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meeting point

# The young and happy marriage of membrane traffic and cell polarity

*Barry J. Thompson, Franck Perez & Thomas Vaccari*

**The ESF–EMBO meeting on 'Cell Polarity and Membrane Traffic' took place in Poland in April 2012. It brought together scientists from two once separate fields and highlighted their emerging interdependence. The wealth of scientific insights and discoveries presented laid a path for future research.**

## Introduction

Catherine Rabouille (Hubrecht Institute, Utrecht, the Netherlands) organized the ESF–EMBO meeting on 'Cell Polarity and Membrane Traffic' with the help of Keith Mostov (UC San Francisco, USA) and additional financial support from The Company of Biologists, the Wellcome Trust, Genentech, Abcam, Springer, *Nature Reviews Molecular Cell Biology* and the Foundation of Polish Research. The meeting was held within the ancient walls of Polonia Castle in the beautiful town of Pultusk, the perfect venue to celebrate, after a very long engagement, the blossoming marriage of the fields of cell polarity and membrane traffic. This was the second instalment in the series that Ian Macara (Vanderbilt U. Medical Center, Nashville, USA) and Anne Spang (Biozentrum, Basel, Switzerland) began two years ago. This exciting gathering naturally featured great science from established investigators, but the organizers also went to great lengths to promote the science of junior principal investigators, to involve younger scientists in the proceedings as session chairs, and to leave plenty of time available for informal discussions. Here we report the highlights, focusing in particular on research that bridges the gap between cell polarity and membrane traffic. We apologize to the investigators whose work is not highlighted here due to space constraints.

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## The engagement

Daniel St Johnston (Gurdon Institute, Cambridge, UK) opened the meeting with the first keynote lecture. St Johnston's lab has been at the forefront of the study of Par-dependent cell polarity (see [1] for review). In keeping with the theme of the meeting right from the start, Johnston showed how polarized movement of an entire organelle—the nucleus of the *Drosophila* oocyte—determines dorsoventral polarity of the future animal. The oocyte nucleus has long been known to migrate dorsally to impart dorsoventral polarity. However, the underlying mechanisms have so far proven elusive. Live imaging allowed St Johnston's group to rule out the hypothesis that dynein pulls the nucleus into position. Instead, he showed that growing microtubules push on the nucleus, essentially cornering it to the future dorsal site. Antoine Guichet (Institut Jacques Monod, Paris, France) presented similar results later in the meeting. Thus, the dorsal part of the animal is probably not set deterministically, but is rather defined as the corner in which the nucleus randomly ends up due to the microtubule growing process [2].

Keith Mostov and Enrique Rodriguez-Boulan (Cornell U., New York, USA) have played seminal roles in merging the fields of cell polarity and vesicular trafficking. Mostov's lecture introduced the Madin–Darby canine kidney (MDCK) epithelial cell culture as the historic model to study how trafficking shapes apico-basal polarity. He described his group's discovery of the polarization of plasma membrane lipids—PIP2 and PIP3—in these cells, and reported new findings using three-dimensional (3D) cultures of MDCKs for wound healing and Hippo signalling studies. Rodriguez-Boulan described the evolution of our knowledge about the trafficking pathways of plasma membrane proteins in MDCK cells, leading to the idea that their post-Golgi routes involve different types of recycling or sorting endosome. He then focused on the role of clathrin and the clathrin adaptors AP1A and AP1B in promoting basolateral sorting at the trans-Golgi network or recycling endosomes, respectively [3]. Finally, he showed that tissues lacking the epithelial-specific adaptor AP1B, such as retinal pigment epithelium and kidney proximal tubule, show marked changes in the transendosomal routes used by basolateral proteins, when compared with tissues expressing AP1B. The seminal efforts of all three of these speakers over the years inspired the research summarized below, which reveals to what extent cell polarity and membrane trafficking are complementary characters of a happy couple.

