# 1 Introduction

1.1 Vesicular transport

Living cells are separated from their surroundings by lipid bilayer membranes which fence off the interior of the cell, the cytosol, from the outside world. In addition, the cells of all higher organisms contain specialized compartments, the organelles, which are also separated from the cytosol by lipid membranes. These organelles include mitochondria, where energy is produced, the endoplasmic reticulum (ER), where proteins are synthesized, the Golgi network involved in protein maturation, endosomes that contain extracellular substances taken up by endocytosis, and lysosomes, which can degrade proteins. Because of their chemical properties membranes constitute a barrier for the exchange of substances. This allows the maintenance of distinct environments for different processes carried out in these areas. Controlled transport across biological membranes is mediated by membrane proteins that can act as specific transporters or regulated channels. In addition, the exchange of proteins and other molecules between different compartments of the cell and the exterior can be achieved by vesicular transport: Small membrane vesicles bud at a donor membrane and load cargo into their interior. The vesicles travel through the cell to their destination compartment where the vesicle is first tethered to the target membrane and then fuses, thus delivering its cargo. Different pathways are established along which substances can be transported in vesicles through the cell.

The secretory pathway represents one example. It is required for the transport and maturation of extracellular proteins. These proteins carry a signal sequence (a helix of 10-15 amino acids length with mainly hydrophobic and few basic residues). During translation of mRNA the nascent peptide chain and the ribosome are bound by the signal recognition particle (SRP) and *via* the SRP receptor to the ER. After cleavage of the signal peptide, the protein is synthesized into the ER lumen through the SRP pore or, if it is a transmembrane protein, diffuses from the pore into the ER membrane. From the ER, proteins are transported in COP II (coat protein complex) coated vesicles to the Golgi network. Here proteins may be further processed, e.g. cleaved by proteases, glycosylated or otherwise post-translationally modified. The next vesicular transport step carries the mature protein to the plasma membrane, where it is either secreted into the intercellular space or integrates into the cell membrane. Alternatively, further signal sequences can guide proteins from the Golgi to e.g. lysosomes (or the yeast equivalent, the vacuole) or back to the ER. For the biogenesis of lysosomes, proteins are modified with mannose 6-phosphate. Receptors (mannose 6-phosphate receptors, MPR) at the *trans*-Golgi membrane bind the marked proteins and concentrate them to budding vesicles designated to

pre-lysosomal compartments. After vesicle fusion and acidification of the endosome, lysosomal proteins are released from the receptor, which then is recycled to the Golgi for a further round of sorting. Retrograde transport to the ER with COP I coated vesicles helps to avoid a depletion of membranes at the starting compartment of the pathway. However, the coexistence of anterograde and retrograde transport as well as the coordination at crossroads like the Golgi exit requires a specifically regulated control of the different trafficking steps.

