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Authors
Melville, David
Gorur, Amita
Schekman, Randy

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Fatty-acid binding protein 5 modulates the SAR1 GTPase cycle and enhances budding of large COPIII cargoes

David Melville, Amita Gorur, and Randy Schekman*
Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA 94720

ABSTRACT COPII-coated vesicles are the primary mediators of ER-to-Golgi trafficking. Sar1, one of the five core COPIII components, is a highly conserved small GTPase, which, upon GTP binding, recruits the other COPIII proteins to the ER membrane. It has been hypothesized that the changes in the kinetics of SAR1 GTPase may allow for the secretion of large cargoes. Here we developed a cell-free assay to recapitulate COPIII-dependent budding of large lipoprotein cargoes from the ER. We identified fatty-acid binding protein 5 (FABP5) as an enhancer of this budding process. We found that FABP5 promotes the budding of particles ∼150 nm in diameter and modulates the kinetics of the SAR1 GTPase cycle. We further found that FABP5 enhances the trafficking of lipoproteins and of other cargos, including collagen. These data identify a novel regulator of SAR1 GTPase activity and highlight the importance of this activity for trafficking of large cargoes.

INTRODUCTION
Lipoproteins traffic insoluble lipids within an apolipoprotein shell. Large lipoproteins, including chylomicrons, very low-density lipoproteins (VLDLs), and its lipolytic conversion product, LDL, are produced in the intestine and liver, respectively, and transport the majority of cholesterol and triglyceride in the bloodstream. High LDL levels are a major risk factor for atherosclerosis and coronary heart disease (Budoff, 2016), one of the leading causes of mortality in developed countries (Heron, 2012); therefore, increased understanding of the production and processing of lipoproteins bears directly on human health.

The pathophysiology of rare genetic diseases can serve as a powerful entry point for understanding of genes that are most physiologically relevant to a biological pathway. Chylomicron retention disease/Anderson’s disease (CMRD) results in an inability to transport newly synthesized chylomicrons out of intestinal epithelial cells and, in some patients fatty liver and hypercholesterolemia, suggesting that the disease affects lipoprotein transport in the liver as well (Nemeth et al., 1995; Charcosset et al., 2008; Silvain et al., 2008; Peretti et al., 2009; Georges et al., 2011), making it relevant to the study of general lipoprotein production. CMRD is caused by mutations in SAR1B (Jones et al., 2003; Charcosset et al., 2008; Silvain et al., 2008; Peretti et al., 2009; Georges et al., 2011), part of the COPII coat, the primary mediator of protein traffic from the endoplasmic reticulum (ER) to the Golgi apparatus (Hicke and Schekman, 1989; Salama et al., 1993; Yoshihisa et al., 1993; Schekman and Novick, 2004). Strikingly, despite disrupting this fundamental cellular pathway, CMRD only affects limited tissues. Loss of function of other COPIII components is associated with other highly specific phenotypes in both humans and animal models (Boyadjiev et al., 2006; Lang et al., 2006; Bianchi et al., 2009; Schwarz et al., 2009; Merte et al., 2010; Sarmah et al., 2010; Niu et al., 2012). Traffic-specific phenotypes associated with COPIII lesions may help explain another significant question in the protein trafficking field, namely, how very large cargoes are transported (Bonfanti et al., 1998; Stephens and Pepperkok, 2002; Canty and Kadler, 2005). Although cargo-free, self-assembled COPIII vesicles are 40–80 nm in diameter, procollagen bundles form stiff 300-nm rods, and...
previous work on the mechanism of lipoprotein traffic from the ER demonstrated a role for COPII vesicles (Gusarova et al., 2003). In this study, we have refined the cell-free vesicle budding reaction and discovered a novel requirement for the capture of lipoprotein particles. Using the unique level of control afforded by in vitro reconstitution, we have identified FABP5 as a factor that enhances large lipoprotein transport. We further found that FABP5 binds Sec12/PREB and modulates the guanine exchange of SAR1, as well as interacting with the active fragment of SEC31 and altering the GTPase activity of SAR1. We found that the effect of FABP5 extends to collagen trafficking. We propose that FABP5 is a novel regulator of the SAR1 GTPase cycle and plays a significant role in large protein trafficking.

**RESULTS**

**In vitro reconstitution of VLDL budding**

To provide a tool for understanding the cellular requirements for budding of large lipoprotein cargoes, we attempted to reconstitute VLDL budding in a cell-free assay. We modified previously published cell-free reactions designed to detect the formation of transport vesicles, including ones that carry vesicles as large as collagen, that bud from ER membranes (Figure 1A; Kim et al., 2005; Merte et al., 2010; Gorur et al., 2017; Yuan et al., 2017).

Donor ER membrane was prepared from McArdle-RH7777, cultured Rattus norvegicus liver hepatoma cells. Membranes were incubated at 37°C with nucleotides and purified recombinant human COPII proteins. McArdle membranes were washed with high salt, the high-salt wash (HSW) was dialyzed and added to a vesicle budding reaction where indicated, heated (“H”) or treated with proteinase K, then heated (“K”) as indicated. Vesicles in 18,000 × g supernatant fractions from budding reactions were isolated by density gradient flotation.

