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ER-to-Golgi transport and cytoskeletal interactions in animal cells

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Abstract. The endoplasmic reticulum (ER)-Golgi system has been studied using biochemical, genetic, electron and light microscopic techniques. We now understand many aspects of trafficking from the ER to the Golgi apparatus, including some of the signals and mechanisms for selective retention and retrieval of ER resident proteins and export of cargo proteins. Proteins that leave the ER emerge in 'export complexes' or ER 'exit sites' and accumulate in pleiomorphic transport carriers referred to sometimes as VTCs or intermediate compartments. These structures then transit from the ER to the Golgi apparatus along microtubules using the dynein/dynactin motor and fuse with the cis cisterna of the Golgi apparatus. Many proteins (including vSNAREs, ERGIC53/p58 and the KDEL receptor) must cycle back to the ER from pre-Golgi intermediates or the Golgi. We will discuss both the currently favored model that this cycling occurs via 50-nm COPI-coated vesicles and in vivo evidence that suggests retrograde trafficking may occur via tubular structures.

Key words. Cytoskeleton; Golgi; endoplasmic reticulum; secretory pathway; membrane trafficking; COPI; COPII; molecular motors.

Introduction

In most cell types the endoplasmic reticulum (ER) is a large membrane-bounded organelle that is spread throughout much of the cytoplasm of the cell and consists of membrane bilayers separating an internal lumen from the cytoplasm [1]. This structure can have a sheetlike or a tubular morphology [1, 2] depending on cell type and on intracellular localization. Large portions (referred to as rough ER) are covered with ribosomes engaged in the synthesis of proteins targeted to the membranes or lumen of the ER [3]. Other portions (referred to as smooth ER) are devoid of ribosomes. Smooth ER is a catchall term for various forms of ribosome-free ER including 'transitional elements' specializing in export of protein from the ER [4] and specialized regions involved in calcium storage such as the sarcoplasmic reticulum in muscle [5]. In this review, only the properties of the ER relevant to its function as part of the secretory pathway will be discussed.

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Proteins that traverse the secretory pathway are synthesized on ribosomes targeted to ER protein channels through a hydrophobic signal peptide and are inserted into the ER during synthesis [3, 6, 7]. The normal itinerary of every protein that traverses the secretory pathway leads then to the Golgi apparatus [8]. There are also cycling proteins that leave the ER for the Golgi apparatus only to return and repeat the journey again [9–13]. These pathways are illustrated in figure 1. Although ER-to-Golgi and the retrograde Golgi-to-ER trafficking pathways are essential for the function of the secretory pathway and for the survival of eukaryotic cells, these pathways are only partially understood.

In this review we will outline briefly the molecular players and mechanisms involved in retention in the ER and trafficking from the ER to the Golgi apparatus in animal cells. We will then focus at length on the formation and morphology of ER-to-Golgi transport intermediates followed by their trafficking to the Golgi apparatus using the microtubule-based cytoskeleton, with the hope of pointing out not only what is already known but some of the unsolved questions in this area waiting for research. We will describe the dynamic self-organizing nature of the

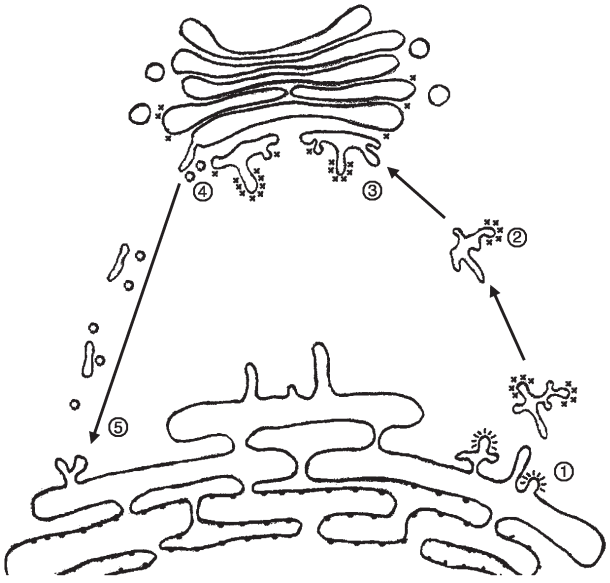

Figure 1. Schematic of trafficking pathways between the ER and the Golgi apparatus. Steps in ER-to-Golgi transport are shown to the right. These are: (1) COPII-mediated formation of pre-Golgi intermediate on transitional elements of ER. (2) dynein/dynactin-mediated transport of pre-Golgi intermediate along microtubules (not shown) to the Golgi apparatus. (3) attachment and fusion of pre-Golgi intermediate with cis-Golgi cisterna via tethering proteins and SNAREs. The retrograde pathway for Golgi-to-ER trafficking is shown to the left. These more poorly understood intermediates probably form from the rims of Golgi cisternae or the cis-Golgi (4). They may be tubules or COPI-coated vesicles and are believed to employ a kinesin motor to move away from the Golgi apparatus to the ER. These intermediates then must fuse with ER membranes (5), although the proteins involved in this fusion are not known.

Golgi apparatus as it relates to ER-to-Golgi trafficking, since many of the experiments that first demonstrated this self-organizing nature and its relationship to ER-to-Golgi trafficking involve pertubations of the cytoskeleton. Finally, we will describe the much more poorly understood Golgi-to-ER trafficking pathway and current favored and alternative models for how this pathway functions. This review will focus on data obtained studying higher eukaryotic systems with the exception of plants, and will only peripherally discuss results obtained in nonvertebrate systems. Reviews of the enormous body of work on ER-to-Golgi trafficking in yeast systems can be found in [14, 15].