1.1.1 Modular organization of vesicular transport

The different trafficking steps in eukaryotic cells are all organized based on the same underlying principle, but differ in their components [5, 10]. The whole process of transporting a vesicle with a specific cargo from a donor compartment to an acceptor compartment can be considered a functional module. The different steps that are required to fulfill this complex task are carried out by submodular entities, which work together in a coordinated concert. These include the sorting of cargo, budding and scission of the vesicle from the donor membrane, uncoating, and tethering, docking and membrane fusion at the acceptor compartment (Figure 1.1a).
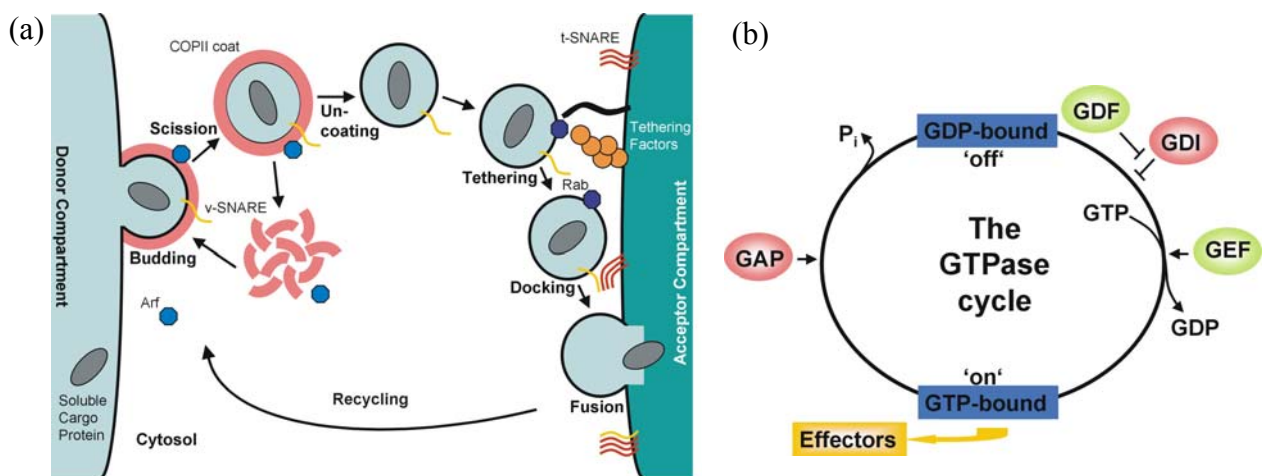
Figure 1.1: Modular organization of vesicle transport. (**a**)The modul can be dissected into submodular activities that carry out sorting of cargo, budding, scission, uncoating, tethering, docking and membrane fusion. (**b**) The GTPase cycle of the Arf and Rab GTPases is a submodular entity that regulates vesicle budding and membrane fusion, respectively (modified from [5]).

A coordination of these processes is achieved through regulation by GTP-binding proteins (GTPases, G-proteins) of the Ras superfamily. For the formation of transport vesicles, GTPases of the Arf (ADP ribosylating factor) family are required [11], membrane fusion is regulated by Rab (Ras-related in brain) GTPases [12]. The GTPases cycle between a GDP-bound 'off' state and a GTP-bound 'on' state, in which they interact with their effectors (Figure 1.1b). The actual GTP hydrolysis activity of the GTPases is low, thus GTPase activ-

ating proteins (GAPs) are required for dephosphorylation of GTP, resulting in inactivation of the GTPase. The GDP state is stabilized by GDP dissociation inhibitors (GDIs) to keep the GTPases switched off. GDI displacement factors (GDFs) need to remove the GDI, and subsequently guanine nucleotide exchange factors (GEFs) can reactivate the GTPase by the replacement of GDP by GTP.

As an example, the different submodular activities required for transport of ER-derived vesicles to the Golgi as they have been described in yeast will be discussed in the following. At the ER exit sites, activation the Arf GTPase Sar1p (small Arf-like) is required for cargo sorting and vesicle budding [13, 14]. Upon GTP binding, a conformational change is induced so that Sar1 is bound to the ER membrane *via* its N-terminal hydrophobic helix. GTP·Sar1p forms a pre-budding complex with a heterodimeric Sec23/24p coat subcomplex. Crystallographic studies revealed a slight curvature of that complex, which might be involved in bending of the membrane [15]. The subunit Sec24p contains a binding site for the DxE motif, which is the sorting signal for ER export. Thus, cargo is concentrated in the budding vesicle. The scission of COP II vesicles is achieved by binding of a Sec13/31p complex to the Sec23/24p-Sar1p complex. Sec13/31p form the outer shell of the coat and mediate the polymerization of COP II, allowing self-assembly of spherical vesicles. Uncoating of the COP II vesicles occurs when GTP of Sar1p is hydrolyzed. Sec23p has GAP activity for Sar1p, and binding of Sec13/31p additionally promotes GTP hydrolysis. Thus, the coat proteins trigger their own disassembly.