(A) Scheme of cell-free vesicle budding reaction. In brief, McArdle RH7777 cells were incubated with oleic acid. Treated cells were used to prepare donor membranes which were then incubated at 37°C with GTP and purified recombinant human COPII proteins. McArdle membranes were washed with high salt, the high-salt wash (HSW) was dialyzed and added to a vesicle budding reaction where indicated, heated (“H”) or treated with proteinase K, then heated (“K”) as indicated. Vesicles in 18,000 × g supernatant fractions from budding reactions were isolated by density gradient flotation.

(B) Fractions from the top of an OptiPrep gradient were analyzed by immunoblot. APOB serves as a marker for large VLDL cargoes and ERGIC53 serves as a marker for small traditional COPII cargoes. Ribophorin serves as a marker for ER contamination.

**FIGURE 1:** Budding of APOB lipoprotein cargoes in a cell-free reaction. (A) Scheme of cell-free vesicle budding reaction. In brief, McArdle RH7777 cells were incubated with oleic acid. Treated cells were used to prepare donor membranes which were then incubated at 37°C with GTP and purified recombinant human COPII proteins. McArdle membranes were washed with high salt, the high-salt wash (HSW) was dialyzed and added to a vesicle budding reaction where indicated, heated (“H”) or treated with proteinase K, then heated (“K”) as indicated. Vesicles in 18,000 × g supernatant fractions from budding reactions were isolated by density gradient flotation.

(B) Fractions from the top of an OptiPrep gradient were analyzed by immunoblot. APOB serves as a marker for large VLDL cargoes and ERGIC53 serves as a marker for small traditional COPII cargoes. Ribophorin serves as a marker for ER contamination.

other cargoes (e.g., chylomicrons) can be 600 nm in size. Certain COPII components appear to have evolved specialized functions to allow for expansion of vesicle size and transport of large cargoes, whereas other proteins such as TANGO1 or KLHL12 may enhance COPII-dependent large cargo budding by influencing the SAR1 GTPase cycle (Siddiqi et al., 2003, 2010; Boyadjiev et al., 2006; Lang et al., 2006; Sarmah et al., 2010; Melville et al., 2011, 2014, 2017; Wilson et al., 2011; Siddiqi and Mansbach, 2012; Santos et al., 2016; Tanabe et al., 2016).

The SAR1 GTPase cycle plays a critical role in COPII coat formation, and may play a key role in regulation of vesicle size in large cargo secretion (Venditti et al., 2012; Ma and Goldberg, 2016). The cycle involves two major processes: exchange of GDP for GTP mediated by the guanine exchange factor (GEF) SEC12 (prolactin regulatory element binding [PREB] in mammals; Barlowe and Schekman, 1993; Weissman et al., 2001) and GTPase activity mediated by the GTPase-activating protein (GAP) SEC23. The GAP activity of SEC23 is substantially stimulated by the proline-rich “active fragment” of SEC31, which binds, overlapping the junction of SEC23/SAR1 (Bi et al., 2007; Fromme et al., 2007).

TANGO1 exemplifies one mechanism by which the SAR1 GTPase cycle may be regulated for secretion of large proteins. In collagen secretion, TANGO1 dampens SAR1 GTPase activity by preventing SEC31 from binding to SEC23 (Ma and Goldberg, 2016), introducing a pause that may allow for the loading of large cargoes and genesis of large vesicular carriers (Raote et al., 2017). As the budding event for large cargoes has not been reconstituted with pure components, other factors may come into play.
dominant-negative form of SAR1B, and found that it indeed inhibited APOB budding (Figure 1B, lane 4). This suggested that a factor in the HSW was enhancing COPII activity to allow for VLDL secretion. To determine whether the active factor in HSW was protein, we heated the HSW in a boiling water bath before aliquots were added to the budding reaction. Surprisingly, extreme heating did not inhibit the activity of this fraction (Figure 1B, lane 5). To distinguish a small molecule effector from a thermostable protein, we treated the HSW fraction with proteinase K before heating. Proteinase-sensitive, heat resistant, and sustained a budding reaction that was inhibited by dominant-negative form of SAR1B, and found that it indeed inhibits APOB budding (Figure 1B, lane 8–10). We subcloned the rat FABP5 gene from McArdle cell mRNA into pGEX-2T vectors, and recombinantly expressed and purified the protein from Escherichia coli. We found that the addition of FABP5 to budding reactions enhanced APOB budding (Figure 2A). The effect was dose dependent and most effective in the presence of COPII (Figure 2B). The addition of FABP5 also appeared to enhance budding of ERGIC53 and SEC22B, although not the severalfold change seen with APOB.

