

# The ER-Golgi intermediate compartment (ERGIC): in search of its identity and function

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## Summary

Protein traffic moving from the endoplasmic reticulum (ER) to the Golgi complex in mammalian cells passes through the tubulovesicular membrane clusters of the ER-Golgi intermediate compartment (ERGIC), the marker of which is the lectin ERGIC-53. The dynamic nature and functional role of the ERGIC have been debated for quite some time. In the most popular current view, the ERGIC clusters are mobile transport complexes that deliver secretory cargo from ER-exit sites to the Golgi. Recent live-cell imaging data revealing the formation of anterograde carriers from stationary ERGIC-53-positive membranes, however, suggest a stable compartment model in which ER-derived cargo is first shuttled from ER-exit sites to

stationary ERGIC clusters in a COPII-dependent step and subsequently to the Golgi in a second vesicular transport step. This model can better accommodate previous morphological and functional data on ER-to-Golgi traffic. Such a stationary ERGIC would be a major site of anterograde and retrograde sorting that is controlled by coat proteins, Rab and Arf GTPases, as well as tethering complexes, SNAREs and cytoskeletal networks. The ERGIC also contributes to the concentration, folding, and quality control of newly synthesized proteins.

Key words: ER-exit sites, ER-to-Golgi transport, ERGIC-53, Protein sorting, COPI, COPII, Tethering, Fusion

## Introduction

The ER-Golgi intermediate compartment (ERGIC) is a complex membrane system between the rough endoplasmic reticulum (ER) and the Golgi that was initially defined following the identification of a 53 kDa membrane protein (ERGIC-53) that is predominantly localized to these membranes (Hauri et al., 2000; Schweizer et al., 1988). Studies of the transport of a temperature-sensitive mutant G protein from vesicular stomatitis virus (tsO45-VSV-G) and the E1 glycoprotein of Semliki forest virus demonstrated that ERGIC-53-positive membranes are intermediates in ER-to-Golgi protein transport (Saraste and Svensson, 1991; Schweizer et al., 1990), and biochemical analysis showed that the protein composition of ERGIC membranes differs from that of the neighboring ER and Golgi (Schweizer et al., 1991).

The discovery of the ERGIC (Fig. 1A) – also referred to as vesiculo-tubular clusters or pre-Golgi intermediates – led to several models for the organization of the ER-Golgi interface (Hauri and Schweizer, 1992). The ERGIC was at one point proposed to be a specialized domain of the ER (Sitia and Meldolesi, 1992) or the cis-Golgi (Mellman and Simons, 1992). Serial sectioning and three-dimensional reconstruction analysis of pancreatic acinar cells at the ultrastructural level, however, reinforced the notion that the ERGIC constitutes an independent structure that is not continuous with the ER or the cis-Golgi (Sesso et al., 1994), as did several subsequent studies (Bannykh et al., 1996; Fan et al., 2003; Klumperman et al., 1998). Nevertheless, the structure of the ERGIC clusters does appear to vary somewhat, depending on how the cells are fixed. In rapidly frozen cells, for example, membranes that possess

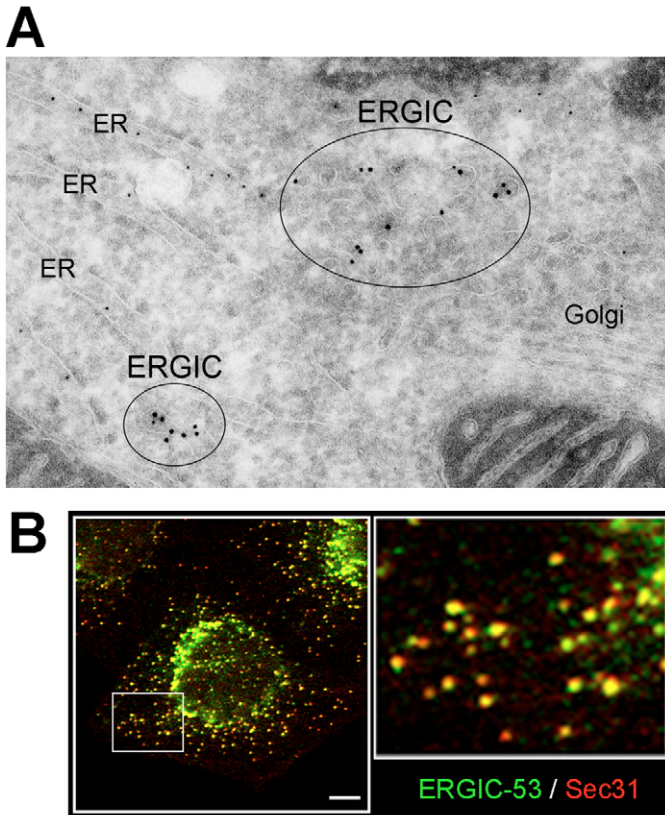
ERGIC-marker proteins appear as large pleiomorphic bodies that have numerous buds at their rims rather than as tubulovesicular clusters (Horstmann et al., 2002).

Recent work has shed new light on the nature and dynamics of the ERGIC. Here, we discuss these findings and propose a new working model for ER-to-Golgi transport via this compartment.

## The role of the ERGIC in ER-to-Golgi transport

The results of genetic and biochemical analyses of trafficking in *S. cerevisiae*, which lacks ERGIC clusters, can be accommodated within a simple model in which vesicles bearing the protein coat COPII mediate transport from the ER to the Golgi (Bonifacino and Glick, 2004). In higher eukaryotes, transport from the ER is also initiated by COPII-mediated budding of vesicular carriers, but this is restricted to specialized, long-lived subdomains of the ER, the ER-exit sites (ERES) (Bannykh et al., 1996; Hammond and Glick, 2000). ERGIC clusters containing ERGIC-53 are close to but clearly distinct from ERES and delineate the subsequent stage in transport to the Golgi. Owing to its limited resolution, however, ERGIC clusters cannot be fully resolved from ERES by light microscopy (Fig. 1B).

What is the role of the ERGIC in ER-to-Golgi transport? Studies of export of a tsO45-VSV-G-GFP fusion protein from the ER have identified large carriers moving from ERES to the Golgi along microtubules in living cells (Presley et al., 1997; Scales et al., 1997). These carriers, typically <1 µm in diameter, were considered to be identical to the ERGIC clusters defined by ERGIC-53, and their characterization gave rise to



**Fig. 1.** Morphology of the ERGIC. (A) Ultrastructure of ERGIC clusters (circled). Immunogold-labeled ultrathin cryosection of a HepG2 cell showing the subcellular distribution of the ERGIC marker ERGIC-53 (10 nm gold) and the rough ER marker protein disulfide isomerase (5 nm gold). Modified and reprinted with permission from Klumperman et al. (Klumperman et al., 1998). (B) Close apposition of ERGIC clusters and ER-exit sites (ERES) visualized by confocal double immunofluorescence microscopy. HeLa cells were fixed, permeabilized and stained for the ERGIC marker ERGIC-53 and the ERES marker Sec31, a subunit of COPII (anti-Sec31 was kindly provided by Wanjin Hong, Institute of Molecular and Cell Biology, Singapore). Note that the majority of ERGIC-clusters are localized close to ERES. The enlargement of the boxed area (right-hand panel) shows partial overlap of ERGIC-53 and Sec31. Bar, 5  $\mu$ m. (Micrograph kindly provided by Houchaima Ben-Tekaya, University of Basel, Basel, Switzerland.)

