5 Discussion

5.1 Structural studies of the TRAPP tethering complex

The structure determination of the TRAPP subunit Tpc6B and the Bet3:Tpc6B subcomplex has provided valuable information on the architecture and assembly of the tethering complex. It was the determination of the crystal structure of Tpc6B that actually led to the identification and characterization of its heterodimeric complex with Bet3.

Comparison of the structures of Tpc6B and Bet3 revealed that both proteins dimerize in a similar manner, and biochemical studies clearly showed that they are able to form hetero-dimers (4.1). Therefore, a model for a putative TRAPP subcomplex was suggested, where Tpc6B and Bet3 form heterodimers, utilizing their closely similar dimer interfaces (Figure 4.7c). This was the first model of a TRAPP subcomplex that might function as a starting point for complex assembly at the Golgi membrane. The heterocomplex would provide specific binding pockets for other TRAPP subunits, which subsequently may lead to the formation of the complete TRAPP complex.
In the following the crystallization of the Bet3:Tpc6B heterodimeric complex of both proteins that had been postulated based on the homodimer structures of the two subunits could be accomplished. The structure of the Bet3:Tpc6B complex indeed closely resembles the model proposed before. The only prominent structural rearrangement is seen in the α1-α2 loop of Tpc6B which leads to the formation of a groove in the heterocomplex that might represent an interaction interface for a binding partner. This binding pocket lies opposite to the flat surface of Bet3:Tpc6B, which is thought to associate with the Golgi membrane. Mutants of the Bet3:Tpc6B complex were generated where residues forming this groove were replaced. These mutations showed only limited effects on the binding of Mum2, a further TRAPP subunit, which was reported to bind to Bet3:Tpc6B. However, binding of Mum2 to the heterocomplex probably represents the next step in TRAPP complex formation. The crystallization of a Bet3:Tpc6B:Mum2 complex was not successful, but synbindin could be identified as an interacting protein for the trimeric complex. This led to the reconstitution of a tetrameric TRAPP subcomplex that was crystallizable.

Considering the high structural similarity between Tpc6B and Bet3 it was presumed that the α/β-plait fold might represent the common fold for all paralogous Bet3 family members. The sequence alignment and circular dichroism spectroscopy of Bet3, Tpc5 and Tpc6B (4.2.2),

taken together, strongly supported the idea that Tpc5 may adopt a similar fold to that of Tpc6B and Bet3. Conserved and similar residues between different family members are predominantly located in the α-helical secondary structure elements in two motifs ($LX_2\#GX_2\#GX_2LXE$ and $G\#_2XGXL$) previously described for the yeast Bet3 family members [39], were they constitute a part of the hydrophobic protein core.

The idea of a similar fold of the three Bet3 family proteins raised the question whether Tpc5 might also be able to form homodimers or heterodimers with Bet3 and Tpc6B. Association studies (Figure 4.8) could show that Tpc5 does indeed interact with Bet3, but not with Tpc6B. A homodimeric interaction of Tpc5 could not be shown with *in vitro* cross-linking experiments. However, this might be due to a methodical problem as successful cross-linking with glutardialdehyde requires the availability of lysine residues in the vicinity of the interaction site. Taken together, the data indicate that Tpc5 might form a heterodimer with Bet3, but does not associate with Tpc6B. This is conistent with data from a yeast-two hybrid analysis [35], that also suggested an interaction between Bet3 and Tpc5.

During the writing of this thesis, the structure of the mammalian TRAPP I complex has been published [6], which now allows to judge some of the predictions made before. In this impressive study structure determination using X-ray crystallography was combined with single-particle electron microscopy (EM) to reconstruct an image of a TRAPP subcomplex consisting of six subunits. Two mammalian TRAPP subcomplexes were identified, the Bet3:Tpc6A:Mum2:synbindin subcomplex and a Bet3:Tpc5:Sedl complex. These subcomplexes could be crystallized, and their structures were determined at a resolution of 2.5 Å and 2.1 Å, respectively.

The Bet3:Tpc6A:Mum2:synbindin subcomplex (Figure 5.1a) is virtually identical to the tetrameric complex that was described in 4.5.3, with the exception that Tpc6B is replaced by Tpc6A. This is consistent with previous observations that both Tpc6 isoforms are interchangeable in TRAPP assembly (4.4.2). The tetramer contains a Bet3:Tpc6A heterodimer that basically has the same structure as the isolated heterodimer. Mum2 and synbindin adopt a longin fold and closely resemble Sedl. These three proteins have been grouped in one family of TRAPP subunits before based on their homology [7]. Mum2 and synbindin form a heterodimer with pseudo-2-fold symmetry via an extensive interaction interface formed by a continuous intermolecular β-sheet and the α1 helices.

