

Modeling new conceptual interpretations of development

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Summary

In April 2011, researchers from diverse background met at the Gulbenkian Institute (Oeiras, Portugal) to discuss the emerging input of biophysics into the field of developmental biology. The scope of the workshop was to bring together scientists working in different model systems and to discuss some of the most recent advances towards understanding how physical forces affect embryonic development. Discussions and talks highlighted two main trends: that many aspects of embryogenesis can be accurately quantified and translated into a limited number of physical forces and biochemical parameters; and that simulations and modeling provide new conceptual interpretations of classical developmental questions.

Key words: Cell biology, Cell migration, Development, *Drosophila*, Force, Zebrafish

Introduction

Morphogenesis is a synchronized process that integrates chemical and physical inputs in time and space. For example, the formation of a tube or the periodicity of segment formation during axis elongation both require the combined activity of cell signaling, migration, proliferation and the expression of cell identity genes. In addition, physical constraints are part of the developmental program, as cells are constantly challenged by their three-dimensional environment. This results in reciprocal mechanical and genetic interactions both at the subcellular and cellular levels, and in whole tissue. Reflecting the complexity of this biological information and the diversity of tissue shapes and types, conferences covering the topics of positional information, cell and tissue shape and morphogenesis are numerous. However, it is now clear that biophysical mechanisms control important aspects of development, a theme that is not always extensively covered in these meetings.

The EMBO workshop on Biophysical Mechanisms of Development (organized by Ana Borges, Ana Certal, Ana Tavares, Filipa Alves and Beatriz Garcia Fernandez) brought together scientists in the field of developmental biology for whom interdisciplinary and quantitative approaches are central to the issues they are investigating. Cell and tissue mechanics were a major focus of the meeting, while live imaging, genetic and reverse genetic

approaches combined with modeling were the predominantly discussed tools. In addition, the meeting featured efforts to combine several disciplines in order to address questions concerning growth, morphogenesis and patterning from a dynamic point of view. Talks were highly diverse, from ion dynamics and regeneration to cellular mechanodetection and transduction. In addition, numerous new conceptual advances in coupling physical forces and morphogenesis, as well as the development of novel technologies to study forces, were discussed. The researchers attending the meeting agreed that the combined use of modeling and imaging approaches is key to ensuring the quality of measurements and the validity of the models developed using these measurements. Another emerging theme was the importance of the physical input and mechanical forces in developmental processes, as well as their interactions with the genetic program at work in the embryo.

The embryo gets biophysical

In growing tissues, cells experience a mix of tensile forces and chemical information. Key to untangling the possible links, as well as the causality, between the two is the ability to measure and map the physical properties of tissues at the cellular level and characterize their biological effects. Overall, these measures need to be made using innovative technologies, the challenge being the ability to explore dynamics of developmental events.

Clues as to how to address some of these issues were provided by Jochen Guck (University of Cambridge, UK), who studies the viscoelastic properties of tissues and their influences on cell behavior during central nervous system (CNS) development. His group has developed an approach using quantitative scanning force microscopy (through atomic force microscopy, AFM) to measure CNS tissue compliance. Owing to the heterogeneities of this tissue, glial cells and neurons can behave rather differently during their migration, as this process is mechano-dependant (Christ et al., 2010). To address the mechanical properties of tissues and cell behavior in response to their physical constraints, Guck's group created compliant polyacrylamide gel surfaces that are of varying stiffness that match or exceeds the stiffness of CNS tissues. They showed that, depending on their identity, cells will react differently to the stiffness of the environment: astrocytes and microglia will change shape if they are in contact with stiff substrates, whereas neurons will do so to a much lesser extent. Nevertheless, the migration of neurons is highly dependent on stiffness, as neurons tend to migrate towards soft tissue. By contrast, microglia move toward stiffer substrates. Importantly, astrocytes and microglia in stiff environments react by upregulating inflammatory mediators (Moshayedi et al., 2010). Guck's results show that the growth cone is also sensitive to its environment, as it retracts when in contact with the mechanical stimulation of the AFM probe. Guck suggested that the mechanical mismatch between neural implants and native tissue might be at the root of foreign body reaction and proposed the use of applied force to trigger better healing. Altogether, his presentation clearly showed that cell function has to be seen in the context of mechanical properties, and that extended and systematic in vivo studies need to be performed to characterize mechanical properties of tissues.