The docking and fusion of ER-derived vesicles is coordinated by the Rab GTPase Ypt1p [10, 16]. Rab proteins contain a variable carboxyl terminus with a cysteine motif where they are modified with one or two hydrophobic geranylgeranyl groups [12]. This region is supposed to mediate specific association of Rabs with different membrane compartments. Rab escort proteins (REPs) and GDIs are able to bind and thus solubilize geranylgeranylated Rab in the GDP form. Displacement of the stabilizing proteins leads to membrane binding of Ypt1p *via* the lipid modifications. Tethering is the early event of vesicle recruitment and is marked by the interaction of tethering factors with Ypt1p. The multi-subunit complex TRAPP I (transport protein particle) is a nucleotide exchange factor for Ypt1p [17, 18], and the long coiled-coil protein Uso1p functions as a Rab effector. After initial contact between incoming vesicle and target membranes has been established, SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) complex assembly can occur. SNAREs are characterized by the SNARE motif, which contains 60-70 amino acids with heptad repeats and allows the formation of coiled coils from four-helical bundles [19]. SNARE proteins are

distributed to the vesicle (v-SNARE) and target (t-SNARE) membrane, whereby at least one SNARE protein has to be anchored to each membrane. Functionally, SNAREs can be divided into Q- and R-SNAREs: The arginine from the R-SNARE Sec22p or Ykt6p interacts with one glutamine from each of three Q-SNAREs (Sed5p, Bos1p, Bet1p). Structural studies showed that these residues form specific hydrogen bonds in the center of the coiled-coil that otherwise consists of hydrophobic residues [20]. The tight SNARE complex brings vesicle and target membranes into close proximity, and the energy set free upon coiled-coil formation is thought to be used to overcome the thermodynamic barrier of membrane fusion, so that a fusion pore can arise. SNARE complexes are very stable and have to be disassembled by the ATPase NSF (N-ethylmaleimide-sensitive factor) and the cofactor SNAP (soluble N-ethylmaleimide-sensitive factor attachment protein) so that SNAREs can be reused for another cycle of membrane fusion.

1.2 Tethering Factors

The term tethering factor stands for a heterogeneous group of proteins that all are required prior to vesicle docking and SNARE-mediated membrane fusion [7]. The concept of vesicle tethering arose from numerous observations that SNARE proteins alone cannot account for the specificity and efficiency of vesicle fusion. Two groups of tethering factors can be distinguished, the long coiled-coil proteins and multi-subunit tethering complexes.

The long coiled-coil tethers form stable homodimers and are associated with the membrane directly or *via* anchor proteins [21]. One of the best characterized representatives is Uso1p and its mammalian homologue, the Golgin p115. The architecture of p115 comprises a C-terminal acidic region, the central coiled-coil core and a large globular N-terminal domain. The central region mediates homodimerisation, and p115 binds to the active GTP-bound form of Rab1 [22]. Rab1 is the membrane receptor for p115, and this interaction is thought to tether COP II vesicles to each other and thus to promote homotypic vesicle fusion [23]. The Rab binding site has been mapped to the coiled-coil region of p115 [24].With its C-terminal region p115 can bind to GM130 and giantin, two further Golgi coiled-coil tethers, which enhances Rab1 binding and establishes a link to the *cis*-Golgi network. Interestingly, p115 also interacts with the v-SNARE GOS28 and the t-SNARE syntaxin-5, representing a joint between tethering and fusion machinery [25].

The coiled-coil tether EEA1 is associated to early endosomes and implicated in fusion with other endososmes or endocytic vesicles. Structural data are available of a C-terminal fragment of the EEA1 homodimer showing a C-terminal dyad-symmetric FYVE domain that binds

phosphoinositol-3-phosphate and supports membrane association on the endosomal vesicle [26]. The crystal structure also shows part of the parallel coiled-coil domain, which is expected to extend ~160-180 nm as a rigid ensemble into the cytoplasm. At the N-terminus, a Rab5 binding domain can establish the contact to vesicles that have Rab5 bound to their surface. Thus, different binding domains at each end of the coiled-coil domain tether the two different vesicles to each other.