The binding to Bet3:Tpc6A by Mum2 is mediated through the association of Bet3's helix α2 with a pocket (termed groove A) formed by α2 and the β4-β5 loop of Mum2. In addition, α2,

α3 and the connecting loop region of Mum2 are bound to a hydrophobic pocket formed by helices α1 and α4 of Tpc6A. This interaction site does not correspond to the region that had been hypothesized as putative Mum2 binding site (4.3.5). Instead of the α1-α2 loop regions, it is rather the helices α1 (of Tpc6) and α2 (of Bet3) that bind Mum2. An explanation for this could be that, although not obvious from the structure of the Bet3:Tpc6B heterocomplex, the mutations in the loops must have caused distortions in the helices. Because the putative binding groove is not occupied in the tetrameric complex it might be responsible for the interaction of TRAPP with another binding partner.

Synbindin contains an additional PDZ-like (PDZL) domain that is inserted between β2 and α1 of the longin-fold domain. The PDZL domain is found only in synbindin homologs from metazoans but not from single-cell eukaryotes, suggesting that this domain was acquired during evolution of higher organisms. A hydrophobic pocket is formed by the PDZL domain which is expected to provide a further protein binding site.

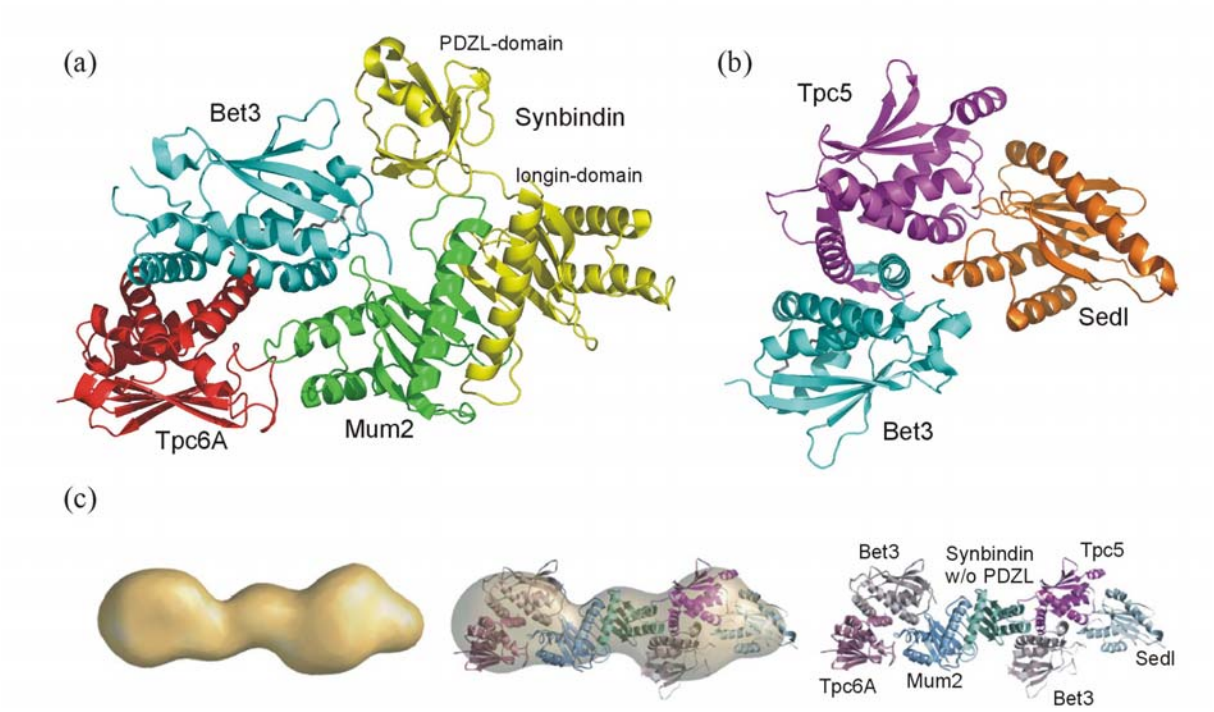

Figure 5.1: Model of the complete TRAPP I complex. (**a**) Crystal structure of a tetrameric complex of Bet3 (cyan), Tpc6A (red), Mum2 (green) and synbindin (yellow). (**b**) Crystal structure of a trimeric complex of Bet3 (cyan), Tpc5 (magenta) and Sedl (orange). (**c**) The crystal structures were docked into a map from single particle EM analysis of recombinant yeast TRAPP I. The PDZL domain of synbindin was omitted.
Figure 5.1c was taken from [6].

