

**Structural and functional studies of the
human TRAPP tethering complex involved
in vesicular transport**

Dissertation zur Erlangung des akademischen Grades

Doktor der Naturwissenschaften

(Dr. rer. nat.)

am Fachbereich Biologie, Chemie und Pharmazie
der Freien Universität Berlin

Vorgelegt von Dipl.-Biochem.

Daniel Kümmel

Berlin, 2007

1. Gutachter: Prof. Dr. Udo Heinemann

2. Gutachter: PD Dr. Michael Veit

Eingereicht am: 18. 01. 2007

Mündliche Prüfung am: 03. 04. 2007

Selbstständigkeitserklärung

Diese Arbeit wurde im Zeitraum von März 2004 bis Januar 2007 in der Forschungsgruppe Kristallographie (Leiter: Prof. Dr. Udo Heinemann) am Max-Delbrück-Centrum für Molekulare Medizin in Berlin-Buch angefertigt.

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und auf Grundlage der angegebenen Hilfsmittel angefertigt habe.

Teile dieser Arbeit sind Bestandteil folgender Publikationen:

Turnbull, A.P., **Kümmel, D.**, Prinz, B., Holz, C., Schultchen, J., Lang, C., Niesen, F.H., Hofmann, K.P., Delbrück, H., Behlke, J., Müller, E.C., Jarosch, E., Sommer, T., Heinemann, U. (2005) Structure of palmitoylated BET3: Insights into TRAPP complex assembly and membrane localization. *EMBO J* **24**, 875-884

Kümmel, D., Müller, J.J., Roske, Y., Misselwitz, R., Büssow, K., Heinemann, U. (2005) The structure of the TRAPP subunit TPC6 suggests a model for a TRAPP subcomplex. *EMBO Rep* **6**, 787-793

Kümmel, D., Müller, J.J., Roske, Y., Henke, N., Heinemann, U. (2006) Structure of the Bet3-TPC6B core of TRAPP: Two TPC6 paralogs form trimeric complexes with Bet3 and Mum2. *J. Mol. Biol.*, **361**, 22-32

Kümmel, D., Heinemann, U., Veit M. (2006) Unique self-palmitoylation activity of the TRAPP component Bet3: A novel mechanism required for protein stability. *Proc. Natl. Acad. Sci. USA*, **103**, 12701-12706

Scheich, C., **Kümmel, D.**, Soumailakakis, D., Heinemann, U., Büssow, K. (2007) Vectors for co-expression of an unlimited number of proteins. *in revision*

Table of contents

1	Introduction	7
1.1	Vesicular transport	7
1.1.1	Modular organization of vesicular transport	8
1.2	Tethering Factors	10
1.2.1	The exocyst.....	12
1.2.2	The transport protein particle	13
1.3	Objectives.....	17
2	Materials	18
2.1	Instruments.....	18
2.2	Chemicals, enzymes, kits	20
2.3	Strains and plasmids.....	22
2.4	Media and buffers	24
2.5	Synthetic oligonucleotides	29
2.5.1	Gene-specific primers.....	29
2.5.2	Vector-specific primers	29
2.5.3	Oligos for QuikChange Mutagenesis	30
2.5.4	Primer for quantitative RT-PCR.....	30
3	Methods.....	31
3.1	Molecular Biology	31
3.1.1	Polymerase chain reaction (PCR).....	31
3.1.2	Restriction digest	32
3.1.3	DNA purification.....	32
3.1.4	Gel electrophoresis of DNA fragments	33
3.1.5	Ligation.....	33
3.1.6	Transformation of chemically competent <i>E. coli</i> cells.....	33
3.1.7	Ligation independent cloning (LIC).....	34
3.1.8	Transformation of <i>S. cerevisiae</i>	35
3.1.9	Plasmid preparations	35
3.1.10	QuikChange mutagenesis (QCM)	35
3.1.11	RNA isolation.....	37
3.1.12	Quantitative real-time (RT) PCR.....	37
3.2	Protein purification and characterization	39
3.2.1	Expression in <i>Escherichia coli</i>	39
3.2.2	Expression in <i>Saccharomyces cerevisiae</i>	39
3.2.3	Production of seleno-methionine labeled protein.....	39
3.2.4	Cell lysis	39
3.2.5	Affinity chromatography	40
3.2.6	Determination of protein concentration.....	41
3.2.7	Tag removal.....	42
3.2.8	Gel-filtration chromatography.....	42

