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Review

Long coiled-coil proteins and membrane traffic

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Abstract

Protein transport between organelles is mediated by vesicles which must accurately dock and fuse with appropriate compartments. Over the past several years a large number of long coiled-coil proteins have been identified on the Golgi and on endosomes, mostly as auto-antigens in autoimmune disorders. Based on their restricted intracellular distributions and their predicted rod-like structure, these proteins have been proposed to play a role in tethering vesicles to target organelles prior to fusion. However, such proteins may also play a structural role, for example as components of a Golgi matrix, or as scaffolds for the assembly of other factors important for fusion. This review will examine what is known about the function of these large coiled-coil proteins in membrane traffic.

Keywords: Coiled-coil; Vesicle tethering; Golgin; Golgi; Endosome; Rab GTPase

1. Introduction

The eukaryotic secretory pathway is made up of a number of distinct organelles through which macromolecules traffic. Anterograde and retrograde transport by means of vesicles, and other carriers, allows organelles to communicate with one another while maintaining their individual homeostasis. Maintenance of a complex network of distinct organelles requires that vesicles only fuse with the correct destination compartment. Thus, organelles must possess docking and fusion machinery that allows specific recognition of incoming vesicles.

Initially, the specificity of vesicle targeting was thought to be mediated by SNARE (soluble NSF-attachment proteins (SNAP) receptors) proteins [1]. The SNARE hypothesis proposes that the pairing of v-SNAREs on vesicles and t-SNARES on target membranes is sufficient to ensure that vesicles recognise and fuse only with the correct acceptor compartments. However, evidence that SNAREs are not the complete picture comes from a variety of sources. The observations that SNARE–SNARE pairing can be promiscuous, and that both v- and t-SNAREs are found on recycling vesicles lead to a conceptual problem of how they can specify a particular compartment [2,3]. In addition, SNAREs often appear to decorate compartments more or less homogeneously, while vesicle fusion is frequently observed at ''hot spots'' or discrete loci. For example during polarised growth in the yeast *Saccharomyces cerevisiae* the syntaxins Sso1p and Sso2p are found evenly distributed throughout the plasma membrane, while exocytic vesicles fuse only at the bud tip [4]. Most strikingly, EM pictures of nerve terminals treated with botulinum and tetanus toxins, which selectively cleave SNARE proteins, show docked vesicles remaining tightly associated with membranes [5]. Finally, when membrane fusion is reconstituted in vitro using SNARE proteins alone, the rate is slower than that observed in vivo, suggesting that additional factors must be available in the cell which stimulate vesicle fusion, possibly by stabilising vesicle docking [6]. Recently, a whole range of proteins have been proposed to act prior to SNARE protein assembly to increase the specificity or efficiency of the initial attachment of vesicles, a process now known as tethering [7,8].

Proteins which have emerged as candidate tethering factors fall into two general classes; those which are components of multi-subunit complexes such as the GARP/VFT complex [9–11], the COG complex [12,13], the TRAPP complex and the Exocyst [7], and those which belong to a class of large coiled-coil proteins [14,15]. Unlike SNARE proteins, putative tethering factors are quite heterogeneous in sequence and structure, although certain similarities are now becoming apparent. For example, some but not all, large

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multi-subunit tethers consist of four or eight related proteins and have been termed quatrefoil complexes [16]. In addition, motifs have been identified that are shared by a number of coiled-coil proteins, as will be discussed below. What is apparent is that many of the proteins have clear homologues in all eukaryotes so far examined. This high degree of conservation implies an important role in some aspect of cellular function, although in many cases the precise nature of this role remains unclear. Since multi-subunit complexes have recently been reviewed elsewhere [7,16], this article will examine the evidence to link large coiled-coil proteins with the process of vesicle tethering and other aspects of membrane traffic. In particular we will concentrate on the large coiled-coil proteins of the Golgi and of endosomes, where most of these proteins have been found. As yet, no role has been proposed for large coiled-coil proteins in membrane traffic at other compartments such as the ER and the plasma membrane. A possible exception is the neuronal synapse, where Rim1, a protein that plays a role in priming synaptic vesicles for fusion, is localised to its site of action by CAST, a neurone-specific, long coiled-coil protein (reviewed in Refs. [17–19]). However, this review will concentrate on proteins of the general trafficking pathways.

2. Coiled-coil proteins as potential vesicle tethers

Genes encoding coiled-coil proteins comprise roughly 5% of the coding sequences of a typical eukaryotic genome, implying an involvement in numerous cellular processes [20,21]. Coiled-coils are autonomous folding units consisting of at least two α-helices that wrap around each other with a slight left-handed superhelical twist [22]. The amino acid sequence which gives rise to this structure consists of a repeat of seven residues, termed a heptad repeat, in which positions 1 and 4 of the sequence are usually hydrophobic [23,24]. Such coiled-coils typically form rod-like structures, and 100 amino acid residues is sufficient when dimerised to extend about 15 nm.

A large number of long coiled-coil proteins have been identified on the Golgi and endosomes, and the current set for human cells is illustrated in Fig. 1 and Table 1. Most of these proteins were not identified by functional assays, but rather by indirect means often as antigens recognised by sera from patients with autoimmune diseases, or as potentially erroneous interactions arising from yeast two-hybrid screens. Therefore, their potential role in membrane traffic has often been inferred simply from their organelle-specific location and their structure. Long rod-like molecules are

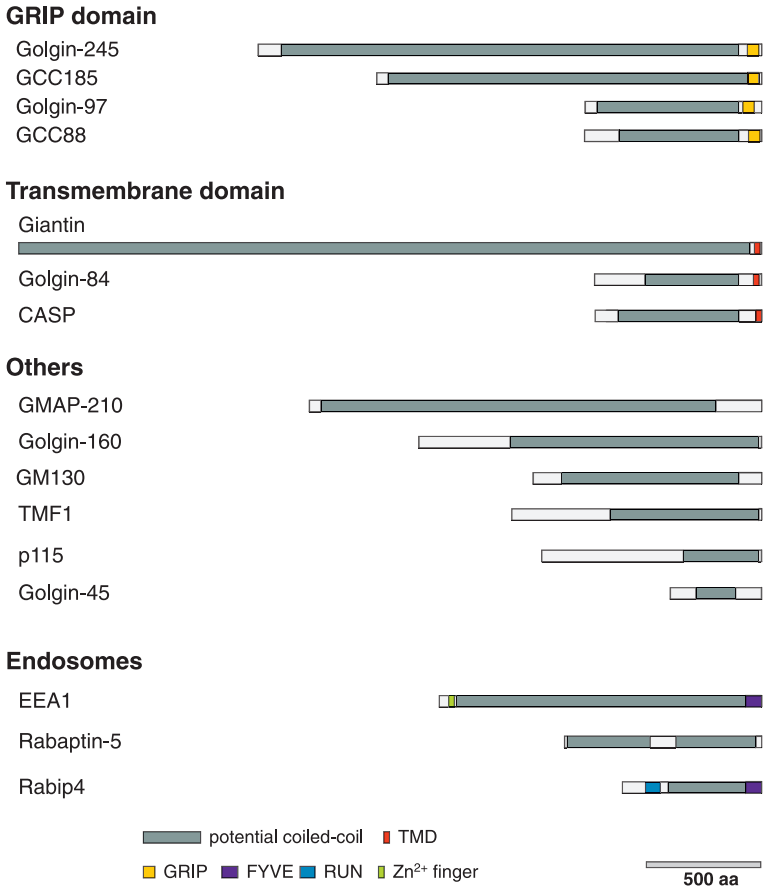

Fig. 1. Long coiled-coil proteins of the Golgi and of endosomes. Schematic representations of known coiled-coil proteins, showing their relative sizes and overall structures. Coiled-coil domains are shown in grey. Domains important for protein function or subcellular targeting are also indicated.

