

# Shape meets polarity in Japan

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A diverse group of developmental biologists who study cell polarity gathered in late March 2009 at the RIKEN Center for Developmental Biology in Kobe, Japan, for a symposium entitled 'Shape and Polarity'. The organizers, Masatoshi Takeichi, Fumio Matsuzaki, Hitoshi Sawa [RIKEN Center for Developmental Biology (CDB), Kobe, Japan] and Carl-Philipp Heisenberg (Max Planck Institute, Dresden, Germany), put together an engaging program that highlighted recent progress towards understanding the mechanisms of cell polarization during development, and the functions of cell polarity in shaping development.

## Introduction

In the past decade, an increasing number of developmental biologists have turned to the tools of cell biology to address longstanding questions about how development works. Cell biology is bridging the gap between important molecular details and some central issues in development, such as how crucial molecules move to one side of a cell, and how forces are generated to position mitotic spindles, shape cells, and to position cells within embryos. As the complexity of systems becomes more apparent, mathematical and computational models are increasingly recognized as being important for testing the feasibility of hypotheses regarding the underlying mechanisms of development. This introduction of cell-biological tools and modeling to diverse areas of developmental biology is changing the field, adding insights that are important for understanding the mechanisms that underpin development. Here, we present highlights from the RIKEN symposium on developmental biology, held from the 23rd to the 25th March, 2009, at the RIKEN CDB in Kobe, Japan, that touch on these themes.

## Fundamental first steps: embryonic axis establishment

Animal embryos often depend on cell polarization mechanisms to establish the initial asymmetries that are essential for development. Asymmetric RNA localization plays a key role in embryonic axis establishment in various organisms. In *Drosophila*, the mRNA of the posterior determinant Oskar is moved along microtubules, mostly toward microtubule plus ends, in a kinesin-dependent, posteriorly biased, nearly random walk (Zimyanin et al., 2008). Are Oskar mRNA-associated granules moving directly along microtubules powered by motors associated with the granules, or are the movements indirect and by way of motor-associated vesicles? Anne Ephrussi (European Molecular Biology Laboratory, Heidelberg, Germany) showed beautiful transmission electron micrographs that

suggest that *oskar* RNA complexes are moving as naked, not as membrane-bound, structures. *Caenorhabditis elegans* also has ribonucleoprotein granules that become localized during axis establishment (Strome and Wood, 1983), but these are thought to move not by associated motor activity but instead passively, in a cytoplasmic flow driven by an opposing cortical actomyosin contraction (Cheeks et al., 2004). Mirjam Mayer (Max Planck Institute, Dresden, Germany) reported new laser-cutting experiments that assessed the forces produced in the cortex during this actomyosin contraction. Analyzing how quickly the cortex opens up after a cut makes it possible to quantify cortical tension in different locations and along specific axes. This method shows promise for investigating the force-generating mechanisms that can drive cortical actomyosin contraction.

The mechanisms that establish initial left-right asymmetry were addressed in three talks on *Drosophila*, chick and mouse. Kenji Matsuno (Tokyo University of Science, Tokyo, Japan) showed how left-right asymmetric morphology develops in *Drosophila*. His group members have conducted a forward screen in which they examined the position of the gut, and have isolated tens of mutants with aberrant gut positions. In a pair of especially interesting mutants, the position of the gut was reversed in one and randomized in the other. Based on these and further findings, Matsuno proposed that a gut epithelial cell shape bias determines the future asymmetric morphology of the organ. Jerome Gros (Harvard University, Cambridge, MA, USA) presented exciting new findings from chick embryos. As the chick embryo does not have motile cilia in the node, as seen during the initial establishment of asymmetry in mice and zebrafish, it must employ a mechanism that differs from that of other vertebrates. By using time-lapse microscopy, Gros discovered a leftward movement of cells that express Shh and Fgf8 around Hensen's node, and proposed that the displacement of these cells is responsible for establishing asymmetric gene expression domains (Gros et al., 2009a). Like chicks, pigs also lack cilia at their node, which suggests that diverse mechanisms for the initial establishment of asymmetry might exist even among mammals. Hiroshi Hamada (Osaka University, Osaka, Japan) talked about the origin of the left-right axis in the mouse, which involves leftward flow at the node, generated by the rotation of motile cilia. A posterior tilt of the cilia, which is responsible for generating the leftward flow, is determined by planar cell polarity (PCP); this involves the polarized localization of Dishevelled proteins in the node cells. He also showed that Lefty1-positive cells in the peri-implantation embryo contribute to the most proximal portion of the anterior visceral endoderm, which suggests that the origin of anteroposterior (AP) polarity can be traced back at least to the Lefty1-positive cells at the peri-implantation stage. Together, these findings highlight that the mechanisms of left-right axis specification are more divergent among animals than previously anticipated.

