Basic principles of polarity establishment and maintenance

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Polarity during bacterial cell division

The mechanisms that are responsible for the localized positioning of the bacterial division machinery were discussed by Jeff Errington (Newcastle, UK) in Bacillus subtilis and Christine Jacobs-Wagner (New Haven, CT, USA) in Caulobacter crescentus. During cytokinesis of B. subtilis, cell-wall growth is spatially restricted to a circular ring, which eventually becomes the division septum. The tubulin-like bacterial protein FtsZ (filamentous temperature-sensitive protein Z), which is usually found in helical-like structures, rearranges as a ring about the division plane where it recruits other proteins that are responsible for cell division. After cytokinesis, rod-shaped bacteria elongate by localizing their growth either to cylindrical regions (e.g. Escherichia coli and B. subtilis) or to the poles (e.g. Corynebacterium). This polarized growth is partly regulated by the bacterial actin homologue MreB (mecillinam resistance gene B). These proteins – found only in non-spherical bacteria – form helical filamentous structures that are involved directly in the control of cell shape. Elongation of B. subtilis requires the co-ordinated activity of MreB and its two homologues, Mbl (MreB-like) and MreBH (MreB homologue). MreB also plays a role in establishing polarity in C. crescentus, where it alternates between a helical structure and a ring-like form defining the division plane. Division in C. crescentus is asymmetric, yielding a swarmer daughter cell that is shorter than its stalked sister. The protein TipN (tip of new pole) localizes at the tip of the new pole that is created by division, and is responsible for establishing and maintaining correct polarization; ΔtipN mutant cells produce swarmer cells that are longer than their stalked brethren. The size of bacteria presents a significant challenge to the understanding of the functional properties of bacterial cytoskeleton proteins such as FtsZ and MreB. Mohan Balasubramanian (Singapore) demonstrated that MreB function can be elucidated by expressing the bacterial protein in fission yeast Saccharomyces pombe, where it results in a linear array extending the length of the cell, which is reminiscent of the yeast microtubules.

Polarity during development

Paul Martin (Bristol, UK) and Peter Lawrence (Cambridge, UK) discussed polarity in the context of Drosophila development. Martin uses dorsal closure, which occurs late in embryogenesis, as a model of epithelial fusion. During this process, filopodia extend from approaching epithelial sheets and meet in an interdigitated pattern known as ‘zippering’. By using flies that express red fluorescent protein (RFP)–moesin and green fluorescent protein (GFP)–moesin, Martin showed that the size of bacteria presents a significant challenge to the understanding of the functional properties of bacterial cytoskeleton proteins such as FtsZ and MreB.
Different promoters, it is possible to establish that distinct recognition mechanisms regulate cell matching during dorsal closure[3]. These filopodia extensions resemble the pseudopod extensions during amoeboid locomotion that were also discussed at the meeting (see below). Lawrence explored a model of planar cell polarity to explain how hair cells acquire their orientation. The consensus view is that Dachsous (Ds) and the two genes fat (ft) and four-jointed (fj) function together to read morphogen gradients, establishing a positive feedback loop that amplifies the external asymmetric signal. Ds and Ft then provide the polarizing signal to the Starry night (Stan) pathway, consisting of the three genes frizzled (fz), Van Gogh (Vang) and starry night (stan), which, in turn, signals to downstream effectors. Lawrence, however, presented evidence that excess Ft, Ds or Fj can polarize adjoining cells that have a complete block in Stan signalling (for example, fz mutant cells; Figure 2D) suggesting that the Ds system can generate planar cell polarity independently of the Stan system. The morphogen gradients necessary for planar cell polarity provide the spatial information required for hair-cell orientation, and represent mechanisms that are likely to be translated into directional movement of migrating cells.

