cell surface to the endosomal/lysosomal system. Experimental data suggest that both these pathways may be used and the preference for either pathway depends on the individual protein being examined[52,54-59]. The molecular nature for recognition of the Tyr-containing or the di-leucine lysosomal targeting sequences is not known, although a particular type of cytosolic adaptins has been proposed to be involved[52-56].

The sequence responsible for the endosomal localization of the invariant chain (a type II integral membrane protein) of the class II major histocompatibility antigen has been mapped to the cytoplasmic domain[60-61]. Furthermore, the transmembrane domain of the invariant chain may also contain an endosomal targeting signal[42]. Further studies are needed to work out the details of the signal and its mechanism.

Protein Sorting in the TGN

Plasma membrane and secretory proteins are routed from the TGN into the constitutive secretory pathway to the plasma membrane. In polarized epithelial cells, the plasma membrane is separated by tight junctions into morphologically, biochemically and functionally distinct apical and basolateral domains[14-16]. Two different routes exist from the TGN to the two surface domains. Proteins may be transported directly to one or both domains and they may travel between these two domains via transcytosis in both directions. The apical route from the TGN (as well as from the endosome) in Madin-Darby canine kidney (MDCK) cells can be selectively inhibited by low doses of brefeldin A which does not affect ER-Golgi transport[62-64]. Basolateral delivery of proteins is not affected by similar doses of BFA.

While protein transport from the TGN to the surface is believed to be a default process[17], recent identification of structural motifs that may mediate selective targeting to different plasma membrane domains in epithelial cells suggests that, at least in these cells, there also exist signal-mediated routes from the TGN to the plasma membrane, in addition to the default pathway. One structural motif involved in this selective routing is the glycosylphosphatidylinositol (GPI) linker[65]. GPI-linked proteins are enriched in the apical plasma membrane domain of several epithelial cell types and it was demonstrated that the GPI linker is sufficient for apical targeting. The mechanism for such selective targeting is unclear, but may involve selective aggregation of this type of protein in the Golgi with the apically-enriched glycosphingolipids[15,66]. Two types of basolateral targeting sequences have been described[14]. The first type is examplified by a basolateral targeting sequence identified in the cytoplasmic domain of the polymeric IgA receptor[67]. A related sequence that is important for the basolateral localization of the low density lipoprotein (LDL) receptor has also been described[68]. Another type of basolateral targeting signal resembles that for rapid endocytosis[14,69-71]. The transferrin receptor and LDL receptor are examples of a family of surface proteins which are rapidly internalized from the cell surface into the endosomal system by clathrin-coated vesicles[72]. The sequence motif for this rapid endocytosis has been identified in several proteins and shown to have a reverse-turn structure with an essential Tyr residue[72-73]. This signal is most likely recognized by plasma membrane associated alpha-adaptin proteins, which promote the assembly of clathrin-coated vesicles at receptor-occupied regions of the plasma membrane[53,72-73]. These basolateral targeting sequences may similarly be recognized by cytosolic proteins (e.g. adaptin-like molecules), resulting in selective basolateral delivery of the proteins. The significance of the resemblence of the second type of basolateral signal to the endocytotic signal is not clear at present and remains to be examined.

Experiments so far have identified signals that mediate selective targeting to both the apical and basolateral surface, especially in polarized MDCK cells. The isolation of similar but distinct vesicles harboring either apical or basolateral cargo suggests strongly that selective transport to either surface is mediated by distinct types of vesicular carriers[74]. As has been previously proposed, transport to the plasma membrane could occur by default[17] This observation raised the intriguing question as to the whereabouts of the default pathway from the TGN to the surface in polarized epithelial cells, such as MDCK cells. Our recent studies employing brefeldin A, in conjunction with other observations, indicate that there exists a default pathway from the TGN to both the apical and basolateral surfaces in MDCK cells[63]. Polarized cells could thus possess both signal-mediated and default pathways to either surface, creating an enigma of how the TGN sorts domain-specific proteins accurately while maintaining a default pathway to both surfaces. A highly speculative model is presented in Fig. 3. We hypothesize that the TGN is struc-

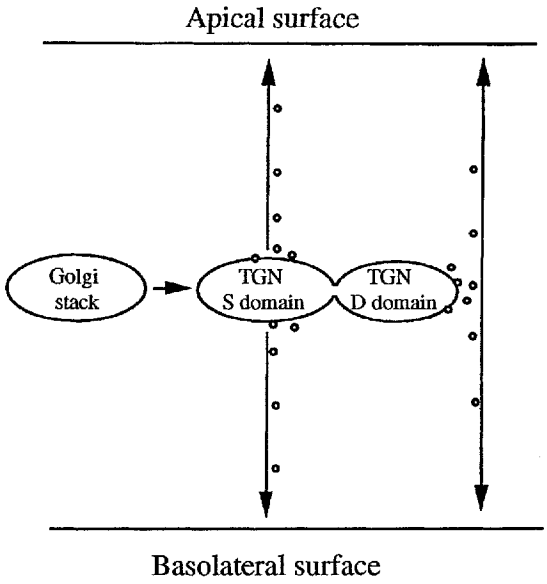

Fig. 3. A hypothetical model for protein sorting in the TGN. We speculate that the TGN is organized into two major sub-domains: the sorting (S) and default (D) domains. Proteins derived from the Golgi stack enter the TGN by fusion of the transport veiscles with the sorting (S) domain, where signal-containing proteins are actively packaged into vesicles destined for selected post-Golgi structures (e.g. the apical or basolateral surface of polarized epithelial cells). Proteins with no sorting signals move readily from the S to the D domain and are then packaged into default vesicles that will fuse with any potential site of the plasma membrane.

