Twenty-five years after coat protein complex II

Charles Barlowe*
Department of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth, Hanover, NH 03755

ABSTRACT In 1994, a convergence of ideas and collaborative research orchestrated by Randy Schekman led to the discovery of the coat protein complex II (COPII). In this Perspective, the chain of events enabling discovery of a new vesicle coat and progress on understanding COPII budding mechanisms are considered.

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SETTING THE STAGE

In late 1974, Randy Schekman was conducting postdoctoral work with Jon Singer at the University of California, San Diego, when he attended the annual meeting of American Society for Cell Biology that was convened nearby. George Palade had shared that year's Nobel Prize and was presenting a special lecture to the society. Randy was impressed with Palade's brilliance, but came away struck by the lack of a molecular mechanism to explain the secretory process (Schekman, 2013). Intrigued by questions on how biological membranes were assembled, this sparked his ideas to molecularly define intracellular transport. Schekman's determined pursuit led to discovery of major components of the transport machinery, including the coat protein complex II (COPII) in 1994, now some 25 years ago.

In 1976, at the age of 27, Schekman set up his lab at the University of California, Berkeley, and began studying intracellular transport in the budding yeast model, fully appreciating the synergy of combined genetic and biochemical approaches from his graduate training in Arthur Kornberg's lab (Schekman *et al.*, 1974). During Schekman's first year at Berkeley, Peter Novick joined the lab as a new graduate student, and they discovered the first temperature-sensitive secretory mutant (*sec1*), which soon led to a powerful density-enrichment screen for isolation of additional *sec* mutants and identification of 23 complementation groups required for the secretory process (Novick and Schekman, 1979; Novick *et al.*, 1980). Further analyses of the *sec* mutants indicated that they function in distinct stages of the secretory process from the endoplasmic reticulum (ER) to the cell surface, as outlined by Palade (Novick *et al.*, 1981; Stevens *et al.*, 1982). Schekman was by no means alone in his quest to define mechanisms underlying trafficking pathways. Pioneers during this period produced groundbreaking discoveries on clathrin-mediated endocytosis (Pearse, 1976; Goldstein *et al.*, 1979) and coatomer-dependent intra-Golgi transport (Orci *et al.*, 1986; Malhotra *et al.*, 1989). A framework for how cytoplasmic protein complexes could produce coated vesicle intermediates was established. Yet transport between the ER and Golgi compartments remained ill-defined as Schekman continued to seek a cell-free assay that could authentically recapitulate the ER–Golgi transport process.

David Baker, a graduate student in the lab, succeeded in developing such an assay using yeast semi-intact cells that measured delivery of labeled alpha-factor precursor to the Golgi complex where Golgi-specific carbohydrate was attached (Baker *et al.*, 1988). The assay was elegant and quantitative and depended on energy and added cytosol. Importantly, Baker and colleagues could demonstrate that cytosol from *sec23* mutant cells was thermosensitive and could be restored by addition of a wild-type cytosol, which provided a functional assay for purification of the complementing activity. Linda Hicke successfully used this biochemical complementation assay to characterize functional Sec23 protein, which was found in complex with p105/Sec24 (Hicke and Schekman, 1989; Hicke *et al.*, 1992). Michael Rexach further refined subreactions in the ER–Golgi transport process, revealing distinct requirements and assays for the vesicle-budding and fusion stages (Rexach and Schekman, 1991). Genetic analyses also identified an interacting set of *SEC* genes that operated in production of ER-derived vesicles, and the prevailing model at this time placed Sec12, Sec13, Sec16, and Sec23 in the vesicle-budding step (Kaiser and Schekman, 1990). With cell-free assays and a clear dependence on *SEC* gene products, Schekman had set the stage to define the mechanism that produced ER-derived transport vesicles.

THE POWER OF COLLABORATIVE RESEARCH

In the early 1990s, the Schekman lab and the University of California, Berkeley, community were brimming with impressive scientists.

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*Address correspondence to: Charles Barlowe (charles.barlowe@dartmouth.edu).

Abbreviations used: COPI, coatomer complex; COPII, coat protein complex II; EM, electron microscopy; ER, endoplasmic reticulum.

