

# Making waves: the rise and fall and rise of quantitative developmental biology

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## Summary

The tenth annual RIKEN Center for Developmental Biology symposium 'Quantitative Developmental Biology' held in March 2012 covered a range of topics from coat colour patterning to the mechanics of morphogenesis. The studies presented shared a common theme in which a combination of physical theory, quantitative analysis and experiment was used to understand a specific cellular process in development. This report highlights these innovative studies and the long-standing questions in developmental biology that they seek to answer.

**Key words:** Morphogenesis, Pattern formation, Quantitative analysis, Systems biology, Cellular computation

## Introduction

Over the last 30 years, advances in genetics and genomics have identified a conserved set of molecules that give rise to the positional identity of cells in developing animals. These include components of cell-cell signalling cascades that act reiteratively to pattern different tissues during animal development, homeostasis, repair and regeneration, many of which appear dysregulated in cancer. The successes in cloning the molecular players involved, however, have yielded few answers to the questions that intrigued the founders of modern embryology regarding the mechanisms that underlie the self-organisation, evolvability and robustness of development. Nevertheless, as was clear from the talks at the tenth annual RIKEN Center for Developmental Biology symposium 'Quantitative Developmental Biology' held in March 2012 in Kobe, Japan, and organised by Suzanne Eaton (MPI-MCBG), Shigeru Kondo (Osaka University), Shigeo Hayashi (RIKEN CDB), Shuichi Onami (RIKEN QBiC) and Tatsuo Shibata (RIKEN CDB), such questions are coming to the fore once more now that it is possible to image developmental processes as they occur in living animals. Moreover, studies tracking the dynamics of fluorescently tagged molecules in individual cells in living animals are beginning to reveal a raft of new phenomena that were invisible to previous generations, and make clear the importance of viewing morphogenesis and patterning as integrated processes.

In bringing together a dynamic mix of biologists, physicists, mathematicians, engineers and computational biologists interested in a quantitative understanding of development, the symposium also revealed how each person's training influences their perspective. Using the 19th century Hokusai woodblock print *The Great Wave Off Kanagawa* as an analogy (Fig. 1A), there are those who like to view development from afar, from the top of Mount Fuji for example, who see mathematical beauty in the regular swell

and fall of the ocean waves. Meanwhile, those with a molecular bent are happiest in the boat, holding tight to their oars as they attempt to keep pace with the movements of the nearest neighbours. And the cell biologists watch from the prow, trying to maintain a steady course in the face of the waves and the erratic behaviour of individual rowers. Just a few years ago, these researchers would never have been seen at the same meeting and their different perspectives of the world would have seemed disconnected and irreconcilable. Now, with the availability of quantitative molecular and cellular data, researchers are beginning to integrate analyses across multiple spatial and temporal scales to produce multi-level explanations of developmental processes. The meeting exemplified this by metaphorically putting physicists in the boat, driving cell biologists to the hills to get a better view, and giving the molecular biologists a rest from their oars.

