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COMMENTARY

ERGIC-53 and traffic in the secretory pathway

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SUMMARY

The ER-Golgi intermediate compartment (ERGIC) marker ERGIC-53 is a mannose-specific membrane lectin operating as a cargo receptor for the transport of glycoproteins from the ER to the ERGIC. Lack of functional ERGIC-53 leads to a selective defect in secretion of glycoproteins in cultured cells and to hemophilia in humans. Beyond its interest as a transport receptor, ERGIC-53 is an attractive probe for studying numerous aspects of protein trafficking in the secretory pathway, including traffic routes, mechanisms of anterograde and retrograde traffic, retention of proteins in the ER, and the function of the ERGIC. Understanding these fundamental processes of cell biology will be crucial for the elucidation and treatment of many inherited and acquired diseases, such as cystic fibrosis, Alzheimer's disease and viral infections.

Key words: ERGIC-53, Endoplasmic reticulum, Golgi intermediate compartment, Lectin, Secretory pathway, Protein traffic, Transport receptor, Targeting signal

INTRODUCTION

Understanding the molecular basis of secretion requires knowledge of how the individual organelles of the secretory pathway are formed and maintained and of how they exchange membranes and proteins in the anterograde (forward) and retrograde (backward) directions. The analysis of these processes involves the use of indicator proteins to define organelle boundaries and the use of reporters to study traffic routes and mechanisms.

A versatile protein for studying various aspects of secretion is a marker for the ER-Golgi intermediate compartment (ERGIC), ERGIC-53, a non-glycosylated type I membrane protein of 53 kDa (Hauri and Schweizer, 1992, 1997; Saraste and Kuismanen, 1992; Farquhar and Hauri, 1997; Hong, 1998). Our group discovered ERGIC-53 in a monoclonal antibody screen for organelle marker proteins, starting with a Golgi-enriched membrane fraction of the human intestinal epithelial cell line Caco-2 as an antigen (Schweizer et al., 1988), and subsequently cloned its cDNA (Schindler et al., 1993). Independently, Saraste and co-workers generated polyclonal antibodies against a Golgi fraction of rat pancreas that recognized among other proteins a 58-kDa protein dubbed p58 (Saraste et al., 1987). The p58 protein was originally believed to be a glycoprotein of the cis-Golgi (Hendricks et al., 1991), but further purification of the antiserum and cloning revealed that p58 is non-glycosylated and is the rat ortholog of ERGIC-53 (Lahtinen et al., 1996). In a third independent approach, Pimpaneau et al. (1991) isolated an intracellular protein designated MR60 in a search for lectins by mannose-column chromatography. Cloning identified MR60 as ERGIC-53 (Arar et al., 1995; Itin et al., 1996). Here, we use the term ERGIC-53 irrespective of species.

ERGIC-53 has several interesting features whose analysis has provided important information on the early secretory pathway, including its organization, traffic routes and the mechanism of protein targeting. Here, we summarize current knowledge of ERGIC-53 and its implications for understanding the basic mechanisms underlying secretion.

FEATURES OF ERGIC-53

Localization

ERGIC-53 is most highly concentrated in characteristic tubulovesicular clusters (also termed VTCs for vesicular tubular clusters; Bannykh et al., 1996) near the Golgi apparatus and in the cell periphery, the sum of which constitutes the ERGIC (Schweizer, 1988; Hauri and Schweizer, 1992; Klumperman et al., 1998). At the ultrastructural level, these clusters are indistinguishable in morphology and stain positive for both COPII, which coats vesicles mediating ER-to-ERGIC anterograde transport, and COPI, which coats vesicles mediating Golgi/ERGIC-to-ER retrograde transport (Aridor et al., 1995; Scales et al., 1997; Klumperman et al., 1998; Shima et al., 1999). In unfixed rapidly frozen cells these membrane entities often appear as single flat cisternae, rather than tubulovesicular clusters, exhibiting numerous budding profiles at their rims (H. Horstmann and W. Hong, unpublished). Lower concentrations of ERGIC-53 are present in the ER and in the first fenestrated cis-cisterna of the Golgi (Schweizer et al., 1988; Chavrier et al., 1990; Klumperman et al., 1998),

reflecting recycling pathways for this protein. At 15°C newly synthesized vesicular stomatitis virus G protein (VSV-G) and other secretory proteins accumulate in the ERGIC-53-positive ERGIC clusters (Schweizer et al., 1990; Saraste and Svensson, 1991; Klumperman et al., 1998). This finding established that the 15°C compartment defined by VSV-G and the ERGIC defined by ERGIC-53 are identical. Note, however, that many newly synthesized secretory proteins are inefficiently transported from the ER to the ERGIC at 15°C. ERGIC-53 has also been used as a marker in purification of the ERGIC by subcellular fractionation (Schweizer et al., 1991, 1994; Annaert et al., 1999). ERGIC membranes exhibit features that are different from those of the ER and cis-Golgi.

Topology and oligomerization

Cloning (Schindler et al., 1993; Arar et al., 1995; Lahtinen et al., 1996) and biochemical analysis (Schweizer et al., 1988) showed that ERGIC-53 is a type I integral membrane protein that has a large luminal domain and a short 12-residue cytosolic domain (Fig. 1). ERGIC-53 forms disulfide-linked homodimers and homohexamers in the ER (Schweizer et al., 1988; Lahtinen et al., 1992). At steady state the two oligomeric forms are present in about equal amounts in total cell extracts (Schweizer et al., 1988), as well as in all membrane fractions resolved on a Nycodenz gradient that harbor ERGIC-53 (unpublished observations). Disulfide-linked oligomerization is mediated by two of the four luminal cysteine residues, Cys 466 and Cys 475. If either of these cysteine residues is mutated to alanine, ERGIC-53 forms dimers but no hexamers (Lahtinen et al., 1999; Appenzeller et al., 1999). Replacement of both cysteine residues by alanine residues prevents all disulfide-linked oligomerization, leaving ERGIC-53 in a monomeric form. Treatment of living cells with dithiothreitol (DTT) breaks down hexamers to dimers but not to monomers, which suggests that in vivo one of the disulfide bonds is more accessible to reducing agents than is the other (unpublished observations). Because high concentrations of DTT (10 mM) are required for this conversion, once formed, hexamers are unlikely to dissociate to form dimers under physiological conditions.

