Formation and Remodeling of Epithelial Polarity

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Polarity is a fundamental property of all eukaryotic cells that underlies many developmental processes. A recent EMBO workshop (March 27–31) organized by Thomas Lecuit, Norbert Perrimon, and Keith Mostov brought cell and developmental biologists together on the Mediterranean coast near Marseille, France, to share views on how epithelium polarity is established and remodeled during development and disease. Participants witnessed and celebrated the emerging convergence of intellectual and experimental approaches to address how individual cells acquire polarity and form polarized tissues in the context of developing embryos.

Morphogenesis of multicellular organisms requires that cells coordinate their individual behavior—proliferation, migration, adhesion, differentiation, and death—across space and time. Cell polarization underlies proper execution and coordination of many of these individual cell behaviors. All eukaryotic cells have an inherent ability to polarize that is embedded in the intrinsic asymmetry of the actin and tubulin polymers. Thus, polarity could conceivably develop without external signals through the stabilization of a stochastically fluctuating asymmetry. In most experimental situations as well as in developing organisms, cell polarity is, however, induced and oriented by extrinsic cues. Thus, understanding how epithelial cells become polarized and contribute to organogenesis requires insight into how extrinsic signals establish a local change at the cell surface, how this local change triggers global changes in the organization of the whole cell, and how this polarization is coordinated across the whole epithelium to form polarized tissues (Figure 1). Furthermore, once polarity is established, it can be further remodeled in response to developmental signals to shape the body and form three-dimensional organs. Oral and poster presentations were of outstanding quality and covered a wide spectrum of experimental approaches and model systems that I will not attempt to fully cover here due to space limitations. Instead, I will summarize a few key insights obtained from various experimental approaches, with a particular focus on approaches combining gene inactivation and high-resolution imaging in living cells.

Extrinsic Signals Establish and Orient Polarity

Plasma membranes of epithelial cells consist of two domains, an apical and a baso-lateral domain, that differ in membrane protein composition. Difference in protein composition is brought about by specific protein transport pathways and by the existence of a diffusion barrier separating these two domains (Nelson and Yeaman, 2001). Cell-cell adhesion may act as a local symmetry-breaking event at the cell surface, inducing global changes in cell organization (Figure 1). Indeed, cell-cell adhesion serves as a spatial landmark for both the transport machinery and the assembly of the diffusion barrier. J. Nelson (Stanford University) discussed the role of cell-cell contact mediated by the clustering of cadherin cell adhesion receptors in providing an initial and local symmetry-breaking event in epithelium-forming MDCK cells. Adhesion results in the assembly, through the Cadherin intracellular domain, of signaling and cytoskeleton-organizing complexes. One activity of this Cadherin-based platform is to recruit the exocyst complex. This complex, first identified in yeast, is composed of eight distinct subunits. It functions by a yet unknown mechanism to target secretory vesicles to the plasma membrane. In MDCK cells, the exocyst complex regulates the targeting of baso-lateral proteins but appears to be dispensable for the proper localization of apical markers. J. Nelson showed that Sec6 localizes in the apical-most region of the lateral membrane in polarizing MDCK cells, close to where secretory vesicles fuse. Consistent with these localization data, Sec6 coimmunoprecipitates with surface-labeled Cadherin and cofractionates with Cadherin in MDCK cell extracts upon Ca²⁺-induced cell-cell adhesion. Moreover, while Sec6 is mostly cytosolic in fibroblasts that do not express Cadherin, it is recruited to the membrane upon Cadherin transfection. Thus, the exocyst complex associates with cell adhesion receptors upon polarization (Yeaman et al., 2004). The recruitment of the exocyst complex to the Adherens Junctions (AJs) may serve to increase fidelity and efficiency of lateral membrane delivery and baso-lateral protein targeting.