Directed cell migration

• For some of the cells, the initial step in the establishment of polarity is binding an external chemical, although the identity of this cue and its associated receptor is not always known. One such setting is the migration of primordial germ cells in Drosophila, as discussed by Ruth Lehmann (New York, NY, USA). Before gastrulation, these cells are found in an organized group that expresses Drosophila E-cadherin at its centre, and during development they dissociate and begin directional migration. A novel G-protein-coupled receptor, Tre-1 (trapped in endoderm-1), the ligand of which has not yet been identified, is required for primordial germ-cell polarization and transepithelial, but not subsequent, migration. During migration along the midgut, two lipid phosphate phosphatases, Wunen and Wunen 2, are expressed along the midline and act as chemorepellants. Primordial germ-cell migration in zebrafish was discussed by Eres Raz (Münster, Germany). These cells are propelled by bleb-like protrusions that are generated by calcium-dependent actomyosin contraction (Figure 2E). The blebs can be either oriented in response to an external chemotactant or uniformly distributed. This alternating pattern generates a movement that is reminiscent of bacterial runs or tumbles. Raz discussed recent evidence demonstrating that an additional chemotrac-
tand-dependent receptor, CXCR7, plays an essential role during cell polarity, primarily in somatic non-migrating cells⁶. Plasma membrane blebbing has also been observed in amoeboid cells; however, these cells migrate primarily by the extension and retraction of pseudopods. Peter van Haastert (Haren, The Netherlands) reported on computer-aided analysis of the patterns of pseudopod extensions in Dicystostelium. New pseudopods are formed in two ways: the splitting of existing pseudopods, which is the predominant method, and de-novo formation. In shallow chemical gradients, cells extend pseudopods in an alternating left–right pattern reminiscent of an ice skater. When the direction of this gradient is changed, the cells skip steps and use consecutive pseudopods on the side of the new direction (left–left or right–right) to reorient themselves. Multiple signalling pathways control Dicystostelium chemotaxis and the regulation of chemotaxis depends on the degree to which cells are polarized⁷. Early in their development, Dicystostelium chemotaxis is regulated by phosphatidylinositol-3-kinase (PI(3)K) and phospholipase A2 (PLA2) pathways. Subsequently, cells become considerably more polarized. At this point, soluble guanylyl cyclase is important for orientation and directional persistence.

For an external gradient of chemoattractant to elicit intracellular polarization, cells must interpret receptor-mediated signals. Dicystostelium and neutrophils sense these gradients spatially: immobilized cells placed in a static chemoattractant gradient respond by selectively and persistently translocating intracellular markers, such as pleckstrin homology (PH) domains, to the side of the cell with the highest receptor occupancy. However, the same cells, when exposed to spatially homogeneous but persistent signals, respond transiently. A local-excitation, global-inhibition mathematical model that explains these two modes of response was presented by Pablo Iglesias (Baltimore, MD, USA). In the model, receptor occupancy triggers a fast excitation, as well as a slower inhibitory response. Diffusion of the inhibitor results in loss of the local information about receptor occupancy, leading to an inhibitory signal that reflects the global level of the stimulus. Iglesias presented simulations in which the model recreates the observed behaviour for both graded and spatially homogeneous stimuli. Though originating in the observed behaviour of Dicystostelium, this model also seems to fit data presented by Matthias Peter (Zurich, Switzerland) for Saccha-

**FIG. 2: Polarization in multicellular organisms.** (A) One-cell Caenorhabditis elegans embryo marked with the early endosomal autoantigen 1 (EEA1; blue) and non-muscle myosin type II (NMY2; red), both of which are enriched at the anterior cortex. (B) Polarized migrating astrocytes showing Golgi (green), centrosome (red) and nuclei (blue). (C) Polarized hippocampal neurons showing F-actin (red) and the axonal marker Tau (green). (D) Disrupted polarization in the Drosophila pleura. The cells at the bottom right (marked by the absence of hairs) over-express FZ. Adjacent FZ-mutant cells are polarized by the over-expressing cells. However, mutant cells far from the clone have a random polarity. (E) Migrating zebrafish primordial germ cells (actin is shown in green and the nucleus is shown in blue). (F) Cytotoxic T lymphocytes with secretory granules (green) and microtubule-organizing centre polarized towards one of two target cells (nuclei are stained in blue and microtubules are red). (G) T cell migrating on ICAM-1, stained for F-actin (red) and microtubules (green). (H) Leaf epidermal cells of plants that express a GFP–tubulin in wild-type background (left) and in plants over-expressing the Rop GTPase scaffold protein ICRI (right). In wild-type cells, the microtubules are oriented in different directions; in ICRI-over-expressing cells, they are arranged in a direction transverse to the long axis of cells (arrowheads). (Images kindly provided by Julie Abringer (A), Sandrine Etienne-Manneville (B), Britta Eickholt (C), Peter Lawrence and Jose Casal (D), Eres Raz (E), Gillian Griffiths (F), Sarah Heasman and Anne Ridley (G) and Shaul Yalovsky (H))
Role of Cdc42 in Polarization