The Bet3:Tpc5:Sedl complex (Figure 5.1b) contains a Bet3:Tpc5 heterodimer that is similar to the Bet3:Tpc6 heterodimers. Tpc5 adopts the Bet3 fold but contains an additional C-terminal helix and dimerizes with Bet3 in a similar manner as Tpc6. This ultimately proves the idea correct that Tpc5 forms heterodimers with Bet3 (4.2.4) and folds like Tpc6 and Bet3 (4.2.2). Sedl binds with its groove A (between α2 and β4-β5 loop) to the α2 helix of Tpc5, which is opposite to the binding site of Mum2 to Tpc6A.

The Bet3:Tpc6A and Bet3:Tpc5 heterodimers both form flat surfaces with positively charged residues which are expected to associate with the membrane. This observation defines the arrangement of TRAPP at the Golgi membrane: The subunits assemble in a side-to-side fashion, forming flat subcomplexes. This notion is supported by the single-particle analysis of recombinant yeast TRAPP I (rTRAPP I), which contains all subunits but Trs85p. No mamalian homolog of Trs85p has been identified, and the subunit is dispesible for yeast cell viability and was not required for the assembly of rTRAPP I. The EM density map at 30 Å resolution revealed an elongated shape of the complex with dimensions of 180 Å x 65 Å x 50 Å. Two distinguishable lobes are observed, connected by a central density. By labeling the complex with α-Trs33p (Tpc6 homolog) Fab anitbody fragment, the protein could be localized to the proximal end of one lobe. The crystal structures of the mammalian TRAPP subcomplexes could be fitted into the EM density of yeast rTRAPP I, but the PDZL domain of synbindin (not present in the yeast homolog Trs23p) had to be omitted from the model (Figure 5.1c). The trimeric and the tetrameric subcomplex seem to be joined by the interaction of synbindin with the Bet3:Tpc5 heterodimer.

5.2 Membrane anchoring of TRAPP

The current model of membrane association of TRAPP envisages that Bet3 is bound with its hydrophobic channel to an acyl chain of an anchor moiety, which is supported by positively charged residues on the flat surface of Bet3 that compensate for the negatively charges of phospholipid headgroups [56]. A number of positively charged residues are also found on the correspomding surface of Tpc5, with five positions conserved as lysine or arginine from yeast to human proteins [6]. This accumulation of positive charges might give rise to a major stabilization of membrane association. Thus, the Bet3:Tpc6 and Bet3:Tpc5 heterodimers are both expected to associates *via* their flat surface with the Golgi membrane.

Several basic and aromatic residues of Tpc6 are found at the putative membrane interacting surface that also might interact with phospholipid head groups or insert into the lipid bilayer, respectively. Of these, Lys66, Phe67, and Lys79 are conserved and could be required for

membrane association of TRAPP. In addition, sequence comparison of Tpc6 paralogs and orthologs led to the identification of a conserved surface patch that, according to the current model, faces towards the membrane (4.4.1). Interestingly, the conserved patch is located at a structurally similar position where the entrance to the hydrophobic channel is found in Bet3.

Biochemical and genetic data show that Trs33p is indeed able to recruit Bet3p to the membrane and to suppress the growth defect of Bet3p mutants lacking proper membrane localization [97]. A Trs33p charge inversion mutant was generated, where two lysine residues proposed to interact with phospholipid headgroups of the membrane were mutated (corresponding to K67E,K71E of human Tpc6A). The mutant Trs33p could rescue the Bet3p charge inversion mutant, but not the Bet3p channel blocking mutant. These studies suggest that Trs33p is not (primarily) involved in charge compensation of the membrane, but helps anchoring Bet3p to the proposed acyl moiety. A similar effect was observed in membrane preparations from HEK293 cells over-expressing Bet3 and Tpc6B, where Bet3 but not Tpc6B seemed to possess an intrinsic affinity for membranes (Figure 4.5b). Interestingly, the mutated lysine residues are located in the conserved patch of Tpc6. It can therefore be assumed that the patch is involved in recruitment of TRAPP to the Golgi membrane *via* the interaction with an anchoring protein. This anchoring protein does not necessarily have to be the same moiety that carries the acyl chain which is expected to occupy the hydrophobic channel of Bet3 (Figure 5.2). This model could explain the additional contribution of Tpc6 for membrane binding of TRAPP, and the identification of the conserved patch might help find the anchor protein for TRAPP at the Golgi.