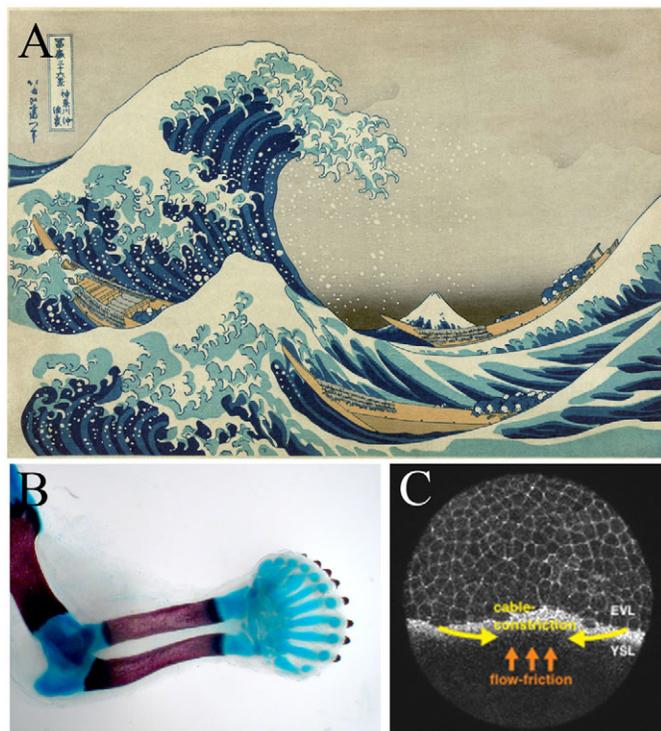
The meeting covered a range of themes, often seeking to understand the emergence of complex form from the activity of individual component cells. Several groups examined the way in which forces are communicated across tissues and embryos to shape the whole. Others discussed how patterns of cell identity and polarity across a tissue are shaped by local processes such as cell-cell communication. Although the integration of signalling, cell shape and mechanics has been most fully realised in studies on single cells, this field remains in its infancy. In studying the responses of individual cells, different researchers variously emphasized the importance of noise, excitability or mechanical coherence in cellular decision making. Finally, we had a brief glimpse of a future in which we would be able to track every cell in a developing animal at high accuracy, measure forces or calculate them based upon strain, watch and manipulate the local concentrations of individual protein molecules in living cells, and rationally engineer tissues.

## Alan Turing at 100

Many modern cell and developmental theorists trace their heritage to the pioneering mathematical theory developed by Alan Turing, who was born 100 years ago this year. In the late 1940s and early 1950s, Turing developed a theory showing how simple systems of partial differential equations could generate a complex pattern out of a uniform field (Turing, 1952). This idea, popularised in biology by Meinhardt and colleagues (Gierer and Meinhardt, 1972), proved to have an extraordinarily broad influence in the study of emergent properties in a wide array of dynamic systems. In the case of embryos, Turing imagined that patterning would be driven by the diffusion of molecules that he called 'morphogens'. With the discovery of diffusible growth factors and elementary gene regulatory networks, Turing's 'morphogen' was recast as a molecule that could pattern tissues based upon concentration, as in Lewis Wolpert's French Flag model (Wolpert, 1969), or elegant patterns of standing waves (Gierer and Meinhardt, 1972). However, self-organisation as a principle in development was for the most part set aside on the grounds that genetic screens suggested that the

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**Fig. 1. Waves, patterning and mechanics at RIKEN.** (A) *The Great Wave Off Kanagawa* by Hokusai. (B) Removal of *Gli3* genes and *Hox* genes from the mouse limb bud results in extensive polydactyly. (C) Zebrafish embryo at 60% epiboly (6.5 hours postfertilisation) expressing Utrophin-GFP for visualisation of F-actin. An actomyosin band forms within the yolk syncytial layer (YSL) adjacent to the margin of the epithelium (enveloping layer, EVL). Analysis of the distribution of cortical tension and the dynamic organisation of the actomyosin network reveals that the force-generating mechanism cannot be solely explained by a simple cable-string constriction. Images courtesy of James Sharpe (B) and Martin Behrndt (C).

individual parts of organisms have distinct molecular identities. With rigid genetic pathways controlling cell identity there would no need for hard-to-tame dynamic processes. It was interesting to see how many of the speakers at this symposium had, in the light of new data, turned back the clock to look again at roles for Turing-type processes in developmental tissue patterning. In doing so, all had to grapple with the issues that arise when mathematical abstractions meet the evolved complexity of biological systems. One clear example of this came from Philip Murray (Maini and Baker laboratories; University of Oxford, UK), who presented mathematical models of somitogenesis and showed how different mathematical frameworks can be used to reach similar conclusions about the working of the somitogenesis clock (Murray et al., 2011).