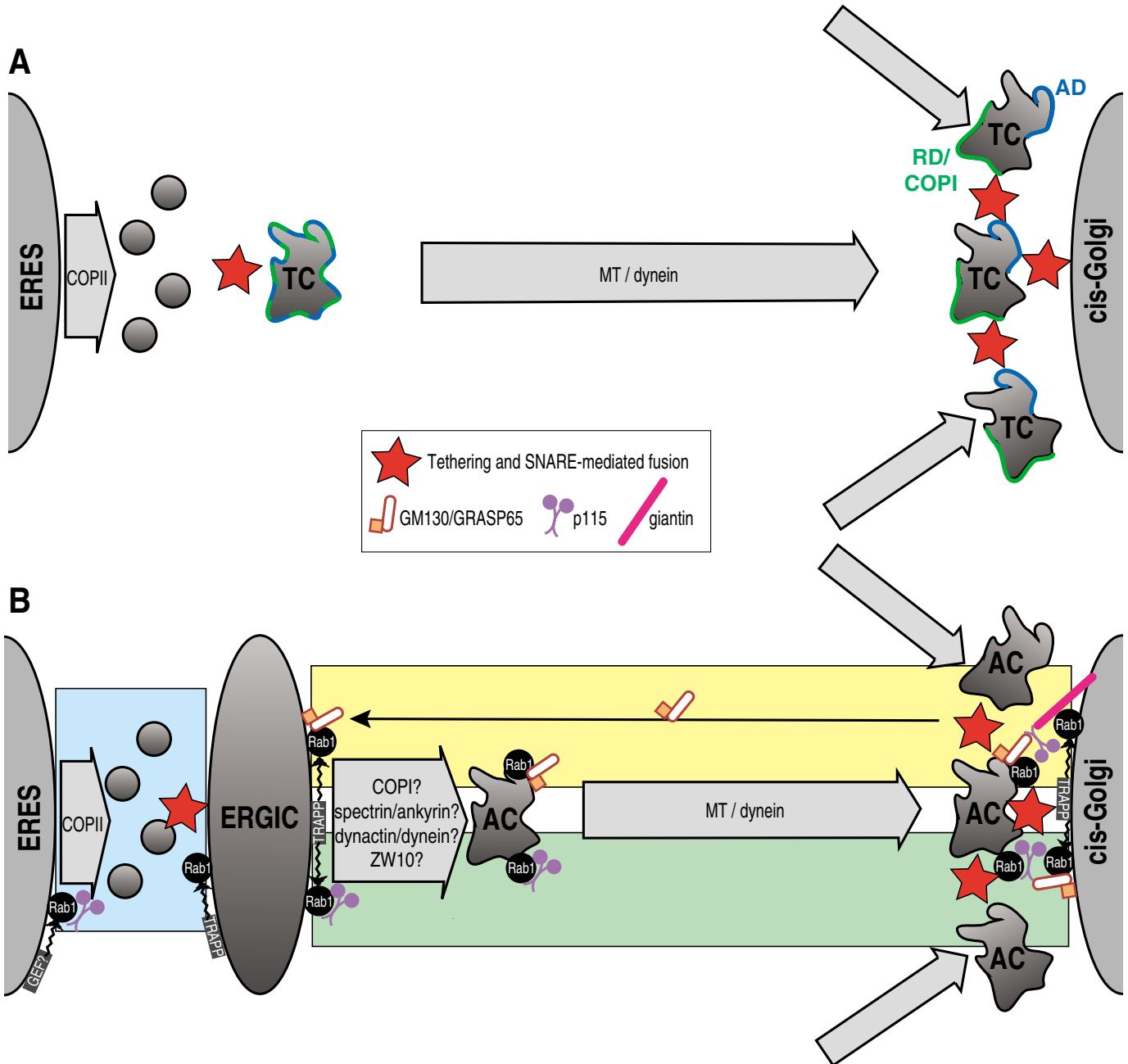
the transport complex (TC) model (Bannykh et al., 1998; Stephens and Pepperkok, 2001) (Fig. 2A). In this model, the ERGIC clusters are transient cargo containers formed *de novo* by the fusion of ER-derived COPII vesicles. These then migrate to and fuse with or give rise to the cis-Golgi. Recently, this model has been extended following the suggestion that under certain circumstances TCs can form directly from the ER by COPII-dependent protrusion of large saccular carriers (Mironov et al., 2003). Notably, in these experiments TC formation was not prevented by conditions that would interfere with the homotypic fusion of ER-derived COPII vesicles (Mironov et al., 2003). The fusion of such vesicles, however, has been reproduced *in vitro* (Xu and Hay, 2004), and ERES-adjacent, discrete COPII-coated vesicles have been detected by immunoelectron microscopy *in situ* (Zeuschner et al., 2006).

The formation and morphology of such carriers is discussed extensively elsewhere (Fromme and Schekman, 2005; Watson and Stephens, 2005).

The TC model is almost exclusively based on studies using a single, overexpressed viral protein (tsO45-VSV-G) that can be reversibly accumulated in the ER by temperature manipulation. Undoubtedly, this work has provided important insights into traffic in the secretory pathway. Nevertheless, there is a considerable lack of studies using cellular proteins expressed at moderate levels to confirm that conclusions based on VSV-G trafficking are more broadly applicable. Temperatures that prevent the export of tsO45-VSV-G from the ER also significantly inhibit COPII vesicle budding (Aridor et al., 1999) and block not only tsO45-VSV-G transport but overall traffic from the ER – as indicated by the ER retention of the KDEL receptor and ERGIC-53 (Trucco et al., 2004). Furthermore, overloading intracellular membranes with a viral membrane protein that is highly specialized for efficient transport (Nishimura and Balch, 1997; Sevier et al., 2000) may distort the secretory pathway to best suit viral production, which may be reflected by the altered COPII dynamics in response to tsO45-VSV-G (Forster et al., 2006). Rather than faithfully representing normal cell physiology, studies using VSV-G thus at best indicate the ability of the secretory pathway to adapt to temporary high-throughput conditions. VSV-G overexpression could also lead to an exaggeration of membrane movement or the suppression of possible post-ER quality-control mechanisms. Clearly, additional proteins need to be studied to address these concerns.