Role of COPII

COPII is the name given to a protein complex required for ER exit [16]. The components of the COPII coat (Sar1p, Sec23/24p, Sec13/31p) and their regulator, the Sar1p exchange factor Sec12p [17], were (with the exception of Sar1p [18]) first identified in the yeast *Saccharomyces cerevisiae* as proteins required for ER exit in a genetic screen [19]. However, it was only many years after the original discovery of these proteins that their physical association and ability to produce COPII vesicles in vitro from ER membranes was shown [16, 20]. Vesicles could be produced from ER membranes [16, 20] or purified liposomes [21] incubated with only the defined proteins Sar1p, the Sec23/24p complex, the Sec13/31p complex and GTP. Uncoated vesicles averaging 58 nm in size were produced in the in vitro assay in the presence of GTP, while coated vesicles were produced in the presence of the nonhydrolyzable analogs GTPγS or GMP-PNP (5′-[β-γ-imido] triphosphate) [16].

These and other experiments (reviewed in [15, 22, 23]) have led to a consensus that Sar1p, which is membrane associated only in the GTP form, is recruited from the cytosol to the membrane by its exchange factor, the integral membrane protein Sec12p [24]. Sar1p-GTP recruits the Sec23/24p subunit [16]. The Sec23p polypeptide stimulates Sar1p GTP hydrolysis [25] which leads to the loss from the membrane of both Sar1p and the associated Sec23/24p complex. How this GTPase activity is regulated so as to stimulate uncoating at the proper time but without blocking coat polymerization is still unclear. However, Sec23p enhancement of Sar1p GTP hydrolysis can be upregulated by Sec13/31p addition in vitro, indicating that Sec13/31p may have a regulatory role as well as being a structural component of the coat [26]. The Sec24p polypeptide is mostly responsible for interactions with specific recognition sequences on cargo molecules of which diacidic (e.g. -DXE- [27]) and diaromatic (i.e. -FF [28, 29]) sequences are best characterized, although one example of interaction of cargo with Sar1p has been postulated [30]. Interestingly, multiple Sec24p isoforms with different apparent cargo specificities and possibly different coat geometries exist [31–34], a similar situation to the adaptin μ chains, which have different cargo-binding specificities.

Sec13/31p binds in the final step, apparently forming an outer layer on the coat [16, 35, 36]. Since the Sec31p subunit can interact with both the Sec23p and Sec24p polypeptides [37], it is likely that Sec31p is responsible for the attachment of the Sec13/31p complex to already polymerized Sec23/24p. Recent structural studies indicate a structure similar to a clathrin/adaptin coat in which Sec13/31p takes the role of clathrin, interacting with the Sec23/24p subunit and with itself to form a polyhedral coat, but not with the membrane or directly with Sar1p [35, 36].

COPII does not attach randomly to ER membranes in vivo but instead is concentrated in ‘export complexes’ on regions of transitional ER [38]. There is abundant evidence by electron microscopy for clustering of COPII in coated buds at these sites, but it is not certain that the 60-nm-diameter vesicles observed in vitro have the same morphology as COPII transport intermediates in intact

cells. Electron microscopic studies of the coat structure indicate potential flexibility for COPII coats greater than for clathrin/adaptin coats, raising the potential that coat geometries other than spherical vesicles could exist (e.g. tubules described in [30] or entire pre-Golgi intermediates [36, 39, 40]).

Cargo recruitment

Proteins inserted into the ER system can be integral membrane proteins, proteins more loosely attached to membranes (e.g. through glycophosphoinositol or other lipid anchors) or proteins that are soluble within the ER lumen. These proteins often consist of multiple polypeptides that must form properly folded multimers prior to exiting the ER [41, 42]. Also, there are many resident ER proteins that must be kept within the early secretory pathway [43]. Thus some proteins must be retained indefinitely in the ER, while other proteins must be kept temporarily within the ER until they are properly folded and assembled. However, proteins that are properly folded and assembled and destined for export must be recognized and sorted into exiting transport intermediates.

Photobleaching studies have found that both membrane-associated and lumenal proteins can diffuse freely within the ER [44]. The many kinds of proteins freely mobile within the ER lumen and membranes include both cargo proteins that exit from the ER [44] and resident proteins that are retained in the ER [43]. This implies that neither retention nor exit mechanisms can function by immobilizing a large portion of the resident protein pool in ER at any one time.

Almost certainly, transient immobilization in ER 'exit sites' plays a role in exit from the ER. Since only a small fraction of new protein is exiting the ER at any one time, exit does not require immobilization of a substantial portion of the protein pool. Integral membrane proteins with cytoplasmic domains containing COPII binding sequences [45, 46] can diffuse into these exit sites, where they are trapped by direct interactions with subunits of COPII, as discussed previously [16]. These interactions with COPII immobilize the cargo protein molecule [46] and trap it in a domain destined to become a transport intermediate that separates from ER membranes [47].

Many cargo proteins (especially lumenal proteins) destined to leave the ER have no cytosolic domain and cannot interact directly with COPII. It has been proposed that some proteins (e.g. ERGIC53/p58 [48, 49] and members of the p24 family [29]) function as 'cargo receptors' for these lumenal proteins. ERGIC53/p58 is a protein that cycles repeatedly between ER and Golgi [50] and contains a cytosolic COPII binding domain [11] and a lumenal lectin domain with mannose-binding activity [49] which has been shown in a chemical crosslinking study to bind to the cathepsin-Z-related protein catZr [51]. It has been proposed that the lectin domain would allow it to recruit any N-glycosylated cargo into COPII domains [48]. Since knockouts of ERGIC53 have only minor defects in ER exit [52, 53], there may be multiple and partially redundant cargo receptors. The p24 family of proteins [29] have also been proposed to be cargo receptors [54], although there is not the kind of direct evidence found for ERGIC53/p58. In addition to the 'professional' cargo receptors such as ERGIC53, there could also be proteins carrying ER exit signals that in addition to their primary function selectively recruit one or more other cargo proteins for ER exit. Thus, evidence to date indicates proteins are selected for ER exit either by direct or indirect interactions with COPII.