Randy was sporting a diamond earring after promising to have his ear pierced in the unlikely event he was selected to become a Howard Hughes Medical Institute investigator. His lab was on the sixth floor of Barker Hall, next to Jeremy Thorner's group. The labs using yeast as a model organism held regular "supergroup" meetings where one could hear about the latest advances and share in generally constructive banter. Between labs and within the Schekman group, collaboration and free exchange of reagents and ideas was constant. I initially worked with Christophe d'Enfert, a postdoc in the lab who was returning to the Pasteur Institute and generously handing off the Sec12/Sar1 project. Sec12 had been characterized as an ER-localized transmembrane protein by Aki Nakano (Nakano *et al.*, 1988). In the process of isolating the *SEC12* gene, Aki had isolated *SAR1* as a dosage suppressor of *sec12* and found that it encoded a small GTPase (Nakano and Muramatsu, 1989). Christophe had shown that Sec12 recruited Sar1 protein to ER membranes and, when overexpressed, could deplete cytosol of Sar1. This served as the starting point to add back and purify functional Sar1 (d'Enfert *et al.*, 1991).

After some missteps at purification, I learned that Sar1 much preferred a buffer containing detergent or lipids to retain full activity. Using overexpression in yeast, low levels of functional Sar1 protein could be purified in a weeklong procedure (Barlowe *et al.*, 1993). The purified Sar1 was active for nucleotide binding and hydrolysis, and we could show that nucleotide exchange on Sar1 was catalyzed by the cytosolic domain of Sec12 (Barlowe and Schekman, 1993). Supplies of purified Sar1 were used up rapidly, so Tom Yeung developed a bacterial expression system to increase stores of pure protein that was as active as the yeast-expressed version. Tohru Yoshishisa continued the Sec23/Sec24 project and devised methods to overexpress and purify each subunit and the functional complex. Torhu also demonstrated that the Sec23 subunit contained potent GAP activity toward Sar1 GTPase (Yoshihisa *et al.*, 1993). Nancy Pryer and Nina Salama tackled the Sec13 protein and devised a cell-free assay that depended on addition of active Sec13, finding that it too was in a hetero-oligomeric complex with a 150-kDa partner protein, later termed Sec31 (Pryer *et al.*, 1993; Salama *et al.*, 1997). At this point, we wondered whether the full cast for the budding reaction had been identified. Indeed, Nina and Tom showed convincingly that these three protein preps (Sar1, Sec23/Sec24, and Sec13/Sec31), now regularly stored away as aliquots in the −70 C freezer, were sufficient to drive the budding of vesicles carrying labeled alpha-factor precursor. Moreover, secretory cargo was efficiently segregated into vesicles and away from ER-resident proteins such as Bip/Kar2 (Salama *et al.*, 1993).

My initial experiments using Sar1 added back to a Sar1-depleted cytosol had shown that GTP hydrolysis was needed for efficient vesicle budding. Now armed with the purified set of factors, I found that budding proceeded efficiently in the presence of nonhydrolyzable GTP analogue (GMP-PNP) as long as saturating amounts of the purified proteins were added to the reaction. It seemed as if the budding factors were consumed in the reaction when supported with GMP-PNP. The next question was to determine how budded vesicles differed in the presence of GTP and nonhydrolyzable GTP analogue. This required a scale-up to obtain sufficient levels for biochemical analyses. Using isolated ER microsomes and ever-increasing amounts of the purified factors due to Tom Yeung's optimization efforts (Yeung *et al.*, 1995), we began to isolate ER-derived vesicles released into the medium-speed supernatant fraction of budding reactions and could detect polypeptide constituents on protein stained gels. Whereas budded vesicles from both reactions contained similar levels of labeled alpha-factor precursor, the reactions reconstituted with GMP-PNP contained substantially higher levels of Sar1, Sec23/Sec24, and Sec13/Sec31.