Another problem with the TC model is that it cannot easily accommodate all the available information regarding ER-to-Golgi traffic. For instance, at steady state, the vast majority of ERGIC clusters lie close to ERES rather than being randomly distributed between ERES and the Golgi, which one would expect if mobile TCs connect the ER and the Golgi (Ben-Tekaya et al., 2005; Stephens, 2003) (see Fig. 1B). Furthermore, low-temperature treatment that blocks ERGIC-to-Golgi but not ER-to-ERGIC transport of ERGIC-53 does not increase the number of ERGIC clusters (Klumperman et al., 1998), which again is contrary to what one would expect if the TC model applies.

Live-cell imaging of GFP-tagged ERGIC-53 calls the current TC model into question (Ben-Tekaya et al., 2005). When GFP-ERGIC-53 is expressed at moderate levels, it oligomerizes and recycles identically to endogenous ERGIC-53. Moreover, it forms hetero-oligomers with endogenous ERGIC-53, which likewise strongly indicates that its behavior mimics that of the endogenous protein. However, GFP-ERGIC-53 localizes to long-lived stationary membrane compartments that are next to but distinct from ERES and correspond to the tubulovesicular ERGIC clusters previously identified by immunoelectron microscopy (Klumperman et al., 1998). Vector field quantification confirms that these clusters show no net movement towards the Golgi whereas TCs carrying tsO45-VSV-G-GFP do (Ben-Tekaya et al., 2005). These observations contradict the TC model. The new work also reveals that the stationary ERGIC membranes are sites of repeated sorting of a fluorescent secretory marker protein, signal-sequence-tagged DsRed (ssDsRed), into large anterograde carriers (ACs) that leave the GFP-ERGIC-53 compartment behind when moving on to the Golgi (Ben-



**Fig. 2.** Models for ER-to-Golgi transport in mammalian cells. (A) The transport complex (TC) model. Upon budding from ER-exit sites (ERES), COPII vesicles tether and fuse to form pleiomorphic TCs, which in turn are transported along the microtubule cytoskeleton (MT) in a dynein-motor-dependent way. Relocation of a TC is accompanied by the progressive, COPI-mediated segregation of an anterograde cargo-rich domain (AD) from a retrograde cargo-rich domain (RD/COPI). At the cis-Golgi, incoming TCs gather and undergo fusion. Depending on the model for intra-Golgi transport (Rabouille and Klumperman, 2005), they either directly fuse with the first cisterna of the cis-Golgi or form a new cis-Golgi cisterna by homotypic fusion. (B) Stable compartment model of anterograde membrane traffic through the ERGIC. Short-range vesicular transport from ERES to the ERGIC depends on COPII but is microtubule independent. Conversely, long-range transport from ERGIC to cis-Golgi requires microtubules and the dynein motor. Fission of anterograde cargo-rich ACs from the ERGIC may involve the COPI coat, the spectrin/ankyrin skeleton, ZW10, as well as dynein and its membrane adaptor complex dynactin. Targeting of membrane carriers to the correct acceptor compartment is orchestrated by the tethering machinery. First, Rab1 is activated and thereby recruited to the membrane by guanine nucleotide exchange factors (GEFs), such as the TRAPP complex (zigzag-arrows, the GEF for Rab1 during ER exit is not known). Activated Rab1 recruits p115 to ERES (Allan et al., 2000), which is responsible for the subsequent docking of ER-derived vesicles to the ERGIC (Cao et al., 1998). For ERGIC-to-cis-Golgi transport there are two possible scenarios. In the first (green box), p115 binds to the ERGIC through activated Rab1, and docking at the cis-Golgi involves a p115-Rab1-GM130-GRASP65 tether (Moyer et al., 2001). In the second (yellow box), the GM130-GRASP65 complex recycles from the cis-Golgi to the ERGIC (Marra et al., 2001), and docking at the Golgi involves the Rab1-coordinated interaction of GM130-p115 with the transmembrane protein giantin (Beard et al., 2005; Sonnichsen et al., 1998). Membrane docking in all cases is followed by SNARE-mediated membrane fusion that requires catalysis by p115 (red star) (Sapperstein et al., 1996; Shorter et al., 2002). For simplicity Golgi-to-ER retrograde pathways are not shown.

Tekaya et al., 2005). These findings thus revitalize the original 'stable compartment' model in which the ERGIC is considered to be a membrane compartment in the true sense (Klumperman et al., 1998) that receives cargo from the ER in a COPII-dependent process and generates ACs destined for the Golgi.

It is important to note that the live-imaging studies of VSV-G-GFP that led to the TC model did not include an ERGIC marker. This explains why the stationary ERGIC was not apparent. What then are the TCs defined by VSV-G-GFP? They may correspond to the ACs defined by ssDsRed (Ben-Tekaya et al., 2005). Alternatively, synchronized VSV-G transport may make retrograde sorting in the ERGIC inefficient or entire ERGIC clusters become mobile and move to the Golgi. Such alterations would explain the observation of ERGIC-53 and VSV-G on the same membranes in VSV-infected, temperature-manipulated cells (Horstmann et al., 2002).

In the stable compartment model (Fig. 2B), the tubulovesicular ERGIC clusters are stationary and operate as the first post-ER sorting stations for anterograde and retrograde traffic. ER-to-Golgi transport is a two-step process involving short-range transport from ERES to the ERGIC followed by long-range transport from the ERGIC to the cis-Golgi. Below, we discuss the molecular basis underlying the anterograde traffic and speculate on the functions of the ERGIC, presenting a working model based on the stable compartment model (Fig. 2B) that can better accommodate published data than the current TC model (Fig. 2A).

### The molecular basis of anterograde traffic at the ER-Golgi interface

Membrane traffic is controlled by many molecular players (Bonifacino and Glick, 2004). The directionality of transport from a donor to an acceptor compartment is defined at several levels. This starts with coat-protein-mediated cargo recruitment and vesicle budding, which is followed by motor/cytoskeleton-mediated vesicle transit and finally vesicle docking and fusion controlled by tethering and SNARE proteins. The greater morphological complexity of the secretory pathway in mammalian cells compared with yeast is reflected by the several-fold higher complexity of the molecular transport machinery. This is best illustrated by the Rab GTPases (Ypt proteins in *S. cerevisiae*), the number of which correlates evolutionarily with increasing membrane complexity. Rab proteins regulate the dynamic assembly and disassembly of multiprotein scaffolds and thereby control various stages of ER-Golgi transport and of vesicular traffic in general (Munro, 2004; Segev, 2001; Zerial and McBride, 2001). *S. cerevisiae* contains 11 Rab proteins, ten of which have an assigned function. Among the 63 Rab proteins found in the human genome (Bock et al., 2001; Gurkan et al., 2005), some share a high degree of sequence and functional identity with their yeast counterparts, but many have unknown functions.