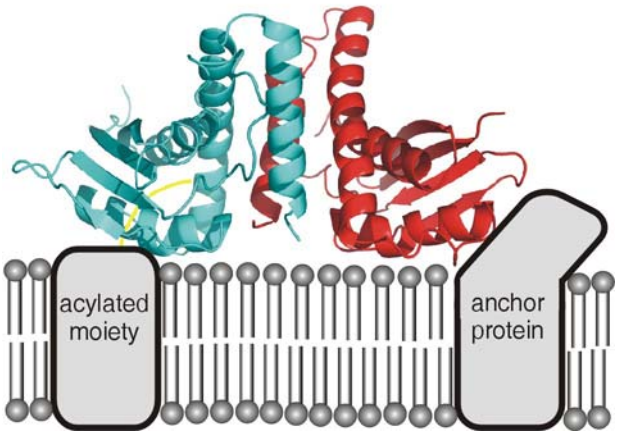

Figure 5.2: Model for membrane association of the Bet3:Tpc6 heterodimer.
An acyl chain (yellow) from a Golgi-specific protein occupies the hydrophobic channel of Bet3 and an anchor protein might associate with the conserved patch of Tpc6, thus facilitating membrane recruitment of TRAPP.

5.3 Isoforms of TRAPP subunits

Crystallographic studies have revealed that both of the existing Tpc6 paralogs can form stable heterodimeric complexes with Bet3. Further experiments showed that both isocomplexes are able to bind to Mum2 and synbindin and should thus both be able to participate in TRAPP complex formation ([6], 4.5.3). The investigation of six different mouse organs revealed that *tpc6b* expression levels are somewhat higher compared to *tpc6a*, but no tissue specificity in the *tpc6* paralog expression pattern could be observed (Figure 4.15b). This indicates that both isoforms coexist.

The duplication of genes involved in vesicular transport is not only restricted to Tpc6 (Table 5.1). Among the TRAPP subunits also two paralogs of Bet3 and Sedl are also found in the human and mice genomes. This has important implications for the X-linked genetic disease SEDT, where the loss of mutated Sedl can be at least in part compensated by the expression of a Sedl variant, leading to only a mild phenotype. In a previous report, the genome analysis of rice (*Oryza sativa*) resulted in the identification of duplicates of Bet3, Sedl, Tpc6 and synbindin homologous genes [107]. Notably, the GTPase Rab1 crucial for the coordination of vesicle fusion is also present in two isoforms in the human genome.

Isoforms (NCBI accession numbers)		identity / similarity
Bet3 (NP_055223)	Bet3-like (CAI12937)	61% / 77%
Sedl (NP_001011658)	Sedl-like (AAI05810)	28% / 50%
Tpc6A (O75865)	Tpc6B (CAD61947)	56% / 72%
Rab1A (NP_004152)	Rab1B (NP_112243)	93% / 96%

Table 5.1. Isoforms of human TRAPP subunits and Rab1.

A detailed study has been performed on the two isoforms of the retromer subunit Vps26 [108]. The retromer complex is required for trafficking of cation-dependent mannose-6-phosphate receptors through the endosomal system in mammals. A phylogenetic study revealed that the two paralogs of Vps26 must have arisen during the evolution of chordates. Whereas the intracellular location of Vps26A is predominantly on endosomal membranes, Vps26B is found in the cytosol and on the plasma membrane. These observations for the retromer subunit Vps26 open up a possible explanation for the findings on the paralogous TRAPP subunits. Different variants might be involved in the formation of different mammalian TRAPP isoforms. In yeast, TRAPP I and II have been characterized which differ in size, cellular localization and function [39]. Only one TRAPP complex could be identified in HeLa cells based on its size [34]. But it cannot be excluded that in higher eukaryotes the repertoire

of TRAPP subunit isoforms might assemble into distinct TRAPP complexes that could differ in their cellular localization or function. This could be mediated by different surface properties, as they are apparent for the not conserved patches between the Tpc6A and Tpc6B paralogs.

