1978	Ph.D. - Ludwig-Maximilians-Universität München, Germany (Max Planck Institute of Biochemistry, Martinsried)
1978 - 1986	PostDoc and Group Leader - University of Regensburg, Germany
1986 - 1988	Visiting Scientist - Dept. of Biochemistry, Stanford University, USA
1988 - 1997	Full Professor and Chairman of Biochemistry I - Heidelberg University, Germany
1991 - 2003	Chairman SFB 352
1997 - 2002	Director - BZH
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2005 - 2007	President of the GBM
2004 - 2015	Chairman SFB 638
2014 - 2015	Director - BZH

Felix Wieland

Molecular mechanisms of COPI transport

Goal

We are interested in the molecular mechanisms that underlie the formation and uncoating of coated vesicles, and the structure of their coats. (Since establishing her own group Britta Brügger continues research on lipid/protein interactions and on lipidomics).

We are characterizing the components of vesicular coats and their coordinate action that allow formation, fission and uncoating of Golgi- or semi-intact cell derived 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. 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 various Adaptor Protein-containing clathrin-coated vesicles operating in the late secretory and endocytic pathways. 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 such vesicular transport..

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. In contrast to COPI, where the heptameric coat component coatomer is recruited to the membrane en bloc, the COPII-and clathrin coats are recruited in two subsequent steps.

Research Highlights

Proteomics of COPI and COPII vesicles

In order to further understand the functions of COP vesicle isoforms, we have cloned and expressed in insect cells the various isoforms of both the mammalian COPI and COPII systems.

Vesicle preparations were obtained from semi-intact cells with the isoformic coats and subjected to quantitative comparing proteomic analysis by SILAC. Whereas clear differences are observed with regard to cargo proteins in isoforms of COPII vesicles, very surprisingly the protein compositions of all four isoforms of COPI vesicles are strikingly similar, even in three different cell types (Adolf et al.,2016, Adolf et al, in prep., Rhiel et al., in prep.). In light of a heterogeneous partition to the various Golgi stacks of COPI isoforms this homogeneity poses a puzzling problem. In this regard, Julien Bethune describes for the first time evidence of a basic cellular function exclusively of COPIγ1 isoforms (see report Bethune group).

Reconstitution of Clathrin coated vesicles (CCVs)

In the last period we have expressed in insect cells clathrin heavy and light chains as well as complete and functional AP1 and AP2 complexes, together with key molecules described for the formation and scission of AP2 CCVs. Presently we are establishing reconstitution of the corresponding vesicles from liposomes, in order to study their mechanisms of scission from their donor membranes. Together with Frank Adolf and John Briggs we plan to solve the structure of AP1 CCVs by cryo EM.

