1 Introduction

1.1 Vesicular transport

All living cells, from bacteria to animal cells, are surrounded by a phospholipid bilayer (the cell membrane, also called the plasma membrane), which separates the interior of the cell (cytosol) from the outside. In addition to the cell membrane, eukaryotic cells also contain membrane-enclosed compartments (organelles). Each organelle has its individual architecture and functions. For instance, the mitochondrion is required to produce cell energy. For proper functioning, it needs to maintain an intact outer and inner membrane structure and a large number of proteins specifically involved in the reactions of the respiratory chain.

On the other hand, several organelles quite often act sequentially in one single pathway, for instance the secretory pathway. Most secretory proteins are synthesized by ribosomes decorating the rough Endoplasmic Reticulum (ER), and then translocated into the ER lumen where they acquire their native fold and some post-translational modifications. These proteins may be further modified and sorted in the Golgi apparatus. Finally, the mature proteins are exposed at or released from the plasma membrane. Damaged or misfolded proteins are degraded before they reach the plasma membrane.

In order to meet the needs of organelles' self-maintenance and communication, the intracellular transport is strictly regulated in all living cells. Small molecule transport across membranes is normally mediated by membrane proteins that can act as specific transporters or channels. As for macromolecules like proteins, the translocation between different compartments can be achieved by vesicular transport. During this process, small membrane vesicles bud at a donor membrane, loaded with soluble or membrane-bound cargo protein, in a process mediated by specific protein coats (clathrin, COPI or COPII coats). After release from the donor membrane, the vesicles are uncoated and travel through the cells to their corresponding acceptor compartment, where they are first tethered and docked to the target membrane, then fuse with the membrane and deliver the loaded cargo.

1.1.1 Modular organization of vesicular transport

In order to understand the principles lying behind complicated reactions and changes happening in living cells, a reasonable approach is to study them at the level of 'functional modules'. It is well known that major reaction pathways in living cells are carried out by specific subsets of proteins. Each of the subsets, either as a compact structure of a macromolecular complex, or as a more dynamic ensemble of molecules, can be understood as a macromolecular machine, which can change its composition and/or organization during function. Another important feature of a functional module is that it is defined by spatial sequestration, chemical specificity and a characteristic time domain within which it goes through specific functional cycles [1].

In the light of this concept, the whole process of vesicular transport from a donor to an acceptor compartment can be considered a functional module. A cargo molecule with appropriate transport signal serves as the input into the module, whereas the same cargo delivered to its acceptor compartment represents the modular output. This modular process can be further divided into several individual steps: cargo sorting, vesicle budding and scission from the donor membrane, uncoating, and then tethering, docking and membrane fusion at the acceptor compartment. Finally, in order to complete a full functional cycle, the carrier proteins must be recycled back to the donor compartment. The different steps are carried out by submodular entities, which work together coordinated in time and space to accomplish the whole complex tasks (Fig 1.1.1 a).

The coordination of these processes is achieved through regulation by members of the Ras superfamily of small GTPases. More precisely, GTPases of the Arf (ADP ribosylation factor) family are involved in the formation of transport vesicles [2], while membrane fusion is regulated by the Rab (Ras analog in brain) GTPase family. The small GTPases can cycle between an inactive GDP-bound state and an active GTP-bound state, thus regulating many important cellular activities as 'molecular switches' (Fig 1.1.1 b). Since the GTPase activity of the small GTPases is intrinsically very low, the hydrolysis of GTP needs to be accelerated by additional GTPase activating proteins (GAPs). The GDP-bound state of small GTPases is stabilized by GDP dissociation inhibitors (GDIs), which need GDI displacement factors (GDFs) to be removed. Finally, the GTPase can be reactivated by guanine nucleotide exchange factors (GEFs).

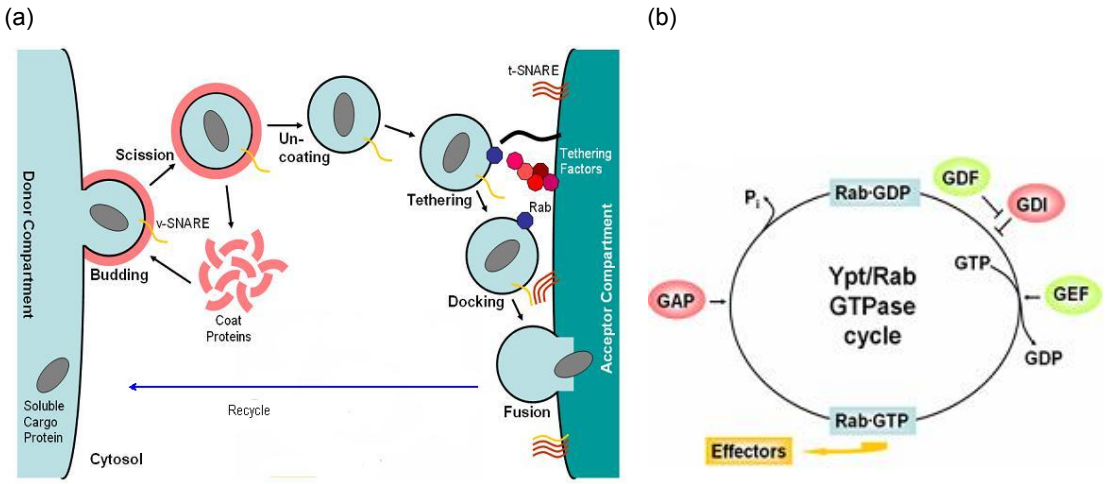

Fig 1.1.1 Modular organization of vesicular transport (modified from [1])
(a) The whole process can be divided into series of submodular activities, such as budding, scission, uncoating, tethering, docking and membrane fusion.
(b) A Ypt/Rab GTPase cycle regulates vesicle transport steps as molecular switch. GEF: guanine nucleotide exchange factor; GAP: GTPase activating protein; GDI: GDP dissociation inhibitor; GDF: GDI displacement factor.

