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MEETING REVIEW

Reverse-engineering growth and form in Heidelberg

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ABSTRACT

The EMBO-EMBL Symposium 'Synthetic Morphogenesis: From Gene Circuits to Tissue Architecture' was held in Heidelberg, Germany, in March 2019, with 150 participants seeking to reverse-engineer embryogenesis, emphasizing quantitative simulation and the use of synthetic systems to test models. This highly dynamic, interdisciplinary mix of quantitative developmental genetics, bioengineering, synthetic biology and artificial life aimed to reveal how evolution exploits physical forces and genetics to implement the cell- and tissue-level decision-making required for complex morphogenesis.

KEY WORDS: Cells, Developmental biology, Engineering, Genetics, Modeling, Synthetic biology

Introduction

Understanding the basic principles by which complex organisms self-assemble, with the idea of reconstructing their component parts *ex vivo*, is a truly fundamental undertaking with applications across many disciplines. Effective control over biological form at multiple scales would revolutionize regenerative medicine, enabling repair of birth defects, traumatic injury, neoplastic defections from the normal body plan and perhaps even aging. The notion of building living systems has permeated mythology, literature and scientific progress over the centuries (Ball, 2011). These efforts saw a golden age in the 17th and 18th centuries, with the development of sophisticated automata in Europe (Riskin, 2016). Contraptions of flutists and, most famously, a duck that would eat, digest and defecate, designed by the famed Jean Vaucanson, created an illusion of living systems as mechanical contraptions of wires and metal plates. In some ways, these are precursors of artificial hearts, limbs and brains, but the field has come a long way toward consilience of efforts focused on the laws governing genes, cells, materials and information processing.

To build functional tissues and organs made of cells, we need to understand how cells do this in the organism and, in particular, what the relationships between genes and cell structure and behavior are. Furthermore, elucidating how genomes encode anatomy and the control circuits that implement dynamic pattern remodeling and robustness would shed crucial new light on cellular decision-making, multicellularity and evolution. If we achieve this, synthetic living machines with desired structure and function could be created (Kamm et al., 2018). Beyond living organisms, the hierarchical plasticity that underlies the cooperation of individual cells toward reliable organism-level anatomical outcomes could be exploited for the engineering of novel inorganic robotics and machine-learning architectures. How morphogenetic control is specified and implemented by biological systems, and how we can exploit this knowledge for novel applications, was the subject of this EMBO-EMBL Symposium organized by Stefano De Renzis (EMBL Heidelberg, Germany), Miki Ebisuya (EMBL Barcelona, Spain), Wendell Lim (University of California San Francisco, USA) and James Sharpe (EMBL Barcelona, Spain). This meeting followed others that, over the last few years, have aimed to capture the synergies that can emerge from the interaction of engineering with cell and developmental biology.

Meeting overview

Speakers showed off work that spanned many scales of size and levels of organization, using a truly unique mix of tools and variety of model organisms. A central theme, to which numerous talks at this meeting referred, is highlighted by the well-known quote by Richard Feynman: 'What I cannot create, I do not understand'. Although the speakers had rather diverging views on its interpretation, it served to focus attention on fundamental conceptual issues in this field.

What does it mean to create a morphogenetic system? Talks at this meeting spanned a continuum of pre-existing building blocks (cells, molecular assemblies and whole tissues) that are being exploited, revealing the range of levels at which 'creation' can take place.

What does it mean to understand a morphogenetic system? Is it sufficient to be able to exert highly accurate predictive control over shape and its deformations (as could be facilitated by current machine-learning approaches), or is it the case that, with sufficient effort, all of the mysteries of complex self-assembling and self-repairing systems will be expressible as straightforward human-understandable models? It is clear that, complementary to state-of-the-art molecular biology and imaging technologies, a workhorse of this field is computational modeling – mathematical analysis and simulation were as centrally integrated into the questions being asked as new molecular tools and reagents. Thus, a computational quantitative simulation is replacing the old 'arrow diagram' paradigm as explanation in this field.

Prominent concepts throughout the meeting included emergent self-organization (James Sharpe asked what complex system is not emergent?), positional information, noise and reliability in both natural and artificial systems, biological time, first-order shape (emergent morphogenesis) versus second-order shape robustness (stability and self-repair of specific shapes after perturbation), and evolutionary search. The opening talk by Wendell Lim set out many of the key topics and meeting themes, including the idea of deconstructing the rules and primitive operations of morphogenesis, not only to rearrange them, but also to construct novel ones.