Cdc42, a member of the Rho family of GTPases, is crucial for the polarization of yeast cells. It plays a key role in the establishment of polarized growth and cell morphology. Cdc42 localizes to the cell cortex in a spatially and temporally regulated manner, facilitating the reorganization of the cytoskeleton and the secretion of vesicles. The activation of Cdc42 is tightly controlled by GTPase-activating proteins (GAPs) and exchange factors (GEFs), which ensure the correct balance between active and inactive forms of the GTPase.

Roles of GTPases in Cell Polarity

- For a polarized morphology to be observed, not only must the cell have a means of sensing spatial heterogeneities through internal or external cues, but these initial cues must also be greatly amplified and spatial information subsequently transduced to the cytoskeleton and secretory apparatus.
- The lytic granules travel on microtubules towards the microtubule-organizing centre, and cortical actin is then cleared away from the site of secretion (Figure 2F).
- In wild-type cells, activated Cdc42 localizes adjacent to the previous bud site, and concentric zones of the GEF Cdc24 and the GAP Rga1 are thought to restrict Cdc42 activation spatially, which could result in a gradient of activated Cdc42.

The Rap1 GTases are the main regulators of plant polarity, which is particularly evident in root hairs and pollen tubes. The main classes of animal and fungal Rap GTase GEFs are absent from plants, and Benedikt Kost (Warwick, UK) discussed the role of the Rac–Rop GTases, which are found at the apical plasma membrane of tobacco pollen tubes. The restriction of their activated form to the pollen-tube tip is crucial for polarized growth. As in yeast, the zone of the activated Rac-Rop GTase Rac5 appears also to be restricted by RhoGAP1, which localizes to the flanks of the pollen-tube tip. Furthermore, the Rho guanine nucleotide-disassociation inhibitor RhoGDI2 can both positively and negatively affect Rac5 activity. Shaul Yalovsky (Tel Aviv, Israel) discussed the roles of Rac–Rop GTases in plant polarity during Arabidopsis development. One such Rho GTase, ATROP6, is transiently palmitoylated and stearoylated (S-acylated) in its activated state, thereby promoting its partitioning into specific membrane domains that might act to amplify polarity signals. Activated ATROPs bind the novel effector protein ICR1 (interactor of constitutive active ROPs), which is a coiled-coil domain protein that also binds itself and the exocyst complex component Sec3. This protein functions as a scaffold linking Rho-GTase signalling to secretion and is likely to be important for amplifying signals that are crucial to polarity (Figure 2H).

The role of the partitioning defective (Par)–Tiam1 GEF complex in various cell polarization processes was discussed by John G. Collard (Amsterdam, The Netherlands). The work that Collard presented emphasized the requirement of the Par–Tiam1 complex for apicobasal polarity of contacting keratinocytes, and for the persistent − and hence chemotactic − migration of non-contacting migratory keratinocytes. The Par complex is also important during Rap1 (Ras-related protein1) and chemokine-induced T-cell polarization. The Rac1 GEF Tiam1 interacts with both the Rap1 GTase and the Par1 complex, indicating that the association of Rac1, Tiam1 and the Par complex is likely to be important to amplify the signals necessary for persistent cell polarization. Ian G. Macara (Charlottesville, VA, USA) discussed the role of the Par3–Tiam1 complex in vesicular trafficking and cell polarity.
complex in hippocampal neurons during spine morphogenesis. Neurons in which Par3 is reduced by RNA interference (RNAi) or in which Tiam1 is over-expressed, form multiple filopodia and lamellipodia. In Par3-knockdown cells, this phenotype can be suppressed upon expression of a constitutively active Rac.