## Diverse roles for planar cell polarity pathways

PCP pathways also function in other animal systems, and act upstream of a remarkable variety of polarized cellular processes. Two talks at the meeting focused on the roles of PCP in the formation of the cilium, a physiologically important organelle that is also involved in axis determination. Chris Kintner (Salk Institute, La Jolla, CA, USA) showed how multiciliated cells in the *Xenopus* larval skin are polarized along the planar axis of the

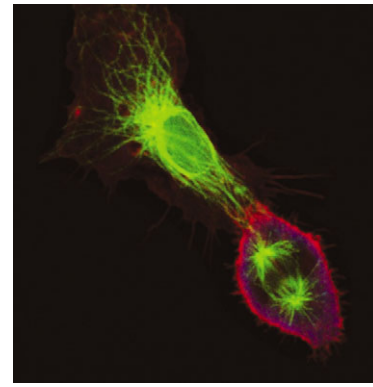
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epithelium. Components of the PCP pathway, such as Dishevelled, Frizzled and Vangl, are required for the organized orientation of the cilia. Interestingly, mutations in Frizzled and Vangl produce non-cell-autonomous effects on wild-type cells located at the edges of mutant clones (Mitchell et al., 2009), an effect that has also been observed in *Drosophila*, where this has been interpreted as a disruption of local signaling upstream of cell polarization (Vinson and Adler, 1987; Adler et al., 2000). Ping Chen (Emory University, Atlanta, GA, USA) presented recent results on sensory hair cells in the mouse inner ear. These cells have a hair bundle composed of microvilli-derived stereocilia and a primary cilium (kinocilium). IFT88 (also known as polaris), an intraflagellar transport component, is present in the kinocilium. In IFT88-deficient mutants, the kinocilium is lost, and stereocilia develop with an abnormal organization. Chen also reported on a new candidate PCP component required for kinocilium formation. PCP pathways orient cells in other ways too. Christophe Marcelle [Developmental Biology Institute of Marseilles-Luminy (IBDML), Marseilles, France] addressed the interesting question of how primitive muscle fibers (myocytes) are oriented in parallel along the AP axis of the embryo. This event also depends on PCP. Wnt11, a non-canonical Wnt pathway ligand, is expressed at the border of each somite, and the specific inhibition of Wnt11 leads to the disorganization of myocytes. Cells that express Wnt11 in an ectopic location can alter the orientation of elongated myocytes, suggesting that Wnt11 acts as a directional cue for the polarized elongation of myocytes (Gros et al., 2009b). In *Drosophila* PCP, Frizzled (Fz) is localized asymmetrically in eye and wing cells. Tadashi Uemura (Kyoto University, Kyoto, Japan) showed a mechanism for such localization: GFP-tagged Fz particles are transported on an array of microtubules that have a bias in orientation, and this bias is controlled by Dachous and Fat, membrane proteins that can act upstream of PCP. Matias Simons (University Hospital Freiburg, Freiburg, Germany) showed that a  $\text{Na}^+/\text{H}^+$  exchanger regulates Fz function, and that it does so by regulating pH, as loss of the exchanger can be rescued by alkalization. He demonstrated that the DEP domain of Fz binds negatively charged lipids. Interestingly, this interaction at the plasma membrane is important for PCP but not for canonical Wnt signaling (Simons et al., 2009).

