Protein complexes in transport vesicle targeting

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The transport of material between membrane-bounded organelles in eukaryotic cells requires the accurate delivery of different classes of carrier vesicles to specific target compartments. Recent studies indicate that different targeting reactions involve distinct protein complexes that act to mark the target organelle for incoming vesicles. This review focuses on the proteins and protein complexes that have been implicated in various targeting reactions.

The specific targeting of different classes of transport vesicles to their distinct membrane destinations is essential to maintain the unique composition of the various eukaryotic organelles. One molecular mechanism for the vesicle targeting reaction has focused on two families of integral membrane proteins (reviewed in Ref. 1). Members of one family are related to the synaptic vesicle protein synaptobrevin/VAMP, and are known as vesicle-SNAREs (v-SNAREs), and members of the other family are related to the neuronal plasma membrane protein syntaxin, and are known as target SNAREs (t-SNAREs). The interaction of v-SNAREs with their cognate t-SNAREs is important for membrane docking and fusion[1–3]. However, several lines of evidence indicate that this interaction cannot be sufficient to confer the necessary targeting specificity. First, the same v-SNARE can reside on both the anterograde- and the retrograde-directed vesicles at any given stage of membrane traffic (reviewed in Refs 4 and 5). Second, the interactions of the SNAREs are promiscuous. In several cases, a single v-SNARE has been shown to interact with several t-SNAREs found on different target membranes in the same cell and a single t-SNARE has been shown to interact with multiple v-SNAREs[4,5]. In addition, specific disruption of the SNAREs in the squid giant synapse[6] and in *Drosophila*[7] does not block vesicle docking. Finally, in exocytosis in neurons and yeast, the t-SNAREs are distributed uniformly over the surface even though vesicle fusion is restricted to limited subdomains of the plasma membrane[8,9]. Although the SNAREs are essential for the fusion of two compartments, the interaction of SNARE pairs is clearly not sufficient to explain the observed specificity of vesicle targeting.

Recent evidence indicates that the interaction of two membrane compartments is a multistage process. The first stage, following membrane recognition, has been termed tethering as the initial interaction is a loose, possibly reversible, connection that leaves the two membranes at some distance from one another. The subsequent interaction of SNAREs on opposing membranes is a more intimate interaction, sometimes termed docking, that leads to membrane fusion. Although targeting specificity would be the product of both tethering and SNARE pairing as tethering is the first step in the process and there is little evidence of mistethered membrane compartments, most of the specificity is probably conferred by the initial recognition and tethering reaction.

Rab GTP-binding proteins regulate different stages of exocytic or endocytic traffic (reviewed in Refs 5 and 10) and are implicated in directing vesicles to their appropriate target compartments. Although rabs might play an important role in the recognition reaction, evidence indicates that they cannot act alone. The rab protein Ypt1p from the yeast *Saccharomyces cerevisiae* appears to function in several early stages of transport along the export pathway[11,12]. Furthermore, a chimera of Ypt1p and Sec4p, a rab protein that acts at the final stage of the yeast exocytic pathway, can fulfil the essential functions of either protein without missorting cargo[13,14]. Nonetheless, rabs are key regulators of membrane traffic and might be involved in recruiting tethering and docking factors.

Numerous proteins and protein complexes have been identified that might play key roles in directing different classes of vesicles to their appropriate target membranes. The sequences of these proteins are often conserved among diverse eukaryotic species, indicating that their functions developed early in eukaryotic evolution. However, in contrast to the rabs and SNAREs, components that function at different stages of transport generally appear to share no detectable sequence similarity, suggesting that their functions are unique for each target compartment. Biochemical and cell-biological evidence suggests that they play key roles in conferring targeting specificity. The various targeting components that act at different stages of membrane traffic are reviewed in the following sections (see Fig. 1).

TRAPP and the Sec34p–Sec35p complex

TRAPP (transport protein particle) is a multiprotein complex of ~1094 kDa that functions in membrane traffic between the endoplasmic reticulum (ER) and the Golgi. The first component of this complex to be identified, *BET3*, was found in a synthetic lethal screen with *bet1-1*, a temperature-sensitive yeast mutant in the SNARE Bet1p. *BET3* encodes a hydrophilic protein of ~22 kDa that functions in the late ER-to-Golgi stage of transport[15,16]. Precipitation of a tagged form of Bet3p from yeast revealed that this complex contains ten subunits, one of which is encoded by *BET5*. The *BET5* gene had been identified as a high-copy suppressor of the *bet3-1* temperature-sensitive mutation[17]. Purification