#### Protein coats

There is general agreement that COPII and the biochemically unrelated protein coat COPI act sequentially along the early secretory pathway (Aridor et al., 1995; Scales et al., 1997; Stephens et al., 2000). Transport from the ER to the ERGIC is controlled by COPII, and subsequent sorting in the ERGIC involves COPI. ERGIC clusters are major sites of COPI

localization (Oprins et al., 1993) and formation of the ERGIC in vitro requires COPI (Lavoie et al., 1999). COPI vesicles also play a well-established role in retrograde traffic from both the ERGIC and Golgi (Klumperman et al., 1998; Letourneur et al., 1994) back to the ER.

Microinjection of antibodies against the COPI subunit  $\beta$ -COP inhibits movement of VSV-G and endogenous proteins from the ERGIC to the Golgi (Pepperkok et al., 1993). Is this block due to an indirect effect? COPI might only be necessary for post-ERGIC anterograde traffic indirectly, because it recycles cargo receptors and other factors required for anterograde transport. However, live-imaging has revealed that VSV-G-containing TCs moving to the Golgi are coated with COPI (Presley et al., 2002; Scales et al., 1997; Stephens et al., 2000) (Fig. 2A). Similarly, a truncated, GFP-tagged version of GlcNAc-transferase is rapidly transported to the Golgi in large COPI-coated structures (Shima et al., 1999). In these experiments, a progressive polarization of anterograde-cargo-rich and COPI-coated, retrograde-cargo-rich domains on the moving TCs was observed (Fig. 2A), which led the authors to propose some function for COPI in anterograde transport of TCs (Shima et al., 1999). Alternatively, these findings could indicate continuous budding of COPI vesicles cycling back to the ER from TCs. These vesicles may either rapidly uncoat or be beyond the limits of detection in light microscopy. However, live imaging of GFP-tagged membrane proteins that cycle between the ER and Golgi, and should therefore be included in retrograde vesicles, has provided no evidence for a net loss of the cycling proteins from the TCs by small vesicles (Stephens and Pepperkok, 2001). Therefore, an anterograde budding function of COPI at the ERGIC has to be considered.

How could COPI selectively package anterograde and retrograde cargo into distinct classes of vesicle budding from the ERGIC? Such segregation would have to be directed by transmembrane cargo. COPI vesicle budding is controlled by the Arf family of small GTPases. One possibility for such selectivity is differential Arf-dependent sorting of proteins by COPI. This is exemplified by the p24 family of putative cargo receptors: in an in vitro system, competing sorting signals act positively and negatively during vesicle budding through a GTPase switch in the COPI coat (Goldberg, 2000). Such a switch can lead to COPI-mediated budding of different types of vesicle (Goldberg, 2000). What determines this switching is unknown.

Alternatively, bidirectional sorting may be mediated by different Arf isoforms; indeed, knocking down different combinations of Arf1, Arf3, Arf4 and Arf5 by RNAi blocks anterograde and retrograde traffic in characteristic ways (Volpicelli-Daley et al., 2005). For instance, knocking down Arf1 and Arf4 causes COPI to dissociate from the ERGIC and Golgi and arrests VSV-G transport in the ER and ERGIC; knocking down Arf1 and Arf3 enlarges ERGIC clusters and blocks ERGIC-to-cis-Golgi transport; knocking down Arf4 and Arf5 blocks retrograde traffic of the KDEL receptor from the cis-Golgi to the ERGIC; and knocking down Arf1 and Arf5 blocks retrograde traffic from the ERGIC to the ER. The Arf effectors responsible for the different traffic phenotypes are presently unknown.

Another mechanism to distinguish COPI-mediated anterograde and retrograde traffic from the ERGIC might be Rab-dependent sorting through different Rab effectors. The

ERGIC harbors two Rabs that have opposing functions. Rab2, presumably in concert with an atypical protein kinase C zeta/lamda and glyceraldehyde-3-phosphate dehydrogenase, promotes formation of COPI vesicles enriched in recycling proteins (Tisdale, 2003), whereas Rab1 (two isoforms are known: Rab1a and Rab1b) is involved in membrane tethering at the ERGIC and cis-Golgi in anterograde transport (Allan et al., 2000; Cao et al., 1998; Moyer et al., 2001) (Fig. 2B) – Rab1b also functions in COPI recruitment (Alvarez et al., 2003). Bidirectional Rab-dependent sorting probably involves different tethering complexes. Although bidirectional budding from the ERGIC has not been studied in the test tube, two different types of COPI vesicle can be isolated in a cell-free budding assay using Golgi membranes (Malsam et al., 2005). One subpopulation of COPI vesicles, defined by the tethering proteins Golgin-84 and CASP, carries Golgi enzymes, and hence has characteristics of intra-Golgi retrograde vesicles. Another subpopulation, defined by the tethering protein p115, has anterograde characteristics.

Finally, molecular variation of the COPI coat itself could dictate anterograde or retrograde directionality. Consistent with this possibility is the observation that different COPI isoforms have differential subcellular localizations (Wegmann et al., 2004).

#### Connection to the cytoskeleton

Specific coupling of membrane compartments to the microtubule cytoskeleton by motor proteins contributes to membrane dynamics and membrane partitioning in the early secretory pathway. The force generated by clusters of individual membrane-linked motor proteins in the presence of microtubules can deform membranes *in vitro*, leading to formation of membrane tubes followed by membrane partitioning (Koster et al., 2003). Since the force barriers for formation of a membrane tube from a flat membrane are too high for a motor protein to overcome (Koster et al., 2005), motor proteins probably rely on initial membrane curvature generated by small GTPases, such as Sar1 (Bielli et al., 2005; Lee et al., 2005), in conjunction with coat proteins.

Bidirectional traffic from the ERGIC to the ER and Golgi is dependent on intact microtubules (Ben-Tekaya et al., 2005) and both the microtubule-plus-end-directed motor kinesin and the microtubule-minus-end-directed motor dynein are associated with the ERGIC (Lippincott-Schwartz et al., 1995; Roghi and Allan, 1999). Moreover, disruption of the multi-subunit complex dynactin that binds dynein to membranes arrests the movement of VSV-G to the Golgi (Presley et al., 1997). The finding that kinesin is present on ERGIC structures is hard to reconcile with the TC model, because the transport of TCs towards the Golgi is minus-end directed. Kinesin on the TCs has therefore been proposed to remain inactive (Lippincott-Schwartz et al., 1995). The stable compartment model provides a more straightforward solution: kinesin that is recruited to the ERGIC would be active and power the microtubule-plus-end-directed retrograde traffic to the ER, whereas dynein/dynactin would be required for transport from the ERGIC to the cis-Golgi. Furthermore, both motor activities could be involved in steady-state positioning of the ERGIC. Precisely which kinesin and dynein isoforms are associated with the ERGIC remains to be shown.

