BIOGRAPHICAL SKETCH

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NAME: Charles Barlowe

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POSITION TITLE: Professor of Biochemistry and Cell Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
College of William & Mary, Williamsburg, VA	B.S.	12/1983	Chemistry
University of Texas, Austin, TX	Ph.D	06/1990	Biochemistry
University of California, Berkeley, CA	Postdoctoral	08/1994	Cell Biology

A. Personal Statement

My research program is focused on defining mechanisms that catalyze protein transport through the early secretory pathway. We combine approaches in biochemistry, cell biology, microscopy, molecular genetics and cell free assays primarily in the model organism *S. cerevisiae* to rigorously investigate underlying mechanisms. My goals are to generate fundamental new knowledge that will ultimately help in the treatment and prevention of human diseases. I also seek to promote a collaborative and inclusive research environment, and to train the next generation of scientists in the methods of experimental research. I am fortunate to have received NIH support of my research program for over 25 years and we have made significant contributions in areas of protein trafficking and membrane biology. Notable advances in elucidating the machinery for selective incorporation of proteins into ER-Golgi transport vesicles is described under the contributions to science section below. During this period I have trained 18 PhD students, 6 postdoctoral scientists and many undergraduate students, visiting scientists and high school students. Most of my former trainees currently hold positions in academics, medicine or industry. I have also served on over 60 PhD thesis committees for graduate students at Dartmouth, Harvard Medical School and MIT. I continually seek new and innovative approaches to mentor PhD trainees for career success. In my administrative responsibilities, I have worked collaboratively across departments, centers and schools in directing the multi-disciplinary Molecular and Cellular Biology Graduate Program (2002-2004), as Dean of Graduate Studies at Dartmouth (2004-2008) and as chair of the Biochemistry & Cell Biology department at Dartmouth's Geisel School of Medicine (2008-current).

B. Positions and Honors

Positions and Employment

1994-1999	Assistant Professor of Biochemistry, Dartmouth Medical School
1999-2004	Associate Professor of Biochemistry, Dartmouth Medical School
2004-present	Professor of Biochemistry & Cell Biology, Geisel School of Medicine at Dartmouth
2002-2004	Director, Molecular and Cellular Biology Graduate Program at Dartmouth
2004-2008	Dean of Graduate Studies, Dartmouth Arts and Sciences Graduate Programs
2008-present	Chair, Department of Biochemistry & Cell Biology, Geisel School of Medicine at Dartmouth
2017-present	James C. Chilcott 1920 Professorship, Geisel School of Medicine at Dartmouth

Other Experience and Professional Memberships

1990-	Member, American Association for the Advancement of Science
1995-	Member, American Society for Cell Biology
1995	American Cancer Society Scientific Review Committee
1998-2002	Ad hoc reviewer, National Science Foundation, Cell Biology Program
1999-2010	Ad hoc reviewer CDF2, CDF4, CSF and MBPP Study Sections, NIH CSR
2000	Co-Chair, Symposium on Vesicle Docking and Fusion, ASCB National Meeting
2001-2017	Member of the Faculty of 1000
2009	Vice-Chair, Molecular Membrane Biology Gordon Conference
2011	Chair, Molecular Membrane Biology Gordon Conference
2011-2015	Member, Membrane Biology and Protein Processing Study Section, NIH CSR
2016-2017	Reviewer, NIH Director's Pioneer Award Program, NIH
2018	Special Emphasis Panel ZRG1-CB-J (55), MIRA Grant Applications
2019	Special Emphasis Panel ZGM-TRN-5 (MR), ESI MIRA Grant Applications
2020	Special Emphasis Panel ZRG1 CB-J (55), MIRA Grant Applications

Honors and Awards

1989	University of Texas Continuing Fellowship
1990	Eakin Biochemistry Award, University of Texas
1990-1993	Damon Runyon-Walter Winchell Cancer Fund Fellow
1995	Hitchcock Foundation Research Award, Dartmouth Medical School
1996-2000	Pew Scholars Program in the Biomedical Sciences
2007-2017	NIH MERIT Award (R37 GM52549 yr 12-22)
2010	Fellow, American Association for the Advancement of Science
2012	Fellow, American Academy of Microbiology

C. Contribution to Science

1. Regulated formation of the COPII coat

As a postdoctoral fellow with Randy Schekman, I made several contributions to the membrane trafficking field. I identified and characterized the first guanine nucleotide exchange factor (GEF) that acts on a secretory pathway GTPase. This work revealed that Sec12, an ER-localized transmembrane protein, catalyzes nucleotide exchange on Sar1 to initiate transport vesicle formation from the ER. In collaboration with Tohru Yoshihisa, we were able to demonstrate that Sec23 acts as a GTPase activating protein (GAP) for Sar1, linking the Sar1 GTPase cycle to coated vesicle formation at the ER. Finally, I discovered the coat protein complex II (COPII), which consists of the proteins Sar1, Sec23-Sec24 and Sec13-Sec31. COPII assembles on ER membranes to produce transport intermediates and incorporates secretory cargo into these coated vesicles. I led the effort to purify COPII coated vesicles for a determination of their protein composition and to document the structural morphology of COPII vesicles by thin section electron microscopy. These findings were highlighted in Schekman's Nobel Lecture in December 2013.