The roles of different Rho GTPases and their activation in T-cell migration and cell stopping were discussed by Anne Ridley (London, UK). T cells undergo persistent migration until they are engaged and movement stops (Figure 2G). Rac1 is required for cell migration, although, surprisingly, this Rho GTPase is activated when cell movement stops whereas RhoA activation levels decrease. When Rac1 is activated there is a decrease in ezrin-radixin-moesin (ERM) protein phosphorylation and adhesion receptors are lost at the back of the cell. Ridley also presented results on the importance of the microtubule cytoskeleton, which is crucial for T-cell motility and polarity. These results highlight that Rho GTPases need to be tightly regulated both for motility and cell-contact responses. Work presented by Sandrine Etienne-Manneville (Paris, France) illustrated the importance of cell interactions with the extracellular matrix, and especially of intercellular contacts, as signals for astrocyte polarization (Figure 2B). Cell contacts – including calcium-dependent cell–cell adhesion – are transduced by N-cadherin to the RhoA GTPase, which probably amplifies the spatial signals, resulting in the orientation of the microtubule organizing centre (or centrosome)–nucleus axis, which establishes the division axis. This response requires the actin and microtubule cytoskeleton, respectively.

Leah Edelstein-Keshet (Vancouver, Canada) presented a mathematical model that shows how the interactions between Rho GTPases can lead to stable polarized movement. In this model, Cdc42 and Rho are mutually antagonistic, and Cdc42 also activates Rho through Rac. This network of interactions induces bistability, which, when coupled to diffusion, leads to a travelling wave. By adding inactive cytosolic components of the three GTPases, the wave can be made to stop, giving the cell a stable polarized form. Edelstein-Keshet presented simulations that recreate the stable migration of fish keratocytes (Figure 3B).

Role of phosphoinositides in polarization

- In some cell types, subtle internal or external cues result in a pronounced spatial asymmetry of plasma membrane lipid phosphoinositide phosphates, including PI(4,5)P₂* and PI(3,4,5)P₃**. The spatial restriction of these phospholipids is likely to be one of the first manifestations of cell polarity, and the steepness of this response relative to a shallow external gradient or small signal hetero-

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**FIG. 3:** Steps in polarization. (A) Polarization begins with an initial cue, which can be intracellular (e.g. cell division; top) or extracellular (e.g. chemotaxis; bottom). This cue is amplified, typically by positive feedback loops, resulting in a polarized morphology. (B) Internal signalling modules (top) were combined to produce local protrusion and retraction of a two-dimensional model cell(11). The level of Rho is used to regulate myosin-based contraction. The cell initiates and maintains persistent motion if stimulated by a small transient gradient of Cdc42 activation. The cell turns in response to a strong (bottom left) or shallow (bottom right) gradient. Images in (B) courtesy of Leah Edelstein-Keshet.

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*phosphatidylinositol 4,5-bisphosphate
**phosphatidylinositol 3,4,5-trisphosphate
genesities indicates that substantial amplification must take place beforehand. Phosphoinositide phosphate asymmetries—which can be observed immediately following exposure to a cue—are a convenient measure of cell polarization. The requirement of PI(4,5)P₂ and PI(3,4,5)P₃ in different cell-polarization processes was the topic of several presentations. For example, PI(4,5)P₂ and PI(3,4,5)P₃ can function by recruiting and activating a range of signalling proteins, in particular small Rho G proteins.