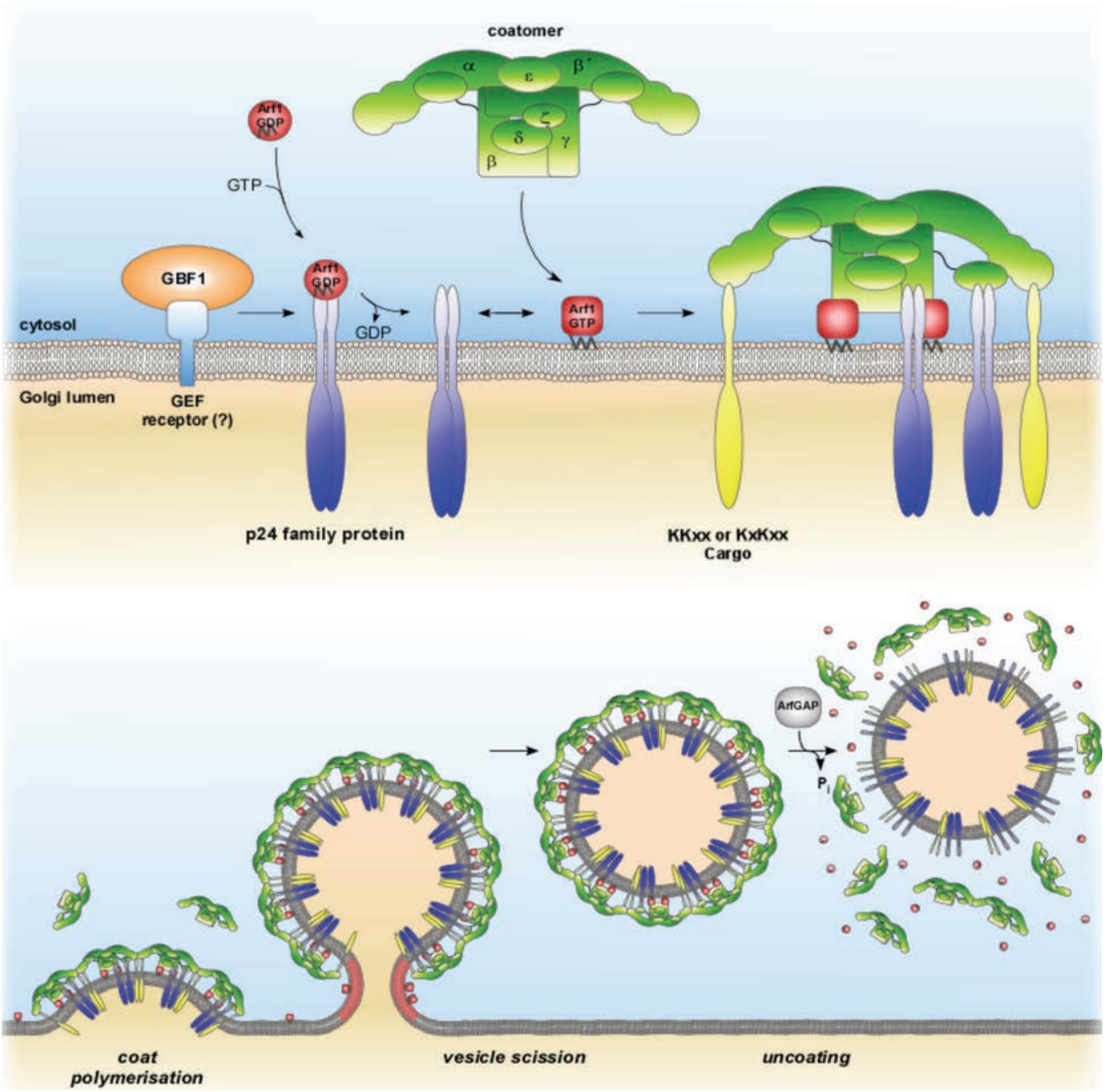

Fig. 1: Upper panel: *Arf-cycle for coat recruitment and dissociation (for details see text).* ***Lower panel:*** *Vesicle scission and ArfGAP-dependent dissociation of the coat.*

Structure of the COPI coat and molecular mechanisms of its dissociation

High resolution structure of the COPI coat

Together with John Briggs' group at the EMBL we are investigating the structure of the coatomer shell on coated vesicles. With the first data of a coat on a membrane, a structure has emerged that is strikingly different from those of the COPII and the clathrin systems as delineated from protein assemblies in solution. The basic unit of the coat lattice turned out to be 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 (Faini et al., 2012 Science). Higher resolution cryo EM (Dodoneva et al., 2015, Science) combined with x-ray crystallography in collaboration with Irmi Sinning's group has allowed us to unequivocally attribute all masses found to individual subunits and structural elements thereof, including a βδ-COP subcomplex resolved by x-ray crystallography at 2.7A°. From these data a stoichometry emerges of 2Arf to 1coatomer, and surprisingly different chemical environments for the two GTPase molecules emerge. We propose that the different sites of Arf within the coat reflect different functions in the process of coating and uncoating (Dodoneva, Aderhold et al. in preparation, and see Fig.2.

