Rab-mediated membrane trafficking and the control of epithelial cell polarity

Maria S. Ioannou and Peter S. McPherson

Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, Quebec, H3A 2B4, Canada

Development of cell polarity requires apical trafficking of podocalyxin; yet the regulation of its transport is unclear. In this issue, Mrozowska and Fukuda (2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201512024) demonstrate that different sets of Rabs and Rab effectors are used to regulate podocalyxin trafficking in two- versus three-dimensional model systems.

Most epithelial organs are composed of a monolayer of highly polarized cells, consisting of a basolateral domain and an apical domain surrounding a lumen. The development of apical-basolateral polarity is essential for the proper function of epithelial tissues, and defects in cell polarity are often associated with diseases such as cancer, yet the mechanisms for establishing and maintaining polarity are not fully defined. One key event in polarity development in many epithelial and endothelial cells is the delivery of podocalyxin (PCX) to the apical surface. PCX is a transmembrane glycoprotein with extensive sialylation of its extracellular domain, which confers a strong negative charge, and this property of PCX is vital for the formation of the apical lumen. For example, repulsive forces resulting from the negative charge of PCX lining the apical surface of endothelial cells is required for the opening of the vascular lumen and the maintenance of vascular permeability (Strilić et al., 2009; Debruin et al., 2014; Fig. 1). Kidney glomeruli, the unit responsible for filtration and urine formation, contain capillary loops that contact epithelial cells called podocytes (Nielsen and McNagny, 2009). Podocytes send out interdigitating foot processes that wrap around capillaries forming the architecture necessary for filtration, and PCX on the apical surface of these cells is required for foot process formation and maintenance (Nielsen and McNagny, 2009). Not surprisingly, the absence of PCX results in perinatal lethality, and, intriguingly, mutations and/or altered expression levels of PCX lead to the development of multiple cancers. Thus, the delivery of PCX to the apical domain is not only a hallmark of cell polarity but is critical for the function of multiple tissues with polarized cells.

MDCK cells are a widely used model of epithelial cell polarity and develop into either 2D monolayers under standard culture conditions or 3D cysts when surrounded by extracellular matrix. In either model system, PCX is polarized exclusively to the apical membrane but the exact route and mechanism of PCX trafficking is poorly understood. In this issue, Mrozowska and Fukuda follow the trafficking itinerary of PCX in MDCK cells during polarity establishment in both 2D and 3D cultures. Before polarization PCX is evenly distributed on the plasma membrane. However, upon plating in a 2D polarization model, PCX is rapidly internalized, transported to perinuclear recycling endosomes, and then selectively delivered on vesicles to the apical membrane located on the dorsal side of the cell. During the formation of 3D cysts, PCX showed a similar trafficking pattern, although internalization was substantially delayed compared with 2D cultures. However, PCX is eventually transported to recycling endosomes and subsequently delivered to the apical membrane where the lumen is created (Fig. 1). The differences in the kinetics of trafficking between the two model systems suggest that the trafficking mechanisms may be different; however, control of PCX trafficking is not well defined.

Rab GTPases are the largest family of small GTPases and are key molecular switches in the regulation of membrane trafficking. Before the Mrozowska and Fukuda (2016) study, it had been established that at least four Rabs—Rab3, Rab8, Rab11, and Rab27—were required for PCX delivery to the apical surface. These Rabs are primarily involved in the transport and tethering of PCX-bearing vesicles to the plasma membrane (Bryant et al., 2010; Gálvez-Santisteban et al., 2012). However, Rabs regulate all steps in membrane trafficking. In addition to transport and tethering, they also control the budding of vesicles at multiple cellular locations, maturation of membrane compartments, and vesicle fusion. To better define the regulation of PCX trafficking, Mrozowska and Fukuda (2016) sought to identify the full complement of Rabs that regulate various trafficking steps as PCX makes it way to the apical membrane during epithelial cell polarization. They first screened a panel of Rabs for their ability to colocalize with PCX at various time points during the polarization process. From there they selected multiple colocalizing Rabs for loss-of-function studies and found that inhibiting different Rabs caused defects in the various trafficking steps of PCX. For example, disruption of Rab14 delayed PCX internalization, Rab8 and Rab13 caused retention of PCX on intracellular vesicles, Rab11 delayed PCX vesicle docking on the apical surface, and depleting Rab5 disrupted lumen formation. Perhaps more interesting is that different Rabs regulate PCX trafficking steps variably depending on whether 2D or 3D culture systems are analyzed. For example, Rab13 and Rab14 are important for PCX trafficking specifically during 2D polarization but did not affect lumen formation in 3D cultures, whereas Rab4, Rab15, Rab19, and Rab25 are only required for efficient PCX trafficking in 3D cultures. Although it is becoming increasingly clear that