Despite the clear implication of Cadherin in generating cell surface asymmetry and promoting epithelial polarity, H. Clevers (Hubrecht Laboratory, Utrecht) provided spectacular evidence that polarity can also be established within single intestine epithelial cells independent of cell-cell contact (Baas et al., 2004). Activation of the Lkb1 kinase by STRAD, a pseudokinase that directly binds Lkb1, is sufficient to promote the formation of an actin-rich domain at one pole of isolated epithelial cells. This actin-rich domain forms villin-containing microvilli and is surrounded by a discontinuous line the tight-junction marker ZO-1. Strikingly, apical markers localize to this actin-rich domain whereas baso-lateral proteins accumulate to the opposite pole. Finally, RNAi-mediated inactivation of Lkb1 function in epithelial cells leads to a loss of polarity. These results establish Lkb1 as a central player in epithelial polarization. This view is further supported by independent genetic screens in Drosophila and C. elegans that have identified Lkb1 as a key regulator of cell polarization (Martin and St Johnston, 2003).

Interestingly, Lkb1-induced polarization of single epithelial cells is not randomly oriented. Microvilli form above the nucleus, opposite to the laminin substrate.*Correspondence: schweisg@wotan.ens.fr
Developmental Cell

Developmental Cell

Figure 1. Morphogenesis of Epithelial Tissues Is a Multistep Process

Local symmetry-breaking events at the cell surface induce global changes in cell organization that stabilize and maintain intracellular asymmetries along one defined axis. During development, signals remodel epithelial tissues to build complex organisms. Physiological signals also trigger adaptive responses in adult epithelia.

Stabilization of Initial Asymmetry at the Cortex

The initial signal-induced asymmetry can be stabilized through the stepwise assembly of protein complexes at cell-cell junctions and the regulated delivery of specific membrane proteins via the secretory pathway (Figure 1), as illustrated above for the Cadherin-mediated polarization of MDCK cells. Both processes were discussed at the meeting.

Recently, a signaling complex of three proteins, Par3, Par6, and atypical protein kinase C (aPKC) has emerged as a key regulator of cell polarization in the different cell types of organisms ranging from worms to mammals (Henrique and Schweisguth, 2003). The Par3-Par6-aPKC complex localizes apically and acts together with another conserved apical protein complex, the Crumbs complex, to regulate epithelial polarity (Knut and Bossinger, 2002; Muller, 2003). Crumbs is a transmembrane protein localizing apical to the AJs that is required to maintain epithelial polarity. The short intracellular domain of Crumbs is highly conserved and contains a C-terminal PDZ binding motif recognized by the PDZ domain of PALS1. Thus, Crumbs recruits PALS1 and its partner PATJ at the apical membrane. Genetic analysis in Drosophila has led to the suggestion that the apically located Par3-Par6-aPKC acts at the top of a genetic hierarchy to regulate the apical localization of the Crumbs-PALS1-PATJ complex. Human Crumbs 3 (hCrb3) is the only human Crumbs homolog expressed in epithelial cells. A. Le Bivic (IBDM, Marseille) reported that, in addition to PALS1, hCrb3 also interacts with Par6 (also via its PDZ domain) (Lemmers et al., 2004). Par6 was recently shown to also bind PALS1 and PATJ, suggesting tight regulatory links between these two complexes (Hurd et al., 2003b). Clearly, the challenge is now to unravel the spatio-temporal interplay between these two complexes as epithelial cells acquire polarity. E. Knust (Dusseldorf University) discussed the roles of Crumbs and Stardust (Std), the fly homolog of PALS1, in regulating epithelial polarity. Std binds dPATJ and Dlin-7, the Drosophila homologs of PATJ and Lin-7, respectively. Dlin-7 also binds the L27 domain of DigS97, a neural-specific isoform of the Membrane Associated GUanylate Kinase homolog (MAGUK) protein Discs-large (Dlg). The main Dig isoform, Dig-A, localizes at septate junctions, basal to AJs, and regulates epithelial polarity. Dig-A contains three PDZ domains, one SH3 domain, and a C-terminal GUK domain but no L27 domain. Dlin-7 colocalizes with Std apically in epithelial cells (that do not express DigS97) and with DigS97 at neuromuscular synapses (Bachmann et al., 2004). This illustrates that protein localization at the cortex can be developmentally regulated via the cell-specific expression of binding partner isoforms.

