Molecular Biology of the Cell
Vol. 21, 845–847, March 15, 2010

MEETING REVIEW

Morphogenesis in Kyoto: A Confluence of Cell and Developmental Biology

Jennifer A. Zallen* and Alpha S. Yap†

*Howard Hughes Medical Institute and the Developmental Biology Program, Sloan-Kettering Institute, New York, NY 10065; and †The University of Queensland and Division of Molecular Cell Biology, Institute for Molecular Bioscience, St. Lucia, Queensland, Australia 4072

Submitted January 4, 2010; Revised January 8, 2010; Accepted January 13, 2010
Monitoring Editor: Doug Kellogg

Understanding morphogenesis is the ultimate multidisciplinary (ad)venture. Three-dimensional tissues are generated from the actions of genes, biochemical pathways, and cells that form multicellular networks and interact with their biomechanical environment. A comprehensive explanation of morphogenetic processes must encompass these different levels of analysis. A recent meeting in Kyoto on "Building the Body Plan: How Cell Adhesion, Signaling, and Cytoskeletal Regulation Shape Morphogenesis" highlighted recent advances in tackling this challenging problem.

INTRODUCTION

Morphogenesis is a fundamental problem in biology that has been slow to reveal its secrets. The generation of three-dimensional tissues from genes and cells to multicellular networks is a problem that encompasses many different aspects of biology. The past few years have witnessed significant progress in this area thanks to multidisciplinary efforts by cell biologists, geneticists, developmental biologists, physicists, and computer scientists. This diversity of approaches has been instrumental in understanding how cell interactions, cytoskeletal dynamics, and mechanical forces are integrated to generate form and structure in development.

This diversity was on display when >150 scientists from Asia, Europe, Australia, and North America met in Kyoto, Japan, in September 2009 to discuss "Building the Body Plan: How Cell Adhesion, Signaling, and Cytoskeletal Regulation Shape Morphogenesis." This joint meeting of the American Society for Cell Biology, the Japan Society for Cell Biology, and RIKEN Center for Developmental Biology was organized by Mark Peifer, Masatoshi Takeichi, and Sachiko Tsukita. A range of approaches and themes were raised during the meeting, several of which we highlight in this report.

Molecular Regulation of Cell–Cell Interactions

The specialized junctions that link cells to their neighbors provide the basis for cell–cell contact and signaling in multicellular tissues. These include cadherin-based adherens junctions and the tight junctions of epithelia. Sachiko Tsukita (Osaka University) talked about the role of the transmembrane protein claudin in tight junction formation. Different claudins are polymerized into tight junction strands in the presence of zona occludens-1/2 in epithelial cells, and knockout studies have revealed roles of claudin-15 in regulating transepithelial conductance and the size of the mouse small intestine (Umeda *et al*., 2006; Tamura *et al*., 2008; unpublished data). Mikio Furuse (Kobe University) discussed the unique molecular organization of tricellular junctions, the distinctive points of contact between multiple cells (usually three) that are found in epithelia. The transmembrane protein tricellulin localizes specifically to tricellular junctions and is required for proper epithelial organization (Ikenouchi *et al*., 2005). Knockdown of tricellulin leads to aberrant cytoskeletal organization at cell contacts. His laboratory is now carrying out localization-based screens to identify new proteins that participate in this aspect of epithelial organization.

Jonathan Pettitt (University of Aberdeen, Aberdeen, United Kingdom) talked about the role of adherens junctions in ventral enclosure in *Caenorhabditis elegans*, the sealing up of the embryonic epidermis at the ventral midline of the embryo. A hypomorphic mutation affecting the *C. elegans* α-catenin homologue, HMP-1, causes a range of defects in epidermal morphogenesis. A genetic interaction screen for genes that enhance or suppress these defects identified several intragenic mutations that affect α-catenin junctional localization and may define key interactions important for α-catenin folding or distribution.

Epithelial junctions are not, of course, the only cell–cell interactions of morphogenetic consequence. This was emphasized by Masatochi Takeichi (RIKEN CDB, Kobe, Japan), who discussed live imaging studies of axon-dendrite interactions in the nervous system. Neurons often form nonspecific synaptic contacts in culture; yet, the neural circuits that form in vivo are precisely wired. For example, the dendrites of cerebellar granule cells receive synaptic input from pontine axons but do not synapse with axons from the inferior olive or hippocampus. When these interactions are recapit-

This article was published online ahead of print in *MBC in Press* (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E09–12–1076) on January 28, 2010.

