6 Summary / Zusammenfassung

Intracellular transport between membrane-enclosed organelles is very important for the proper functioning of all eukaryotic cells. Small molecule transport across membranes is normally mediated by transmembrane transporters or channels. As for macromolecules like proteins, the translocation through membranes can be achieved by vesicle trafficking.

The objective of the first part of my PhD project was to study the structure and organization of the mammalian TRAPP (transport protein particle) complex. TRAPP is a multi-subunit protein complex involved in tethering of vesicles to the Golgi network. In yeast, at least two different types of TRAPP were identified by their different sizes in affinity purification, the small TRAPP I and the larger TRAPP II complex. In mammalian cells only one type of TRAPP was identified so far, with a subunit composition similar to yeast TRAPP II. To continue our group's work on TRAPP I complex, I studied the less well-understood TRAPP II complex, mainly focussing on the two proteins NIBP and Ehoc-1, human orthologs of yeast Trs120p and Trs130p, respectively. A fragment of Ehoc-1 could be produced as recombinant protein in *E. coli*, but could not be studied further with X-ray crystallography, because suitable crystals could not be grown.

Based on co-immunoprecipitation experiments on NIBP and Ehoc-1 fragments and the yeast TRAPP II model, a preliminary model for the mammalian TRAPP complex could be proposed. In this model, the central TRAPP-I like subcomplex is capped on both ends by NIBP and Ehoc-1 and forms a triangular structure. This structure may be further stabilized by interactions between conserved peptide segments from the Ehoc-1 N-terminus and the NIBP C-terminus.

In the second part of this work, a formerly little known yeast TRAPP-associated protein, Tca17, was studied using biochemical and biophysical methods, as well as X-ray crystallography. Though not previously identified as a TRAPP subunit, Tca17 as well as its mammalian ortholog TRAPPC2L was proposed to be a sub-stoichiometric component of TRAPP II and promote the assembly and stability of TRAPP complexes. The crystal structure at 1.8 Å resolution shows that Tca17 adopts the longin fold characteristic for the Bet5 subfamily of small TRAPP subunits. This fold is composed of a central β-sheet formed by five antiparallel β-strands flanked by one α-helix on one side (α1) and two α-helices on the other side (α2, α3). A disulfide–linked dimer-like arrangement was observed in the crystal structure of Tca17. In order to

clarify its oligomerization state in solution, several biophysical experiments were performed. It was found that a small fraction (< 5%) of Tca17 dimerizes in non-reducing buffer, whereas it stays predominantly monomeric in reducing buffer, resembling the reducing environment of the cellular cytoplasm, its natural localization *in vivo*.

On the sequence and structure level, Tca17 is most closely related to the TRAPP subunit Trs20p/sedlin. It can bind the TRAPP subunits Bet3p and Trs33p *in vitro*, and might regulate the function of TRAPP by transiently integrating into TRAPP. It remains unclear how the integration of Tca17 into TRAPP might be controlled and promoted. If Tca17 were integrated into TRAPP in place of sedlin/Trs20p, the membrane association of TRAPP might be expected to be weakened, since Tca17 would introduce a negatively charged patch into the presumed membrane association interface of TRAPP.

Der intrazelluläre Transport zwischen membranumschlossenen Organellen ist essentiell für die einwandfreie Funktion aller eukaryontischen Zellen. Der Transport kleiner Moleküle durch Membranen wird üblicher Weise durch transmembrane Transportproteine oder Kanäle vermittelt. Der Transport von Makromolekülen, zum Beispiel Proteinen, zwischen Membranen jedoch, wird durch den Vesikeltransport bewerkstelligt.

Die Zielsetzung des ersten Teils meiner Arbeit befasste sich mit der Untersuchung der Struktur und des Aufbaus des TRAPP (*transport protein particle*)-Komplexes aus Säugetieren. TRAPP ist ein multimerer Anheftungskomplex, welcher die Anheftung von Vesikeln an das Golgi-Netzwerk vermittelt. In Hefe wurden mindestens zwei unterschiedliche Arten des TRAPP-Komplexes aufgrund ihrer Größe durch Affinitätsreinigung identifiziert, der kleinere TRAPP I- und der größere TRAPP II-Komplex. In Säugetierzellen wurde bisher nur eine Art des TRAPP-Komplexes identifiziert, mit einer Organisation der Untereinheiten, welche dem TRAPP II-Komplex der Hefe ähnt. Im Zuge der fortführenden Arbeiten zum TRAPP I-Komplex in unserer Arbeitsgruppe, untersuchte ich den bisher wenig charaketrisierten TRAPP II-Komplex mit Schwerpunkt bei den humanen Proteinen NIBP und Ehoc-1, den orthologen Proteinen zu Trs120p und Trs130p aus Hefe. Ein Fragment des Proteins Ehoc-1 konnte als rekombinantes Protein aus *E. coli* gewonnen, jedoch nicht weiter für die Röntgenstrukturanalyse verwendet werden, da keine brauchbaren Kristalle erhalten wurden.

Analysen von NIBP- und Ehoc-1-Protein-Konstrukten mittels Co-Immunopräzipitation in Verbindung mit einem publizierten Modell von TRAPP II aus Hefe, erlaubten, ein vorläufiges Modell des TRAPP-Komplexes aus Säugerzellen vorzuschlagen. Dieses Modell postuliert, dass der zentrale TRAPP I-ähnliche Subkomplex durch NIBP und Ehoc-1 an seinen beiden Enden bedeckt wird und eine dreischenkelige Struktur bildet. Interaktionen zwischen konservierten Peptidbereichen nahe dem N-Terminus von Ehoc-1 und dem C-Terminus von NIBP könnten diesen Aufbau zudem stabilisieren.