Six different mult-subunit tethering complexes have been identified with roles in different trafficking steps (Figure 1.2) [7]: The exocyst is located at the plasma membrane at sites of active secretion and functions in endocytosis. In yeast, the HOPS (homotyptic fusion and vacuole protein sorting) complex is required for proper sorting of proteins to the vacuole, and its mammalian homolog has been found to be located at late endosomes and lysosomes. The recycling of proteins from the endosome to the late Golgi is attributed to the GARP (Golgi-associated retrograde protein) complex. For the retrograde transport through the Golgi, COG (conserved oligomeric Golgi) has been shown to be required by interacting with SNARE proteins and COP I vesicles. TRAPP (transport protein particle) is present in two isoforms in yeast, the seven-subunit TRAPP I complex involved in the recruitment of ER-derived vesicles to the *cis*-Golgi, TRAPP II contains three additional subunits and is located at the *trans*-Golgi, where it is required for retrograde transport from endosomes. Finally, the Dsl1p complex has been implicated in Golgi-to-ER transport.
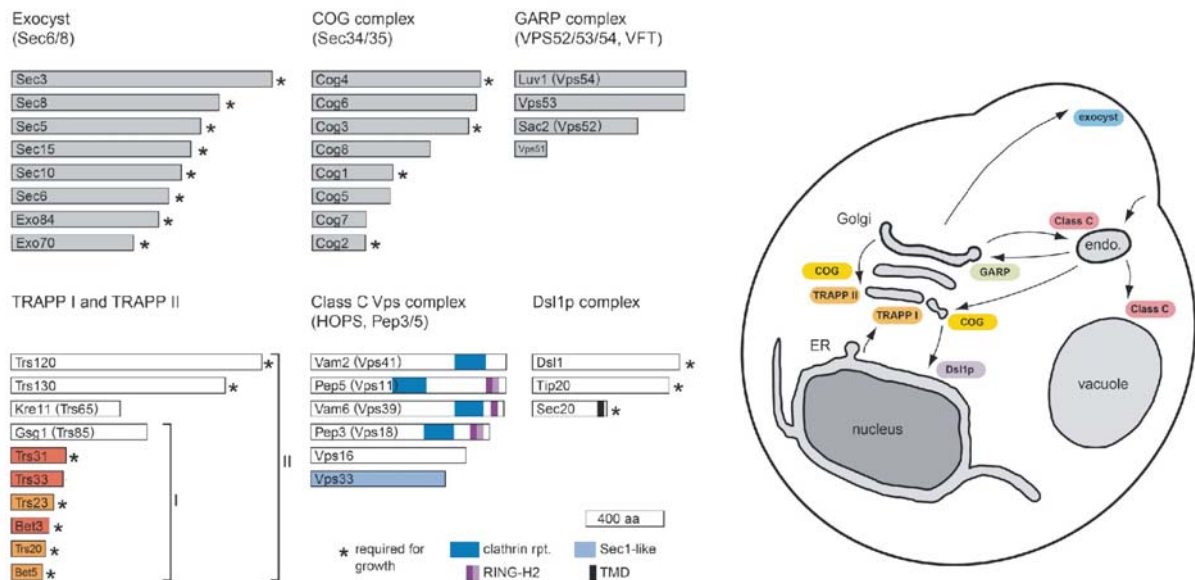
Figure 1.2: Subunit composition of six tethering complexes and their place of action in yeast. Proteins essential for growth are marked by an asterisk, and identified domains are marked. Two protein families within TRAPP with three members each are colored red and orange (from [7]).

The biochemical and structural characterization of multi-subunit tethering complexes has made rapid progress and provides valuable information for understanding the tethering process. However, these studies reveal that the different tethering complexes seem to be quite diverse in their functions, the underlying molecular mechanisms as well as their structure.

1.2.1 The exocyst

The exocyst tethering complex is composed of eight subunits which are conserved from yeast to humans (Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p and Exo84p) [27]. The complex is required before SNARE-mediated membrane fusion of secretory vesicles to the plasma membrane at polarized sites of endocytosis. In animal epithelial cells the exocyst participates in transport to lateral, but not apical membranes. In addition, mammalian exocyst was shown to be involved in neurite branching, membrane recycling and exit from the *trans*-Golgi network.