The recruitment of motors to membranes is believed to be

orchestrated by Rab GTPases. This is based on data showing that the Golgi/endosome-associated Rab6 binds both dynactin (Matanis et al., 2002; Short et al., 2002) and the kinesin Rab6-KIFL (Echard et al., 1998; Hill et al., 2000). However, neither Rab1 nor Rab2 can bind dynactin (Short et al., 2002), and the Rab6-binding domain in Rab6-KIFL (Echard et al., 1998) is unique to this particular kinesin form. Therefore, Rab-mediated binding of motors to the ERGIC seems unlikely. The recruitment of dynactin and the concomitant selection of anterograde cargo at the ERGIC may instead involve the spectrin/ankyrin skeleton that is thought to bridge integral membrane proteins, cytosolic proteins and certain phospholipids to form a versatile adaptor scaffold (De Matteis and Morrow, 2000).  $\beta$ III spectrin not only immunolocalizes to the ERGIC but is also required for the trafficking of the  $\text{Na}^+/\text{K}^+$ -ATPase from the ER to the Golgi (Devarajan et al., 1997; Godi et al., 1998). Furthermore,  $\beta$ III spectrin binds to the dynactin subunit centractin (Arp1) both *in vitro* and *in vivo* (Holleran et al., 2001; Holleran et al., 1996), which suggests that spectrin and microtubules physically interact via dynein/dynactin. Since spectrin is a known actin-binding protein, these data point to a role for the actin cytoskeleton in ER-to-Golgi transport. This is further supported by a recent study of the GTP-binding protein Cdc42, which regulates actin dynamics. A constitutively active Cdc42 mutant greatly reduces Arf1-stimulated recruitment of dynein to COPI vesicles *in vitro* and blocks VSV-G transport through the ERGIC of cultured cells (Chen et al., 2005). In addition to spectrin/ankyrin, the connection of membranes to the microtubule cytoskeleton in the early secretory pathway may depend on the non-classical interphase function of the kinetochore/cell-cycle checkpoint protein ZW10 that binds dynactin (Starr et al., 1998) and may regulate dynein-driven membrane movement throughout the cell cycle (Varma et al., 2006). Knocking down ZW10 delays ER-to-Golgi transport of VSV-G, and its overexpression severely disturbs the morphology of the ER-Golgi interface without disrupting the dynein-dynactin complex (Hirose et al., 2004).

Dynactin also binds to COPII proteins through a C-terminal fragment of its p150<sup>Glued</sup> subunit (Watson et al., 2005). Expression of this fragment increases the turnover of COPII on ER membranes and delays the formation of VSV-G-YFP-containing TCs, but – as opposed to ectopic expression of dynamitin (Presley et al., 1997) – leaves the dynactin complex intact. Transport velocity of TCs is unchanged, as is the organization of the early secretory pathway (Watson et al., 2005). Stabilization of the COPII coat by p150<sup>Glued</sup> must thus lead to efficient ER export. Although GFP-p150<sup>Glued</sup> predominantly labels the growing plus ends of microtubules that migrate towards and through ERES (Watson et al., 2005), COPII-associated dynactin is unlikely to reflect a role of microtubules in ERES-to-ERGIC transport, because short-range vesicular transport is known to be microtubule independent. Because it is the ERGIC-to-cis-Golgi step (presumably involving a  $\beta$ III-spectrin-dynactin-dynein complex) that requires intact microtubules, we propose that the transitory interaction between the COPII coat and p150<sup>Glued</sup> is used to maintain the kinetic stability and steady-state localization of ERES by a COPII-mediated microtubule-trap mechanism, which in turn facilitates the association of the

juxtaposed ERGIC with microtubules. Additionally, a direct pulling force from the ER along microtubules may be required to accommodate large cargo proteins such as procollagen (Fromme and Schekman, 2005; Watson and Stephens, 2005; Zeuschner et al., 2006).

### Tethering and fusion

Can the ERES-to-ERGIC and ERGIC-to-Golgi transport steps also be distinguished by their SNARE complexes (Chen and Scheller, 2001) and tethering proteins (Gillingham and Munro, 2003; Lupashin and Sztul, 2005; Whyte and Munro, 2002)? It is currently not possible to localize precisely where individual SNAREs act at the mammalian ER-Golgi interface, because they cycle between these two compartments (Chao et al., 1999; Miller et al., 2003; Mossessova et al., 2003; Rein et al., 2002). Nevertheless, there is now general agreement that – by analogy with yeast – the major ER-Golgi SNARE complex is composed of syntaxin 5, Sec22b, membrin, and Bet1. This complex operates at two interfaces: the fusion of ER-derived vesicles at the ERGIC, and the subsequent fusion of ACs at the cis-face of the Golgi (Hay et al., 1998). The discovery of a long isoform of syntaxin 5 in mammalian cells that localizes more prominently to the ERGIC than to the Golgi (Hui et al., 1997) may point to some variation between the fusion complexes at the ERGIC and these at the cis-Golgi. Moreover, a syntaxin-5–Bet1–GOS-28–Ykt6 SNARE complex is also involved at a late stage of ER-to-Golgi transport (Zhang and Hong, 2001). The specificity of fusion does not appear to depend entirely on the ER-Golgi SNARE machinery. In contrast to the number of Rab proteins, the number of evolutionarily divergent members of the SNARE family has increased only modestly from yeast to human (1.7-fold) (Bock et al., 2001).

Tethering proteins bridge membranes before formation of SNARE complexes and contribute to the specificity of fusion. They include large multi-protein complexes and long coiled-coil proteins that can link membranes (Gillingham and Munro, 2003; Lupashin and Sztul, 2005; Whyte and Munro, 2002). The tethering proteins also assist in SNARE assembly and activate Rab proteins. An important tethering molecule controlling ER-Golgi traffic at various sites is the coiled-coil, myosin-shaped molecule p115 (Uso1 in yeast). P115 is a Rab1 effector (Allan et al., 2000) that serves as a membrane-tethering factor (Cao et al., 1998; Sonnichsen et al., 1998), a catalyst for formation of SNARE complexes (Sapperstein et al., 1996; Shorter et al., 2002), and a modulator of COPI function (Garcia-Mata and Sztul, 2003). P115 first acts during COPII vesicle biogenesis by recruiting a select set of SNARE proteins required for subsequent vesicle fusion (Allan et al., 2000) and determines the focused positioning of ERES within the cytoplasm (Kondylis and Rabouille, 2003). The guanine nucleotide exchange factor (GEF) that activates Rab1 at ERES has not yet been identified.