One mechanism by which the Par3-Par6-aPKC complex is maintained at the apical cortex relies on lateral exclusion of the Ser/Thr kinase Par1 (Benton and St Johnston, 2003; Hurd et al., 2003a). Importantly, Par1 has also been shown to phosphorylate Lkb1 and to regulate microtubule stability, and it is so far unclear to what extent microtubule stabilization by Par1 contributes to cell polarization. T. Vaccari (EMBL, Heidelberg) studied the localization at the EM level of a functional Par1-GFP protein in the follicular epithelium of Drosophila and showed that Par1 associates with intracellular membranes of the secretory pathway and accumulates at the lateral plasma membrane. In vivo structure/function analysis of Par1 revealed that the spacer domain of Par1 is necessary and sufficient for lateral membrane localization and that the spacer-dependent accumulation of Par1 at the lateral membrane is required for apical-basal polarity. In contrast, the stabilization of microtubules by Par1 does not require the presence of the spacer domain. Thus, microtubule stabilization and regulation of apical-basal polarity are two separable functions of Par1. The polarizing activity of Par1 may involve the Par1-mediated phosphorylation of the Par3-Par6-aPKC complex and the exclusion of this complex from the lateral membrane. Conversely, phosphorylation of
Par1 by aPKC may contribute to restrict its localization to the lateral domain (Hurov et al., 2004).

Amplification of Initial Asymmetry via Directed Sorting
As mentioned above, amplification and stabilization of initial asymmetry also involves polarization of the secretary pathway. Localization of membrane proteins to the apical or baso-lateral domain relies on sorting signals recognized by specific cytosolic adaptor complexes that direct secretion at specific sites on the plasma membrane. A simple, certainly too simple, pathway linking specific adaptor complexes to the exocyst complex and secretion to junction-associated proteins is emerging.

A first link between an adaptor complex and the exocyst was described by H. Folsch (Northwestern University, Evanston, IL). AP-1B is known to regulate the baso-lateral targeting of Tyrosine-based signal-containing proteins. H. Folsch showed that the AP-1B adaptor complex associates with the exocyst. It colocalizes with the exocyst in recycling endosomes, and DAB-mediated inactivation of recycling endosomes blocked surface delivery of a baso-lateral marker. H. Folsch therefore proposed that the recycling endosomes are a necessary intermediate during biosynthetic delivery and that AP-1B facilitates the recruitment of the exocyst complex to the recycling endosomes for subsequent fusion events. This view was further strengthened by data presented by E. Rodriguez-Boulan (Cornell University, Ithaca, NY) showing that a function-blocking antibody against AP-1B blocked biosynthetic delivery of VSVG protein in an endosomal compartment. However, E. Rodriguez-Boulan also presented data indicating that two other baso-lateral proteins, LDL-R and Transferrin Receptor, follow an AP-1B-independent biosynthetic route from the Trans-Golgi Network (TGN) to the plasma membrane but utilize AP-1B after internalization into endosomes, for recycling back to the cell surface.