To test the cellular function of FABP5, we used CRISPR/Cas9 to generate McArdle cell lines lacking FABP5 expression. We also used a tetracycline-inducible lentivirus expression system to overexpress FABP5. We found that whereas all three cell lines had similar levels of APOB when secretion was blocked with brefeldin A (BFA; Figure 2C), the amount of APOB detected in the medium was increased or decreased with the increase or decrease of FABP5 levels (Figure 2, D and E). Consistent with this observation, the amount of intracellular APOB100 remaining in cells after 3 h of incubation with oleic acid showed the opposite trend, although the changes were not statistically significant (P = 0.1). These data are consistent with what we found in the cell-free budding reactions, suggesting that FABP5 enhances the secretion of large VLDL cargoes.

**FABP5 increases budded vesicle size**
We next examined the size of the APOB-containing carriers in relation to traditional COPII cargo carriers. During optimization of the cell-free vesicle budding assay, we observed that with low concentrations of OptiPrep in self-forming gradients (Figure 3A), we were able to separate APOB100-positive fractions from the fractions containing other cargoes, presumably due to the high lipid content and buoyancy of VLDL (Figure 3B). To visualize and measure the particles contained in fractions 1 and 3, we used negative staining and transmission electron microscopy (TEM). Images of the ERGIC53-containing fractions displayed characteristic cup-shaped vesicular particles ∼100 nm in diameter, consistent with COPII budded vesicles (Figure 3C, bottom). These appeared unchanged by the addition of FABP5. In the APOB100-containing fractions, there were vesicular particles ∼150 nm in diameter only in the FABP5-containing reaction (Figure 3C, top). These data suggest that the FABP5-enhanced APOB100-containing cargos are vesicular and larger than traditional COPII vesicles.

Whereas TEM provides a snapshot on the size and appearance of a fairly small number of vesicles, it does not provide much information on a population level. To test what portion of vesicles were proteins in the heated HSW that did not bind to the MonoQ column. From this analysis we detected several candidate proteins, one of which was a fatty-acid binding protein, FABP5, chosen in part because FABP5 is a known heat-stable protein that has been implicated in chylomicron secretion (Siddiqi et al., 2003; Siddiqi and Mansbach, 2012). We subcloned the rat FABP5 gene from McArdle cell mRNA into pGEX-2T vectors, and recombinantly expressed and purified the protein. From this analysis we detected several candidate proteins, one of which was a fatty-acid binding protein, FABP5, chosen in part because FABP5 is a known heat-stable protein that has been implicated in chylomicron secretion (Siddiqi et al., 2003; Siddiqi and Mansbach, 2012). We subcloned the rat FABP5 gene from McArdle cell mRNA into pGEX-2T vectors, and recombinantly expressed and purified the protein.

**FIGURE 2:** FABP5 enhances lipoprotein secretion. (A) Immunoblot of top fraction of OptiPrep gradient from budding reactions with McArdle donor membrane. (B) Immunoblot of budding reaction for dose response to FABP5. (C) Immunoblot of APOB in wild-type McArdle cells (WT), McArdle transformed by lentivirus to overexpress FABP5 (tgFabp5) or CRISPR/CAS9-mediated knockout cells (Fabp5−/−). β-Actin used as a loading control. Brefeldin A (BFA) added to inhibit secretion and show baseline levels of cellular APOB. (D) APOB secreted into the medium of those cells after 3 h and Ponceau-S stain of BSA in membrane for loading control. (E) Quantification of collagen secreted into media (n = 3).

**FABP5 modulates SAR1 in COPII budding**
large within the overall population of budded membranes, we utilized a NanoSight NS300 particle analyzer, which employs a measure of Brownian motion to compute nanoparticle size distributions. We found that FABPS-containing budding reactions had an extra population peak of ~150 nm, consistent with the TEM data, and that this peak accounted for between 5 and 10% of the overall vesicle population (Figure 3D).

We wanted to assess whether this larger size vesicle peak was due to the presence of FABPS alone or required the large APOB100 cargo. We therefore performed NanoSight analysis on a budding reaction where HeLa cells, which do not secrete lipoproteins (or collagen), were used as a source of donor membrane. In this reaction, we did not see a distinct peak at 150 nm, but we did see a shoulder of increased size in the curve (Figure 3E), suggesting that FABPS may increase the range of COPII sizes, but that cargo may also be an important vesicle size determinant.

Given its role as a fatty-acid carrier protein, it seemed reasonable to consider that FABPS may interact directly with membranes to influence the COPII vesicle budding reaction. To test this, we fluorescently labeled FABPS and SAR1B using amine-reactive dyes NHS-Alexa 680 and NHS-rhodamine, respectively. We performed a budding reaction, and then used a LSRFortessa cell analyzer to select SAR1-positive membrane particles. Of those particles, we determined which were FABPS positive. The number of FABPS-positive SAR1-negative particles was negligible. We found that although only a small percentage of particles were FABPS positive (<2%), those particles were larger on average than FABPS-negative particles (Figure 3F). This suggests that FABPS directly interacts with or helps to form large COPII vesicles. The low percentage of FABPS-positive particles among the population of large particles, as observed by NanoSight, may reflect a transient interaction of this peripheral protein with membranes.

