Sec3-containing Exocyst Complex Is Required for Desmosome Assembly in Mammalian Epithelial Cells

Nicholas J. Andersen* and Charles Yeaman**†

*Department of Anatomy and Cell Biology and **Program in Molecular and Cellular Biology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242

Submitted June 5, 2009; Revised October 2, 2009; Accepted October 22, 2009
Monitoring Editor: Patrick J. Brennwald

The Exocyst is a conserved multisubunit complex involved in the docking of post-Golgi transport vesicles to sites of membrane remodeling during cellular processes such as polarization, migration, and division. In mammalian epithelial cells, Exocyst complexes are recruited to nascent sites of cell–cell contact in response to E-cadherin–mediated adhesive interactions, and this event is an important early step in the assembly of intercellular junctions. Sec3 has been hypothesized to function as a spatial landmark for the development of polarity in budding yeast, but its role in epithelial cells has not been investigated. Here, we provide evidence in support of a function for a Sec3-containing Exocyst complex in the assembly or maintenance of desmosomes, adhesive junctions that link intermediate filament networks to sites of strong intercellular adhesion. We show that Sec3 associates with a subset of Exocyst complexes that are enriched at desmosomes. Moreover, we found that membrane recruitment of Sec3 is dependent on cadherin-mediated adhesion but occurs later than that of the known Exocyst components Sec6 and Sec8 that are recruited to adherens junctions. RNA interference-mediated suppression of Sec3 expression led to specific impairment of both the morphology and function of desmosomes, without noticeable effect on adherens junctions. These results suggest that two different exocyst complexes may function in basal–lateral membrane trafficking and will enable us to better understand how exocytosis is spatially organized during development of epithelial plasma membrane domains.

INTRODUCTION

Protein complexes involved in membrane trafficking are structurally conserved from yeast to mammals. One such complex is the hetero-octameric Exocyst complex, which comprises Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 (Hsu et al., 1996; TerBush et al., 1996). Exocyst complexes are enriched at sites of active membrane expansion and are hypothesized to function as molecular tethers that anchor transport vesicles to the plasma membrane before soluble N-ethylmaleimide-sensitive factor attachment protein receptor-mediated fusion. In budding yeast, the Exocyst is recruited to presumptive bud sites and the tips of small-budded cells during early apical growth and to the mother-bud neck during cytokinesis (TerBush and Novick, 1995; Finger et al., 1998). In plants, this complex accumulates at the apex of growing tobacco pollen tubes and is required for cellular morphogenesis (Hala et al., 2008). In mammals, it is enriched at sites of membrane remodeling, such as neuronal growth cones (Hazuuka et al., 1999), podocyte foot processes (Simons et al., 1999), the leading edges of migrating cells (Rosse et al., 2006; Zuo et al., 2006; Spiczka and Yeaman, 2008) and apical junctional complexes (AJs), which include adherens junctions and tight junctions, of expanding lateral membranes such as those of polarizing epithelial Madin-Darby canine kidney (MDCK) cells (Yeaman et al., 2004).

The earliest studies of Exocyst function in polarized epithelial cells showed it to be required for basal–lateral delivery of newly synthesized low-density lipoprotein receptors but not for apical delivery of p75 neurotrophin receptors (Grindstaff et al., 1998). Subsequent studies showed that additional pools of Exocyst complexes localized to and functioned within the endocytic system (Folsch et al., 2003; Langevin et al., 2005; Oztan et al., 2007), endoplasmic reticulum (ER) (Lipschutz et al., 2003), primary cilia/basal bodies (Rogers et al., 2004; Park et al., 2008; Overgaard et al., 2009; Zuo et al., 2009), and apical plasma membrane (Shin et al., 2000; Beronja et al., 2005; Blankenship et al., 2007) of certain polarized epithelial cell types. In addition, an essential role for the Exocyst at the cleavage furrow and midbody of dividing cells has been described previously (Fielding et al., 2005; Gromley et al., 2005; Cascone et al., 2008). Although it is clear that Exocyst activities are required for efficient membrane delivery to multiple destinations within the exocytic and endocytic systems, it is not known whether the same Exocyst holocomplex mediates each of these trafficking events, or whether distinct Exocyst complexes specify disparate tethering events at different target membranes.

Of the eight Exocyst subunits, Sec3 is exceptional in many respects. Under certain conditions, the budding yeast orthologue Sec3 is nonessential for growth and secretion (Finger and Novick, 1997; Wiederkehr et al., 2004). However, cells lacking Sec3 have polarity defects. Indeed, Sec3 was hypothesized to be a spatial landmark for sites of polarized secretion because Sec3-green fluorescent protein (GFP) fusion proteins localized to presumptive bud sites even in the face of disruption of the actin cytoskeleton, secretion, and the function of other Exocyst subunits (Finger et al., 1998). In photobleaching recovery experiments, Sec3 was the only Exocyst component that was not, at least in part, delivered to...
secretion sites on transport vesicles (Boyd et al., 2004). Although the polarized localization of Sec3 in budding yeast was insensitive to blocks in membrane traffic and disruption of the actin cytoskeleton, it depended on direct interactions with active Rho1 and Cdc42 GTPases (Guo et al., 2001; Zhang et al., 2001). Sec3 associates with plasma membranes directly by binding phosphatidylinositol 4,5-bisphosphate (Zhang et al., 2008), a property that is shared with the Ezo70 subunit (He et al., 2007; Liu et al., 2007). Finally, sec3 mutants are unique among yeast Exocyst mutants because they display an aberrant ER distribution (Finger and Novick, 1997). Sec3 was recently shown to be required for inheritance of the cortical ER during yeast cell division, and its role there may be to stabilize associations between the ER tubules and the bud as they are delivered to it (Wiedekehr et al., 2004). Collectively, these results show that Sec3 is an important and potentially multifunctional protein in budding yeast, with roles in both cell polarity and organelle inheritance.

In contrast to yeast Sec3, very little is currently known about its mammalian orthologue, which is the last subunit of the mammalian Exocyst complex to have been identified (Brymora et al., 2001; Matern et al., 2001). Unlike yeast Sec3, the mammalian protein lacks an amino-terminal Rho binding domain, so it is unlikely to directly bind GTPases of the Rho family (Matern et al., 2001). Nevertheless, Sec3 interacts with the polarity protein IQGAP1 to facilitate the targeted delivery of matrix metalloproteinases to tumor cell invadopodia, and this interaction seems to be regulated by the RhoA and Cdc42 GTPases (Sakurai-Yageta et al., 2008). It is possible that in metazoans accessory proteins link Sec3 to Rho GTPases. In fact, ICRI, a tropomyosin-related protein, was recently suggested to serve this function in Arabidopsis (Lavy et al., 2007). A GFP fusion of human Sec3 failed to assemble into Exocyst holocomplexes and remained cytosolic when expressed in MDCK cells (Matern et al., 2001) but was recruited to plasma membranes when coexpressed with GLY1, a glycinetransporter with which Sec3 interacted in vitro (Cubelos et al., 2005). However, the localization and function of endogenous mammalian Sec3 have not been investigated. Because this subunit is important for ensuring correct polarized localization of Exocyst complexes in budding yeast, this represents an important gap in our knowledge of the mammalian Exocyst complex.