A second site of action of p115 is in vesicle tethering at the ERGIC: antibodies against p115 inhibit VSV-G transport at the level of the ERGIC (Alvarez et al., 1999). Because the two p115-interacting coiled-coil proteins GM130 and giantin (see below) are not present in *S. cerevisiae*, we propose that delivery of ER-derived vesicles to the ERGIC in higher eukaryotes (Fig. 2B, blue box) is mechanistically related to the docking of ER-derived vesicles to the fragmented Golgi in *S. cerevisiae*, which requires Uso1p/p115. This process depends on Ypt1p (yeast

Rab1) (Cao et al., 1998), which is activated by the GEF activity of the Golgi-localized TRAPP complex (Wang et al., 2000). TRAPP is a multi-protein tethering assembly on Golgi membranes essential for an early step of COPII vesicle tethering in yeast (Barrowman et al., 2000; Sacher et al., 2001). In mammalian cells, a subunit of TRAPP localizes to both the ERGIC and the Golgi when transiently expressed in COS7 cells (Gecz et al., 2000); TRAPP-catalyzed Rab1 activation might therefore occur in both compartments (Fig. 2B).

A third site of action is the Golgi, where p115 mediates tethering in conjunction with two other Rab1-binding tethering factors: giantin and GM130 (Beard et al., 2005; Sonnichsen et al., 1998). Where precisely GM130 and giantin act remains to be established. Kinetic staging experiments revealed that the tethering activity of the Rab1-GM130 complex (Moyer et al., 2001) precedes the giantin-requiring stage (Alvarez et al., 2001). One scenario is that the p115-GM130 interaction is needed for the anterograde delivery of p115-primed ACs into an early Golgi compartment marked by GM130 (Fig. 2B, green box) whereas a trimeric GM130-p115-giantin complex controls retrograde vesicle transport of Golgi-resident proteins back to that compartment (Alvarez et al., 2001; Beard et al., 2005; Sonnichsen et al., 1998). Indeed, giantin is concentrated at the rims of Golgi cisternae (Martinez-Menarguez et al., 2001), which are involved in retrograde vesicle biogenesis.

In an alternative scenario, a subfraction of GM130 might reside in the ERGIC and giantin might tether GM130-primed ACs through the GM130-p115-giantin complex to the cis-Golgi (Fig. 2B, yellow box). GFP-tagged GM130, in complex with its membrane anchor GRASP65, is present on membrane tubules that mark a Golgi-to-ERGIC recycling pathway. This suggests that GM130 shuttles between the ERGIC and the cis-Golgi (Marra et al., 2001). Although the mechanism underlying retrieval of GM130 back to the ERGIC is unknown, these data are difficult to reconcile with the TC model, because this model postulates that fully fusion competent TCs arise from the ER at ERES in a one-step process.

### Protein sorting in the ERGIC

The function of the ERGIC as the first post-ER sorting station for anterograde and retrograde protein traffic is now beyond dispute (Appenzeller et al., 1999; Aridor et al., 1995; Ben-Tekaya et al., 2005; Bu et al., 1995; Klumperman et al., 1998; Martinez-Menarguez et al., 1999) – regardless of whether its clusters are stationary or moving. How does bidirectional sorting of proteins occur in the ERGIC? Sorting into the retrograde pathway requires a transport signal, such as KDEL in soluble proteins. This binds to a receptor that contains a dibasic cytosolic signal, which in turn is recognized by COPI. Little is known about anterograde sorting from the ERGIC. As in the case of export from the ER (Barlowe, 2003), anterograde exit from the ERGIC might be signal mediated or occur by default. Clearly, the simplest solution would be non-selective packaging by default. Although this is a likely mechanism, a recent study of the trafficking of GABA transporter 1 mutants indicates that anterograde traffic from the ERGIC can be signal mediated (H. Farhan, V. Reiterer, V. M. Korkhov, H.-P. H., E. Sztul, H. H. Sitte, M. Freissmuth, unpublished observations). This study identified a C-terminal motif required for export of GABA transporter 1 from the ERGIC. Note that signal-mediated sorting into both anterograde and retrograde

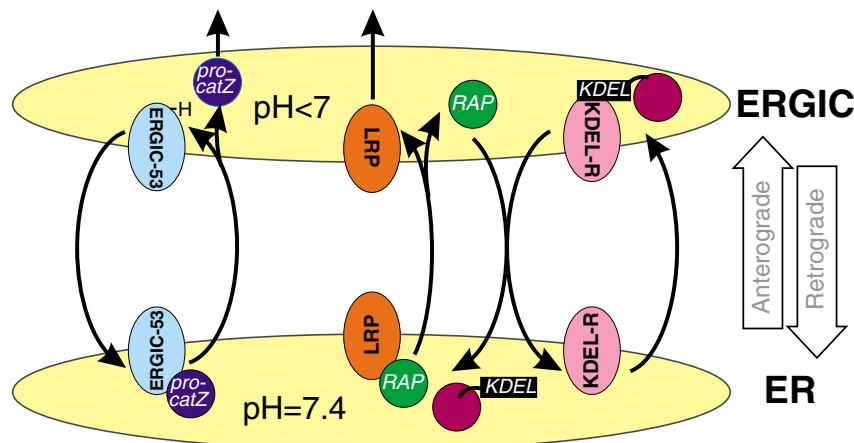
directions is a strong argument in support of the stable-compartment model.

The issue of protein sorting in the ERGIC becomes more complex in the case of cargo proteins that require the assistance of recycling transport receptors during ER export (Appenzeller et al., 1999; Appenzeller-Herzog et al., 2005; Belden and Barlowe, 2001; Muniz et al., 2000; Otte and Barlowe, 2004; Powers and Barlowe, 2002). For successful anterograde delivery from the ERGIC to the Golgi, cargo proteins need to dissociate from their receptors in the ERGIC, which suggests that the ERGIC exhibits features different from those of the ER, where cargo-receptor interaction occurs. One of these is pH. Acidification of cells in culture inhibits the association of ERGIC-53 with its cargo procathepsin Z, and neutralization of organelle pH by chloroquine specifically impairs the dissociation of this glycoprotein in the ERGIC (Appenzeller-Herzog et al., 2004). Whereas the lumen of the ER is neutral (pH 7.4) (Wu et al., 2001), the ERGIC is the earliest low-pH site in the secretory pathway (Fig. 3). Although its exact pH is unknown, it is unlikely to be lower than that of the trans-Golgi network (pH 6.4) (Machen et al., 2003). Even if only slightly lower than in the ER, the reduced pH in the ERGIC may act as a general trigger for the dissociation of cargo-receptor complexes in combination with other factors.