Address correspondence to: Jennifer A. Zallen (zallenj@mskcc.org) or Alpha S. Yap (a.yap@uq.edu.au).

ulated in culture, the correct partners establish synapses with proper morphology that form correctly at the distal dendrite tips (Ito and Takeichi, 2009). By contrast, synapses between nonphysiological partners are abnormal in morphology and aberrantly localized throughout the dendrite. These results reveal that several features of synaptic specificity are maintained in culture, reflecting intrinsic properties of the presynaptic and postsynaptic cells.

Identification of New Molecules through Genetic Approaches

Genetic approaches in model organisms provides a powerful way to identify new genes involved in morphogenesis. Maria Leptin (University of Cologne, Cologne, Germany) described genetic screens for molecules required for mesoderm invagination in *Drosophila*. Mesoderm invagination occurs through the coordinated apical constriction within a group of cells to form a furrow. This process requires the conserved transcription factor Twist (Leptin and Grunewald, 1990). Several important targets of Twist have been identified through forward genetic screens, including the transmembrane protein T48 (Kolsch *et al*., 2007). She showed evidence that T48 activates apical cell constriction through the apical localization of RhoGEF2, an upstream activator of myosin contraction. In addition, the tumor necrosis factor receptor-associated factor 4 plays a distinct role in mediating the apical relocalization of adherens junctions in constricting cells (Matthew *et al*., 2009). These results suggest that contractile and junctional proteins are coordinately regulated to alter cell shape during apical constriction.

Consistent with this idea, Mark Peifer (University of North Carolina, Chapel Hill, NC) showed that contractile structures must be actively linked to adherens junctions to regulate cell shape during apical constriction. In *Drosophila* embryos lacking Canoe/Afadin, apical constrictions initiate in the mesoderm but myosin eventually pulls away from the cortex and contracts independently, without further changing the shape of the cells (Sawyer *et al*., 2009). Canoe can associate directly with E-cadherin, suggesting that Canoe may provide a functional link that transmits the force of actomyosin contraction to the plasma membrane. Shigeo Hayashi (RIKEN CDB) discussed the role of apical constriction in forming epithelial tubes in the *Drosophila* trachea. Using a computer modeling approach, his laboratory showed that the cell invagination behaviors in embryos can be simulated with a three-dimensional vertex simulation, suggesting that many of the mechanical parameters that govern this process may be accurately defined.

Organizing Cells into Tissues: Live Imaging of Cell Polarity and Protein Dynamics

A key issue in morphogenesis is to understand how cellular and molecular mechanisms function in the context of whole organisms. Technological advances in live imaging were prominently featured as tools to explore protein localization and dynamics in vivo, most notably to elucidate the basis of cell and tissue polarity.

Several talks discussed how cells organize into sheets of cells that share a common polarity in the plane of the tissue, a feature known as planar cell polarity (PCP). In the *Drosophila* wing, one of the early signs of planar polarity is the asymmetric transport of the Frizzled transmembrane protein to the distal-most surface of each cell (Shimada *et al*., 2006). Tadashi Uemura (Kyoto University, Kyoto, Japan) presented live imaging studies showing that this polarized transport involves an alignment of microtubules along the proximal-distal axis. Quantitative analysis of high-resolution movies revealed that the directional preference of Frizzled transport is spatially regulated within cells. His group is addressing how this regulated vesicle trafficking contributes to the establishment of cell polarity during development.

Lila Solnica-Krezel (Vanderbilt University) discussed the role of the Frizzled/PCP pathway in establishing cell polarity in zebrafish. In the elongating dorsal mesoderm of the zebrafish gastrula, the Frizzled binding partner Dishevelled is enriched at the posterior surface of each cell, whereas other proteins such as Prickle (Yin *et al*., 2009) and its transmembrane binding partner Trilobite/Vangl2 localize to the anterior cell surface. These polarities are proposed to define interfaces between anterior and posterior cells as preferential sites for polarized radial and planar cell intercalations. By screening for recessive enhancers of mutations in a gene required for polarized cell intercalation, her laboratory is working to identify new components that regulate cell polarity in this dynamic cell context.

Mechanobiology of Cell Populations

Much has been learnt about the genetic pathways that control morphogenesis and the cellular mechanisms that execute those genetic orders. In the three-dimensional context of an embryo, those genetic and biochemical pathways are also affected by the physical reality of cells and tissues that interact in a constrained mechanical environment. Several talks at the meeting highlighted this emerging nexus between biomechanics, cellular mechanisms, and developmental organization.

Jennifer Zallen (Sloan-Kettering Institute, New York, NY) discussed the cell rearrangements and mechanical forces that lead to elongation of the body axis in *Drosophila*. The myosin II motor protein is enriched at boundaries between anterior and posterior cells (Zallen and Wieschaus, 2004), resulting in the generation of increased contractile force along the dorsal–ventral axis. In addition to generating force, myosin itself is also regulated by force, resulting in a positive feedback loop that stabilizes myosin localization at the cortex in regions of increased tension (Fernandez-Gonzalez *et al*., 2009). This mechanical signal leads to the formation of multicellular cables that propagate away from the initial site of contraction, recruiting additional cells to engage in the behaviors that drive elongation.