In Dictyostelium, PI(3,4,5)P₃ is tightly restricted to the front or leading edge, as is the kinase responsible for its generation, PI(3)K. However, PTEN*, crucial for PI(3,4,5)P₃ hydrolysis, accumulates at the rear of the cell. During cytokinesis, these enzymes also polarize to either the pole (PI(3)K) or the furrow (PTEN; Figure 1B). Peter Devreotes (Baltimore, MD, USA) reported that PI(3,4,5)P₃ induces the translocation of the PH-domain-containing protein kinase B (PKB) to the front of the cell. He also showed that TORC₂ (target of rapamycin complex) activates PKB as well as a second related kinase, PKBR₁, which lacks a PH domain yet is myristylated, suggesting that its localization is not PI(3,4,5)P₃-dependent. Intriguingly, a PI(4)P-5-kinase homologue is a substrate for protein kinase B (PKB), raising the possibility that PI(4,5)P₂ might also be important for chemotaxis.

Cortical polarity during asymmetric cell division in Caenorhabditis elegans was discussed by Carrie Cowan (Vienna, Austria) and Julie Ahhringer (Cambridge, UK). Approximately 30 min after fertilization, PAR proteins segregate to two distinct cortical domains establishing an anteroposterior axis in the one-cell embryo (Figure 2A). The initial division is asymmetric because of higher posterior pulling forces on the mitotic spindle. Gβ subunits of heterotrimeric G proteins and their Goloco-domain-containing non-receptor regulators GPR-1/2 control the spindle pulling forces, and PAR-directed posterior enrichment of GPR-1/2 leads to higher posterior forces. Ahhringer discussed new proteins that are required for spindle positioning identified in an RNAi screen. After knockdown of a casein kinase homologue (CSNK-1), GPR-1/2 asymmetry is abolished, indicating that this kinase is upstream of heterotrimeric G-protein signalling. As the yeast orthologue of CSNK-1 phosphorylates PI(4)P-5-kinase, the Ahhringer laboratory studied the worm homologue PIP kinase 1 (PPK-1), which was found to localize to the posterior end of the embryo, and to be required for cortical GPR-1/2 association and the generation of pulling forces. These results suggest that PI(4,5)P₂ might have a role in transducing the spatial signal from PAR protein asymmetry to heterotrimeric G-protein signalling, which is crucial for asymmetric cell division.

The PI(3)K pathway also has an important role in controlling axon specification and elongation, as discussed by Britta Eickholt (London, UK). Hippocampal neurons extend several short processes (neurites) of equal length after being plated. Polarity is achieved through the restriction of PI(3)K activation to one of these neurites, leading to its lengthening and rapid growth (Figure 2C). PI(3)K regulation is achieved through two pathways; PI(3)K activates Rho GEFs, leading to the activation of the Rho GTPases that regulate the actin cytoskeleton, and also activates AKT leading to the downstream inhibition of glycogen synthase kinase 3 (GSK3), which, in turn, controls microtubule dynamics.

**Conclusion and perspectives**

- A forte of this meeting was that it brought together participants with a wide range of scientific backgrounds to examine the broad variety of molecules and mechanisms that govern the establishment and maintenance of cell polarity, and to compare them in model systems ranging from unicellular bacteria to complex multicellular organisms. Owing to the diverse nature of the topics considered, it is not surprising that a consensus was not reached as to where the polarity field is headed. Nevertheless, some common themes emerged from the meeting. In most cases, the establishment of polarity requires the initial sensing of subtle spatial heterogeneities (internal or external), their subsequent amplification and stabilization, and the eventual transduction of this spatial information to various outputs, for example, cytoskeleton and secretory apparatus. The presence of positive feedback loops—in particular those involving small GTPases and phosphoinositides—as a

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**FIG. 4:** The participants of the 97th International Titisee Conference of the Boehringer Ingelheim Fonds on ‘Mechanisms of Cell Polarity’ in Titisee, Germany.
means of amplifying signals was reported in a range of systems. Another common feature was the highly redundant nature of the systems, with multiple pathways co-operating to achieve their function. The inherent beauty of polarized cells and organisms merged with that of Lake Titisee and the Black Forest. The winds over the lake that prevent the surface from freezing in the winter time are reminiscent of the continued excitement and movement in the cell-polarity field and the discoveries that lie ahead.

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