Michael Rexach had extensively characterized the properties of ER-derived vesicles produced with cytosol and shared his knowledge of density-gradient procedures. Isolation of budded vesicles based on their buoyant density permitted biochemical analyses and added confidence that the vesicles generated in the reconstituted reactions were similar to those generated in the cytosol-driven reaction. At Michael's urging, we took initial preparations of GTP- and GNP-PNP–derived vesicles down to the electron microscopy (EM) room in the basement of Barker Hall to capture negative-stain EM images. While the preparations showed a relatively uniformly sized population of smaller membrane vesicles, the best description I could give at the next group meeting was of deflated raisin-like structures. Purification on gradients was disrupting the structure of the vesicles. I turned to a gentler gel-filtration procedure after reading how large virus particles could be purified on Sephacryl S-1000. With gel-filtration, budded vesicles were cleanly separated from contaminating membranes and protein factors used for their synthesis. Purified vesicles synthesized with GTP were fully functional for fusion with Golgi membranes, whereas GMP-PNP vesicles were inactive for Golgi fusion. Again Sar1, Sec23/Sec24, and Sec13/Sec31 remained tightly bound to the purified GMP-PNP vesicles, presumably in a manner that prevented vesiclar engagement with Golgi fusion machinery. After fixation and sedimentation of gel-filtered vesicles, Susan Hamamoto at Berkeley took the first thin-section EM images of purified vesicles in the summer of 1993, which revealed an electron-dense material surrounding budded membrane vesicles. However, our images showed a sparse population of vesicles with variable preservation of coat structures.

Randy quickly perceived the need for additional expertise and reinitiated collaboration with Lelio Orci at the University of Geneva. The two labs had produced striking immunolabeling EM images of mammalian Sec23 showing localization to transitional ER cisternae and vesicles (Orci *et al.*, 1991). We began collaboration with Orci's group to more clearly define the morphology and composition of purified ER-derived vesicles. For the next several weeks, we would prepare GTP and GMP-PNP vesicles, fix them with glutaraldehyde at various stages of purification, and ship them off to Geneva by FedEx. A few days later, our fax machine would start churning out pages of images with comments and a Lelio happy face or, on some occasions, a blank sheet with a Lelio sad face and a request for more samples. After iterations and improvements on purification and fixation procedures, the Geneva group was able to collect spectacular thin-sectioned EM images showing fields of coated vesicles (Figure 1). Cryo-sectioning and immuno-EM documented the composition of this new vesicular coat structure. The ER-derived membrane vesicles were 60–65 nm in diameter with a 10-nm-thick protein coat structure. All three of the budding factors were detected in the coat, with Sar1 density more than 10-fold higher in GMP-PNP vesicles.

The initial publication came together quickly at this point (Barlowe *et al.*, 1994). There was some consideration that the coatomer complex (COPI) characterized by Rothman and colleagues contributed to ER budding and that sufficient coatomer was present on membranes to support vesicle production. Dori Hosobuchi was focused on yeast coatomer complex through her studies on Sec21 protein and its identification as the gamma-subunit of coatomer (Hosobuchi *et al.*, 1992). With antibodies and reagents in hand, Dori showed that low levels of coatomer could be extracted from membranes by mild urea treatment, yet reconstituted budding with purified factors continued efficiently. It was clear that this new coat was molecularly distinct from coatomer. Randy proposed that the