Inspired by Turing, efforts are underway to couple the physics of reaction-diffusion with the mechanics of cell growth and movement. Plants offer a key example of patterning that is free from the confounding influence of cell movement. Using this system, Przemyslaw Prusinkiewicz (University of Calgary, Canada) showed how leaf shapes could be determined by programmes of growth under the control of auxin levels and distribution. In contrast to the original Turing model, plant tissues are patterned as a result of competition for a limited pool of the morphogen auxin, the local concentration of which depends on its

production, degradation and polar transport via transmembrane channels (Bilsborough et al., 2011). Also looking at the role of morphogens in large-scale patterning, Cheng Ming Chuong (University of Southern California, Los Angeles, USA) revealed how secreted ligands drive propagating waves of stem cell activity as hairs are formed and turned over in a coordinated fashion across the entire animal body (Chuong et al., 2012; Plikus et al., 2011). These dynamic patterns were made visible simply by using barber's clippers to shave mice and rabbits daily. Remarkably, a similar set of molecules, BMPs and Wnts, expressed in the intradermal tissue has been found to underlie species-specific propagating patterns, with a form (travelling waves in mice and fractal patterns in rabbits) that depends primarily on the rate of signal propagation across the tissue and which comes to a halt in pregnant mice. Shigeru Kondo (Osaka University, Japan) was able to zoom in and investigate the cellular basis of the signalling events by which cells communicate to generate skin patterns in zebrafish. Kondo, who in previous years established compelling analogies between the behaviour of Turing-like patterning in silico and events seen in real organisms, here presented new data that owe as much to the descriptive approaches of the pioneering cell biologist Michael Abercrombie as to Turing. Through an elegant set of in vitro experiments examining the events that generate pigment pattern in zebrafish, he showed that lateral stripes are likely to be generated via a process of xanthophore protrusion-mediated contact inhibition between migratory yellow and black pigmented cells that lie under the surface of the skin. Contact-mediated unidirectional cell polarisation causes differently coloured cells to repel one another, so that yellow and black cells become segregated. This process is dependent upon an ion channel encoded by the *jaguar* gene (*potassium inwardly-rectifying channel, subfamily J, member 13*); in *jaguar* mutants, cells of different colour remain separate (Inaba et al., 2012). Remarkably, the protrusions of melanophores appear to mediate long-range Notch-Delta signalling to refine the developing pattern as a result of signal-induced cell death. Buzz Baum (University College London, UK) discussed a very similar observation in the developing fly pupa, where basal protrusions mediate intermittent Notch-Delta signalling in cells within a monolayer epithelium to optimise the pattern of bristles across the entire tissue (Cohen et al., 2010), while James Sharpe (Centre for Genomic Regulation, Barcelona, Spain) described similar protrusions in cells of the developing limb bud in mouse, although their function in this context remains unclear. Thus, whatever the mathematics underlying patterning, the equations driving it appear to be encoded both in the diffusion of morphogens and in the dynamic contacts between individual cells.

Another cellular mechanism by which patterns may emerge in developing animals was discussed by Stuart Newman (New York Medical College, USA). He explained how the condensation of mesenchymal cells, which is one of the earliest steps in the formation of cartilage and bone in the developing limb, can be recapitulated in vitro as a uniform layer of embryonic cells self-organises into patterned condensations that are first visible as patterned differences in the concentration of extracellular matrix components and galectins, without the influence of external signals (Bhat et al., 2011). This suggests a radical revision of the prevailing model, such that the conserved morphogens and cell state regulators, which have been the focus of so much attention in the limb field in the past 30 years, function to refine and sharpen underlying patterns generated by self-organising processes similar to those envisioned by Turing. Thus, returning to the Hokusai print, we, sitting in the boat manning the oars, might have lost sight of

the waves. A specific case in point was discussed by James Sharpe who, with his collaborators, has undertaken an impressive effort to reveal the role of Hox genes in patterning digits in the developing limb bud. The results of their analysis are both striking and strikingly simple. The sequential removal of distally expressed Hox genes results in the generation of a progressively greater number of smaller, but evenly spaced, digits, the precise number of which depends on the size of the developing limb bud (Fig. 1B). This is exactly what is expected from a simple Turing-like patterning process, in which patterns have a wavelength that can be smoothly 'tuned' by a molecular parameter (Hox dosage) but is independent of the field size.