Turnover

ERGIC-53 has a long half-life of several days (Schweizer et al., 1988), which is not surprising in light of its proposed function as a transport receptor for glycoproteins (see below).

Lectin features

A remarkable feature of ERGIC-53 is an ~200-residue segment in the luminal domain that shares homology with the carbohydrate-recognition domain (CRD) of several leguminous plant lectins. The closest relative of ERGIC-53, the mammalian cis-Golgi protein VIP36, exhibits similar homologies (Fiedler et al., 1994; Füllekrug et al., 1999a). Fiedler and Simons (1994) suggested therefore that ERGIC-53 and VIP36 define a new class of animal lectins. Subsequent findings that ERGIC-53 can selectively bind to mannose columns and that point mutations of conserved amino acids in the putative CRD abolish this binding confirm this notion (Arar et al., 1995; Itin et al., 1996). Moreover, isolated VIP36 binds

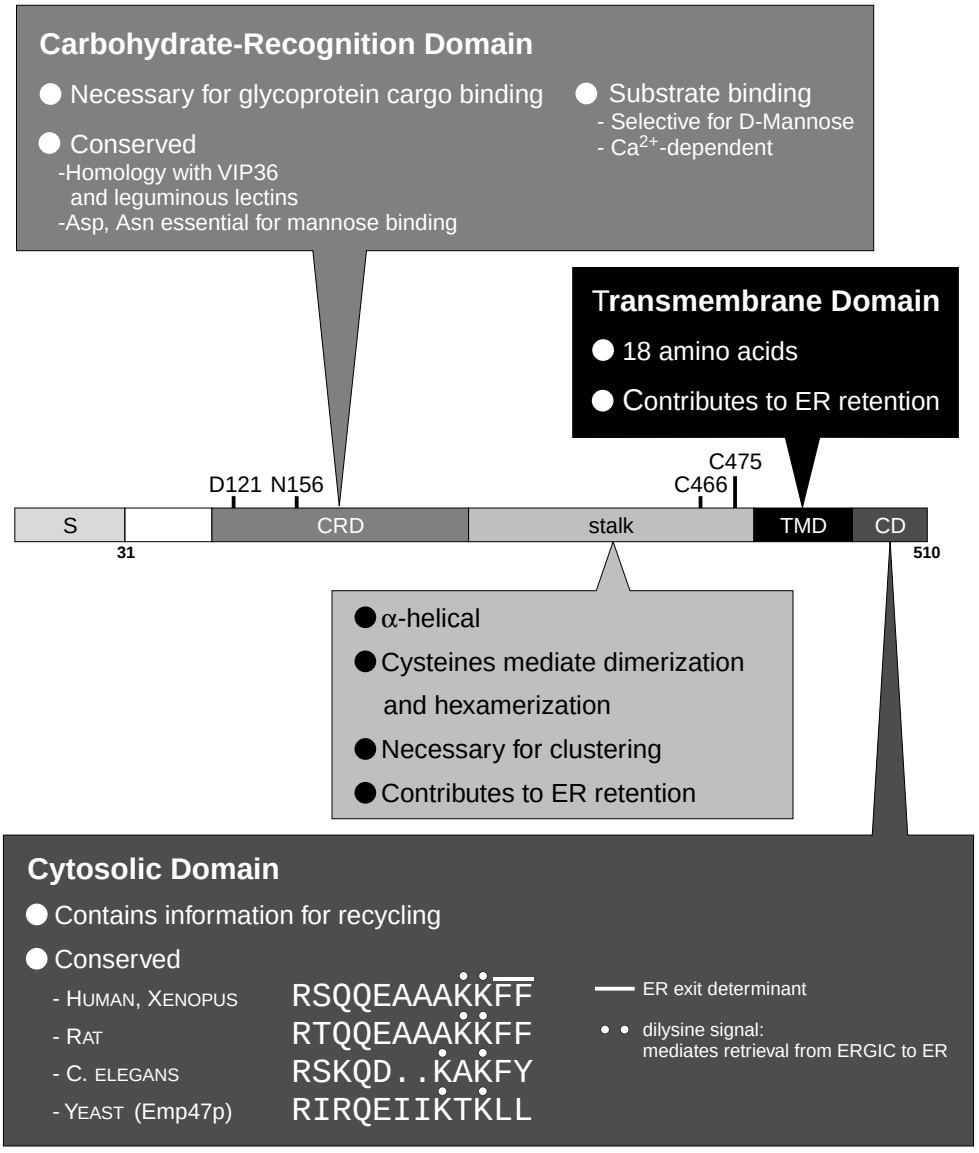

Fig. 1. Schematic diagram of ERGIC-53 and outline of its functional domains. CD, cytosolic domain; S, signal sequence; TMD, transmembrane domain. Some functionally important amino acids (single letter code) are indicated.

to high-mannose-type glycans (Hara-Kuge et al., 1999) and GalNAc residues (Fiedler and Simons, 1996). A notable difference between the two lectins is that binding of sugars to ERGIC-53 requires Ca^{2+} ions (Itin et al., 1996; Appenzeller et al., 1999) whereas binding of sugars to VIP36 is Ca^{2+} independent (Hara-Kuge et al., 1999). This indicates that the two lectins have different modes of action.