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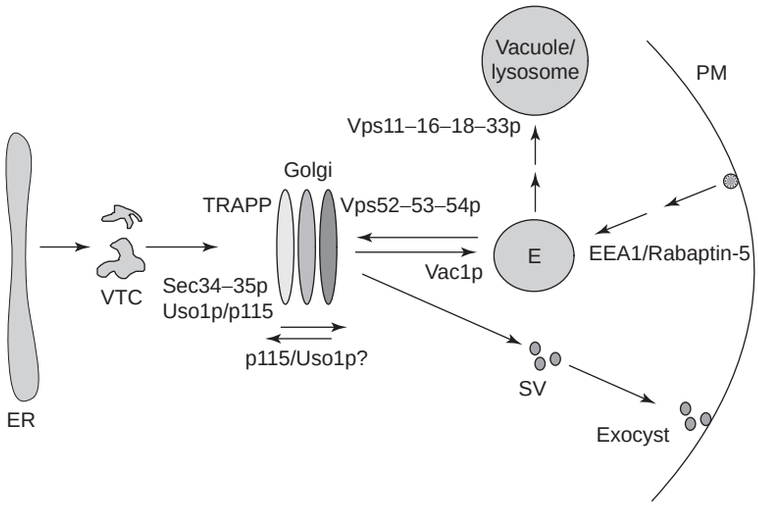

FIGURE 1

Putative localization of the proteins and protein complexes that function in various stages of membrane traffic. TRAPP, Uso1p/p115 and the Sec34–35p complex are implicated in ER-to-Golgi targeting and tethering; p115, and probably Uso1p, play tethering roles in intra-Golgi and other transport stages; the exocyst complex targets post-Golgi secretory vesicles to the plasma membrane; EEA1 and Rabaptin-5 are involved in both homotypic endosome fusion and fusion of early endosomes with endocytic vesicles; Vac1p is implicated in docking of vesicles to endosomes; the Vps52/53/54p complex functions in docking vesicles that recycle from the prevacuolar compartment to the late Golgi; and finally, the Vps11–16–18–33p complex is implicated in docking various vesicle types to the vacuole. Not all pathways are shown here. ER, endoplasmic reticulum; VTC, vesicular tubular cluster; SV, secretory vesicles; E, endosomes of different kinds; PM, plasma membrane.

of the remaining TRAPP subunit (Trs) proteins and sequence analysis demonstrated that they are all products of either uncharacterized open reading frames or gene products of unknown function[16,18]. Seven of the 10 subunits are essential for the vegetative growth of yeast cells. Orthologues of most of the TRAPP subunits have been identified in mammalian species, including humans, underscoring the conserved role that these proteins play in membrane traffic. In addition, a high-molecular-mass complex containing these orthologues has been detected in HeLa extracts[18]. Although the Trs proteins in yeast are present exclusively in an assembled complex, in HeLa cells only a small fraction of the total pool is assembled.

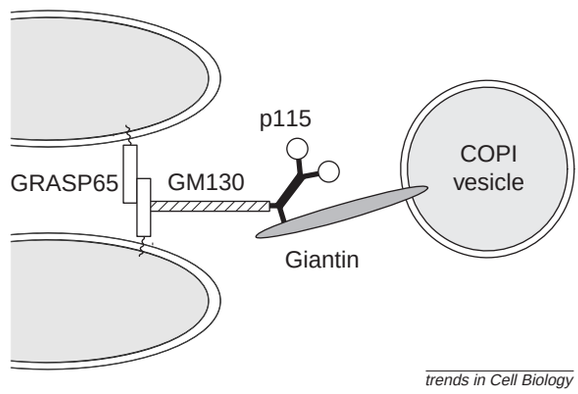

FIGURE 2

Protein interactions involving GRASP65, GM130, p115 and giantin mediate the tethering of COPI vesicles to the Golgi membrane.

Bet3p and several other TRAPP subunits have been localized to early Golgi membranes in yeast[19]. In both yeast and HeLa extracts, the Trs proteins are found in a Triton X-100-insoluble fraction, suggesting that these complexes might be bound to a detergent-resistant Golgi matrix[18]. In an *in vitro* assay, depletion of Bet3p from cytosol and membranes blocked the targeting and/or fusion of ER-derived vesicles with the Golgi, but not vesicle formation[16]. A refinement of this assay indicated that the block was at the level of vesicle targeting rather than fusion[19].