### D'Arcy Thompson at 150

In a seemingly parallel universe to the mathematical world of Turing, at the end of the 19th century early zoologists such as D'Arcy W. Thompson (born 1860) and embryologists such as Thomas Hunt Morgan were influenced by contemporary successes in the physical sciences to propose that morphogenesis could be treated as a deterministic process that was driven by physical principles. In doing so they hoped to explain the beautiful forms seen in nature (Thompson, 1917). Many early embryologists were drawn to these concepts but were frustrated by the technical challenges of testing theories with experiments. Thus, although the work of 20th century embryologists such as Johannes Holtfreter, Yukio Hiramoto, John Trinkaus and many others paved the way, developmental mechanics had to await the successes of genetics and advances in cell and tissue mechanics before taking off. Now, Morgan's intellectual heirs are returning in strength to elucidate the role of mechanics in development using embryos of *Xenopus*, where physical perturbations can be used to probe tissue mechanics, zebrafish, *Drosophila* and *C. elegans* as models in which live cell imaging, mechanical perturbations and genetics can be readily combined.

The large-scale tissue movements that shape the early embryo are the product of forces generated by multiple tissues and are guided by their mechanics and architecture. Just how these forces integrate and interact across the embryo is largely unknown but a fully integrated biomechanical explanation of embryogenesis requires detailed measurements of in toto movements and the physical mechanics of embryonic structures. Martin Behrndt (Heisenberg laboratory; Institute for Science and Technology, Klosterneuburg, Austria) presented an elegant series of experiments combining laser cutting, in toto imaging and micromanipulation to study the movement of an epithelial monolayer during zebrafish epiboly. This work rules out an exclusive role for a leading edge purse string, akin to a belt tightening through loops, in driving tissue movement. Instead, he presented a model in which the contraction of the actomyosin network at the surface of the yolk cell connects to the leading edge of the epithelium to pull the enveloping layer forwards (Fig. 1C). Jerome Solon (Centre for Genomic Regulation, Barcelona, Spain) added another level of complexity by showing how the forces generated during head formation in the developing fly influence dorsal closure. This accounts for the accelerated closure of the anterior end, a previously mysterious feature of the process. Solon also observed both apical and basal waves of actomyosin contraction in the amnioserosa that joins the margins of the closing epithelium, emphasizing that, even in a monolayer epithelium, it is crucial to image events in three dimensions. Also in *Drosophila*, Shigeo Hayashi (RIKEN CDB) demonstrated how a combination of forces leads to the formation of tracheal pits in the developing embryo, as

rounded mitotic cells serve as buckling points that initiate invagination of the contracting epidermis. Finally, Buzz Baum showed how the mechanical effects of cell crowding lead to live cell delamination to ensure proper cell packing in the notum of the fly pupa (Marinari et al., 2012).

### How cells integrate mechanical and biochemical cues

Another key theme at the meeting was the way in which cell and tissue shape and mechanics influence patterning. To generate a well shaped and patterned tissue, cells must interpret both mechanical and chemical cues from their local environment to direct their behaviours, trigger mechanical responses and organise their motility. Several groups described their efforts to elucidate the nature of these cues, in the form of secreted signals and mechanical stresses, and the processes by which morphogenetic and patterning information are integrated.