**MATERIALS AND METHODS**

**Antibodies and Fluorescent Probes**

Mouse monoclonal antibodies (mAbs) against Sec6 (H915 and 8A5) and Sec8 (2E9, 2E12, 5C3, 7E8, 8F12, 10C2, and 17A10), and rabbit polyclonal antibodies against Sec3CT, Sec3C, Exo70, and Exo84 have been described previously (Hsu et al., 1996; Kee et al., 1997; Yeaman, 2003). Rabbit polyclonal antibodies against Sec3NT were generated by immunizing rabbits with a His6/V5-tagged fragment of human Sec3 (residues 2-205) that was expressed in baculovirus. Rabbit polyclonal antibodies against Sec3CT1 were generated by immunizing rabbits with a HisA/V5-tagged fragment of human Sec3 (residues 2-205) that was expressed in baculovirus and purified by nickel-nitrilotriacetic acid agarose. Rabbit polyclonal antibodies against Sec3C were generated by immunizing rabbits with a Sec3CT subfragment (residues 226-205) that was expressed in baculovirus and purified by nickel-nitrilotriacetic acid agarose. Rabbit polyclonal antibodies against Sec3NT were generated by immunizing rabbits with a His6/V5-tagged fragment of human Sec3 (residues 2-205) that was expressed in baculovirus and purified by nickel-nitrilotriacetic acid agarose. Rabbit polyclonal antibodies against Sec3NT were generated by immunizing rabbits with a His6/V5-tagged fragment of human Sec3 (residues 2-205) that was expressed in baculovirus and purified by nickel-nitrilotriacetic acid agarose.

**Immunofluorescence Labeling**

Cells were prepared for immunofluorescence by fixation in methanol at −20°C for 5 min or, alternatively, preextraction in 1% Triton X-100 in buffer containing 10 mM piperazine-N,N′-bis(2-ethanesulfonic acid), pH 6.8, 50 mM NaCl, 300 mM sucrose, and 3 mM MgCl2 (CSK) before fixation in methanol at −20°C for 5 min, or 4% parafomaldehyde at 4°C for 20 min. After cells were quenched in buffer containing 50 mM NH4Cl, they were blocked in 0.2% fish-skin gelatin in Ringer's saline (10 mM HEPES, pH 7.4, 154 mM NaCl, 72 mM KCl, and 1.8 mM CaCl2) for 20 min at room temperature. Antibodies were diluted in blocking buffer and applied to cells for 2 h at 4°C. After five washes in blocking buffer, FITC and Texas Red conjugated secondary antibodies were applied for 1 h at 4°C. Coverslips were washed five times and mounted in VECTORSHIELD containing 4,6-diamidino-2-phenylindole (Vysis, Inc., Downers Grove, IL) or in Evinol/ProLong Gold (Molecular Probes, Inc., Eugene, OR) or in ProLong Gold Antifade Kit (Molecular Probes, Inc., Eugene, OR). Samples were viewed with either a Microphot-FX microscope (60× objective; Nikon, Tokyo, Japan) or a Leica TCS SP2 confocal microscope (5× objective; Carl Zeiss, Thornwood, NY), or by using a krypton/argon laser with 488 nm (FITC) and 543 nm (Texas Red) laser lines, both tuned to 633 nm (long-pass) in the figure legends. Digital images data collected from the Microphot-FX microscope (Nikon) were obtained with a Kodak DCS 760 digital camera.

**Hanging Drop Assay**

Hanging drop assays were performed as described previously (Kim et al., 2008; Zhang et al., 2008). Human foreskin fibroblasts (HFF) were purchased from ATCC. Samples of primary HFF and Sec3 knockdown (MCF-10A) cells were suspended by trypsin treatment, centrifuged, and resuspended at a concentration of 2.5 × 10⁴ cells/ml. Fifty-microliter droplets were added to the sides of a 10-cm tissue culture plate lid. Five milliliters of culture medium (Opti-MEM; Invitrogen) were added to the bottom of the plate to reach final concentration of 200 μl. The lids were inverted and suspensions were either left untreated or triturated by pipetting twice with a 20-μl pipette tip that had been prewashed with phosphate-buffered saline (PBS) containing 0.1% Triton X-100 PBS and rinsed with

Vol. 21, January 1, 2010 153

Sec3 Is Required for Desmosome Assembly

Medicine, Chicago, IL). Fluorescein isothiocyanate (FITC)-goat-antimouse, and Texas Red-donkey-antirabbit immunoglobulin (IgG) were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Alexa594-goat-antichicken IgY was purchased from Biocytex (Carlsbad, CA). Horseradish peroxidase-conjugated rabbit-antichicken IgY was purchased from Promega (Madison, WI). 125I-Labeled goat anti-rabbit IgG and 125I-Labeled goat anti-rabbit IgG were purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA).
Gel Electrophoresis and Immunoblotting

Protein samples were incubated in SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer for 10 min at 65°C before separation in 7.5, 10, or 12.5% SDS-polyacrylamide gels. Proteins were electroblotted onto PVDF membranes. Bands were visualized using a Phosphorimager (Typhoon; GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) and ImageQuant software, version 5.0 (GE Healthcare).

Detergent Solubility Analysis

MDCK cells were washed three times with Ringer’s saline on ice, and lysed in either CSK or radioimmune precipitation assay (RIPA) buffer (50 mM Tris-HCl, pH 7.5, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, and 1 mM EDTA) containing protease inhibitors (10 μg/ml each of peptatin A, leupeptin, and antipain). Cell lysates were collected in 1.5-ml Eppendorf tubes and incubated on ice for 20 min. Detergent insoluble fractions were pelleted at 20,000 × g (Eppendorf 5417C) for 10 min at 4°C and extracted by repeated passages through 18-, 23-, and 25-gauge needles, in 1% SDS. Equal volumes of soluble and insoluble fractions were resolved by SDS-PAGE. Proteins were transferred to Immobilon P membranes for immunoblotting with antibodies specific for each Exocyst subunit, and signals were quantified with a phosphorimager, as described above.

Exocyst Fractionation

Cells were homogenized in isotonic sucrose buffer [0.25 M sucrose in 20 mM HEPES-KOH, pH 7.2, 90 mM KCl, 2 mM Mg(OAc)₂, and protease inhibitors] by repeated passage through a ball bearing homogenizer (Variable, Stanford University, Stanford, CA). Separation of different membrane compartments was achieved by centrifugation in three-step 10–20–30% (wt/vol) iodixanol gradients (Yeaman et al., 2001). One third of the postnuclear supernatant was mixed with Opti-Prep (60% [wt/vol] iodixanol; Nycomed, Oslo, Norway) and homogenization buffer to generate solutions containing 10, 20, or 30% iodixanol. Equal volumes of these solutions were layered in centrifuge tubes, and samples were centrifuged at 353,000 × g for 3 h at 4°C, in an NV85 rotor (Beckman Coulter, Fullerton, CA). Fractions (0.5 ml) were collected, refractive indices were read, and proteins were separated by SDS-PAGE. Proteins were electroblotted from gels to Immobilon P membranes for immunoblotting, as described above. For gel filtration analysis, confluent monolayers of MDCK cells were extracted for 10 min at 4°C, in Tris-saline buffer containing 0.5% (vol/vol) NP-40 and protease inhibitors. Cell lysates were centrifuged at 15,000 × g for 1 h. After five washes, 10 min each, in TBS containing 0.1% Tween 20, the blots were incubated with [125I]-labeled goat anti-mouse or goat anti-rabbit secondary antibody for 1 h at room temperature. Blots were washed as described above and then twice in TBS and exposed to phosphorimager screens. The amount of labeled antibody bound to the blots was determined directly using a Phosphorimager (Typhoon; GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) and ImageQuant software, version 5.0 (GE Healthcare).