When vesicle budding from the ER is blocked in a *sec12* yeast mutant, proteins that cycle continuously between the ER and the Golgi, such as Sed5p and Emp47p, redistribute to the ER[20]. In *sec12* and mutants that block the tethering and fusion of ER-derived transport vesicles to the Golgi, Bet3p remains tightly bound to Golgi membranes in the absence of anterograde traffic and does not recycle to the ER[19]. Taken together, the localization and biochemical analysis of Bet3p indicates that TRAPP plays a crucial role in docking transport vesicles to the Golgi and might mark this membrane for incoming vesicle traffic.

Two other factors acting in ER-to-Golgi transport in yeast are Uso1p[21,22] and the Sec34p–Sec35p complex[23–25]. Uso1p is a soluble protein that forms homodimers that possess two N-terminal globular heads and an elongated coiled-coil tail[26,27]. Uso1p acts prior to the SNAREs and is required for tethering ER-derived vesicles to Golgi membranes[28]. Extraction of membrane-bound rab proteins by guanine nucleotide dissociation inhibitor (GDI) removes membrane-bound Uso1p and inhibits tethering[28], suggesting that Ypt1p mediates the attachment of Uso1p. Another complex acting in ER-to-Golgi transport in yeast contains the products of two nonessential genes, *SEC34* and *SEC35*, and several unidentified polypeptides. In contrast to TRAPP, this complex is largely soluble[23]. *In vitro* assays indicate that Sec34p and Sec35p also function in vesicle tethering[24,25]. A deletion of either *USO1*, *SEC35* or *SEC34* can be bypassed by a dominant allele of *SLY1* or the overexpression of *YPT1*[21,24,25], suggesting that these tethering factors act upstream of the small GTPase Ypt1p and the t-SNARE-associated protein Sly1p. By contrast, deletion of *BET5* (whose product is a component of TRAPP) cannot be bypassed by any gene tested to date[19]. Although TRAPP, Uso1p and the Sec34p–Sec35p complex are required in the same vesicle-docking event, their interrelationship is unknown.

p115

p115 was first identified as a peripheral membrane component required for intra-Golgi transport in mammalian cells[29]. Subsequent findings suggest that p115 functions as a vesicle-tethering factor at several stages of traffic. First, p115 is identical to transcytosis-associated protein (TAP), a factor required for the

binding of transcytotic vesicles to the plasma membrane[30]. p115 has also been localized to peripheral vesicular tubular clusters (VTCs) and to the *cis*-Golgi membrane and is implicated in docking/fusing VTCs with the *cis*-Golgi[31]. p115/TAP is a homologue of Uso1p[26,30].

A role for p115 in the binding of COPI-coated vesicles to mammalian Golgi membranes has been described (Fig. 2)[32]. Studies into the role of p115 in intra-Golgi vesicle docking/tethering originated with the examination of the binding characteristics of p115 to mitotic and interphase Golgi membranes. During mitosis, Golgi membranes fragment into vesicles and tubules, a process that might be due to an inhibition of vesicle docking and fusion[33]. p115 exhibited reduced binding to mitotic Golgi membranes when compared with interphase Golgi membranes[34]. p115 binds to GM130, a component of the Golgi matrix that itself is bound to the Golgi through a tight association with GRASP65[35,36]. p115 also binds to giantin on COPI vesicles, thus serving as the bridge between giantin on the vesicles and GM130/GRASP65 on the Golgi[32]. The binding of p115 to Golgi membranes under interphase conditions appears to be regulated by the phosphorylation of p115, which results in a shift of p115 from membrane fractions to the cytosol[37] and consequently might play a role in regulating the assembly or function of this tethering complex. During mitosis, phosphorylation of GM130 by Cdc2 kinase inhibits the binding of p115 to GM130[35,38], but not to giantin on the vesicles[32], thereby preventing the docking and fusion of COPI-coated transport vesicles. p115 also plays a role in the tethering of Golgi cisternae prior to the action of GRASP65, a component required for Golgi stacking in a cell-free assay, which reconstitutes the reformation of Golgi cisternae from postmitotic fragments[36,39].