Table 1
Human long coiled-coil proteins proposed to play a role in membrane traffic

	Name	Aliases	Size (aa)	Relatives in:	
				Drosophila	*S. cerevisiae*
Golgi					
TMD	Giantin	Macrogolgin GCP364 GCP372	3260	–	COY1
	Golgin-84		731	CG17785	–
	CASP		678	–	–
GRIP	Golgin-245	p230	2230	CG3493	IMH1
	Golgin-97		767	CG4840	
	GCC88	GCC1	775	CG10703	
	GCC185	KIAA0336	1684[a]	CG3532	
Others	p115	TAP	962	CG1422	USO1
	GMAP210	Trip230	1979	CG7821	–
	TMF1	ARA160	1093	CG4557	SGM1
	Golgin-45	JEM-1	400	CG9356	–
	GM130	Golgin-95	990	CG11061	–
	Golgin-67		631	–	–
	Golgin-160	MEA2	1498	–	RUD3?[b]
Endosomes					
	EEA1	p162	1410	–	–
	Rabaptin-5	Rabaptin-4	862	CG4030[c]	–
	Rabip4	RUFY1	606	CG31064	–

[a] GCC185 has been reported to be 1583 residues in length [101], but our examination of ESTs suggests that it has an additional 101 residues at the N terminus.

[b] Rud3p has been noted as being related to mammalian golgin-160 [78], but this is over the coiled-coil regions of the proteins.

[c] Fly protein CG4030 has an FYVE domain at its C terminus that is absent in the putative mammalian homologues.

attractive candidates for factors that form contacts between membranes at a distance, either for structural purposes or to capture transport vesicles in the proximity of an organelle prior to fusion. As illustrated in Fig. 2, several roles of this type have been suggested for these proteins. Indeed, with such a number and diversity of proteins, it is of course possible that they do not all have the same type of function.

Most of these proteins are peripheral membrane proteins, and most have been found to be associated with the Golgi (termed "golgins") or with endosomes (Fig. 3) [25–28]. This restriction to just a subset of organelles may be a clue as to their precise role, and is in contrast to the large multi-subunit complexes, which are found on most organelles [16]. Perhaps coiled-coil proteins are specifically localised to organelles where a high degree of vesicle transport necessitates the actions of more than one tethering factor. Indeed, the proposed rod-like structure of coiled-coil proteins may enable them to assemble into arrays along a membrane, increasing their local concentration. This tight meshwork of tethers could then act to ensure that vesicles are selectively captured or repelled at a particular membrane.

Examination of the surface of the mammalian Golgi by freeze fracture electron microscopy has provided evidence for the existence of fibrous elements, which may represent coiled-coil proteins, associated with vesicles and between Golgi stacks [29]. Usually more than one of these elements is observed making contact with a given vesicle. Such multivalent interactions might be required to add specificity to the tethering process, in that several proteins may be needed to act together to capture a vesicle via a number of weaker individual interactions. This could, perhaps, account for the high degree of redundancy which seems evident in many transport steps (see below, Sections 3.1 and 3.4), and may also explain those cases where loss or mutation of one tethering factor can be compensated for by overexpression of another, or by overexpression of putative downstream components such as SNAREs.

Secretory pathway coiled-coil proteins often have discrete domains at their N or C termini, which may mediate organelle-specific targeting or interactions with other proteins. In addition, many of the proteins have small stretches of non-coiled-coil sequence interspersed among the coiled-coil regions which could act as hinges, enabling vesicles docked at one end of the tether to be physically moved closer to the membrane via some sort of mechanical action. Moreover, the coiled-coiled structure requires that the proteins either multimerise with themselves to form homodimers or other oligomers, or form heterodimers with other proteins. In theory these associations may be in parallel or anti-parallel orientations, or as recently described for the Rad50 zinc hook protein, coiled-coil proteins can fold in half so that their N and C termini are in close apposition [30]. However, as discussed below, all putative tethering factors examined so far exist as parallel homodimers.

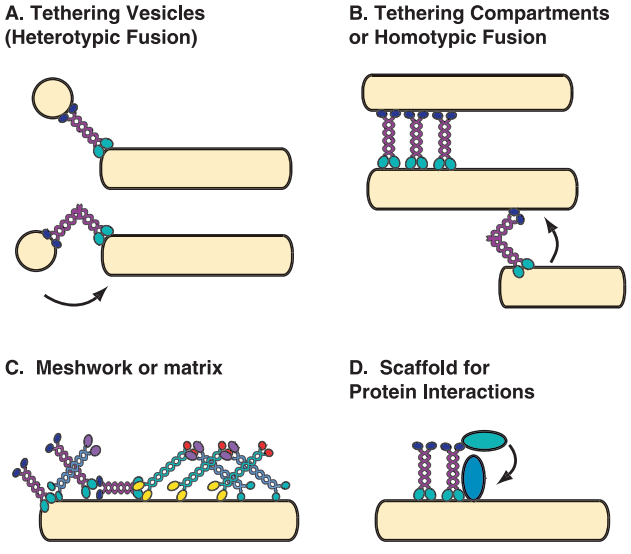

Fig. 2. Possible roles for long coiled-coil proteins in the secretory pathway. Coiled-coil proteins have been implicated in both heterotypic vesicle tethering (A) and homotypic tethering of compartments (B). In addition, coiled-coil proteins may form a meshwork over the surface of organelles (C), which could mediate the selectivity of tethering by retaining or repelling vesicles, and/or play a structural or 'matrix' role. Coiled-coil proteins have also been suggested to form scaffolds or platforms for the assembly of other factors required for membrane fusion (D).

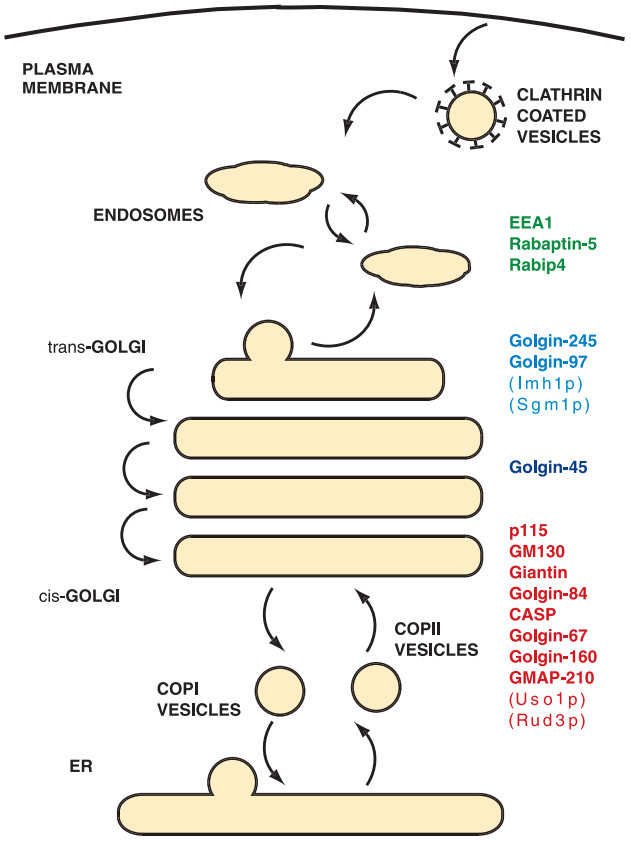

Fig. 3. Location of known long coiled-coil proteins in the mammalian secretory pathway. Individual proteins are indicated next to those compartments in which they have been localised, or in which a function has been defined. Coiled-coil proteins from *S. cerevisiae* have also been included and are shown in brackets and italics.

Dimerisation of identical domains at the N or C termini may be required to increase avidity for a specific binding partner.

As well as their role in vesicle tethering, large coiled-coil proteins have been implicated in the assembly of a Golgi "matrix" or scaffold in mammalian cells [15,31]. This matrix has been proposed by different studies to consist either of members of the GRASP and "golgin" families (Section 3.1), or of a spectrin/ankyrin meshwork [15]. Of course vesicle tethering and maintenance of Golgi structure are not mutually exclusive functions, since the latter can be thought of as the tethering together of two compartments, either with, or without, subsequent fusion. It is important to note that some fungi and yeast, including *S. cerevisiae*, have a Golgi composed of separate compartments, which are not stacked and as such are unlikely to require the formation of a Golgi matrix that facilitates stacking. *S. cerevisiae* does, however, contain homologues of many of the proteins implicated in the formation of the mammalian Golgi matrix, implying that they perform functions besides their structural role in stacking. These functions may include the formation of a meshwork over the surface of the organelle which could "sample" incoming vesicles.