Second, as described above, a link exists between the exocyst and adhesion receptors localized at AJs. A linear function between secretion and a junction-associated protein Scribble (Scrib) was proposed by J.-P. Borg (INSERM, Marseille). Scrib belongs to the LAP protein family that has been shown to regulate epithelial polarity and formation of cell-cell junctions in worms, flies, and, potentially, mammals. J.-P. Borg first showed that a GFP-tagged version of human Scrib (hScrib) was recruited at the lateral membrane of MDCK cells as these cells develop epithelial polarity. Membrane recruitment of hScrib critically depends on its LRR domain. Genetic analyses have suggested that Drosophila Scrib promotes lateral domain formation by antagonizing the activity of the apical Par3-Par6-aPKC complex (Henrique and Schweisguth, 2003) but the precise mechanism of Scrib action remains to be determined. J.-P. Borg reported that hScrib directly associates with the Rac1 GEF βPIX (Audebert et al., 2004). Using an exocystosis assay based on the secretion by PC12 cells of growth hormone (GH), overexpression of βPIX was shown to promote GH release whereas expression of a cytosolic version of hScrib that sequesters βPIX in the cytoplasm significantly decreased GH release. Thus, these data indicate that an hScrib-βPIX complex regulates exocytosis and further raise the possibility that Scrib regulates epithelial polarity by directing secretory vesicles to the baso-lateral membrane domain.

Polarization of exocytosis was also studied by P. Chavrier (Institut Curie, Paris), who showed that Sec10, a component of the exocyst, binds Arf6-GTP and localizes to the TGN and recycling endosomes in nonpolarized MDCK cells and fibroblastic cells (Prigent et al., 2003). Expression of a truncated version of Sec10 lacking the Arf6 binding domain results in the appearance of tubular endocytic structures. Moreover, siRNA-mediated inactivation of several exocyst components perturbs the subcellular organization of the recycling endosome in HeLa cells. These results suggest that interaction of Arf6 with the exocyst may contribute to the addition of new membranes to dynamic region of the plasma membrane. These findings suggest a mechanism whereby Arf6 that is known to control phagocytosis and cell migration may regulate membrane delivery to the forming phagocytic cup and to the leading edge.

Finally, changes in gene expression may also have a role in the long-term stabilization of epithelial polarity. J. Nelson used microarray to show that epithelium formation does not simply rely on the redistribution of pre-existing gene products but rather involves changes in gene expression. Gene expression profiles were analyzed in caco2 cells induced to polarize in vitro. Not surprisingly, cell cycle genes are switched off and cell cycle inhibitors were switched on as cells stop proliferating. However, changes in the expression of other genes, encoding for instance structural components of tight junctions, are more likely to reflect polarity acquisition.

Epithelial Remodeling
The above studies suggest that the key molecular mechanisms of cell polarization are beginning to be unraveled. In contrast, the subsequent mechanisms that orchestrate the actions of epithelial cells during morphogenesis are poorly understood (Figure 1). The Drosophila embryo is well suited to study the molecular basis of simple morphogenetic events in the context of a living organism. Prior to gastrulation, the Drosophila embryo is formed by a single layer of polarized cells. At gastrulation, the ventral cells constrict their apex and invaginate to form the mesoderm. The transcription factor Twist is expressed in ventral cells and directly regulates epithelial folding (Leptin, 1999). Since twist has a unique phenotype, M. Leptin (Koln University) suggested that Twist regulates invagination via the transcriptional regulation of multiple downstream targets that, when mutated, would only produce subtle or transient defects. Consistent with this hypothesis, the forced expression of either Snail or Fog, two direct targets of Twist, in a twist mutant embryo is sufficient to rescue epithelial folding, although the process is slow and inefficient. Invagination of the ventral furrow thus appears to be a good system to study robustness in epithelium remodeling.

Mesoderm invagination is followed by a 2-fold anterior-posterior elongation of the embryo. This process does not depend upon cell division but rather relies on cell intercalation. T. Lecuit (IBDM, Marseille) used 4D microscopy to describe this cell intercalation process. By focusing his analysis on the precise geometry of...
the cell-cell contacts established by each group of four neighboring cells, T. Lecuit realized that elongation relies on a single round of irreversible and unidirectional, hence polarized, junction remodeling. Embryos with reduced Myosin II activity exhibit elongation defects and reduced cell intercalation. This indicates that this polarized remodeling of the junctions is regulated by Myosin II (Bertet et al., 2004). Regulation of cell intercalation may be further modulated by the cell-specific expression of specific Cadherin isoforms. Consistent with this hypothesis, A. Jacinto (Gulbenkian, Oeiras) showed that four Drosophila Cadherin genes exhibit unique and specific expression patterns in the posterior openings of the respiratory tracheal system, the formation of which involves cell intercalation.