Immunoprecipitation

RIPA extracts of MDCK cells were pre-cleared with Pansorbin (Calbiochem, La Jolla, CA) and incubated overnight with specific primary antibodies prebound to protein A-Sepharose (GE Healthcare). Beads were pelleted by gentle centrifugation, and supernatant was transferred to fresh antibody-coupled beads. This was repeated for a total of three rounds (anti-Exoc6 mAbs 2E12, 5C3, 10C2) or four rounds (anti-Exoc5 mAbs NT3) of immunoprecipitations. Then, 10% of the starting extract and the final depleted supernatant were removed for analysis. For analysis of Exocyst complexes lacking Sec3, lysates depleted of Sec3 were subjected to immunoprecipitation with anti-Exoc8 antibodies overnight at 4°C. Samples were resolved by SDS-PAGE and immunoblotted with antibodies specific for Sec3, Sec6, and Sec8, after electrophoretic transfer to PVDF membranes as described above.

To determine relative expression levels of Sec3 and Sec8 and MDCK cell surface proteins, cells were cultured in serum-free medium, metabolically labeled with [35S]methionine/cysteine (EasyTag; PerkinElmer Life and Analytical Sciences) overnight, and amounts of each radiolabeled subunit were compared after immunoprecipitation with specific antibodies. To correct for immunoprecipitation efficiency differences, a non-radioactive reference lystate was prepared, and recoveries of Sec3 and Sec8 were determined by quantitative immunoblotting with specific antibodies, as described above. This revealed that anti-Sec3 and anti-Exoc8 immunoadsorbents recovered 93% and 95% of the Sec3 and Sec8 in the lystate, respectively. In addition, the relative masses of canine Sec3 (102,017 Da) and Sec8 (110,627 Da), as well as differences in methionine/cysteine content of the two proteins, were considered.

Surface Repopulation Assay

Control and Sec3 knockdown MCF-10A cells were seeded at confluent densities on 12-mm Transwell 0.45-μm polycarbonate filters (Corning Life Sciences, Lowell, MA) in LM. At various time points after a calcium switch, cells were plated on ice and washed five times with Ringer’s saline. Sulfot-NHS-S-S-Biotin (Pierce Chemical, Rockford IL) (0.5 mg/ml in Ringer’s saline) was applied to both apical and basal-lateral surfaces (0.5 ml apical/1 ml basal-lateral), and the cells were left for 1 h at room temperature. After washing, the biotinylated membranes were incubated with 0.2% (vol/vol) iodixanol gradients (Yeaman et al., 2001). The localization of several endogenous Exocyst subunits in mammalian epithelial cells has been reported previously (Grindstaff et al., 1998; Lipschutz et al., 2000; Folsch et al., 2003; Prigent et al., 2003; Reiners et al., 2004; Oztan et al., 2007; Zuo et al., 2009) but that of Sec3 has yet to be examined. Although a Sec3-GFP fusion protein was shown to have a cytosolic distribution, it is unclear whether this reflects the localization of endogenous Sec3 (Matern et al., 2001). We approached this question by preparing polyclonal antibodies against fusion proteins representing either N- or C-terminal regions of human Sec3 (Figure 1A). Both antibodies were specific, as determined by a variety of criteria (Figure 1, B–G, and Supplemental Figure S1). Antibodies to both N- and C-terminal domains labeled pools of Sec3 distributed in a punctate, discontinuous pattern along the plasma membrane and in the cytoplasm of MDCK cells (Figure 1, E and F). Preimmune serum did not label either pool (Figure 1D), and immune serum depleted of Sec3-specific antibodies showed decreased levels of plasma membrane and cytoplasmic labeling (data not shown). Antibodies against the N terminus of Sec3 additionally labeled a perinuclear compartment in subconfluent MDCK cultures (Figure 1F). Therefore, in contrast to ectopic GFP-Sec3, endogenous Sec3 was, at least in part, associated with the plasma membrane and cytoplasmic organelles.

To determine whether Sec3 colocalized with other Exocyst subunits on lateral plasma membranes, we colabeled MDCK cells with anti-Exoc3 C-terminal antibodies and monoclonal Sec6 and Sec8 antibodies. Specific Sec3 immunolabeling was coincident with that of Sec6 when one specific mAb (8A5), but not another (9H5), was used (Figure 2, A and B). In polarized MDCK cells, structures labeled by anti-Sec3 and anti-Sec8(8A5) were concentrated at two distinct sites along lateral membranes (Figure 2B). In contrast, anti-Sec3 antibodies did not label structures recognized by anti-Sec6(9H5) (Figure 2A), in spite of the fact that this antibody was pre-
Figure 1. Antibodies to Sec3 are specific. (A) Schematic representation of human Sec3. Three coiled-coil motifs (amino acids 152-176, 205-257, and 742-764) are predicted by the COILS program. Lines indicate regions of the protein that were used to generate antibodies. The N-terminal Sec3 (Sec3NT) peptide encompassed amino acids 2-205, and the C-terminal Sec3 (Sec3CT) peptide consists of amino acids 692-818. (B) The specificity of the anti-Sec3CT antibody was examined by Western blotting. A 100,000 g postnuclear membrane pellet (p) and supernatant (s) were resolved by SDS-PAGE, and immunoblotted with Sec3CT preimmune serum, postimmune serum, and postimmune serum depleted of either glutathione transferase (GST) or GST-Sec3CT antibodies. Specific Sec3 signal was present only in the membrane fraction (p). (C) The specificity of the anti-Sec3NT antibodies was examined by Western blotting. 15 µg of RIPA lysates of MDCK, HBEC, Caco-2, IMCD-3, MCF-10A, and HeLa cells and also BBC, were resolved by SDS-PAGE and blotted against anti-Sec3NT preimmune, postimmune, and postimmune serum depleted of anti-GST-Sec3NT antibodies. In all cell lines and the BBC, a band corresponding to the predicted mass of Sec3 (102 kDa) was present. The smaller band present in some samples might represent a splice variant or be a consequence of protein degradation. (D and E) The specificity of anti-Sec3CT antibodies was examined by immunofluorescence. MDCK cells were fixed and labeled with anti-Sec3CT preimmune serum, postimmune serum, and postimmune serum depleted of anti-GST-Sec3CT antibodies. All cell lines and the BBC, a band corresponding to the predicted mass of Sec3 (102 kDa) was present. The smaller band present in some samples might represent a splice variant or be a consequence of protein degradation. (F) Anti-Sec3CT antibodies specifically labeled puncta on the plasma membranes and in the cytoplasm. (G) Anti-Sec3NT antibodies also labeled the plasma membranes in a punctate pattern but, in addition, labeled the perinuclear region. (G) 0.5 µg of the Sec3NT immunogen (Sec3NT-His/V5) was resolved by SDS-PAGE and immunoblotted with anti-Sec3NT and anti-V5 antibodies. Both the anti-V5 and anti-Sec3NT antibodies recognized the immunogen.

Previously shown to label Exocyst pools associated with the AJC (Yeaman et al., 2004), Sec3 localized to lateral membrane sites that were more basal than those detected by anti-Sec6(9H5). In addition, although the labeling pattern of anti-Sec6(9H5) was continuous and even, that of anti-Sec3 and anti-Sec6(8A5) was discontinuous and punctate. Therefore, although localization of Sec3 to lateral membranes was similar to that of other Exocyst subunits reported previously, Sec3 did not colocalize with previously characterized Sec6/8 complexes there. To confirm that the novel punctate labeling pattern observed with anti-Sec3 and anti-Sec6(8A5) antibodies represented bona fide Exocyst localization, the distribution of several other Exocyst subunits was examined. Because prior studies of Sec8 localization in MDCK cells used a mAb (8F12) that did not colabel structures decorated by anti-Sec3 antibodies, we screened several fixation conditions and a panel of 10 mAbs to Sec8 to determine whether these proteins were colocalized in cells. We found that a cocktail of anti-Sec8 mAbs (2E9, 5C3, 7E8, and 17A10) applied to methanol-fixed cells labeled a pool of Sec8 that colocalized with Sec3 at the lateral plasma membrane (Figure 2C). Permeabilization of cells with Triton X-100 before fixation revealed that this Sec8 pool was, like Sec3, distributed in a punctate and discontinuous manner on the membrane (Supplemental Figure S2).