Myosin activity is also required for the proper organization of adherens junction complexes in culture and in vivo, but the mechanisms by which adherens junctions respond to contractile forces are not well understood. Shigenobu Yonemura (RIKEN CDB) presented evidence supporting a mechanism for myosin regulation of adherens junctions through a force-dependent interaction between vinculin and α-catenin that could stabilize adherens junctions in regions of high contractile activity.

Ultimately, the function of adhesion receptors and cytoskeleton at cell–cell junctions must also be coordinated by cell signaling. Alpha Yap (University of Queensland, Queensland, Australia) discussed the role of Src family kinases in E-cadherin signaling. He reported evidence that the cytoskeletal scaffolding protein cortactin is a target of E-cadherin–activated Src signaling that is necessary for the integrity of the zonula adherens and the apical ring of actin filaments in cultured epithelial cells (Ren *et al*., 2009). An interesting issue for future work will be to understand how junctional signals may control, and respond to, the signaling pathways and mechanical forces that play on cells in tissues.

Holding On and Letting Go: Regulation of Cell Migration In Vivo

The regulation of cell migration in vivo remains a key to understanding morphogenesis. Doris Wedlich (University of Karlsruhe, Karlsruhe, Germany) discussed the role of the mesenchymal *Xenopus* Cadherin11 (XCad11) in neural crest migration. In wild-type cells, XCad11 localizes to the basal cell surface, suggesting a role in mediating adhesion to the substrate rather than between cells (Kashef *et al*., 2009). Her laboratory found that cells injected with an XCad11 morpholino fail to migrate and do not form filopodia or lamellipodia. The extracellular domain of XCad11 is not required to rescue these defects, suggesting that the migration promoting activity of XCad11 is an intrinsic property of the cytoplasmic domain.

Turning to soluble cellular signals, Pernille Rorth (Institute of Molecular and Cell Biology, Singapore) discussed the regulation of collective cell movement during border cell migration in *Drosophila*. Border cells migrate tens of cell diameters through a complex tissue environment in response to signaling by the Pvf1 and EGF ligands and their corresponding Pvr and epidermal growth factor receptor receptors (Duchek *et al*., 2001; Duchek and Rorth, 2001). Using an antibody specific for the phosphorylated form of Pvr, she showed that activated Pvr is enriched at the leading edge of the migrating cell cluster, providing a signaling readout that correlates with directional cell movement.

Morphogenesis and Disease

It is tempting to speculate that the molecular mechanisms that determine morphogenesis might contribute to disease when they go awry. Two talks at the meeting indicated that this speculation might, indeed, be good enough to be true.

One intersection between morphogenesis and disease lies with the role of cell–cell adhesion in the organization and function of the stem cell niche. Work in the laboratory of Valeri Vasioukhin (Fred Hutchinson Cancer Research Center, Seattle, WA) showed that mammalian α-E-catenin is required not only for cell adhesion but also to regulate the proliferation of epidermal and neural progenitor cells (Vasioukhin *et al*., 2001; Lien *et al*., 2006). A new study from the Vasioukhin lab demonstrates that deletion of α-E-catenin in the skin stem cell compartment results in expansion of stem and progenitor cells and development of skin tumors in the adult mice. The tumor suppressor function of α-E-catenin is only beginning to be understood. However, although α-E-catenin can bind β-catenin, which is a known oncogene, β-catenin signaling does not play a crucial role in the development of tumors in skin cells lacking α-E-catenin. These findings provide the first genetic demonstration of the tumor suppressor function of α-E-catenin and emphasize the critical role of cell adhesion pathways in the stem cell niche.

The interplay between pathology, development, and molecular mechanism was also highlighted by Richard Vallee (Columbia University, New York, NY) who used the example of LIS1 to give us a glimpse of ongoing efforts to build a comprehensive model of how molecular mechanism can determine tissue architecture. LIS1 is a regulatory subunit of the dynein motor complex that is mutated in lissencephaly, a disorder of neural precursor proliferation. Disruption of LIS1 leads to abnormal oscillatory movement of nuclei during the cell cycle of neuroepithelial cells, suggesting that it participates in microtubule-based movement of nuclei. Yet, dynein is best understood for its role in transport of much smaller structures, such as vesicles and complexes. Excitingly, Vallee reported the results of single molecule studies in collaboration with Steve Gross's lab showing that LIS1 can lock dynein in a state that generates sustained force, suggesting a mechanism for dynein to move large cellular structures.