Der zweite Teil meiner Arbeit befasste sich mit dem bisher wenig bekannten TRAPP-assoziierten Protein Tca17. Zur Charakterisierung des Proteins wurchen biochemische und biophysikalische Methoden sowie die Röntgenstrukturanalyse herangezogen. Obwohl Tca17 nicht ursprünglich als TRAPP-Untereinheit identifiziert wurde, wurde es zusammen mit dem homologen TRAPPC2L aus Säugern als unterstöchiometrische Komponente des TRAPP II-Komplexes vorgeschlagen,

welches den Aufbau und die Stabilität von TRAPP vermittelt. Die Kristallstruktur bei einer Auflösung von 1,8 Å zeigt einen *Longin*-Faltungstyp des Proteins Tca17, welcher charakteristisch ist für die Bet5-Unterfamilie der kleinen TRAPP-Untereinheiten. Der Aufbau besteht aus einem fünfstängigen antiparallelen β-Faltblatt, flankiert von einer α-Helix auf einer Seite des Faltblatts (α1) und zwei weiteren α-Helices auf der anderen Seite (α2, α3). Ein durch eine Disulfidbrücke vermittelter dimer-ähnlicher Aufbau konnte für Tca17 im Kristall beobachtet werden. Um den tatsächlichen Oligomeren-Zustand des Proteins in Lösung zu untersuchen, fanden unterschiedliche biophysikalische Methoden Anwendung. Es zeigte sich, dass ein kleiner Anteil des Proteins (< 5%) Tca17 nur in nicht reduzierenden Puffersystemen dimerisiert, wobei es hauptsächlich monomer in reduzierenden Puffern auftritt, welche dem reduzierenden Medium des Zytoplasmas entspricht, der natürlichen Umgebung des Tca17 *in vivo*.

Sowohl strukturell, als auch in seiner Sequenzähnlichkeit ist Tca17 der TRAPP-Untereinheit Trs20p/Sedlin am nächsten verwandt. Es interagiert mit den TRAPP-Untereinheiten Bet3p und Trs33p *in vitro*, und könnte die Funktion des TRAPP-Komplexes durch transiente Anbindung an den Komplex regulieren. Obwohl die Art der Anbindung von Tca17 und deren Regulierung an TRAPP weiterhin unklar ist, erscheint es möglich dass eine Bindung an TRAPP Auswirkungen auf die Membran-Assoziation hat. So könnte der Ausstauch von Trs20p/Sedlin durch Tca17 negativ geladene Oberflächenregionen in TRAPP einführen, welche die Membran-Assoziation schwächen.

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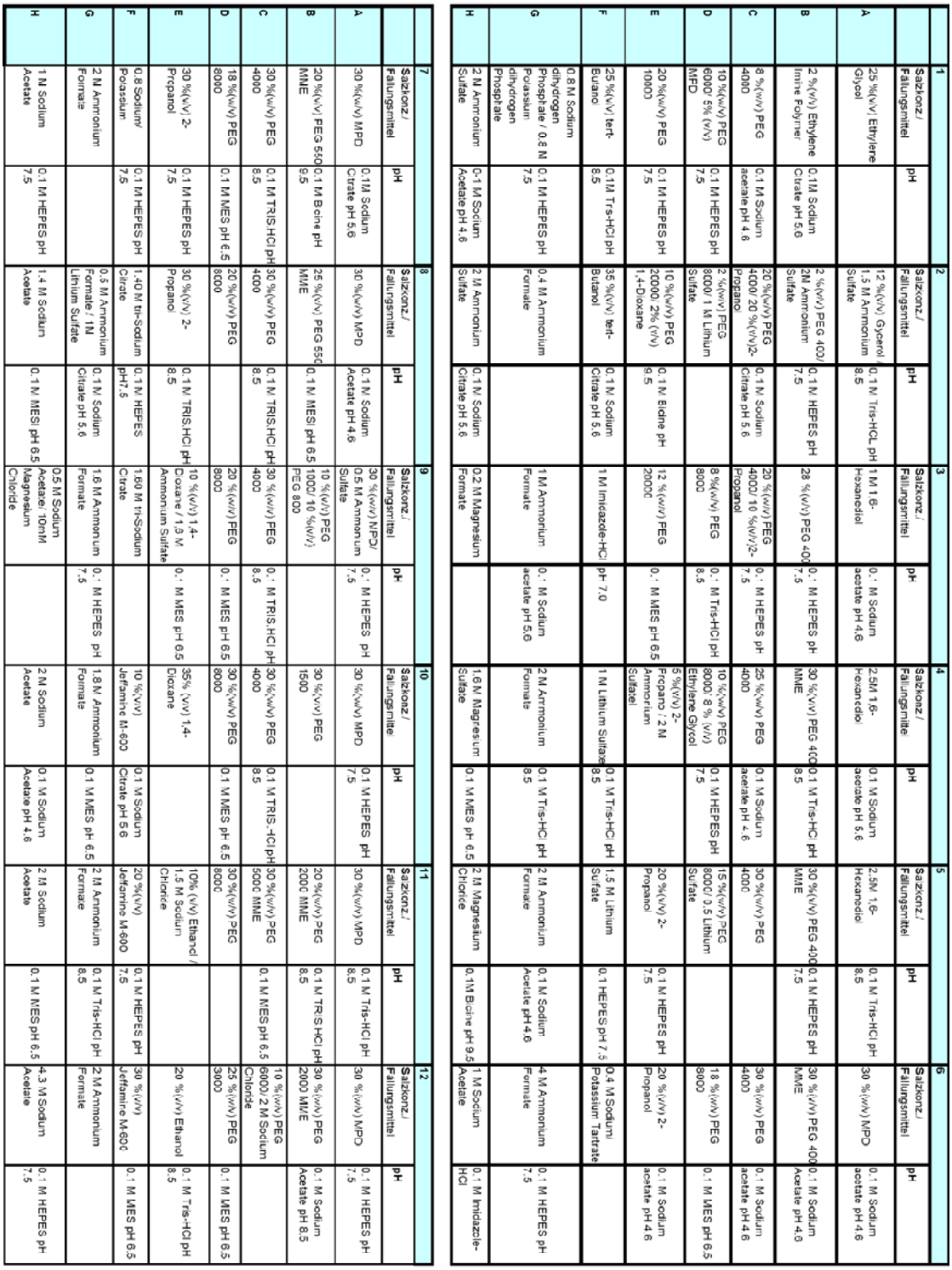
Appendix B: Crystallization screen formulations