On the other hand, two variants of the same protein might help to secure such an essential process as vesicle transport. Considering the fact that Tpc6B is better conserved across species than Tpc6A, this leads to the possibility that Tpc6A might have a specialized function different from classical TRAPP function in the secretory pathway. A knockout mouse of *tpc6a* shows only a mild phenotype with defects in pigmentation of the coat and eye (namely the retinal pigmented epithelial). The expressing level of tpc6a was found to be very high in melanoytes in wild-type mice, pointing towards a role of Tpc6A in melanosome biogenesis [54].

Further studies will be required to elucidate the role of different TRAPP isoforms. The use of specific antibodies for each Tpc6 paralog could be an easy and efficient tool to determine their intracellular localization and to answer some of the remaining questions.

5.4 Palmitoylation of the TRAPP subunit Bet3

The most unusual feature revealed by the structural analysis of TRAPP subunits is the palmitoylation of Bet3. In all crystal structures of Bet3 – from different species [56, 57] and in different subcomplexes [6, 97, 106] – a palmitoyl group is found to be covalently linked to a conserved cysteine residue and buried within a hydrophobic channel in the interior of the protein.

Palmitoylation of Bet3 does not seem to be essential for the survival of yeast cells or to be involved in membrane association in yeast (4.6.1). However, a number of observations point towards a physiological role of Bet3 palmitoylation.

In vitro studies of the enzymology of the palmitoylation revealed an autocatalytic mechanism of the reaction that has an optimum at physiological pH and requires proper folding of Bet3. The pseudo-K_M value is in a range that corresponds to the intracellular concentration of the substrate Pal-CoA, and the kinetics of the reaction are rapid (4.6.2, [100]). Bet3 is the only known eukaryotic protein containing covalently bound fatty acids when purified from *E. coli* which lack enzymes for palmitoylation [56], indicating that even in cells acylation of Bet3 does not require an exogenous enzyme. This is a unique feature, as a proteomics analysis of palmitoylated proteins in *S. cerevisiae* identified Bet3 as the only protein that does not require

DHHC-proteins for its palmitoylation, strongly arguing in favour of an autocatalytic mode of acylation [63].

Several spontaneous palmitoylation reactions are inhibited by the acyl-CoA binding protein (ACBP) [109, 110], but human Bet3 is also palmitoylated in yeast [57] which contain functional ACBP [111]. Consistently, palmitoylation of Bet3 has been shown to naturally occur inside mammalian [100] and yeast cells (4.6.1, [63]).

Finally, the palmitoylation site of Bet3 is strictly conserved in all species and does not only include the palmitoylated cysteine residue (Cys68 in human Bet3), but also the neighboring arginine (Arg67), which is required for the efficiency of the autocatalytic reaction (4.6.2). This indicates that an evolutionary pressure exists that prevents the loss of self-palmitoylation activity. Thus, the self-palmitoylating activity observed *in vitro* is likely to be of physiological significance.

A proven function of Bet3 palmitoylation is the stabilization of the protein shown here (4.6.3). This is manifested in a reduced melting temperature of deacylated Bet3 *in vitro* and the more rapid degradation of unpalmitoylated Bet3 mutants *in vivo*. Degradation *in vivo* has been reported before for other proteins, notably for SNARE proteins, upon removal of their palmitoylation sites [112]. The lack of a ligand in the hydrophobic tunnel might lead to a partial misfolding and subsequently to the destabilization of the protein. It can be concluded that depalmitoylation of Bet3 might result in a disturbance of Bet3's tertiary structure, because unpalmitoylated Bet3 mutants fail to bind Tpc6B *in vivo*, although binding interface and mutations sites are spatially far apart. This raises the question why only the channel-blocking mutant (A82L) but not the mutation C68S of Bet3 does cause conditional lethality in yeast [56]. An explanation could be that non-acylated Bet3 with an open hydrophobic tunnel might still bind to the acyl chain of a Golgi-resident membrane protein and would therefore be stabilized by the postulated insertion of an external fatty acid [56]. This would not be possible if the tunnel is too narrow. As a consequence, Bet3 C68S could be recruited to the Golgi and the tertiary structure be restored, ensuring proper trafficking through the secretory pathway and cell viability, but Bet3 A83L is neither recruited nor stabilized and thus lethal.