Selective retention and quality control

Proteins selectively retained within the ER include misfolded and partially folded proteins [41, 42], proteins containing a carboxy terminal -KKXX motif (type I integral membrane proteins) and the sequence KDEL (ER lumenal proteins) [43]. Selective retention of these proteins in the ER is less well understood than selective exit, especially given that photobleach experiments show that green fluorescent protein (GFP)-tagged versions of ER-resident proteins (both lumenal and integral membrane) and ER-retained incompletely folded proteins are generally freely mobile in the ER membranes [44]. We will look separately at the case of incompletely folded proteins and true resident proteins.

Proteins inserted into ER membranes must undergo a folding process and are normally screened by a quality control process capable of retaining incompletely folded proteins in the ER membranes. This involves sequential binding to chaperonins (BiP, calnexin and calreticulin), which recognize and stabilize folding intermediates [55]. The protein will be retained within the ER by binding to a chaperonin until it is completely folded. These chaperonins have ER retention sequences, including KDEL for BiP and -RKPRRE for calnexin. Misfolded proteins can be immobilized by binding to a mutant BiP [44], indicating that immobilization by chaperonins bound to a postulated lumenal ER matrix [56] could be a possible mechanism for retention of incompletely folded proteins in the ER. However, the ts045 mutant of the G glycoprotein of vesicular stomatitis virus, which is temperature sensitive for ER exit (ts045-VSVG), can freely diffuse while retained in the ER by association with the chaperonin calnexin [44]. Thus the inability of misfolded proteins to exit the ER appears primarily to derive from their attachment to ER-resident chaperonins which are retained within the ER lumen or membrane through any of a variety of means.

Retention of chaperonins and other resident ER proteins can occur through a variety of mechanisms which can be conceptually divided into two categories: (i) direct retention and (ii) retrieval/recycling mechanisms. Direct retention could involve aggregation of the protein, binding to the ER matrix or other immobile protein complexes or exclusion from COPII vesicles. Retrieval/recycling mechanisms, in which proteins escaped from the ER are captured in vesicular-tubular complex (VTC) or Golgi membranes and returned to the ER have been proposed most frequently since two retention signals -KKXX and KDEL are believed to function primarily as retrieval/recycling signals.

The most-studied ER retention sequence is KDEL (HDEL in yeast) [57]. This motif is sufficient to confer ER retention if transferred to a lumenal reporter protein that normally exits the ER [44]. There is a seven-transmembrane spanning protein which is considered a receptor for KDEL which is known to cycle between the ER and Golgi membranes [58, 59]. A pH-dependent association has been shown in vitro between the KDEL receptor and KDEL-containing proteins [60]. These experiments have suggested a widely accepted mechanism for KDEL-conferred ER retention in which the KDEL receptor cycles continuously between the ER and Golgi membranes [43]. In this model, KDEL-containing proteins are not actively excluded from Golgi-destined transport vesicles and are sometimes transported into the Golgi apparatus by mistake. However, since the Golgi lumenal pH is acidic, they are thought to bind with high affinity to the KDEL receptor which transports KDEL proteins specifically back to the ER where the KDEL protein is released because of the neutral lumenal pH. Thus KDEL retention in the ER is normally thought to require its retrieval from later compartments [43]. However, some KDEL-containing proteins, including the chaperonin calreticulin, can be held in the ER by KDEL-independent mechanisms that are not clearly known and appear to involve direct retention rather than retrieval [61].

Many ER-retained transmembrane proteins contain a cytosolic carboxy-terminal dilysine (-KKXX) motif similar to the COPI binding sequence [62, 63]. This motif is found also in proteins that cycle between the Golgi and ER system [63, 64], although the cycling proteins differ in that the final two amino acids are often phenylalanines, which appear to direct binding to COPII [28, 29, 65]. In yeast that possess mutations in either of two COPI subunits (α and β'), dilysine-containing proteins leak to the surface of the cell, and direct interactions have been shown between dilysine motifs on proteins or on peptides and COPI [63, 64]. These experiments have shown a role for COPI in retaining -KKXX proteins in the early secretory pathway.

It is generally believed that -KKXX motifs do not result in active retention of -KKXX proteins in ER membranes but rather the proteins that exit the ER and enter pre-Golgi intermediates or the Golgi apparatus are sorted into COPI-coated vesicles which are targeted back to the ER [66]. However, while the yeast experiments are compelling in showing a role for COPI in retention of -KKXX proteins in the early secretory pathway, there is evidence that -KKXX motifs can also mediate direct retention of proteins. Proteins containing an engineered -KKAA motif in their cytoplasmic tails fail to undergo processing of their N-linked oligosaccharides, indicating that they never advance as far as the cis-Golgi [67]. This ER retention mechanism was not saturable and did not depend on the presence of functional COPI, indicating that current models for retention in the early secretory pathway may be overly simplistic. In addition to -KKXX motifs, there is evidence that motifs in transmembrane domains of integral membrane proteins, specifically, hydrophilic amino acids within these hydrophobic domains, can mediate ER retention of yeast and mammalian proteins through an unknown mechanism [68–70].