At the tissue scale, Darren Gilmour (EMBL, Heidelberg, Germany) showed how the directional migration of a group of ~100 cells that colonise the lateral line of the developing zebrafish influences patterning of the sensory organs that form along the way (Lecaudey et al., 2008). In this case, crosstalk between chemokine signalling from the underlying substrate, which drives cluster migration, and FGF signalling between cells of the migrating cluster, which induces epithelial polarisation and cell differentiation, creates a dynamic polarity that helps to ensure the unfolding developmental pattern of the lateral line. In a similar vein, Tadashi Uemura (Kyoto University, Japan) showed how conserved regulators of planar cell polarity (PCP) define the polarisation of cilia in the developing mouse oviduct epithelium, which, in turn, drive fluid flow that influences morphogenesis of the entire tissue. In another example of the role of flow in development, Tetsuya Nakamura (Osaka University, Japan) demonstrated a clear link between left-right patterning in the mouse node (Nakamura et al., 2006) and fluid flow, where directionally beating cilia drive morphogens across the epithelium to bias the left-right axis of the developing animal.

Continuing on the theme of coordinating local and global events across a tissue, Suzanne Eaton (MPI-MCBG, Dresden, Germany) and her colleagues have begun to unravel the complex polarity code in the fly wing that leads to the alignment of hairs along the proximal and distal axes and along wing veins (Aigouy et al., 2010). Notably, her talk emphasized the importance of looking early in development. By examining polarity in developing wild-type and mutant wing discs, the group was able to show that polarity develops very early during the growth of the wing disc, and that its global pattern is controlled by the organiser regions that also specify growth and patterning. Their evidence indicates that the polarity pattern is not directly specified by morphogen gradients, and the group hypothesizes that it is the growth pattern, rather than signalling gradients, that directly specifies the pattern of PCP (Sagner et al., 2012). Her longtime collaborator, Frank Jülicher (MPI for Physics of Complex Systems, Dresden, Germany), described work carried out with the Gonzalez-Gaitan group that addressed a parallel process in the same tissue over the same time period (Wartlick et al., 2011). He showed how patterning within the developing wing is scaled as the tissue grows, through a precise matching of the decay length of the Dpp gradient with wing disc size as a result of matched changes in the Dpp degradation rate. Importantly, this work also suggests a mechanism whereby changes in the levels of Dpp experienced by a single cell over time can induce uniform cell division across the growing disc,

and so define the tissue's correct final size. Similar theoretical arguments were used by Hidehiko Inomata (RIKEN CDB) to explain how, during development of the early *Xenopus* embryo, crosstalk between diffusing morphogens ensures that dorsal-ventral patterning scales with embryo size, such that half-size embryos (identical twins) can form and hatch.

Processes that localise proteins, direct transport and organise contractility within a single cell can also result in complex phenomena. Masako Tamada (Zallen laboratory; Sloan-Kettering Institute, New York, USA) described how the localisation of a tyrosine kinase can polarise junctional dynamics in individual cells to drive changes in tissue form during *Drosophila* embryonic axis elongation (Tamada et al., 2012), and Orion Weiner (University of California, San Francisco, USA) showed how membrane mechanics can contribute to the polarisation of individual migrating cells (Houk et al., 2012). Taking the analysis of polarity in single cells further, both Edwin Munro (University of Chicago, USA) and Philipp Khuc Trong (Goldstein laboratory; University of Cambridge, UK) discussed the importance of macroscopic flows in both helping and hindering the generation of polarity in single cells. Khuc Trong described how weak cytoplasmic flows are generated as a result of kinesin-mediated cargo transport on a microtubule network during the establishment of cytoplasmic polarity within the normal developing *Drosophila* oocyte. He then explained how these flows would come to dominate and destroy polarity if the network was more ordered – a finding that helps to explain the previously puzzling observation of only weak bias in the microtubule network (Khuc Trong et al., 2012). Similarly, Munro demonstrated how the proper maintenance of polarity in the *C. elegans* zygote requires that cortical flows driven by CDC-42-dependent actomyosin contraction are counterbalanced by CDC42-dependent, Arp2/3-mediated actin polymerisation at the anterior pole. In this way, CDC-42 appears to act through distinct effector pathways to enable the system to precisely regulate cortical flows, ensuring the dynamic maintenance of a robust axis of polarity that is properly aligned with the long axis of the egg.