**FABPS alters the kinetics of SAR1 GTPase activity**

We next set out to determine the mechanism by which FABPS interacts with COPII to increase vesicle size. Because the influence of SEC31 on GTP hydrolysis by SAR1 has been implicated in the COPII packaging of large cargoes (Venditti et al., 2012; Ma and Goldberg, 2016; Raote et al., 2017), we hypothesized that FABPS may interact with the GTPase stimulatory active fragment of SEC31A (Fromme et al., 2007). We performed immunoprecipitation (IP) reactions with a StrepII-tagged fragment of SEC31A and found that regardless of the presence of SAR1, FABPS coprecipitated with a large or small fragment of SEC31 (Figure 4A, lanes 1–4 and lanes 7 and 8), suggesting a direct interaction between the two proteins.

We considered the possibility that FABPS interacting with SEC31 may influence the recruitment of other COPII components. The recruitment of COPII components to membranes was assessed using a liposome flotation assay with or without FABPS. Purified recombinant human COPII proteins were incubated with synthetic liposomes and the nonhydrolyzable GTP analogue GTPγS at 37°C for 20 min and applied to the bottom of a sucrose density gradient. After a brief high-speed centrifugation step, floated liposomes were
Because FABP5 increases the rate of GTP loading of SAR1, we decided to test whether FABP5 could increase SAR1 activity with the intrinsic tryptophan fluorescence of SAR1-GTP substantially increased over that of SAR1-GDP (Jin et al., 2017). We performed with purified proteins (Antonny et al., 2001; Futai et al., 2004; Fromme et al., 2007). In this assay, the nucleotide-bound state of Sar1 is monitored by relative fluorescence measurements. The intrinsic tryptophan fluorescence of SAR1-GTP is significantly higher than that of SAR1-GDP.

We first wanted to test whether FABP5 altered the kinetics of SAR1 GTP loading. To this end, we measured the fluorescence of reactions containing purified soluble SAR1 (missing the amphipathic helix), GTP, and combinations of FABP5 or SEC12. We found, as expected, that SEC12 significantly increased the efficiency of GTP loading into SAR1 (Figure 5A). We further found that FABP5 also increased GTP loading efficiency, although not to the same extent as SEC12. When both FABP5 and SEC12 were present, the GTP loading efficiency increased further than either one alone.

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We performed a budding reaction using membranes from IMR90 human lung fibroblasts, which secrete high levels of collagen. The budding reaction was performed in the presence of collagenase to minimize a background of non-membrane-encased collagen likely arising from membranes that rupture during the preparation procedures. We found FABP5 stimulated the production of a population of procollagen-containing vesicles that sedimented at a higher equilibrium density position of an OptiPrep gradient (Figure 6, C and D).
Although the data from the cell-free budding reaction suggest that FABP5 could alter collagen trafficking, we sought a test of this protein in cells by small interfering RNA (siRNA) to knockdown of FABP5 expression in IMR90, as well as U2OS, a human osteosarcoma. We used immunoblotting to analyze the levels of processed collagen secreted into the media, as well as of intracellular procollagen, which should accumulate if secretion is diminished. We found that, upon knockdown of FABP5, processed collagen in the medium decreased, whereas intracellular procollagen increased for both cell lines (Figure 6, D and E). Conversely, when we overexpressed FABP5 through a lentivirus-mediated tetracycline-inducible system in IMR90 cells, the processed collagen in the medium increased, while the intracellular procollagen decreased (Figure 6, F and G).

We used immunofluorescence microscopy to visualize the subcellular localization of procollagen and FABP5 in the inducible IMR90 cells. Although the cytosolic nature of FABP5 made
FABP5 acts through both binding and regulation of GTPase activity, even in the absence of SEC23, the SAR1 GAP regulator of that COPII function, especially pertaining to the large cargoes that pose a special challenge to COPII. We have found that FABP5 appears to modulate the GTPase cycle of SAR1 and allows for formation of large COPII vesicles.

A model of FABP5’s role in COPII-mediated trafficking of large cargoes

Our data present three potential areas where COPII, and its ability to respond to diverse cellular needs for secretion of cargoes of different quantities and sizes, may be regulated. One is through the regulation of GTPase activity, as also seen with other proteins like Sedlin and TANGO1 (Venditti et al., 2012; Ma and Goldberg, 2016), where the presence of FABP5 stimulates a low level of SAR1 GTPase activity, even in the absence of SEC23, the SAR1 GAP (Figure 5C).

A second potential area of regulation is through modulation of GDP/GTP exchange, which FABP5 enhances (Figure 5A), and this leads to an increase in SAR1’s ability to bud particles from a GUV (Figure 5B). It has been found previously that SAR1 binds membranes of high curvature (Hanna et al., 2016), therefore, a slight enhancement in the initiation of membrane curvature as may occur if FABP5 is present, may lead to a forward feedback loop and a further increase in SAR1 recruitment.