Two subunits, Sec3p and in part Exo70p, are located at regions of active fusion and thus might be markers for these sites. The remaining subunits arrive on the vesicles at the plasma membrane. Several subunits interact with Ras-related GTPases which regulate exocyst function. Consistent with their different localization, Sec15p interacts with the Rab GTPase Sec4p on the vesicle, whereas Sec3p and Exo70p bind to Rho GTPases (Rho1p, and Cdc42p and Rho3p, respectively) at the plasma membrane. In animals further interactions with the GTPases Arf6 (Sec10) and RalA (Exo84 and Sec5) have been detected. Thus, signals from a variety of pathways might possibly be integrated by the exocyst.

The crystal structures of four exocyst subunits and subunit domains revealed that, surprisingly, these domains adopt the same novel protein fold [28]. Two right-handed antiparallel/parallel helical bundles are packed in an end-on arrangement (Figure 1.3a). All helices on either side of the tandem bundle face the same direction. Despite sequence identity between the different domains being less than 10%, the unique topology of the fold strongly argues for a divergent evolution from a common ancestor protein. The crystallized construct of Exo70p [29], lacking only 62 N-terminal residues, contains two such domains forming an elongated rod. Of Sec15 [30] and Exo84 [29], only C-terminal parts could be crystallized, but they closely resemble the N-terminus of Exo70p. The same is true for the C-terminus of Sec6p, but here three additional helices are present, forming an extra helix-bundle [28].

The rod-like shape seems to be the underlying motif of exocyst subunit structures, however, they differ in the arrangement of the helix-bundles in respect to each other and the distribution of charged and hydrophobic patches over the protein surface. Interestingly, mapped interfaces

for the interaction with other exocyst subunits as well as for the GTPase Rab11 are lined along the bundle side. This suggests a side-to-side association within the exocyst complex. Cryo-EM pictures indeed showed such a elongated rod-shape of purified exocyst preparations, supporting the notions from the crystal structures [27].
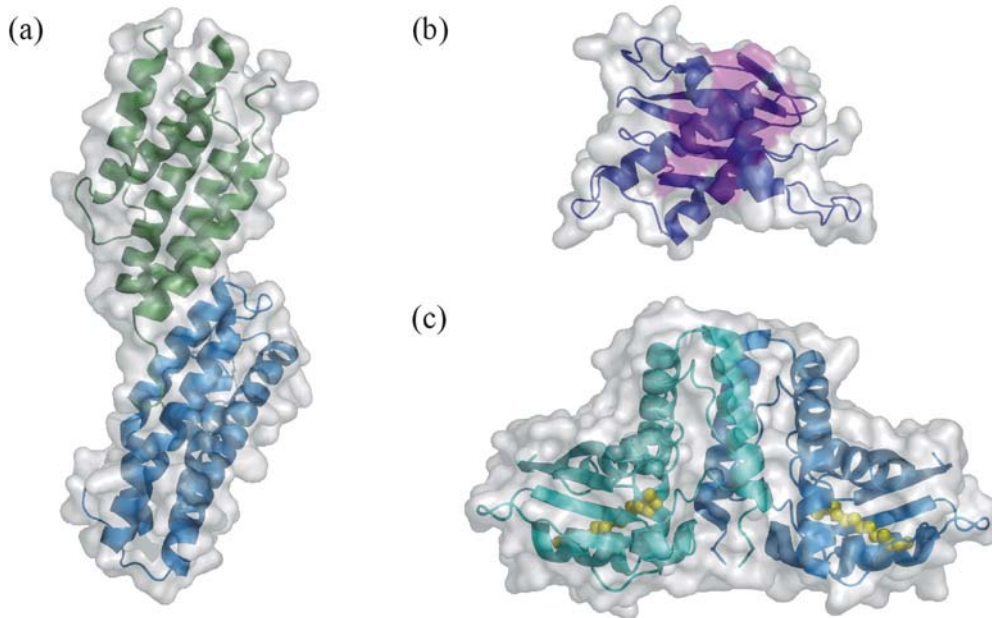
Figure 1.3: Crystal structures of tethering complex subunits. (**a**) The Sec15 C-terminal domain forms two helical bundles related in an end-on fashion. (**b**) Sedl belongs to the longin protein superfamily. Hydrophobic solvent exposed residues are labeld purple on a semi-transparent surface. (**c**) Bet3 adopts a novel protein fold, forms a dimer and is modified with a palmitoyl group (shown as yellow spheres) that is buried in the interior of the protein.
PDB entry codes: 2A2F, 1H3Q, 1SZ7.