Thus, the structure of large coiled-coil proteins appears well suited to their serving a number of roles, but in particular that of vesicle tethers, attaching to organelles via their non-coiled-coil domains and extending into the cytoplasm to capture incoming vesicles. So what is the evidence?

3. ER to Golgi and intra-Golgi traffic

3.1. p115, GM130 and giantin

3.1.1. p115

p115 was initially identified as a factor required in an in vitro assay that reconstituted intra-Golgi vesicular transport [32]. It is a 115-kDa peripheral membrane protein localised predominantly to the cis-Golgi, and to structures between the ER and the Golgi known as vesicular tubular clusters (VTCs) [32,33]. Rotary shadowing shows that p115 is an elongated homodimer with two globular N-terminal head domains [34]. These head domains are unusually large compared to the N-terminal regions of other Golgi coiled-coil proteins, and it seems likely that they have a key role in the function of p115, although so far this role remains unclear. The tail domain of p115 is predicted to form four stretches of coiled-coils (CC1–4) with a length of about 45 nm. Analysis of detergent solubilised Golgi membranes revealed interactions between p115 and two other major protein species, p130 and p400 [35]. These proteins were subsequently identified as GM130 and giantin, and are now discussed.

3.1.2. GM130 and GRASP65

GM130, also known as golgin-95, was identified as a binding partner for p115 by Western blotting [35]. It is a component of a TritonX-100 insoluble Golgi "matrix", and binds to p115 via its first 75 amino acids [35]. GM130 associates with Golgi membranes by an interaction between its C terminus and the protein GRASP65, a 65-kDa protein involved in the reassembly of Golgi stacks following mitosis (Fig. 4) [36,37]. Deletion analysis localises the binding site for GRASP65 to the extreme C terminus of GM130, and it comprises a short region ending in four hydrophobic residues. Indeed, a fusion of green fluorescent protein (GFP) to the last 170 amino acids of GM130 is specifically targeted to the Golgi apparatus [37]. GRASP65 attaches to membranes via an N-terminal myristate anchor, although other factors must be responsible for specifying a Golgi localisation, since in theory a myristoylated protein can associate with any available membrane [36]. The first 202 amino acids of GRASP65 target GFP to the Golgi, although mutation of Gly2, which prevents myristoylation, abolishes targeting.

GRASP65 itself contains two repeats that are distantly related to the PDZ domain that mediates a number of protein–protein interactions. The binding site for GM130 on GRASP65 consists of a region of the second repeat that

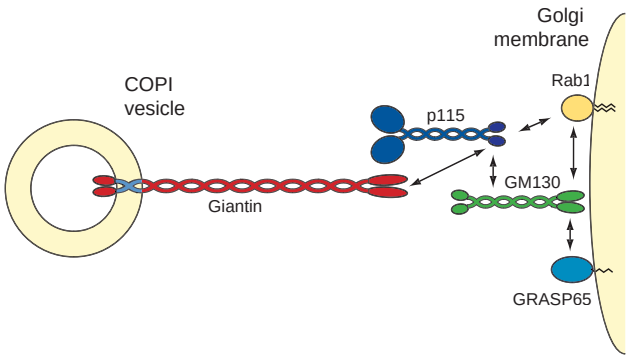

Fig. 4. The interactions between Golgi coiled-coil proteins that have been proposed to mediate the tethering of COPI vesicles to the early Golgi [39,56]. Giantin on COPI vesicles binds to GM130 on the Golgi membrane via p115. GM130 is itself localised to the cis-Golgi by interaction with GRASP65, which attaches to membrane via an N-terminal myristate anchor. Both p115 and GM130 have been shown to interact with the small GTPase Rab1. It should be noted that one study has reported that giantin and GM130 compete for the same binding site on p115 [42].

is highly conserved between different organisms (amino acids 194–201 in the rat sequence), and this region contains the sequence GYGY, which is very similar to the conserved binding site motif of the PDZ domain, GLGF [37,38].

3.1.3. Giantin

Giantin, also known as macrogolgin or GCP372, is the second p115-binding partner, p400 [39]. It was first identified using monoclonal antibodies raised against Golgi membranes, and cloning and sequencing showed it to be a very large (400 kDa) type II integral membrane protein with a C-terminal transmembrane domain (TMD) and the majority of its mass projecting into the cytoplasm [25,40]. Giantin is found both at the rims of the Golgi stacks and on COPI vesicles [39]. The binding site for p115 on giantin has been mapped to the N-terminal 448 amino acids [41].

3.1.4. Functional analysis of p115, giantin and GM130

So what are the functions of p115, GM130 and giantin? The presence of giantin on COPI vesicles, the length of its coiled-coil domain and the localisation of GM130 and p115 to membranes make them good candidates to act together to tether COPI vesicles on to the Golgi. Indeed it has been proposed that this type of long tether may enable vesicles budding at one cisterna to become loosely attached to an adjacent cisterna, preventing them from migrating away from the vicinity of the Golgi [39]. In support of this model are experiments which show that giantin on COPI vesicles can be cross-linked to p115, and that binding of p115 to COPI vesicles is inhibited by pre-incubation with anti-giantin antibodies. An in vitro vesicle docking assay shows that increasing the amount of added p115 increases the rate of COPI vesicle docking. Furthermore, pre-incubation of COPI vesicles with anti-giantin antibodies inhibits docking, but pre-incubation of Golgi membranes has no effect. In contrast, pre-incubation of Golgi membranes, but not vesicles, with anti-GM130 antibodies blocks docking [39]. Collectively this data suggests a model in which giantin on COPI vesicles is bound to GM130 on Golgi membranes with p115 acting as a linker (Fig. 4) [39].

Although the above experiments appear to show quite clearly the interactions between giantin, p115 and GM130, there is some debate. A study by Linstedt et al. [42] suggests that GM130 and giantin actually compete for the same binding site on p115. Furthermore, using antibodies against p115, GM130 and giantin and an in vitro transport assay, vesicle trafficking between the ER and the Golgi has been dissected to show that the three proteins act at temporally distinct stages; p115 antibody inhibition occurring at the level of VTCs, and GM130 and giantin antibody inhibition occurring at the Golgi [43], although this does not exclude p115 from also acting in later steps in the Golgi. Furthermore, analysis of the kinetics of inhibition suggests that GM130 is required prior to giantin, implying that they may not function simultaneously [43]. GM130 and giantin also localise to different regions of the Golgi, the majority of GM130 is found at the cis-face of the stack, while giantin is found predominantly at the Golgi rims. One possible explanation for this is that giantin plays a dual role in Golgi transport, playing both an essential role in a post-GM130 step, but an additional role in a GM130-dependent step, in which it is not essential. In this respect it is interesting to note that two other coiled-coil, integral membrane proteins with TMDs related to that of giantin have recently been identified (golgin-84 and CASP, see Section 3.3). These proteins may be able to substitute for giantin at the GM130-mediated step, although no direct evidence for this has been reported.

Since p115 is a peripheral membrane protein recruited to the Golgi by GM130, and since giantin on COPI vesicles apparently enters them passively as it is present at the same density as on Golgi membranes [39], the obvious question is what is different about giantin on cisternae from giantin on COPI vesicles, as only the latter appears to be a receptor for p115. It is conceivable that the association of additional proteins with stack-localised giantin, for example GCP60 (Section 3.1.7), enables the cell to differentiate between the two giantin populations.