### Cellular computation

A number of groups were interested in the fascinating process by which individual cells make decisions. Several groups used *Dictyostelium* as a model to explore how cells compute an appropriate axis of polarity in response to noisy and variable external cues. In all cases, it was clear that the extracellular signal was subject to intracellular processing (boosting and filtering). As a result, cells can polarise in response to extremely low levels of a cAMP signal, rapidly adapt to changes in cAMP levels and gradient, and can polarise in the absence of a directional cue. More specifically, Tatsuo Shibata (RIKEN CDB), using microfluidic devices and a mathematical model, described how oscillatory and excitable behaviours of PIP3 couple positive and negative feedbacks to produce a robust response to inherently noisy cAMP signals (Arai et al., 2010). Similarly, Satoshi Sawai (RIKEN CDB) described the use of a microfluidic device to deliver precise amounts of cAMP and then analyse the kinetics of actin filament formation in responding cells. From these studies they were able to develop a model of cellular computation that could explain propagating waves of PIP3 and actin filament formation and the consequences of wave collisions within single migrating cells. Kees Weijer (University of Dundee, UK) made clear the parallels between cAMP signalling in *Dictyostelium* and the swirling movements of cells during chick gastrulation (Chuai et al., 2006). Also dealing with the question of the influence of noise on

developmental processes, Dina Faddah (Jaenisch laboratory; MIT, Cambridge, USA) discussed the noisy path that somatic cells take on their route back to pluripotency during reprogramming.

A picture is emerging from this type of work in which noise, which is inevitably associated with molecular processes in living cells based upon small numbers of molecules, is not simply a problem that cells (and synthetic biologists) need to overcome. Rather, noisy intracellular signals and the molecular networks that process these signals lie at the very heart of the particular way in which biological systems sense and respond to the world and implement the genetically encoded developmental programme to yield an animal with a reproducible final form.

### A glimpse of the future

The great advances in cell and developmental biology that have come with new imaging techniques present both opportunities and difficulties. The good news is the growing availability of commercial microscopes with unparalleled resolving power in space and time. The problem is that the analysis and the interpretation of the datasets generated are enormously challenging. For instance, complete 4D recordings of the development of a single embryo over just a few hours can produce a terabyte or more of image data, necessitating the development of novel computational tools. *C. elegans* represents one of the simpler models, with a deterministic cell lineage. But even here, as Eugene Myers (MPI Systems Biology Center, Dresden, Germany) highlighted, there is a need for better microscopes and for analytical techniques that can deliver cell lineage maps of high accuracy. Two other talks described significant progress in the quantitative analysis of *C. elegans* development. Ralf Schnabel (Technical University Braunschweig, Germany) demonstrated the use of automated 4D DIC microscopy to track cells to identify a novel mechanism that is capable of guiding misplaced cells to their correct destination in wild-type and mutant embryos (Schnabel et al., 2006). Shuichi Onami (RIKEN QBiC) combined live cell imaging and automated image processing (Hamahashi et al., 2005) with systematic RNAi to identify the set of genes influencing cleavage patterns of early *C. elegans* embryos. He also presented new computational methods to infer the sequence of morphogenetic events and the genes that determine this order using large-scale quantitative cell division dynamics data of RNAi embryos.

Meanwhile, in *Drosophila*, Kaoru Sugimura (Kyoto University, Japan) working together with the physicist Shuji Ishihara, showed how it might be possible to take image analysis further, working together with the physicist Shuji Ishihara (University of Tokyo, Japan) to develop methods to infer maps of mechanical forces from patterns of epithelial cell shapes and their connectivity, using laser-cutting experiments to support their predictions. It will be important to verify this type of approach in systems in which one can directly measure tissue mechanics, and Lance Davidson (University of Pittsburgh, USA) showed how classical amphibian microsurgery and explant techniques can be used to do just this and how, in the process, they made the surprising discovery that morphogenesis in *Xenopus* is robust to natural twofold variations in mechanical properties (von Dassow and Davidson, 2009) and to significant variation in applied forces (von Dassow et al., 2011).