FABP5 may have the opposite effect, stimulating the GTPase activity of SAR1, but at a slower rate than that achieved by SEC23, therefore acting in a mechanism similar to TANGO1, slowing the kinetics of SAR1 to allow sufficient time for loading of large cargoes into vesicles (Figure 7B).

The proposed mechanism would likely have an effect on COPII budding beyond large cargoes. Indeed, we do see an increase in the budding of other cargoes, namely, ERGIC53 and SEC22B, although the effect on these smaller cargoes does not appear to be as pronounced.

The many roles of FABP5

Fatty-acid binding proteins, such as FABP5 (also known as E-FABP or Mal1), are intracellular carriers for fatty acids and lipids and have been shown to play a surprisingly diverse role in multiple cellular processes including important roles in metabolism (Hotamisligil and Bernlohr, 2015), neurogenesis (Matsumata et al., 2016), inflammation (Berger et al., 2012; Gally et al., 2013; Bogdan et al., 2018), and cancer (Levi et al., 2013). FABP5 acts through both binding and transport of fatty acids and other lipids such as retinoic acid (Schug et al., 2007; Bando et al., 2014, 2017), and as an indirect regulator of gene expression through interactions with the PPAR class of nuclear receptors (Berger et al., 2012; Gally et al., 2013; Thumser et al., 2014).

The diverse roles of lipids in cellular processes, may, in part, account for the diversity of roles of proteins that carry them. That fatty-acid binding proteins may play a role in membrane trafficking should not be surprising. FABP5 has recently been found to bind the cytoplasmic domain of calnexin as well (Jung et al., 2017), further suggesting a relationship with the ER.

FABP5 is not the first fatty-acid binding protein to be implicated in lipoprotein trafficking. FABP1 has been proposed to play a role in chylomicron budding, and even that it has a binding partner in chylophilic lipoproteins.
SAR1B (Siddiqi and Mansbach, 2012), although the proposed mechanism of action for FABP1 appears unrelated to what we have observed with FABP5.

It is not surprising that FABP5 would have a role in lipoprotein trafficking, as the delivery of fatty acids from the cytoplasm is an integral part of the biogenesis of lipoproteins assembled in the lumen of the ER. Why FABP5 would also have a role in secretion of other large cargoes is less apparent, except insofar as the function of FABP5 relates to the generation of larger vesicles capable of packing unusual cargo complexes.

Many of the roles of FABP5 have functional overlap with its most closely related parologue, FABP4. In fact, FABP5 knockout (KO) mice do not have reduced levels of APOB-containing lipoprotein in serum (Babaev et al., 2011), as would be expected given the partial effect we observe on lipoprotein and collagen secretion in FABP5 knockout cells. However, FABP4/5 double knockout mice (in an ApoE−/− background) do secrete lower levels of APOB lipoproteins (Boord et al., 2004). This suggests that there may be significant overlap in the function of the two proteins in their role in lipoprotein trafficking as well. The many roles of FABP5 make it difficult to parse what role of the protein is responsible for which function. Our direct biochemical approach has allowed us to focus on one aspect of its function in regulating traffic of lipoprotein particles from the ER.

MATERIALS AND METHODS
Antibodies
Commercially available antibodies used for IP, immunoblotting, and immunofluorescence were as follows: Goat anti-apolipoprotein B (EMD, Hayward, CA; #178467 1:500 for immunoblot); rabbit anti–FABP5 human (Proteintech, Rosemont, IL; #12348-1-AP 1:2000 for immunoblot); rat anti-FABP5 monoclonal antibody (Thermo Fisher Scientific, Walther, MA; ma5-24029 1:300 for immunofluorescence); rabbit anti–PC1 LF-41 was a gift from L. Fisher (National Institute of Dental and Craniofacial Research, Bethesda, MD [Fisher et al., 1989, 1995; Bernstein et al., 1996]; 1:5000 for immunoblot); rabbit anti-collagen type I (Millipore, Hayward, CA; #AB7451 1:300 for immunofluorescence); Alexa Fluor 488 donkey anti-rabbit immunoglobulin G (IgG) (Invitrogen, Carlsbad, CA; #A-21206 1:250 for immunofluorescence); Alexa Fluor 546 goat anti-rat IgG (Invitrogen #A-11081 1:250 for immunofluorescence).

Cell culture, transfection, and drug treatments
Rat liver hepatoma MrArdle-RH7777 and human osteosarcoma U2OS were obtained from the American Type Culture Collection. Human lung fibroblasts IMR-90 were obtained from Coriell Cell Repositories at the National Institute on Aging, Coriell Institute for Medical Research, Camden, NJ. IMR-90 and U2OS were maintained in DMEM plus 10% fetal bovine serum (FBS; GE Healthcare). MrArdle-RH7777 were maintained in DMEM plus 20% FBS (GE Healthcare). Cells were kept in a 37°C incubator with 5% CO2. Transfection of DNA constructs into IMR-90 U2OS cells was performed using Lipofectamine 2000 as described in the manual provided by Invitrogen. Oleic acid treatment was used at 0.75 mM oleic acid complexed to fatty-acid–free bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO). Ascorbate treatment was used at 0.25 mM ascorbic acid (Sigma) and 1 mM ascorbic-2-phosphate (Sigma-Aldrich). This concentration was in addition to the amount of ascorbic acid present in FBS (0.08 mM), as supplied by the manufacturer. Doxycycline (Sigma-Aldrich) was used at 1 µg/ml. BFA (Sigma-Aldrich) was used at 10 µg/ml.