A number of speculative but plausible mechanisms for ER retention can be proposed. One potential mechanism is based on competition for the finite amount of membrane or lumenal space in a COPII vesicle. Since the rate of COPII vesicle formation appears to depend on cargo load, it is quite possible that the vesicles are always densely packed with protein containing ER exit signals. In this case, since proteins not targeted for exit will have to compete for space with proteins containing strong COPII binding motifs, they will be incorporated at a reduced rate relative to bulk membrane. It is also possible that the membrane in ER transitional elements or COPII-coated areas has a lipid composition or membrane thickness which favors some proteins over others. Other possibilities for direct retention could be specific interactions with cytoplasmic proteins (including possibly COPI), ER matrix or aggregate formation. Unfortunately, these and other models, while conceptually simple, are difficult to test experimentally.

ER transitional elements and export complexes

Proteins do not exit the ER at random locations, at least in mammalian cells. The original electron microscopic autoradiography investigations of the secretory pathway in pancreatic cells [4, 71] identified small regions of tubular ribosome-free ER, which were termed transitional elements. These transitional elements appeared to be specialized domains where formation of exiting vesicles took place [4]. Recent light microscopy studies by Lippincott-Schwartz and co-workers [72] and Storrie and co-workers [73] have shown that in cells in which microtubules are depolymerized (immobilizing material exiting the ER at the exit point), a fixed number of ‘exit sites’ can be iden-

tified (usually about 150/cell) at which cargo proteins accumulate and form Golgi 'ministacks' [72]. This lends strong additional support to the notion that the ER has stable specialized exit areas found both adjacent to the central Golgi apparatus [4] and throughout the cell [72].

Scales and co-workers identified nascent transport intermediates using light microscopy and found that immobile intermediates (presumably found at ER exit sites) were initially COPII coated and then appeared to exchange the COPII coat for COPI before moving along microtubules to the Golgi apparatus [74]. Glick and co-workers and Balch and co-workers found GFP-labeled COPII on punctate structures throughout the cytoplasm [30, 75] which corresponded to these exit sites and provided light-microscopic evidence of vesicular tubular complexes (VTCs) repeatedly leaving the same sites. These data taken together indicate that ER exit sites persist for substantial lengths of time and can form multiple Golgi-targeted VTCs.

Bannykh and co-workers conducted a detailed electron microscopic study of the morphology of these exit structures [38]. They found clusters of adjacent COPII buds often on two or more adjacent but noncontiguous pieces of ER membrane [38]. These budding zones cupped a space containing a VTC [38]. This VTC consisted of a complex of tubules apparently not contiguous with ER membranes and enriched in cargo proteins [38, 50]. Many of the tubular profiles were capped by a COPI coat [38, 50]. These VTC/transitional element complexes were termed export complexes [38] and almost certainly are the same structures described by Glick and co-workers in their light microscopic studies [75]. Interestingly, if COPII was locked onto membranes with GTPγS in permeabilized cells, COPII coated 'necklaces' consisting of chains of what were described as unseparated vesicles were produced [38].

These data can be synthesized into a model in which specialized COPII-coated domains in ER, which appear in electron micrographs as small regions containing large numbers of COPII-coated buds [38], trap cargo directly or indirectly through specific interactions with the COPII coat [46]. It is generally assumed that the buds transform into COPII vesicles which then uncoat and fuse with each other in the open area of the export complex to form a VTC [38], and that COPI is not involved directly in ER exit [66]. COPII-coated vesicles continue to fuse with the COPI-containing VTC, which becomes larger and eventually binds to microtubules and uses the dynein/dynactin complex to leave the ER exit site. The exit site remains after the VTC has left and synthesis of a new VTC commences. While in vitro production of COPII vesicles has been well demonstrated, it has also been considered that in vivo COPII-coated regions of ER membrane could directly transform into VTCs [40] and that COPI could play a direct role in this transformation [39].

VTC structure and the role of COPI

The discontinuous carriers that move from ER membranes to Golgi membranes have been given various names including intermediate compartment [50], pre-Golgi intermediate [76] and VTC [38]. These carriers consist of a large convoluted mass of tubules individually 50 nm in diameter with an average diameter for the cluster of 0.4 micron [38, 77]. It has proven technically difficult to determine whether the tubules form a structure with a single continuous lumen or whether a VTC consists of multiple intertwined but unconnected tubules [38]. The VTC consists of subdomains which are enriched in cargo or in proteins such as ERGIC53 which are destined for recycling back to ER membranes [77]. The tubules that cluster to make the VTC are often capped on the ends with COPI [77].

Klumperman and co-workers have performed a detailed quantitative study of the localization of soluble and integral membrane cargo proteins as well as ERGIC53 and COPI on VTC membranes [77]. They have confirmed the earlier reports [38] that COPI tends to be found on the ends of VTC tubules [77]. While they do not show concentration of proteins to be recycled in the COPI-coated membrane areas, active exclusion of cargo proteins from these areas was found, as was exclusion of soluble proteins [77]. They interpret these data with a detailed model involving multiple rounds of COPI vesicle budding from VTCs during their formation, returning integral membrane proteins with COPI-binding sequences to the ER, while leaving much of the soluble and membrane-incorporated cargo to move on to the Golgi apparatus [77].

It is currently widely accepted based on the above-described observations that interactions between motifs on the cytoplasmic tails of transmembrane proteins to be recycled and COPI lead to the recruitment of this subset of proteins into COPI-coated buds. These buds then transform into COPI-coated vesicles which uncoat and fuse with ER membranes. However, sorting of the dilysine-containing ERGIC53/p58 from VTCs at least under some circumstances (after release of a 15 °C block) appears to involve formation of tubules which exclude cargo protein but do not appear to have a COPI coat [50]. Thus, while there is clear evidence for the existence of sorting of recycling proteins from cargo in VTCs, more work needs to be done to establish the mechanism(s) for this sorting.