1.1.2 Vesicle transport from ER to Golgi

As an example of the modular organization of vesicular transport, the transport of ER-derived vesicles to the Golgi apparatus has been well studied in yeast.

Firstly, COPII (coat protein complex-II) coated vesicles start budding on ER membrane. In *Saccharomyces cerevisiae*, budding occurs across the entire ER, but in *Pichia pastoris* and mammalian cells, COPII dependent vesicle exit is observed at specific sites only, so called ER exit sites [3]. The COPII coat is one of the three known coat protein complexes (CPCs), which are structurally organized as multi-layered complexes that are assembled through sequential protein interactions. The organization of CPCs requires both the adaptor protein (AP) complexes, which recognize and select different types of cargo, and cage proteins (CPs). In the case of COPII vesicles, cargo recognition is facilitated by the heterodimeric AP complex Sec23p-Sec24p, and the CP scaffold is formed by the Sec13p-Sec31p complex.

Coordination of the COPII assembly is regulated by the small GTPase Sar1p. Firstly, Sar1p is activated by its GEF Sec12p, then it is attached to and disturbs the ER membrane via an N-terminal amphipathic helix [4]. Afterwards, Sar1p recruits the hetero-dimer Sec23p-Sec24p by direct interaction with Sec23p [5]. The majority of cargo is captured through interaction with Sec24p [6]. Finally, the heterotetramer

Sec13p-Sec31p (2 copies of either protein) forms a cage-like structure around the vesicle [7]. For a review of COPII coat formation, see [8] & [9].

Soon after budding, uncoating of COPII vesicles is promoted by GTP hydrolysis of Sar1p. Sec23p has GAP activity for Sar1p, and binding of Sec13p-Sec31p additionally enhances the GAP activity. Thus, the coat proteins trigger their own disassembly [10].

After uncoating, the vesicles are first tethered (brought into close proximity) to the cis-Golgi membrane by tethering factors (long coiled-coil tethers or multisubunit protein complexes), and then docked and fused with the membrane through formation of SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) complexes. The formation of tight SNARE complexes not only brings vesicle and target membrane into even closer proximity, but also leads to initiation of the membrane fusion.

Finally, the ER resident proteins involved in this process are selectively retrieved and recycled from cis-Golgi to ER by COPI (coat protein complex - I) coated vesicles [11]. The small GTPase that regulates the docking and fusion of ER-derived vesicles with the Golgi membrane is Ypt1p of the Ypt/Rab GTPase family [12]. The Rab GTPases are associated with their specific target membranes by prenylation at or near the C-terminus [13]. All Rabs contain an unfolded hypervariable C-terminal domain. This region is highly divergent between all Rab sequences and is supposed to have an impact on targeting of a Rab to its specific compartment [14]. Switching between GTP-bound and GDP-bound states leads to major structural changes within two regions in the G-domain of Rabs, termed switch I and switch II regions. Only in the active, GTP-bound form, can Rabs fulfill their function by interacting with effector proteins. To ensure correct membrane association, Rabs need to be prenylated by the Rab geranylgeranyltransferase (Rab-GGTase), a multisubunit enzyme consisting of a catalytic heterodimer and an accessory component, named Rab escort protein (REP) [15]. Like other small GTPases, the cycle between the GTP-bound active form and the GDP-bound inactive form of Rabs is also strictly regulated by many factors (see Fig 1.1.1 b).

1.2 SNARE proteins

SNAREs are a superfamily of membrane proteins involved in membrane fusion. Until now, 25 members of this family were found in yeast and more than 35 in mammals [16]. Different SNARE proteins vary widely in size and structure, but all share one central homologous sequence, named the SNARE motif. The SNARE motif contains 60-70 amino-acid residues that include eight heptad repeats typical for coiled coils [17].

In vesicle fusion, SNARE proteins either locate to a vesicle (thus termed v-SNARE) or a target membrane (t-SNARE). Pairing of v- and t-SNAREs is the first step before formation of a tight SNARE complex. Functionally, SNAREs can be divided into R- and Q-SNAREs. The core SNARE complex is a four-helix bundle made up from 4 SNARE motifs. In the center of this complex is one arginine (R) from an R-SNARE interacting with three glutamines (Q) from Q-SNAREs, flanked by leucine zippering on both sides [18-19]. During the formation of SNARE complexes, the residues in the center of the coiled-coil regions form specific hydrogen bonds with each other, and seal the otherwise exposed hydrophobic residues [19] (Fig 1.2.1). The free energy released during this process is used to overcome the thermodynamic barrier of membrane fusion.