Lentivirus production and adipocyte transduction
Rat FABP5 was subcloned into pLenti-puro (Addgene Cambridge, MA; plasmid #39481). The plasmid containing FABP5 was transfected with Lipofectamine 2000 (Invitrogen), following the manufacturer’s recommendations, into HEK293T cells at 50% confluence the day of transfection along with lentiviral packaging plasmids pVSVG (3.5 µg) and psPAX2 (6.5 µg; Addgene). Transfection was performed using one 10-cm dish. After a 24-h transfection, the medium was changed, and after an additional 24 h, the medium was removed and filtered through a 0.45-µm low-protein binding membrane (WVR International, Radnor, PA). McArdle-RH7777 or IMR-90 was then transduced with the virus with 8 µg/ml polybrene (Sigma-Aldrich). After 24 h, medium was replaced with fresh medium, and after an additional 24 h, 2 µg/ml puromycin (Sigma-Aldrich) was added to select transduced cells.

siRNA transfection
We obtained three distinct siRNAs targeting FABP5 Hs_FABP5_8 FlexiTube siRNA (Qiagen, Hilden, Germany; SI04210941), Hs_FABP5_9 FlexiTube siRNA (Qiagen SI04210948), and Hs_FABP5_5 FlexiTube siRNA (Qiagen SI03145835). IMR90 and U2OS cells were transfected with these siRNAs at a final concentration of 20 nM using the Lipofectamine RNAiMAX reagent (Life Technologies, Carlsbad, CA) according to the manufacturer’s instructions. Owing to the high stability of FABP5 protein, after 48 h the process was repeated, and after a further 48 h cells were harvested for immunoblot.

HSW preparation and purification for tandem mass spectrometry
Plates (3 × 15 cm) of McArdle-RH7777 cells were cultured to 95% confluence, incubated with 10 µg/ml BFA and 0.75 mM oleic acid for 1 h. Cells were treated with 20 µg/ml digitonin (5 min on ice) in B88 (20 mM HEPES, pH 7.2, 250 mM sorbitol, 150 mM potassium acetate, and 5 mM magnesium acetate) and washed with 10 ml 1 M KOAc. The HSW was collected as a supernatant fraction after centrifugation at 300 × g for 5 min. It was then desalted through dialysis in HKM overnight at 4°C at a final protein concentration of 0.5 mg/ml.

For further purification of the active HSW fraction, HSW was, where indicated, heated in a boiling water bath for 10 min. Precipitates were centrifuged at 20,000 × g for 10 min and the supernatant was dialyzed in MonoQ Buffer A (20 mM Tris-HCl, pH 7.5, 1 mM MgOAc,10 mM dithiothreitol) overnight at 4°C and then loaded onto an anion exchange column (Mono Q 10/100 GL) on an AKTA FPLC system (GE Healthcare). Flowthrough (unbound) fraction was collected and stored at –80°C at a final protein concentration of 50 ng/µl.

For proteinase K treatment, HSW was incubated with 10 µg/ml proteinase K (Sigma-Aldrich) and stored on ice for 30 min. The reactions were stopped by heating for 10 min in a boiling water bath. For tandem mass spectrometry analysis, 2 µg of heated monoQ unbound fraction of HSW was used. In-solution trypsin digestion, desalting, and liquid chromatography with tandem mass spectrometry were performed by the QB3/Chemistry Mass Spectrometry Facility at the University of California, Berkeley (UC Berkeley) (Strader et al., 2006; Ciciorva et al., 2007; Hervey et al., 2007; Rebecchi et al., 2011).