### Polarity systems that listen to neighboring cells

It is becoming increasingly clear that Wnt signaling and other cell-cell signaling systems can induce cell polarity, with regard to both the localization of intracellular proteins and the orientation of the mitotic spindle. In *C. elegans*, a Wnt/ $\beta$ -catenin asymmetry pathway is involved in most asymmetric cell divisions. Hitoshi Sawa (RIKEN CDB, Kobe, Japan) showed by fluorescence recovery after photobleaching (FRAP) experiments that asymmetric nuclear export is the source of an induced  $\beta$ -catenin asymmetry in a postembryonic cell. In an embryonic cell, a newly recognized asymmetry in microtubule numbers is responsible for this  $\beta$ -catenin asymmetry. Hiroki Nishida (Osaka University, Osaka, Japan) reported that a Wnt signal can also induce asymmetric division in ascidian embryos. In addition, FGF signaling induces asymmetric cell divisions; FGF produced by endodermal precursors induces both anterior and posterior neighboring cells to divide asymmetrically. Cell interactions also function in asymmetric division in the mammalian immune system. To establish adaptive immunity, a single cell undergoes asymmetric cell division, and its daughter cells will adopt diverse cell fates (Chang et al., 2007). Sarah Russell (Peter MacCallum Cancer Centre, Melbourne,



**Fig. 1. T cell polarity.** Activation of naïve cells by interaction with a cognate antigen-presenting cell can lead to asymmetric cell division, influencing the generation of immunological memory. The T cell is on the lower right; green marks microtubules. Image courtesy of S. Russell.

Australia) reported that the asymmetric division of naïve T cells involves conserved polarity proteins, such as Par3, aPKC and Numb. Naïve T cells use prolonged attachment to antigen-presenting cells to orient their axis of cell division (Fig. 1). This allows daughter cells to inherit different cell fate determinants and to adopt different fates.

### Mitotic spindle orientation in neuroepithelial polarity

Neural progenitors undergo planar cell divisions, but how is the orientation of the mitotic spindle controlled in neural progenitors, and to what extent does this orientation determine the fates of daughter cells as proposed over a decade ago (Chenn and McConnell, 1995)? Fumio Matsuzaki (RIKEN CDB, Kobe, Japan) presented beautiful time-lapse imaging data of neural progenitor cells in the mouse neural tube. When the orientations of normally horizontal divisions were randomized or changed to vertical by knockout of the G protein regulator LGN, the fates of daughter cells shifted: daughter cells contributed to neurons at ectopic sites (Konno et al., 2008). Matsuzaki proposed that planar mitosis ensures the self-renewal of neuroepithelial progenitors by one daughter inheriting both apical and basal compartments during neurogenesis. Xavier Morin (IBDML, Marseilles, France) showed how such spindle orientations are controlled using chick neuroepithelial cells. G-protein  $\alpha$ -i ( $\text{G}\alpha\text{i}$ ) subunits, LGN and the dynein/dynactin interactor NuMA localize as laterally nested ring-shaped cortical domains in neural progenitors, and are essential for correct planar spindle orientation, which suggests that the mechanism used is conserved between fly and worm. Morin proposed that a gradient of  $\text{G}\alpha\text{i}$ -GDP might be formed to restrict the distribution of LGN and NuMA to a narrow ring within a wider  $\text{G}\alpha\text{i}$  distribution. Jon Clarke (King's College London, London, UK) reported on his group's analysis of polarized divisions of neuroepithelial cells in the zebrafish. In the transparent zebrafish embryo, the researchers were able to use in vivo time-lapse imaging to analyze the symmetry of inheritance of apical and basolateral proteins, and to correlate this with symmetric or asymmetric fates of the daughter cells. Together, these three talks highlighted that conserved mechanisms align mitotic spindles in neuroepithelia, and that inheritance of apical versus basal domains correlates with the determination of daughter cell fates.