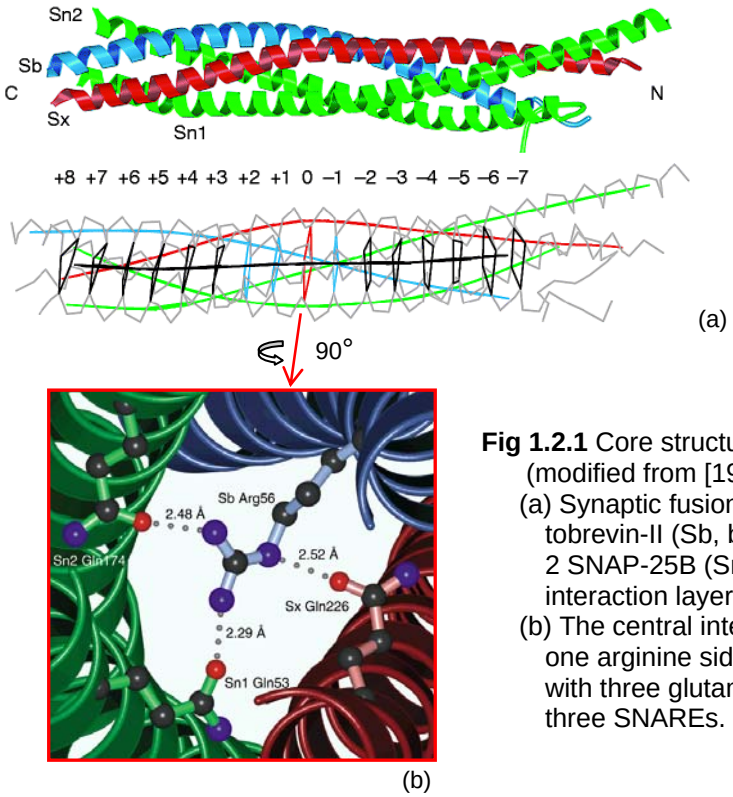

Fig 1.2.1 Core structure of SNARE complex (modified from [19])
(a) Synaptic fusion complex formed by synaptobrevin-II (Sb, blue), syntaxin-1A (Sx, red) and 2 SNAP-25B (Sn1 and Sn2, green), with interaction layers +8 to –7 marked out.
(b) The central interaction layer is composed of one arginine side chain from Sb inter-acting with three glutamine side chains from the other three SNAREs.

Finally, the recycling of SNAREs is achieved through dissociation of the helical bundle, which is mediated by the AAA+ (ATPases Associated with diverse cellular Activities) protein *N*-ethylmaleimide-sensitive factor (NSF).

1.2.1 Longin domain of SNARE proteins

Unlike the conserved central SNARE motif, SNAREs have quite different types of N-terminal domains, which often serve as regulatory domains by intramolecular interaction with the SNARE motif. Many Q-SNAREs, for instance syntaxin-1, have N-terminal antiparallel three-helix bundles, which can fold back and pack extensively with the helices in SNARE motifs, thus preventing the formation of a SNARE complex [20]. By contrast, the N-terminal domains of many R-SNAREs have a profilin-like fold. These domains are also referred to as longin domains. Although the sequence similarity is quite low, the longin domain of R-SNAREs shares a similar fold with several well-known protein domains which are involved in molecular interactions. For instance, it resembles a circular permutation of GAF/PAS domains, widely used as small-molecule binding regulatory modules, and profilin, a widely expressed protein that can bind diverse ligands including polyphosphoinositides (PPIn) [21-23] (Fig 1.2.2). Although the organization of secondary elements is slightly different, they all have a five-stranded antiparallel β-sheet, sandwiched by α-helices on both sides.

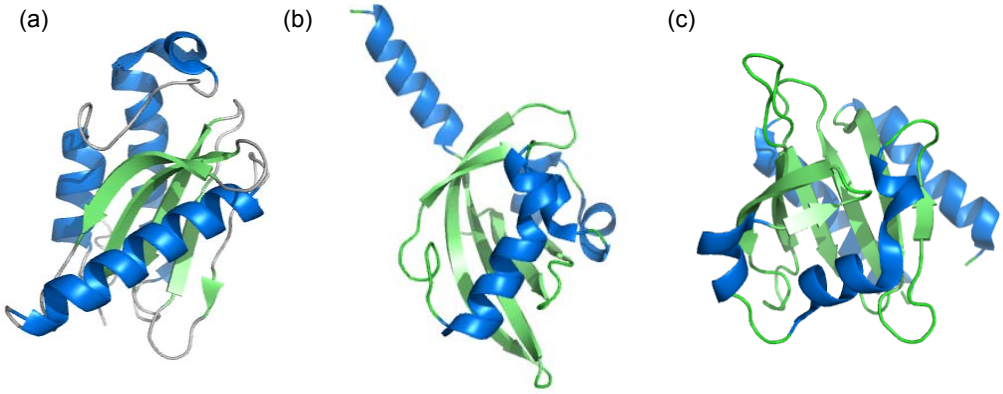

Fig 1.2.2 Longin domain of R-SNAREs, compared with similar folds
(a) Longin domain of Ykt6p, (b) PAS domain, (c) rat profilin 2A.
(a), (b) and (c) were generated from PDB entries 1H8M, 1Y28, 2VK3, respectively.

As a good example for R-SNAREs with longin domain, the yeast SNARE Ykt6p has been well studied [24]. Ykt6p is a SNARE that works in multiple membrane fusion reactions at the Golgi, vacuoles and endosomes. Unlike most other SNAREs, Ykt6p

does not contain a C-terminal transmembrane domain for stable membrane association. Instead, it contains a "CCAIM" motif that can be palmitoylated at the first Cys and farnesylated at the second Cys. Both lipid modifications are required for its stable membrane association, and hence its ability to facilitate membrane fusion [25]. If it is only farnesylated and not palmitoylated, Ykt6p stays in the cytosol and adopts an autoinhibited conformation via farnesyl-dependent interaction between its SNARE core and longin domain [26]. The detail of this interaction is revealed by an X-ray crystallographic structure of a C-terminally truncated Ykt6p in complex with the long-chain fatty acid DPC (dodecylphosphocholine) (Fig 1.2.3). The additional palmitoylation increases the partition coefficient of Ykt6p to the hydrophobic membrane bilayer, thus shifts the protein from the cytosol to membranes [27].

