Functional characterization of the secretory pathway and the role of COPI vesicles

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Avhandlingen baseras på följande delarbeten:

I. **Quantitative Proteomics Analysis of the Secretory Pathway**
Annalyn Gilchrist*, Catherine E. Au*, Johan Hiding*, Alexander W. Bell, Julia Fernandez-Rodriguez, Souad Lesimple, Hisao Nagaya, Line Roy, Sara J.C. Gosline, Michael Hallett, Jacques Paiement, Robert E. Kearney, Tommy Nilsson, and John J.M. Bergeron
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* These authors contributed equally to this work

II. **PI(4,5)P_2 is Required for the Fusion of COPI-Derived Vesicles with Golgi Cisternae**
Frédéric Laporte, Johan Hiding, Fredrik Kartberg, François Lépin, Markus Grabenbauer, Anirban Siddhanta, Dennis Shields, Joachim Ostermann, John J.M. Bergeron and Tommy Nilsson
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III. **Membrane Binding and Fusion Ability of COPI Vesicles upon Protease Treatment**
Frédéric Laporte*, Johan Hiding*, Joachim Ostermann*, Fredrik Kartberg, Joel Lanoix, John J.M. Bergeron and Tommy Nilsson
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Functional characterization of the secretory pathway and the role of COPI vesicles

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In the secretory pathway, proteins synthesized in the endoplasmatic reticulum move through the Golgi apparatus in a cis-to-trans direction and then sorts into different types of vesicles at the trans-Golgi network. How intracellular transport takes place has been the focus of cell biology research for a long time. In order to understand how the organelles within the pathway functions it would be very helpful to know all the proteins from which it is composed. However there is no complete and accurate map of the proteins within the organelles of the secretory pathway.

In order to achieve such a cell map, isolated subcellular organelles from rat liver were analyzed by tandem mass spectrometry. Redundant peptide counting was used to determine the relative abundance of proteins in the highly enriched organelle preparations. Peptide counting is based on the idea that when normalized for protein size, abundant proteins will generate more peptides than relatively rare proteins. The method was validated by comparing the results from quantitative western blotting and standard enzyme assays. The study resulted in a quantitative proteomic map of more than 1400 proteins with spatial information and relative presence of each protein of the rough ER, smooth ER and Golgi apparatus. Of the 345 proteins of unknown function discovered the subcellular locations for 230 of these were predicted. Extending the study to biochemical subfractions and organelle subcompartments resulted in the observation that COPI vesicles were enriched with Golgi-resident proteins while largely excluding secretory cargo. This would support a function for COPI vesicles in retrograde transport from trans to cis within the Golgi stack, which is in line with the cisternal maturation model of biosynthetic protein transport.

Coatomer is recruited to Golgi membranes in an ARF1-dependent manner that couples protein sorting to vesicle formation. Using a modified intra-Golgi transport assay with highly purified COPI vesicles, demonstrated that fusion of retrograde-directed vesicles with Golgi membranes requires phosphatidylinositol-(4,5)-bisphosphate (PI(4,5)P2). The dependency on $PI(4,5)P_2$ appears to be COPI vesicle associated since pre-treatment of vesicles with either a specific 5-phosphatase or a specific 5-kinase, resulted in inhibition or gain of fusion, respectively. As ARF1 recruits the kinase responsible for $PI(4,5)P_2$ synthesis in a GTP-dependant manner to Golgi membranes, ARF1 can effectively prime COPI vesicles for fusion during vesicle formation.

The rate whereby functional COPI vesicles bind to Golgi membranes can be measured independently of the fusion reaction, *in vitro*. Proteinase K was used to investigate the effect on vesicle binding and fusion when proteins are removed by proteolysis. While binding was accelerated by protease-accessible factors such as SNARE proteins, these are not required for fusion. That proteins on COPI vesicles where removed was confirmed by Western blotting as well as by quantitatively determining NSF and SNAP requirements for vesicle fusion before and after proteolysis. As shown for vacuolar fusion, we propose that the principal role of SNARE proteins is to ensure the recruitment of cytoplasmic fusion proteins to the docking site rather than to mediate the actual fusion event of COPI vesicles to Golgi membranes.

Keywords: Golgi, Phosphoinositides, Fusion, COPI, ARF1, Proteomics ER
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