Correspondence to Peter S. McPherson: peter.mcpherson@mcgill.ca

The Rockefeller University Press $30.00
J. Cell Biol. Vol. 213 No. 3 301–303
www.jcb.org/cgi/doi/10.1083/jcb.201604076

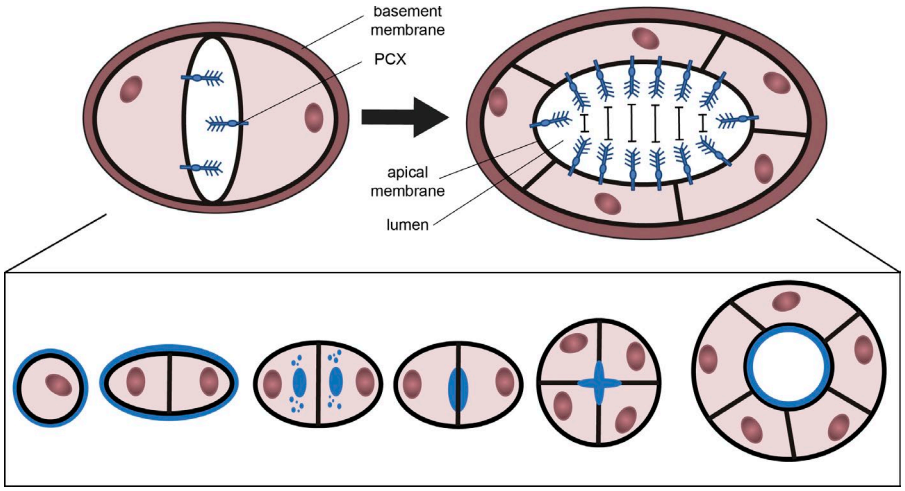

Figure 1. **Development of a lumenal vessel as the surrounding epithelial cells divide.** PCX is indicated and the protrusions from the protein represent negatively charged sialylation. The lines indicate repulsive charge interactions that help to open and stabilize the vascular lumen. The inset shows the redistribution of PCX (blue) from the plasma membrane on single cells to recycling endosomes and finally the apical membrane as the lumen of a 3D structure (cyst, blood vessel, etc.) develops.

the 3D situation will better mimic the developmental paradigms found in vivo, this study highlights the molecular differences that arise between the two culture systems.

The most striking example of differences in the role of Rabs in 2D versus 3D culture was seen with Rab35. Rab35 loss of function during 2D polarization caused PCX accumulation on large, actin-rich structures, whereas during 3D polarization PCX accumulated on small vesicles near the apical surface, largely free of actin. Thus, even though Rab35 regulates PCX trafficking during both polarization paradigms, there must be differences in the activity/effectors of the GTPase. Rab35 is an important regulator of multiple cellular activities, including control of the levels of phosphatidylinositol (4,5)-bisphosphate in endosomes after clathrin-coated vesicle scission from the plasma membrane (Nández et al., 2014; Cauvin et al., 2016), cargo recycling from early/sorting endosomes, dynamics of the actin cytoskeleton (Allaire et al., 2010; Chaineau et al., 2013), and cytokinetic abscission (Kouranti et al., 2006). Early in the formation of a 3D cyst, the location of the cytokinetic bridge between the first two daughter cells specifies the site where the apical membrane will initiate and thus specifies the central position of the future apical lumen. Rab35 is crucial for this lumen formation as it couples cytokinesis with the tethering of vesicles carrying lumen-promoting PCX at the emerging apical membrane (Klinkert et al., 2016). In fact, PCX acts as an effector for Rab35, interacting directly with Rab35 in its active GTP-bound form (Klinkert et al., 2016). It is common, however, for a single Rab to recruit different effectors depending on the cellular context, allowing a single Rab to mediate various cellular responses. For instance, Rab35 interacts with six effectors in addition to PCX (Chaineau et al., 2013). Mrozowska and Fukuda (2016) now discover that the differences in PCX trafficking mediated by Rab35 in 2D versus 3D cultures are a result of the engagement of different effectors. Rab35 requires oculocerebrorenal syndrome of Lowe (OCRL) protein for proper PCX trafficking in 2D monolayers. OCRL is an inositol polyphosphate 5-phosphatase and is mutated in Lowe's syndrome. In dividing cells, active Rab35 recruits OCRL to hydrolyze phosphatidylinositol (4,5)-bisphosphate and stimulate F-actin clearance in the intracellular bridge (Dambournet et al., 2011). Therefore, the accumulation of PCX on large actin-rich structures in the absence of Rab35 during 2D polarization could be because of the inability of OCRL to stimulate actin clearance on these structures similar to that observed in the intracellular bridge of dividing cells.

