Ultracentrifugation-based approaches to study regulation of Sec6/8 (exocyst) complex function during development of epithelial cell polarity

Charles Yeaman*
Department of Anatomy and Cell Biology, University of Iowa, Iowa City, IA 52242, USA
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Abstract

The Sec6/8 (exocyst) complex is an essential component of the exocytic apparatus and plays an evolutionarily conserved role in polarized membrane growth. During development of epithelial cell polarity, this cytosolic protein complex is recruited to plasma membrane sites of cell–cell contact, where it facilitates exocytosis to the lateral membrane domain. However, the identity of membrane binding sites for Sec6/8 complex, mechanisms regulating association of Sec6/8 complex with these sites, and the precise function of the complex in polarized trafficking are not known. Biochemical strategies involving differential, rate-zonal, and isopycnic density gradient ultracentrifugation are providing clues to these questions.

Keywords: Sec6/8 (exocyst) complex; Rate-zonal centrifugation; Isopycnic density centrifugation; Iodixanol; Plasma membrane

1. Introduction

One of the major goals of research in cell polarity is to define mechanisms that direct exocytic trafficking to different plasma membrane domains. During development of cell polarity de novo, mechanisms must be established that ensure not only the fidelity of trafficking (i.e., that a particular transport vesicle is delivered to the correct membrane domain) but also enhance the efficiency of trafficking. For polarized membrane growth to occur, the rate of membrane insertion must be greater than that of membrane removal within an emerging plasma membrane domain. Efficient exocytic trafficking relies on cytoskeletal elements and associated motor proteins to move cargo from the trans-Golgi network to the growth site, and an active exocytic apparatus at the growth site to facilitate docking and fusion of transport carriers when they arrive there. An important component of this exocytic machinery is the Sec6/8 (exocyst) complex [1,2].

Sec6/8 holocomplex is a ~750-kDa T-shaped particle that is assembled from eight cytosolic subunits (Sec3, 5, 6, 8, 10, and 15, Exo70, Exo84), each present in 1:1 stoichiometry [3,4]. The overall composition of the complex is conserved in budding yeast [3], neurons [5], and epithelial cells [6] (Fig. 1). It is not known how assembly of Sec6/8 holocomplex is controlled, whether partially assembled subcomplexes are present in epithelial cells and whether these subcomplexes or individual subunits have unique functions outside of the holocomplex. Insight into these questions is provided by differential and rate-zonal ultracentrifugation experiments, described here, that resolve different exocyst complexes on the basis of size.

Whether or not holocomplex assembly is regulated, it is clear that activity of Sec6/8 complex is controlled, in part, by controlling its recruitment to sites of membrane growth on receipt of polarizing spatial cues. In budding yeast, the complex is recruited to presumptive bud sites at the beginning of each cell cycle through a signaling pathway involving Rho GTPases [7,8]. In neurons, Sec6/8 complex is recruited to sites of synapse formation and to the growth cone by a NGF-activated MAP kinase

* Fax: 1-319-335-7198.
E-mail address: charles-yeaman@uiowa.edu.
signaling pathway [9,10]. In epithelial cells, induction of E-cadherin-mediated cell–cell adhesion triggers redistribution of Sec6/8 complex from cytosolic and, possibly, TGN-associated fractions to contacting plasma membranes and eventually to the apical junctional complex [6,11,12]. Evidence has also been presented for a regulatory interaction between Sec5 and Ral GTPase, but the mechanism of this regulation is not understood [13].

Considering that the function of Sec6/8 complex in directing polarized membrane trafficking is tightly coupled to regulated assembly and/or recruitment of this complex to specific plasma membrane sites, methods that distinguish different pools of Sec6/8 complexes in epithelial cells are valuable tools. The procedures described here outline how the ultracentrifuge is being used to analyze biochemical changes that accompany the activation of Sec6/8 complex function following induction of epithelial polarity development.

2. Materials and methods

2.1. Antibodies

Mouse monoclonal antibodies against Sec6 (9H5 for immunofluorescent staining and 10D11 for Western blotting) and Sec8 (8F12) have been described previously [5,14]. Rabbit polyclonal antibodies to Sec3, Sec15, Exo70, and Exo84 were generated by immunization with bacterially expressed protein fragments (corresponding to amino acids (aa) 692–818 (Sec3), 239–386 (Sec15), 190–308 (Exo70), and 143–312 (Exo84)) fused to glutathione S-transferase (GST). Affinity-purified rabbit polyclonal antibodies to Sec5 (aa 484–504) and Sec10 (aa 366–386) were generated by immunization with synthetic peptides coupled to keyhole limpet hemocyanin.