The VTC contains molecular motors (i.e. dynein and kinesin) as well as cargo proteins and recycling proteins. As will be discussed later, the binding and regulation of these motors is only beginning to be worked out. The motor dynein is required for microtubule-mediated transport of the entire VTC to the Golgi apparatus. Once the VTC has translocated to the Golgi region, it fuses with the cis-Golgi cisterna [74, 76] or in the cisternal maturation model with other VTCs to form a cis cisterna [66]. This

fusion requires first recognition of the target membrane and then activation of a fusion machinery. While the process of specific recognition followed by fusion is not completely understood, it is now believed to involve multiple proteins conferring several layers of specificity. v-SNARE (small N-ethylmalemide-sensitive factor attachment protein receptor) and t-SNARE proteins were formerly proposed to mediate attachment between transport intermediate and target membranes. However, Rab-regulated 'tethering' or 'docking' proteins, long protein molecules which usually contain coiled-coiled domains, appear to mediate the initial recognition event between two membranes, and correct recognition (although not fusion) can occur in the absence of SNAREs. SNAREs show structural similarity to viral fusion proteins, and mediate membrane fusion along with cofactors NSF and SNAP-25. In mammalian cells, at least Bet1p, ERS-24/Sec22b, GOS-28 and syntaxin 5 appear to be involved in ER-Golgi transport [78]. For detailed reviews, see ([79–81]).

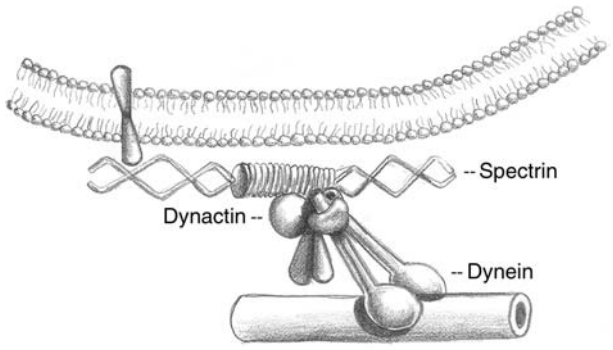

Figure 2. The dynein/dynactin complex. The molecular motor dynein does not attach directly to the plasma membrane of a transport intermediate but is attached indirectly. Dynein binds to the protein complex dynactin. While details are not completely clear, dynactin may attach to a spectrin lattice anchored to integral membrane proteins in the transport intermediate.

Interaction of ER and VTCs with microtubules

The ER can have various morphologies. It can be sheet-like, typically in the cell center or in professional secretory cells, or it can consist of a network of interconnecting tubules [82]. This is typically the case near the periphery of the cell. It has been shown that extension of the ER to the cell periphery requires the presence of microtubules, as the ER gradually collapses towards the cell center after treatment with microtubule depolymerizing agents [82]. The extension of ER towards the cell periphery requires the (+)-end directed motor kinesin [83]. Work by Waterman-Storer and co-workers has directly visualized the extension of ER tubules towards but not into the actin cortex at the cell periphery [84]. ER tubules that make contact can undergo SNARE-mediated fusion [85], and ER tubules can break [82]. Thus the ER network in the cell periphery is in a dynamic equilibrium between fission and homotypic fusion.

VTCs also interact with microtubules, but unlike the ER they come from, the interactions involve primarily the (–)-end-directed motor dynein [86] complexed with dynactin (fig. 2; [87]). Dynein is a (–)-end directed microtubule motor which moves VTCs towards the Golgi apparatus [76, 88, 89], which in most mammalian cell types is located near the centrosome. VTCs contain a kinesin as well that later serves as a Golgi-to-ER motor [90] although this (+)-end directed motor activity is presumably inactive during translocation of the VTC to the centrosome. There are multiple organelle-associated cytoplasmic dyneins [91]. Various dynein subunits have been identified on Golgi and on pre-Golgi intermediates. Vaisberg and co-workers have identified dynein heavy chain 2 on Golgi by immunofluorescence and membrane fractionation [91], while dynein heavy chain 1a [92] has been identified by Roghi and co-workers on pre-Golgi intermediates and on Golgi. The reasons for the discrepancies between these observations are not clear, and the identity of the dynein responsible for ER-to-Golgi trafficking is not resolved at this time. Dynein/dynactin remains active at the Golgi apparatus and is partly responsible for the centrosomal localization of this organelle [88].

Dynactin is a membrane-associated multi-subunit dynein-binding complex that is required to bind dynein to membranes. It is a protein complex composed of multiple polypeptides, including at one end a 37-nm-long filament composed of the actin homologue Arp-1, p150^{Glued} and p50/dynamitin (reviewed in [93–95]). Overexpression of p50/dynamitin results in the failure of the dynactin complex to assemble properly. Both ER-to-Golgi trafficking [76, 88] and the intracellular distribution of endocytic organelles [88, 96] are disrupted after p50/dynamitin overexpression. While dynactin has been shown to bind directly to dynein, the mechanism for binding of dynactin to the membrane compartment has not been determined. Recently, a spectrin/ankyrin skeleton has been proposed on the Golgi apparatus and on pre-Golgi transport intermediates [97]. Since binding of the Arp-1 subunit dynactin to βIII spectrin has been shown [98], a plausible mechanism for binding of dynein to membranes can be postulated.