During the formation of 3D cysts, however, Rab35 relies more heavily on ArfGAP with coiled-coil ankyrin repeat and PH domain 2 (ACAP2), also known as centaurin β2, for proper PCX trafficking. In addition to serving as an effector for Rab35, ACAP2 can inactivate Arf6, another small GTPase found on the same endosomal compartment as Rab35 (Kobayashi and Fukuda, 2012; Allaire et al., 2013). Conversely, when Arf6 is active, it recruits its effector TBC1D10, which inactivates Rab35 (Chesneau et al., 2012). This coordinated regulation ensures that only one of the GTPases, Rab35 or Arf6, is active at a time, as the two GTPases promote opposing physiological functions. Rab35 recycles E-cadherin to the plasma membrane to facilitate cell–cell adhesion, whereas Arf6 recycles integrins to the surface in favor of cell migration (Allaire et al., 2013). Thus, rather than the defects in actin dynamics observed in 2D polarization after Rab35 disruption, defects in 3D lumen formation are likely caused by the failure to promote cell–cell adhesion. What remains unknown is why certain effectors are more important for polarization in the two model systems.

The importance of 2D cell culture models in understanding cellular physiology cannot be overstated. However, it is clear that cells behave differently depending on the model system being used. Most studies of integrin-based focal adhesions are performed using 2D culture systems; however, focal adhesions are considerably different when observed in a 3D system. For example, 3D matrix adhesions differ from 2D focal and fibrillary adhesions in their molecular composition, morphology, and physiology (Cukierman et al., 2001). The mode of cellular migration also differs depending on the dimensionality of the culture system being used. Compared with cells in 2D, cells in 3D cultures migrate faster, depend more on myosin II contractility and microtubules, and depend less on the extracellular matrix ligand density (Doyle et al., 2009). Finally, 3D cultures greatly increase the efficiency of reprograming human somatic cells into induced pluripotent stem cells (Caiazzo et al., 2016). Moreover, human neural stem cell–derived 3D culture systems better recapitulate hallmark pathologies of Alzheimer's disease compared with various cell-based and mouse models (Choi et al., 2014). In this issue, Mrozowska and Fukuda (2016) extend this notion to provide a molecular mechanism as to how cells behave differently in the two culture systems. These studies partly challenge the use of traditional 2D cell culture systems to extrapolate how a cell may behave in vivo. However, we cannot say for certain that conclusions drawn even in 3D

models accurately represent the behavior of cells in vivo. To truly understand how a cell behaves in an organism will ultimately require studying its behavior in the most native context within the organism itself.

Acknowledgments

The authors declare no competing financial interests.

Submitted: 18 April 2016
Accepted: 18 April 2016

References

Allaire, P.D., A.L. Marat, C. Dall'Armi, G. Di Paolo, P.S. McPherson, and B. Ritter. 2010. The Connecdenn DENN domain: a GEF for Rab35 mediating cargo-specific exit from early endosomes. *Mol. Cell.* 37:370–382. http://dx.doi.org/10.1016/j.molcel.2009.12.037

Allaire, P.D., M. Seyed Sadr, M. Chaineau, E. Seyed Sadr, S. Konefal, M. Fotouhi, D. Maret, B. Ritter, R.F. Del Maestro, and P.S. McPherson. 2013. Interplay between Rab35 and Arf6 controls cargo recycling to coordinate cell adhesion and migration. *J. Cell Sci.* 126:722–731. http://dx.doi.org/10.1242/jcs.112375

Bryant, D.M., A. Datta, A.E. Rodríguez-Fraticelli, J. Peränen, F. Martín-Belmonte, and K.E. Mostov. 2010. A molecular network for de novo generation of the apical surface and lumen. *Nat. Cell Biol.* 12:1035–1045. http://dx.doi.org/10.1038/ncb2106

Caiazzo, M., Y. Okawa, A. Ranga, A. Piersigilli, Y. Tabata, and M.P. Lutolf. 2016. Defined three-dimensional microenvironments boost induction of pluripotency. *Nat. Mater.* 15:344–352. http://dx.doi.org/10.1038/nmat4536

Cauvin, C., M. Rosendale, N. Gupta-Rossi, M. Rocancourt, P. Larraufie, R. Salomon, D. Perrais, and A. Echard. 2016. Rab35 GTPase triggers switch-like recruitment of the Lowe syndrome lipid phosphatase OCRL on newborn endosomes. *Curr. Biol.* 26:120–128. http://dx.doi.org/10.1016/j.cub.2015.11.040

Chaineau, M., M.S. Ioannou, and P.S. McPherson. 2013. Rab35: GEFs, GAPs and effectors. *Traffic.* 14:1109–1117.