### Mechanisms of apicobasal polarity

The polarization of cells into apical and basal domains was explored in a few systems. Madin-Darby canine kidney (MDCK) cells that are deprived of cell-cell contacts form intracellular storage compartments for apical membrane proteins called vacuolar apical compartments (VACs). VACs can fuse either with each other to form apical lumens (Vega-Salas et al., 1987; Vega-Salas et al., 1988) or with the plasma membrane to produce an apical surface (Martin-Belmonte and Mostov, 2008). Shigeo Ohno (Yokohama City University, Yokohama, Japan) showed that knocking down Par3 in MDCK cells caused the mislocalization of apical luminal surface proteins, and, in many cases, VACs formed. Therefore, Par3 might serve as a key apical indicator for the fusion of VACs.

Apicobasal cell polarity is essential for the organogenesis of the liver, in which intrahepatic conduits are evenly spaced. Takuya Sakaguchi [University of California (UC) San Francisco, San Francisco, CA, USA] visualized the structure of intrahepatic vascular networks in the developing liver and found that endothelial cells migrate along the basal surfaces of hepatocytes, whereas the biliary system is formed at the apical sides of hepatocytes. Genetic and transplantation experiments suggest that the polarization of hepatocytes is influenced by adjacent endothelial cells: the vascular network invades hepatocyte populations and induces a cell polarity that puts bile network markers on the opposite side of each hepatocyte (Sakaguchi et al., 2008). The epithelial polarity regulators Scribble, Dlg and Lgl are normally localized to basolateral cell-cell junctions and regulate apicobasal polarity. They also control cell proliferation and migration and act as tumor suppressors. Patrick Humbert (Peter MacCallum Cancer Centre, Melbourne, Australia) reported on the role of the Scribble-Dlg-Lgl complex in development and cancer, addressing whether the loss of cell polarity can cause tumorigenesis. Loss of Scribble function together with oncogenic Ras mutations resulted in cell invasion through the extracellular matrix via the deregulation of the Ras/Raf/MAPK pathway (Dow et al., 2008). RNAi screening has been used to identify novel interactions between Scribble and a number of polarity complexes in the regulation of transformation and invasion. These talks and others at the meeting highlighted that only a few cell polarization pathways are known to function to polarize cells in diverse animal systems.