Evolution of ERGIC-53

ERGIC-53 is conserved from *C. elegans* to *Xenopus*, rat and man, and is present in all cell types. Likewise, VIP36 is conserved from *C. elegans* to man. The non-essential yeast protein Emp47p appears to be the yeast homolog of ERGIC-53 (Schröder et al., 1995). Unlike CRD in ERGIC-53 from multicellular organisms, the putative CRD of Emp47p lacks some residues known to be critical for sugar binding. This raises some doubts as to whether Emp47p has a lectin function. However, Emp47p also recycles in the early secretory pathway.

TRAFFIC ROUTES

As a rather abundant recycling protein, particularly in actively secreting cells such as HepG2 cells, ERGIC-53 is an attractive protein for visualizing anterograde and retrograde traffic routes. Although the ER harbors considerable amounts of ERGIC-53, the concentration of ERGIC-53 in this organelle is low, except in budding structures at the transitional elements, where ERGIC-53 is concentrated (Klumperman et al., 1998). This concentration might indicate that ERGIC-53 is actively recruited by COPII proteins (see below). Low-temperature/rewarming experiments in which synchronized recycling can be followed from the ERGIC (Lippincott-Schwartz et al., 1990) revealed that a direct recycling pathway from the ERGIC to the ER bypasses the cis-Golgi (Aridor et al., 1995; Itin et al., 1995c; Tang et al., 1995a). Morphological and biochemical analyses indicate that for ERGIC-53 this is in fact the major recycling route, and only a minor fraction of ERGIC-53 recycles via the cis-Golgi (Fig. 2; Itin et al., 1995c; Kappeler et al., 1997; Klumperman et al., 1998).

The recycling of ERGIC-53 can be blocked either in the ER or in the ERGIC by various agents (Fig. 2; Hauri and Schweizer, 1997). Exit from the ER is inhibited by lowering of the culture temperature to 10°C, by lowering of the ATP levels in deoxyglucose/azide-treated cells (Appenzeller et al., 1999), by the phosphatase inhibitor okadaic acid (Pryde et al., 1998), by a GTP-restricted dominant negative mutant of the small GTPase Sar1 (which is required for COPII vesicle formation; Shima et al., 1998; Andersson et al., 1999), or by osmotically induced cell-volume change (Lee and Linstedt, 1999). ER exit is also inhibited during mitosis (Farmaki et al., 1999). Exit from the ERGIC in both the anterograde and retrograde directions is reversibly blocked both at 15°C (Lippincott-Schwartz et al., 1990; Saraste and Svensson, 1991; Klumperman et al., 1998) and in the presence of aluminum fluoride (AlF_4^-, which inhibits trimeric GTPases; Kappeler et al., 1997; Klumperman et al., 1998). Exit from the ERGIC is also blocked by the vacuolar H^+-ATPase inhibitor bafilomycin A1 (Palokangas et al., 1999) and by the proton ionophore monensin (unpublished observations); this suggests that acidification plays a role in the recycling process.

Remarkably, ERGIC-53 also accumulates in the ERGIC in the presence of brefeldin A (BFA; Lippincott-Schwartz et al., 1990; Saraste and Svensson 1991). This contrasts with most Golgi-resident proteins, which are transported to the ER under these conditions. As a general rule, rapidly recycling proteins of the early secretory pathway accumulate in the ERGIC rather than in the ER upon BFA treatment, irrespective of whether they localize more to the Golgi than to the ERGIC at steady state (Hauri and Schweizer, 1997). Examples include the cis-Golgi proteins KDEL receptor (Itin et al., 1995c; Tang et al., 1995b), VIP36 (Füllekrug et al., 1999a) and gp74 (Alcade et al., 1994), and members of the p24 protein family (Blum et al., 1999; Füllekrug et al., 1999b; Gommel et al., 1999). BFA-induced accumulation in the ERGIC is therefore a diagnostic test for rapidly recycling, as opposed to resident, Golgi proteins.

The different behavior of resident and recycling Golgi proteins in BFA-treated cells suggests that two different retrograde pathways from the Golgi to the ER exist: a direct one bypassing the ERGIC, which might be followed by Golgi-resident trimming enzymes and glycosyltransferases; and an indirect one, presumably via the ERGIC, that is followed by rapidly recycling proteins, including ERGIC-53. There is indeed evidence that Golgi-resident proteins slowly recycle through the ER in unperturbed cells during interphase and mitosis (Cole et al., 1996; Presley et al., 1998; Storrie et al., 1998), although the extent of this recycling remains unclear (see Jesch and Linstedt, 1998 and Shima et al., 1998, for opposite views). Very recent evidence supports the notion that two independent retrograde routes from the Golgi back to the ER exist (Girod et al., 1999). Microinjection of anti-COPI antibodies or expression of a GDP-restricted Arf1 mutant inhibits the recycling of ERGIC-53 and KDEL receptor but not Golgi-to-ER transport of Golgi-resident glycosylation enzymes and Shiga toxin.

It has not been possible to block ERGIC-53 selectively in the cis-Golgi, although Fujiwara et al. (1998) have claimed that the lipoxygenase inhibitor nordihydroguaiaretic acid blocks this pathway before it also inhibits ER export of ERGIC-53. Fujiwara et al. based this conclusion on an ultrastructural study using the non-quantitative peroxidase technique, which complicates the differentiation of major and minor recycling pathways. Moreover, the high mobility of the ERGIC clusters, which can accumulate close to the Golgi (Klumperman et al., 1998), has to be taken into account. Such clusters can be mistaken for cis-Golgi elements. The study by Fujiwara et al. (1998) is at variance with biochemical findings (Kappeler et al., 1997) and immunogold-labeling data (Klumperman et al., 1998) that suggest that the cycling of ERGIC-53 through the cis-Golgi is only a minor pathway. It takes at least 12 hours before N-glycosylation-site-tagged ERGIC-53 molecules quantitatively acquire cis-Golgi-mediated glycan trimming down to the Man5 structure. This inefficient recycling complicates the use of traffic inhibitors to block recycling in the cis-Golgi. By contrast, accumulation of ERGIC-53 in the ERGIC in the presence of AlF_4^- takes only 30 minutes (Kappeler et al., 1997; Klumperman et al., 1998). These observations suggest that cycling of ERGIC-53 through the cis-Golgi is more than one order of magnitude less efficient than cycling through the ERGIC.