Not surprisingly, the meeting was permeated with ion dynamics research, and this fact was best illustrated by talks on the roles of ion flux during migration. As the group of Guck showed, cells need to

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overcome and generate forces in order to migrate. The lab of Christian Stock (University of Munster, Germany) is interested in addressing the interactions between migrating cells and their environment, and his talk focused on the study of local ion flux in intracellular subdomains that are in contact with cell substrates. His group found that the Na^+/H^+ exchanger NHE1 is necessary for the migration of cultured human melanoma cells and acts through $\beta 1$ integrin at the front of the cell to promote attachment to the substrate. This attachment is necessary for cell movement (Krahling et al., 2009). NHE1 is part of the focal adhesion contacts and accumulates at the cell front where its activity causes acidification at the cell surface. By measuring the local pH ratiometrically in live cells, Stock found that the cytosolic pH is higher at the plasma membrane of focal adhesion contacts than it is in the rest of the cell. As integrin structure can vary with pH, Stock proposed that NHE1 is active at focal adhesions where it contributes to the generation of a pH nano-environment that is needed to modulate the strength of cell-matrix contacts.

Cell environments are complex, and endogenous electrical environments constitute another source of cell-guiding cues. The influence of bioelectricity on cell migration has been best studied in tissue regeneration, where it acts as a guidance signal that cells at wound sites receive to indicate that damage has occurred (Zhao, 2009). In human skin wounds, the damaged center can generate up to 150 mV/mm (Zhao, 2009). Human skin cells will migrate directionally in an applied electrical field, suggesting that endogenous fields act as attracting centers. The group of Christine Pullar (University of Leicester, UK) studies bioelectric guidance, a process also known as galvotaxis. To determine the roles of ion channels in electrical sensing, her group uses zebrafish as a model organism. In fish, wound inflammation can be studied easily, as neutrophils are attracted through a variety of pathways to the wound site within hours of injury. The data Pullar presented suggest that ion channels play a role in electrical sensing.

Cell motility and ion dynamics were also discussed in a slightly different context by Alberto Darszon (Universidad Nacional Autónoma de México, Mexico). His lab studies marine animals that undergo external fertilization and their strategies to enhance sperm-egg encounter. His presentation focused on a diffusible chemoattractant, Speract, which is released from the egg's envelope to attract sperm before fertilization. He showed that sea urchin Speract induces non-chemotactic motility responses in *Strongylocentrotus purpuratus* spermatozoa, while it is chemotactic in *Lytechinus pictus* sperm. Based on their experimental results, Darszon's group derived a signaling network model that corroborated previous empirically determined responses and predicted the involvement of an unexpected K^+ channel that modulates the flagellar Ca^{2+} fluctuations and regulates sperm swimming.

These talks stimulated discussions about how important these observations might be to other model organisms studied in developmental biology. It will be interesting to follow up how the combination of ion imaging with physical measurements can help to improve our understanding of morphogenesis.

Coupling mechanodetection, tissue growth and stochastic mechanics

Cell migration and division usually result in mechanically induced rearrangements within developing tissues. Accurate and precise patterning must thus be tightly coupled with these mechanical forces. The *Drosophila* wing imaginal disc provides a good model system to study the coupling between cell division and tissue elongation; clonal analysis in the developing wing disc has previously suggested that the direction of tissue growth is controlled by the orientation of cell

division (Baena-López et al., 2005). In his talk, Barry Thompson (Cancer Research UK, London, UK) went a step further and showed that orientation of cell division in the wing imaginal disc requires planar polarization of an atypical myosin, Dachs. Time-lapse analysis performed by his group revealed that cell shape orients cell division, suggesting that shape could determine the orientation of the mitotic spindle. Using modeling approaches based on a vertex model that takes into account cell elasticity and junctional forces arising from cortical contractility and adhesion, he showed that polarized cell tension can be sufficient to orient cell shape and cell division when compared with isometric apical tension. His model thus suggests that oriented tissue growth in the extending wing is the result of the activity of Dachs in constricting cell-cell junctions to lead to altered cell shape geometry and oriented cell division (Mao et al., 2011).

Cellular mechanodetection and mechanotransduction are essential because they provide cells with information about the surrounding physical environment. It is still unclear in most cases what these mechanodetectors are and how mechanotransduction is coupled to other developmental programs. The talk by Florence Janody (Gulbenkian Institute, Oeiras, Portugal) addressed this question from a very interesting angle. Her lab is interested in the Hippo tumor suppression pathway and recently showed that proper regulation of the actin microfilament system is required to suppress inappropriate tissue growth through the regulation of Hippo (Fernandez et al., 2011; Sansores-Garcia et al., 2011). This work suggests that a regulatory loop exists between actin and cell proliferation that is mediated by Yorkie. Thus, it seems that tension exerted by neighboring cells can be sensed at the cell membrane by the actin cytoskeleton and is translated into cell proliferation by the Hippo pathway.