2.2. Establishment of “contact-naïve” MDCK cell cultures and calcium switch paradigm

Low-passage Madin–Darby canine kidney (MDCK) clone II cells are maintained in Dulbecco’s modified Eagle’s medium (DMEM, containing 1.0 g/liter sodium bicarbonate) supplemented with 10% fetal bovine serum (FBS), penicillin, streptomycin, and kanamycin (PSK). Cells are passaged at low density (2 x 10^6 cells/150-mm-diameter dish) on 2 consecutive days to prepare “contact-naïve” cultures [15]. Following trypsinization on the third day, cells are centrifuged at 1000g for 5 min and resuspended in low-calcium medium (LCM: DMEM containing 5 μM Ca^{2+}, suppl
dialyzed FBS and PSK). Cells are then plated in LCM at confluent density (~2 × 10⁷ cells/well) on 75-mm Transwell 0.45-µm polycarbonate filters (Costar Corp., Cambridge, MA, USA) or at ~2 × 10⁷ cells/150-mm dish and incubated for 3 h at 37 °C. “Contact-naive” cultures are harvested at the end of this incubation period. Alternatively, cell–cell contacts are synchronously induced by replacing LCM with DMEM (1.8 mM Ca²⁺) for the desired lengths of time. “Polarized” cultures are harvested after incubation for 5 days in DMEM. This tissue culture method permits careful analysis of biochemical changes accompanying Sec6/8 complex localization and interactions at defined stages during cell contact-induced epithelial polarity development.

2.3. Domain-specific cell surface biotinylation

Polarized MDCK cells cultured on Transwell filters are placed on ice and washed five times with Ringer’s saline (154 mM NaCl, 1.8 mM Ca²⁺, 7.2 mM KCl, 10 mM Hepes, pH 7.4). Sulfo-NHS-LC-LC-biotin (Pierce, Rockford IL; 0.5 mg/ml in Ringer saline buffer then twice with ice-cold homogenization buffer) are layered on top of a parallel gradient. Biotinylation is quenched by washing cells in five changes of TBS (120 mM NaCl, 10 mM Tris–HCl, pH 7.4) containing 50 mM NH₄Cl and 0.2% BSA.

2.4. Homogenization of cells and preparation of postnuclear supernatant

Cells are placed on ice and washed twice with ice-cold Ringer’s saline buffer then twice with ice-cold homogenization buffer (0.25 M sucrose in 20 mM Hepes–KOH, pH 7.2, 90 mM KOAc, 2 mM Mg(OAc)₂). Cells are scraped from plates or filters in homogenization buffer, pelleted by centrifugation at 1000g for 5 min, and resuspended in 1–3 ml homogenization buffer containing protease inhibitors (1 mM pefabloc, and 10 µg/ml each of aprotinin, antipain, leupeptin, and pepstatin A). Cells are passed through a 22-gauge needle (six times) and then through a 26-gauge needle (six times) to generate a single-cell suspension, and then homogenized by 10 passages through a ball bearing homogenizer, fitted with a 0.3747-in. stainless-steel ball bearing, in which the difference between chamber and ball bearing diameters is 10 µm (Varian Physics, Stanford University). Cell homogenates are transferred to microcentrifuge tubes and postnuclear supernatants (PNSs) are recovered after centrifugation at 3000g for 10 min. Note that this is a relatively gentle homogenization method, but it is important to maintain consistency in technique to avoid fractionation artifacts due to variability in homogenates from batch to batch.

2.5. Differential centrifugation

To separate membranes and large cytosolic protein particles from the bulk of cytosolic proteins, PNS is prepared as described and centrifuged at 100,000g for 45 min in TLA 100.3 rotor. Pellet and supernatant fractions are separately collected and analyzed by SDS–PAGE and Western blotting with specific antibodies.