JBS classic (JBScreen Basic 1-4)



	1		2		3		4		5		6	
	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH
A	25 %(v/v) Ethylene Glycol		12 %(v/v) Glycerol / 1.5 M Ammonium Sulfate	0.1 M Tris-HCL pH 8.5	1 M 1,6-Hexanediol	0.1 M Sodium acetate pH 4.6	2.5M 1,6-Hexanediol	0.1 M Sodium acetate pH 5.6	2.5M 1,6-Hexanediol	0.1 M Tris-HCl pH 8.5	30 %(v/v) MPD	0.1 M Sodium acetate pH 4.6
B	2 %(v/v) Ethylene Imine Polymer	0.1M Sodium Citrate pH 5.6	2 %(v/v) PEG 400/ 2M Ammonium Sulfate	0.1 M HEPES pH 7.5	28 %(v/v) PEG 400	0.1 M HEPES pH 7.5	30 %(v/v) PEG 400/ MME	0.1 M Tris-HCl pH 8.5	30 %(v/v) PEG 400/ MME	0.1 M HEPES pH 7.5	30 %(v/v) PEG 400/ MME	0.1 M Sodium Acetate pH 4.6
C	8 %(w/v) PEG 4000	0.1 M Sodium acetate pH 4.6	20 %(w/v) PEG 4000/ 20 %(v/v) 2-Propanol	0.1 M Sodium Citrate pH 5.6	20 %(w/v) PEG 4000/ 10 %(v/v) 2-Propanol	0.1 M HEPES pH 7.5	25 %(w/v) PEG 4000	0.1 M Sodium acetate pH 4.6	30 %(w/v) PEG 4000		30 %(w/v) PEG 4000	0.1 M Sodium acetate pH 4.6
D	10 %(w/v) PEG 6000/ 5% (v/v) MPD	0.1 M HEPES pH 7.5	2 %(w/v) PEG 8000/ 1 M Lithium Sulfate		8 %(w/v) PEG 8000	0.1 M Tris-HCl pH 8.5	10 %(w/v) PEG 8000/ 8 % (v/v) Ethylene Glycol	0.1 M HEPES pH 7.5	15%(w/v) PEG 8000/ 0.5 Lithium Sulfate		18 %(w/v) PEG 8000	0.1 M MES pH 6.5
E	20 %(v/v) PEG 1000	0.1 M HEPES pH 7.5	10 %(w/v) PEG 2000/ 2% (v/v) 1,4-Dioxane	0.1 M Bicine pH 9.5	12 %(w/v) PEG 2000	0.1 M MES pH 6.5	5 %(v/v) 2-Propanol / 2 M Ammonium Sulfate		20 %(v/v) 2-Propanol	0.1 M HEPES pH 7.5	20 %(v/v) 2-Propanol	0.1 M Sodium acetate pH 4.6
F	25 %(v/v) tert-Butanol	0.1M Tris-HCl pH 8.5	35 %(v/v) tert-Butanol	0.1 M Sodium Citrate pH 5.6	1 M Imidazole-HCl	pH 7.0	1 M Lithium Sulfate	0.1 M Tris-HCl pH 8.5	1.5 M Lithium Sulfate	0.1 M HEPES pH 7.5	0.4 M Sodium Potassium Tartrate	
G	0.8 M Sodium dihydrogen Phosphate / 0.8 M Potassium dihydrogen Phosphate	0.1 M HEPES pH 7.5	0.4 M Ammonium Formate		1 M Ammonium Formate	0.1 M Sodium acetate pH 5.6	2 M Ammonium Formate	0.1 M Tris-HCl pH 8.5	2 M Ammonium Formate	0.1 M Sodium Acetate pH 4.6	4 M Ammonium Formate	0.1 M HEPES pH 7.5
H	2 M Ammonium Sulfate	0.1 M Sodium Acetate pH 4.6	2 M Ammonium Sulfate	0.1 M Sodium Citrate pH 5.6	0.2 M Magnesium Formate		1.6 M Magnesium Sulfate	0.1 M MES pH 6.5	2 M Magnesium Chloride	0.1M Bicine pH 9.5	1 M Sodium Acetate	0.1 M Imidazole-HCl