Polyclonal anti-Sec15 antibodies also labeled plasma membrane structures that were indistinguishable from those labeled by anti-Sec6(8A5) (Figure 2D). This labeling was enriched at sites of cell–cell contact and absent from free cell borders, consistent with Sec3 labeling at the plasma membrane. Furthermore, antibodies to Sec15 and two other Exocyst subunits, namely, Exo70 and Exo84, produced identical labeling patterns in A431 cells (Supplemental Figure S3). Thus, at least six different Exocyst subunits localized to similar punctate, lateral plasma membrane structures that were distinct from the sites at which previously identified AJC-associated Sec6/8 complexes localized.

Because several Exocyst subunits were distributed in a characteristic spot-like pattern at sites of cell-cell contact, the possibility that these sites represented desmosomes was examined. Anti-Sec6(8A5) and anti-desmoplakin antibodies colabeled identical structures enriched in two regions of the lateral plasma membrane (Figure 2E). Moreover, Sec3 colocalized with desmoglein-GFP at these lateral membrane puncta (Figure 2F). These data show that Exocyst complexes containing Sec3 are enriched at desmosomes.

Not only this desmosome-associated Sec3 fraction but also an additional Sec3 pool was observed (Figures 2A and 3). Specifically, Sec3 colocalized with γ-tubulin (Figure 3A) and kendrin (pericentrin-B) (Figure 3B) at centrioles. Given that either one or two dots were conspicuous in polarized MDCK cells, Sec3 seems to associate with both maternal and daughter centrioles. In polarized cells, the majority of centrioles are located in the apical cytoplasm in a higher focal plane than desmosomes, so it is not surprising that not all optical sections showed both pools of Sec3 protein (Figure 3). Antibodies to Sec15, Exo70, and Exo84 also labeled centrosomes in A431 cells, suggesting that a Sec3-containing Exocyst complex is associated with this organelle (Supplemental Fig. S3).

Sec3 Occupies a Subset of Exocyst Complexes in Epithelial Cells

In contrast to published localizations of Sec6 and Sec8 at the AJC, Sec3 colocalized with Sec15, Exo70, and Exo84 and immunologically distinct pools of Sec6 and Sec8 at desmosomes. To determine whether distinct Exocyst complexes are associated with desmosomes and the AJC, we compared the biochemical properties of Sec3 and Sec8. First we examined the detergent solubility of each subunit. Sec3 solubility in buffer containing Triton X-100 was nearly identical to that of every other Exocyst subunit examined, except Exo84 (Figure
Supplemental S4). Sec3 was, like Sec8, more soluble in deoxycholate-containing RIPA buffer (Figure 4). However, no significant differences in solubility were observed between Sec3 and Sec8, regardless of the solubilization buffer used.

Because the Exocyst associates with membranes, the buoyant densities of Sec3- and Sec8-associated membrane domains were analyzed by isopycnic density gradient centrifugation. Sec8 had been shown previously to cofractionate with proteins associated with the AJC—such as zona occludens (ZO)-1, ZO-2, nectin-2, and E-cadherin—at 1.16 g/ml (Yeaman et al., 2004). We found that membrane-associated Sec3 also cofractionated with Sec8 at 1.16 g/ml (Figure 5A). In addition, the desmosome-associated protein desmplakin was enriched in these fractions, consistent with immunolocalization of Sec3 to desmosomes. A secondary peak of Sec3 and Sec8 corresponded to cytosolic fractions (δ = 1.20 g/ml).

To determine whether Sec3 assembles into large, multimeric complexes similar in size to those containing Sec8, we fractionated detergent extracts of MDCK cells by Superose 6 fast-performance liquid chromatography. Most of the Sec3 coeluted with Sec8 in a single peak at fraction 12, corresponding to a protein complex with an apparent molecular size of >3000 kDa, based on the elution of globular protein standards (Figure 5B). In addition to this major peak, an overlapping peak of Sec8, centered at fraction 14 and corresponding to a protein complex of ~1000 kDa, was observed. Sec3 was not associated with this Sec8 fraction, but a minor pool of Sec3 was observed in fraction 19, as part of a protein complex of ~450 kDa. No Sec3 was recovered in fractions that eluted from the column at later time points, indicating that monomeric Sec3 was not present in detectable quantities in MDCK cells (data not shown). Instead, all of the Sec3 was assembled into high-molecular-weight complexes. In addition, these data hint that Sec8 may exist in at least two distinct complexes that either contain or lack Sec3.

To directly test this possibility, we performed coimmunoprecipitation studies. We performed three consecutive rounds of Sec8 immunoprecipitation in order to deplete this protein from MDCK RIPA lysates (Figure 6A). Sec3 was almost completely cleared from the column at later time points, indicating that monomeric Sec3 was not present in detectable quantities in MDCK cells (data not shown). Instead, all of the Sec3 was assembled into high-molecular-weight complexes. In addition, these data hint that Sec8 may exist in at least two distinct complexes that either contain or lack Sec3.

To directly test this possibility, we performed coimmunoprecipitation studies. We performed three consecutive rounds of Sec8 immunoprecipitation in order to deplete this protein from MDCK RIPA lysates (Figure 6A). Sec3 was almost completely cleared from the column at later time points, indicating that monomeric Sec3 was not present in detectable quantities in MDCK cells (data not shown). Instead, all of the Sec3 was assembled into high-molecular-weight complexes. In addition, these data hint that Sec8 may exist in at least two distinct complexes that either contain or lack Sec3.

To directly test this possibility, we performed coimmunoprecipitation studies. We performed three consecutive rounds of Sec8 immunoprecipitation in order to deplete this protein from MDCK RIPA lysates (Figure 6A). Sec3 was almost completely cleared from the column at later time points, indicating that monomeric Sec3 was not present in detectable quantities in MDCK cells (data not shown). Instead, all of the Sec3 was assembled into high-molecular-weight complexes. In addition, these data hint that Sec8 may exist in at least two distinct complexes that either contain or lack Sec3.

To directly test this possibility, we performed coimmunoprecipitation studies. We performed three consecutive rounds of Sec8 immunoprecipitation in order to deplete this protein from MDCK RIPA lysates (Figure 6A). Sec3 was almost completely cleared from the column at later time points, indicating that monomeric Sec3 was not present in detectable quantities in MDCK cells (data not shown). Instead, all of the Sec3 was assembled into high-molecular-weight complexes. In addition, these data hint that Sec8 may exist in at least two distinct complexes that either contain or lack Sec3.

To directly test this possibility, we performed coimmunoprecipitation studies. We performed three consecutive rounds of Sec8 immunoprecipitation in order to deplete this protein from MDCK RIPA lysates (Figure 6A). Sec3 was almost completely cleared from the column at later time points, indicating that monomeric Sec3 was not present in detectable quantities in MDCK cells (data not shown). Instead, all of the Sec3 was assembled into high-molecular-weight complexes. In addition, these data hint that Sec8 may exist in at least two distinct complexes that either contain or lack Sec3.
complexes are present in MDCK cells, and that these are distinguished by the presence or absence of Sec3.