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## Phospholipids as apical determinants

Lipids provide membrane identity, a prerequisite for direct trafficking and compartment-specific interactions. At the meeting, the role of phospholipids in regulating apical polarity was prominent, particularly phosphatidylinositol 4,5-bisphosphate (PIP2). Fernando



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Martin-Belmonte (CSIC, Madrid, Spain) described how the synaptotagmin-like proteins 2a and 4a (Slp2a/4a) act during the formation of an apical lumen in 3D cultures of MDCK cells. His lab found that Slp2a localizes to the luminal membrane in a PIP2-dependent manner, where it helps to guide polarized delivery of Rab27-containing vesicles. Vesicle tethering and fusion is then regulated by Slp4a in conjunction with Rabs and the SNARE protein syntaxin 3 [4]. Martijn Gloerich (with James Nelson at Stanford U., USA) spoke about work he performed in Hans Bos' lab (UMC Utrecht, The Netherlands) on the regulation of brush border formation. This process involves Ezrin, a FERM-domain protein that, when active, binds to both actin and PIP2. Gloerich showed that, on generation of phosphatidic acid at the apical membrane of human intestinal cells, the small GTPase Rap2A acts through the TNIK and MST4 kinases to phosphorylate and activate Ezrin. Finally, Antoine Guichet reported on the role of the PI-kinase Skittles in the *Drosophila* follicle cell epithelium. By producing PIP2, Skittles controls epithelial polarity by allowing targeting of PAR3 to the apical membrane. Guichet also showed that the PIP phosphatase PTEN, which can also produce PIP2 from PI(3,4,5)P3, surprisingly does not seem to be required for apical-basal polarity.

### AP adaptors for polarized trafficking

The early work on trafficking in MDCKs pointed to the extensive role of the AP trafficking adaptors in imparting correct polarization of membrane proteins, both at the Golgi and plasma membrane. In relation to this, Barry Thompson (London Research Institute, UK) reported that AP2/clathrin-mediated endocytosis from the basolateral membrane, and recycling through Rab11-containing endosomes to the apical domain, are required to localize the transmembrane polarity determinant Crumbs in the *Drosophila* follicle cell epithelium. Thompson also showed that the process is regulated by self-recruitment of the GTPase Cdc42 to the apical membrane [5]. Gregoire Michaux (Rennes Institute of Genetics and Development, France) showed a requirement for AP1 in polarization of apical determinants, including Cdc42, in the *Caenorhabditis elegans* intestinal epithelium. This is the first evidence that AP1 is implicated not only in basolateral, but also in apical sorting [6]. Ben Margolis (U. Michigan Medical School, USA) had previously shown that transmembrane proteins, such as Crumbs3, can be trafficked into cilia in MDCK cells. In collaboration with the neighbouring laboratory of Kristen Verhey, he has found that cilia trafficking is strikingly similar to nuclear import [7]. Finally, Roman Polishchuk (TIGEM, Naples, Italy) described polarized trafficking of the copper transporters ATP7A and ATP7B, which are mutated in Menkes and Wilson disease, respectively. In MDCK cells, ATP7A/B move out of the Golgi apparatus towards the basolateral and apical membranes, respectively, in an AP1B-independent fashion. Roman has also found that the most frequent Wilson disease-causing mutants of ATP7B mislocalize to the endoplasmic reticulum, and that this aberrant retention is reverted by inhibition of the MAP kinase p38, suggesting a strategy to ameliorate the effects of the disorders.