1.2.2 The transport protein particle

The TRAPP (transport protein particle) complexes are tethering complexes that play an important role for the recruitment of vesicles to the Golgi network. TRAPP I binds ER-derived vesicles to the *cis*-Golgi whereas TRAPP II is involved in trafficking from endosomes to the *trans*-Golgi.

The first TRAPP subunit *BET3* was discovered in a synthetic lethality screen with *BET1* and was shown to genetically interact with the SNARE proteins *BOS1*, *SEC22* and the Rab GTPase *YPT1* [31]. A *BET3* mutant (*bet3-1*) did block ER-to-Golgi transport but had no effect on Bos1p/Sec22p, pointing towards a role before SNARE action. With a high-copy suppressor screen for *bet3-1* a second TRAPP subunit, *BET5*, was identified [32]. Bet3p and Bet5p physically interacted and were ultimately shown to be part of a large protein complex [33]. The purification of the yeast TRAPP complex [34] led to the identification of a total of ten subunits [33, 35]: Bet3p, Bet5p, Trs20p, Trs23p, Trs31p, Trs33p, Trs65p, Trs85p,

Trs120p and Trs130p. Using database searches [33, 35, 36] and TAP (tandem-affinity purification) with human Bet3 from cell culture [37], mammalian counterparts of the yeast TRAPP subunits could be assigned (Table 1.1). Whereas no orthologs are found for Trs85p and Trs65p, two isoforms of Trs33p have been identified in mammals.

yeast TRAPP	human TRAPP subunits	identical aa [%]
Bet5p	Bet5, **Mum2** [b,d]	30
Trs20p	Trs20, **Sedl** [a,d]	40
Trs23p	Trs23, PTD009, **synbindin** [a,d]	30
Bet3p	**Bet3** [a,d]	54
Trs31p	Trs31, **Tpc5** [c,d]	27
Trs33p	Trs33A, **Tpc6A**, R32611_2 [a,d,e] Trs33B, **Tpc6B** [e,f]	24 33
Trs85p	-	
Trs65p	-	
Trs120p	**NIBP** [f]	7.5 (20 over 338 aa)
Trs130p	**Ehoc1**, GTT334 [d]	5 (20 over 325 aa)

Table 1.1: Human orthologs of the yeast TRAPP subunits identified with databank searches and purification of human TRAPP ([a][33]; [b][32]; [c][35]; [d][37]; [e][36]; [f]this study). The names of the mammalian proteins used in the following are printed bold.

It is interesting to note that distant sequence similarity among some of the TRAPP subunits defines two protein families within the complex (Figure 1.2) [33, 36]. One family includes Bet5p, Trs20p and Trs23p. Bet3p together with Trs31p and Trs33p constitutes a second family, identified by two sequence motifs in these proteins, $LX_2\#GX_2\#GX_2LXE$ and $G\#_2XGXL$ (X represents any amino acid, # a hydrophobic residue; [35]).

The characterization of yeast TRAPP showed that the complex localizes to the Golgi network [35, 38]. Only a small fraction of TRAPP protein is detected in the cytosol, and membrane anchored TRAPP is resistant to extraction with Triton X-100, indicating tight membrane association [35]. Bet3p resides on the Golgi and does not cycle between ER and Golgi, but ER-derived vesicles fail to bind to the Golgi in the absence of Bet3p. Considering that Bet3 action takes place prior to SNARE-meditated membrane fusion, TRAPP can be classified as tethering complex of the Golgi network.