Binding of ERGIC-53 to its substrates requires calcium (Appenzeller et al., 1999; Itin et al., 1996), and *in vitro* binding experiments show that the pH sensitivity of ERGIC-53 is increased when calcium concentrations are low (Appenzeller-Herzog et al., 2004). Accordingly, a mechanism that maintains lower calcium levels in the ERGIC could promote pH-induced cargo release. Imaging of total calcium (i.e. the sum of free and bound calcium) in quick-frozen, freeze-dried PC12 cells has revealed high levels of calcium in the ER and Golgi, but

calcium cannot be detected in ERGIC elements (Pezzati et al., 1997). Although this does not necessarily indicate a drop in free calcium (which is the physiologically relevant fraction for calcium-dependent processes), it underlines the distinct identity of the ERGIC. It will be interesting to determine the changes in free calcium and pH from the ER to the ERGIC.

The acidification of the ERGIC and cis-Golgi serves additional important purposes. pH-dependent binding of the ER-resident protein RAP to LDL-receptor-related protein, which prevents premature ligand interactions, ceases in the ERGIC, where the two proteins segregate (Bu et al., 1995) (Fig. 3). Moreover, the lowered pH is thought to contribute to the effective recognition and retrieval of KDEL-bearing proteins from post-ER compartments by the KDEL receptor, because binding of the KDEL sequence to the KDEL receptor *in vitro* is greater at pH <7 than at pH 7.4 (Scheel and Pelham, 1996). Most interestingly, pH has opposing effects on binding of ligands to the KDEL receptor (Scheel and Pelham, 1996) and ERGIC-53 (Appenzeller-Herzog et al., 2004), which reflects their reciprocal modes of action (Fig. 3). The steady-state localizations as well as the recycling pathways of the two receptors, however, slightly differ: the KDEL receptor localizes to the ERGIC and cis-Golgi, occasionally preferentially to the cis-Golgi (Tang et al., 1995). The binding of KDEL ligands is required to stimulate the COPI-mediated retrograde transport of the KDEL receptor to the ER (Lewis and Pelham, 1992) by a process involving receptor homooligomerization (Aoe et al., 1998) and/or phosphorylation (Cabrera et al., 2003). Acidification along the ER-Golgi pathway may occur gradually, by selective recruitment of H<sup>+</sup>-v-ATPases during partition of ACs from the ERGIC, and the optimal pH for KDEL ligand binding may be achieved only at the cis-Golgi. Indeed, staining of acidic compartments with the weak base



**Fig. 3.** pH-dependent sorting between the ER and ERGIC. Proteins travel between the ER and the ERGIC in anterograde and retrograde directions. The pH of the ER is 7.4 (Wu et al., 2001), whereas that of the ERGIC is more acidic. The scheme shows three different pH-dependent sorting mechanisms of anterograde-directed and/or recycling proteins in the early secretory pathway. ERGIC-53 functions as a transport receptor for the secretory/lysosomal protein procathepsin Z (pro-catZ). Association between ERGIC-53 and pro-catZ occurs in the ER at neutral pH, and dissociation occurs in the ERGIC following protonation of the ligand-binding site in ERGIC-53 (Appenzeller-Herzog et al., 2004). ERGIC-53 is then recycled back to the ER; pro-catZ proceeds through the secretory pathway. The LDL receptor-related protein (LRP) forms a complex with the escort protein RAP (Bu et al., 1995), which inhibits receptor-ligand interactions in the early secretory pathway. Again, low pH in the ERGIC triggers the dissociation of the two proteins. Subsequently, RAP is recycled back to the ER, probably by the KDEL receptor (KDEL-R), and LRP travels through the Golgi to the cell surface. By contrast, binding of the KDEL receptor to escaped ER proteins containing a C-terminal KDEL signal is thought to require the acidified pH in post-ER compartments (Scheel and Pelham, 1996). Ligand binding in turn triggers the retrograde transport of KDEL-receptor–ligand complexes from ERGIC and cis-Golgi to the ER.

DAMP showed partial overlap with perinuclear (i.e. late) pre-Golgi structures only (Palokangas et al., 1998).

### Additional functions of the ERGIC

Does the function of the ERGIC go beyond sorting? One additional function is initial concentration of secretory cargo that leaves the ER by a non-selective process, such as pancreatic enzymes. Amylase and chymotrypsinogen in pancreatic acinar cells are concentrated not in COPII buds but at the level of the ERGIC, and this appears to result from the exclusion of these proteins from retrograde COPI vesicles (Martinez-Menarguez et al., 1999).

Increasing evidence indicates that the ERGIC is also involved in conformation-based quality control of proteins and, possibly, protein folding. A proteomic approach to search for proteins cycling in the early secretory pathway, revealed – in addition to cargo receptors and membrane-trafficking proteins – that several chaperones are enriched in ERGIC membranes isolated from brefeldin-A-treated cells (Breuza et al., 2004). Likewise, an ultrastructural study using quantitative immunoelectron microscopy localized the highest levels of the glycoprotein folding sensor UDP-glucose:glycoprotein glucosyltransferase to the ERGIC (Zuber et al., 2001). If the maturation of some proteins indeed occurs beyond the ER, we would expect that permanently misfolded variants of these proteins are re-routed back to the ER, the major site where terminally misfolded proteins are cleared from the biosynthetic pathway by ER-associated degradation (ERAD). A non-classical retrieval pathway for some ERAD substrates has been discovered in *S. cerevisiae* (Caldwell et al., 2001; Taxis et al., 2002; Vashist et al., 2001). This pathway is distinct from the conventional ERAD pathway that is based on ER retention of misfolded proteins (Haynes et al., 2002). The retrieval pathway may serve as a backup system that can be upregulated when ER retention of some proteins is saturated.

A similar recycling pathway may also operate in mammalian cells. When tsO45-VSV-G is overexpressed in CHO cells at the non-permissive temperature, which prevents its folding, some moves to the ERGIC and cis-Golgi, rather than being retained in the ER (Hammond and Helenius, 1994). It undergoes ER export in a complex with the soluble folding chaperone BiP, and returns to the ER by retrograde transport from the Golgi (Hammond and Helenius, 1994). In HepG2 cells, tsO45-VSV-G is rapidly degraded at the non-permissive temperature, but acquires cis-Golgi-specific modifications ahead of ERAD, which again points to a recycling event (Spiro and Spiro, 2001). Likewise, considerable amounts of unfolded VSV-G have access to the ERGIC in Vero cells, but are not transported as far as the cis-Golgi (Schweizer et al., 1990). More recently, Mezzacasa and Helenius showed that unfolded VSV-G is restricted to the ER of Vero cells when expressed at lower levels (Mezzacasa and Helenius, 2002). When they allow it to move into the ERGIC and then artificially unfold it by using DTT, they observed that it now moves on to the Golgi rather than back to the ER (Mezzacasa and Helenius, 2002). The ERGIC of Vero cells thus cannot recycle misfolded VSV-G, even though the formal possibility exists that DTT also harms the quality-control machinery in the ERGIC.