Epithelial cells can also form internal three-dimensional organs consisting of tubes and cysts (Affolter et al., 2003). The tracheal respiratory system of the fly consists of a network of interconnected tubes that may either be multicellular (polarized cells connected by intercellular AJs), unicellular (each cell forms a tube by looping around the luminal surface that is closed up by an autocellular AJ), or intracellular (the tube is not enclosed by AJs). M. Affolter (Biozentrum, Basel) described how paired cells intercalate to produce unicellular tube and how AJs zip up to form autocellular junctions. Genetic analysis suggests that this zipping process needs to be stopped; otherwise tracheal cells would transform their intercellular AJs into autocellular AJs and detach from each other. Termination of the zipping process requires secreted Zona-Pellucida domain proteins that may form a rigid luminal structure antagonizing final zipping. This detailed description provides a firm ground to study how this intercalation process is developmentally regulated to produce unicellular and how AJs zip up to form autocellular junctions. Genetic analysis suggests that this zipping process needs to be stopped; otherwise tracheal cells would transform their intercellular AJs into autocellular AJs and detach from each other. Termination of the zipping process requires secreted Zona-Pellucida domain proteins that may form a rigid luminal structure antagonizing final zipping. This detailed description provides a firm ground to study how this intercalation process is developmentally regulated to produce unicellular and beautiful network of interconnected tubes of different shapes and cellular arrangements seen in the embryo.

The vertebrate embryo also provides simple, easy to observe, and essential morphogenetic events, such as neural tube closure as nicely described by J. Wallingford (University of Texas at Austin) (Wallingford et al., 2002). Proper closure of the neural tube depends in part on the actin binding protein Shroom. Detailed analysis of Shroom function in Xenopus indicated that it is not required for convergent extension movements but is specifically required to induce apical constriction of the neural plate cells that are located along the hinge and where shroom is expressed (Haigo et al., 2003). This suggests that Shroom expression may be sufficient to promote bending of an epithelial sheet.

**Epithelial Integrity and Growth**

Morphogenetic movements are always accompanied, and sometimes caused, by forces generated outside or within the tissue that may challenge the integrity of the rearranged epithelium. It is thus important to consider what possible mechanisms may protect the tissue from these forces. Three different studies illustrate various levels of control of epithelial integrity. N. Perrimon (Harvard University) showed that large clones of cells mutant for the type I receptor for the TGF-β family member Decapentaplegic (Dpp) are eliminated from the developing wing epithelium through a process of epithelial extrusion. Some of the mutant clones actually survive extrusion and persist as discrete epithelial cysts with inverted polarity. Dpp signaling was thus proposed to ensure long-range tissue integrity, possibly through a regulation of the apical microtubule network. Evidence that the actin cytoskeleton is also required for epithelial integrity was provided by a genetic analysis of the Ste20-like kinase Slik in Drosophila. D. Hipfner (EMBL, Heidelberg) showed that silk mutant epithelial cells produce actin-rich protrusions and actively leave the epithelium. This phenotype can be suppressed by the expression of an activated phospho-mimetic form of Moesin. Furthermore, Slik is required for the phosphorylation of Moesin, and forced expression of the kinase domain of Slik is sufficient to induce Moesin phosphorylation. These results thus indicate that Slik maintains epithelial integrity by regulating Moesin activity. Finally, work by M. Labouesse (IGBMC, Illkirch) suggests that all three major cytoskeletal elements, i.e., microtubules, microfilaments, and intermediate filaments, contribute to maintain tissue integrity in the worm embryo. Toward the end of embryogenesis, the C. elegans embryo elongates 4-fold along its anterior-posterior axis in the absence of cell division. Elongation of the epidermis is associated with epithelial cell shape changes that result in part from forces exerted by the contracting muscles onto the attached epidermis. In a screen designed to isolate genes required for epithelial integrity, M. Labouesse identified mutations in the spectraplakin locus that encodes several isoforms with the potential to link actin filaments, microtubules, and intermediate filaments. Loss of spectraplakin results in the disorganization of all three cytoskeletal networks and in the detachment of the epidermis from the extracellular matrix upon muscle contraction. Thus, spectraplakin protects the epidermis from forces exerted by the muscles and therefore helps maintain epithelium integrity (Boscher et al., 2003).