turally organized into two major domains: the S (sorting) and D (default) domains. The S domain is mainly involved in signal-mediated packaging of proteins to various types of transport vesicles destined for selected post-Golgi structures (e.g. the apical or basolateral domains of polarized cells); while the D domain is mainly involved in the formation of default transport vesicles which could fuse with any area of the plasma membrane (both the apical and basolateral surfaces in polarized cells). The D domain is more distal than the S domain from the Golgi stack. Therefore, proteins derived from the Golgi stack encounter the S domain first and signal-containing proteins are sorted immediately to either the apical or the basolateral vesicles. Proteins containing no specific signals move readily from the S to the D domain by diffusion either in the lumen (for secretory proteins) or along the lipid bilayer (for membrane proteins), where they are packaged into vesicles for the default pathway to both cell surfaces. One important requirement for this model is the organization of the TGN into two structurally and functionally related but distinct domains. This complex membrane organization could readily be compared to the ER system, which consists of several domains, including the nuclear membrane, the rough and smooth ER, and possibly the ERES[2]. This model would explain the experimental results accumulated so far. For example, the BFA effect on MDCK cells[62-63] could be resolved by proposing that BFA selectively inhibits the packaging of apical proteins into apical vesicles in the S domain. Unpackaged apical proteins then diffuse into the D domain where they are included into default vesicles that will fuse with both the apical and basolateral domains. The identified basolateral signals may mediate protein targeting into vesicles destined for the basolateral surface in the S domain, ensuring that the targeting is accurate and efficient even though there is a default pathway to both surfaces. If the signal was to be destroyed by mutagenesis, the mutant proteins would no longer be sorted in the S domain and, therefore, eventually end up in the D domain for the default transport to both surfaces[14,69]. Alternatively, a protein may contain an apical signal whose function is masked by the presence of a dominant basolateral signal in the same protein, and such a protein is sorted to the basolateral surface in the S domain. Mutagenesis of the dominating basolateral signal will unmask the apical signal, which will then target the protein to the apical vesicles[14,70]. Introducing a dominant basolateral signal could target an otherwise apical protein to the basolateral surface by selective packaging into basolateral vesicles in the S domain[59,70]. Similarly, we could also predict that an apical protein carrying a mutated signal would be transported to both surfaces from the D domain.

Proteins destined for the endosomal/lysosomal system may also be actively sorted in the S domain and targeting from the TGN to the endosomal/lysosomal system would therefore be accurate despite the presence of a default pathway to the surface. The efficiency of sorting in the S domain depends on the affinity of the signal for the sorting machinery. As mentioned above, there are two pathways for the targeting of proteins into the endosomal/lysosomal system: direct targeting from the TGN or indirectly from the surface. Proteins with high affinity signals will be sorted efficiently so that they are mainly targeted intracellularly to the endosomal/lysosomal system. Proteins with weak signals are sorted poorly in the S domain and a significant fraction of the proteins is able to diffuse into the D domain where these molecules are transported to the surface by default. These proteins could then be retrieved from the surface back to the endosomal/lysosomal system by the sorting machinery on the cell surface. Proteins normally sorted intracellularly may be sorted on the cell surface if their high affinity signals were mutated into weak ones[54,56]. If packaging into sorting vesicles is prevented by either mutagenesis to destroy the sorting signal or by agents (e.g. NH_4Cl) that interfere with sorting, endosomal/lysosomal proteins will end up in the D domain and are then transported by default to the surface[52,55]. The identification and characterization of more TGN components will be essential to test whether the TGN is indeed organized into subdomains.

Conclusions

Although significant progress has been made in understanding the structural organization of the exocytotic pathway as well as in trafficking signals and the underlying molecular mechanisms, many more questions await to be answered. Understanding the regulation of the dynamic nature of the exocytotic pathway and the balance between the retrograde and anterograde pathways will be essential for studies on other aspects of protein trafficking. The pathway and molec-

ular mechanisms responsible for the ER retention of type I membrane proteins and for transmembrane domain-mediated Golgi localization of glycosyltransferases remain to be determined. Little is known about the ER retention of other types of integral membrane proteins. The mechanisms and compartments (or subcompartments) involved in the efficient ER-Golgi transport remain to be investigated further. Much of the composition and structure of the TGN as well as the mechanism of sorting in this Golgi subcompartment is not understood. The hypothesis that the TGN is organized into sub-domains will need to be tested by future experiments. The nuclear envelope has a unique structure and composition but is still in continuity with the ER membrane. The underlying mechanism for this segregation is also not known. Although BFA has widespread effects on the entire exocytotic pathway but its direct effector molecule(s) have not yet been recognized. The multi-faceted approaches employed by investigators in this field and the combination of powerful molecular techniques with those of classical biochemistry and yeast genetics will undoubtedly reveal much more about the fundamentals of subcellular protein trafficking.

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