Sorting Vangl2 at the Golgi

Planar cell polarity (PCP) co-organizes the polarity of individual epithelial cells and is critical in development, but exactly how PCP components such as Vangl2 are correctly distributed remains unclear. Schekman and colleagues demonstrate that Vangl2 is transported from the *trans* Golgi network (TGN) in a manner dependent on the small GTPase Arfrp1 and the clathrin adaptor AP-1 (*eLife* http://doi.org/j7c; 2013).

An siRNA screen of TGN-localized Arf proteins demonstrated that the loss of Arfrp1 resulted in defective exit of Vangl2 from the TGN. Clathrin and AP-1 vesicle coat proteins are involved in protein sorting at the Golgi, and the authors found that AP-1 preferentially binds to the GTP-bound form of Arfrp1. A known AP-binding basolateral sorting motif in Vangl2 was shown to mediate export of Vangl2 from the TGN and to directly interact with the μ1 subunit of AP-1. Furthermore, knockdown of AP-1 subunits caused Vangl2 to accumulate at the TGN. Experiments in which purified components were incubated with synthetic liposomes led the authors to propose that an interaction between Arfrp1–GTP and AP-1 recruits Vangl2 through its sorting motif, and this in turn stabilizes AP-1 membrane localization.

Interestingly, the authors also found that Frizzled 6, a PCP component localized on the opposite side to Vangl2 in the cell, leaves the TGN independently of Arfrp1 and AP-1. Thus, different TGN-sorting mechanisms may influence the opposing localization of PCP components. CKR

Scaling somitogenesis

The sequential formation of vertebrate somites is under the control of signalling pathways that are comprised of Notch, Wnt and FGF, and involve oscillatory gene activity. Earlier studies showed that scaling operates in somitogenesis: if parts of an embryo are removed, somites still arise in the correct place and in the expected numbers, but are smaller than usual. Aulehla and colleagues have developed an *ex vivo* model for segmentation, and combined it with real-time imaging of gene activity to show that a shift in the phase of gene expression oscillations drives scaling (*Nature* **493,** 101–105; 2013).

Phase shifts in oscillatory gene activity between neighbouring cells have been described in the pre-somitic mesoderm (PSM) but their functional importance was unclear. The authors used a fluorescence reporter to follow the cyclic expression of the segmentation gene Lunatic fringe, in embryos and in a dissected monolayer of PSM cells placed in a dish. They first observed that the appearance of segments with specific gene expression patterns is recapitulated in their *ex vivo* system. They showed that larger samples exhibit fewer phase differences in the reporter gene activity between PSM cells than the small samples. Through detailed quantifications, they identify phase differences as a novel single predictor for future segment sizes. The authors also formulated a mathematical model that emphasizes the predicted value of the differences in phase shifts to define scaling parameters. NLB

Interrogating Lkb1 function

The kinase Lkb1 regulates cell polarity and is mutated or deleted in many cancers. Lkb1 substrates include the kinase AMPK, but whether AMPK is a relevant Lkb1 target in polarity and tumorigenesis remains unclear. Mellman and colleagues now use elegant chemical genetics approaches to address this issue (*J. Cell Biol.* **199,** 1117–1130; 2012).

The authors generated a knock-in mouse expressing an analogue-sensitive kinase allele (ASKA) of Lkb1; this strategy enables Lkb1 to be inhibited by the compound 1NMPP1. Mice homozygous for mutant Lkb1 were embryonic lethal, but embryonic tissues harvested from these mice could be used for *ex vivo* analyses. Acute Lkb1 inhibition in the lung led to branching defects but not loss of apical–basal polarity. Pancreatic explants similarly retained apical–basal polarity following Lkb1 inhibition, but formed dynamic cysts lined with rapidly proliferating epithelial cells.

To determine if these defects resulted from aberrant Lkb1–AMPK signalling, the authors applied an allosteric AMPK activator, A-769662, to lung and pancreatic explants. A-769662 suppressed the lung branching defects but not pancreatic cyst formation, suggesting that this phenotype is independent of AMPK. Furthermore, prolonged Lkb1 inhibition in pancreatic explants elicited lesions resembling early-stage pancreatic intraepithelial neoplasias. These data reveal the power of chemical genetics approaches and *ex vivo* analyses in understanding protein function, and suggest that Lkb1 affects morphology and tumorigenesis through AMPK-dependent and -independent pathways. EJC

Focal adhesions tug at matrix for rigidity sensing

Durotaxis is the process of directed cell migration towards areas of increased extracellular matrix (ECM) stiffness. Waterman and colleagues now report that cells use force fluctuations in individual focal adhesions to sample different ECM rigidities and migrate towards stiff ECM (*Cell* **151,** 1513–1527; 2012).

Using high-resolution time-lapse traction force microscopy, the authors analysed mouse embryonic fibroblasts plated on matrices of different rigidities, and observed that single focal adhesions exhibited asymmetrically distributed traction stress and were not mechanically coupled. They found that individual focal adhesions adopted a state of either stable or fluctuating traction, similarly to a pattern of intermittently 'tugging' at the ECM. Tugging and stable focal adhesion tractions were shown to be supported by soft and rigid ECMs, respectively, in a mechanosensitive response dependent on Rho kinase (ROCK). The authors showed that force transmission and tugging traction dynamics in focal adhesions were regulated by a previously identified signalling axis involving FAK-dependent phosphorylation of paxillin and its subsequent interaction with vinculin. Manipulation of paxillin and ROCK activities in cells plated on ECMs of different rigidities demonstrated that focal adhesion traction dynamics did not affect their maturation or directed migration towards diffuse or substrate-bound cues (PDGF and fibronectin, respectively). In contrast, following the generation of a local rigidity gradient, focal-adhesion-mediated sensing of ECM stiffness by tugging traction dynamics was shown to be necessary for durotaxis. AIZ

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