In total yeast cell lysates, two forms of the TRAPP complex have been separated with gel-filtration chromatography [39]. The smaller TRAPP I complex is composed of seven subunits (Bet3p, Bet5p, Trs20p, Trs23p, Trs31p, Trs33p, Trs85p), whereas TRAPP II contains three additional subunits (Trs65p, Trs120p and Trs130p). Interestingly the TRAPP II specific

subunits can not suppress the growth defect of *bet3-1*, but all TRAPP I subunits could, indicating a functional difference between the protein subsets. TRAPP I, but not TRAPP II, was shown to be required for tethering of ER-derived vesicles to the Golgi and to bind COP II vesicles [39]. Thus, the additional subunits mask the binding site on TRAPP for a yet un-identified ligand on the vesicle. TRAPP I co-fractionates with early Golgi compartments, but Trs120p localizes to late Golgi/early endosome membranes. Questions remain about the role of TRAPP II. Yeast *trs130* mutants block general secretion [39] pointing at a function in traffic within the Golgi, but *trs120* mutants do not. However, mutants of both genes show defects in the recycling from the early endosome to the late Golgi [40]. In addition, subunits of the COP I coat are mislocalized. The mechanism of TRAPP function at the late Golgi/early endosome remains to be clarified.

A different pictures emerges for the role of TRAPP in mammalian cells. Here only one TRAPP complex according to its size could be identified [36], and Bet3 was found mostly in the cytosol, with only a small fraction (~15%) bound to membranes. Although mammalian like yeast TRAPP was shown to act after COP II and prior to Rab1 and the SNARE machinery [36], Bet3 is mostly localized to the transitional ER (tER), where COP II vesicles bud, and to some extend to endosomes [41]. Inhibiting Bet3 still allowed the budding of COP II vesicles but interfered with formation of vesicular tubular clusters (VTC, a mammalian pre-Golgi compartment) by homotypic fusion of COP II vesicles. This ultimately disrupts the structure of the VTCs and the Golgi network.

A molecular function assigned to the TRAPP complexes in yeast is a role as guanine nucleotide exchange factor for the Rab GTPase Ypt1p and Ypt31/32p [17, 18]. TRAPP I promotes the nucleotide exchange of Ypt1p, but this activity is lost upon binding of Trs120p/Trs130p. TRAPP II now acts as a GEF for Ypt31/32p, and Trs130p was shown to bind to Ypt31p, but only in the nucleotide free form [42]. Thus TRAPP represents a tunable GEF, where TRAPP I and TRAPP II might represent two separate pools, or early-Golgi localized TRAPP I might be converted to TRAPP II during Golgi cisternal maturation [43, 44] when Trs120p and Trs130p join the complex.

Besides their role in vesicle transport through the Golgi (as part of the TRAPP complexes), several subunits have been identified to be involved in a variety of cellular functions. Spondyloepiphyseal Dysplasia Tarda (SEDT) is an X-linked genetic disorder caused by mutations in the *SEDL* gene [45]. Patients have short trunk, short stature, but no systemic complication. The mild phenotype can be explained by the expression of a *SEDL* pseudogene that can compensate for the loss of Sedl. In addition, Sedl was found to interact with the c-

myc promotor-binding protein 1 (MPB-1) [46] and cloride intercellular channels [47]. Mum2 was originally described to be point mutated in a melanoma cell line, and this mutation is recognized by autologous cytolytic T cells leading to strong antitumor response [48]. Murine synbindin was found to be involved in the function of dendritic spine synapses and to bind to syndecans [49]. Trs85p has been reported to be required in the formation of autophagosomes [50, 51]. The human orthologs of Trs120p (NIBP, [52]) and Trs130p (Ehoc-1, [53]) share significant similarity only over a limited part of their sequence. The non-conserved regions might explain why NIBP has been implicated in cytokine-induced signaling and Ehoc-1 was assigned a function as membrane channel. In a mouse model, Tpc6A was shown to be required for biogenesis of melanocytes [54]. Whether these diverse functions can be attributed to the TRAPP complex or represent specialized functions of the individual subunits remains to be seen.