	7		8		9		10		11		12	
	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH
A	30 %(w/v) MPD	0.1M Sodium Citrate pH 5.6	30 %(w/v) MPD	0.1 M Sodium Acetate pH 4.6	30 %(w/v) NPG/ 0.5 M Ammonium Sulfate	0.1 M HEPES pH 7.5	30 %(w/v) MPD	0.1 M HEPES pH 7.5	30 %(w/v) MPD	0.1 M Tris-HCl pH 8.5	30 %(w/v) MPD	0.1 M HEPES pH 7.5
B	20 %(v/v) PEG 550 MME	0.1 M Bicine pH 9.5	25 %(v/v) PEG 550 MME	0.1 M MES pH 6.5	10 %(w/v) PEG 1000/ 10 %(w/v) PEG 800		30 %(v/v) PEG 1500		20 %(w/v) PEG 2000 MME	0.1 M TRIS-HCl pH 8.5	30 %(w/v) PEG 2000 MME	0.1 M Sodium Acetate pH 8.5
C	30 %(w/v) PEG 4000	0.1 M TRIS-HCl pH 8.5	30 %(w/v) PEG 4000	0.1 M TRIS-HCl pH 8.5	30 %(w/v) PEG 4000	0.1 M TRIS-HCl pH 8.5	30 %(w/v) PEG 8000	0.1 M TRIS-HCl pH 8.5	30 %(w/v) PEG 5000 MME	0.1 M MES pH 6.5	10 %(w/v) PEG 6000/ 2 M Sodium Chloride	
D	18 %(w/v) PEG 8000	0.1 M MES pH 6.5	20 %(w/v) PEG 8000		20 %(w/v) PEG 8000	0.1 M MES pH 6.5	30 %(w/v) PEG 8000	0.1 M MES pH 6.5	30 %(w/v) PEG 8000		25 %(w/v) PEG 3000	0.1 M MES pH 6.5
E	30 %(v/v) 2-Propanol	0.1 M HEPES pH 7.5	30 %(v/v) 2-Propanol	0.1 M TRIS-HCl pH 8.5	10 %(v/v) 1,4-Dioxane / 1.5 M Ammonium Sulfate	0.1 M MES pH 6.5	35% (v/v) 1,4-Dioxane		10% (v/v) Ethanol / 1.5 M Sodium Chlorice		20 %(v/v) Ethanol	0.1 M Tris-HCl pH 8.5
F	0.8 Sodium/ Potassium	0.1 M HEPES pH 7.5	1.40 M tri-Sodium Citrate	0.1 M HEPES pH7.5	1.60 M tri-Sodium Citrate		10 %(v/v) Jeffamine M-600	0.1 M Sodium Citrate pH 5.6	20 %(v/v) Jeffamine M-600	0.1 M HEPES pH 7.5	30 %(v/v) Jeffamine M-600	0.1 M MES pH 6.5
G	2 M Ammonium Formate		0.5 M Ammonium Formate/ 1N Lithium Sulfate	0.1 M Sodium Citrate pH 5.6	1.6 M Ammonium Formate		1.6 M Ammonium Formate	0.1 M MES pH 6.5	2 M Ammonium Formate	0.1 M Tris-HCl pH 8.5	2 M Ammonium Formate	
H	1 M Sodium Acetate	0.1 M HEPES pH 7.5	1.4 M Sodium Acetate	0.1 M MES pH 6.5	0.5 M Sodium Acetate/ 10mM Magnesium Chloride		2 M Sodium Acetate	0.1 M Sodium Acetate pH 4.6	2 M Sodium Acetate	0.1 M MES pH 6.5	4.3 M Sodium Acetate	0.1 M HEPES pH 7.5

	1	2	3	4	5	6
	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH
A	2 M Ammonium sulfate	0.1 M Citric acid pH 3.5	2 M Ammonium sulfate	0.1 M BIS-TRIS pH 5.5	2 M Ammonium sulfate	0.1 M BIS-TRIS pH 6.5	2 M Ammonium sulfate	0.1 M HEPES pH 7.5	2 M Ammonium sulfate	0.1 M TRIS pH 8.5
B	0.3 M Magnesium formate	0.1 M BIS-TRIS pH 5.5	0.5 M Magnesium formate	0.1 M BIS-TRIS pH 6.5	0.1 M HEPES pH 7.5	0.5 M Magnesium formate	0.1 M TRIS pH 8.5	0.3 M Magnesium formate	1.25 M Sodium phosphate	0.14 M Potassium phosphate	0.49 M Sodium phosphate	0.91 M Potassium phosphate
C		3.5 M Sodium formate pH 7.0		1.1 M Ammonium tartrate pH 7.0		2.4 M Sodium malonate pH 7.0		0.56 M Sodium citrate pH 7.0		0.96 M Sodium citrate pH 7.0	0.1 M Sodium chloride / 1.5 M Ammonium sulfate	0.1 M BIS-TRIS pH 6.5
D	25 %(w/v) PEG 1500	30 %(v/v) Jeffamine M-600 pH 7.0	30 %(v/v) Jeffamine M-600 pH 7.0	0.1 M HEPES pH 7.0	30 %(v/v) Jeffamine EDR-2001 pH 7.0	0.1 M HEPES pH 7.0	25 %(w/v) PEG 3350	0.1 M Citric acid pH 3.5	25 %(w/v) PEG 3350	0.1 M Sodium acetate pH 4.5	25 %(w/v) PEG 3350	0.1 M BIS-TRIS pH 5.5
E	0.2 M Calcium chloride/45 %(v/v) MPD	0.1 M BIS-TRIS pH 6.5	0.2 M Ammonium acetate/ 45 %(v/v) MPD	0.1 M BIS-TRIS pH 6.5	0.2 M Ammonium acetate/ 45 %(v/v) MPD	0.1 M HEPES pH 7.5	0.2 M Ammonium acetate/ 45 %(v/v) MPD	0.1 M TRIS pH 8.5	0.2 M Ammonium chloride/ 30 %(v/v) PEG 550 MME	0.1 M BIS-TRIS pH 6.5
F	0.2 M L-Proline / 10 %(w/v) PEG 3350	0.1 M HEPES pH 7.5	0.2 M TMAO / 20 %(w/v) PEG 2000 MME	0.1 M TRIS pH 8.5	0.064 M Sodium citrate pH 7.0 / 10 %(w/v) PEG 5000 MME	0.1 M HEPES pH 7.0	0.005 M Cobalt chloride/0.005 M Magnesium chloride / 12 %(w/v) PEG 3350/0.005 M Nickel chloride/	0.1 M HEPES pH 7.5	0.1 M Ammonium acetate/17 %(w/v) PEG 10,000	0.1 M BIS-TRIS pH 5.5	0.2 M Ammonium sulfate/25 %(w/v) PEG 3350	0.1 M BIS-TRIS pH 5.5
G	0.2 M Sodium chloride/25 %(w/v) PEG 3350	0.1 M TRIS pH 8.5	0.2 M Lithium sulfate/25 %(w/v) PEG 3350	0.1 M BIS-TRIS pH 5.5	0.2 M Lithium sulfate/25 %(w/v) PEG 3350	0.1 M HEPES pH 7.5	0.2 M Lithium sulfate/25 %(w/v) PEG 3350	0.1 M TRIS pH 8.5	0.2 M Ammonium acetate/25 %(w/v) PEG 3350	0.1 M BIS-TRIS pH 5.5
H	0.2 M Magnesium chloride/25 %(w/v) PEG 3350	0.1 M TRIS pH 8.5	20 %(w/v) PEG 3350	0.2 M Potassium Sodium tartrate	20 %(w/v) PEG 3350	0.2 M Sodium malonate pH 7.0	20 %(w/v) PEG 3350	0.2 M Ammonium citrate pH 7.0	15 %(w/v) PEG 3350	0.1 M Succinic acid pH 7.0	20 %(w/v) PEG 3350	0.2 M Sodium formate