How tissues might balance cell division and cell loss was discussed by Buzz Baum (University College London, UK), who reported his group's investigation of the role of mechanics in the maintenance of epithelial tissue homeostasis through the regulation of cell delamination. By looking at the developing fly notum, his group has found that tissue organization increases as development proceeds (Cohen et al., 2010). Through time-lapse imaging, he showed that epithelial cells leave crowded regions of the tissue via basal delamination. This process appears to help the system achieve a final well-ordered state once growth stops and intra-tissular forces stabilize. Using laser ablation and modeling approaches, he showed that cells delaminate in a stochastic manner from mechanically compressed tissues through loss of cell-cell junctions. Cells then die underneath the tissue. Epithelial growth is thus balanced by a process that regulates cell delamination in response to tissue mechanics. This could be a generic mechanism that buffers epithelia against variation in growth.

Stochasticity is also at work at the transcriptional level. Alfonso Martinez Arias (University of Cambridge, UK) is interested in the stochasticity of gene expression and reported that transcriptional fluctuations in Nanog expression in cultured ES cells are an essential element of the pluripotent state. He also presented evidence that these stochastic events correspond to transient states that may correspond to a phase in which cells are available or competent to be recruited into different cell lineages (Kalmar et al., 2009). This process could be part of the cell fate decision mechanism and certainly forms the basis of the variability of fate observed in differentiated cell culture.

Assessing the impact of physical forces in embryonic tissues

One important developmental event that has successfully been used to investigate the role of mechanical forces in development is the process of dorsal closure in the *Drosophila* embryo. Dorsal closure

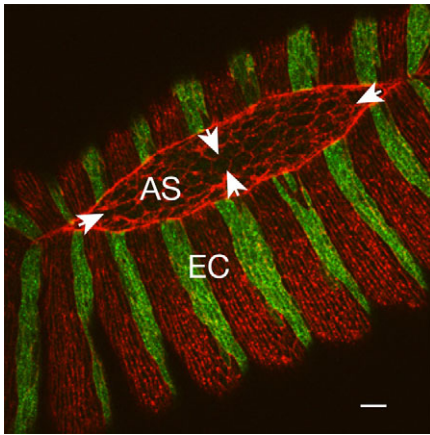


Fig. 1. Dorsal closure in a *Drosophila* embryo. A *Drosophila* embryo undergoing dorsal closure as epidermal cells (EC) cover the amniosera (AS). The white arrows underline the motion of closure. Cell outlines are visualized by phalloidin staining (red). Spastin tagged with EGFP (a microtubule-severing protein, green) is expressed under the control of the *engrailed* promoter to eliminate microtubules specifically in a subset of epidermis cells to address the roles of microtubules in force generation during dorsal closure. Image courtesy of Damian Brunner (University of Zurich, Switzerland). Scale bar: 25 μ m.

occurs during gastrulation and relies on the joining of the epidermis on either side of a dorsal opening. The amniosera is replaced as closing occurs (Fig. 1). Pioneering work has identified several signaling pathways involved in dorsal closure via genetic and reverse genetic approaches, and the forces involved have been characterized by using live imaging and physical interference experiments, such as laser microsurgery (Franke et al., 2005; Hutson et al., 2003). It turns out that both tissues involved, the dorsal epidermis and the amniosera (Fig. 1), generate and respond to forces, and a number of mathematical models published in the past have tried to integrate these forces and model the outcome (see e.g. Layton et al., 2009). How the forces at work are controlled and how they couple to cell signaling remain somewhat mysterious. Two talks discussed the most recent concepts emerging from studying these questions.

First, Dan Kiehart (Duke University, NC, USA) addressed specific features of the first- and second-generation models, making the point that there is a certain model hierarchy that ultimately aims to provide a definitive account of the dynamics of dorsal closure. He illustrated the pivotal role of nonmuscle myosin II in all force-producing cells. He also reported on a first investigation of the role of mechanically gated channels in dorsal closure, using chemical interference and genetic approaches. In both cases, closure was blocked to various extents, indicating that the actinomyosin network is regulated by mechanically gated channels. Using optogenetics, Kiehart furthermore showed that experimental manipulations of Ca^{2+} levels induce apical constriction, one of the features of the force-producing amniosera cells. It was clear from this talk that dorsal closure will continue to be one of the leading models used for the integration of biophysics and biology. However, much work remains to be carried out in order to finally link the signaling cascades to the underlying cell biology.