Numerous pieces of evidence point toward a link between loss of epithelial polarity and integrity and cancer. Whether polarity and tissue growth are causally linked is, however, a question difficult to address experimentally. In Drosophila, scrib acts cooperatively with dlg and lethal giant larvae (lgd) to regulate both epithelial polarity and proliferation (Bilder et al., 2000). Polarity and growth could indeed be causally linked if, for instance, polarity defects lead to a disorganization of the signaling complexes that keep the cell cycle in check. Alternatively, polarity and growth could be two distinct and separable outputs of the activity of these genes. D. Bilder (University of California Berkeley) addressed this important issue through a detailed structure/function analysis of the multidomain protein Scrib. This study indicates that distinct domains of Scrib have separable activities. Specifically, regulation of cell polarity only required the LRR domain of Scrib whereas the presence of at least one PDZ domain was required to limit tissue growth. Thus, at least in this context, excessive proliferation does not simply result from overall polarity loss.

**Epithelial Polarity and Cell Fate Determination**

Cell polarization is also used during development to regulate cell fates. Two key developmental processes that depend on epithelial cell polarity were discussed...
at the meeting: the formation of morphogen gradients and the regulation of binary cell-fate decisions during asymmetric cell division.

Morphogens are secreted signaling molecules that are produced at compartment boundaries and that act in a concentration-dependent manner to pattern fields of undifferentiated cells. A central and debated issue is whether a stable concentration gradient of morphogens can be established based solely on passive diffusion or whether more active mechanisms participate in this process (Vincent and Dubois, 2002). In *Drosophila*, Wingless (Wg; a Wnt family member) and Dpp are two well-known secreted proteins that act as morphogens to pattern the wing single-layered epithelium along the anterior-posterior and dorsal-ventral axes, respectively. M. Gonzalez-Gaitan (MPI, Dresden) showed that Dynamin activity is required for the propagation of Dpp away from its source. This indicates that formation of the Dpp gradient is an active process that involves the dynamin-dependent endocytosis of Dpp (Entchev and Gonzalez-Gaitan, 2002). Consistently, both the Dpp receptor and an HRP-Dpp fusion protein localized at the cell-cell junction area as well as in endocytic compartments. One important implication of these findings is that the slope of the morphogen gradient can be finely tuned by responding cells as they regulate the rate of secretion versus degradation of internalized morphogens. J.-P. Vincent (NIMR, London) showed that the range of action of Wg in the wing imaginal disc is limited by Wg degradation. He described how both Wg receptors, Arrow and Frizzled-2, contribute to degradation. He suggested that the involvement of both signaling receptors in Wg trafficking could allow for degradation and recycling to be modulated independently, thereby affording optimal signaling by limiting amounts of Wg.