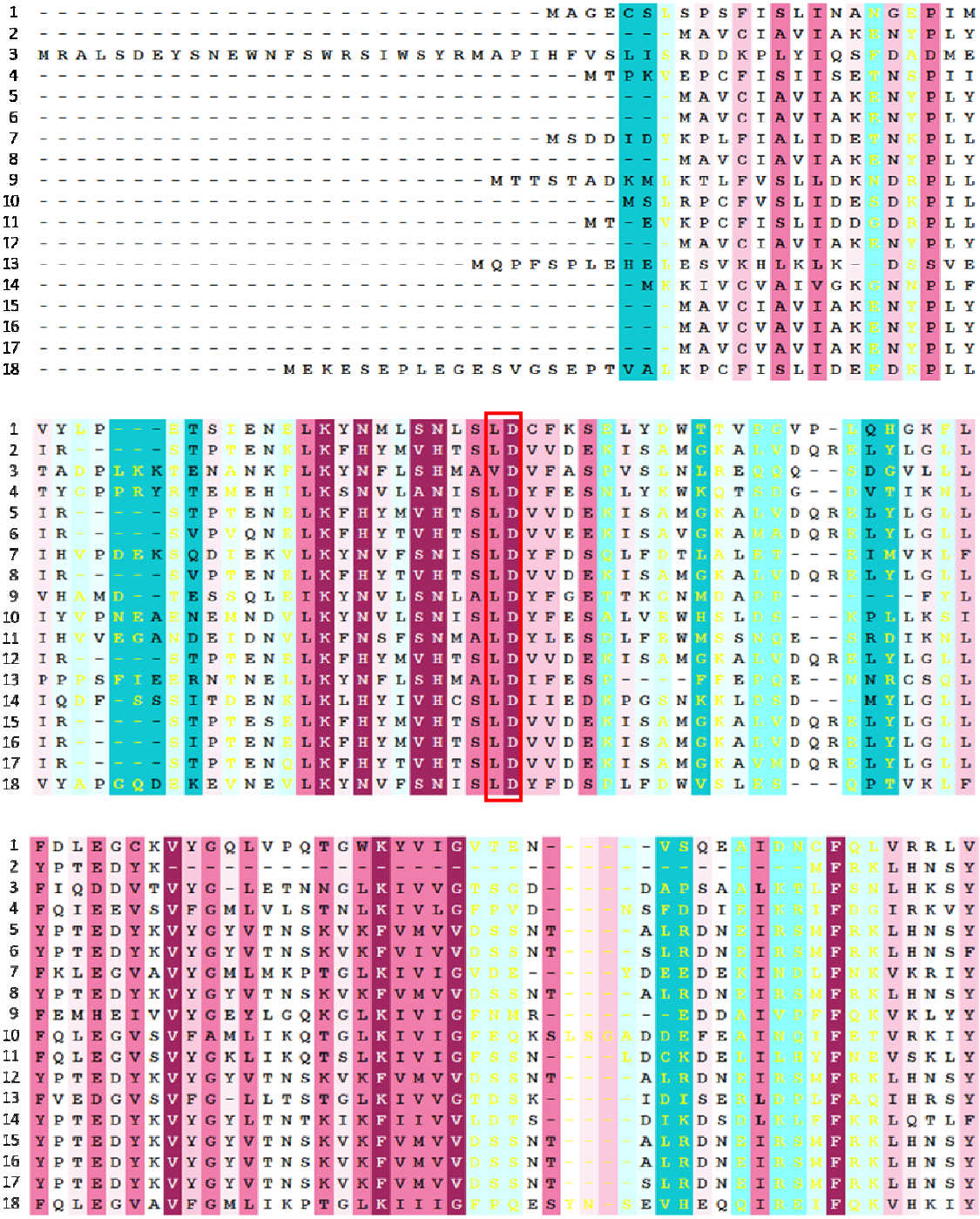
	7	8	9	10	11	12
	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH
A	3 M Sodium chloride	0.1 M Citric acid pH 3.5	3 M Sodium chloride	0.1 M BIS-TRIS pH 5.5	3 M Sodium chloride	0.1 M BIS-TRIS pH 6.5	3 M Sodium chloride	0.1 M HEPES pH 7.5	3 M Sodium chloride	0.1 M TRIS pH 8.5
B	1.344 M Potassium phosphate	0.056 M Sodium phosphate	1.4 M Sodium citrate	0.1 M HEPES pH 7.5	1.8 M Ammonium citrate pH 7.0	0.8 M Succinic acid pH 7.0	2.1 M DL-Malic acid pH 7.0	2.8 M Sodium acetate pH 7.0
C	0.8 M Sodium/Potassium tartrate /0.5 %(v/v) PEG 6000 MME	0.1 M TRIS pH 8.5	1 M Ammonium sulfate / %(w/v) PEG 3350	0.1 M BIS-TRIS pH 5.5	malonate pH 7.0/3.5 %(v/v) Jeffamine EDR-2101 pH 7.0	0.1 M HEPES pH 7.0	1 M Succinic acid pH 7.0/1 %(v/v) PEG 2000 MME	0.1 M HEPES pH 7.0	1 M Ammonium sulfate/ 0.5 %(v/v) PEG 8000	0.1 M HEPES pH 7.0	0.191 M Sodium citrate pH 7.0/ 2 %(w/v) PEG 3350	0.1 M HEPES pH 7.0
D	25 %(w/v) PEG 3350	0.1 M BIS-TRIS pH 6.5	25 %(w/v) PEG 3350	0.1 M HEPES pH 7.5	25 %(w/v) PEG 3350	0.1 M TRIS pH 8.5	20 %(w/v) PEG 5000 MME	0.1 M BIS-TRIS pH 6.5	28 %(w/v) PEG 2000 MME	0.1 M BIS-TRIS pH 6.5	0.2 M Calcium chloride/45 %(v/v) MPD	0.1 M BIS-TRIS pH 5.5