### How cells and tissues are positioned in morphogenesis

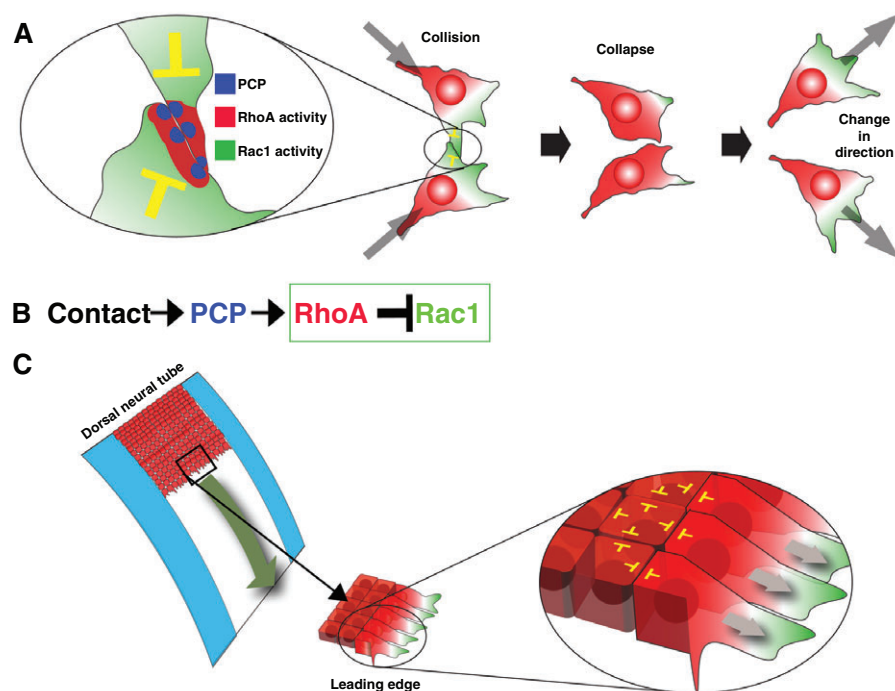
The forces that drive cell and tissue movements are being explored in a number of systems, by combining the tools of cell and developmental biology and, in many cases, by directly assessing and modeling the forces involved. Here, cell polarity and cell signaling often function as upstream inputs into force-generating systems. Bob Goldstein (University of North Carolina, Chapel Hill, Chapel Hill, NC, USA) showed that gastrulating cells in *C. elegans* are positioned by the contraction of apical actomyosin networks, with myosin II being activated by a Wnt/Fz/Dsh signaling pathway (Lee et al., 2006). Surprisingly, network contraction occurs for several minutes before neighboring cells begin to move in concert, suggesting that a regulatable link might couple contracting actomyosin networks to the resulting movements of neighboring cells. Shigeo Hayashi (RIKEN CDB, Kobe, Japan) showed that the invagination of tracheal placodes in *Drosophila* also involves myosin-dependent movements; in this context, it is EGF signaling that coordinates movements by inducing beautiful ring-like patterns of myosin accumulation around the sites where invagination will subsequently occur.

The motile and adhesive forces that drive morphogenetic movements can be measured either directly or through the analysis of rapid responses to breaks in force-carrying elements. Thomas Lecuit (IBDML, Marseilles, France) showed that specific cell-cell junctions shorten during the convergent extension of the *Drosophila* germband, and that this shortening is driven by myosin. Myosin was shown to move into the junctions from an apical network. Laser-induced breaks in the shortening junctions, without breaks in associated membranes, revealed that these shortening junctions are under tension – more tension than is the case for non-shortening junctions – which suggests that the shortening forces work actively against an elastic resistance. Carl-Phillip Heisenberg (Max Planck Institute, Dresden, Germany) measured the adhesive forces of individual progenitor cells of the zebrafish embryo using a modified atomic force microscope and found that mesoderm and endoderm cells are more cohesive than ectoderm cells. Differences in adhesive forces between the germ layers could not predict tissue-sorting behaviors, but, interestingly, differences in cell cortex tension could, reviving an old model according to which differential surface contraction is proposed to position cells (Harris, 1976). Heisenberg showed that perturbing cortex tension by pharmacologically perturbing actomyosin networks prevents cell sorting, suggesting, along with computational modeling, that differences in cortex tension, rather than differences in cohesive forces, might be responsible for germ layer organization. Rudi Winklbauer (University of Toronto, Toronto, Canada) showed roles for both random and non-random (oriented) cell movements in the morphogenesis of *Xenopus* gastrulae (Ninomiya and Winklbauer, 2008). Random cell movement, which provides tissues with surface tension and interfacial tensions, affects epithelial-mesenchymal interactions. The forces generated by random and oriented cell motility are similar in magnitude. To permit directional cell intercalation and tissue elongation, however, the effects of random movement must be constrained. The combination of tools being applied to understand morphogenesis mechanisms is making this an interesting area of research.