Assuming that all Sec3 and Sec8 were present in Exocyst complexes at a 1:1 stoichiometry, but additional Sec8-containing complexes lacking Sec3 were also present in cells, Sec8 should be more abundant than Sec3. To test this prediction, we immunoprecipitated Sec3 and Sec8 from radiolabeled cells (Figure 6D). After correcting for immunoprecipitation efficiencies and differences in the methionine and cysteine contents of the two proteins, we determined that MDCK cells had ∼65% more Sec8 than Sec3.

Finally, the spatiotemporal redistribution of Sec3 during early stages of cell–cell contact formation was compared with that of Sec6 and Sec8. The rationale for this experiment was that if distinct Exocyst complexes that either contain or lack Sec3 are associated with desmosomes and AJCs, respectively, it might be possible to observe differences in how they become associated with plasma membranes during polarity development. Contact-naive MDCK cells were allowed to adhere to a collagen substrate at low plating density in LCM for 1 h, and then either maintained in LCM (Figure 7A) or switched to high calcium medium (HCM) for 1 h (Figure 7B). In cells cultured in LCM for 2 h, nascent E-cadherin–containing intercellular contacts formed, and these were labeled with anti-Sec6(9H5) and anti-Sec8(8F12) antibodies (Figure 7A). Although Sec3 was not present at these nascent cellular contacts in LCM, it did colocalize with Sec6, Sec8 and desmosomal cadherins in cytoplasmic puncta (Figure 7, A and C). Only after culturing cells in HCM for 1 h was Sec3 observed to accumulate at sites of intercellular contact (Figure 7B). Therefore, membrane recruitment of the Sec3 protein, which ultimately becomes associated with desmosomes, was slower than that of the Sec6/8 complexes that associate with the AJC.
Sec3 Is Required for Desmosome Assembly

To study the role of Sec3 in epithelial cells, we reduced its expression in human MCF-10A cells by transduction with lentiviral vectors containing a puromycin selection cassette and shRNAs targeting both splice variants of human Sec3. It was necessary to switch to these cells because multiple efforts to stably reduce Sec3 to levels below 50% of control levels in MDCK cells were unsuccessful. MCF-10A cells form a polarized, cuboidal epithelium with well-developed desmosomes and adherens junctions (Tait et al., 1990). We identified two hairpins that reduced Sec3 protein levels by >90%, as assessed by Western blotting (Figure 8A). These also led to greatly reduced immunofluorescence labeling intensity after selection in puromycin (Figure 8B).

Because Sec3 accumulated at desmosomes, we determined whether it was required for the structural integrity of these junctions. Pan-specific desmoglein antibodies labeled spot-like desmosomes along plasma membranes of control cells, but this plaque-like labeling was disrupted in Sec3 knockdown cells. In these cells, most of the membrane-proximal desmoglein-positive structures were organized into linear arrays that ran perpendicular to cell–cell contacts (Figure 8, C and E). Similar results were observed with antibodies specific for Dsg1, Dsg2, and Dsc2/3 (data not shown). Furthermore, anti-plakoglobin antibodies labeled cell–cell contacts in both control and Sec3 knockdown cells, but the morphology of these contacts was abnormal when Sec3 expression was reduced (Figure 8C). In addition, plasma membrane labeling of desmoplakin and the Exocyst [as defined by Sec15 and Sec6(8A5) labeling] was substantially reduced in Sec3 knockdown cells (Figure 8C). In contrast, E-cadherin localization was largely unchanged when Sec3 expression was suppressed (Figure 8, D and E). On reexpression of Sec3 in these cells, desmoplakin and Sec15 were returned to the plasma membrane, and normal desmosomal morphology was restored (Figure 8C). Therefore, the absence of Sec3 seems to affect the morphology of desmosomes specifically and not that of adherens junctions.

Because the Exocyst has been implicated in vesicle tethering to target membranes, we investigated the possibility that Sec3 was required for trafficking of desmoglein-containing vesicles to the plasma membrane. The delivery of Dsg2 to the plasma membrane was assayed by biotin accessibility in a surface repopulation assay. Cells were suspended in trypsin to dissociate them and remove preexisting surface proteins, and then replated in LCM at confluent density. At various time points after transferring cells to HCM, Dsg2...
expression at the plasma membrane was quantified by cell surface biotinylation, followed by streptavidin precipitation of biotinylated proteins. In control cells, Dsg2 gradually accumulated at the cell surface over a 72-h time course (Figure 9, A and B). Surprisingly, in Sec3-knockdown cells the rate of Dsg2 accumulation at the plasma membrane was increased relative to that in control cells, reaching steady state within 15–30 h of the calcium switch (Figure 9, A and B). However, the Dsg2 that was delivered to the plasma membrane did not seem to assemble into desmosomes (Figure 8, C and E). In contrast, E-cadherin transport was not altered after the suppression of Sec3 expression (Figure 8C). Therefore, loss of Sec3 expression is associated with changes in transport or incorporation of Dsg2 into desmosomes but not with trafficking of E-cadherin to developing adherens junctions.

To gain further insight into how a reduction in Sec3 expression affected desmosome structure and function, we quantified overall expression levels of several desmosomal and adherens junction-associated proteins in two independent clones of MCF-10A cells expressing hairpin 2. These clones exhibited an ~80–90% reduction of Sec3 expression (Figure 10A). In both clones, as well as in pools of cells expressing either hairpin 1 or hairpin 2, the levels of all desmosomal cadherins examined were elevated relative to those in control cells (Figure 10B and Supplemental Figure S5). Dsg1 expression was 1.4- and 3.2-fold higher in clones 1 and 2, respectively. Likewise, Dsg2 expression was 1.9- and 4.2-fold higher, and Dsc2/3 expression was 13- and 23-fold higher, in the Sec3 knockdown clones (Figure 10B). In addition, plakoglobin protein levels were increased, but desmoplakin expression was not substantially altered (Figure 10B). Expression of Sec3HR in these cells partially reversed these effects, leading to a substantial reduction in Dsc2/3 expression and a more modest reduction in plakoglobin expression (Figure 10E). Adherens junction components, such as E-cadherin and α-catenin, were not consistently affected, indicating that effects of Sec3 knockdown were specific to desmosomes (Figure 10, C and D, and Supplemental Figure S5).

To determine whether observed changes in desmosome composition and morphology in Sec3-knockdown cells had functional consequences, we evaluated intercellular adhesion strength by hanging drop assay (Kim et al., 2000; Huen et al., 2002; Lorch et al., 2004). After 20 h in suspension, both control and Sec3-knockdown cells had formed cellular aggregates of similar size and distribution (Figure 11). How-
ever, application of an external force revealed a clear difference in relative adhesive strengths of the two cultures. Although the frequency of large aggregates ($\geq 50$ cells) was reduced after trituration of either population, the extent to which each culture was affected was not the same. Whereas essentially all of the large cell aggregates were disrupted in Sec3-knockdown cultures, nearly 50% of such clusters were resistant to trituration in the control cultures (Figure 11). Consistent with the disruption of large aggregates, the number of single cells and doublets in Sec3-knockdown cultures was higher than that of larger aggregates (Figure 11). Collectively, these findings indicate that a Sec3-containing Exocyst localizes to desmosomes and is required for the assembly of functioning intercellular junctions between epithelial cells.