a. **Barlowe, C.**, d'Enfert, C. and Schekman, R. (1993) Purification and characterization of Sar1p, a small GTP-binding protein required for transport vesicle formation from the endoplasmic reticulum. J. Biol. Chem. 268, 873-879.

b. Yoshihisa, T., **Barlowe, C.** and Schekman, R. (1993) Requirement for a GTPase-activating protein in transport vesicle budding from the endoplasmic reticulum. Science 259, 1466-1468.

c. **Barlowe, C.**, and Schekman, R. (1993) Sec12p encodes a guanine nucleotide exchange factor essential for transport vesicle formation from the ER. *Nature* 365, 347-349.

d. **Barlowe, C**., Orci, L., Yeung, T., Hosobuchi, D., Hamamoto, S., Salama, N., Rexach, M., Ravazzola, M., Amherdt, M., and Schekman, R. (1994) COPII: A membrane coat formed by Sec proteins that drive vesicle budding from the ER. *Cell* 77, 895-907.

2. Reconstitution of ER-Golgi transport to define Uso1-dependent vesicle tethering
With purified COPII vesicles in hand that were fully functional for transport, I directed a research effort in my new lab to purify the soluble factors needed to reconstitute targeting and fusion of vesicles with Golgi membranes. We found that the extended coiled-coil domain protein Uso1 in addition to Sec18/NSF and LMA1 supported fusion of vesicles with washed Golgi membranes. Moreover, we could drive overall ER-Golgi transport with washed semi-intact cell membranes and 6 purified proteins (Sar1, Sec23-Sec24, Sec13-Sec31, Uso1, Sec18 and LMA1). This allowed for a full dissection of transport stages. Uso1 and the membrane bound Rab GTPase Ypt1 produced a dilution resistant tethered vesicle intermediate. The SNARE proteins (Sed5, Bos1, Bet1 and Sec22) in addition to the SM-protein Sly1 catalyzed fusion of tethered vesicles. These results fit well with genetic analysis of the fusion stage and contribute to the our general understanding of distinct steps in intracellular membrane fusion.

a. **Barlowe, C.** (1997) Coupled ER to Golgi transport reconstituted with purified cytosolic proteins. *J. Cell Biol.* 139, 1097-1108.
b. Cao, X., Ballew, N., and **Barlowe, C.** (1998) Initial docking of ER-derived vesicles requires Uso1p and Ypt1p but is independent of SNARE proteins. *EMBO J.* 17, 2156-2165.
c. Cao, X., and **Barlowe, C.** (2000) Asymmetric requirements for a Rab GTPase and SNARE proteins in fusion of COPII vesicles with acceptor membranes. *J. Cell Biol.* 149, 55-65.
d. Liu, Y., and **Barlowe, C.** (2002) Analysis of Sec22p in Endoplasmic Reticulum/Golgi Transport reveals cellular redundancy in SNARE protein function. *Mol. Biol. Cell* 13, 3314-3324.

3. Proteomic analysis of COPII vesicles identifies new components of budding and fusion machinery
We could scale up the reconstituted COPII budding reaction to purify biochemical quantities of the vesicles for analysis of the polypeptide constituents. As mass spectrometry methods of protein identification improved, we have been able to inventory the major proteins that are packaged into these vesicles. In addition to secretory cargo, which are relatively in abundant, we identified proteins involved in vesicle budding and cargo packaging as well as vesicle targeting and membrane fusion machinery. We were the first to identify new proteins involved in ER-Golgi trafficking, including Erv14, Erv24, Erv26, Erv29, Erv41 and Erv46 that have been studied in our lab and now in many other labs and cell types. Our studies have shown that the major membrane proteins in COPII vesicles have potent COPII and COPI binding signals that drive the cycling of these proteins between the ER and Golgi compartments to catalyze specific processes.

a. Belden, W. J., and **Barlowe, C.** (1996) Erv25p, a component of COPII-coated vesicles, forms a complex with Emp24p that is required for efficient ER to Golgi transport. *J. Biol. Chem.* 271, 26939-26946.
b. Otte, S., Belden, W., Heidtman, M., Liu, J., Jensen, O. and **Barlowe, C.** (2001) Erv41p and Erv46p: New components of COPII vesicles involved in transport between the ER-Golgi complex. *J. Cell Biol.* 152, 503-517.
c. Heidtman, M., Chen, C.Z., Collins, R.N., and **Barlowe, C.** (2003) A role for Yip1p in COPII vesicle biogenesis. *J. Cell Biol.* 163, 57-69.
d. Margulis, N.G., Wilson, J.D., Bentivoglio, C.M., Dhungel, N., Gitler, A.D., and **Barlowe, C**. (2016) Analysis of COPII vesicles indicates a role for the Emp47-Ssp120 complex in transport of cell surface glycoproteins. Traffic 17, 191-210.