Exocyst

The exocyst complex is thought to be responsible for targeting secretory vesicles to the appropriate exocytic sites on the plasma membrane (Fig. 3). The exocyst complex contains one copy each of Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p and Exo84p[40–43]. In yeast, loss of function of any of these components results in a block in secretion and the accumulation of secretory vesicles. Unlike the t-SNAREs, components of the exocyst are concentrated in subdomains of the plasma membrane that represent sites of active exocytosis in both yeast and mammalian cells[42–47]. The localization of Sec3p to these sites in yeast is independent of both ongoing secretion and the function of the other components of the complex, suggesting that Sec3p represents a spatial landmark for exocytosis and that it might be the component of the exocyst complex most proximal to the target membrane[47]. Another component, Sec15p, associates with the secretory vesicle and interacts with the Rab GTPase Sec4p in its activated, GTP-bound form. This crucial interaction might trigger further interactions between Sec15p and the other components of the exocyst, leading to the productive association of secretory vesicles with specific

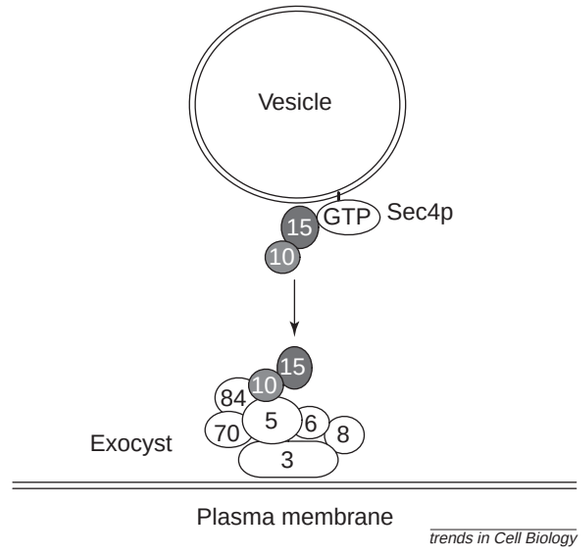

FIGURE 3

Activated Sec4p interacts with Sec15p and controls the assembly of the exocyst complex upon vesicle docking. Sec3p is a landmark for vesicle docking at specific domains of the plasma membrane. The assembly of the exocyst complex is involved in targeting secretory vesicles to specific domains of the plasma membrane.

sites on the plasma membrane marked by Sec3p[44]. The exocyst complex might assemble during the process of vesicle docking[44]. This is suggested by the observation that other components of the complex require a functional secretory pathway for proper localization to exocytic sites in yeast[43,47].

This multiprotein complex could have multivalent interactions with other proteins, such as components of the cell-cycle machinery[48], cell polarity proteins[49], the actin cytoskeleton and the SNAREs. The assembly of the exocyst complex might integrate various sources of cellular information to ensure that exocytosis occurs at the right time and place.

EEA1 and the Rabaptin-5–Rabex-5 complex

EEA1 (an early endosome-associated protein) and Rabaptin-5[50,51] are mammalian proteins that interact with the GTP-bound form of Rab5, the GTPase involved in both homotypic endosome fusion and the fusion of early endosomes with endocytic vesicles (Fig. 4). EEA1 binds to phosphatidylinositol 3-phosphate in the endosome membrane through its 'FYVE' domain and tethers endosomal membranes independent of *trans*-SNARE pairing[52,53]. Rab5 enhances the tethering function of EEA1, and high levels of EEA1 can bypass the requirement of Rab5. Interestingly, phosphotidylinositol 3-kinases are found to be Rab5 effectors[54]. Although Rab5 does not stimulate the activity of these kinases, it might confine their activities to microdomains of the endosome, thus coupling the local production of phosphoinositides to EEA1 function. Rabaptin-5 binds to Rab5–GTP and also complexes with Rabex-5, a GDP–GTP exchange factor (GEF) for Rab5, and is essential for endocytic membrane fusion[55]. This