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### Trafficking of cadherins and function

Cadherins have long been known to mediate several aspects of adhesion between polarized cells. At the meeting, several researchers showed how control of cadherin trafficking is crucial to their functions. Ian Macara examined E-cadherin trafficking in MDCK cells, in which it localizes to the lateral membrane together with the basolateral determinant Scribble. Loss of Scribble caused endocytosis of E-cadherin to late endosomes and multivesicular bodies, which was then recycled through the retromer to the Golgi. Interestingly, loss of p120 catenin also caused endocytosis of E-cadherin, but resulted in degradation in lysosomes rather than recycling. Catherine Rabouille and Adam Grieve (Hubrecht Institute, Utrecht, the Netherlands) described a mechanism that specifically leads to the endocytosis of truncated E-cadherin and to a special set of lysosomes for degradation. This pathway seems to involve the Golgi resident protein GRASP65. Roland Le Borgne (Rennes Institute of Genetics and Development, France) reported that septins are required for asymmetrical cell division in *Drosophila* sensory organs. Septins localize at the cytokinesis ring and mutants fail to reform adherens junctions after division, as Roland's lab assessed by following trafficking of E-cadherin *in vivo*. Finally, Matias Simons (U. Freiburg, Germany) showed that the atypical cadherin Flamingo recruits VhaPRR—also called ATP6AP2—to the planar cell polarity complex. VhaPRR is an accessory subunit of vacuolar ATPase that also functions in endosomal trafficking.

### Organelles and cytoskeleton in polarity

Rab GTPase, ESCRT proteins and cytoskeletal components are required for the biogenesis of organelles that support trafficking, yet their role in regulating polarity is poorly understood. Several contributors at the meeting discussed how these factors influence cell polarity. Thomas Vaccari (IFOM, Milan, Italy) showed that the ESCRT components Hrs and Stam are jointly required for polarity of *Drosophila* epithelia. Their combined loss also enhances proliferative signalling, suggesting that they might act as tumour suppressors. Pankaj Dhonukshe (U. Utrecht, the Netherlands) described a new mechanism for oriented cell division in *Arabidopsis* stem cells. His group has found that the microtubule regulators MAP65 and CLASP act to reorient the cortical microtubular array during cell morphogenesis. This process also redirects endocytic trafficking, and its study will allow us to resolve the connections between microtubule organization and cell polarity [8]. Marino Zerial (MPI-CBG, Dresden, Germany), who gave the final lecture of the meeting, summarized his extensive work on the organizational principles of the endocytic system, and on Rab5 function in membrane fusion and endosome maturation. He then showed how Rab5 activity

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is required for the biogenesis and homeostasis of endosomes in the liver, which is necessary to deliver molecules in a polarized fashion [9]. Other fascinating work from Zerial's lab presented at the meeting included the development of quantitative image analysis approaches to unravel how hepatic cells build the liver tissue.

## New tools for new discoveries

Cutting edge technology has allowed the field of trafficking and polarity to flourish. At the meeting, two talks were about new tools that promise further advancements. Franck Perez (Institut Curie, Paris, France) described a new method, 'retention using selective hook' (RUSH), for conditional induction of synchronous secretion of virtually any streptavidin-binding protein (SBP)-tagged transmembrane protein from the Golgi, after biotin-mediated release of the protein from a Golgi-resident streptavidin 'hook' in mammalian-cultured cells. This approach might be used for real-time analysis or cellular screening approaches and will be valuable for the study of trafficking in polarized cells [10]. Marko Brankatschk (MPI-CBG, Dresden, Germany) presented a new Rab library consisting of *Drosophila* fly lines in which individual Rab proteins have been tagged with fluorescent protein by homologous recombination. These knock-in lines can allow precise determination of the endogenous localization of the vast array of *Drosophila* Rab proteins. Each Rab was knocked out, or engineered to be silenced at the RNA level, or to be cleaved by insertion of TEV cleavage sites. The library is complemented by a web-based platform for data annotation and sharing. Such an approach will allow tissue-specific functional studies *in vivo*.

## End of the honeymoon

Overall, this excellent meeting highlighted how, in many tissues and organisms, polarity and trafficking are interdependent, as good couples usually are in working marriages. However, although much of the basic machinery of polarity and trafficking is widely shared across different systems, its precise organization seems to differ, possibly reflecting functional differences. Thus, careful analysis and close comparison of different model systems will be needed in the future for this union to last, and to bear as fruit a deeper understanding of tissue physiology and pathology.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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