Molecular mechanisms of COPI transport

Goal
Our research interests comprise two converging fields:
i) Molecular mechanisms of coated vesicle formation and uncoating, and
ii) Specificity and structural basis for protein-lipid interactions within a bilayer that regulate membrane protein activity.
These research interests have led us to develop and maintain
iii) A platform for quantitative lipidomics.
We are characterizing the components and their coordinate action that allow formation, fission and uncoating of Golgi-derived COPI-coated vesicles. This includes proteomics and lipidomics, functional in vitro assays and reconstitution of individual functional steps in a chemically defined liposomal system.

Background
In the eukaryotic cell, vesicular transport represents the basic mechanism for i) maintaining the homeostasis of the endomembrane system, ii) biosynthetic transport of newly synthesized proteins and lipids, and iii) the uptake and intracellular transport of exogenous macromolecules. The mechanism of fusion of vesicles as well as their role in neurotransmission has been recognized by the 2013 Nobel Prize for Physiology and Medicine. Three classes of coated vesicles are well established to mediate transport in the exo- and endocytic pathway: COPII vesicles for ER export, COPI vesicles for retrograde Golgi-ER and bidirectional intra-Golgi transport, and clathrin-coated vesicles operating in the late secretory and endocytic pathway. Coat components are involved in multiple tasks such as cargo selection, curvature formation at the donor membrane, vesicle fission and initiation of uncoating.

We are interested in the molecular mechanisms underlying vesicular transport by COPI vesicles. In contrast to COPII and clathrin coats, the heptameric large COPI coat component coatomer is recruited en bloc to the membrane, so that both the inner and outer shell of the vesicle are formed at the same time.

In our view, the formation of a COPI transport vesicle involves the following minimal set of components: donor membranes with transmembrane proteins acting as coat and/or cargo receptors (e.g. members of the p24 family), cytosolic Arf1, cytosolic coatomer and auxiliary enzymes that serve activation on the membrane of Arf1 (GBF1) and the activation of GTP hydrolysis by Arf1 (Arf GAPs).
A schematic view of individual steps in COPI vesicle biogenesis is given in Fig. 1.

Research Highlights

Molecular mechanisms of COPI vesicle biogenesis

1) Roles of Arf1 in vesicle formation and fission

Formation of coated vesicles requires two striking manipulations of the lipid bilayer. First, membrane curvature is induced to drive bud formation. Second, a scission reaction at the bud neck releases the vesicle. Using a reconstituted system for COPI vesicle formation from purified components we find that a non-dimerizing Arf1 mutant, which does not display the ability to modulate membrane curvature in vitro or to drive formation of coated vesicles, is able to recruit coatamer to allow formation of COPI-coated buds, but does not support scission. Chemical cross-linking of this Arf1 mutant restores vesicle release. These studies show that initial curvature of the bud is driven primarily by coatamer, whereas the mem-

Britta Brügger

Ph.D. - University of Frankfurt, Germany 1998
PostDoc - Memorial Sloan Kettering Cancer Center, New York, USA (Prof. James E. Rothman) 1998 - 2000
PostDoc - BZH (Prof. Felix Wieland) 2000 - 2002
Research Fellow - BZH since 2002
Habilitation in Biochemistry, University of Heidelberg, Medical Faculty 2007
brane curvature potentiating activity of dimeric Arf1 is required for membrane scission. Using a semi-intact cell system we have shown that during the scission reaction Arf does not hydrolyse its bound GTP.

2) Structures of coatomer and of the COPI coat
Together with John Briggs’ group at the EMBL we investigate the structure of soluble coatomer by single particle electron microscopy, and of the coatomer shell on coated vesicles. With the first data of a coat on a membrane, a structure emerges that is strikingly different from those of the COPII and the clathrin systems as delineated from protein assemblies. The basic unit of the lattice is a coatomer triade. Triades can be arranged on the vesicular membrane in various patterns that are defined by variable vertices at the contact sites of triades.

3) Regulation of COPI transport by a unique sphingolipid/cargo-receptor complex
We have discovered a specific binding of the sphingomyelin molecular species SM 18:0 to the transmembrane domain of one member of the p24 family, p24. SM 18:0 binding favors dimerization of p24. Dimeric p24, in turn, recruits coatomer and triggers a conformational change of the complex resulting in polymerization, initiating COPI bud formation. Thus, a membrane lipid molecular species can serve as a cofactor in controlling vesicle budding. We have defined steps in vesicular transport in vivo that depend on this specific interaction.

Structural principles of transmembrane protein/membrane lipid interactions
1) A signature within the p24 transmembrane domain for recognition of a sphingolipid molecular species
We have discovered a peptide signature for sphingolipid binding within the transmembrane span of p24. When transplanted, the signature confers sphingolipid binding to a non-sphingolipid binding transmembrane domain. Results from a data mining approach indicate that this signature represents a conserved binding site for sphingolipids in several transmembrane proteins.

2) We found that a functional sphingomyelin-binding signature in the influenza neuraminidase is necessary for optimal transport of this protein to the host’s plasma membrane.

3) Capitalizing on fluorescent sphingomyelin species containing pentaenyl fatty acids we have developed a simple assay to assess specific lipid-lipid recognition in liposomal membranes.

Lipidomics platform
Using our lipidomics platform, we have elucidated lipidoms of organisms and subcellular systems (see lipidomics by Britta Brügger on page 52).
Our investigations are based on a wide range of methods, including live cell imaging (with Rainer Pepperkok; EMBL), bioinformatics (with Gunnar von Heijne and Arne Elofsson, Stockholm), molecular dynamics simulations (with Erik Lindahl, Stockholm), in vivo and in vitro FRET studies, cryo-electron microscopy (with John Briggs, EMBL), protein chemistry, molecular biology, and quantitative nano-mass spectrometry of lipids, as well as chemical biology approaches.

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Selected Publications 2011 - 2013


Awards and Honors Felix Wieland

1993 Honorary Member of Charité, Medical Faculty of the Humboldt University, Berlin since 2000 Elected EMBO Member 2001 Heinrich-Wieland Award since 2003 Member of Deutsche Akademie der Naturforscher Leopoldina 2006 Feldberg Foundation Award 2011 Elected Member of the Academia Europea 2013 HMLS Award

Felix Wieland
Phone: +49 (0)6221-54 4150 E-mail: felix.wieland@bzh.uni-heidelberg.de

Britta Brügger
Phone: +49 (0)6221-54 5426 E-mail: britta.bruegger@bzh.uni-heidelberg.de

Fig. 3: Structure of a SM 18-binding motif. Molecular dynamics simulation of p24 TMD (blue, with the motif highlighted in red) and SM 18:0 (green, hydrocarbon chains; yellow, headgroup of SM 18:0) in a POPC bilayer.