In mammalian cells the Golgi apparatus undergoes fragmentation and reassembly during mitosis, and inhibition of the action of p115 in docking of COPI vesicles appears to play an important role in this process [44,45]. Reduction of p115 to below detectable levels by antibody induced proteasome-mediated degradation, leads to Golgi breakdown and an increase in vesicles similar to the changes observed during mitosis [46]. The inhibition of p115 action during mitosis appears to be mediated by modification of GM130 during the cell cycle. At the G2/M transition, GM130 is phosphorylated by cyclin-dependent kinase I [47]. This reduces the affinity of GM130 for p115, which results in a loss of p115 from Golgi membranes, and an accumulation of COPI vesicles in the cytoplasm. This, ultimately, results in

the disintegration of the Golgi apparatus, as vesicles continue to bud but cannot fuse [35,45]. This can be recapitulated in tissue culture cells, by microinjecting an N-terminal peptide of GM130 corresponding to the p115 binding site. This peptide blocks the association of p115 with Golgi membranes and leads to an accumulation of vesicles. Under these conditions, protein transport is reduced but not completely blocked, suggesting that alternative pathways may be utilised [48].

3.1.5. The association of p115 with SNAREs

Is the tethering process coupled to fusion? Analysis of the sequence of p115 has suggested the presence of a degenerate SNARE motif in coiled-coil-1 [49]. When this region was used to probe detergent solubilised Golgi extracts, interactions were identified with the SNARE proteins involved in intra-Golgi transport, i.e. syntaxin 5, GOS28, GHS15, Ykt6, rSec22 and membrin, as well as with the syntaxin 5-binding protein Sly1 [49]. In an in vitro assay to measure the formation of syntaxin 5-containing SNARE complexes, sub-stoichiometric amounts of p115 stimulated the formation of complexes. In addition, p115 could be removed from the resulting complexes without affecting their integrity. These observations suggest that p115 may catalyse the formation of specific SNARE bundles during docking and fusion of transport vesicles to the Golgi [49].

How could p115 act to stimulate SNARE assembly? It has been suggested that p115 reduces the activation energy for SNARE complex formation, or stabilises an assembly intermediate, perhaps playing a similar role to complexin, which binds in the groove of the syntaxin 1:SNAP23:VAMP SNARE bundle, thereby stabilising it [49,50]. p115 binds to the cytoplasmic domains of both syntaxin 5 and GS28, but does not bind to SNARE proteins that are involved in post-Golgi transport steps (e.g. syntaxin 1), indicating a degree of specificity in p115's interactions with SNAREs [49]. Nonetheless, it should be noted that the analysis of SNARE interactions is made particularly challenging by the fact that the α-helical domains in SNAREs that form the SNARE bundle are unfolded prior to assembly, which means they have a propensity to bind to any protein which can stabilise the formation of an α-helix [51,52].

3.1.6. The association of p115, GM130 and Rab proteins

In addition to its role in COPI vesicle transport within the Golgi, p115 is also a key factor in the tethering of ER-derived COPII vesicles to the Golgi. Indeed, inhibition of p115 binding to COPII vesicles prevents them from docking in an in vitro assay [53,54]. Along with tethering factors, members of the Rab family of small GTPases have also been implicated in the docking of vesicles with membranes [55]. Recently, Allan et al. [54] showed that p115 binds to Rab1-GTP, and that this is important for recruiting p115 into a cis-SNARE complex on COPII vesicles (Fig. 4).

In addition to p115, the GM130:GRASP65 complex is also a Rab1 effector, binding to Rab1-GTP in a p115 independent manner (Fig. 4). This binding is required for COPII vesicle docking or fusion at the cis-Golgi, presumably at a step downstream of Rab1-p115 tethering [56,57]. One interesting observation is that GM130 and GRASP65 remain on membranes in the presence of Rab-GDI, whereas most Rab effectors, including p115, are extracted from membranes under these conditions. Thus one role of Rab1 may be to co-ordinate budding on donor membranes, with docking and fusion at the acceptor compartment, by interaction with sequential tethers [56].

The *S. cerevisiae* homologue of Rab1 is Ypt1p, a protein localised to vesicles and Golgi membranes. Using an in vitro fusion assay, and a temperature sensitive allele of Ypt1p that can block ER to Golgi transport in vivo, Cao and Barlowe [58] dissected the role of Ypt1p on vesicles and on the Golgi. Vesicles bearing mutated Ypt1p were fusion-competent but acceptor membranes containing the same mutation were not, implying an asymmetry in the function of Ypt1p [58]. Furthermore, a TMD-anchored chimera of Ypt1p, restricted to the acceptor compartment, could support fusion of vesicles depleted of Ypt1p [58]. However, several things remain unclear. How is Uso1p (the *S. cerevisiae* homologue of p115, see below), or indeed any other tether, attached to vesicles if this is not mediated by Ypt1p? And how is the Ypt1p on vesicles distinguished from the Ypt1p on acceptor membranes? In mammalian cells, perhaps, the GDI-insensitivity of GM130 means that Rab1 (the Ypt1p homologue) has a longer residence time on acceptor compartments than on vesicles, but this does not explain the paradox in yeast. Presumably other proteins, either on vesicles or on the Golgi, play a role in specifying the identity of the two membranes.

3.1.7. Giantin interacting proteins

In addition to p115, other proteins have been identified which interact with giantin; one such protein is GCP60 [59,60]. GCP60 was isolated in a yeast two-hybrid screen using the cytoplasmic C-terminal region of giantin as bait [59,60]. It is a ubiquitously expressed peripheral membrane protein containing a domain related to acyl-CoA binding proteins, and a C-terminal GOLD domain, a feature of unknown function found in several otherwise unrelated proteins [60,61]. The exact role of GCP60 is unclear but it localises to Golgi rims via its C terminus, the region of the protein that also binds giantin. Overexpression of GCP60 results in Golgi disassembly and blocks transport of the viral membrane protein VSV-G at the point of ER exit. Homologues of GCP60 have been identified in *C. elegans* and *Drosophila* (but not in yeasts) which suggests an additional role for GCP60, since neither worms nor flies contain an obvious homologue of giantin [60].

3.1.8. Golgin-67

The golgin-67 gene was serendipitously isolated in the process of screening a human cDNA expression library with antibodies against SAM68, an otherwise unrelated protein

that is a target of mitotic Src [62]. Full-length golgin-67 has a predicted size of 51.4 kDa but the native protein runs as a 67-kDa species [62]. Antibodies raised against recombinant golgin-67 decorate the Golgi apparatus, and the C terminus of golgin-67 is essential for Golgi targeting [62]. This region was reported to encode a TMD, but the sequence does not appear particularly hydrophobic, and current TMD prediction programmes (e.g. TMHMM 2.0) do not suggest the existence of a TMD. There is, however, an unusual cysteine-rich sequence (CCVPCFC) very close to the C terminus which could act as a lipidated membrane anchor.

Golgin-67 is related to GM130 across much of its length [62,63] but does not contain the binding site for GRASP65 found at the C terminus of GM130. Database analysis reveals that golgin-67 is part of an extensive family of GM130-like genes in humans, many of which are also found in the EST database and are therefore not pseudogenes. Indeed, at least 10 copies of a short duplicated region, each containing a GM130-related protein, are found on chromosome 15 [64]. The exact function of these GM130-like proteins remains to be established, but it is interesting to note that duplication of this gene locus appears to be a recent event in evolution, since it is not present in mouse which only has GM130 itself.

3.2. S. cerevisiae Uso1p

Uso1p is the *S. cerevisiae* homologue of p115. It is an essential 1790-amino-acid (200-kDa) protein, which has been shown by electron microscopy to consist of a globular head domain and a long (approximately 150 nm) C-terminal α-helical coiled-coil with some hinge regions [65]. Uso1p and p115 share homology in their head domain but the coiled-coil region of Uso1p is at least twice the size of that of p115. Since yeasts do not appear to have an obvious homologue of GM130, and since the C-terminal region of Uso1p is, perhaps, related to GM130, it has been suggested that Uso1p represents a fusion of p115 and GM130 [35]. It will be interesting to determine whether the recently identified *S. cerevisiae* homologue of GRASP65, encoded by the nonessential gene *GRH1*, associates with Uso1p in a similar manner to that seen with GM130 and GRASP65 in mammalian cells [37].