Vesicle budding reaction
Vesicle budding reactions were performed as previously described (Kim et al., 2005; Gorur et al., 2017; Yuan et al., 2017) with the following modifications: McArdle donor ER membrane was prepared by permeabilizing cells (95% confluent in 3 × 10-cm dishes) that had
been incubated with 10 µg/ml BFA and 0.75 mM oleic acid for 1 h. Cells were treated with 20 µg/ml digitonin (5 min on ice) in B88 and washed with 1 M KOAc in B88 then B88 and resuspended in B88-0 (20 mM HEPES, pH 7.2; 250 mM sorbitol, and 150 mM potassium acetate) to a final concentration of OD_{600} = 2-6. Each 100-µl reaction contained an ATP regeneration system (1 mM ATP, 40 mM creatine phosphate, and 0.2 mg/ml creatine phosphokinase), 3 mM GTP, purified human COPII proteins (0.2 µg SAR1B for McArdiele-RH7777, 1 µg for other cell types, 1 µg SEC23A/24D, and 1 µg SEC13/31A), 1 µg FABP5 (or 10 µg of HSW protein) as indicated, and a final concentration of 0.5 OD_{600}/ml donor ER membrane in B88-0. IMR90 membranes were mixed with 0.1 U/µl collagenase (Sigma-Aldrich). Donor membranes were sedimented by centrifugation (7000 × g for IMR90, 13,000 × g for other cell types) at 4°C. For McArdiele-RH7777 donor membrane, the supernatant was then mixed with 60% OptiPrep (Sigma-Aldrich) gently until homogeneous, for a final concentration of 25% OptiPrep. This was overlaid with 18% OptiPrep and B88. In brief, insect cell lysates were centrifuged at 185,000 × g for 1 h, and 30% ammonium sulfate was added to the supernatant fraction at 4°C. The precipitant was collected by centrifugation at 30,000 × g for 30 min and solubilized in no-salt buffer (20 mM HEPES, pH 8, 10% glycerol, 250 mM sorbitol, 0.1 mM ethylene glycol-bis-β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 5 mM β-mercaptoethanol, and 10 mM imidazole). The solubilized 30% ammonium sulfate precipitant was cleared at 30,000 × g for 20 min, and the supernatant was incubated with prewashed Ni-NTA resin (1.25 ml slurry/l insect cells; Thermo Fisher Scientific) for 1 h at 4°C. Ni-NTA wash buffer (20 mM HEPES, pH 8, 10% glycerol, 250 mM sorbitol, 0.1 mM EGTA, 5 mM β-mercaptoethanol, and 50 mM imidazole) and eluted with 250 mM imidazole. Ni-NTA-eluted SEC13/31A protein was further purified using an anion exchange column (MonoQ) on an AKTA FPLC system (GE Healthcare). Purified fractions were pooled and stored at −80°C at final protein concentrations of 500 ng/µl (for SEC23/24) and 250 ng/µl (for full-length SEC31).

Human SEC13/31A and SEC23A/24D were purified from lysates of baculovirus-infected insect cells, as described previously (Kim et al., 2005). In brief, insect cell lysates were centrifuged at 185,000 × g for 1 h, and 30% ammonium sulfate was added to the supernatant fraction at 4°C. The precipitant was collected by centrifugation at 30,000 × g for 30 min and solubilized in no-salt buffer (20 mM HEPES, pH 8, 10% glycerol, 250 mM sorbitol, 0.1 mM ethylene glycol-bis-β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 5 mM β-mercaptoethanol, and 10 mM imidazole). The solubilized 30% ammonium sulfate precipitant was cleared at 30,000 × g for 20 min, and the supernatant was incubated with prewashed Ni-NTA resin (1.25 ml slurry/l insect cells; Thermo Fisher Scientific) for 1 h at 4°C. Ni-NTA wash buffer (20 mM HEPES, pH 8, 10% glycerol, 250 mM sorbitol, 0.1 mM EGTA, 5 mM β-mercaptoethanol, and 50 mM imidazole) and eluted with 250 mM imidazole. Ni-NTA-eluted SEC13/31A protein was further purified using an anion exchange column (MonoQ) on an AKTA FPLC system (GE Healthcare). Purified fractions were pooled and stored at −80°C at final protein concentrations of 500 ng/µl (for SEC23/24) and 250 ng/µl (for full-length SEC31).

**Flow cytometry**

Vesicle budding reactions were scaled up 2x for flow cytometry analyses. Budding reactions were performed as described above, with FABP5 and SAR1 labeled with Alexa Fluor 680 NHS ester (Invitrogen A37567) and NHS-rhodamine (Invitrogen 46406) according to manufacturer’s protocol. Aliquots (40 µl) were taken from the top of an OptiPrep gradient. Particles (100,000) were collected for each sample. FSC-A and FSC-H were used to gate single particles (singlets), which were used for further analysis. Gating of each fluorescent channel was determined by comparing a control sample without any fluorescence labeling and a control that was labeled in a single channel. Data were collected on a BD LSR Fortessa (Becton Dickinson, Franklin Lakes, NJ) and analyzed by FlowJo software and Flowing software. Instruments and software were provided by the LKS flow core facility at UC-Berkeley.

**Nanoparticle tracking analysis**

Sizes of vesicles budded in vitro were estimated using a NanoSight NS300 instrument equipped with a 405-nm laser (Malvern Instruments, Malvern, United Kingdom). Particles were analyzed in the scatter mode without a filter. Silica 100-nm microspheres (Poly-sciences, Warrington, PA) were analyzed to check instrument performance and determine the viscosity coefficient of B88. Aliquots (20 µl) of vesicles were collected from the top of the flotation gradient as described in the vesicle budding reaction section and diluted 50x with 980 µl filtered B88 (0.02 µm; Whatman). The samples were automatically introduced into the sample chamber at a constant flow rate of 50 (arbitrary manufacturer unit, −10 µl/min) during five repeats of 60-s captures at camera level 11 in scatter mode with Nanosight NTA 3.1 software (Malvern Instruments). The particle size was estimated with detection threshold 5 using the Nanosight NTA 3.1 software, after which “experiment summary” and “particle data” were exported. Particle numbers in each size...
category were calculated from the particle data, in which “true” particles with track length >3 were pooled, binned, and counted with Excel (Microsoft).