At the beginning of this PhD project, structural information from two mammalian TRAPP subunits was available. The mouse Sedl protein (homolog Trs20p, [55]) is a monomeric (α+β) protein adopting a longin fold and thus shares, among others, structural similarity with the N-terminal regulatory domain of the SNAREs Ykt6p and Sec22p (Figure 1.3b). A large number of hydrophobic surface residues, forming two prominent hydrophobic pockets, suggests the involvement in multiple protein-protein interactions that, considering the similar fold, might include binding to SNARE proteins. The structure also revealed that the mutations causing SEDT will all lead to misfolded and unfunctional protein, which is expected to reduce the functionality of TRAPP and thus vesicle transport.

Furthermore, the crystal structures of Bet3 from mouse [56] and man [57] had been determined (Figure 1.3c). Bet3 was shown to form a dimer in the crystal and in solution and carries an unusual modification. The protein is acylated with palmitate at a conserved cysteine residue and the fatty acid moiety is buried in a hydrophobic tunnel in the interior of the protein.

Palmitoylation, in contrast to other hydrophobic modifications like myristoylation or isoprenylation, is a dynamic event with cycles of acylation and deacylation [58, 59]. The acyl-chain transfer (either enzyme mediated or chemical) occurs through nucleophilic attack of a cysteine in the substrate protein on the carbonyl of the C-S bond in the Palmitoyl-Coenzym A (Pal-CoA) molecule [60]. Previous work suggested that protein palmitoylation is involved in vesicular transport. Pal-CoA, the lipid donor in the palmitoylation reaction, stimulates the Golgi transport assay, supporting budding as well as fusion of vesicles [61]. This effect might be due to palmitoylation of a protein, which is required for activation or functionality of that

protein in the transport reaction. Most palmitoylated proteins and peptides can be autoacylated *in vitro*. This often requires unphysiologically high pH or occurs with slow rates, so that these events are unlikely to happen *in vivo*, suggesting a requirement for acyltransferases. Recently, the family of DHHC-CRD proteins (polytopic membrane proteins with the sequence DHHC and a cysteine-rich domain) has been identified as enzyme class that catalyses palmitoylation of a variety of substrate proteins in yeast and mammals [59, 62]. However, in a proteomics study Bet3 was the only protein in yeast that did not require any DHHC-CRD protein for its palmitoylation [63].

It was demonstrated that Bet3 is membrane-anchored, however, acylation appeared dispensable for this process [57]. The Bet3 dimer forms a flat, positively charged surface that might interact with the negative headgroups of phospholipids and could represent a membrane interaction interface. Indeed, a Bet3 K13E,K84E mutant, where two conserved positive charges are replaced by glutamate, failed to bind to the membrane. A channel blocking mutant, that has a closed hydrophobic tunnel due to the substitution of an alanine to leucine, still bound to membranes [56]. However, this protein was no longer recruited specifically to the Golgi, but is found on all membrane compartments. Based on these findings, the current model of membrane association of Bet3 and thus the entire TRAPP complex postulates a two-step mechanism. Initial membrane association is mediated by a pattern of positively charged residues on the flat surface of Bet3, followed by the selective anchoring through the insertion of the acyl chain from an Golgi-specific moiety into the hydrophobic tunnel of Bet3.

1.3 Objectives

The TRAPP complexes were shown to be essential for the proper trafficking from the ER to the Golgi in both yeast and mammalian cells and are expected to function in the tethering process prior to membrane fusion. TRAPP possesses GEF activity for GTPases of the Rab class, which coordinate membrane fusion at the Golgi membrane. However, little is known about the function of TRAPP on a molecular level.

The goal of my work was to elucidate the molecular mechanisms underlying the tethering event mediated by TRAPP. Therefore the purification, crystallization and structural characterization of individual TRAPP subunits was pursued. In addition the identification of TRAPP subcomplexes and their preparation for structure analysis was attempted. An further focus was on the functional characterization of the palmitoylation of Bet3. The unusual way this post-translational modification occurs defines a unique feature of this TRAPP subunit and is expected to have important implications for its function.