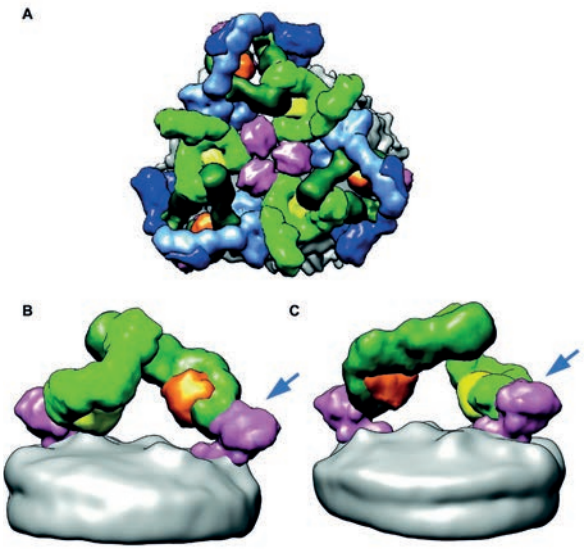

Fig. 2: 2)Top: *A triade of coatomers with Arf molecules in pink (three center Arfs linked to γ-COPs, and three peripheral Arfs linked to β-COPs), viewed from top.* ***Bottom:*** *Side views of β-Arf (arrow in B) and of γ-Arf (arrow in C).*

Role of Arf1 in coat dissociation

Fusion of a transport vesicle with its target membrane requires prior dissociation of its protein coat. As outlined above, the small GTPase Arf in its GTP-loaded state anchors coatomer to the membrane by different interactions: one Arf via the trunk domain of β-COP together with δ-COP (Dodoneva et al. in preparation), and another Arf by the trunk domain of γ-COP. According to the current view of the field, auxilliary Arf-GTP-hydrolysis activating proteins (ArfGAPS) stimulate GTP hydrolysis in Arf and thus render the G-protein soluble to dissociate from the membrane, followed suit by coatomer. This concept does not explain how during uncoating the various interactions of coatomer within and between triads are dissociated and how the membrane-associated conformation(s) of the complex is reversed to its soluble form. We have investigated ArfGAP mediated coat dissociation in a fluorescence burst analysis in real time, with Arf and coatomer labeled with different fluorescent dyes. Full length ArfGAPs efficiently cause dissociation of Arf, followed by coatomer after a lag time. The catalytic domain of the ArfGAPs alone also efficiently dissociates Arf from the vesicles. Strikingly, however, the coatomer network remains on the membrane in a meta-stabile state. Subsequent addition of the non-catalytic domain or of full length ArfGAPs did not dissociate coatomer. Thus, ArfGAP together with Arf must connect to the coat in order to drive coatomer dissociation at the expense of GTP hydrolysis (Ganeva et al., in preparation, see Fig.3). Specific interactions of ArfGAP non-catalytic domains with coatomer described in the literature are candidates for such "point levers". (see Fig.3)

Various mechanistic aspects

Vincent Popoff during his postdoctoral work in our group has revealed a mechanism by which cargo

can be taken up efficiently into a COPI vesicle by bridging to coatomer via Golgi phospho protein 3 (Eckert et al. 2014).

Based on our recent finding of an unexpected specificity of trans-membrane segments of proteins with lipid molecular species, Andreas Ernst during his postdoctoral work has discovered that the transmembrane span of the Ebola virus glycoprotein specifically binds cholesterol. Cells expressing this transmembrane domain drastically shrink when cholesterol is depleted. We conclude that cytotoxicity of the virus can be alleviated by cholesterol inhibiting drugs (Hacke et al., 2015).

Further results on protein-lipid interactions are described in the report of Britta Brügger.