Asymmetric cell division is a conserved mechanism for generating cell fate diversity during development (Bardin et al., 2004; Wodarz and Hutters, 2003). Over the past several years, significant progress has been made in our understanding of how unequal segregation of cell-fate determinants at mitosis leads to the adoption of distinct cell fates by the two daughter cells. In particular, genetic studies in *C. elegans* and *Drosophila* have linked cell polarity to the asymmetric distribution of cell-fate determinants. In *Drosophila*, asymmetric cell division is one of the primary mechanisms to generate cell-fate diversity in the developing nervous system. Embryonic neuroblasts delaminate basally from the surface epithelium and divide asymmetrically in a stem-cell like manner to generate a small basal neuron-generating cell and a large apical neuroblast. Asymmetry of this division requires the activity of the apical Par3-Par6-aPKC complex that orients the mitotic spindle, directs the basal localization of cell-fate determinants, and contributes to determine daughter cell size asymmetry. A. Wodarz (Düsseldorf University) showed that complete loss of aPKC activity in embryonic neuroblasts results in the mislocalization of Par6 and strongly decreases but does not abolish asymmetric localization of Par3. In contrast, apical localization of both Par6 and Par3 in the overlying epithelial cells is completely abolished upon loss of aPKC activity. This suggests that a neuroblast-specific activity promotes Par3 asymmetric localization in the absence of aPKC activity. One key function of apical aPKC in neuroblasts is to phosphorylate, and thereby inactivate, Lgl such that active Lgl is restricted to the basal cortex where it acts by a yet unknown mechanism to localize cell-fate determinants (Betschinger et al., 2003).

In the sensory organ lineage, fate asymmetry depends on the unequal segregation of Numb and Neuralized, two regulators of Notch receptor signaling. The pII cell divides asymmetrically to generate an anterior pIIb cell that specifically inherits Numb and Neuralized and a posterior pIIa cell that has no Numb and Neuralized at its birth. Numb acts in the pIIb cell to antagonize Notch receptor signaling by promoting the endocytosis of Sanpodo (Spdo), a positive regulator of Notch. Neuralized is an E3 ubiquitin ligase that also acts in the pIIb cell to upregulate endocytosis of and signaling by the Notch ligand Delta. Both Numb and Neuralized therefore contribute to turn Notch signaling OFF in the pIIb cell and ON in the pIIa cell. J. Knoblich (IMP, Vienna) investigated the function of the endocytic regulator Rab11 in generating fate asymmetry. Using a Rab11-GFP fusion protein, he observed that Rab11 remains dispersed in the pIIa cell whereas it transiently concentrates around the centrosome of the pIIb cell. This centrosomal accumulation of Rab11-GFP is also seen in the two daughters of symmetrically dividing epidermal cells, suggesting that localization of Rab11 at the centrosome is specifically suppressed in the pIIa cell. Rab11 colocalizes with the Rab11 binding-protein Nuf in the pIIb cell, and overexpression of Nuf leads to a symmetric distribution of Rab11 that correlates with an increase in Notch receptor activation. This study underscores the importance of membrane trafficking in the regulation of Notch signaling during asymmetric cell division.

**Cell Migration**

Cell polarization is also used during development to regulate cell migration. Genetic analysis in zebrafish and functional studies in *Xenopus* have revealed that convergent-extension movements in gastrulating vertebrate embryos are regulated by a noncanonical Wnt signaling pathway. C.-P. Heisenberg (MPI, Dresden) presented a detailed analysis of the zebrafish Wnt11 mutant phenotype and showed that cells within the mesendodermal cell layer fail to produce oriented processes and show defective velocity and directionality of migration during early stages of gastrulation. Live imaging analysis indicated that this defective migratory behavior resulted from increased adhesion of mesendodermal cells to the overlying ectodermal cells onto which they migrate (Ulrich et al., 2003). C.-P. Heisenberg proposed that this increased adhesion might result from a lower turnover of AJ complexes in Wnt11 mutant mesendodermal cells. Establishing a mechanistic link between noncanonical Wnt signaling and cell adhesion will clearly have a broad impact on our understanding of the role of Wnt in cell polarity.

Cell migration can also be genetically studied in *Drosophila* using germ cell as a model system. Germ cells form at the posterior pole of the embryo and are internalized in the lumen of the gut. Germ cells then undergo a trans-epithelial migration across the gut epithelium and then migrate as single “amoeboid” cells toward the...
developing gonads. R. Lehmann (Skirball Institute and HHMI, NYU, New York) described a genetic dissection of the processes of germ cell formation and migration. Genetic analysis revealed that a conserved G protein-coupled receptor, called Tre1 in *Drosophila* and CXCR4 in the mouse, regulates germ cell migration in both species. Germ cell-specific rescue experiment indicates that the activity of the *tre1* gene is only required in germ cells to promote germ cell dispersion prior to trans-epithelial migration across the gut (Kunwar et al., 2003). Further analysis of this process should reveal how germ cells cross the gut epithelium.