**Electron microscopy of budded vesicles**

For morphological analysis of the budded vesicles, the top 40 µl of the OptiPrep gradient from a 2X scaled budding reaction (as described above) was fixed with 2% paraformaldehyde (PFA) and 0.2% glutaraldehyde in BB8 buffer for 15 min at 4°C. Samples were spread onto a nickel grid coated with Formvar (Plano, Wetzlar, Germany). Excess liquid was blotted off with filter paper, the grid was stained with 1% aqueous uranyl acetate, and excess staining solution was blotted off. Dried specimens were examined on a ZEISS EM 900 transmission electron microscope.

**Coimmunoprecipitation assay**

Purified protein (1 µg SAR1A, 1 µg SAR1B, 1 µg FABPS, 1 µg active fraction SEC31A, and 3 mM GTP or GTPyS) was added to 100 µl HKM buffer (20 mM HEPES, pH 7.2, 150 mM KOAc, 1 mM Mg(OAc)₂) and reactions were incubated at 37°C for 30 min. An aliquot (20 µl) of equilibrated Strept-Tactin Sepharose slurry (IBA Lifesciences, Goettingen, Germany; 2-1201-010) was added to each reaction, and the protein-bead mixture was incubated for 1 h at 4°C with gentle mixing. Beads were washed five times in HKM by centrifugation at 3000 x g for 5 min at 4°C. Protein was eluted by incubation with 2.5 mM desthiobiotin (Sigma D1411) in HKM, and then analyzed by SDS-PAGE followed by silver staining with a Pierce Silver Stain Kit (Thermo Fisher 24612) according to the manufacturer’s protocol.

**GTPase activity assay**

The tryptophan fluorescence GTPase activity assay was performed at 37°C as described (Antonny et al., 2001; Futai et al., 2004; Fromme et al., 2007), using a stirred-cell cuvette. In HKM buffer, we added soluble SAR1B to a final concentration of 1.33 µM and where indicated SEC31 active fragment (2 µM; Fromme et al., 2007), the cytosolic domain of SEC12 (2 µM), or FABPS (2 µM). Five minutes later, GTP was added to 30 µM. For Figure 5C, SEC23A-SEC24D complex was added to 250 nM after GTP exchange was complete (∼10 min) and FABPS was added simultaneously where indicated.

**Liposome binding assay**

The liposome binding assay was performed as described for yeast COPII proteins (Matsuoka et al., 1998; Kim et al., 2005) using 10% cholesterol major-minor mix liposomes with the addition of DGSNTA(Ni) (Avanti 790404) for binding of his-tagged SEC12. Following a 20 min incubation at 37°C, the protein–liposome mixture was separated by flotation through a sucrose density step gradient (in HKM buffer) achieved by centrifugation at 391,000 x g for 25 min at 22°C.

**GUV budding assay**

GUVs were prepared by electroformation as previously described using major-minor mix (Matsuoka et al., 1998) synthetic liposomes containing 10% cholesterol (Angelova and Dimitrov, 1986; Bacia et al., 2011). Briefly, lipids were combined in chlorform:methanol (2:1, volume ratio) at 10 mg/ml total concentration and dried as a thin layer on indium-tin-oxide–coated glass slides. The slides were assembled to form a chamber by using 3-mm-thick silicon spacers and holding the slides together with office clips. The chamber was filled with sucrose solution (540 mOsml/kg) and electroformation was performed over 2 h using a 1.4 V, 10 Hz sinusoidal voltage. GUVs (of 5 µl) were added to 45 µl BB8 containing 5 µg SAR1 and 2 µg FABPS as indicated. Reactions were incubated for 30 min at 37°C and diluted 1:100 for particle analysis on NanoSight NS300 as described above. All lipids were obtained from Avanti Polar Lipids, Alabaster, AL.

**Immunofluorescence**

Cells growing on glass coverslips were fixed in 4% PFA for 20 min at RT, washed five times with phosphate-buffered saline (PBS), and incubated with permeabilization buffer (PBS containing 0.1% Triton X-100 and 0.2 M glycine) at RT for 15 min. Cells were incubated with blocking buffer (0.5% BSA in PBS) for 30 min at room temperature followed by incubation for 1 h each at RT with primary antibody and then secondary antibody. Antibody incubations were followed by five washes with PBS. Coverslips were mounted in ProLong-Gold antifade mountant with DAPI (Thermo Fisher Scientific) overnight, before imaging. Images were acquired using Zen 2010 software on an LSM 710 confocal microscope system (ZEISS, Oberkochen, Germany). The objectives used were Plan-Apochromat 100x, 1.4 NA.

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