Just as developmental biologists are assimilating new imaging tools, a wide range of new technologies are being developed that should enable the direct visualisation of tissue stresses as well as strains, and that enable light to be used to control the local activity of proteins in living cells. Orion Weiner showed how it was

possible to use optogenetic tools together with an optical clamp to reliably probe and manipulate cell signalling in a manner similar to how a voltage clamp can be used to both accurately measure and control axonal activity (Toettcher et al., 2011). Finally, Christopher Chen (University of Pennsylvania, Philadelphia, USA) described technological advances in microfabrication that allow the control of cell shape and mechanics to direct the development of tissues, such as heart muscle, from pluripotent stem cells (Wozniak and Chen, 2009). One can now begin to imagine how, by combining these tools and via a better understanding of cellular computation, one might engineer cells and tissues.

### Developmental biology as a predictive science?

In summary, although the future of developmental biology will continue to involve large-scale, high-throughput ‘-omics’ approaches, advances will increasingly depend on the integration of the diverse datasets into model ‘pictures and narratives’ of development. As never before, theorists and experimentalists are working closely together to channel the huge crashing waves of data into predictive models of pattern generation and morphogenesis. As Suzanne Eaton said at the close of the meeting, there is hope that this work might lead to the definition of a handful of broadly applicable principles in developmental biology, akin to those in the physical sciences. Although the end results of these efforts are hard to foresee, they will no doubt make a great splash in fields such as stem cell biology, cancer and regenerative biology that have turned to developmental biology for guidance.