Dynein heavy chain 1a appears highly stably associated with Golgi and pre-Golgi membranes, not coming off even when the Golgi apparatus is disrupted and tubulated with the drug brefeldin A [92]. This would suggest that at least some dyneins cycle between the ER and Golgi apparatus along with their associated membrane [92]. If that is the case, dyneins are not only on (–)-end-directed transport intermediates such as pre-Golgi intermediates but also on (+)-end directed retrograde transport interme-

diates. Thus the dynein activity must be regulated. How this would be done is not clear, but there is evidence that Rab proteins can regulate binding or activity of microtubule motors as well as membrane recognition. An interaction between Rab6 and the p150^{Glued} subunit of the dynactin complex has been shown that may be involved in Golgi-to-ER trafficking [99]. An interaction between the late endosomal Rab, Rab7 and dynein has been proposed. Rab1 and Rab2 are the predominant Rabs known to be involved in ER-to-Golgi trafficking; however their role in regulation of molecular motors is not well studied.

The dynamic nature of the Golgi apparatus

It has been known for a long time that that the Golgi apparatus fragments in cells treated with agents such as nocodazole or colchicine that depolymerize microtubules [100]. Since the Golgi apparatus is located near the microtubule-organizing center (MTOC) in most mammalian cells (fig. 3; [100]) and morphologically is often a ribbon composed of stacks of cisternae connected by a tubular network [101, 102], it was believed that the microtubules play a role in tethering the cisternae and holding the Golgi apparatus together [100]. Golgi fragmentation would result from loss of this microtubule tethering followed by the individual cisternae drifting throughout the cytoplasm of the cell [100].

Experiments from several different laboratories have recently painted a dramatically different picture. Careful experiments using immunofluorescence to follow early Golgi markers such as KDEL receptor and late Golgi markers such as TGN38 showed that after microtubule depolymerization, the early Golgi markers rapidly dispersed to ER exit sites and labeled ~150 punctate structures. These structures were initially weakly labeled but became more heavily labeled with time [72]. Late Golgi markers colocalized in the same punctate structures only after several hours [72]. The central Golgi structure remained intact and localized to the cell center in cells in which microtubules were depolymerized but lost protein content with time as these proteins redistributed to the peripheral punctae [72]. Electron microscopic analysis showed that at early times, the punctate structures were morphologically VTC-like but that later they resembled Golgi and showed a typical stacked morphology [72]. These structures were referred to as Golgi mini-stacks [72]. The hypothesis was proposed based on this data and an in vivo assay for Golgi-to-ER cycling [72, 103] that there is continuous cycling of Golgi resident proteins between the Golgi and the ER. The Golgi-to-ER step may normally require the motor protein kinesin [90] but is not blocked in the presence of nocodazole [104], presumably because there are large amounts of ER immediately adjacent to Golgi. However, since ER exit sites are scattered

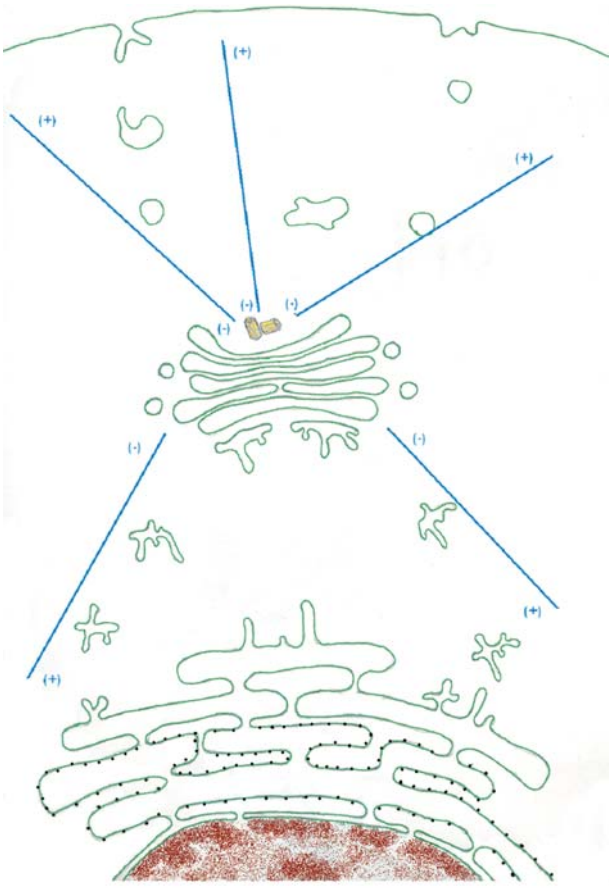

Figure 3. Schematic showing the relationship of the early secretory pathway to the microtubule cytoskeleton. The Golgi apparatus is near the centrioles and the (–) ends of microtubules in many cell types. This localization has important implications for targeting of transport intermediates: transport to the Golgi (i.e. ER-to-Golgi trafficking) uses (–)-end-directed microtubule motors such as cytoplasmic dynein, while transport from the Golgi apparatus to the ER or cell surface generally employs (+)-end-directed motors in the kinesin family.

throughout the cytoplasm [72] and VTCs require dynein/dynactin [76] and microtubules [105] to leave their site of origin, return of these proteins to the central Golgi apparatus is blocked, and they accumulate in VTCs at ER exit sites that continue to acquire more exiting material. The outcome is that as the VTCs acquire more Golgi proteins, they transform into functional though small Golgi stacks [72] that support posttranslational modification of N-linked oligosaccharides [72] and trafficking of cargo proteins to the cell surface [106].