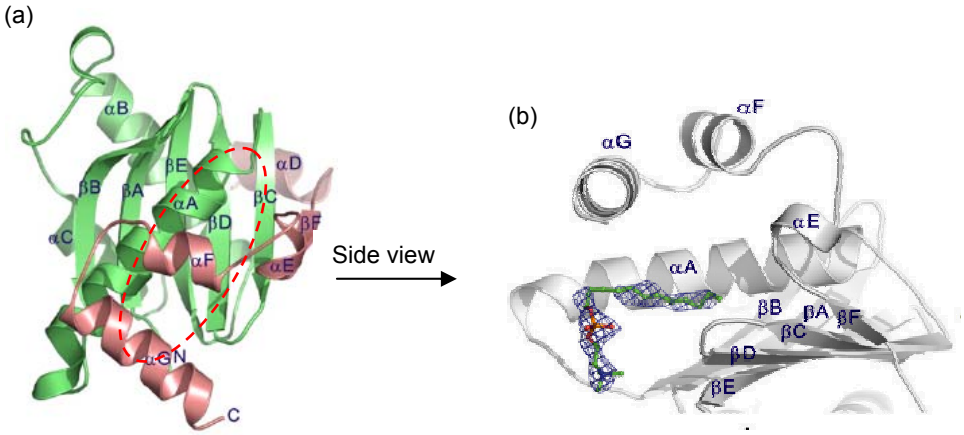

Fig 1.2.3 Farnesyl-dependent autoinhibition of Ykt6p (modified from [26])
(a) Structure of Ykt6pΔC, with longin domain in green and SNARE motif in pink;
(b) Side view into the hydrophobic groove where DPC (dodecylphosphocholine) is bound ($2F_o$-F_c electron density map for DPC is drawn in blue).

1.3 Tethering factors

The term tethering factor stands for a group of loosely related proteins that work as facilitators of membrane recognition before fusion. In vesicular transport, this task is rather challenging: the tethers need to bring the vesicle and target membrane close and then need to step aside so that SNAREs can interact and form a tight complex. Also, tethers are necessary to ensure the specificity and efficiency of vesicle targeting, which cannot be provided by the SNAREs alone [28].

Until now two different types of tethering factors have been described: proteins with long coiled-coil domains and large multi-subunit protein complexes.

1.3.1 Coiled-coil tethers

Coiled-coil tethers are characterized by long stretches of heptad repeats, which have a propensity to support the formation of superhelically intertwined α-helices, called coiled coils. Many (if not all) coiled-coil tethers form dimers, and are associated with the membrane directly or via anchor proteins [29]. Structurally, they appear as long, extended rod-like molecules. Among the best characterized examples of coiled-coil tethers are the yeast tether protein Uso1p and its mammalian homolog p115.

P115 is a parallel homodimer with two globular heads, followed by an extended central region composed of four coiled-coil domains, and a C-terminal acidic tail [30]. It is recruited to membranes in a nucleotide-dependent manner by the small GTPase Rab1 and believed to function in homotypic vesicle fusion of ER-derived COPII vesicles to generate later transport intermediates [31-32]. The C-terminal region of p115 binds to GM130 and giantin, two further coiled-coil tethers and Golgi marker proteins [33]. The yeast homolog of p115, Uso1p, was also shown to be required for tethering COPII vesicles to Golgi membrane [34].

Besides its role in exocytotic transport, p115 also functions in retrograde transport from Golgi to ER, intra-Golgi transport and Golgi biogenesis [35], due to essential interactions with the COPI subunit β-COP [36], and the subunit COG2 of the hetero-multimeric tether complex COG [37].

1.3.2 Multi-subunit tethering complexes

In addition to coiled-coil proteins, six different multi-subunit complexes (COG, DSL1, exocyst, GARP, HOPS, and TRAPP) have been shown to regulate distinct membrane trafficking steps. The subunit organization of five of these is shown in Fig 1.3.1 a. The tethering complexes working in the yeast secretory pathway are shown in Fig 1.3.1 b. Like coiled-coil tethers, these tethering complexes also show interactions with SNAREs and small GTPases, suggesting a conserved mechanism of action.

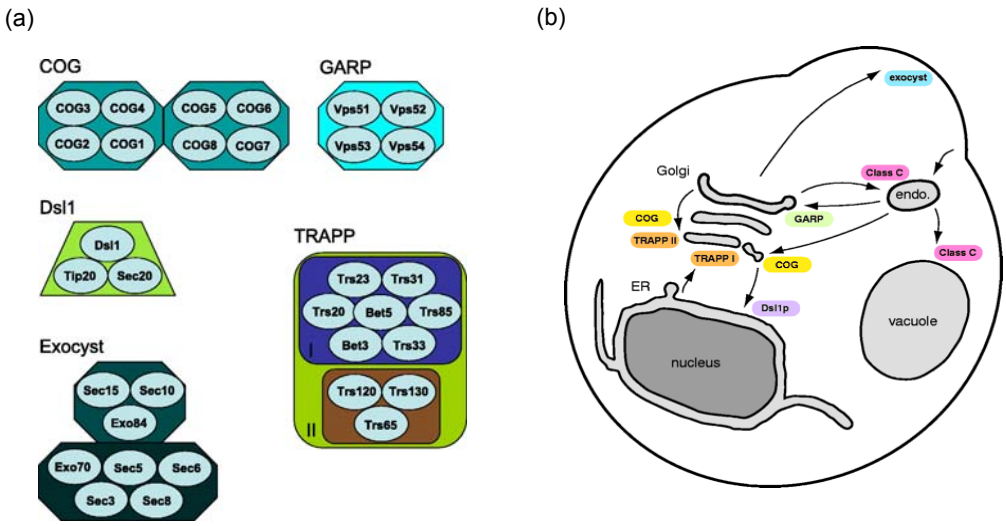

Fig 1.3.1 Multisubunit tethering complexes
(a) Subunit organization of multi-subunit tethering complexes COG, GARP, Dsl1, exocyst and TRAPP, taken from [38].
(b) Tethering complexes in the yeast secretory pathway (Class C = HOPS), taken from [28].