ER–Golgi transport in yeast can be reconstituted in vitro by incubating salt-washed membranes with purified COPII, Uso1p, LMA1, ATP and GTP [53]. In this assay COPII vesicles that have budded from the ER only dock with Golgi membranes upon addition of Uso1p. Vesicle docking is sensitive to GDI (Sec19p in *S. cerevisiae*) which removes Ypt1p (yeast Rab1) from both vesicles and Golgi membranes. In addition, the degree of membrane-bound Uso1p correlates closely with the amount of membrane bound Ypt1p, suggesting that Ypt1p is important for Uso1p targeting [8].

Genetic evidence from yeast demonstrates that, as expected, SNARE proteins act downstream of both Uso1p and Ypt1p. Temperature-sensitive mutations in the yeast SNAREs Sed5p, Bet1p, Bos1p and the syntaxin-5 interacting protein Sly1p do not prevent Uso1p mediated tethering of vesicles, but block ER–Golgi transport by inhibiting fusion [66]. Interestingly, overexpression of the same SNAREs rescues deletion of Ypt1p and temperature-sensitive mutations in Uso1p [66]. Therefore, tethering is dependent on Uso1p and Ypt1p but overexpression of SNAREs can bypass this requirement. This latter observation is perhaps due to weak inefficient SNARE-mediated interactions with the vesicle which, when present in increased numbers, can overcome the need for a semi-docked ‘‘tethered’’ intermediate [8,53,66].

In addition to its putative role in vesicle tethering, Uso1p may also play a role in protein sorting during the formation of COPII vesicle on the ER [67]. Morsomme and Reizman [67] showed recently, using an assay which reconstitutes ER-derived vesicle budding in vitro, that sorting of GPI-anchored proteins from non-GPI-anchored proteins is defective in a temperature-sensitive mutant of *USO1* (*uso1-1*).

3.3. Integral membrane proteins related to giantin

Two integral membrane proteins have recently been identified which share sequence homology with giantin in their C-terminal TMDs, and which localise to the cis/medial Golgi. These proteins are known as golgin-84 [68] and CASP (CDP/cut alternatively spliced protein) [69]. Golgin-84 is an 84-kDa protein, which is predicted to form coiled-coils over the majority of its length, and is capable of forming dimers as shown by chemical cross-linking. Interestingly, an oncogenic chromosome translocation has been identified that results in the coiled-coil domain of golgin-84 becoming fused to the cytoplasmic portion of the receptor tyrosine kinase c-ret [68]. Presumably, dimerisation by the golgin-84 derived sequence leads to constitutive activation of the kinase domain.

Like golgin-84, CASP is a large coiled-coil protein, which shares homology with giantin in its C-terminal TMD. Indeed, all three of these proteins possess conserved histidine and tyrosine residues within this region [69]. In contrast to the other two proteins, CASP has a clear homologue in *S. cerevisiae*, known as Coy1p [69]. Indeed, all eukaryotes so far examined have at least one of these related proteins, although only mammals have all three, suggesting that they might all be involved in the same transport steps and are able to compensate for one another. The identification of Coy1p in yeast has allowed genetic analysis of the protein which has revealed a genetic interaction with the yeast SNARE proteins Gos1p (the *S. cerevisiae* homologue of GS28) and Sec22p. Mutation of the conserved histidine residue in the TMD of Coy1p does not affect the localisation of HA-tagged Coy1p but does disrupt the genetic interaction with Gos1p. Therefore the TMD of CASP (and by analogy those of giantin and golgin-84) may mediate cross-talk between events at the cytoplas-

mic surface, including vesicle capture, and processes occurring in the membrane.

3.4. Golgin-160

Golgin-160, also known as GCP170, was identified by screening a human cDNA library with an antiserum from a patient with the autoimmune disorder systemic lupus erythematosus, and was shown to reside on the cis-Golgi compartment [26,70]. Golgin-160 is predicted to form coiled-coils over two thirds of its length, with an N-terminal head domain (residues 172–257) that is responsible for targeting the protein to Golgi membranes. Golgin-160 does not have any clear homologues in *Drosophila* or *C. elegans*, and indeed the abundance of the murine homologue of golgin-160 (MEA-2) in testis may indicate a specialised function in this tissue. Homozygotes of a transgenic mouse line in which golgin-160/MEA-2 is disrupted are sterile due to an apoptotic degeneration of spermatocytes [71,72]. Since the Golgi apparatus is thought play an important role in acrosome formation, these results implicate golgin-160 in this process [71]. Interestingly, golgin-84 is also particularly abundant in testis [68], suggesting that a number of golgins may be required for acrosome formation.

3.5. GMAP-210

GMAP-210 was independently identified in a screen of an expression library with a human auto-antiserum, and by showing an apparent interaction with retinoblastoma protein in a yeast two-hybrid screen [73,74]. On the basis of the latter interaction, the protein was suggested to be a possible coactivator of thyroid hormone receptor and so named Trip230 [75], but subcellular localisation showed the protein to be associated with the cis-side of the Golgi apparatus [74]. Deletion analysis reveals that the C-terminal region of GMAP-210, a region suggested to bind thyroid hormone receptor, is responsible for Golgi targeting [75]. This region was also reported to interact with the minus end of microtubules [76], although the significance of this is unclear as the intracellular distribution of the protein does not resemble that of microtubule minus-ends. At present, the function of GMAP-210 on the Golgi is unknown, and so possible nuclear or microtubule binding roles cannot be formally excluded. Overexpression of GMAP-210 induces an enlargement of the Golgi apparatus and perturbations in the microtubule network [74]. In addition, anterograde transport of both a soluble form of alkaline phosphatase and the integral membrane protein hemagglutinin between the ER and the cis/medial Golgi stacks is inhibited, suggesting a role in Golgi function [77].

3.6. S. cerevisiae Rud3p

In addition to Uso1p and Coy1p, a further *S. cerevisiae* coiled-coil protein has been implicated in membrane traffic processes in the early Golgi. This is Rud3p, also known as Grp1p, which was independently identified in two genetic screens for proteins which, when overexpressed, could suppress the temperature-sensitive growth defects of the mutants *uso1-1* or *sec34-2* [78,79]. These are both conditional mutations in proteins proposed to be involved in vesicle tethering in the Golgi, and overexpression of Rud3p can also suppress several other mutations that inhibit membrane traffic in the early Golgi including *sec35-1*, *sec22-3* and *bos1-1*, strongly suggesting some role for the protein in these processes [78]. Rud3p is a nonessential, 484-amino-acid, peripheral membrane protein with a molecular mass of 56 kDa, and is predicted to form a coiled-coil, particularly in the central ~200-amino-acid region. The coiled-coil region of Rud3p has been suggested to be related to that of the mammalian Golgi protein, golgin-160, but is also related to many other coiled-coil proteins in this region [78]. At present no proteins have been identified which interact with Rud3p, and its precise role remains unclear.

4. Medial Golgi transport steps

As well as transport to and from the cis-Golgi, transport also occurs within the Golgi stack. At present it is still debated whether vesicles traffic in an anterograde manner between cisternae or whether cargo moves from the cis- to trans-side of the Golgi by a process of cisternal maturation [80,81]. What is clear is that retrograde transport within the Golgi exists, and that this is mediated by COPI vesicles. These vesicles are responsible for trafficking enzymes and Golgi components back to their resident cisternae, ensuring that they are not depleted at their site of action [82].