Unassembled T-cell antigen receptor  $\alpha$  chains follow a recycling route together with BiP. Retrieval occurs via the KDEL receptor/COPI pathway, presumably through the

recognition of the C-terminal KDEL motif in BiP (Yamamoto et al., 2001). Other folding substrates potentially subject to quality control in the ERGIC include unassembled MHC class I molecules (Hsu et al., 1991), mutant forms of sucrase-isomaltase and lysosomal  $\alpha$ -glucosidase (Moolenaar et al., 1997), the mutant cystic fibrosis transmembrane conductance regulator (CFTR)  $\Delta$ F508 (Gilbert et al., 1998), connexin E186K (VanSlyke et al., 2000), tissue-non-specific alkaline phosphatase N153D (Ito et al., 2002), soluble IgM molecules (Elkabetz et al., 2003), misfolded proinsulin in pancreatic  $\beta$ -cells of Akita mice (Zuber et al., 2004), and certain mutants of V<sub>2</sub> vasopressin receptors (Hermosilla et al., 2004). A wide range of incompletely folded proteins that escape the ER quality control stage because of system overload or failed recognition can therefore be caught by a subsequent checkpoint in the ERGIC.

ERGIC clusters not only sort anterograde and retrograde protein cargo but also generate short-lived, rapidly moving vesicles that seem to connect the stationary ERGIC clusters laterally (Ben-Tekaya et al., 2005). These can only be visualized by imaging at high temporal resolution (at least 5 frames per second). Many of them appear to be unsorted, because they carry both anterograde and retrograde cargo. Their movement is microtubule dependent, and their number increases in response to protein overexpression. The role of these vesicles remains to be elucidated. They may functionally link the ERGIC clusters by exchanging crucial components or represent incompletely sorted transport vesicles that can fuse with neither the Golgi nor the ER.

### Conclusion and Perspectives

Here, we argue that ERGIC clusters defined by ERGIC-53 constitute a stationary compartment closely apposed to ERES. This view must incorporate two vesicular transport steps from the ER to the Golgi: a short COPII-dependent step to the ERGIC followed by a second, longer step from the ERGIC to the cis-Golgi. The stable compartment model can better accommodate published data on ER-to-Golgi traffic than the earlier TC model, in which ERGIC clusters are assumed to operate as mobile transport complexes. TCs originally defined by VSV-G-GFP may correspond to ACs that segregate from stationary GFP-ERGIC-53 and move to the Golgi. This possibility needs to be tested by simultaneous recording of VSV-G and ERGIC-53 trafficking in the same cell.

What is the advantage of having a stationary sorting compartment in between the ER and Golgi? A stationary ERGIC may increase the efficiency of extraction of components destined for recycling such as ERGIC-53. For instance, in *S. cerevisiae* the vast majority of Emp47p, a cargo receptor closely related to ERGIC-53 (Sato and Nakano, 2002), localizes to the Golgi at steady state (Schroder et al., 1995). In more highly developed cells featuring an ERGIC sorting compartment, energy-consuming transport of the recycling transmembrane machinery and of lipids back and forth along microtubules is elegantly prevented. It is conceivable that the ERGIC evolved in parallel with the increasing complexity of the secretory pathway from small to large eukaryotic cells. Furthermore, it is possible that sorting in the ERGIC contributes to the maintenance of the perinuclear Golgi apparatus in interphase cells as a centralized platform for modification and signaling. In some types of eukaryotic cells



that lack a classical ERGIC, such as *Pichia pastoris* (Rossanese et al., 1999) or *Drosophila* S2 cells (Kondylis and Rabouille, 2003), several Golgi ministacks are positioned next to ERES, which obviously results in subcellular splitting of the secretory apparatus. The maintenance of a constitutive low-Ca<sup>2+</sup>/low-pH site within the secretory pathway may also be essential for the productive folding and/or ERAD of a subset of substrate proteins. In this light, the unique milieu in the ERGIC has a cytoprotective function given the harmful consequences of attenuated degradation of certain ERAD substrates (Haynes et al., 2004).

A stationary ERGIC would have important implications for future studies of the early secretory pathway. One prediction is that a mechanism must keep ERGIC clusters stationary close to the ERES. We also postulate a three-way sorting mechanism in the ERGIC that leads to formation of ACs destined for the Golgi, retrograde carriers destined for the ER and fast lateral carriers connecting individual ERGIC clusters. Defects in this sorting process may lead to accumulation of cargo in the ERGIC in some traffic diseases (Gilbert et al., 1998; Hermsilla et al., 2004; Ito et al., 2002; Moolenaar et al., 1997; VanSlyke et al., 2000; Zuber et al., 2004). The fact that ERGIC clusters can fuse with each other and divide (Ben-Tekaya et al., 2005) indicates that specific mechanisms for this must exist. They are entirely unknown, but the scenario is reminiscent of early endosomes. Another interesting aspect relates to the differential mobility of ERGIC clusters and their vesicular products. Although stationary, ERGIC clusters hover around a fixed location through a microtubule-dependent mechanism. Microtubules also mediate bidirectional traffic from these clusters to the Golgi or ER, as well as rapid lateral traffic between ERGIC clusters (Ben-Tekaya et al., 2005). These different movements imply that different molecular motors are involved.

Future progress in our understanding of the ERGIC can be expected from dual- and multi-channel live imaging in conjunction with RNA interference and from cell-free systems that allow more detailed studies of the roles of Rabs, tethers and coat proteins, as well as cytoskeletal components and motors. Rab proteins whose functions are currently unknown may be essential for the proper organization and function of ERGIC membranes, but the role of Rab1 and Rab2 also needs to be scrutinized by knock-down experiments rather than dominant-negative mutants – as illustrated by a recent study of Arf isoforms that reported more specific effects of Arf knock-downs than dominant-negative Arfs, which may affect the activity of more than one isoform (Volpicelli-Daley et al., 2005). Proteomic analysis of purified native ERGIC membranes and analysis of ERGIC lipids (Schweizer et al., 1994), may also yield functional insights. Furthermore, it will be important to elucidate the function of saccular versus vesicular budding in the early secretory pathway and to localize the subcellular activities of SNARE molecules. Other interesting directions for research concern the change in the luminal environment from the ER to the ERGIC and cis-Golgi and the molecular determinants in ERAD substrates that require transport to the ERGIC to be properly degraded. Clearly, more than 15 years after its discovery, the ERGIC has not entirely revealed its identity and function.

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