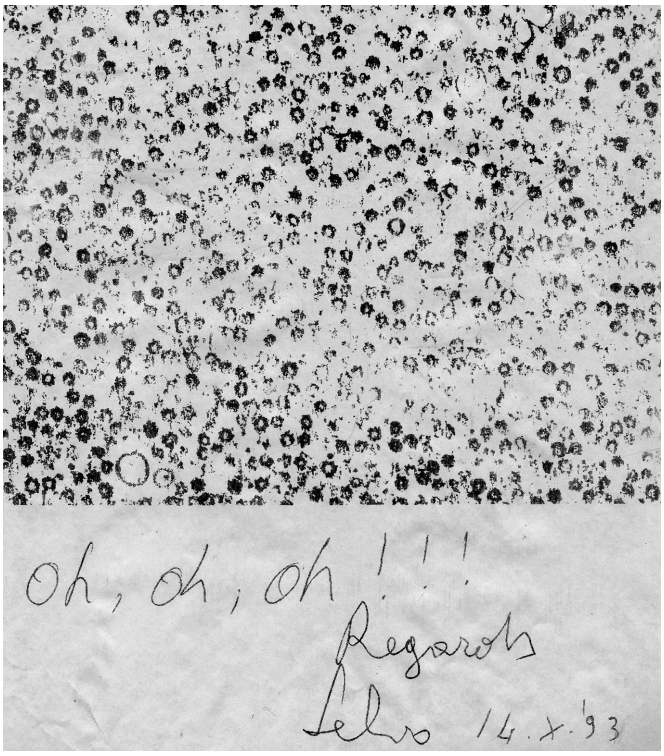

FIGURE 1: Fax sent from Geneva at 19:31 on October 14, 1993, showing a field of COPII-coated vesicles purified by Sephacryl S-100 and caption from Lelio Orci.

new coat structure be called COPII and that coatomer be termed COPI due to its mechanistically similar budding cycles. This terminology is likely from earlier days in Stanford's biochemistry department, where distinct polymerases were discovered and numbered on a regular basis. The naming was prescient, as further understanding of the COPI, COPII, and clathrin coat complexes has revealed conserved functional and structural elements (Lee and Goldberg, 2010).

TOWARD AN UNDERSTANDING OF COPII BUDDING MECHANISMS

Characterization of COPII-coated transport vesicles was a testament to Schekman's vision and to the collaborative scientific approach. Yet this was only another chapter in defining ER export mechanisms, as the findings raised new questions that have engaged many research programs. Notable milestones include minimal reconstitution of COPII vesicle budding from synthetic liposomes with the purified set of coat proteins (Matsuoka *et al.*, 1998). The selective cargo-packaging mechanism was advanced by showing that GTP-locked Sar1 formed tight prebudding complexes with cargo and the Sec23/Sec24 complex (Kuehn *et al.*, 1998; Nishimura *et al.*, 1999). Transmembrane cargo receptors have been identified that link soluble cargo proteins to the COPII coat (Appenzeller *et al.*, 1999; Belden and Barlowe, 2001). Structural approaches provided a molecular basis for how Sec24 subunits recognize and bind specific ER exit motifs for their selective uptake into vesicles (Miller *et al.*, 2003; Mossessova *et al.*, 2003). Crystallographic analyses have also explained how recruitment of the outer layer Sec13/Sec31 complex stimulates Sec23/Sec24 GAP activity toward Sar1 (Antonny *et al.*, 2001; Bi *et al.*, 2007; Fath *et al.*, 2007). Cryo-EM approaches revealed the structures of coassembled Sec23/Sec24 and Sec13/Sec31 coat complexes (Stagg *et al.*, 2008) as well as the architecture of the COPII coat assembled on synthetic membranes (Zanetti *et al.*, 2013; Hutchings *et al.*, 2018).

In current reviews (Hutchings and Zanetti, 2019), models for COPII budding propose that activated Sar1-GTP is produced by ER-localized Sec12 to initiate assembly of inner-layer Sec23/Sec24 complexes bound to selected export cargo. This inner cargo-adaptor layer also interacts with membrane phospholipids and forms a structural scaffold to recruit extended Sec13/Sec31 oligomers. Recruitment and polymerization of the Sec13/Sec31 outer coat subunits produces a flexible cage that drives membrane deformation. Assembly of the Sec13/Sec31 cage then accelerates Sar1-GTP hydrolysis in a process that leads to vesicle scission and coat disassembly. In this manner, the COPII budding cycle reversibly links cargo packaging and membrane deformation to produce transport vesicles.

Nonetheless, several open questions remain regarding COPII-mediated budding. For example, how are COPII proteins organized at ER exit sites and what limits entry of ER-resident proteins into these vesicles? How is vesicle size controlled and vesicle scission catalyzed? Moreover, we have limited understanding of the regulatory circuits that control COPII budding rates in coordination with other cellular processes. It is also unclear to what extent COPII vesicles transport membrane lipids from the ER relative to ER–organelle contact sites. Hopefully bright young scientists are currently viewing this field with new ideas to spark the next big advances.

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