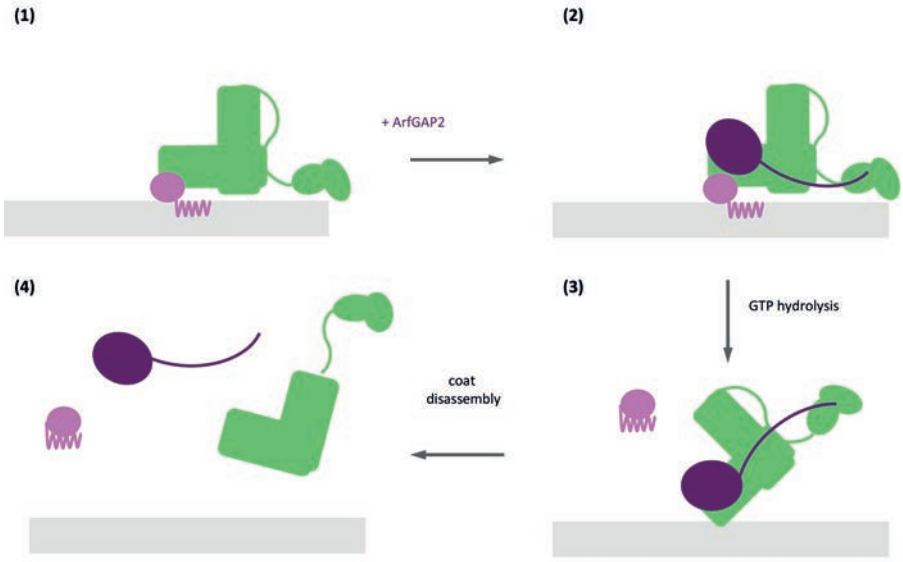

Fig. 3: Model of point-lever action of ArfGAPs. 1: *detail of COPI coat with Arf (pink) on the membrane (gray) in contact with γ-COP trunk domain (green triangle) and appendage domain (green rest).* ***2:*** *ArfGAP2 (dark red) binding to Arf with its catalytic domain (ball), and to γ-COP appendage domain (bow).* ***3:*** *Hydrolysis of GTP in Arf converts Arf into its soluble form and concomitantly induces a change in conformation in γ-COP. As a consequence the coat is disassembeled and coatomer is rendered soluble.*

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Selected Publications 2014 - 2016

E.S. P. Eckert , I. Reckmann, A. Hellwig , S. Röhling ,A.E.Battari, F.T. Wieland, V. Popoff (2014) Golgi phosphoprotein 3 triggers signal-mediated incorporation of glycosyltransferases into coatomer-coated (COPI) vesicles. J. Biol. Chem.289 (45): 31319-31329

M.J. Gerl, T. Sachsenheimer, M. Grzybek, U. Coskun, F.T. Wieland, B. Brügger. (2014) Analysis of transmembrane domains and lipid modified peptides with matrix-assisted laser desorption ionization-time-of- flight mass spectrometry ACS Publ. 86 (8):3722-3726

P. Björkholm, A.M. Ernst, M. Hacke, F. Wieland, B. Brügger, G. von Heijne, (2014) Identification of novel lipid-binding motifs in mammalian membrane, Biochimica et Biophysica Acta-Biomembranes 1838, 2066-2070

M. Hacke, P. Björkholm, A. Hellwig, P. Himmels, C. Ruiz de Almodóvar, B. Brügger, F. Wieland, A.M. Ernst (2015) Inhibition of Ebola virus glycoprotein-mediated cytotoxicity by targeting its transmembrane domain and cholesterol. Nat Commun. July 9; &.7688. doi:10.1038/ncomms8688 PMID2615

S.O. Dodonova, P. Diestelkoetter-Bachert, A. von Appen, W.J.H. Hagen, R. Beck. M. Beck, F. Wieland, J.A.G. Briggs (2015) A structure of the COPI coat and the role of coat proteins in membrane vesicle assembly Science 349 (6244), 195-198.

F. Adolf, M. Rhiel, I. Reckmann, F.T. Wieland. (2016) Sec24C/D-isoform-specific sorting of the preassembled ER-Golgi Q-SNARE complex. Mol Biol Cell. 27(17):2697-707. doi: 10.1091/mbc.E16-04-0229. Epub 2016 Jul 13.

Awards and Honors

1991-92	Dean of the Faculty of Science in Medicine, Heidelberg University
1993	Honorary Member of Charité, Medical Faculty of the Humboldt University, Berlin
2000	Elected EMBO Member
2001	Heinrich-Wieland Award
2003	Member of Deutsche Akademie der Naturforscher Leopoldina
2006	Feldberg Foundation Award
2011	Elected Member of the Academia Europea
2013	HMLS Award

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