The efficient retrograde transport of ERGIC-53 from the

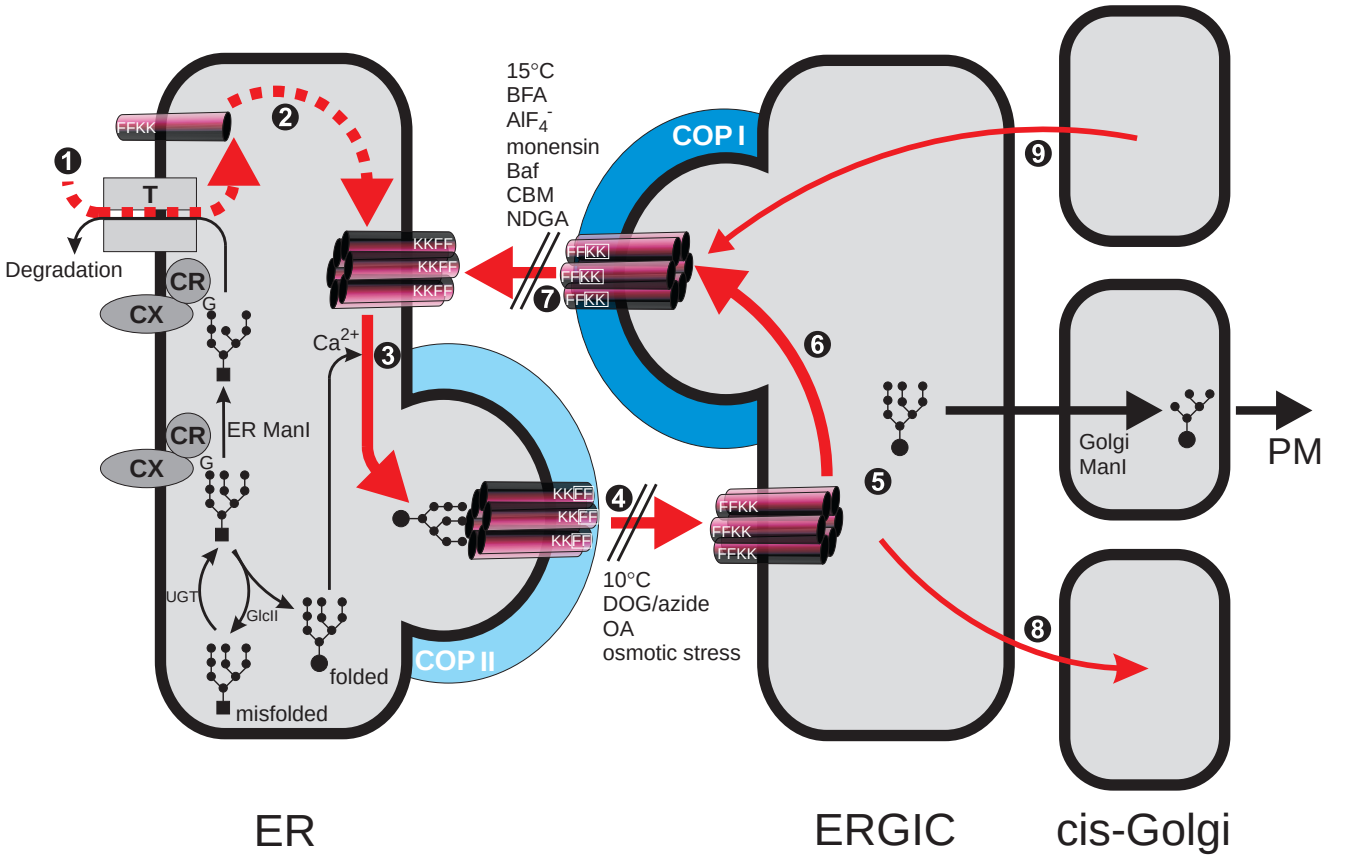

Fig. 2. Cargo receptor function and recycling of ERGIC-53. (1) Translocation of newly synthesized ERGIC-53 monomers into the lumen of the rough ER and anchored in the ER membrane by their single transmembrane domain. (2) Formation of disulfide-linked homo-oligomers that bind to Man9-N-glycans of correctly folded cargo glycoproteins in the presence of Ca^{2+}. (3) Recruitment of the cargo-ERGIC-53 complex to budding sites of the transitional elements by direct interaction between the cytosolic FF motif and components of the COPII coat. (4) COP II-vesicle budding and vesicular transport to the ERGIC. (5) Release of cargo in the ERGIC. (6) Recruitment of free ERGIC-53 to COPI vesicle-budding sites by direct interaction of the cytosolic di-lysine signal with components of the COPI coat. (7) COPI-vesicle budding and retrograde transport to the ER. (8) Escape of a subpopulation of ERGIC-53 molecules to the cis-Golgi. (9) Retrieval of this subpopulation to the ERGIC by COPI vesicles. Incompletely folded glycoproteins bind to calnexin (CX) and/or calreticulin (CR) after removal of the two outermost glucose residues (Trombetta and Helenius, 1997). Subsequently they are trimmed by glucosidase II (Glc II) and, if incompletely folded, reglucosylated by UDP-glucose:glycoprotein glucosyltransferase (UGT). After folding is completed, the glycoprotein cargo molecule can bind to ERGIC-53. After a prolonged time, ER α1,2-mannosidase (ER Man I) acts on the middle branch of the glycan, removing one mannose residue. Mono-glucosylated Man8 is a targeting signal for calnexin-dependent retranslocation of the misfolded protein to the cytosol and subsequent degradation possibly due to attenuated deglucosylation by Glc II (Liu et al., 1999). Inhibitors of anterograde and retrograde traffic are indicated: DOG, deoxyglucose; OA, okadaic acid; BFA, brefeldin A; Baf, bafilomycin; CBM, 1,3-cyclohexane-bis(methylamine); NDGA, nordihydroguararetic acid. Additional abbreviations: COP I, type I coat protein complex; COP II, type II coat protein complex; ER, endoplasmic reticulum, ERGIC, ER-Golgi intermediate compartment, PM, plasma membrane; T, translocation channel; •, mannose; G, glucose.