2.6. Rate-zonal centrifugation in glycerol gradients

Assembly of Sec6/8 complexes is analyzed by rate-zonal centrifugation in glycerol gradients. To determine sedimentation coefficients of cytosolic Sec6/8 complexes, PNS is prepared as described above and centrifuged at 20,000g for 10 min to pellet large membranes, and the supernatant is kept for analysis. To generate gradients, glycerol solutions of 22.5, 24, 25.5, 27, 28.5, 30, 31.5, 33, 34.5, and 36% are prepared by mixing the following:

<table>
<thead>
<tr>
<th>[Glycerol]</th>
<th>48% Glycerol stock</th>
<th>4× Homogenization buffer</th>
<th>H₂O</th>
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<tr>
<td>36%</td>
<td>750 µl</td>
<td>250 µl</td>
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<tr>
<td>34.5%</td>
<td>719 µl</td>
<td>250 µl</td>
<td>31 µl</td>
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<tr>
<td>33%</td>
<td>688 µl</td>
<td>250 µl</td>
<td>62 µl</td>
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<tr>
<td>31.5%</td>
<td>656 µl</td>
<td>250 µl</td>
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<tr>
<td>30%</td>
<td>625 µl</td>
<td>250 µl</td>
<td>125 µl</td>
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<tr>
<td>28.5%</td>
<td>594 µl</td>
<td>250 µl</td>
<td>156 µl</td>
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<tr>
<td>27%</td>
<td>562 µl</td>
<td>250 µl</td>
<td>188 µl</td>
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<tr>
<td>25.5%</td>
<td>531 µl</td>
<td>250 µl</td>
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<tr>
<td>24%</td>
<td>500 µl</td>
<td>250 µl</td>
<td>250 µl</td>
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<tr>
<td>22.5%</td>
<td>469 µl</td>
<td>250 µl</td>
<td>281 µl</td>
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*80 mM Hepes–KOH, pH 7.2, 360 mM KOAc, 8 mM Mg(OAc)₂, 4 mM pefabloc, and 40 µg/ml each of aprotinin, antipain, leupeptin, and pepstatin A. Starting with the 36% glycerol, 120 µl of each solution is carefully layered stepwise in 1.4-ml thick-walled polycarbonate tubes for the TLS-55 rotor. Samples (120 µl) are loaded on top of the 22.5% glycerol step. Molecular weight standards (100 µg each of BSA (4.3S), β-amylase (11.2S), and thyroglobulin (19.2S) in 120 µl of homogenization buffer) are layered on top of a parallel gradient. Gradients are centrifuged at 91,000g (43,000 rpm) for 16 h at 4 °C, with slow acceleration and deceleration. Fractions (120 µl) are collected from the top using a pipettor and analyzed by SDS–PAGE and Western blotting with specific antibodies.

2.7. Nonequilibrium density gradient centrifugation in iodixanol (Opti-Prep) gradients

Starting with a single concentration of Opti-Prep (60% (w/v) iodixanol, Nycomed, Oslo, Norway), it is possible to generate nonlinear density gradients with steep regions at the top and bottom separated by a
shallow middle region following centrifugation for a short time (1 h) in a vertical or near-vertical rotor (Fig. 2C). With longer centrifugation times, gradients become linear, but the sigmoidal shape achieved under non-equilibrium conditions optimizes separation of particles with densities less than or greater than that of the mid-section. For separation of membrane-bound Sec6/8 complexes ($\delta < 1.18 \text{ g/ml}$) from cytosolic Sec6/8 com-

![Fig. 2. Separation of membrane-bound and cytosolic pools of Sec6/8 complex by density gradient centrifugation.](image)

(A) Flotation of membrane-bound Sec8 and E-cadherin in linear gradients. MDCK cell PNS, prepared from contact-naive or polarized cultures, was mixed with iodixanol (35% w/v), overlaid stepwise with equal volumes of 30, 25, 20, 15, and 10% iodixanol solutions in homogenization buffer, and centrifuged at 350,000 g for 3 h. Fractions were assessed for Sec8 and E-cadherin by SDS–PAGE and Western blotting. Sec8 blots are shown above gradient profiles. (B) Separation of membranes and cytosol in nonlinear gradients. MDCK cell PNS, prepared from contact-naive or polarized cultures, was mixed with iodixanol (30% w/v) and centrifuged at 350,000 g for 1 h. The presence of Sec6, Sec8, and E-cadherin in each gradient fraction was assayed by SDS–PAGE and Western blotting. (C) Density profiles of nonequilibrium gradients produced with different starting concentrations of iodixanol (in w/v).
plexes ($\delta > 1.22 \text{ g/ml}$) (Fig. 2B), gradients are prepared by mixing equal volumes of PNS and Opti-Prep to yield a sample containing $30\%$ (w/v) iodixanol. Samples are loaded into quick-seal polyallomer centrifuge tubes for the VTi 65.1 rotor and centrifuged at 350,000 g (61,000 rpm) for 65 min. Fractions (1 ml) are collected from the top and analyzed by SDS–PAGE and Western blotting with specific antibodies. Refractive indices are measured and density is determined for each fraction.