Second, Damian Brunner (University of Zurich, Switzerland) illustrated the pulsing behavior of the amniosera cells that his lab recently discovered and discussed its important role in dorsal closure (Solon et al., 2009). As he stressed a number of times, modeling

provides a helpful means of exploring parameters and of analyzing their effects on the modeled process. This allows the collection of ideas that can be tested *in vivo* and helps to improve on one's model. Brunner made the point that the understanding of such dynamic processes was not possible without the information obtained by three-dimensional data at high resolution. He then illustrated the efforts of his lab to reconstruct the cells involved in dorsal closure by correlative serial electron tomography, shedding more light on the distribution of the actual cellular players involved in this process, such as microtubules and actin bundles, which provide the scaffold for force production. Such high-resolution data will add yet another layer of information, which ultimately will have to be linked to the highly dynamic processes that underlie the balanced production of force during dorsal closure.

Mapping forces in the living embryo remains an experimental challenge. Julien Vermot's group (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France) is interested in addressing the roles of fluid mechanics during embryogenesis. He presented a method using optical tweezers, which allow his group to probe microscopic flow fields in developing organs that are mediated by motile cilia. He showed experiments designed to address the flow forces generated in the zebrafish inner ear that are involved in building up the otoliths, a biomineral structure necessary for hearing and balance. By analyzing the motion of endogenous small mineral particles after optical trapping and mathematical modeling, he described the hydrodynamics modulated by beating cilia operating in the system (Wu et al., 2011).

Using high-resolution time-lapse microscopy in zebrafish embryo, Markus Affolter (University of Basel, Switzerland) described the sequence of cell rearrangements that are needed to connect and generate functional, lumenized vascular networks. Careful analysis of the behavior of individual cells has allowed his group to define two clearly separated mechanisms of lumenization that occur in the presence or absence of pre-existing pressure, respectively. This also allowed Affolter to speculate on the possible role that forces exert on blood vessels as they connect during anastomosis.

The force of modeling

Of course, modeling is not a new addition to biological research. Hans Meinhardt and others have had a long-term interest in modeling the behavior of biological processes in order to better understand how axes and patterns form during development. In the opening talk of this meeting, Meinhardt (Max Planck Institute for Development Biology, Tübingen, Germany) focused on the achievements and the problems in modeling axis formation in higher organisms. In vertebrates, usually only one organizer, the Spemann-type organizer, is assumed to exist and to drive axis formation. In his modeling of the axial organization of higher organisms, Meinhardt showed that the ancestral hydra-type organizer acts in vertebrates as an organizer of the anterior-posterior (AP) axis, and is based, as in hydra, on the Wnt pathway. He also showed that the Spemann-type organizer does not pattern the dorsal-ventral (DV) axis directly but gives rise to the midline (notochord, floor- and prechordal plate), which then acts as a line of positional reference. Both systems together generate a near-Cartesian positional information system (Meinhardt, 2004; Meinhardt, 2008). His modeling of midline formation revealed that the generation of a single stripe-like organizing region is a subtle patterning problem. In vertebrates, midline formation occurs by elongation, which is accomplished by a dorsal spot-like organizer, for example, in the wake of the moving Hensen's node. In insects, an inhibition that spreads from a dorsal

organizer forces the midline to appear at the ventral side. The midline in insects is fully extended along the AP axis but sharpens along the DV axis, in agreement with the observations from modeling. Meinhardt suggested that the different modes of midline formation might represent the point of no return in the major divergence of axial patterning and also raised new questions about the concept of an ‘urbilaterian’. Through these examples, Meinhardt showed that models allow for an integrated view of early axes formation in higher organisms, bringing differences and similarities into an evolutionary perspective and helping to resolve several controversially discussed issues, such as the DV-VD reversal in insects and vertebrates.