4.1. Golgin-45 and GRASP55

GRASP55, a homologue of GRASP65, is localised to the medial Golgi where it is important for the maintenance of the Golgi matrix and for Golgi re-stacking in vitro [83]. Like GRASP65, GRASP55 appears to be a receptor for a coiled-coil protein, in this case golgin-45, a protein with a central coiled-coil region that is conserved in flies and worms, but apparently not in yeasts. Golgin-45 was also identified in a screen for genes up-regulated by retinoic acid treatment, and termed JEM-1 [84]. Although JEM-1 was initially proposed to be a potential nuclear cofactor, golgin-45 shows a clear localisation to the Golgi apparatus. Depletion of golgin-45 from cells by RNA interference results in a marked redistribution of the medial Golgi enzyme GlcNac transferase I back to the ER, along with a disruption of early and medial Golgi morphology [85]. In addition, transport of the marker protein VSV-G is abrogated, and the protein remains in the ER. Like other golgins, golgin-45 binds specifically to a small GTPase protein, in this case Rab2, a GTPase implicated in early Golgi transport [85,86].

5. Transport between the trans-Golgi and endosomes

5.1. GRIP domain proteins

The GRIP domain is a sequence of approximately 50 residues, which is shared by a number of proteins, and has been named according to the first letter of several of these (golgin-97, *R*an-binding protein 2α, *I*mh1 and *p*230/golgin-245) [87–89]. This domain, predicted to form three short α-helices, is sufficient to target GFP to the trans-Golgi. It contains only one invariant residue, a tyrosine near the start of the domain, which is critical for Golgi targeting in both mammals and protists [90]. Mammalian GRIP domains have been shown to bind to Rab6 on proteins blots, and mutations which prevent Golgi targeting, also disrupt Rab6 binding [88]. However, Rab6 does not appear to be the only small GTPase capable of binding to the GRIP domain, and may not be the physiologically relevant interacting partner (see below).

5.1.1. Golgin-245

Golgin-245, also known as p230, was identified in two independent studies that probed cDNA libraries with sera from patients with the autoimmune disorder Sjogrens syndrome [91,92]. The protein has 2230 amino acids with a predicted molecular mass of 261 kDa, and Western blotting with the original human sera detects a protein of 245 kDa [91]. Immunogold labelling shows that golgin-245 specifically localises both to tubulovesicular structures and to TGN-derived non-clathrin-coated vesicles, which do not contain Rab6 [93,94]. Overexpression of the golgin-245 GRIP domain, which has the potential to displace endogenous golgin-245, does not affect the number of vesicles budding from the TGN. This implies that golgin-245 is not involved in the budding process but may be required for downstream events [93].

The localisation of golgin-245 to the trans-side of the Golgi complex can be stimulated both by activation of heteromeric G proteins using AlF_4^-, and by activation of small G-proteins with GTPγS [94]. This implies a role for GTPases in recruiting the GRIP domain to the Golgi. This might be consistent with a role for Rab6, but recently Van Valkenburgh et al. [95] reported that GRIP domain proteins were among those isolated in a yeast two-hybrid screen which used the activated form of the GTPase ARL1 as bait. These GRIP domain proteins were golgin-245 and RanBP2α, along with a small Golgi localised, coiled-coil protein called SCOCO. ARL1 is a member of a family of ARF-like GTPases which share about 50% identity with ARF GTPases, and of which there are at least 10 in humans. ARFs have a well-characterised role in vesicle coat recruitment [55], but in contrast little is known about the function of ARLs, although ARL1 has been found to be localized to the Golgi apparatus [95–97]. Mutation of the conserved tyrosine in the GRIP domain, responsible for Golgi targeting, disrupted the yeast two-hybrid interaction between golgin-245 and activated ARL1, indicating that the GRIP domain is important for this interaction [95]. Moreover, it has recently been shown in *S. cerevisiae* that Arl1p is required for the Golgi targeting of the one *S. cerevisiae* protein with a GRIP domain (Imh1p, see below), and that the domain and the GTPase bind directly in a GTP-dependent manner [98,99].

5.1.2. Golgin-97, GCC88 and GCC185

Golgin-97, like golgin-245, was also identified using serum from a patient with Sjogrens syndrome to screen a cDNA library [100]. Although little is known about its function, golgin-97 has been implicated in vesicle tethering based on its coiled-coil structure and the presence of its GRIP domain, which is sufficient to target GFP to the trans-side of the Golgi [87–89].

Database searching reveals two additional mammalian proteins with predicted coiled-coil sequence and a C-terminal GRIP domain, GCC88 and GCC185 (originally termed GCC1 and KIAA0336, respectively) [101]. These proteins are also localised to the TGN, and overexpression of GCC88 results in the formation of electron-dense extensions of this compartment, [101]. The human genome encodes a fifth GRIP domain protein, RanBP2α, but this appears to be the result of a recent gene duplication and rearrangement that has attached the C terminus of GCC185 to the nuclear transport protein RanBP2 and so its biological significance is unclear.

In addition to the mammalian proteins described above, many organisms have proteins with putative GRIP domains [87–89]. Indeed the *Drosophila* genome appears to encode four such proteins (Table 1), whereas one has been identified in each of yeast (Section 5.1.3), plants and protozoa [90]. This conservation across species suggests an important role for GRIP domain proteins in cellular function, and in the case of higher eukaryotes, the conservation of four distinct GRIP proteins suggests a possible diversity of roles.

5.1.3. S. cerevisiae Imh1p

Imh1p, also called Sys3p, is the only protein in the yeast *S. cerevisiae* that contains a discernible GRIP domain. It is a 105-kDa, nonessential protein predicted to form coiled-coils over most of its length [87–89,102]. It was independently identified by two different laboratories as a suppressor of the temperature sensitivity of a yeast strain lacking functional Ypt6p (the yeast homologue of Rab6) [102,103]. Deletion of both *IMH1* and *YPT6* leads to a severe growth defect or inviability of the strain at all temperatures tested, an accumulation of vesicles, and a defect in α-pheromone maturation [102,103]. The latter effect is probably due to the missorting of recycling Kex2p, a protease that is required for α-pheromone processing [102]. In the absence of Imh1p and Ypt6p, vesicles containing Kex2p returning to the Golgi can no longer dock with membranes, and the protein is therefore mistargeted to the vacuole where it is degraded. Indeed, levels of Kex2p in strains in which both YPT6 and

IMH1 are deleted are severely reduced. All this evidence indicates a role for both *Imh1p* and *Ypt6p* in vesicle tethering; however, as yet, no physical interaction between these proteins has been found [102]. It may be that the two proteins both contribute to the same process in a manner that allows one or the other to be lost but not both. Alternatively, it may be that the proteins act in distinct transport pathways that are sufficiently related to be redundant under laboratory growth conditions. As mentioned above, it has recently been found that the Golgi targeting of Imh1p is mediated by a direct association with the GTP-bound form of the GTPase Arl1p [98,99].

5.1.4. TMF/ARA160 and S. cerevisiae Sgm1p

Recent biochemical studies have shown that activated Ypt6p binds to the GARP/VFT complex, a putative vesicle tethering complex required for the retrieval of late Golgi proteins and also to another coiled-coil protein called Sgm1p [9,10]. Sgm1p is a nonessential protein which contains two substantial regions of predicted coiled-coil [9]. At present, the exact function of Sgm1p remains unknown, but the mammalian homologue TMF/ARA160 has recently been localised to the Golgi apparatus [104], despite being originally proposed to be a transcription factor [105,106].

6. Endosomal transport steps

6.1. EEA1

As in the Golgi, long coiled-coil proteins appear to play an important role in membrane fusion events in the endosomal system. The most well-characterised endosomal tethering protein is EEA1, which was identified as an autoantigen in subcutaneous systemic lupus erythematosus [27,107]. It is predicted to consist mostly of coiled-coil, and chemical cross-linking indicates that it forms homodimers. EEA1 has been localised to early endosomes (EEs), and by electron microscopy to filamentous material that extends about 50 nm into the cytoplasm, but it is not found on clathrin-coated vesicles (CCVs). EEA1 localisation has been further defined by the observation that it appears only on a subset of EEs in polarised epithelial cells, such as MDCK cells, where it appears to play a specific role in basolateral endocytic sorting pathways [108]. Even in nonpolarised cells such as fibroblasts, EEA1 labels a subpopulation of endosomes distinct from those targeted by a transfected apical marker protein, endotubin. This restricted localisation implies that EEA1 may be important for specifying the directionality of tethering and fusion [108].