The COG (conserved oligomeric Golgi) complex consists of eight subunits, which associate to form a dumbbell-like structure of two globular subcomplexes connected by a short extension [39] (Fig 1.3.1). COG seems to be involved in different aspects of membrane trafficking: the intra-Golgi recycling of COPI vesicles [40], the cargo sorting during exit from the ER [12], vesicle tethering in anterograde ER-to-Golgi traffic [41-43], and proper locating of yeast enzymes in the trans-Golgi network (TGN) [44].

DSL1 is a trimeric tethering complex from yeast (Fig 1.3.1). The ER-localized DSL1 complex as well as its mammalian counterpart, the syntaxin 18 complex [45], are

suggested to bind the COPI vesicle coat and function in retrograde traffic to the ER [46-47].

The exocyst complex consists of eight subunits (Fig 1.3.1). It is peripherally associated with the cytoplasmic side of the plasma membrane at the sites of polarised exocytosis and plays a central role in tethering secretory vesicles to the plasma membrane [48]. In order to ensure a tightly controlled exocytosis, the exocyst acts as an effector for five small GTPases Sec4, Rho1, Rho3, Cdc42, and RalA [49-50]. These small GTPases are able to regulate exocyst assembly by regulating recruitment of exocyst subunits to the vesicle membrane and the plasma membrane.

The GARP (Golgi-associated retrograde protein) complex is a tetrameric complex localized to the late Golgi and the trans-Golgi network (TGN) [51]. It has a dual function in tethering vesicles derived either from early endosomes or from prevacuolar/lysosomal compartments to Golgi/TGN membranes. For proper function and regulation, GARP can bind the late Golgi/TGN SNARE Tlg1p and the GTP-bound form of Ypt6p [52-53].

The HOPS (homotyptic fusion and vacuole protein sorting) complex, also known as the Class C Vps complex, is required for proper sorting of proteins to the vacuole in yeast. It consists of six subunits: Pep3p, Pep5p, Vps16p, Vps33p, Vps41p and Vps39p. Vps39p is a GEF for the Rab GTPase Ypt7p [54], while Vps33p is able to bind the unpaired vacuolar SNARE Vam3p [55]. It is believed that during the assembly of the HOPS complex, Vps39p is recruited to both the vacuole and incoming vesicles. Vps39p activates Ypt7p, which in turn acts on HOPS complex to promote tethering. Then the SNARE Vam3p is no longer inhibited, which allows the formation of the SNARE complex and hence vesicle fusion.

1.3.3 TRAPP complex

The TRAPP (transport protein particle) complex is a multi-subunit protein complex involved in tethering of vesicles to the Golgi network [56] (Fig 1.3.1). With affinity purification, TRAPP complexes were isolated from yeast and mammalian cells [57-58], and their subunits were subsequently identified. Two types of TRAPP complexes were found in yeast: TRAPP I is ~300 kDa in size, and contains seven subunits (Bet3p, Bet5p, Trs20p, Trs23p, Trs31p, Trs33p, Trs85p) whereas TRAPP II is ~1000 kDa and contains three additional subunits (Trs65p, Trs120p, Trs130p). Functionally these complexes are also involved in different tethering processes: TRAPP I recognizes and binds ER-derived COPII vesicles to the Golgi [59], whereas TRAPP II recognizes the Golgi-derived COPI vesicles, and is involved in trafficking within the Golgi [60]. In mammalian cells, only one type of TRAPP complex of ~670 kDa could be identified by its size [57, 61]. Using database searches [61-63] and tandem-affinity purification (TAP) with human Bet3 from cell culture [57], mammalian counterparts of the yeast TRAPP subunits could be assigned (Table 1.3.1).

Most TRAPP I subunits are essential for yeast and highly conserved between yeast and mammals (~30% sequence identity), with the exception of Trs85p, which is non-essential and shows sequence similarity between the yeast and mammalian orthologs only in discontinuous patches along the sequence. The three TRAPP II-specific subunits are also not highly conserved from yeast to mammals. Trs65p is only conserved among fungi, no ortholog is identified in mammals.

<table>
<tr><th colspan="3">Yeast</th><th colspan="2">Human</th></tr>
<tr><td rowspan="10">TRAPP II
(~1000 kDa)</td><td rowspan="7">TRAPP I
(~300 kDa)</td><td>Trs23p</td><td>Trs23</td><td rowspan="10">TRAPP
(~670 kDa)</td></tr>
<tr><td>Trs31p</td><td>Trs31</td></tr>
<tr><td>Trs20p</td><td>sedlin</td></tr>
<tr><td>Bet5p</td><td>Bet5</td></tr>
<tr><td>Bet3p</td><td>Bet3</td></tr>
<tr><td>Trs33p</td><td>Tpc6A,
Tpc6B</td></tr>
<tr><td>Trs85p</td><td>Trs85</td></tr>
<tr><td rowspan="3"></td><td>Trs120p</td><td>NIBP</td></tr>
<tr><td>Trs130p</td><td>Ehoc-1</td></tr>
<tr><td>Trs65p</td><td>-</td></tr>
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Table 1.3.1 Yeast TRAPP subunits and their human homologs

In yeast, TRAPP I was shown to act as a GEF for the Rab GTPase Ypt1p [56]. The minimal subcomplex required for Ypt1p GEF activity (Bet5, Trs23, Trs31, and two copies of Bet3) is present in both TRAPP I and II [64]. However, there is conflicting evidence about whether the addition of the TRAPP II-specific subunits Trs120p and Trs130p would inhibit its Ypt1p GEF activity and switch its GEF activity to the late Golgi Rabs Ypt31p and Ypt32p [64-67].