E	0.05 M Magnesium chloride / 30 %(v/v) PEG 550 MME	0.1 M HEPES pH 7.5	0.2 M Potassium chloride / 35 %(v/v) Pentaerythritol propoxylate	0.05 M HEPES pH 7.5	0.02 M Ammonium sulfate / 30 %(v/v) Pentaerythritol ethoxylate	0.05 M BIS-TRIS pH 6.5	45 %(v/v) PEG P 400	0.1 M BIS-TRIS pH 6.5	0.02 M Magnesium chloride / 22 %(w/v) Sodium Polyacrylate 5100	0.1 M HEPES pH 7.5	0.01 M Cobalt chloride / 20 %(w/v) PVP K15	0.1 M TRIS pH 8.5
F	0.2 M Ammonium sulfate/25 %(w/v) PEG 3350	0.1 M BIS-TRIS pH 6.5	0.2 M Ammonium sulfate/25 %(w/v) PEG 3350	0.1 M HEPES pH 7.5	0.2 M Ammonium sulfate/25 %(w/v) PEG 3350	0.1 M TRIS pH 8.5	0.2 M Sodium chloride/25 %(w/v) PEG 3350	0.1 M BIS-TRIS pH 6.5	0.2 M Sodium chloride/25 %(w/v) PEG 3350	0.1 M BIS-TRIS pH 6.5	0.2 M Sodium chloride/25 %(w/v) PEG 3350	0.1 M HEPES pH 7.5
G	0.2 M Ammonium acetate/25 %(w/v) PEG 3350	0.1 M BIS-TRIS pH 6.5	0.2 M Ammonium acetate/25 %(w/v) PEG 3350	0.2 M Ammonium chloride / 30 %(v/v) PEG 3350	0.2 M Magnesium chloride/25 %(w/v) pH 6.5	0.1 M BIS-TRIS pH 6.5	0.2 M Magnesium chloride/25 %(w/v) PEG 3350	0.1 M BIS-TRIS pH 6.5	0.2 M Magnesium chloride/25 %(w/v) PEG 3350	0.1 M BIS-TRIS pH 6.5	0.2 M Magnesium chloride/25 %(w/v) PEG 3350	0.1 M HEPES pH 7.5
H	20 %(w/v) PEG 3350	0.15 M DL-Malic acid pH 7.0	15 %(w/v) PEG 3350	0.1 M Magnesium formate	20 %(w/v) PEG 3350	0.05 M Zinc acetate	20 %(w/v) PEG 3350	0.2 M Sodium citrate	30 %(w/v) PEG 2000 MME	0.1 M Potassium thiocyanate	30 %(w/v) PEG 2000 MME	0.15 M Potassium bromide