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### References

- Aigouy, B., Farhadifar, R., Staple, D. B., Sagner, A., Roper, J. C., Julicher, F. and Eaton, S. (2010). Cell flow reorients the axis of planar polarity in the wing epithelium of *Drosophila*. *Cell* **142**, 773-786.
- Arai, Y., Shibata, T., Matsuoka, S., Sato, M. J., Yanagida, T. and Ueda, M. (2010). Self-organization of the phosphatidylinositol lipids signaling system for random cell migration. *Proc. Natl. Acad. Sci. USA* **107**, 12399-12404.
- Bhat, R., Lerea, K. M., Peng, H., Kaltner, H., Gabius, H. J. and Newman, S. A. (2011). A regulatory network of two galectins mediates the earliest steps of avian limb skeletal morphogenesis. *BMC Dev. Biol.* **11**, 6.
- Bilborough, G. D., Runions, A., Barkoulas, M., Jenkins, H. W., Hasson, A., Galinha, C., Laufs, P., Hay, A., Prusinkiewicz, P. and Tsiantis, M. (2011). Model for the regulation of *Arabidopsis thaliana* leaf margin development. *Proc. Natl. Acad. Sci. USA* **108**, 3424-3429.
- Chuai, M., Zeng, W., Yang, X., Boychenko, V., Glazier, J. A. and Weijer, C. J. (2006). Cell movement during chick primitive streak formation. *Dev. Biol.* **296**, 137-149.
- Chuong, C. M., Randall, V. A., Widelitz, R. B., Wu, P. and Jiang, T. X. (2012). Physiological regeneration of skin appendages and implications for regenerative medicine. *Physiology (Bethesda)* **27**, 61-72.
- Cohen, M., Georgiou, M., Stevenson, N. L., Miodownik, M. and Baum, B. (2010). Dynamic filopodia transmit intermittent Delta-Notch signaling to drive pattern refinement during lateral inhibition. *Dev. Cell* **19**, 78-89.
- Gierer, A. and Meinhardt, H. (1972). A theory of biological pattern formation. *Kybernetik* **12**, 30-39.
- Hamahashi, S., Onami, S. and Kitano, H. (2005). Detection of nuclei in 4D Nomarski DIC microscope images of early *Caenorhabditis elegans* embryos using local image entropy and object tracking. *BMC Bioinformatics* **6**, 125.
- Houk, A. R., Jilkine, A., Mejean, C. O., Boltyanskiy, R., Dufresne, E. R., Angenent, S. B., Altschuler, S. J., Wu, L. F. and Weiner, O. D. (2012). Membrane tension maintains cell polarity by confining signals to the leading edge during neutrophil migration. *Cell* **148**, 175-188.
- Inaba, M., Yamanaka, H. and Kondo, S. (2012). Pigment pattern formation by contact-dependent depolarization. *Science* **335**, 677.
- Khuc Trong, P., Guck, J. and Goldstein, R. E. (2012). Coupling of active motion and advection shapes intracellular cargo transport. *Phys. Rev. Lett.* (in press).
- Lecaudey, V., Cakan-Akdogan, G., Norton, W. H. and Gilmour, D. (2008). Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. *Development* **135**, 2695-2705.
- Marinari, E., Mehonic, A., Curran, S., Gale, J., Duke, T. and Baum, B. (2012). Live-cell delamination counterbalances epithelial growth to limit tissue overcrowding. *Nature* **484**, 542-545.
- Murray, P. J., Maini, P. K. and Baker, R. E. (2011). The clock and wavefront model revisited. *J. Theor. Biol.* **283**, 227-238.
- Nakamura, T., Mine, N., Nakaguchi, E., Mochizuki, A., Yamamoto, M., Yashiro, K., Meno, C. and Hamada, H. (2006). Generation of robust left-right asymmetry in the mouse embryo requires a self-enhancement and lateral-inhibition system. *Dev. Cell* **11**, 495-504.
- Plikus, M. V., Baker, R. E., Chen, C. C., Fare, C., de la Cruz, D., Andl, T., Maini, P. K., Millar, S. E., Widelitz, R. and Chuong, C. M. (2011). Self-organizing and stochastic behaviors during the regeneration of hair stem cells. *Science* **332**, 586-589.
- Sagner, A., Merkel, M., Aigouy, B., Gaebel, J., Brankatschk, M., Julicher, F. and Eaton, S. (2012). Establishment of global patterns of planar polarity during growth of the *Drosophila* wing epithelium. *Curr. Biol.* (in press).
- Schnabel, R., Bischoff, M., Hintze, A., Schulz, A. K., Hejnowicz, A., Meinhardt, H. and Hutter, H. (2006). Global cell sorting in the *C. elegans* embryo defines a new mechanism for pattern formation. *Dev. Biol.* **294**, 418-431.
- Tamada, M., Farrell, D. L. and Zallen, J. A. (2012). Abl regulates planar polarized junctional dynamics through beta-catenin tyrosine phosphorylation. *Dev. Cell* **22**, 309-319.
- Thompson, D. A. W. (1917). *On Growth and Form*. Cambridge, UK: Cambridge University Press.
- Toettcher, J. E., Voigt, C. A., Weiner, O. D. and Lim, W. A. (2011). The promise of optogenetics in cell biology: interrogating molecular circuits in space and time. *Nat. Methods* **8**, 35-38.
- Turing, A. M. (1952). The chemical basis of morphogenesis. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **237**, 37-72.
- von Dassow, M. and Davidson, L. A. (2009). Natural variation in embryo mechanics: gastrulation in *Xenopus laevis* is highly robust to variation in tissue stiffness. *Dev. Dyn.* **238**, 2-18.
- von Dassow, M., Strother, J. A. and Davidson, L. A. (2011). Surprisingly simple mechanical behavior from a complex embryonic tissue. *PLoS ONE* **5**, e15359.
- Wartlick, O., Mumcu, P., Kicheva, A., Bittig, T., Seum, C., Julicher, F. and Gonzalez-Gaitan, M. (2011). Dynamics of Dpp signaling and proliferation control. *Science* **331**, 1154-1159.
- Wolpert, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. Theor. Biol.* **25**, 1-47.
- Wozniak, M. A. and Chen, C. S. (2009). Mechanotransduction in development: a growing role for contractility. *Nat. Rev. Mol. Cell Biol.* **10**, 34-43.