The TRAPP complexes include three nonessential subunits, Trs33p, Trs65p and Trs85p [56, 63]. Among these, Trs33p is not involved in Ypt1p binding, raising suspicion that it might have other still unknown functions in TRAPP complexes. Another subunit Trs65p, which is TRAPP II specific, exhibits synthetic lethality with Trs33p, i.e. a combination of mutations in Trs65p and Trs33p leads to cell death, whereas a mutation in only one of the two genes does not. This observation suggests a joint function for the two TRAPP subunits. Further experiments found that this lethality can be suppressed by over-expression of Ypt31p/Ypt32p, suggesting that Trs33p and Trs65p share a function related to Ypt31p/Ypt32p [68]. There is also evidence suggesting that Trs33p interacts directly with Trs65p, and that either Trs33p or Trs65p is required for TRAPP II assembly, through interactions with Trs120p and Trs130p [69].

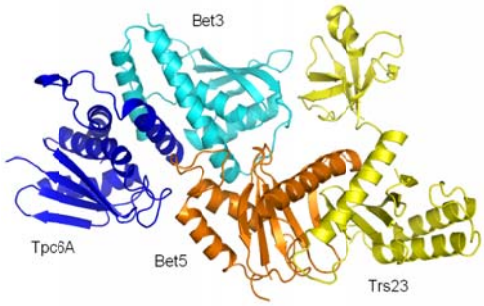
Another nonessential I subunit, Trs85p, was first identified as TRAPP I subunits through affinity purification [62], but its function in TRAPP I was not identified. Later researches suggest that Trs85p marks a third type of TRAPP complexes, TRAPP III, which is composed of all seven subunits formally identified as TRAPP I (see Table 1.3.1), whereas the real TRAPP I complex consists only six subunits. Also an Ypt1p GEF, TRAPP III acts specifically in autophagy, and is targeted to the phagophore assembly site (PAS) by Trs85p [70].

1.3.3.1 Subunits organization of TRAPP I complex

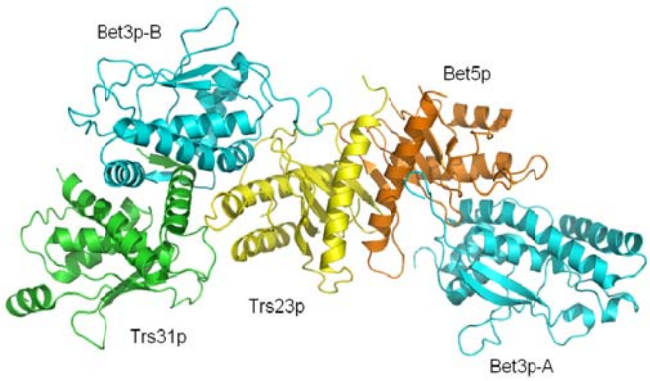
Researchers from laboratories around the world have studied the structure and organization of TRAPP subunits and subcomplexes. Through their efforts, a model of the yeast TRAPP I structure (Fig 1.3.2 d&e) can now be obtained by combining three crystal structures of TRAPP subcomplexes: 1) the heterotrimeric structure of mammalian Bet3-Trs31-sedlin (Fig 1.3.2 a, [71]), 2) the heterotetrameric structure of mammalian Bet3-Tpc6A-Bet5-Trs23 (Fig 1.3.2 b, [71]), and 3) the yeast TRAPP subcomplex Bet3p(A)-Bet5p-Tr23p-Bet3p(B)-Trs31p in complex with Ypt1p (Fig 1.3.2 c, Ypt1p not shown here in the figure, [64]).

(a)

(b)



(c)