This method is convenient and has been used extensively to quantify association of Sec6/8 complex with membranes at different stages of epithelial polarity development [6], to compare Sec6/8 complex distribution in fibroblasts and epithelial cells [12], and to analyze the biochemical characteristics of membrane binding [6]. In principle, nonequilibrium density gradient centrifugation can be used to analyze recruitment of any cytosolic protein to the membrane. However, under nonequilibrium centrifugation conditions, sedimentation rate depends on particle size, in addition to density. Consequently, small cytosolic proteins do not sediment to their equilibrium density under the conditions used to study Sec6/8 complex because their sedimentation rate is much slower than that of large protein complexes. Therefore, it is necessary to optimize centrifugation conditions (iodixanol concentration, centrifugation speed and time) to achieve desired separation.

### 2.8. Isopycnic centrifugation in linear iodixanol (Opti-Prep) gradients

Separation of different membrane compartments is achieved by centrifugation in three-step 10–20–30% (w/v) iodixanol gradients (Fig. 3). One-third of the PNS is mixed with Opti-Prep (60% (w/v) iodixanol) and homogenization buffer to generate solutions containing 10, 20, and 30% (w/v) iodixanol. Equal volumes of these three solutions are layered in centrifuge tubes, and samples are centrifuged at 353,000 g for 3 h at 4 °C in a Beckman VTi65 rotor. Fractions (0.5 ml) are collected, refractive indices are read, and proteins are analyzed by SDS–PAGE and immunoblotting. Bradford assays are performed on each fraction to determine total protein concentrations.

Although this method, in which PNS is loaded uniformly throughout the gradient, separates different membrane compartments and effectively resolves large cytosolic protein complexes from all membranes, small (<100 kDa) cytosolic proteins contaminate membrane fractions recovered in denser regions of the gradient. To avoid this problem, samples are loaded in a high-density zone (containing 35% iodixanol) at the bottom of the gradient, overlain stepwise with Opti-Prep solutions of 30, 25, 20, 15, and 10% (w/v), and centrifuged at 353,000 g for 3 h at 4 °C in a Beckman VTi65 rotor. Membranes float up to their equilibrium density during the centrifugation run, but cytosolic proteins remain in the high-density...
medium at the bottom of the gradient. For Sec8, the fractionation profile obtained when sample is bottom-loaded (Fig. 2A) is very similar to that obtained when the sample is distributed throughout the gradient (Fig. 3).

3. Results

3.1. Analysis of Sec6/8 (exocyst) holocomplex assembly by differential and rate-zonal centrifugation

Plasma membrane association of many proteins is assayed by differential centrifugation. Sedimentation at 100,000g is reflective of membrane association and presence in supernatant is indicative of cytosolic localization. This approach fails to resolve membrane-bound and cytosolic pools of Sec6/8 complex, however. Immunofluorescence staining of MDCK cells for Sec6 shows that this protein is diffusely distributed throughout the cytoplasm of contact-naive cells, but is associated with lateral plasma membranes of polarized cells (Fig. 4). Staining for other Sec6/8 complex subunits gave similar results (data not shown). However, this striking redistribution is not apparent in differential centrifugation profiles of Sec6/8 complex subunits. Although each was recovered primarily in the high-speed pellet in

![Fig. 4. Analysis of Sec6/8 complex by differential centrifugation. Top: Contact-naive and polarized MDCK cultures were fixed and permeabilized with ice-cold methanol and stained with anti-Sec6 mAb. Bound antibodies were visualized with FITC anti-mouse IgG. Bottom: PNS from contact-naive and polarized MDCK cultures was centrifuged at 100,000g for 45 min. Supernatant (s) and pellet (p) fractions were collected and resolved by SDS-PAGE, and immunoblots were probed with specific antibodies. Fractionation profiles of occludin and green fluorescent protein (GFP) are included to show distributions of transmembrane and cytosolic proteins, respectively.](image_url)
polarized cells, as expected for membrane-bound proteins, the same distribution was observed in contact-naive cultures, in which a cytosolic distribution was expected (Fig. 4). One possible explanation for this finding is that Sec6/8 complex is associated with small vesicles that are distributed throughout the cytoplasm of contact-naive cells, and these have sufficient mass to pellet during differential centrifugation. This possibility is unlikely, however, because results of isopycnic density gradient centrifugation are inconsistent with membrane association of Sec6/8 complex in contact-naive cells (Fig. 2). It is more likely that the holocomplex is simply so large, or that it is associated with even larger non-membranous structures in contact-naive cells, that it sediments at 100,000g.