Chesneau, L., D. Dambournet, M. Machicoane, I. Kouranti, M. Fukuda, B. Goud, and A. Echard. 2012. An ARF6/Rab35 GTPase cascade for endocytic recycling and successful cytokinesis. *Curr. Biol.* 22:147–153. http://dx.doi.org/10.1016/j.cub.2011.11.058

Choi, S.H., Y.H. Kim, M. Hebisch, C. Sliwinski, S. Lee, C. D'Avanzo, H. Chen, B. Hooli, C. Asselin, J. Muffat, et al. 2014. A three-dimensional human neural cell culture model of Alzheimer's disease. *Nature.* 515:274–278. http://dx.doi.org/10.1038/nature13800

Cukierman, E., R. Pankov, D.R. Stevens, and K.M. Yamada. 2001. Taking cell-matrix adhesions to the third dimension. *Science.* 294:1708–1712. http://dx.doi.org/10.1126/science.1064829

Dambournet, D., M. Machicoane, L. Chesneau, M. Sachse, M. Rocancourt, A. El Marjou, E. Formstecher, R. Salomon, B. Goud, and A. Echard. 2011. Rab35 GTPase and OCRL phosphatase remodel lipids and F-actin for successful cytokinesis. *Nat. Cell Biol.* 13:981–988. http://dx.doi.org/10.1038/ncb2279

Debruin, E.J., M.R. Hughes, C. Sina, A. Lu, J. Cait, Z. Jian, M. Lopez, B. Lo, T. Abraham, and K.M. McNagny. 2014. Podocalyxin regulates murine lung vascular permeability by altering endothelial cell adhesion. *PLoS One.* 9:e108881. Published erratum appears in *PLoS One*. 2014. 9:e116613. http://dx.doi.org/10.1371/journal.pone.0108881

Doyle, A.D., F.W. Wang, K. Matsumoto, and K.M. Yamada. 2009. One-dimensional topography underlies three-dimensional fibrillar cell migration. *J. Cell Biol.* 184:481–490. http://dx.doi.org/10.1083/jcb.200810041

Gálvez-Santisteban, M., A.E. Rodriguez-Fraticelli, D.M. Bryant, S. Vergarajauregui, T. Yasuda, I. Bañón-Rodríguez, I. Bernascone, A. Datta, N. Spivak, K. Young, et al. 2012. Synaptotagmin-like proteins control the formation of a single apical membrane domain in epithelial cells. *Nat. Cell Biol.* 14:838–849. http://dx.doi.org/10.1038/ncb2541

Klinkert, K., M. Rocancourt, A. Houdusse, and A. Echard. 2016. Rab35 GTPase couples cell division with initiation of epithelial apico-basal polarity and lumen opening. *Nat. Commun.* 7:11166. http://dx.doi.org/10.1038/ncomms11166

Kobayashi, H., and M. Fukuda. 2012. Rab35 regulates Arf6 activity through centaurin-β2 (ACAP2) during neurite outgrowth. *J. Cell Sci.* 125:2235–2243. http://dx.doi.org/10.1242/jcs.098657

Kouranti, I., M. Sachse, N. Arouche, B. Goud, and A. Echard. 2006. Rab35 regulates an endocytic recycling pathway essential for the terminal steps of cytokinesis. *Curr. Biol.* 16:1719–1725. http://dx.doi.org/10.1016/j.cub.2006.07.020

Mrozowska, P.S., and M. Fukuda. 2016. Regulation of podocalyxin trafficking by Rab small GTPases in 2D and 3D epithelial cell cultures. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201512024

Nández, R., D.M. Balkin, M. Messa, L. Liang, S. Paradise, H. Czapla, M.Y. Hein, J.S. Duncan, M. Mann, and P. De Camilli. 2014. A role of OCRL in clathrin-coated pit dynamics and uncoating revealed by studies of Lowe syndrome cells. *eLife.* 3:e02975.

Nielsen, J.S., and K.M. McNagny. 2009. The role of podocalyxin in health and disease. *J. Am. Soc. Nephrol.* 20:1669–1676. http://dx.doi.org/10.1681/ASN.2008070782

Strilić, B., T. Kucera, J. Eglinger, M.R. Hughes, K.M. McNagny, S. Tsukita, E. Dejana, N. Ferrara, and E. Lammert. 2009. The molecular basis of vascular lumen formation in the developing mouse aorta. *Dev. Cell.* 17:505–515. http://dx.doi.org/10.1016/j.devcel.2009.08.011