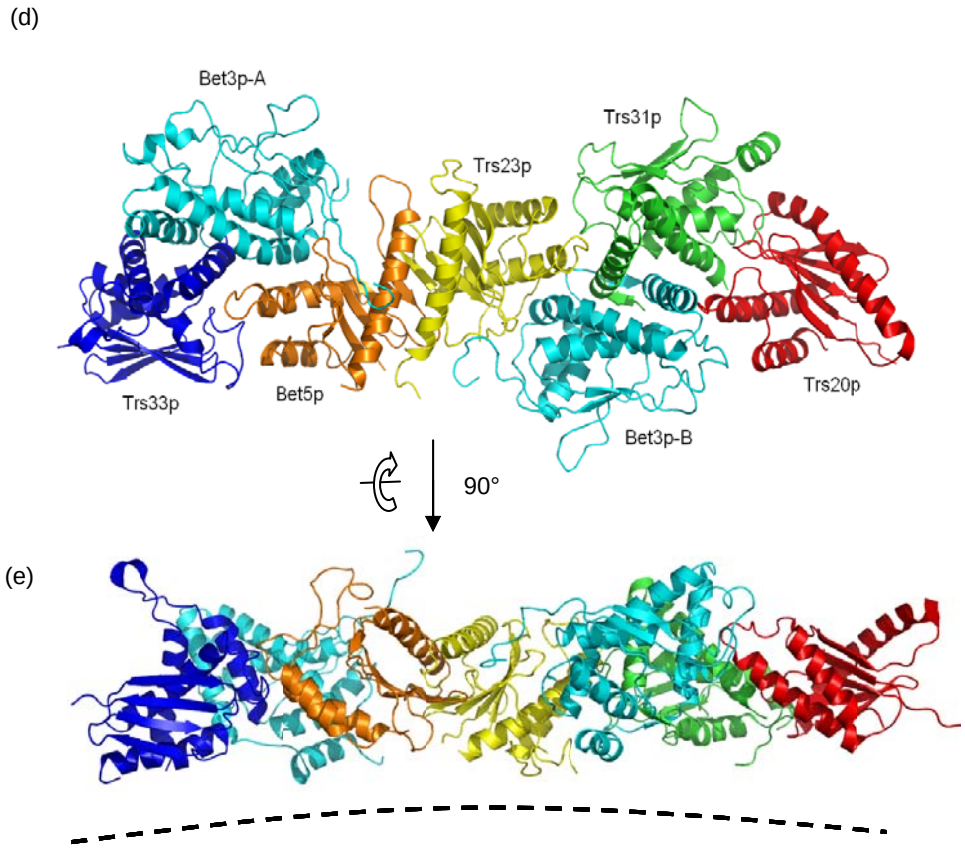


Fig 1.3.2 Structure and subunit organization of yeast TRAPP I.
(a) Mammalian Bet3-Trs31-sedlin, PDB ID 2J3W;
(b) Mammalian Bet3-Tpc6A-Bet5-Trs23, PDB ID 2J3T;
(c) Minimal subcomplex of yeast TRAPP I required for Ypt1p GEF activity, Bet3p(A)-Bet5p-Tr23p-Bet3p(B)-Trs31p, PDB ID 3CUE;
(d) Yeast TRAPP I modelled by combining (a), (b) and (c), looking from cytosolic face;
(e) Yeast TRAPP I model, side view.

In the model, yeast TRAPP I assembles into an elongated structure of ~180 Å, sitting flat on the cytosolic side of the Golgi membrane. Since no anchoring protein was identified so far for TRAPP, it is proposed to associate with the membrane through Bet3p [72-74]. In earlier studies on mammalian Bet3 protein, it was found that 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 [72]. Another interesting feature of Bet3 is a hydrophobic tunnel found near this membrane interaction interface, occupied in the crystal structure by a covalently bound palmitoyl group [74]. In the yeast TRAPP I model, the two Bet3p molecules are positioned in such a way that both their putative membrane-interacting surface and the hydrophobic tunnel are exposed on the same side. Based on current knowledge, a two-step mechanism is proposed for the membrane association of

TRAPP [74]. Firstly the initial membrane association is mediated by the positively charged residues on Bet3. This membrane association of Bet3 is probably enhanced by binding to an anchor protein or another modification of Bet3. Then the selective anchoring is accomplished through insertion of the acyl chain from a Golgi-specific moiety into the hydrophobic tunnel of Bet3. Before the acyl chain insertion can happen, the palmitoyl group would be excluded from the hydrophobic tunnel, probably through a conformational change in Bet3 [74].

1.3.3.2 Subunits organization of TRAPP II complex

The larger and less well-studied TRAPP II complex consists of all the subunits of TRAPP I and three additional proteins, the non-essential protein Trs65p, and two large subunits Trs120p and Trs130p (with a length of 1289 and 1102 AAs, respectively). Experiments on mammalian TRAPP complex found that the two large subunits on one hand shift its tethering specificity from COPII to COPI coated vesicles, presumably through direct interaction with γ1COP, a COPI coat adaptor subunit [60, 75], and on the other hand retain the Ypt/Rab GTPase GEF activity, although the identity of the target GTPase remains unclear [64-65].

Unlike TRAPP I, very little is known about the subunits organization of the TRAPP II complex. A recent publication using single-particle electron microscopic (EM) analysis of native TRAPP II isolated from yeast lysate shed some light on this question [76]. With tandem affinity purification (TAP) experiments, a full TRAPP II complex (all TRAPP subunits detected, with the only exception of Trs85p) could be extracted from yeast strains containing TAP-tagged Trs120p or Trs130p. A three-dimensional structure of TRAPP II could be reconstructed from EM images obtained from cryo-negatively stained TRAPP II, which is shown in Fig 1.3.3.

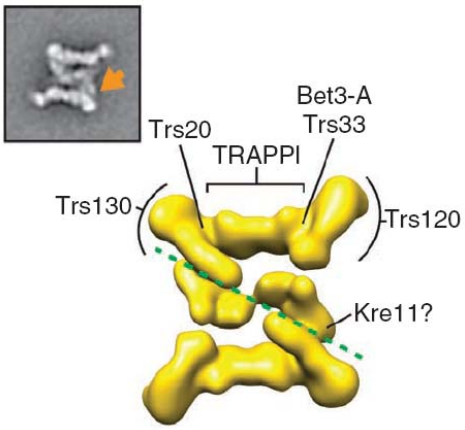


Fig 1.3.3 Subunit organization model of yeast TRAPP II [76]
Left, averaged EM image of TRAPP II; Right, 3D reconstruction in the same orientation. Orange arrow, possible position of Kre11 (Trs65p). Green line, dimer interface.

From the result, a model was proposed for TRAPP II, where the nine components form a core complex that dimerizes into a diamond-shaped structure (Fig 1.3.3). The TRAPP I subunits assemble into the normal rod-like TRAPP I complex, with two ends capped by Trs120p and Trs130p. Trs65p is required to mediate the dimerization of TRAPP II. However, the fact that Trs65p is non-essential for yeast and only found in fungi suggests that the dimeric formation of TRAPP II complex might not be necessary for its function *in vivo* [76].

1.3.3.3 Bet3 and Bet5 families of TRAPP subunits

By sequence similarity and structural features, the six smallest TRAPP subunits (all subunits of the TRAPP I complex with the exception of Trs85) fall into two protein families: the Bet3 family including Bet3, Trs33 and Trs31, and the Bet5 family including Bet5, sedlin and Trs23 (Fig 1.3.4) [71, 77].