Among the classical developmental systems addressed during this meeting, the limb bud appeared to be one of the earliest developing structures to have been modeled. James Sharpe (Centre for Genomic Regulation, Barcelona, Spain) reminded us that some of the most powerful computers of the 1960s were used to simulate limb growth. However, although theoretically plausible, most of the available models were in two dimensions and were not validated *in vivo*. To match the challenge to combine modeling with quantitative biological data sets, his team associated thorough modeling [which included a numerical description of the developing limb’s shape (Boehm et al., 2011)] with a quantitative three-dimensional mapping of the cell division rate. They merged this information with a three-dimensional finite element model of tissue growth. Against many predictions, he could show that the observed proliferation rates do not appear to play a significant role in controlling distal elongation of the limb bud. Rather, the model suggests that directional cell migration (or cell displacement) is more likely to explain limb growth; experimental data seem to support this idea (Boehm et al., 2010).

We also heard about the dynamics of gene regulatory circuitry, where numerous parameters are acting together to define spatial and temporal patterns seen in the developing embryo. Clearly, as soon as more than two parameters are needed to explain a phenomenon (which means in virtually all cases), it becomes rather difficult to anticipate the reaction of a system. Modeling becomes extremely useful in these situations. Julian Lewis (Cancer Research UK, London, UK) illustrated this issue during his talk about the developmental gene circuit that governs the formation of vertebrate body segments. Through modeling, Lewis understood that delays in the negative feedback loop that controls the expression of a pair of auto- and cross-regulative genes can define the periodicity of the clock, and thereby the pace of segmentation. His group used zebrafish as an experimental system to perform a brilliant quantitative analysis of the elongation rate of RNA polymerase 2 for their favorite oscillating gene. The results showed that the rate of transcription is much faster than expected, suggesting that delays necessary for the clock to oscillate could be due to other processes, such as splicing rates.

Quite obviously, morphogens could not escape from being discussed at the meeting. Two talks dealt with morphogen gradients and their role in tissue growth using experimental and theoretical approaches. Tinri Aegerter-Wilmsen (Institute of Molecular Life Science, University of Zurich, Switzerland) reported on her efforts and progress to integrate force-sensing and signaling pathways in a model of size regulation of the *Drosophila* wing imaginal disc. She incorporated hypothetical interactions between mechanical forces and specific growth regulators into a network of known morphogen signaling interactions. The combined action of growth factors, their spatial gradients and mechanical forces leads to a regulatory feedback mechanism that is capable of reproducing the most important dynamics of the system, as well as many additional

experimental results. Marcos Gonzalez-Gaitan (University of Geneva, Switzerland) described the remarkable efforts of his lab to take a quantitative approach towards elucidating the formation and function of the Decapentaplegic (Dpp) morphogen gradient in the developing *Drosophila* wing. The large quantity of data his lab has collected has been used to produce a quantitative model that predicts that the rate of tissue growth, and hence the rate of cell division, is proportional to the relative increase in Dpp activity (Wartlick et al., 2011). This means that cells divide when the Dpp activity has increased by roughly 50%. Gonzalez-Gaitan went on to describe his group’s first efforts to understand how the increase in Dpp can be measured at the cellular level in time, and the results points towards an integration between the Dpp Smad proteins (which mediate Dpp signaling activity) and the Smad proteins of the *Drosophila* Activin signaling pathway. The lively discussion following both talks showed both the broad interest in morphogen gradients in the field of biology, and the power of modeling in explaining the growth properties of an entire organ system. It will be very interesting to follow the work of Aegerter-Wilmsen and Gonzalez-Gaitan in future years to see how the different models evolve, and whether they can at some point be merged in a meaningful manner.

Conclusions

In essence, key discoveries in developmental biology have always emerged from a mix of ingenious approaches and precise observations, in earlier days using grafting experiments [such as those by Spemann and Mangold (Sander and Faessler, 2001)] or genetic screens [such as those conducted by Nüsslein-Volhardt and Wiehaus (Nüsslein-Volhardt and Wiehaus, 1980)]. This combination of approaches is still a motor in the field and becomes even more valuable when accompanied by modeling and simulations, generating a back and forth between model and experimental data. Overall, participants agreed that we should build on quantitative analyses and reinforce biophysical approaches to studying development. As optical and imaging tools increasingly allow us to manipulate tissues and cells, and to measure with greater precision biological and physical parameters, it is the novel interactions between fields of research, as well as the proper training of young researchers, that will set the pace of the advances to come. Thus, more meetings and workshops of this kind, as well as the study of other model systems (both biological and theoretical), will be instrumental in a fuller exploration of the biophysical processes that underlie development.

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Competing interests statement

The authors declare no competing financial interests.

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