4. Molecular definition of anterograde cargo receptors in the early secretory pathway
For efficient export of soluble secretory proteins in COPII vesicles, we hypothesized that transmembrane receptors would bind to the soluble cargo for linkage to COPII coat subunits. Until discovery of COPII, a non-specific “bulk-flow” model had been the prevailing explanation for ER export of soluble cargo. To test our receptor-dependent export model, we have undertaken comprehensive proteomic analyses of purified COPII vesicles. We reasoned that the purified vesicles should contain transmembrane cargo receptors in addition to proteins required for vesicle formation and vesicle targeting/fusion. Our proteomic analysis led to the discovery of several conserved transmembrane cargo receptors (Erv14, Erv25, Erv26 and Erv29) that are required for efficient ER export of soluble cargo as well as certain integral membrane secretory cargo that have type II topology or have lengthy transmembrane domains. These findings have provided significant new mechanistic

insights on the biogenesis of secretory proteins. Moreover, the binding and release of secretory cargo by these receptors is likely a site for cellular regulation and may provide opportunities for therapeutic intervention.

a. Powers, J., and **Barlowe, C.** (1998) Transport of Axl2p depends on Erv14p, an ER-vesicle protein related to the Drosophila cornichon gene product. *J. Cell Biol.* 142, 1209-1222.

b. Belden, W.J., and **Barlowe, C.** (2001) Role of Erv29p in collecting soluble secretory proteins into ER-derived transport vesicles. *Science* 294, 1528-1531.

c. Otte, S., and **Barlowe, C.** (2004) Sorting signals can direct receptor-mediated export of soluble proteins into COPII vesicles. *Nat. Cell Biol.* 6, 1189-1194.

d. Bue, C.A., Bentivoglio, C.M., and **Barlowe, C.** (2006) Erv26p directs pro-alkaline phosphatase into ER-derived COPII transport vesicles. *Mol. Biol. Cell* 17, 4780-4789.

5. Molecular definition of retrograde cargo receptors in the early secretory pathway

While purified COPII vesicles were expected to contain anterograde cargo receptors required for efficient ER export, we had not anticipated that retrograde receptors would also be enriched in these intermediates. Forward transport from the ER to the Golgi complex is balanced by a retrograde pathway that is carried out by COPI coated vesicles. Therefore, most constituents of COPI vesicles are also present in COPII vesicles as these proteins cycle dynamically between the ER and Golgi compartments. In our characterization of the Erv41 and Erv46 vesicle proteins, we determined that this complex functions as a retrograde receptor to retrieve specific ER resident proteins that have escaped to Golgi compartments. The KDEL-receptor had been previously characterized as an important retrograde cargo receptor, we propose that the Erv41-Erv46 complex plays a key role as a retrograde receptor for non-KDEL bearing ER resident proteins. This story is currently evolving as the mechanism by which Erv41-Erv46 binds and releases cargo appears to be quite novel. Recent findings indicate conserved vicinal cysteine pairs are required for the cargo binding cycle and that Erv41-Erv46 functions in retrieval of misfolded cargo from Golgi compartments.

a. Otte, S., Belden, W., Heidtman, M., Liu, J., Jensen, O. and **Barlowe, C.** (2001) Erv41p and Erv46p: New components of COPII vesicles involved in transport between the ER-Golgi complex. *J. Cell Biol.* 152, 503-517.

b. Otte, S. and **Barlowe, C.** (2002) The Erv41p-Erv46p complex: Multiple export signals are required in trans for COPII-dependent transport from the ER. *EMBO J.* 21, 6095-6104.

c. Shibuya A., Margulis N., Christiano R., Walther T.C., and **Barlowe C.** (2015) The Erv41-Erv46 complex serves as a retrograde receptor to retrieve escaped ER proteins. *J Cell Biol.* 208, 197-209.

d. Keiser, K.J. and **Barlowe, C.** (2020) Molecular dissection of the Erv41-Erv46 retrograde receptor reveals a conserved cysteine-rich region in Erv46 required for retrieval activity. *Mol. Biol. Cell 31, 209-220.*

Complete List of Published Work in PubMed:
https://www.ncbi.nlm.nih.gov/pubmed/?term=Barlowe+C

D. Research Support

Project Number: R01 GM52549-27 05/01/2021-04/30/2025
Principal Investigator: Charles Barlowe
Source: NIGMS
Title of Project: Mechanisms of COPII-dependent Transport

The focus of this research program is to elucidate molecular mechanisms that catalyze protein sorting and transport through the early secretory pathway. Defects in biogenesis and sorting of secretory proteins have wide-ranging effects on human health and disease including atherosclerosis, blood clotting disorders, kidney disease and cystic fibrosis.