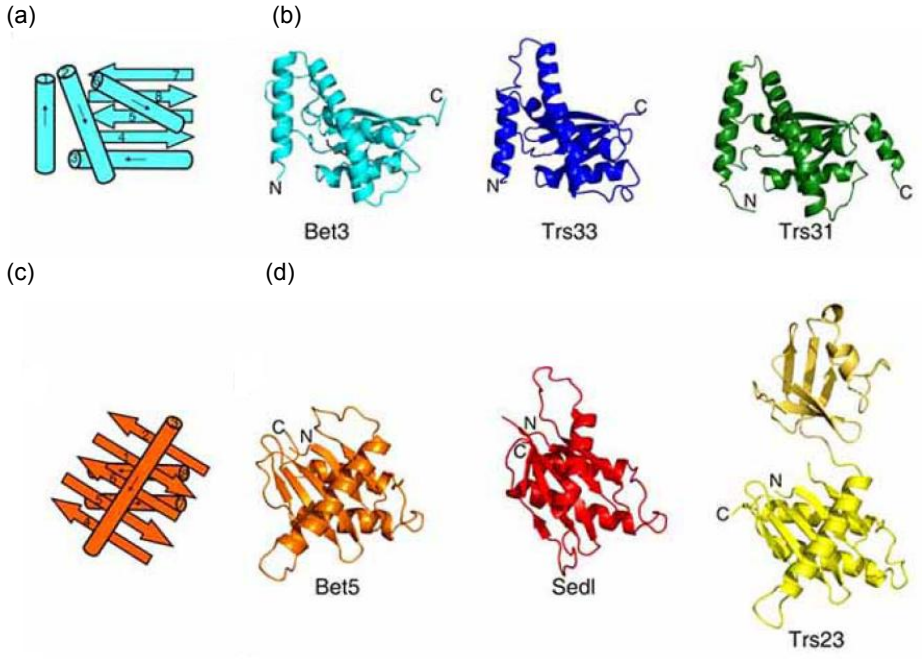


Fig 1.3.4 Bet3 and Bet5 family of TRAPP subunits, from [78].
(a) Schematic drawing of the Bet3 fold;
(b) Crystal structures of Bet3, Trs33 and Trs31:
(c) Schematic drawing of the Bet5 longin fold;
(d) The crystal structures of Bet5, sedlin and Trs23. Trs23 contains an additional PDZL domain, which is only found in metazoan Trs23 homologs.

Interestingly, the structure of the Bet5 family proteins was shown to adopt a longin fold, a protein fold commonly involved in protein binding (see 1.2.1). A 2.4 Å reso-

lution crystal structure of mouse sedlin revealed an unexpected structural similarity with the N-terminal regulatory domain of the SNAREs Ykt6p and Sec22p, despite the lack of any detectable sequence homology to these proteins [79]. This suggests a possible interaction between a TRAPP subunit and SNARE protein. However, no direct binding of TRAPP to any SNARE protein has been documented so far.

1.3.4 Regulation of tethering factors

As important factors working in tethering processes, both the coiled-coil tethers and the multi-subunit tethering complexes are believed to work under tight regulation in order to ensure the required selectivity and efficiency. However, the regulation mechanisms are often not well understood.

In the case of the coiled-coil tether p115, a conformational transition is proposed to regulate its recruitment to the Golgi and its tethering functions [80]. In this hypothetical model, the C-terminal acidic tail of the p115 coiled-coil region can bind to its own middle region and lead to a closed conformation of p115. P115 can also go into an open conformation, when it C-terminal acidic tail is bound to the Golgi proteins GM130 and/or giantin. At an open conformation, Rab1a-binding site is exposed to be involved in the tethering process. It is still not clear how this conformational change is triggered and controlled.

In mammalian cells, only one type of TRAPP can be identified by size, although there are distinct TRAPP I and TRAPP II isocomplexes identified in yeast cell. However, distinct from yeast, the mammalian TRAPP subunit Tpc6 (homologous to yeast Trs33p) has two isoforms: Tpc6A and Tpc6B. A size exclusion chromatography analysis of a HEK293 cell lysate, combined with Western blots and immunodetection using isoform-specific antibodies, showed that the elution profiles of the two Tpc6 isoforms were slightly different [81]. This finding suggests that both proteins are part of human TRAPP and thus mark two distinct TRAPP isocomplexes. Besides Tpc6, several other proteins involved in vesicular transport between the ER and the Golgi have multiple isoforms in mammals, for instance the COPII adaptor proteins Sec23/24, and the small GTPase Rab1. It is tempting to speculate that different variants of these factors assemble into different groups, each serving to transport distinct subsets of cargo or vesicles from ER to Golgi.

1.4 Objectives

To continue our group's work on TRAPP I complex, the first part of my PhD project was directed toward the less well-understood TRAPP II complex. The work on TRAPP II mainly focused on two human proteins NIBP and Ehoc-1, human orthologs of yeast Trs120p and Trs130p, respectively. The aim was to produce full-length or truncated forms of these two proteins for structural research, and study their function in TRAPP complexes.

My work was also directed toward the yeast protein Tca17, a newly identified binding partner of the yeast TRAPP complex. Tca17 was proposed to be a substoichiometric component of TRAPP II and promote the assembly and stability of TRAPP II [82]. A similar result was also reported for its human ortholog TRAPPC2L [83]. Based on sequence similarity, TRAPP2L is remotely related to the TRAPP subunit sedlin, which is grouped into the same family of TRAPP subunits as Bet5 and Trs23 [28].

As the second part of my PhD project, I focused on analyzing the structure of the yeast TRAPP associated protein Tca17 and its probable functions in regulating the organization and function of TRAPP complexes.