However, yeast and human Bet3 might also have different requirements for palmitoylation. Most of mammalian Bet3 is not membrane anchored [36], and it can be speculated that this pool of Bet3 might serve a different function beyond acting as a tethering factor in the framework of the TRAPP complex. Although increased palmitoylation of proteins upon over-expression of Bet3 was not detected in cell lysate [100], it is intriguing to speculate that Bet3 acylates proteins of lower abundance or lipids. Interestingly, the fatty acid synthetase (FASN)

was identified to bind to Bet3 in a TAP (tandem affinity purification) tag analysis from HEK293 cells [37]. Thus, a role of Bet3 in fatty acid metabolism or transport, e.g. a function as carrier of activated fatty acids, is also conceivable.

5.5 Implications for the function of TRAPP and its subunits

The structure determination of mammalian TRAPP subcomplexes and the model of a partial recombinant yeast TRAPP I complex allow an initial description of its architecture and provide a starting point for assessing its function: In yeast, TRAPP I is required for tethering of ER-derived vesicles to the Golgi and binds COP II vesicles at the early Golgi compartment [39]. Yeast TRAPP II specific subunits function at the late Golgi/early endosome and are implicated in the recycling pathway from the early endosome to the late Golgi [40]. in contrast, mammalian Bet3 is mostly localized to the transitional ER (tER) and to some extend to endosomes. It mediates the formation of vesicular tubular clusters by homotypic fusion of COP II vesicles [41]. This defines an essential role for TRAPP in vesicular traffic from the ER to the Golgi.

The assembly of the TRAPP subunits in a side-to-side fashion in respect to the membrane leads to the formation of an extended protein patch on the Golgi surface. The complex is composed of subunits that belong to two protein families, the Bet3 fold and the longin-like Sedl fold. The Bet3-like proteins form heterodimeric complexes, which always contain a Bet3 subunit and are considered the membrane-proximal units involved in TRAPP anchoring. Longin-fold proteins are usually involved in multiple protein-protein interactions. It thus has been suggested that these proteins connect TRAPP with associating partners [6, 55].

To fully characterize TRAPP as a submodular entity in vesicular transport, which is the comprehensive functional module [5], requires the knowledge of the molecular properties of TRAPP and its subunits. Therefore, some crucial points remain to be clarified. The membrane facing side of TRAPP must be responsible for the proper anchoring of the complex, a process that still awaits its molecular elucidation (as discussed in 5.3). Whereas most functional data on TRAPP was obtained from yeast, structural studies have been carried out with mammalian proteins, and it is unclear how these results relate. For some of the yeast subunits (Trs65p, Trs85p), no mammalian paralogs have been identified. Their function was either lost during evolution or has been taken over by other TRAPP subunits (e.g. synbindin contains an additional PDZL domain) or by functional homologs that share no sequence similarity. The small yeast subunits assemble into one complex (rTRAPP I), but the mammalian homologs are not able to form a stable complex *in vitro*, indicating that additional factors could be

required for mammalian TRAPP formation. Consistent with this observation, only one large TRAPP complex was identified in mammalian cells, whereas a small TRAPP I and the larger TRAPP II are found in yeast. These differences in complex assembly have important implications for TRAPP function, as the minimal GEF TRAPP subcomplex described for yeast could not be reconstituted with mammalian proteins [6] (see below).

Understanding the molecular interactions within TRAPP is only the first step in elucidating its function. It is of major interest to characterize the interactions between TRAPP and other submodular entities of vesicular transport. Submodular entities contribute to the output of a functional module through mediating the amplification of signals, intracellular regulation and/or localization of components *via* complex interactions between the biomolecules [5]. The activity must be initiated, the information processed and signals are relayed. In the case of the TRAPP complex, the different tethering functions might include the interaction with other tethering factors (e.g. the coiled-coil protein p115 in human cells), binding of incoming vesicles, activation of the GTPase Rab1 and the coordination of SNARE assembly.

Most of these interactions remain to be established. Although TRAPP was shown to recognize incoming vesicles by binding to COP II vesicles [41], the vesicle receptor protein was not identified. There are no data showing a direct interaction of TRAPP with a coiled-coil tether or a SNARE protein. However, the longin-fold of Sedl resembles that of the regulatory domain of the SNARE Ykt6p, which is able to bind to its own helical domain. In analogy it can be presumed that Sedl could bind to a SNARE domain and thus regulate SNARE function [6, 55].