ERGIC will be of great help in the future: it should allow us to visualize retrograde and anterograde traffic from the ERGIC simultaneously in living cells and to elucidate the mechanisms of protein sorting in the ERGIC.

THE ORGANIZATION OF THE EARLY SECRETORY PATHWAY

Although it is now generally accepted that the ERGIC defined by ERGIC-53 is an obligatory intermediate compartment in protein transport from the ER to the Golgi, the question of whether the ERGIC is only a transient membrane structure (the maturation hypothesis) or a true compartment (the stable-compartment hypothesis) remains unanswered. According to the maturation hypothesis, the ERGIC clusters would be formed by homotypic fusion of ER-derived, initially COPII-coated vesicles and subsequently fuse with each other to form a new cis-Golgi cisterna. This model has gained increasing popularity (Bonfanti et al., 1998; Presley et al., 1998; Glick and Malhotra, 1998; Allan and Balch, 1999). It is based mainly on studies at the light microscope level of the anterograde reporter GFP-tagged VSV-G protein in living cells (Presley et al., 1997; Scales et al., 1997). Although intriguing, the results of these studies could also be accommodated by the stable-compartment hypothesis, assuming the ERGIC clusters are highly dynamic. According to the stable-compartment hypothesis, the ER-derived vesicles would fuse with ERGIC clusters rather than with themselves. Anterograde and retrograde traffic from the ERGIC would not entirely consume

the clusters, but part of them would remain and function as an acceptor for new membrane traffic from both the ER and the cis-Golgi. A detailed immunofluorescence and ultrastructural study (Klumperman et al., 1998) showed that the ERGIC clusters tend to concentrate near the Golgi at 15°C and accumulate twice as much ERGIC-53 as normal. 10 minutes after rewarming to 37°C, cluster distribution within the cytoplasm was almost normal again, and the clusters contained normal amounts of ERGIC-53. The average number of clusters was constant throughout the 15°C-rewarming period, and there was no indication that ERGIC clusters fuse with each other or the cis-Golgi. A straightforward interpretation of these findings is that the ERGIC clusters are permanent but highly mobile entities. However, the data can also be accommodated by the maturation model, if one assumes that the swift normalization is due to rapid recycling of ERGIC-53 into ERGIC clusters formed de novo from the ER. A simultaneous visualization of anterograde and retrograde markers both in living cells and at the ultrastructural level in rapidly frozen cells will be required if we are to differentiate between the two hypotheses. Moreover, we must test whether ER-derived vesicles can fuse with each other or only with existing ERGIC clusters.

A major role of the ERGIC is the sorting of anterograde and retrograde traffic (Klumperman et al., 1998; Bannykh et al., 1998; Martinez-Menarguez et al., 1999). COPI coats play a fundamental role in this segregation process (Shima et al., 1999). Not only retrograde traffic but also anterograde traffic from the ERGIC appears to depend on the action of COPI coats. Indeed, conditions that interfere with COPI coat assembly or disassembly block the transport of cargo from the ERGIC to the Golgi (Pepperkok et al., 1993; Hauri and Schweizer, 1997; Tisdale et al., 1997). Moreover, in living cells, non-functional COPI reduces ERGIC dynamics in a similar way to the microtubule-active drug nocodazole (Shima et al., 1999). We hypothesize that the motility of ERGIC clusters toward and away from the Golgi is driven by microtubule-associated motor proteins (Klumperman et al., 1998). Both minus-end-directed (toward the Golgi) and plus-end-directed (toward the cell periphery) motor activities are associated with the ERGIC (Lippincott-Schwartz et al., 1995; Presley et al., 1997). The binding of these motors to the ERGIC might involve rab GTPases such as rab2. An emerging view is that rab-GTPases – molecules that are critical for membrane tethering and fusion – can also link vesicles and organelles to motor proteins (Pfeffer, 1999). Selective recruitment of as-yet-unidentified adaptor proteins for plus-end-directed motors into COPI-coated domains of the ERGIC might couple membrane segregation and maturation. Such a mechanism would explain the inhibitory effect of COPI-inactivating agents on anterograde transport.

TARGETING SIGNALS AND TARGETING MACHINERIES

The cycling of ERGIC-53 is determined by a complex interplay of numerous targeting determinants and two different coat complexes (Figs 1 and 2). All three domains of ERGIC-53 (luminal, transmembrane and cytosolic) appear to contribute to its correct targeting. The molecular analysis of recycling is still in progress but important insight has already been obtained.