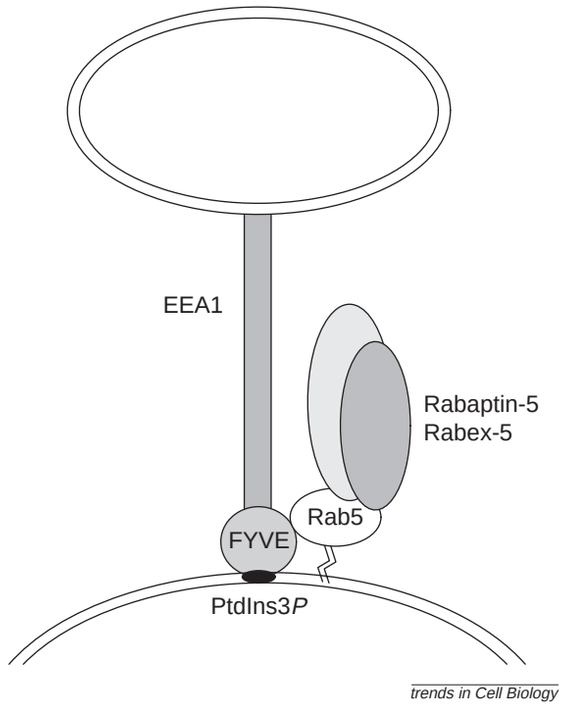

FIGURE 4

Proteins involved in endosome–endosome and endocytic vesicle–early endosome tethering. The Rabaptin-5–Rabex-5 complex activates Rab5, which recruits EEA1. EEA1, through its 'FYVE' domain, binds PtdIns3*P* produced in microdomains of the membrane. Activated Rab5 might also recruit phosphatidylinositol 3-kinases for local production of PtdIns3*P*.

complex could help to keep Rab5 in its active form and cluster active Rab5 to a defined region of the membrane. The Rabaptin-5–Rabex-5 complex works coordinately with the EEA1 complex for membrane docking and fusion. EEA1, upon recruitment to the endosome membrane, assembles into macromolecular complexes with Rabaptin-5, Rabex-5 and NSF, and this EEA1 complex interacts transiently with the local endosome t-SNARE syntaxin 13, which might lead to SNARE assembly and membrane fusion[56]. These findings provide a path of molecular interactions leading from the activated Rab protein to assembly of the SNARE complex.

Vac1p, an EEA1 homologue, is required for Golgi-to-endosome transport in budding yeast[57,58]. Vac1p binds to the GTP-bound form of the Rab protein Vps21p, and interacts with phosphatidylinositol 3-phosphate; it also binds to Vps45p, a member of the Sec1p family of proteins. These interactions suggest that Vac1p plays a targeting role in Golgi-to-endosome transport.

Other multiprotein complexes involved in docking

Three new vacuolar protein sorting (*VPS*) gene products of yeast, Vps52p, Vps53p and Vps54p, were shown recently to form a stable, 300-kDa complex[59]. This multimeric complex localizes to the late Golgi, but not the prevacuolar (PVC/endosomal) compartment. In *vps52*, *vps53* and *vps54* mutants, the carboxypeptidase Y receptor (Vps10p), that normally cycles between the late Golgi and the PVC/endosome, is missorted to the vacuole, consistent with a defect in retrieval of these proteins from a post-Golgi compartment. Proteins that cycle continuously between the late Golgi and the PVC/endosome can be trapped in an aberrant structure, called the class E compartment, in several *vps* mutants, such as *vps27*. However, the stable localization of Vps52p, Vps53p and Vps54p to the late Golgi in *vps27* cells indicates that this complex does not cycle between the Golgi and the PVC/endosome. These data imply that this novel complex plays a role in docking vesicles that recycle from the PVC to the late Golgi.

The class C *VPS* gene products of yeast, Vps11p, Vps16p, Vps18p and Vps33p, were shown to be components of a hetero-oligomeric complex[60]. However, the existence of this complex is revealed only after chemical crosslinking, indicating that the interaction of these subunits is of a transient nature. The accumulation of vesicles, autophagosomes and vacuolar precursor proteins in a *vps18*^{tsf} mutant suggests that this complex might play a role in docking various vesicle types to the vacuole.

Concluding remarks

Numerous proteins have been implicated in vesicle targeting. Unlike the rabs and SNAREs, these evolutionarily conserved proteins share no significant sequence similarity. Perhaps it is the diversity of these proteins that confers the specificity for vesicle targeting at different stages of traffic. Many of these proteins interact physically or genetically with the rab protein that acts at the same transport step. The rab proteins, although not the physical docking/targeting machinery, clearly play an important role in regulating the targeting reactions.

In principle, formal proof that any given component confers targeting specificity will require an experiment in which the component in question is moved to another compartment and targeting specificity is shown to be reprogrammed. To date, no component implicated in targeting has been tested. Whether such formal proof will ever be obtained depends to a large extent on the degree to which targeting specificity resides in a single component. If many components work in concert to control specificity, a clear response from such an experiment might not be possible.

Vesicle targeting might be regulated by additional factors, and the complexity of the docking machinery might provide cells with multiple levels of regulatory control. In some cases these proteins could communicate with the cell-cycle machinery, the cytoskeleton and other proteins in the cell, integrating various sources of cellular information to ensure that vesicles are targeted and docked at the right time and place. Identification of the proteins that interact with the targeting machinery should help to clarify the regulation of membrane traffic. Future studies are needed to further dissect and reconstitute all of the docking reactions.

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