### Mechanisms of directional cell crawling

The directional migration of certain cells involves ‘contact inhibition of locomotion’, a term coined many years ago to describe a behavior of fibroblast cells in culture: some cells stop crawling or change direction upon contact with other cells (Abercrombie and Heaysman, 1953). Roberto Mayor (University College London, London, UK) demonstrated, using beautiful imaging techniques, that contact inhibition of locomotion can account for the directional migration of neural crest cells in *Xenopus* and zebrafish embryos. When two migrating neural crest cells contact each other, they stop and change direction. When two frog embryos are placed in contact on their ventral sides, neural crest cells from one embryo can invade another embryo only if the latter lacks its own neural crest. Inhibition of non-canonical Wnt signaling abolishes contact inhibition of locomotion: components of the PCP pathway such as Frz7 and Dsh are localized at the sites of cell-cell contact, where, as fluorescence resonance energy transfer (FRET) assays show, they locally activate RhoA (Fig. 2). Mayor showed a computational model that suggests that contact inhibition of locomotion can make cell spreading more efficient than random migration alone. Hiroko Sano (Ochanomizu University, Tokyo, Japan) showed that *Drosophila* germ cells polarize, disperse and migrate across an epithelium in their journey towards the somatic gonad. Trans-epithelial migration depends in part on the downregulation of an epithelial cadherin, which is regulated by a G protein-coupled





**Fig. 2. Contact inhibition of locomotion of *Xenopus* neural crest cells.** (A) The result of contact between two migrating neural crest cells. (B) Signaling pathways. (C) Contact inhibition of locomotion of groups of cells can result in directional migration. Neural crest cells are initially densely packed at the dorsal neural tube and surrounded by negative signals (light blue). Contact inhibition of locomotion (yellow) results in polarization and migration of leading edge cells. Image courtesy of C. Carmona-Fontaine and R. Mayor.

receptor (GPCR) that also functions in the polarization of germ cells. Interestingly, the adhesion-independent functions of GPCR signaling appear to be important for cell migration in this system, as migration was not rescued in GPCR mutants that also had an epithelial cadherin mutation or that had only one germ cell (Kunwar et al., 2008). Popular models for such directional crawling of cells often propose that gradients of chemoattractants drive localized actin polymerization, leading to directional cell crawling, but Rob Insall (Beatson Institute, Glasgow, UK) showed that this need not be the case, at least not when cells are presented with signaling molecules in shallow gradients. Crawling cells in shallow gradients made pseudopods in random orientations, and changing the orientation of a gradient did not change the rate of pseudopod formation. Instead, pseudopods that formed away from a source of chemoattractant tended to retract, and pseudopods that formed towards a source of chemoattractant were more often stabilized. A computational model confirmed that such a mechanism could result in the observed crawling behaviors without signaling molecules driving local actin polymerization.

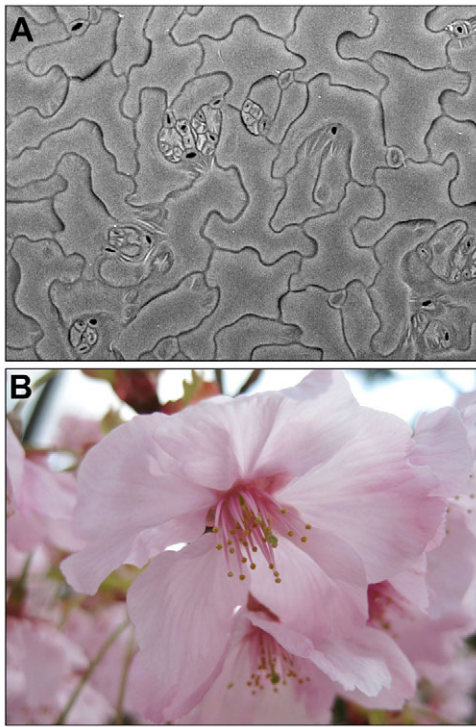
### How plant cells polarize

Diverse types of plant cells become polarized, and plants have generated another set of solutions to some of the same challenges that animal cells face, as well as to some challenges that are unique to plants. Some of the common mechanisms used by animal cells can be excluded as possibilities in plants, given that plant cells polarize without canonical PAR or CDC42 proteins and without tight junctions. Auxin transporters called PINs localize asymmetrically in plant cells. To determine how PINs localize asymmetrically, Pankaj Dhonukshe (Utrecht University, Utrecht, Netherlands) tagged a PIN with a photoconvertible fluorophore. Locally photoconverted fluorophores diffuse very little laterally, suggesting that lateral diffusion of this PIN to one side of each cell could not explain its asymmetric localization. Instead, fluorescence mobility and genetic experiments demonstrated a role for polarized endocytic recycling. After synthesis, PINs initially associate with