ER exit

In transfected cells, efficient exit of ERGIC-53 from the ER requires the two C-terminal phenylalanine residues of the cytosolic domain (Fig. 1; Kappeler et al., 1997). The two residues mediate binding of ERGIC-53 to COPII coats in vitro. The di-phenylalanine motif by itself is not sufficient for efficient ER exit of reporter proteins, which suggests that it is part of a larger ER-exit signal. The entire signal is currently unknown. Interestingly, members of the p24 protein family, which recycle between the ER and post-ER compartments and which have been proposed to operate as transport receptors (Schimmöller et al., 1995) and COPI receptors (Stamnes et al., 1995; Bremser et al., 1999), also possess a di-phenylalanine motif that mediates binding to COPII coats (Dominguez et al., 1998). These findings strongly suggest that ER export of at least some membrane proteins is signal mediated. In *C. elegans* the most C-terminal phenylalanine residue of ERGIC-53 is replaced by a tyrosine, and in yeast the di-phenylalanine motif is replaced by two leucine residues. This suggests that the ER-exit determinant tolerates other hydrophobic residues. Corresponding mutants in human ERGIC-53 indeed indicate that these residues function in ER export (unpublished observations). Although the di-phenylalanine motif is clearly required for efficient ER exit, the precise mechanism by which it mediates interaction of ERGIC-53 and COPII coats is unknown. Some other proteins, including VSV-G protein, carry a di-acid ER-exit motif in their cytoplasmic domain (Nishimura and Balch, 1997), but there is no evidence for a direct interaction of these proteins with COPII coats.

ER retrieval and retention

ERGIC-53 carries a C-terminal KKXX di-lysine ER-targeting signal (Jackson et al., 1990, 1993; Schindler et al., 1993) that binds to COPI proteins in vitro and is required for recycling (Fig. 2; Tisdale et al., 1997; Kappeler et al., 1997). Inactivation of the di-lysine signal by mutagenesis abolishes retention of ERGIC-53 in the recycling pathway (Itin et al., 1995a), and microinjection of anti-COPI antibodies (which block COPI function) inhibits recycling of ERGIC-53 (Pepperkok et al., 1993; Girod et al., 1999). COPI-mediated recycling is saturable: when ERGIC-53 is overexpressed, some leaks to the cell surface (Kappeler et al., 1994; Itin et al., 1995b). Detailed immunogold analysis by electron microscopy showed partial co-localization of ERGIC-53 and β-COP in ERGIC clusters and in COPI-coated vesicles, which further supports the notion of a COPI-dependent recycling mechanism for ERGIC-53 (Klumperman et al., 1998).

An additional mechanism contributing to correct targeting of ERGIC-53 is protein retention. The luminal and transmembrane domains together mediate retention of ERGIC-53 in the ER. This is apparent when the cytoplasmic domain is replaced by a sequence of alanine residues of the same length (Kappeler et al., 1997). If, in this construct, the transmembrane domain is replaced by that of the plasma membrane protein CD4, ER export is increased (Itin et al., 1995a). Further analysis of this retention mechanism might provide novel information about the mechanism by which transmembrane domains can mediate ER retention. Cycling of ERGIC-53 in the early secretory pathway is therefore determined by at least three signals, which mediate ER retention, ER exit and retrieval from the ERGIC.

Mutational analysis of targeting determinants in ERGIC-53 has also broadened our view of the functions of di-lysine signals. Until recently, all cytosolic di-lysine signals of type I proteins were believed to be defined by two lysines, either in positions -3 and -4 or in position -3 and -5, that exclusively function in COPI-mediated retrieval from post-ER compartments (Jackson et al., 1993; Teasdale and Jackson, 1996; Cosson and Letourneur, 1994; Letourneur et al., 1994). However, if the KKFF signal of ERGIC-53 chimeric proteins is mutated to KKAA, the altered protein is unable to leave the ER. Further analysis showed that KKAA is a true ER retention signal and that this retention is independent of COPI (Andersson et al., 1999). These findings strongly suggest that di-lysine signals can mediate both ER retention and retrieval. Contrary to di-lysine-signal-mediated retrieval, KKAA-mediated retention is not saturable and the retention mechanism is unknown.

Another surprising finding concerns the fate of ERGIC-53 mislocalized to the plasma membrane upon overexpression. Surface ERGIC-53 is efficiently endocytosed (Kappeler et al., 1994). This endocytosis depends on the di-lysine signal, and like the ER-retrieval signal, the signal must occupy a C-terminal position. The minimal consensus sequence for endocytosis is K-K/R-F/Y-F/Y (Itin et al., 1995b). VIP36 contains a C-terminal tetrapeptide KRFY that matches the consensus sequence and can also mediate endocytosis (Itin et al., 1995b). The relatedness of the KKFF targeting determinant and the K-K/R-F/Y-F/Y endocytosis signal suggests that there are mechanistic similarities between signal-coat interactions in the early secretory pathway and those at the cell surface.

THE FUNCTION OF ERGIC-53 IN GLYCOPROTEIN TRAFFICKING

Two different models for how secretory and membrane proteins (collectively termed cargo proteins) are transported from the ER to the Golgi have been proposed. According to the bulk-flow model (Wieland et al., 1987), cargo moves by default and requires no export signal – in contrast to ER-resident proteins, which possess retention or retrieval signals. According to the second model, receptor-mediated export (Lodish, 1988; Kuehn and Schekman, 1997), cargo is selectively packaged into budding COPII vesicles by a signal-mediated process. Membrane cargo could directly interact with the COPII coat, whereas soluble cargo would require transport receptors for efficient ER export.