Like some Golgi coiled-coil proteins, EEA1 interacts with a member of the Rab family of GTPases, in particular with the activated (GTP-bound) form of Rab5 which is localised to endosomes [109]. EEA1 appears to contain two spatially distinct Rab5 binding sites, one at either end of the protein [109]. Both appear to be functional, since a point mutation in the N-terminal domain is sufficient to abrogate Rab5 binding to this region, and the C-terminal Rab5 binding domain is 29% identical to the Rab5 binding domain in Rabaptin-5, another Rab5 effector protein [109,110]. However, Rab5 is not the sole determinant of EEA1 targeting to membranes, since mutation of the Rab5 binding sites does not affect the localisation of EEA1 [111]. Moreover, Rab5 is not only located to early endosomes, but is also found on the plasma membrane and on CCVs, while EEA1 localisation is restricted to early endosomes. This appears to be because the targeting of EEA1 is primarily dependent on a second determinant, the phosphoinositide PI(3)P [112]. Treatment of cells with the PI 3-kinase inhibitor, wortmannin, leads to dissociation of EEA1 from membranes [109]. The region of EEA1 responsible for binding PI(3)P is a specialised form of the zinc finger RING domain, known as the FYVE domain, a name derived from the first four proteins in which it was identified (*F*ab1, *Y*OTB/ZK632.12, *V*ac1 and *E*EA1) [113–115].

The C-terminal FYVE domain of EEA1 alone is not sufficient to target GFP to early endosomes. Instead, a stretch of about 130 residues, including the FYVE domain, the Rab5 binding site and a short stretch of coiled-coil sequence, is required [113]. This additional sequence is important for dimerisation of the C termini resulting in the association of two FYVE domains that mediate binding. Indeed, the affinity of a single FYVE domain for PI(3)P is of the order of 130 μM, while by contrast, the affinity for bivalent binding by the homodimer would be predicted to be the square of this value, i.e. about 17 nM. This figure is close to the experimentally observed value of 50 nM [116]. Thus bivalency of the FYVE domain serves to overcome both the low affinity and selectivity for PI(3)P exhibited by the monomeric domain. EEA1 is one of the few coiled-coil proteins to be crystallised, at least in part. Crystallisation of these proteins is inherently difficult due to the elongated nature of their structure, but it has proven possible with the C-terminal 123 residues of EEA that include the FYVE domain. The structure of this region bound to $I(1,3)P_2$ reveals an ordered parallel coiled-coil ending in a dyad symmetric FYVE domain homodimer [117].

So what is the evidence that EEA1 is involved in membrane tethering at the early endosome, and how is this regulated? First, anti-EEA1 antiserum inhibits an in vitro assay for early endosome fusion by 80% [109]. Likewise, depletion of EEA1 from cytosol used in the assay results in a decrease in endosome fusion that can be rescued by adding back recombinant EEA1. As well as homotypic fusion, EEA1 also tethers CCVs derived by endocytosis from the plasma membrane on to early endosomes [118]. Thus EEA1 appears to play a role in specifying the target organelle for both homotypic and heterotypic fusion. The specification of endosomal membranes by EEA1 is thus analogous to the specification of Golgi membranes by p115 for docking of both

COPI and COPII vesicles, as well Golgi reassembly following mitosis, i.e. homotypic Golgi fusion.

Since EEA1 is a long molecule with binding sites at both ends, one can easily imagine that it binds to endosomes via its C-terminal FYVE domain, and to Rab5 positive compartments via its N terminus, bringing them into close apposition for fusion. The putative coiled-coils, which make up the majority of EEA1, are interrupted by several proline–glycine motifs, which may introduce flexible kinks close to the N-terminal Rab5 binding site. One could therefore envisage a scenario where a conformational change within the flexible region of EEA1 brings the two membranes closer together. One puzzling observation is that free EEA1 homodimers in the cytoplasm do not seem to bind to CCVs. This may be because the clathrin coat blocks EEA1 binding to the vesicle. However, this is unlikely since in an in vitro assay, CCVs, which are uncoated by the actions of the ATPase Hsc70, still do not bind EEA1 [118]. Thus an alternative explanation is that the low affinity of the Rab5 interaction requires that EEA1 oligomerises on endosomal membranes before CCV binding can be stabilised through the engagement of multiple N-terminal Rab5 binding domains [118].

Recently, the PI 3-kinase hVPS34 has been shown to be a Rab5 effector. This may be one way of linking PI(3)P production with Rab5 localisation. Furthermore, hVPS34 is absent from CCVs, and thus may provide an insight into the asymmetric localisation of EEA1. Of course this raises the question of how hVPS34 is itself localised to EEs, and there is evidence that this is mediated by association with other factors such as Vps15p/p150 and beclin [119–121]. There is no clear homologue of EEA1 in *S. cerevisiae*. The FYVE domain protein Vac1p, a protein involved in endosome and vacuole fusion, was initially proposed as the yeast homologue; however, this protein appears more closely related to the mammalian protein Rabenosyn-5 [122].

6.1.1. EEA1 interaction with SNAREs

As with the early Golgi tethering protein p115, EEA1 has also been suggested to couple tethering with SNARE engagement. In an in vitro fusion assay, addition of EEA1 alone is sufficient to support a minimal level of homotypic endosome fusion [107]. One possibility is that EEA1 not only acts as a kinetic tether, but also stimulates the formation of SNARE complexes.

A yeast two-hybrid approach, using full-length EEA1 as bait and a number of SNAREs as prey, showed a specific interaction between EEA1 and syntaxin 6 [123]. This interaction was mediated by the C terminus of EEA1; however, neither the Rab5-binding site nor the FYVE domain alone was sufficient. GST-syntaxin 6 was shown to bind EEA1, both from cytosol, as well as recombinant protein, the latter result indicating a direct association [123]. Interestingly, Rab5-GTP and syntaxin 6 appear to compete for binding to EEA1, implying that their binding sites partially overlap. Thus EEA1 may mediate tethering via sequential interaction with Rab5 and syntaxin 6.

In addition to syntaxin 6, other SNARE proteins have been implicated in early endosome fusion. In particular, the soluble domain of syntaxin 13 has been shown to specifically inhibit this process in vitro [124]. Furthermore, a direct interaction between EEA1 and syntaxin 13 has been demonstrated using a biosensor assay [124]. Interestingly, although soluble syntaxin 6 had no effect on the in vitro endosome assay, both syntaxin 6 and syntaxin 13 are predicted to bind to approximately the same region of EEA1 [123,124].

EEA1 has been suggested to exist on endosomal membranes in a large oligomeric complex which contains the Rab5 effectors Rabaptin-5, Rabex-5, as well as NSF, the ATPase activity of which modulates assembly of the complex [124]. How might this complex mediate or stimulate fusion? One suggestion is that the formation of higher order EEA1/NSF oligomers is analogous to the large oligomers formed by viral glycoproteins, which mediate the formation of a viral fusion pore [124], thus linking vesicle tethering with fusion. Another possibility is that EEA1 simply provides a large platform for other proteins involved in fusion to assemble. This may then allow local activation of SNARE complexes containing syntaxin 13 and possibly syntaxin 6.

6.2. Rabaptin-5

Rabaptin-5 is a large coiled-coil protein, which was identified as a Rab5 effector using a yeast two-hybrid screen [125]. It has two long regions predicted to form coiled-coils separated by a flexible linker. Rabaptin-5 is recruited to early endosomes from the cytosol in a Rab5-dependent manner, and overexpression stimulates endosome fusion in vivo [125,126]. Interestingly, Rabaptin-5 is not functional by itself in an vitro endosome–endosome fusion assay and in fact inhibits the assay [127]. This is because, in vivo, it is found in a complex with another protein, known as Rabex-5. Rabex-5 is a guanine nucleotide exchange factor for Rab5, and has been proposed to link recruitment of Rabaptin-5 with the subsequent activation of Rab5 [127,128]. Indeed, overexpression of Rabex-5 can bypass the requirement for Rabaptin-5. Thus, Rabaptin-5 and Rabex-5 perhaps create a region of activated Rab5 on early endosomes, which may act as a target for incoming vesicles or as a binding domain for other proteins involved in vesicle docking.