PEGs suite

	1	2	3	4	5	6
	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH
A	40 %(v/v) PEG 200	0.1 M HEPES pH 7.5	30 %(v/v) PEG 300	0.1 M Sodium acetate pH 4.6	30 %(v/v) PEG 400	0.1 M Sodium acetate pH 4.6	25 %(v/v) PEG 1000	0.1 M Sodium acetate pH 4.6	25 %(v/v) PEG 2000 MME	0.1 M Sodium acetate pH 4.6
B	40 %(v/v) PEG 200	0.1M Na-Hepes pH 7.5	30 %(v/v) PEG 300	0.1 M HEPES pH 7.5	30 %(v/v) PEG 400	0.1 M HEPES pH 7.5	25 %(v/v) PEG 550 MME	0.1 M HEPES pH 7.5	25 %(v/v) PEG 1000	0.1 M HEPES pH 7.5	25 %(v/v) PEG 2000 MME	0.1 M HEPES pH 7.5
C	25 %(v/v) PEG 3000	0.1 M Sodium acetate pH 4.6	25 %(v/v) PEG 4000	0.1 M Sodium acetate pH 4.6	25 %(v/v) PEG 6000	0.1 M Sodium acetate pH 4.6	25 %(v/v) PEG 8000	0.1 M Sodium acetate pH 4.6	20 %(v/v) PEG 10000	0.1 M Sodium acetate pH 4.6	15 %(v/v) PEG 20000	0.1 M Sodium acetate pH 4.6
D	25 %(v/v) PEG 3000	0.1 M HEPES pH 7.5	25 %(v/v) PEG 4000	0.1 M HEPES pH 7.5	25 %(v/v) PEG 6000	0.1 M HEPES pH 7.5	25 %(v/v) PEG 8000	0.1 M HEPES pH 7.5	20 %(v/v) PEG 10000	0.1 M HEPES pH 7.5	15 %(v/v) PEG 20000	0.1 M HEPES pH 7.5
E	20 %(v/v) PEG 3350	0.2 M Sodium fluorice	20 %(v/v) PEG 3350	0.2 M Potassium fluoride	20 %(v/v) PEG 3350	0.2 M Ammonium fluoride	20 %(v/v) PEG 3350	0.2 M Lithium chloride	20 %(v/v) PEG 3350	0.2 M Magnesium chloride	20 %(v/v) PEG 3350	0.2 M Sodium chloride
F	20 %(v/v) PEG 3350	0.2 M Sodium thiocyanate	20 %(v/v) PEG 3350	0.2 M Potassium thiocyanate	20 %(v/v) PEG 3350	0.2 M Lithium nitrate	20 %(v/v) PEG 3350	0.2 M Magnesium nitrate	20 %(v/v) PEG 3350	0.2 M Sodium nitrate	20 %(v/v) PEG 3350	0.2 M Potassium nitrate
G	20 %(v/v) PEG 3350	0.2 M Magnesium acetate	20 %(v/v) PEG 3350	0.2 M Zinc acetate	20 %(v/v) PEG 3350	0.2 M Sodium acetate	20 %(v/v) PEG 3350	0.2 M Calcium acetate	20 %(v/v) PEG 3350	0.2 M Potassium acetate	20 %(v/v) PEG 3350	0.2 M Ammonium acetate
H	20 %(v/v) PEG 3350	0.2 M K/Na tartrate	20 %(v/v) PEG 3350	0.2 M di-Ammonium tartrate	20 %(v/v) PEG 3350	0.2 M di-Sodium phosphate	20 %(v/v) PEG 3350	0.2 M di-Sodium phosphate	0.1 M Na-Hepes pH 7.5	20 %(v/v) PEG 3350	0.2 M di-Potassium phosphate	0.2 M di-Ammonium tartrate

	7	8	9	10	11	12
	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH	Salzkonz./ Fällungsmittel	pH
A	40 %(v/v) PEG 200	0.1 M MES pH 6.5	30 %(v/v) PEG 300	0.1 M MES pH 6.5	30 %(v/v) PEG 400	0.1 M MES pH 6.5	25 %(v/v) PEG 550 MME	0.1 M MES pH 6.5	25 %(v/v) PEG 1000	0.1 M MES pH 6.5	25 %(v/v) PEG 2000 MME	0.1 M MES pH 6.5
B	40 %(v/v) PEG 200	0.1 M TRIS HCl pH 8.5	30 %(v/v) PEG 300	0.1 M TRIS-HCl pH 8.5	30 %(v/v) PEG 400	0.1 M TRIS HCl pH 8.5	25 %(v/v) PEG 550 MME	0.1 M TRIS-HCl pH 8.5	25 %(v/v) PEG 1000	0.1 M TRIS-HCl pH 8.5	25 %(v/v) PEG 2000 MME	0.1 M TRIS-HCl pH 8.5
C	25 %(v/v) PEG 3000	0.1 M MES pH 6.5	25 %(v/v) PEG 4000	0.1 M MES pH 6.5	25 %(v/v) PEG 6000	0.1 M MES pH 6.5	25 %(v/v) PEG 8000	0.1 M MES pH 6.5	20 %(v/v) PEG 10000	0.1 M MES pH 6.5	15 %(v/v) PEG 20000	0.1 M MES pH 6.5
D	25 %(v/v) PEG 3000	0.1 M TRIS HCl pH 8.5	25 %(v/v) PEG 3000	0.1 M TRIS-HCl pH 8.5	25 %(v/v) PEG 3000	0.1 M TRIS HCl pH 8.5	25 %(v/v) PEG 3000	0.1 M TRIS-HCl pH 8.5	25 %(v/v) PEG 3000	0.1 M TRIS-HCl pH 8.5	25 %(v/v) PEG 3000	0.1 M TRIS-HCl pH 8.5
E	20 %(v/v) PEG 3350	0.2 M Calcium chloride	20 %(v/v) PEG 3350	0.2 M Potassium chloride	20 %(v/v) PEG 3350	0.2 M Ammonium chloride	20 %(v/v) PEG 3350	0.2 M Sodium iodide	20 %(v/v) PEG 3350	0.2 M Potassium iodide	20 %(v/v) PEG 3350	0.2 M Ammonium iodide
F	20 %(v/v) PEG 3350	0.2 M Ammonium nitrate	20 %(v/v) PEG 3350	0.2 M Magnesium formate	20 %(v/v) PEG 3350	0.2 M Sodium formate	20 %(v/v) PEG 3350	0.2 M Potassium formate	20 %(v/v) PEG 3350	0.2 M Ammonium formate	20 %(v/v) PEG 3350	0.2 M Lithium acetate
G	20 %(v/v) PEG 3350	0.2 M Lithium sulfate	20 %(v/v) PEG 3350	0.2 M Magnesium sulfate	20 %(v/v) PEG 3350	0.2 M Sodium sulfate	20 %(v/v) PEG 3350	0.2 M Potassium sulfate	20 %(v/v) PEG 3350	0.2 M Ammonium sulfate	20 %(v/v) PEG 3350	0.2 M di-Sodium tartrate
H	20 %(v/v) PEG 3350	0.2 M Ammonium phosphate	20 %(v/v) PEG 3350	0.2 M di-Ammonium phosphate	20 %(v/v) PEG 3350	0.2 M Tri-Lithium citrate	20 %(v/v) PEG 3350	0.2 M tri-Sodium citrate	20 %(v/v) PEG 3350	0.2 M tri-Potassium citrate	20 %(v/v) PEG 3350	0.2 M di-Ammonium citrate