Overall, we hope that this brief report gives a sense of the diverse approaches that now energize the study of morphogenesis. These multidisciplinary approaches augur well for rapid progress in the very near future.

ACKNOWLEDGMENTS

We thank all the speakers who allowed us to discuss their work. J.A.Z. is funded by National Institutes of Health National Institute of General Medical Sciences grant R01GM-079340 and a W. M. Keck Foundation Distinguished Young Scholar in Medical Research Award. J.A.Z. is an Early Career Scientist of the Howard Hughes Medical Institute. A.S.Y. is funded by the National Health and Medical Research Council of Australia.

REFERENCES

Duchek, P., and Rorth, P. (2001). Guidance of cell migration by EGF receptor signaling during *Drosophila* oogenesis. Science *291*, 131–133.

Duchek, P., Somogyi, K., Jekely, G., Beccari, S., and Rorth, P. (2001). Guidance of cell migration by the *Drosophila* PDGF/VEGF receptor. Cell *107*, 17–26.

Fernandez-Gonzalez, R., Simoes, S., Roper, J. C., Eaton, S., and Zallen, J. A. (2009). Myosin II dynamics are regulated by tension in intercalating cells. Dev. Cell *17*, 736–743.

Ikenouchi, J., Furuse, M., Furuse, K., Sasaki, H., Tsukita, S., and Tsukita, S. (2005). Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. J. Cell Biol. *171*, 939–945.

Ito, S., and Takeichi, M. (2009). Dendrites of cerebellar granule cells correctly recognize their target axons for synaptogenesis in vitro. Proc. Natl. Acad. Sci. USA *106*, 12782–12787.

Kashef, J., Kohler, A., Kuriyama, S., Alfandari, D., Mayor, R., and Wedlich, D. (2009). Cadherin-11 regulates protrusive activity in *Xenopus* cranial neural crest cells upstream of Trio and the small GTPases. Genes Dev. *23*, 1393–1398.

Kolsch, V., Seher, T., Fernandez-Ballester, G. J., Serrano, L., and Leptin, M. (2007). Control of *Drosophila* gastrulation by apical localization of adherens junctions and RhoGEF2. Science *315*, 384–386.

Leptin, M., and Grunewald, B. (1990). Cell shape changes during gastrulation in *Drosophila*. Development *110*, 73–84.

Lien, W. H., Klezovitch, O., Fernandez, T. E., Delrow, J., and Vasioukhin, V. (2006). alphaE-catenin controls cerebral cortical size by regulating the hedgehog signaling pathway. Science *311*, 1609–1612.

Matthew, S. J., Kerridge, S., and Leptin, M. (2009). A small genomic region containing several loci required for gastrulation in *Drosophila*. PLoS One *4*, e7437.

Ren, G., Helwani, F. M., Verma, S., McLachlan, R. W., Weed, S. A., and Yap, A. S. (2009). Cortactin is a functional target of E-cadherin-activated Src family kinases in MCF7 epithelial monolayers. J. Biol. Chem. *284*, 18913–18922.

Sawyer, J. K., Harris, N. J., Slep, K. C., Gaul, U., and Peifer, M. (2009). The Drosophila afadin homologue Canoe regulates linkage of the actin cytoskeleton to adherens junction during apical constriction. J. Cell Biol. *186*, 57–73.

Shimada, Y., Yonemura, S., Ohkura, H., Strutt, D., and Uemura, T. (2006). Polarized transport of Frizzled along the planar microtubule arrays in *Drosophila* wing epithelium. Dev. Cell *10*, 209–222.

Tamura, A., *et al*. (2008). Megaintestine in claudin-15-deficient mice. Gastroenterology *134*, 523–534.

Umeda, K., Ikenouchi, J., Katahira-Tayama, S., Furuse, K., Sasaki, H., Nakayama, M., Matsui, T., Tsukita, S., Furuse, M., and Tsukita, S. (2006). ZO-1 and ZO-2 independently determine where claudins are polymerized in tight-junction strand formation. Cell *126*, 741–754.

Vasioukhin, V., Bauer, C., Degenstein, L., Wise, B., and Fuchs, E. (2001). Hyperproliferation and defects in epithelial polarity upon conditional ablation of alpha-catenin in skin. Cell *104*, 605–617.

Yin, C., Kiskowski, M., Pouille, P. A., Farge, E., and Solnica-Krezel, L. (2009). Cooperation of polarized cell intercalations drives convergence and extension of presomitic mesoderm during zebrafish gastrulation. J. Cell Biol. *180*, 221–232.

Zallen, J. A., and Wieschaus, E. (2004). Patterned gene expression directs bipolar planar polarity in *Drosophila*. Dev. Cell *6*, 343–355.