DISCUSSION

In Mammalian Epithelial Cells, Sec3 Associates with a Subset of Exocyst Complexes That Are Enriched at Desmosomes and Centrosomes but Not AJCs

Immunolocalization studies of endogenous Exocyst complexes in mammalian epithelial cells have revealed that sub-units of these complexes are present at the AJC (Sec6 and Sec8) (Grindstaff et al., 1998; Charron et al., 2000; Yeaman et al., 2004), recycling endosomes (Sec8, Sec10, and Exo70) (Folsch et al., 2003; Prigent et al., 2003; Oztan et al., 2007), and centrosomes/ciliary basal bodies (Sec6, Sec8, and Sec10) (Rogers et al., 2004; Zuo et al., 2009). Our study extends these findings to Sec3, antibodies to which labeled most of the same organelles, although Sec3 was notably absent from the AJC-associated Exocyst complex (see below). Findings from
the current study have identified the desmosomes of epithelial cells as additional sites of Exocyst assembly and function; six subunits (Sec3, Sec6, Sec8, Sec15, Exo70, and Exo84) were detected there. In addition, the current study adds a subset of the same protein group (Sec3, Sec15, Exo70, and Exo84) to the Exocyst subunits that are known to be present at centrosomes. Collectively, these immunolabeling studies suggest that, in epithelial cells, desmosomes and centrosomes are likely to be the principle sites at which fully assembled Exocyst holocomplexes accumulate.

Our data also have revealed that the Sec6/8 complex assembled at the AJC is distinct from the Exocyst complex assembled at desmosomes. Polyclonal antibodies to Sec3, Sec10, Sec15, Exo70, and Exo84 did not label the AJC (Prigent et al., 2005; Zuo et al., 2009; this study). Although differential epitope accessibility has been offered as an explanation for the organelle-specific labeling patterns observed with several anti-Sec6 mAbs (Yeaman et al., 2001), it is unlikely that epitope masking accounts for our inability to detect five distinct components (Sec3, Sec10, Sec15, Exo70, and Exo84) at the AJC, especially because polyclonal antibodies to each of these proteins were used in these studies. It is more likely that these subunits simply do not accumulate there. It thus remains unclear whether the AJC-associated Sec6/8 complex recruited to nascent E-cadherin–positive adhesion sites in advance of the assembly of Sec3-containing Exocyst complexes at desmosomes represents a by-product of the dynamic assembly and disassembly of Exocyst complexes in a variety of functional states or a truly distinct functional unit. It should be noted that a recent study identified, within yeast Sec6, conserved sequences that were capable of targeting the Exocyst to sites of polarized membrane trafficking, indicating that a functional Sec6/8 subcomplex could potentially target to the AJC in the absence of Sec3 (Songer and Munson, 2009).

Several lines of evidence support the existence of multiple Exocyst complexes or subcomplexes with distinct functions. First, antibodies to different subunits have been shown to differentially label subcellular compartments in many cell types (Yeaman et al., 2001, 2004; Vik-Mo et al., 2003; Beronja et al., 2005; Mehta et al., 2001, 2004; Vik-Mo et al., 2003). Second, distinct phenotypes have been associated with loss-of-function mutations in a variety of Drosophila Exocyst genes, and RNA interference (RNAi)-mediated silencing of different subunits in cell culture has likewise led to distinct phenotypes (Mehta et al., 2005; Chien et al., 2006; Zuo et al., 2009). Third, mammalian genomes include two distinct Sec15-like genes, and as many as five Sec6-like genes; many or all of these encode proteins that are simultaneously expressed in the same cell and that interact with other Exocyst subunits, further increasing the potential diversity of Exocyst complexes in cells (Brymora et al., 2001; Saito et al., 2008; Spiczka and Yeaman, unpublished data). Therefore, the Sec3-containing Exocyst complex associated with desmosomes may represent only one member of a family of related tethering complexes that are expressed by epithelial cells to ensure targeted delivery of cargo-laden transport vesicles to the correct destination at the plasma membrane.

Sec3 Is Required for Desmosome Assembly

Using an RNAi approach, we investigated the role for the Sec3 protein in epithelial cells. Suppression of Sec3 expression resulted in a loss of Exocyst from desmosomes, consistent with conclusions from budding yeast that Sec3 directly binds plasma membrane phospholipids, and from this location coordinates the assembly of other subunits arriving on transport vesicles (Boyd et al., 2004; Zhang et al., 2008). The fact that the Exocyst was lost from desmosomes upon Sec3 knockdown suggests that transport vesicles were no longer targeted to these sites. However, this did not result in intracellular accumulation of the desmosomal cadherin Dsg2, which continued to be exocytosed in the absence of Sec3. This indicates that Dsg2-containing transport vesicles, once relieved of the spatial constraint to fuse only at desmosomes, were able to efficiently dock and fuse elsewhere on the plasma membrane.

However, in Sec3 knockdown cells, the desmosomes had a distorted morphology, and hanging drop assays revealed that they were less adhesive than those in control cultures. Perhaps in response to these weakened junctions, cells increased the overall expression of many other desmosomal proteins. Importantly, effects of Sec3 knockdown were most acute at desmosomes, with AJCs not being noticeably impacted.
How might a Sec3-containing Exocyst complex function in desmosome assembly? Formation of this intercellular junction is a complex process, involving multiple phases of microtubule-dependent vesicular trafficking events that deliver membrane components (e.g., desmocollins, desmogleins, and plakoglobin) to the plasma membrane, in coordination with multiple phases of actin-dependent movement of plaque components (e.g., desmoplakin and plakophilin) (reviewed by Green and Simpson, 2007). Sec3, and by extension the Exocyst, could contribute to desmosome assembly in several different ways. As a putative vesicle tether, the Sec3-Exocyst might be required for the delivery of transport vesicles carrying desmosomal membrane components. Electron microscopy has shown that desmosome assembly involves two phases of vesicle delivery (Burdett and Sullivan, 2002). At early stages, small (60-nm) vesicles carrying mostly desmogleins were delivered to the plasma membrane in a nonpolarized manner. At later stages, larger (200-nm) vesicles carrying mostly desmogleins were targeted, in a polarized manner, to basal–lateral membranes at which desmosomes were forming. Live-cell imaging showed that vesicles carrying Dsg2-GFP explored different regions of the plasma membrane, before fusing with existing desmosomal puncta (Glouchankova et al., 2003). It is possible that Sec3 is required for the second, polarized trafficking step, but not the first. Hence, the diffuse pattern of surface Dsg2 labeling observed in Sec3 knockdown cells might reflect vesicles that were delivered via a Sec3-independent, nonpolarized pathway.

An alternative possibility for Sec3 function at the desmosome is that it may organize microtubule tracks required for the efficient delivery of membrane components to desmosomes. This is consistent with previous suggestions of a role for the Exocyst in regulating microtubule dynamics (Vega and Hsu, 2001; Wang et al., 2004; Liebl et al., 2005). Furthermore, Sec3 was recently shown to bind IQGAP1, a Cdc42/Rac effector that interacts with the plus-end binding protein CLIP-170 to capture microtubules at the leading edge of migrating cells (Hart et al., 1996; Kuroda et al., 1996; Fukuta et al., 2002). Although a role for IQGAP1 at desmosomes has not been established, it is notable that CLIP-170 is associated with the plus ends of microtubules at desmosomes (Wacker et al., 1992). It will be interesting to determine whether Sec3 contributes to the polarized delivery of cargo to developing desmosomes by facilitating microtubule organization.

Finally, it is possible that a desmosome-associated Exocyst complex helps to organize the actin cytoskeleton at these sites to facilitate the movement of plaque proteins. Careful live-cell imaging analysis of the dynamics with which desmoplakin-GFP was incorporated into forming desmosomes revealed that three phases of movement take place (Godsel et al., 2005). After the rapid accumulation of one pool of desmoplakin at nascent cell–cell contact sites, a second pool was observed to accumulate in cortical puncta that were not membrane bound. Subsequently, these puncta moved in an actin-dependent manner, and with slower kinetics, to the membrane, and then were incorporated into preexisting desmosomes. The organization of actin-based structures that facilitate the incorporation of desmoplakin into maturing desmosomes could be regulated by the Exocyst, which is known to regulate actin dynamics in other cell types through its interactions with the Arp2/3 complex (Zuo et al., 2006).