3.2.9	Sodium dodecylsulfate polyacrylamide-gel electrophoresis (SDS-PAGE)	43
3.2.10	Staining of PAGE gels	44
3.2.11	Concentration of protein samples	44
3.2.12	Circular Dichroism	44
3.2.13	Dynamic light scattering	45
3.3	Protein crystallography	46
3.3.1	Crystallization	46
3.3.2	Collection of diffraction data	48
3.3.3	Data processing	50
3.3.4	Determination of electron density	51
3.3.5	Model building	55
3.3.6	Refinement	55
3.4	Biochemical studies	58
3.4.1	Cell culture	58
3.4.2	Transient expression in eukaryotic cells	58
3.4.3	Association studies	58
3.4.4	Western blot	59
3.4.5	Immunodetection	59
3.4.6	Metabolic labeling with [³ H]palmitate	59
3.4.7	Membrane preparations and extractions	60
3.4.8	Tetrad analysis	60
3.4.9	Palmitoylation assay and depalmitoylation of Bet3	60
4	Results	61
4.1	The structure of the TRAPP subunit Tpc6B	61
4.1.1	Expression and purification of recombinant Tpc6B	61
4.1.2	Crystallization of Tpc6B	62
4.1.3	Data collection and structure determination	62
4.1.4	The structure of Tpc6B	64
4.1.5	Structural comparison of Tpc6B and Bet3	65
4.2	Studies on the Bet3 protein family	68
4.2.1	Purification of Bet3 and Tpc5	68
4.2.2	A common fold for the Bet3 protein family	68
4.2.3	Interaction of Tpc6B and Bet3	70
4.2.4	Tpc5 interacts with Bet3, but not Tpc6B	71
4.3	The structure of the Bet3:Tpc6B subcomplex of TRAPP	72
4.3.1	Expression and purification of the Bet3:Tpc6B heterodimer	72
4.3.2	Crystallization of 6×His-Bet3:Tpc6B	72
4.3.3	Structure determination of Bet3:Tpc6B with molecular replacement	73
4.3.4	Structure of the Bet3-Tpc6B complex	74
4.3.5	Mum2 binding to the Bet3:Tpc6B heterodimer	77
4.4	Two variants of Tpc6 are identified in some organisms	79
4.4.1	A hydrophobic surface patch is conserved between Tpc6 homologs	79

4.4.2	Functional comparison of both Tpc6 paralogs	82
4.5	Identification and characterization of TRAPP subcomplexes.....	84
4.5.1	Purification and characterization of Mum2	84
4.5.2	Reconstitution of the Bet3:Tpc6B:Mum2 complex and its interaction with synbindin	85
4.5.3	Purification and crystallization of the Bet3:Tpc6B:Mum2:synbindin complex	87
4.6	Palmitoylation of Bet3	90
4.6.1	Palmitoylation is not required for membrane association of Bet3 or yeast cell viability	90
4.6.2	Bet3 possesses strong auto-palmitoylation activity.....	92
4.6.3	Palmitoylation is required for Bet3 protein stability <i>in vitro</i> and <i>in vivo</i>	95
5	Discussion.....	97
5.1	Structural studies of the TRAPP tethering complex	97
5.2	Membrane anchoring of TRAPP	100
5.3	Isoforms of TRAPP subunits	102
5.4	Palmitoylation of the TRAPP subunit Bet3	103
5.5	Implications for the function of TRAPP and its subunits	105
5.6	Conclusion.....	108
6	Summary / Zusammenfassung.....	109
	Appendix A: References.....	113
	Appendix B: Initial crystal screen formulations.....	120
	Appendix C: Abbreviations.....	126
	Curriculum Vitae	128