The contribution of proteins and lipids to COPI vesicle formation and consumption

Akademisk avhandling

som för avläggande av medicine doktorsexamen
vid Sahlgrenska Akademin vid Göteborgs Universitet
kommer att offentligen försvaras i hörsal Arvid Carlsson,
Medicinaregatan 3, Göteborg, fredagen den 5 december 2008 kl. 13.00

av

Fredrik Kartberg

Fakultetsopponent:
Professor Dan Cassel
Technion Israel Institute of Technology
Haifa, Israel

Avhandlingen baseras på följande delarbeten:

I: Early stages of Golgi vesicle and tubule formation require diacylglycerol
Lennart Asp, Fredrik Kartberg, Julia Fernández-Rodríguez, Maria Smedh, Markus Elsner, Frédéric Laporte, Montserrat Bárcena, Karen A Jansen, Jack A Valentijn, Abraham J Koster, John J.M. Bergeron and Tommy Nilsson.
In press, Molecular Biology of the Cell

II: ARFGAP2 and ARFGAP3 are essential for COPI coat assembly on the Golgi membrane of living cells
Fredrik Kartberg, Lennart Asp, Maria Smedh, Julia Fernández-Rodríguez and Tommy Nilsson.
Submitted

III: PI(4,5)P_2 promotes fusion of COPI-derived vesicles with Golgi cisternae, *in vitro*
Frédéric Laporte, Fredrik Kartberg, Johan Hiding, Francois Lepine, Markus Grabenbauer, Anirban Siddhanta, Dennis Shields, Joel Lanoix, Joachim Ostermann, John J.M. Bergeron and Tommy Nilsson.
In manuscript

IV: Anomalous subdiffusion is a measure for cytoplasmic crowding in living cells
Matthias Weiss, Markus Elsner, Fredrik Kartberg and Tommy Nilsson.
Biophysical Journal vol 87 November 2004 3518-3524

Göteborg 2008

UNIVERSITY OF GOTHENBURG

The contribution of proteins and lipids to COPI vesicle formation and consumption

Fredrik Kartberg
Institute of Biomedicine, Department of Medical and Clinical Genetics
The Sahlgrenska Academy at the University of Gothenburg 2008

Abstract:

In the secretory pathway, movement of proteins between compartments occurs through small (50-100 nm) vesicles. The coat of COPI vesicles is composed the small GTPase ARF1 and the large coatomer complex. In the secretory pathway, these vesicles mediate Golgi-to-ER and intra-Golgi transport. During vesicle formation, recruitment of cytosolic coatomer to the membrane by ARF1 represents the key step. This generates a bud, which subsequently separates from the donor membrane as a vesicle. During consumption, the vesicle tethers to the target membrane, followed by docking and fusion, resulting in the merging of the two bilayers. This thesis has been devoted to COPI vesicle formation and consumption.

We examined the role of the lipid diacylglycerol (DAG) in bud formation. We demonstrate that efficient inhibition of DAG synthesis by the addition of the inhibitor Propranolol causes rapid dissociation of the ARFGAP1 protein from the membrane. Upon electron microscopy examinations of treated cells, we find that this results in smooth Golgi membranes devoid of budding profiles. Washout of Propranolol resulted in a marked increase of buds and associated vesicles. Cells expressing low amounts of ARFGAP1 were treated similarly. In such cells, removal of the inhibitor caused an increase in membrane buds but not vesicles. This suggests that DAG is needed at an early stage of bud formation whereas ARFGAP1 is required at a later step.

We investigated the function of two new ARFGAPs in COPI vesicle formation in living cells. We demonstrate that stimulation of vesicle budding by addition of aluminum fluoride causes accumulation of ARFGAP2, ARFGAP3, and coatomer on the Golgi, but not of ARFGAP1. Fluorescence recovery after photobleaching (FRAP) analysis of the association with the Golgi demonstrates that this accumulation also reflects irreversible binding of ARFGAP2 and ARFGAP3 with the membrane. The degree of immobilization was close to that of coatomer, suggesting a closer role than of ARFGAP1. The ability to generate the COPI coat lattice in cells lacking different combinations of ARFGAP1-3 was investigated. Absence of the ARFGAP2 and ARFGAP3 pair but not ARFGAP1 prevented coat lattice formation. This suggests that these two ARFGAPs play an overlapping role in COPI vesicle formation in the Golgi.

We looked into the factors that influence the docking and fusion of COPI vesicles with Golgi cisternae using an *in vitro* assay for the reconstitution of intra-Golgi transport. We find that vesicle fusion is regulated by the presence of $PI(4,5)P_2$ on vesicles. The pre-treatment of vesicles with a kinase stimulated fusion and treatment with a phosphatase inhibited fusion. The ability of ARF1 to generate $PI(4,5)P_2$ on the Golgi membrane may therefore prime vesicles for the following fusion event.

We analyzed a property of the cytosol, molecular crowding, and its consequences for diffusion by fluorescence correlation spectroscopy. We find that fluorescent dextrans diffuse normally in water but become subdiffusive upon microinjection into cells or in artificially crowded solutions. This phenomenon can have important consequences for the function of proteins, such as coatomer, that depend on diffusion for the association with the membrane.

Keywords: ARF1, COPI, ARFGAP, vesicle, fusion, diffusion

ISBN: 978-91-628-7631-9