the plasma membrane uniformly, and endocytosis and polar recycling are required for their polarity (Dhonukshe et al., 2008). Zhenbiao Yang (UC Riverside, Riverside, CA, USA) showed that members of the plant Rho GTPase family, known as ROPs, regulate the coordinated cell expansions and indentations that give leaf pavement cells their beautiful, interdigitated shapes (Fig. 3A). ROP2 promotes actin-dependent localized expansion on one side of a cell wall, whereas ROP6 promotes microtubule-dependent indentation on the other side of the cell wall. The cross-wall coordination and the intracellular counteraction of these two ROP signaling pathways generate the interdigitated cell shapes.

### Identifying new molecular players for future studies

Although screens for signaling components and for the cell polarization machinery began decades ago, important molecular players continue to be found every year. Large-scale RNAi screens have a special potential to identify large numbers of key players. Jürgen Knoblich (Institute of Molecular Biotechnology, Vienna, Austria) presented the results of a genome-wide RNAi screen for *Drosophila* Notch pathway genes, in which GAL4/UAS promoters were used to drive tissue-specific expression of transgenic dsRNAs (Dietzl et al., 2007). *Drosophila* lines were examined for external sensory organ defects on the notum (the dorsal surface of a fly's thoracic segment), and a secondary screen looked for the Notch phenotype on wings. Among the new genes found were some that functioned upstream of the expression of a Notch pathway component, and some that functioned in asymmetric cell divisions. Interestingly, also isolated were some nuclear lamin integrity genes, which suggested possible ways forward, along with existing approaches (Scaffidi and Misteli, 2008), to understanding how laminopathies are linked to Notch signaling. Julie Ahringer (University of Cambridge, Cambridge, UK), whose lab established bacterial feeding RNAi screens in *C. elegans* several years ago (Fraser et al., 2000), reported screens for suppressors of temperature-sensitive (ts) lethal mutations in cell polarity genes. Adapting any



**Fig. 3. Plants.** (A) The 'jigsaw puzzle' shapes of *Arabidopsis* pavement cells. Scanning electron micrograph courtesy of Z. Yang. (B) Cherry blossoms near Kobe (photograph courtesy of B.G.).

reverse genetic screen to genome scale takes effort, but this kind of screen is a great candidate: screens for the suppression of lethality in *C. elegans* have been called 'screens from heaven' (Jorgensen and Mango, 2002), as looking for live worms among plates of dead worms is a cinch, and one of the great joys of screening by RNAi is forgoing tedious cloning and instead just checking a table of gene designations to learn the identity of each hit. Ahringer reported 11 suppressors of a *ts* mutation in a non-muscle myosin II gene, and screens in 29 other embryonic *ts* mutants are underway. Perhaps surprisingly, these screens and a similar screen for dynein *ts* suppressors (O'Rourke et al., 2007) are successfully identifying even essential genes, which can often produce only low penetrance lethality when targeted by dsRNAs in the worm's bacterial food source. The discovery of large numbers of new molecular players in these fields is likely to open up new avenues towards understanding the mechanisms and functions of signaling and cell polarity.

## Conclusions

The symposium ended just as cherry blossoms began to open in areas around Kobe (Fig. 3B), giving attendees an opportunity to reflect on biological mechanisms in the presence of one of biology's great displays. It has become increasingly clear that a handful of mechanisms are used repeatedly to polarize cells during development, mechanisms that depend, for example, on small GTPases, on PAR proteins and on PCP pathways. Currently, the mechanisms used by these and other molecular pathways to polarize cells are of great interest. The ongoing dissection of cellular and subcellular mechanisms used by these common pathways, together with the identification of new pathways and molecular players, should allow us to make headway in understanding how cells polarize in the future.

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