A prediction of this hypothesis is that if ER exit is blocked, Golgi mini-stacks will not form and Golgi integral membrane proteins such as enzymes will accumulate in the ER. Storrie and co-workers [73] and Ward and coworkers [107] tested this prediction by expressing a mutant Sar1p which acts as a dominant negative and prevents the assembly of the COPII complex at ER exit sites, leading to a block in ER exit. As predicted, Golgi resident pro-

teins accumulated in the ER rather than in mini-stacks [73, 107]. Conversely, if the Golgi pool of protein was rendered invisible by photobleaching the Golgi region at the beginning of nocodazole treatment, mini-stack formation was not prevented [108], indicating that mini-stack proteins can originate from the ER. Time-lapse imaging established that mini-stacks appear randomly and rapidly on ER membranes after nocodazole addition and gradually accumulate the cargo protein VSVG [76].

An even more dramatic example of de novo reconstitution of Golgi from ER membranes is given by the reversibility of the drug brefeldin A. Brefeldin A causes the loss of COPI and Arf1 from Golgi membranes [109–112], followed by profuse tubulation of Golgi membranes [9, 113], subsequent fusion of the Golgi with the ER [104, 113] and with endosomes [114, 115] and the complete loss of Golgi identity within a few minutes. Brefeldin A is reversible, and if it is washed out, the Golgi apparatus is reconstituted even after several hours of exposure to this drug [113].

Self-organization of the Golgi entirely from ER membranes has been questioned, and a need for a permanent cytoplasmic scaffold has been postulated by Warren and co-workers. According to their model this scaffold is composed of golgins, including GM130 and members of the GRASP family (see [116, 117]). They report that these proteins remain behind after redistribution of Golgi enzymes to the ER by expression of a dominant-negative Sar1 or by treatment with brefeldin A [117]. In contrast, Ward and co-workers find that at least one of these peripheral membrane proteins (GRASP-65) can rapidly exchange between the Golgi and the cytosol in photobleach experiments and is rapidly distributed between the central Golgi and mini-stacks after microtubule disruption with nocodazole [107].

From the experiments described above, it is clear that the Golgi is a transient structure. Unlike the ER, which could not be reconstituted by a cell if destroyed, the information implicit in the ER and its proteins and lipids is largely sufficient to build a Golgi apparatus (even assuming the need for a cytoplasmic scaffold), and this must involve a large element of self-organization. The mechanisms and principles behind this self-organization are almost completely unknown, although there are implications for the normal functioning of the Golgi apparatus, which retains its structure in the face of enormous membrane flux through the system [118, 119]. These experiments demonstrating the self-organizing capacity of the Golgi apparatus have given a boost to cisternal maturation models [43, 120, 121] and even to models of the Golgi as a single simply connected self-organizing compartment [122], and have helped in the recent fall from favor of the more static model of a Golgi apparatus consisting of stable disconnected cisternae acting as essentially a series of separate compartments that retain differing resident proteins over time through which cargo must be transported by a series of discontinuous vesicular transport steps [123].

Recycling and Golgi-to-ER transport

The possibility of retrograde trafficking from the Golgi to the ER was first shown under two sets of circumstances: delivery of certain toxins that bind receptors or lipids on the plasma membrane (including cholera toxin to the ER; [13, 124]); and rapid tubule-based delivery of Golgi proteins to the ER after treatment with the drug brefeldin A [9, 113].

It was primarily the brefeldin A experiments that called attention to a retrograde pathway from the Golgi to the ER. Brefeldin A inhibits the GTP-exchange factor (GEF) required to localize the small GTPase Arf1 to Golgi membranes [125]. Since Arf1 normally is cycling on and off of Golgi membranes [110, 126], inhibiting new recruitment of Arf1 causes its rapid loss from these membranes [111, 112, 126, 127]. Since Arf1 is required to recruit the coat protein COPI to Golgi membranes, the result is that after a short time (60 s in HeLa cells), both COPI and Arf1 relocate from Golgi membranes to the cytosol [109, 110, 128]. Elements of a Golgi-associated spectrin/ankyrin network are also lost from Golgi membranes [129]. After the loss of these components, there is profuse microtubule-dependent tubulation of Golgi membranes [9], and eventually the Golgi contents appear in the ER [9, 113]. As brefeldin A blocks exit of some proteins from the ER as well [44, 113] (although not ERGIC53; [9]), Klausner and Lippincott-Schwartz [130] proposed that brefeldin A upregulates a preexisting retrograde pathway from the Golgi apparatus to the ER. This and the block in ER exit results in a net massive relocalization of Golgi proteins to the ER and eventual destruction of the Golgi apparatus [9, 104, 113]. The microtubule motor kinesin was shown to be responsible for the extension of the brefeldin A-induced tubules from the Golgi apparatus towards the cell periphery [90], and it was proposed that a COPI coat on Golgi membranes stabilizes the Golgi apparatus and inhibits the kinesin motor required to form the tubular retrograde transport intermediates in non-brefeldin A-treated cells [90, 130]. More recently, it has been shown that brefeldin A-mediated delivery of Golgi proteins to the ER is not a gradual process but occurs explosively, apparently after the fusion of a single tubule with ER [104]. Sciaky and co-workers have suggested that delivery of Golgi proteins to ER could be a flow process driven by energetically favorable mixing of ER and Golgi lipids [104].