Although differential centrifugation does not resolve membrane-bound and cytosolic pools of Sec6/8 complexes, this method is useful for identifying partial complexes and individual subunits that exist outside of the holocomplex. For example, a significant fraction of Sec15 is recovered in the high-speed supernatant fraction from both contact-naive and polarized cells, suggesting that this subunit is only partially assembled into the Sec6/8 holocomplex. Other subunits, such as Sec8 and Sec10, are partially recovered in supernatant fractions of polarized cells, when most other subunits pellet quantitatively. This result suggests that Sec8, Sec10, and Sec15 may be in excess over the other subunits, and fail to pellet because interactions with the holocomplex are limiting. Interestingly, a subcomplex consisting of Sec10 and Sec15 was reported to exist outside the Sec6/8 holocomplex in yeast [16], and Sec10 and Sec15 from both yeast and mammals show strong interactions in the yeast two-hybrid assay [16,17].

Sec6/8 complex assembly is also analyzed by rate-zonal centrifugation, in which the fully assembled complex has a characteristic sedimentation coefficient of 17S [6,14]. This method has been employed to determine whether holocomplex assembly is dependent on membrane recruitment. Mouse L-cell fibroblasts are nonpolarized cells, but express an amount of Sec6/8 complex similar to that of MDCK cells. Immunofluorescence staining with anti-Sec6 antibodies reveals a diffuse staining pattern in these cells (Fig. 5), and fractionation through density gradients has confirmed that Sec6/8 complex subunits are present in a cytosolic pool (data not shown). Thus, the complex behaves similarly in L cells and contact-naive MDCK cells. Unlike MDCK cells, L cells lack cadherins, do not engage in cell-cell adhesion, and do not undergo polarized membrane growth. Therefore, it is unlikely that Sec6/8 complex is ever recruited to the plasma membrane of L cells. When L-cell PNS was fractionated by rate-zonal centrifugation through glycerol gradients, both Sec6 and Sec8 fractionated as a single peak at 17S (Fig. 5). Neither protein sedimented as a monomer or in a partially assembled complex, despite the fact that L cells are nonpolarized and do not accumulate a significant pool of active, membrane-associated Sec6/8 complex. Therefore, assembly of Sec6/8 holocomplex appears to occur independently of spatial cues that recruit the complex to plasma membrane growth sites.

3.2. Separation of membrane-bound and cytosolic Sec6/8 (exocyst) complexes by nonequilibrium isopycnic gradient centrifugation

Because the function of Sec6/8 complex in directing polarized exocytosis is tightly coupled to its recruitment to specific membrane sites, methods to distinguish cytosolic from membrane-bound forms are essential to studying regulation of Sec6/8 function. Centrifugation approaches, in which particles are separated according to their buoyant density, are useful for this purpose. Cytosolic and membrane-associated Sec6/8 complexes have different densities in iodixanol gradients (Fig. 2).
When PNS of contact-naive MDCK cells was placed in a dense medium at the bottom of a 10–35% iodixanol gradient, membranes (detected by the presence of E-cadherin) floated up through the gradient to their equilibrium density, but Sec6/8 complex remained in the load zone with the bulk of cytosolic proteins near the bottom of the gradient (Fig. 2A). The cytosolic pool of Sec6/8 complex has an equilibrium density of ~1.21 g/ml in these gradients. By contrast, when PNS of polarized MDCK cells was similarly fractionated, most of the Sec6/8 complex floated with membranes to an equilibrium density of ~1.16 g/ml (Fig. 2A). Note that Sec6/8 cofractionated with only a subset of E-cadherin-containing membranes in polarized cells, and that the majority of E-cadherin floated to regions of lower density that contained little Sec6/8. Thus, it is possible to separate different plasma membrane domains by equilibrium density gradient centrifugation (see also Fig. 3).