In conclusion, this study shows that Sec3 targets Exocyst complexes to desmosomes in mammalian epithelial cells, and that these complexes are required for assembly of functional desmosomes. Moreover, we show that this complex is distinct from a previously characterized Sec6/8 complex at the AJC. Future studies will focus on elucidating the mechanisms by which these complexes regulate the development and maintenance of specialized membrane domains in polarized epithelial cells.

**ACKNOWLEDGMENTS**

We thank Krystle Spiczka for technical assistance. We also thank Stephen Dossey (University of Massachusetts Medical Center) for the Kndrin antibody; Kathleen Green (Northwestern University) for Dsg2-GFP adenovirus and antibodies to Dsg1, Dsg2, Dsc2/3, and plakoglobin; Ryits Prekeris (University of Colorado Denver School of Medicine) for MCF-10A cells; and Vann Bennett (Duke University) for Human Bronchial Epithelia cells. This work was supported by National Institutes of Health grant GM-067002.

**REFERENCES**

Beronja, S., Laprise, P., Papoulas, O., Pelliikka, M., Sisson, J., and Tepass, U. (2005). Essential function of *Drosophila* Sec6 in apical exocytosis of epithelial photoreceptor cells. J. Cell Biol. 169, 635–646.

Blankenship, J. T., Fuller, M. T., and Zallen, J. A. (2007). The *Drosophila* homolog of the Exo84 exocyst subunit promotes apical epithelial identity. J. Cell Sci. 120, 3099–3110.

Boyd, C., Hughes, T., Pypaert, M., and Novick, P. (2004). Vesicles carry most exocytic subunits to exocytic sites marked by the remaining two subunits, Sec3p and Exo70p. J. Cell Biol. 167, 889–901.

Brymora, A., Valova, V. A., Larsen, M. R., Roufogalis, B. D., and Robinson, P. J. (2001). The brain exocyst complex interacts with RaIA in a GTP-dependent manner: identification of a novel mammalian Sec3 gene and a second Sec15 gene. J. Biol. Chem. 276, 29792–29797.

Burdett, I. D., and Sullivan, K. H. (2002). Desmosome assembly in MDCK cells: transport of precursors to the cell surface occurs by two phases of vesicular traffic and involves major changes in centrosome and Golgi location during a Ca2+ shift. Exp. Cell Res. 276, 296–309.

Cascone, I., Selimoglu, R., Ozdemir, C., Del Nery, E., Yeaman, C., White, M., and Camonis, J. (2008). Distinct roles of RaIA and Rabl in the progression of cytokinesis are supported by distinct RabGFEs. EMBO J. 27, 2575–2587.

Charron, A. J., Nakamura, S., Bacallao, R., and Wandinger-Ness, A. (2000). Compartmentalized cytoarchitecture and polarized trafficking in autosome dominant polycystic kidney disease cells. J. Cell Biol. 149, 111–124.

Chien, Y., et al. (2006). Rab1B GTPase-mediated activation of the IkappaB family kinase TBK1 couples innate immune signaling to tumor cell survival. Cell 127, 157–170.

Cubelas, B., Gimenez, C., and Zafra, F. (2005). The glycoside transporter GLYT1 interacts with Sec3, a component of the exocyst complex. Neuropharmacology 49, 935–944.

Debnath, J., Muthuswamy, S. K., and Brugge, J. S. (2003). Morphogenesis and oncogenesis of MCF-10A mammary epithelial acini grown in three-dimensional basement membrane cultures. Methods 30, 256–268.

Fielding, A. B., Schonteich, E., Matheson, J., Wilson, G., Xu, X., Hickson, G. R., Srivastava, S., Baldwin, S. A., Prekeris, R., and Gould, G. W. (2005). Rab11-FIP3 and FIP4 interact with Arf6 and the exocyst to control membrane traffic in cytokinesis. EMBO J. 24, 3389–3399.

Finger, F. P., Hughes, T. E., and Novick, P. (1998). Sec3p is a spatial landmark for polarized secretion in budding yeast. Cell 92, 559–571.

Finger, F. P., and Novick, P. (1997). Sec3p is involved in secretion and morphogenesis in *Saccharomyces cerevisiae*. Mol. Biol. Cell 8, 647–662.

Flory, M. R., Moser, M. J., Monnat, R. J., Jr., and Davis, T. N. (2000). Identification of a human centrosomal calmodulin-binding protein that shares homology with pericentrin. Proc. Natl. Acad. Sci. USA 97, 5919–5923.

Folsch, H., Pypaert, M., Maday, S., Pelletier, L., and Sellman, I. (2003). The AP-1A and AP-1B clathrin adaptor complexes define biochemically and functionally distinct membrane domains. J. Cell Biol. 163, 351–362.

Fukata, M., Watanabe, T., Noritake, J., Nakagawa, M., Yamaga, M., Kuroda, S., Matsuura, Y., Iwamatsu, A., Perez, F., and Kaibuchi, K. (2002). Rac1 and Cdc42 capture microtubules through IQGAP1 and CLIP-170. Cell 109, 873–885.

Gaudry, C. A., Palka, H. L., Dusek, R. L., Huen, A. C., Khandekar, M. J., Hudson, L. G., and Green, K. J. (2001). Tyrosine-phosphorylated plakoglobin is associated with desmogleins but not desmoplakin after epidural growth factor receptor activation. J. Biol. Chem. 276, 24871–24880.
Mostov, K. E. (2000). Exocyst is involved in cystogenesis and tubulogenesis.

Lipschutz, J. H., Guo, W., O’Brien, L. E., Nguyen, Y. H., Novick, P., and Mostov, K. E. (2000). Exocyst is involved in cystogenesis and tubulogenesis.

Sec6/8 complex is recruited to cell-cell contacts and specifies transport vesicle delivery to the basal-lateral membrane in epithelial cells. Cell 93, 731–740.

Gromley, A., Yeaman, C., Rosa, J., Redick, S., Chen, C. T., Mirabelle, S., Guha, M., Stillbourne, J., and Dosshey, S. J. (2001). Centrosol anchoring of exocyst and SNARE complexes at the midbody is required for secretory-vesicle-mediated abscission. Cell 123, 75–87.

Guo, W., Roth, D., Walch-Solimena, C., and Novick, P. (1999). The exocyst is an effector for Sec63, targeting secretory vesicles to sites of exocytosis. EMBO J. 18, 1071–1080.

Guo, W., Tamanoi, F., and Novick, P. (2001). Spatial regulation of the exocyst complex by Rho1 GTPase. Nat. Cell Biol. 3, 353–360.

Hala, M., et al. (2008). An exocyst complex functions in plant cell growth in Arabidopsis and tobacco. Plant Cell 20, 1330–1345.

Hart, M. J., Callow, M. G., Souza, B., and Polakis, P. (1996). IQGAP, a calmodulin-binding protein with a rasGAP-related domain, is a potential effector for cc2d4Hs. EMBO J. 15, 2997–3005.

Hakuya, C. D., Foletti, D. L., Hsu, S. C., Kee, Y., Hopf, F. W., and Scheller, R. H. (1999). The sec6/8 complex is located at neurite outgrowth and axonal synapse-assemble domains. J. Neurosci. 19, 1324–1334.

He, B., Xi, F., Zhang, X., Zhang, J., and Guo, W. (2007). Exo70 interacts with phosphorylids and mediates the targeting of the exocyst to the plasma membrane. EMBO J. 26, 4053–4065.