**New Approaches, New Questions, New Models ...**

As always, part of the excitement at meetings comes from the development of new experimental approaches on old questions, from new experimental models, or from unexpected observations that bring new questions. While many of the presentations fit one or more of these criteria, three presentations nicely illustrate this point. J. Axelrod (Stanford University) has taken a novel mathematical approach to investigate how planar cell polarity (PCP) is established in the *Drosophila* epidermis (see Strutt, 2003, for a review on PCP). J. Axelrod described a model that integrates (1) the minimal key features of experimentally derived relationships between four core PCP proteins (Fzrll, Dishevelled, Prickle, and Strabismus/Vang) that localize at two opposite domains at the apical cell cortex and coordinate PCP between neighboring cells and (2) a global upstream orientation signal. The model worked in producing oriented asymmetry reproducibly and faithfully along a defined axis and reproduced known PCP phenotypes. Importantly, this modeling approach indicates that no diffusible factor X needs be hypothesized to explain observed PCP phenotypes. In a different vein, H. López-Schier (Rockefeller University, New York) argued that the lateral line of the zebrafish may be a useful addition to the fly epidermis for the analysis of PCP, in particular for the study of PCP maintenance through phases of growth, remodeling, and regeneration. Each sensory organ of the lateral line is composed of a group of sensory hair cells surrounded by supporting cells. Each sensory cell possesses a single apical kinocilium localizing asymmetrically within the surface plane. H. López-Schier described how the precise orientation of PCP in hair cells follows complex, yet stereotyped, rules that depend in part on the timing of neuromast formation and on its migration pattern. Further analysis of this system will certainly provide novel insights into how PCP is developmentally regulated.

As a final note, this meeting provided the participants with a whole zoo of beautiful polarity patterns that can be recognized at the single-cell level. Understanding the molecular mechanisms underlying this amazing diversity in polarity pattern is certainly one of the challenges ahead of us. F. Pichaud (MRC, University College London) argued convincingly that *Drosophila* photoreceptor cells offer a beautiful and powerful model system to approach this question. Photoreceptor cells are highly specialized and polarized neurons endowed with an apical light gathering structure called a rhabdome. Each ommatidium of the compound eye is composed of eight distinct photoreceptor cells that differ by their stereotyped, cell-specific organization of a specific Crumbs/Par3-positive cortical domain that connects the apical rhabdomere to the Zonula Adherens. F. Pichaud described a genetic screen for mutations affecting photoreceptor morphogenesis and has presented mutants in which the cell-specific organization of the subapical membrane is perturbed. Molecular analysis of these mutations should provide important clues as to how diversity can be introduced in the organization of apical-basal polarity in specialized cells.

**Epilogue**

A clear indication of this meeting is that the combined use of cell biology and developmental genetic approaches to both cultured cells and living organisms greatly fosters cultural integration of cell and developmental biologists. Live imaging of cells and embryos, genetic analysis in model organisms, and siRNA-mediated gene inactivation in cultured cells are obviously shaping the future of this research field. While developmental geneticists are continuously learning from cell biologists about cell dynamics, cell biologists in return may experience some of the complexity in the regulation of cell polarity that can be gained from looking at cells inside the embryo. Joining forces will help us understand the logic and the dynamics of the molecular interactions involved in the regulation of junction assembly, membrane trafficking, and/or cytoskeleton dynamics. Additional help may be gained in the future from applying biophysical approaches to the study of polarity in living organisms. Yet another exciting cultural challenge for developmental geneticist!

**Selected Reading**