Because membrane-bound and cytosolic forms of Sec6/8 complex have different buoyant densities, a convenient method to resolve these pools was developed. Sigmoidal density gradients were formed by centrifugation of gradients composed of single initial iodixanol concentrations (Fig. 2C). Inspection of a series of blank gradients revealed that those formed by centrifugation of 30% iodixanol for 1 h consisted of a steep region of relatively low density (d < 1.18 g/ml) at the top of the gradient separated from a steep region of relatively high density (d > 1.20 g/ml) at the bottom of the gradient by a shallow region of intermediate density. Fractionation of PNS from contact-naive MDCK cells in these gradients resulted in the quantitative recovery of cytosolic Sec6/8 complex at the bottom of the gradient and of membrane-associated E-cadherin at the top of the gradient [6] and (Fig. 2B). By contrast, fractionation of PNS from polarized MDCK cells under identical conditions resulted in the recovery of ~75% of the Sec6/8 complex in low-density membrane fractions at the top of the gradients. This redistribution is quantitatively similar to that observed in equilibrium flotation gradients (Fig. 2A), but use of nonequilibrium density gradients to study membrane recruitment of Sec6/8 has several advantages: (1) higher resolution of cytosolic and membrane-bound pools; (2) relative ease of gradient preparation; (3) shorter centrifugation time (1 h vs 3 h).

3.3. Fractionation of Different Plasma Membrane Domains by Equilibrium Gradient Centrifugation

Isopycnic centrifugation in linear iodixanol gradients is an effective method to separate different organelles, and this method was recently used to identify distinct pools of Sec6/8 complex associated with plasma membrane and trans-Golgi network in fibroblasts [12]. Equilibrium density flotation gradients (Fig. 2A) indicated that it is possible to resolve different plasma membrane domains on the basis of density. Most of the E-cadherin, which is distributed along the entire length of the lateral plasma membrane, was recovered in a low-density region of the gradient (d ~ 1.06–1.12 g/ml), whereas Sec6/8 complex, which is restricted to the apical junctional complex [6], is recovered in a peak of higher density (d ~ 1.16 g/ml). Methods to resolve plasma membrane subdomains are useful for analysis of specific membrane binding sites for Sec6/8 complex and, potentially, for reconstituting in vitro functional assays for Sec6/8 complex function.

To further explore the potential of linear iodixanol gradients to separate different plasma membrane domains, polarized MDCK cells were biotinylated on either the apical or the basal-lateral surfaces and samples were centrifuged through three-step (10–20–30% (w/v) iodixanol) gradients (Fig. 3). Western blotting with peroxidase-conjugated streptavidin revealed the distributions of all membrane proteins that had been accessible to biotin. This analysis showed that many apically biotinylated proteins were recovered in a region of relatively low density (d ~ 1.07 g/ml). Western blotting for two apical membrane glycoproteins, gp114 and gp135, showed that these proteins were also recovered primarily in this peak. A second peak of apical membrane, enriched in a distinct set of biotinylated proteins, was recovered in a region of higher density (d ~ 1.16 g/ml). This peak cofractionated with marker proteins of tight junctions (data not shown) and the Sec6/8 complex (Fig. 3), and might represent a subdomain of the apical plasma membrane that remains associated with apical junctional complex following homogenization of cells.

Fractionation of basal-laterally biotinylated cells revealed that many proteins cofractionated with Sec6/8 complex in the peak at 1.16 g/ml, and thus had a fractionation profile different than that of the low-density apical membrane peak (Fig. 3). In contrast to Sec8, most basal-lateral proteins were not tightly focused within the peak at 1.16 g/ml, but were spread broadly throughout the middle of the density gradient. Western blotting for two specific lateral membrane proteins, E-cadherin and the α subunit of the Na/K-ATPase, revealed that these proteins were recovered both in the peak at 1.16 g/ml (~40%) and within a broader region of the gradient with an average density of 1.10 g/ml (~60%). Therefore, isopycnic density centrifugation through linear iodixanol gradients appears to resolve subdomains of basal-lateral membranes into distinct membrane fractions containing intercellular junctions (d ~ 1.16 g/ml) and nonjunction-associated basal-lateral membranes (d ~ 1.10 g/ml).

4. Conclusions

The behavior of particles in a centrifugal field is described by the equation
in which \( v = \text{velocity of sedimentation}, \ d = \text{diameter of particle}, \ \delta_p = \text{density of particle}, \ \delta_l = \text{density of liquid}, \ g = \text{relative centrifugal force}, \) and \( \mu = \text{viscosity of liquid}. \) Therefore, “classic” ultracentrifugation methods that separate particles on the basis of size or density are ideally suited for analyzing the assembly and membrane recruitment of Sec6/8 (exocyst) complex during development of epithelial polarity. The biochemical changes accompanying Sec6/8 complex activation following E-cadherin-mediated adhesion are important events in the early stages of polarity development. With the advent of iodixanol as a versatile density gradient medium, it has become possible to isolate distinct cellular pools of Sec6/8 complex and begin detailed analysis of this process.

References