ERGIC-53 has several hallmarks of a transport receptor for glycoproteins, including a functional CRD, anterograde and retrograde transport signals that interact with vesicular coats, and a long half-life (Hauri and Schweizer, 1997; Herrmann et al., 1999). But what is the cargo? Recent evidence suggests that ERGIC-53 is required for efficient transport of a limited set of glycoproteins. A first indication that ERGIC-53 is involved in glycoprotein transport came from a study of a case of congenital sucrase-isomaltase (SI) deficiency (Ouwendijk et al., 1996). In this patient a point mutation in SI led to the accumulation of the altered enzyme in the high-mannose form in the ERGIC and Golgi, and the entire Golgi, rather than the ERGIC, labeled positive for ERGIC-53. The data are consistent with a prolonged association of the mutant SI with ERGIC-53, although a direct interaction between the two proteins could not be demonstrated (unpublished observations). Interestingly, other cases of SI deficiency, and a case of lactase deficiency in which there is a selective block in transport of the corresponding enzymes in the Golgi, showed the same unusual Golgi localization of ERGIC-53 (unpublished observations). Evidence for a putative receptor function came from the identification of the gene responsible for the autosomal recessive bleeding disorder combined deficiency of coagulation factors V and VIII (F5F8D), which encodes ERGIC-53 (Nichols et al., 1998, 1999; Neerman-Arbez et al., 1999). In F5F8D, plasma levels of the two coagulation factors are reduced to 5-30% of the normal level. F5F8D is caused by several different mutations in the gene that encodes ERGIC-53 that lead to a truncated, and hence non-functional, protein or no protein at all. These studies suggest that ERGIC-53 is required for efficient secretion of coagulation factors V and VIII.

An entirely different approach also revealed a rather limited effect of non-functional ERGIC-53 on secretion. If endogenous ERGIC-53 in HeLa cell cultures is locked in the ER by overexpression of a dominant negative mutant of human ERGIC-53 (which terminates in KKAA) in an inducible manner, the major effect is inefficient secretion of the lysosomal enzyme cathepsin C. This suggests that efficient trafficking of this protein requires a functional ERGIC-53-recycling path (Vollenweider et al., 1998). As in the case of cathepsin C, the secretion of coagulation factors V and VIII expressed in this HeLa cell culture system is also inefficient when cycling of ERGIC-53 is blocked (Moussalli et al., 1999). In both cases, however, a direct interaction between these secretory proteins and ERGIC-53 could not be shown.

The most direct evidence for a cargo receptor function of ERGIC-53 was obtained through a chemical crosslinking approach in which a glycoprotein related to human cathepsin Z (termed catZr for cathepsin Z related) was identified in hamster cells (Santamaria et al., 1998; Appenzeller et al., 1999). Binding of catZr to ERGIC-53 occurs in the ER, is carbohydrate- and Ca^{2+}-dependent, and is affected by untrimmed glucose residues. Dissociation of catZr occurs in the ERGIC and is delayed if ERGIC-53 is mislocalized to the ER. Mannose trimming is not required for catZr binding or catZr release. Collectively these data suggest that ERGIC-53 functions as a cargo receptor facilitating ER-to-ERGIC transport of some soluble cargo proteins. These data suggest the following model for ERGIC-53 function (Fig. 2). After synthesis, ERGIC-53 oligomerizes in the ER to form disulfide-linked dimers and hexamers, and perhaps forms higher-order assemblies (Vollenweider et al., 1998). The actual oligomeric state of ERGIC-53, however, is unknown. Assembled ERGIC-53 then binds to (some) newly synthesized glycoproteins in a Ca^{2+}-dependent manner. Binding might start before the cargo is completely folded and released from the deglucosylation/reglucosylation cycle (Trombetta and Helenius, 1998), but maximal binding occurs after glucose trimming (Appenzeller et al., 1999). The cargo-receptor complex is then actively recruited into COPII vesicles by direct interaction between the cytoplasmic domain of ERGIC-53 and COPII coat proteins through the di-phenylalanine motif. In the ERGIC, dissociation of cargo occurs by an unknown mechanism, perhaps involving a drop in Ca^{2+} concentration. Free ERGIC-53 is then

recognized by COPI coats through its di-lysine signal and recycles to the ER for a next round of transport.

Although ERGIC-53 is clearly required for efficient transport of some glycoproteins, note that the absence of functional ERGIC-53 only slows down but does not entirely block their secretion. This might be due to an underlying bulk-flow mechanism. Alternatively, other lectins might operate as cargo receptors that are not necessarily related to ERGIC-53 and remain to be discovered. The same reasoning applies for the question of why only few secretory glycoproteins depend on ERGIC-53. It is possible that abundant secretory proteins are transported by bulk flow (Martinez-Menarguez et al., 1999; Warren and Mellman, 1999) whereas some less abundant proteins need efficient extraction from the ER by cargo-transport receptors such as ERGIC-53 – be it to protect them from degradation or from deleterious interactions with other proteins in the early secretory pathway. It should be emphasized, however, that the evidence for bulk flow operating as a major transport mechanism is still not compelling and will be difficult to provide in a convincing manner. The mere fact that soluble cargo, such as amylase in pancreatic cells, cannot be seen by immunoelectron microscopy to concentrate in vesicles budding from the ER (Martinez-Menarguez et al., 1999) does not necessarily indicate passive transport. For instance, binding of cargo to a receptor might lead to epitope shielding and thereby selective cargo packaging could be missed by this technique.

Successfully folded cargo must be efficiently sequestered from the folding machinery and from the degradation pathway. Prolonged residence in the ER can lead to Man8 trimming of the newly synthesized glycoproteins by ER mannosidases (Herscovics, 1999). The Man8 configuration can direct the selection of misfolded glycoproteins for degradation by the proteasome (Jakob et al., 1998; Liu et al., 1999; Fig. 2). It is also conceivable that, in normal cells, efficient transport of a majority of glycoproteins is mediated by ERGIC-53 and that ERGIC-53-defective cells would largely bypass this requirement by increasing the synthesis of the ERGIC-53-dependent glycoproteins. Virtually normal amounts of these proteins would then leave the ER by bulk flow. In that sense, ERGIC-53, and selective transport from the ER in general, although not essential, would serve as an energy saver.