Hinck, L., Nelson, W. J., and Papkoff, J. (1994). Wnt-1 modulates cell-cell adhesion in mammalian cells by stabilizing beta-catenin binding to the cell adhesion protein cadherin. J. Cell Biol. 124, 729–741.

Hsu, S. C., Ting, A. E., Hakuya, C. D., Davanger, S., Kenny, J. W., Kee, Y., and Scheller, R. H. (1996). The mammalian brain sec6/8 complex. Neuron 17, 1209–1219.

Huen, A. C., et al. (2002). Intermediate filament-membrane attachments function synergistically with actin-dependent contacts to regulate intercellular adhesive strength. J. Cell Biol. 155, 1005–1017.

Kee, Y., Yao, J. S., Hakuya, C. D., Peterson, K. E., Hsu, S. C., and Scheller, R. H. (1997). Subunit structure of the mammalian exocyst complex. Proc. Natl. Acad. Sci. USA 94, 14438–14443.

Kim, J. B., Islam, S., Kim, Y. J., Prudoff, R. S., Sass, K. M., Wheelock, M. J., and Johnson, K. R. (2000). N-Cadherin extracellular repeat 4 mediates epithelial to mesenchymal transition and increased motility. J. Cell Biol. 151, 1193–1206.

Kizhatil, K., and Bennett, V. (2004). Lateral membrane biogenesis in human bronchial epithelial cells requires 190-kDa ankyrin-G. J. Biol. Chem. 279, 16706–16714.

Klesner, J. L., Desai, B. V., Amargo, E. V., Getson, S., and Green, K. J. (2009). EGFR and ADAMs cooperate to regulate shedding and endocytic trafficking of the desmosomal cadherin desmoglein 2. Mol. Biol. Cell 20, 328–337.

Kuroda, S., Fukuta, M., Kobayashi, K., Nakafuku, M., Nomura, N., Iwamatsu, A., and Kaibuchi, K. (1996). Identification of IQGAP as a putative target for the small GTPases, Cdc42 and Rac1. J. Biol. Chem. 271, 23363–23367.

Langevin, J., Morgan, M. J., Sibarita, J. B., Aresta, S., Murthy, M., Schwarz, T., Camonis, J., and Bellaiche, Y. (2005). Drosophila exocyst components Sec5, Sec6, and Sec15 regulate DE-Cadherin trafficking from recycling endosomes to the plasma membrane. Dev. Cell 9, 365–376.

Lavry, M., Bloch, D., Hazak, O., Gutman, I., Poraty, L., Sorek, N., Sternberg, H., and Oron, Y. (2005). The mammalian Sec6/8 complex interacts with Cac2(+) signaling complexes and regulates their activity. J. Cell Biol. 150, 1101–1112.

Simons, M., and Munson, M. (2009). Sec6p anchors the assembled exocyst complex at sites of secretion. Mol. Biol. Cell 20, 973–982.

Spiczka, K. S., and Yeaman, C. (2008). Rae1-regulated interaction between Sec5 and paxillin targets Exocyst to focal complexes during cell migration. J. Cell Sci. 121, 2880–2891.

Stewart, D. B., and Nelson, W. J. (1997). Identification of four distinct pools of catenines in mammalian cells and transformation-dependent changes in catenin distributions among these pools. J. Biol. Chem. 272, 29652–29662.

Tait, L., Soule, H. D., and Russo, J. (1990). Ultrastructural and immunocytochemical characterization of an immortalized human breast epithelial cell line, MCF-10. Cancer Res. 50, 6087–6094.

Sec63 Is Required for Desmosome Assembly

and acts by modulating synthesis and delivery of basolateral plasma membrane and secretory proteins. Mol. Biol. Cell 11, 4259–4275.
TerBush, D. R., Maurice, T., Roth, D., and Novick, P. (1996). The Exocyst is a multiprotein complex required for exocytosis in Saccharomyces cerevisiae. EMBO J. 15, 6483–6494.

TerBush, D. R., and Novick, P. (1995). Sec6, Sec8, and Sec15 are components of a multisubunit complex which localizes to small bud tips in Saccharomyces cerevisiae. J. Cell Biol. 130, 299–312.

Vega, I. E., and Hsu, S. C. (2001). The exocyst complex associates with microtubules to mediate vesicle targeting and neurite outgrowth. J. Neurosci. 21, 3839–3848.

Vik-Mo, E. O., Oltedal, L., Hoivik, E. A., Kleivdal, H., Eidet, J., and Davanger, S. (2003). Sec6 is localized to the plasma membrane of mature synaptic terminals and is transported with secretogranin II-containing vesicles. Neuroscience 119, 73–85.

Wacker, I. U., Rickard, J. E., De Mey, J. R., and Kreis, T. E. (1992). Accumulation of a microtubule-binding protein, pp170, at desmosomal plaques. J. Cell Biol. 117, 813–824.

Wahl, J. K., 3rd. (2002). Generation of monoclonal antibodies specific for desmoglein family members. Hybrid Hybridomics 21, 37–44.

Wahl, J. K., Sacco, P. A., McGranahan-Sadler, T. M., Sauppe, L. M., Wheelock, M. J., and Johnson, K. R. (1996). Plakoglobin domains that define its association with the desmosomal cadherins and the classical cadherins: identification of unique and shared domains. J. Cell Sci. 109, 1143–1154.

Wang, S., Liu, Y., Adamson, C. L., Valdez, G., Guo, W., and Hsu, S. C. (2004). The mammalian exocyst, a complex required for exocytosis, inhibits tubulin polymerization. J. Biol. Chem. 279, 35958–35966.

Wiederkehr, A., De Craene, J. O., Ferro-Novick, S., and Novick, P. (2004). Functional specialization within a vesicle tethering complex: bypass of a subset of exocyst deletion mutants by Sec1p or Sec4p. J. Cell Biol. 167, 875–887.

Yeaman, C. (2003). Ultracentrifugation-based approaches to study regulation of Sec6/8 (exocyst) complex function during development of epithelial cell polarity. Methods 30, 198–206.

Yeaman, C., Grindstaff, K. K., and Nelson, W. J. (2004). Mechanism of recruiting Sec6/8 (exocyst) complex to the apical junctional complex during polarization of epithelial cells. J. Cell Sci. 117, 559–570.

Yeaman, C., Grindstaff, K. K., Wright, J. R., and Nelson, W. J. (2001). Sec6/8 complexes on trans-Golgi network and plasma membrane regulate late stages of exocytosis in mammalian cells. J. Cell Biol. 155, 593–604.

Yeaman, C., Grindstaff, K. K., Wright, J. R., and Nelson, W. J. (2001). Sec6/8 complexes on trans-Golgi network and plasma membrane regulate late stages of exocytosis in mammalian cells. J. Cell Biol. 155, 593–604.

Zhang, X., Bi, E., Novick, P., Du, L., Kozminski, K. G., Lipschutz, J. H., and Guo, W. (2001). Cdc42 interacts with the exocyst and regulates polarized secretion. J. Biol. Chem. 276, 46745–46750.

Zhang, X., Orlando, K., He, B., Xi, F., Zhang, J., Zajac, A., and Guo, W. (2005). Membrane association and functional regulation of Sec3 by phospholipids and Cdc42. J. Cell Biol. 169, 145–158.

Zuo, X., Guo, W., and Lipschutz, J. H. (2009). The exocyst protein Sec10 is necessary for primary ciliogenesis and cystogenesis in vitro. Mol. Biol. Cell 20, 2522–2529.

Zuo, X., Zhang, J., Zhang, Y., Hsu, S. C., Zhou, D., and Guo, W. (2006). Exo70 interacts with the Arp2/3 complex and regulates cell migration. Nat. Cell Biol. 8, 1385–1388.