Rabaptin-5 has a C-terminal Rab5 binding domain and a distinct N-terminal Rab4 binding domain [129]. The finding that Rabaptin-5 binds Rab4 is intriguing since Rab4 is important for recycling from early endosomes to the cell surface, while Rab5 is involved in endocytosis, suggesting that Rabaptin-5 could be involved in co-ordinating the actions of both these pathways at endosomes [129].

6.3. Rabip4

Rabip4 is a 600-amino-acid protein, which was identified as an effector of Rab4 [130]. In addition to two regions of coiled-coil, it contains an N-terminal RUN domain, through which it associates with endosomes, and a C-terminal FYVE domain [130,131]. As discussed earlier, FYVE domains bind PI(3)P [115], while RUN domains are less well defined but are found in various proteins whose functions are linked to those of the Ras families of GTPases [132].

Co-expression of Rabip4 and activated Rab4 leads to the expansion of early endosomes [130] and an increased co-localisation of markers for sorting and recycling endosomes [130]. Furthermore, the protein GLUT1, which normally recycles through the plasma membrane, now accumulates within the endosomal system [130]. This suggests that Rabip4 may promote retrieval from recycling to sorting endosomes; thus, overexpression causes components to be trapped in a recycling loop between the two compartments, and hence they become mixed.

7. Coiled-coil proteins and apoptosis

Cells undergoing apoptosis undergo a series of characteristic changes including DNA cleavage, nuclear breakdown, blebbing, and fragmentation of organelles [133]. These events ultimately lead to the disintegration of cells to form apoptotic bodies, which are engulfed by neighbouring cells. As discussed earlier (Section 2), a number of coiled-coil proteins thought to act as tethering proteins were originally identified as autoantigens in systemic autoimmune diseases [25,26]. A number of these Golgi and endosomal autoantigens appear to be specific targets for the proteolytic actions of caspases during apoptosis. The exact purpose of these events remains to be established, but its is interesting to speculate that cleavage of proteins responsible for vesicle transport/docking would be an ideal way to disassemble organelles in a controlled manner. This mechanism may be similar to that used during mitosis, where phosphorylation of GM130 results in loss of p115 binding to membranes which in turn inhibits fusion of COPI vesicles with the Golgi [35,44]. However, there is at least a mechanistic difference in that GM130 remains unphosphorylated during apoptosis [134].

7.1. Golgins

Both giantin and golgin-160 (Sections 3.1.3 and 3.4) are susceptible to caspase cleavage during apoptosis [135,136]. Treatment of Jurkat cells with staurosporine to induce apoptosis generates three specific giantin fragments and two golgin-160 derived fragments. In contrast, golgin-97 and GM130 appear relatively resistant to cleavage under these conditions. These results indicate that either a subset of golgins are targets for caspases or that there are differences in the kinetics of golgin degradation, and that giantin and golgin-160 are early targets for caspases during apoptosis [135]. Golgin-160 is acted upon by caspase-2, -3 and -7. The first cleavage is mediated by caspase-2 and this proteolytic event appears to be critical since blocking it by mutation of the cleavage site, prevents the subsequent actions of caspase-3 and -7, and delays Golgi fragmentation [136]. Recently, a nuclear localisation signal (NLS) has been identified in the N-terminal head domain of golgin-160 [137]. During apoptosis, cleavage of golgin-160 results in the release of fragments that are specifically imported into the nucleus [137]. The function of these fragments remains to be established, although it has been speculated that they could regulate transcription during apoptosis. p115 is also cleaved by caspases during apoptosis, and like golgin-160, the resulting C-terminal 30-kDa fragment specifically translocates into the nucleus [134]. Significantly, expression of this C-terminal fragment also induces apoptosis and leads to Golgi fragmentation, suggesting a role for p115 in the propagation of the apoptotic signal [134]. Rosen and Casciola-Rosen [138] have proposed that modifications of auto-antigens during apoptosis may be crucial for the generation of auto-antibodies in autoimmune disorders. The alteration of coiled-coil proteins by cleavage during apoptosis, along with their extended and repetitious structure, may perhaps explain why so many have been identified as antigens recognised by the sera of patients with autoimmune diseases.

7.2. GRASP65

As well as the long coiled-coil proteins, it appears that their membrane receptors can also be targets for caspases during apoptosis. Recently, GRASP65 (Section 3.1.2) was shown to be a specific target for caspase-3, being cleaved at a conserved site close to the C terminus at an early stage in apoptotic cell death [139]. Interestingly, expression of a caspase-resistant mutant of GRASP65 partially delayed the loss of integrity of the Golgi ribbon in apoptotic cells, consistent with GRASP65 normally playing a role in the maintenance of Golgi structure [139].

7.3. Rabaptin-5

Endosomal coiled-coil proteins are also targets for caspases during apoptosis. As discussed above (Section 6.2), Rabaptin-5 consists of two large coiled-coil domains linked by a non-coiled-coil region. Although this linker region is not particularly well conserved, it contains two potential caspase cleavage sites which are targets for caspase-3 [140,141]. Cleavage of Rabaptin-5 during apoptosis has been shown to prevent vesicles fusing with endosomes, ultimately leading to fragmentation of endosomes and the inhibition of the endocytic pathway [140]. This is intriguing since cleavage by caspase-3 generates two coiled-coil proteins, the C-terminal of which is still

capable of binding Rabex-5 [141], implying that binding Rab5 and its effector protein Rabex-5 may not be the only function of Rabaptin-5 in endosomal docking or fusion. These results also suggest that Golgi and endosomal membranes are fragmented during apoptosis by similar mechanisms.

8. Summary

Some common themes are emerging from studies of the long coiled-coil proteins found in the secretory pathway, but much remains to be resolved. Many of these proteins are recruited to organelles by members of the Rab and Arf families of GTPases, but if they are acting to tether vesicles it is not yet clear how the specific binding to vesicles is mediated. Furthermore, in the cases of p115 and EEA1, the proteins have been suggested to participate in SNARE complex assembly, implying that vesicle tethering could be directly linked to downstream events. The use of large proteins, which can act not only as initial vesicle tethers but also as scaffolds for the assembly of other proteins required for fusion, would seem a logical way of integrating the processes of docking and fusion. Furthermore, one can imagine that captured vesicles could be passed sequentially from longer to shorter tethers bringing them closer to the membrane surface for fusion. As well as their role in tethering cognate vesicles, the fibrous nature of the coiled-coil proteins could also allow them to potentially act as a mesh that opposes those vesicles not appropriate for fusion with a given compartment. Tethers could also play a role in the stabilisation of organelles, as is the case with GM130 and GRASP65, which have been proposed to be components of a Golgi matrix.

The roles discussed above for the large coiled-coil proteins are not dissimilar to those suggested for the multi subunit complexes, such as the exocyst, the COG complex and TRAPP, that have also been proposed to play a role in vesicle tethering [16]. Both sets of proteins can be recruited to membranes by small GTPases, and both have been suggested to interact with SNAREs. One obvious difference is that the coiled-coil proteins have the potential to form interactions over larger distances and thus, in the case of vesicle tethering, could provide a kinetic enhancement to membrane fusion by trapping vesicles in the vicinity of other factors that make a thermodynamic contribution to fusion. Another difference is that the coiled-coil proteins do not generally form stable interactions with other proteins, unlike the components that make up the multisubunit complexes, but rather they appear to rely upon labile or low affinity interactions. This may indicate that the coiled-coil proteins are more suited to forming transient reversible interactions to mediate the initial stages of vesicle tethering or to allow organelles to have highly defined and yet dynamic structures. What seems certain, however, is that further investigation of the role of coiled-coil proteins in membrane traffic will reveal much that is interesting and surprising.

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