CONCLUSIONS AND PERSPECTIVES

The studies on ERGIC-53 summarized in here point to a novel function of lectins in the secretory pathway: facilitation of ER-to-Golgi transport of secretory proteins. It is clear now that lectins are important at many sites along the secretory pathway. Lectin-dependent mechanism include the quality control of newly synthesized glycoproteins in the ER, which involves the glucose-specific lectins calnexin and calreticulin in conjunction with glucosyltransferase (Trombetta and Helenius, 1998), the sorting of lysosomal enzymes by mannose-6-phosphate receptors in the Golgi and at the plasma membrane (Dahms et al., 1989), and presumably the sorting of apical glycoproteins in the trans-Golgi network of polarized epithelial cells (Matter and Mellman, 1994; Fiedler and Simons, 1995; Scheiffele et al., 1995; Keller and Simons, 1997; Simons and Ikonen, 1997; Gut et al., 1998; Rodriguez-Boulan and Gonzalez, 1999). Additional lectin-dependent processes might operate in the Golgi. For instance, the lectin VIP36 might play a role in intra-Golgi glycoprotein trafficking – as indicated by its recycling within this organelle (Füllekrug et al., 1999a).

Despite some progress, many questions regarding the function of ERGIC-53 remain unanswered. What is the carbohydrate specificity of ERGIC-53 and how does it compare with that of its cis-Golgi relative VIP36 (Fiedler and Simons, 1994; Hara-Kuge et al., 1999)? The interaction of cargo with ERGIC-53 might depend on both sugar and peptide moieties; this would explain why not all glycoproteins bind. So far, no common feature in the four cargo proteins that would explain their selective binding to ERGIC-53 has been found. How is cargo released in the ERGIC, and is further transport of ERGIC-53-dependent cargo through the Golgi also receptor-mediated? What is the function of ERGIC-53 in primitive organisms? Although a low level of factors V and VIII is the only known consequence in patients lacking functional ERGIC-53, it is safe to say that the original importance of ERGIC-53 in evolution is unrelated to blood coagulation, because ERGIC-53 is present in *C. elegans*. Inactivation of the gene that encodes ERGIC-53 in *C. elegans* might reveal the original function of ERGIC-53 in multicellular organisms. Is there a compensatory mechanism for the lack of functional ERGIC-53, and what is wrong in F5F8D patients who have normal ERGIC-53 (Neerman-Arbez et al., 1999; Nichols et al., 1999)?

ERGIC-53 will be increasingly important as a reporter protein in future studies of various mechanistic aspects of secretion. For instance, ERGIC-53 might help us to identify and characterize novel ER-retention, ER-exit and ER-retrieval signals. It might contribute to the elucidation of the role of the different subunits/isoforms of the COPII coat in vesicle formation (Gimeno et al., 1995; Kuehn and Schekman, 1997; Roberg et al., 1999; Pagano et al., 1999; Tani et al., 1999). In these studies it will be important to assess the mechanism by which the di-phenylalanine motif mediates interaction of ERGIC-53 and COPII coats. This analysis will require purified COPII components. The use of ERGIC-53 in conjunction with anterograde markers in trafficking studies could also clarify the mechanisms of anterograde and retrograde protein sorting in the ERGIC and might answer the question of whether the maturation model or the vesicle-only model of secretion is correct.

As a membrane marker for the ERGIC, ERGIC-53 could be crucial in searching for additional functions of the ERGIC that are relevant for understanding a diverse range of human disease states. Many important proteins initially believed to localize exclusively to the ER now appear to recycle through the ERGIC. An interesting recent addition to this list is the Sec61 complex (Greenfield and High, 1999). Oligomers of the Sec61 complex form a transmembrane channel through which nascent proteins are translocated from the cytosol into the ER or misfolded proteins are exported to the cytosol for degradation. For an increasing number of genetic or acquired human diseases, disease-relevant normal or misfolded proteins have been reported to localize to the ERGIC – for example, presenilin 1 (Culvenor et al., 1997; Lah et al., 1997; Annaert et al., 1999) and the amyloid precursor protein fragment beta(1-42) (Cook et al., 1997; Skovronsky et al., 1998). Both

molecules are implicated in the pathogenesis of Alzheimer's disease. Another example is ΔF508 CFTR, which causes cystic fibrosis. This mutant chloride channel is trapped in the ER and ERGIC and, hence, cannot exert its normal function at the cell surface (Gilbert et al., 1998; Riordan, 1999). Because ΔF508 CFTR retains some channel activity, it is of therapeutic importance to identify its ER and ERGIC retention/recycling signals. In addition to molecular chaperones that contribute to ΔF508 CFTR retention in the ER, the additional ERGIC localization might indicate that the mutant, incompletely folded protein carries an ER-recycling signal. Interference with the corresponding recognition machinery might help to restore surface expression of the channel. In other diseases caused by disorders of protein conformation that affect the ER (Aridor and Balch, 1999), recycling of proteins through the ERGIC could also play an important role.

The ERGIC defined by ERGIC-53 also participates in the maturation of (or is target for) several viruses such as corona virus (Nguyen and Hogue, 1997; Salanueva et al., 1999), cytomegalovirus (Ziegler et al., 1997), flavivirus (Mackenzie et al., 1999), poliovirus (Sandoval and Carrasco, 1997), Uukuniemi virus (Jantti et al., 1997), and vaccinia virus (Salmons et al., 1997; Sodeik et al., 1993). Understanding the targeting of viruses and viral proteins to the ERGIC could lead to development of general approaches for viral interference.

Further analysis of the function of ERGIC-53, and the use of ERGIC-53 as a marker and reporter protein, should provide novel surprises about the mechanisms controlling traffic in the secretory pathway.

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