Brefeldin A treatment creates a highly nonphysiological condition leading to the collapse of the secretory pathway. However, rapid cycling of Golgi proteins (e.g. ERGIC53/p58, KDEL receptor and some SNARES) be-

tween ER and Golgi membranes has been shown through a variety of techniques under physiological conditions as well [10, 51, 103, 131, 132]. In one of these studies, Lippincott-Schwartz and co-workers fused the temperature-sensitive domain of ts045-VSVG with Golgi-resident proteins and were able to trap them in the ER at the non-permissive temperature [103]. Other experiments used photobleaching techniques to show cycling of GFP-tagged Golgi resident proteins ([73, 107, 108]; discussed in Ward and Brandizzi in this issue) as well as ER exit blocks induced with dominant-negative Sar1p [73, 107] and with nocodazole [73, 107]. The existence of this cycling between ER and Golgi membranes is now firmly established. It must involve both ER-to-Golgi trafficking of the proteins (already discussed) and a retrograde Golgi-to-ER trafficking pathway.

The role of COPI in Golgi-to-ER trafficking

COPI was identified initially by Kreis, Klausner and co-workers as a protein dispersed from Golgi membranes by brefeldin A [109] and by Rothman as the coat protein found on vesicles in an intra-Golgi transport assay [133]. Based on the brefeldin A studies, Klausner proposed that COPI stabilizes Golgi membranes and negatively regulates the formation of transport intermediates [130]. In support of this, a brefeldin A-like phenotype in which Golgi tubulates and then merges partially with ER was produced in a mutant CHO cell line (ldlF; [134]) in which the epsilon subunit of COPI was degraded [135]. Also, brefeldin A-induced tubulation could be prevented with an antibody against COPI [136].

Rothman and co-workers identified COPI as a required factor in an in vitro Golgi transport system and as a vesicle coat which was originally proposed to mediate ER-to-Golgi trafficking and intra-Golgi anterograde trafficking [133, 137]. The in vitro transport system was designed to assay transport of the VSVG protein from early to middle Golgi cisternae [138]. VSVG is a typical cargo protein targeted to the cell surface. COPI was proposed to be a coat for transport vesicles carrying the cargo protein forwards in the Golgi apparatus [123]. Unfortunately, with some exceptions [139], investigators have found COPI vesicles identified in vivo consistently devoid of cargo proteins [77, 140–142], and most investigators have abandoned the view that COPI vesicles are anterograde transport intermediates.

However, the COPI vesicles could be shown to contain Golgi resident proteins [140] and proteins that cycle between the Golgi and the ER [77]. Many of the recycled proteins contain a COPI-binding -KKXX motif [63] or other COPI binding motif. Thus it has been presumed that COPI is involved in the formation of Golgi-to-ER transport intermediates [66]. COPI-coated vesicles containing Golgi enzymes and cycling molecules have been identified in the work of Klumperman and co-workers [77, 142] and Nilsson and co-workers [140]. These vesicles are depleted in both soluble [77] and membrane-associated cargo proteins [140].

The supporting evidence that COPI is involved in Golgi-to-ER trafficking comes primarily from yeast genetics and in vitro binding experiments. Cosson and Letournier [63, 64] supplied two pieces of data that were taken as evidence that COPI is involved in retrograde trafficking. First, in yeasts with mutations in certain COPI subunits (α and β' but not γ), -KKXX proteins appeared on the cell surface, the same result as if the -KKXX was deleted [64]. Second, direct interactions could be shown between some COPI subunits and -KKXX peptides bound to affinity columns [63]. These results, taken together with the observations made by many laboratories that COPI vesicles seem to exclude forward-directed cargo molecules [77, 140–142], while generally consistent with a role for COPI in organizing Golgi-to-ER trafficking were not consistent with the previously dominant model of the secretory pathway in which COPI vesicles carried cargo in an anterograde direction within the secretory pathway [123]. These observations led to a revival of the cisternal maturation model [120, 121] of intra-Golgi trafficking, with retrograde COPI vesicles involved in relocation of Golgi enzymes to compensate for the anterograde flow of cisternae [121]; and also to the proposal that Golgi-to-ER trafficking is mediated by 50-nm COPI-coated vesicles [66].

As a result of these developments, there is currently a consensus that COPI binds to -KKXX and other motifs on the cytoplasmic tails of recycling transmembrane proteins, including ERGIC53, KDEL receptor and members of the p24 family, and the coat protein polymerizes to form COPI-coated pits, as outlined previously. These pits then pinch off and detach from ER/VTC membranes as COPI coated vesicles, uncoat and fuse with ER membranes. For reviews describing this model, see [143, 144].

Work from several laboratories indicates that ERGIC53 and other recycling proteins, including KDEL receptor, are recycled directly from VTCs to ER as well as from Golgi to ER in mammalian cells (probably through similar mechanisms). Hong and co-workers [145] and Hauri and co-workers [50] showed that ERGIC53 accumulated in VTCs with 15 °C block exited the VTCs rapidly after warmup to 37 °C; however, cargo proteins were retained. On the other hand, Hauri [50] reported that ERGIC53 appeared to exit the VTCs in tubules rather than 50-nm vesicles. These tubules did not possess a COPI coat [50, 128]. White and co-workers [13], and Sciaky and co-workers [104] were able to directly image the trafficking of GFP constructs of molecules (KDEL receptor, Rab6 and cholera toxin) that utilize the Golgi-to-ER retrograde pathway, and reported tubular transport intermediates.

White and co-workers found that trafficking of KDEL receptor from the Golgi to the ER could be blocked by microinjection of antibodies against COPI, while trafficking of Rab6 and cholera toxin was not affected [13]. They proposed two Golgi-to-ER transport pathways, one COPI independent and the other dependent on COPI [13]. The laboratory of Lippincott-Schwartz was able to image apparent tubular transport intermediates using p58-GFP and coming from the Golgi apparatus [128]. These, like the VTC-originating ERGIC53-containing tubules observed by Klumperman and co-workers [50], often lacked COPI. Thus there is currently a discrepency between in vivo and in vitro data which has not yet been resolved.

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