The interaction of TRAPP with Rab GTPases is well established. TRAPP I is a nucleotide exchange factor for Rab1/Ypt1p, whereas TRAPP II (with the additional subunits Trs120p and Trs130p) has nucleotide exchange activity for Ypt31p [17, 18, 42, 113, 114]. Trs130p has also been genetically linked to the GTPase Arf1 [114], but the significance of this observation has not been investigated.

The minimal TRAPP I subcomplex that was able to promote nucleotide exchange of Ypt1p *in vitro* was a Bet3p:Trs33p:Trs23p:Bet5p complex of yeast [6]. The paralogous mammalian complex could not be reconstituted, indicating a different mode of action. A conserved cytosol-exposed surface patch in the interaction interface of Bet5:Trs23p/Mum2:synbindin is postulated to mediate the GEF activity of TRAPP. How this process is triggered and the underlying mechanism of Rab activation by the TRAPP complex remains to be established.

TRAPP and the exocyst, a well studied tethering complex located at the plasma membrane, share functional homology as they both have been implicated in the tethering of vesicles to a

donor compartment. However, the comparison of TRAPP with the exocyst reveals striking differences between both complexes. TRAPP and the exocyst interact with different classes of GTPases [27, 42], indicating different modes of action. Furthermore, TRAPP acts in concert with coiled-coil tethers, whereas no such factors have been described to be involved in tethering at the plasma membrane [21]. To current knowledge, both complexes share no similarity on a structural level. Exocyst subunits adopt a unique fold and assemble in a rod-like framework [27]. TRAPP proteins belong to the Bet3-like and the longin-like family and are expected to form an extended patch on the Golgi membrane [6]. Therefore, the conservation of activity between the complexes does not coincide with structural similarity. A convergent evolution of function from different scaffolds has been observed in several cases, e.g. for enzymes like phytase, of which a propeller-fold class [115] and an acid-histidine class [116] have been identified that carry out identical function. Even for such a fundamental process as tRNA activation two classes of aminoacyl-tRNA synthetases exist, which do not share any similarity in primary or tertiary structure [117]. Such a mechanism is also conceivable for the evolution of tethering complexes, or tethering might be organized in different ways at different sites.

In addition to their role in vesicle transport, several subunits of TRAPP have been implicated in a variety of cellular processes in higher eukaryotes. Interestingly, TRAPP subunits are found in a monomeric state besides their presence in the TRAPP complex in mammalian cells [34, 36], which might allow their participation in special events apart from tethering.
The genetic disease Spondyloepiphyseal Dysplasia Tarda (SEDT) caused by Sedl mutations could be explained by a general defect in secretion with conditional distortion of cartilage development [118], or Sedl might be associated with transcriptional regulation which is deregulated upon mutation of the *SEDL* gene [46]. Furthermore, there are indications that TRAPP subunits might be involved in biogenesis of melanocytes (Tpc6A, [54]), function of dendritic spine synapses (synbindin, [49]), formation of autophagosomes (Trs85p [50, 51]), fatty-acid metabolism (Bet3, [100]), cytokine-induced signaling (NIBP, [52]) and might function as membrane channel (Ehoc-1, [53]).
Whether these diverse functions can be attributed to the TRAPP complexes or the individual subunits remains to be seen. However, these specialized roles might be of great interest in examining the regulation of vesicular transport by cross-talk with other cellular modules involved in signaling and development.

5.6 Conclusion

The results presented in this work have made a contribution to elucidate the tethering event mediated by the TRAPP complex on a molecular level. The structural characterization of the TRAPP subunit Tpc6B and a Bet3:Tpc6B heterodimer has provided a starting point for biochemical analysis of the proteins' functions. This led to the identification of TRAPP subcomplexes and suggestions for their role in TRAPP assembly. In addition, the functional characterization of Bet3 palmitoylation identified a novel enzymatic mechanism required for protein stability.

My data together with several recent publications now provide a much better understanding of TRAPP and its role in vesicular transport. The emerging picture, however, leaves many questions unanswered. The task for future work will be to elucidate the interplay of the subunits, subcomplexes and the whole TRAPP complex with the other components of vesicular transport.