Appendix C: Multiple sequence alignment



Variable Average Conserved

Tca17 orthologs included in alignment:

1. Q753D1: Hypothetical protein from *Ashbya gossypii* (yeast).
2. Q5RBK9: TRAPPC2L from *Pongo abelii*.
3. C4XWA9: Hypothetical protein from *Clavispora lusitaniae* (yeast).
4. Q6FLS1: Tca17-like protein from *Candida glabrata* (yeast).
5. Q9UL33: TRAPPC2L from *Homo sapiens*.
6. B5XGE7: TRAPPC2L from *Salmo salar*.
7. A7TN14: Hypothetical protein from *Vanderwaltozyma polyspora*.
8. B5FXJ6: TRAPPC2L from *Taeniopygia guttata*.
9. Q6CJQ8: Hypothetical protein from *Kluyveromyces lactis* (yeast).
10. P32613: Tca17 from *Saccharomyces cerevisiae* (Baker's yeast).
11. C5DDR2: Hypothetical protein from *Lachancea thermotolerans* (yeast).
12. B2RYU6: TRAPPC2L from *Rattus norvegicus*.
13. C4R7P1: Hypothetical protein from *Pichia pastoris* (yeast).
14. Q54CU7: TRAPPC2L from *Dictyostelium discoideum*.
15. Q9JME7: TRAPPC2L from *Mus musculus*.
16. A6H7F7: TRAPPC2L from *Bos taurus*.
17. Q5M8X5: TRAPPC2L from *Xenopus tropicalis*.
18. C5DXJ3: Hypothetical protein from *Zygosaccharomyces rouxii*.

Appendix D: Abbreviations

AA	Amino acid
AAA+	ATPases associated with diverse cellular activities
APS	Ammonium persulfate
Arf	ADP ribosylation factor
AUC	Analytical ultracentrifugation
βME	β-mercaptoethanol
bp	Base pair
CD	Circular dichroism
COG	Conserved oligomeric Golgi complex
CoIP	Co-immuno-precipitation
COPI	Coat protein complex – I
COPII	Coat protein complex - II
CP	Cage protein
CPC	Coat protein complex
dNTPs	Deoxyribonucleotide triphosphates
DPC	Dodecylphosphocholine
DSF	Differential scanning fluorimetry
DTT	1,4-dithiothreitol
E. coli	*Escherichia coli*
EM	Electron microscope
ER	Endoplasmic Reticulum
FPLC	Fast protein liquid chromatography
GAP	GTPase activating protein
GARP	Golgi-associated retrograde protein
GDF	GDI displacement factor
GDI	GDP dissociation inhibitor
GEF	Guanine nucleotide exchange factor
GSH	Glutathion
GST	Glutathion-S-transferase
HOPS	Homotypic fusion and vacuole protein sorting complex
HRP	Horseradish peroxidase
IPTG	Isopropyl-thiogalactoside
Ni-NTA	Ni-nitrilotriacetate
NMR	Nuclear magnetic resonance
NSF	*N*-ethylmaleimide-sensitive factor
PAGE	Polyacrylamide gel electrophoresis
PAS	Phagophore assembly site

PCR	Polymerase chain reaction
PDZL	PDZ like domain
PEG	Polyethylene glycol
pI	Isoelectric point
PPIn	Polyphosphoinositides
PVDF	Poly-vinylidene fluoride
Rab	Ras analog in brain
Rab-GGTase	Rab geranylgeranyltransferase
REP	Rab escort protein
S. cerevisiae	*Saccharomyces cerevisiae*, baker's yeast
SDS	Sodium dodecyl sulfate
SeMet	Seleno-methionine
SLS	Static light scattering
SNARE	Soluble N-ethylmaleimide sensitive factor attachment protein receptor
SV	Sedimentation velocity
TAP	Tandem-affinity purification
TEMED	N,N,N´,N´-tetramethylenediamine
TGN	Trans-Golgi network
TRAPP	Transport protein particle complex
TSA	Thermal shift assay

Curriculum vitae

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

Publication

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