Several speakers presented important methodological advances, including new cell types, materials, computational tools and culture systems that facilitate the study of physical forces. Seraphine Wegner (Max Planck Institute for Polymer Research, Germany), for example, showed novel optogenetic methods for reversibly controlling cell-cell interactions with tight spatio-temporal control, and Christopher Chen

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(Boston University, USA) presented new organotypic models that exploit control of stress and other physical properties of the microenvironment to build biomedically relevant tissues out of stem cells. Daniel Corbett (University of Washington, USA) exploited temperature as a control modality, using stereolithography bioprinting to make hydrogels in which control of fluid temperature regulates gene expression. Silas Boye Nissen (University of Copenhagen, Denmark) discussed new theoretical modeling approaches that help understand emergent tissue polarity and its integration with proliferation control, illustrating applications to gastrulation and the origin of folds and tubes. This theme was also addressed by Keisuke Ishihara (Max Planck Institute of Molecular Cell Biology and Genetics, Germany), who used a tissue reconstitution system to explore how chemical cues regulate the mechanical properties and 3D topology of epithelia.

The model systems, data and approaches could be classified along three orthogonal continua. One is the axis of basic science versus applications, exemplified by Jiandong Huang's (University of Hong Kong, China) investigations of the fundamental rules of self-organizing patterns in programmed bacterial colonies and Milica Radisic's (University of Toronto, Canada) work on human organoids for cardiovascular biomedicine, respectively. The second axis for the presentations concerns different degrees of micromanagement versus reliance on the system's self-assembly properties that were utilized or revealed; for example, Ron Weiss (Massachusetts Institute of Technology, USA) used microfluidics and synthetic transcriptional circuits to implement specific dynamics directly, whereas Anne Grapin-Botton (DanStem and University of Copenhagen, Denmark) tried for guided self-assembly by steering self-organizing properties of organoids. The third axis spans the range of study of specific developmental events, such as Sabina Kanton's (Max Planck Institute for Evolutionary Anthropology, Germany) work on the differences in single cell expression dynamics across various primate brain organoids, to the implementation of developmental principles in entirely novel media. The latter include biochemicals, such as Dora Tang's (Max Planck Institute of Molecular and Cell Biology and Genetics, Germany) engineering of synthetic coacervates, lipid vesicles and proteinosomes, and Joachim Spatz's (Max Planck Institute for Medical Research, Germany) use of microfluidics and DNA nanotechnology to recombine cell parts into synthetic endomembrane systems with organelles and cytoskeleton. The considerable diversity of work presented can be grouped according to the following five themes.

Modeling

Anna Kicheva (Institute of Science and Technology, Austria) used mouse spinal cord dorso-ventral patterning to understand how the sizes of neural progenitor domains are established. Coordination of BMP and Shh signals (gradients of which change over time) controls cell identity switches. Once the cell identities are established, the domains expand by anisotropic growth. When initially exposed to opposing signals, individual cells were seen to be decisive, allowing the decoding of position from the levels of the two morphogen signals. She demonstrated a three-node network for interpreting gradient encoding and computationally screened for parameters that reproduce stripes, discovering an optimal decoding strategy that maximizes precision. Naama Barkai (Weizmann Institute of Science, Israel) focused on the robust biological timer of neurogenesis in *Drosophila melanogaster*, which creates the necessary diversity of neurons. She showed the cycling and decay of repressors operating as part of this timer, suggesting that evolution selects circuits with minimal dependency on parameters. She searched for a circuit model in this large parameter space that was consistent with the data; comparing perturbations enabled her to identify the robust few. Miki Ebisuya continued the theme of time, by looking at the segmentation clock in human versus mouse organoids. Using a combination of modeling and experiments, she dissected the oscillating Notch components, discovering that different biochemical reaction speeds between human and mouse cells can explain the species-specific oscillation periods of the human (5 h) and mouse (2 h) segmentation clocks. Veronica Grieneisen (Cardiff University, UK) addressed plant patterning – a welcome and very interesting complement to the animal work, as plants have a defined anatomical structure of organs but not an invariant target morphology at the organism level as most animals do. Also, plant cells have to communicate indirectly via secretion through a cell wall. She stressed the environment as a key input into morphogenesis and discussed quantitative modeling of a one cell minimal symmetry-breaking circuit and how it might be scaled to the tissue level. She showed how coupling of polarity circuits of adjacent cells allows planar polarity and alignment of cell fields and used a shape analysis pipeline to test various mutants to reveal robustness to perturbation of the various components. Thomas Sokolowski (Institute of Science and Technology, Austria) constructed and analyzed a generic, yet uniquely biophysically accurate, model of (stochastic) gene expression dynamics in the *Drosophila* early embryo. By optimizing the information content of modeled transcriptional patterns *ab initio*, he showed that precision in transcriptional patterns can be achieved with simple biochemical processes and realistic parameters of developmental time and protein/RNA copy number. Showing that multiple solutions exist, their system makes predictions for evolutionarily plausible alternative embryo classes.

James Sharpe discussed local (reaction-diffusion) self-organization and global coordinate (gradient and positional information) systems in the vertebrate limb. His models of gene regulatory networks driving morphogenesis were augmented by 'analog models' – patterning via swarms of small robots with noise and limited sensing. This highlighted the importance not only of identifying elements of biological systems that are required for specific phenomena, but also of attempts to identify and test algorithms that are sufficient to implement them. It also takes the concept of synthetic morphogenesis beyond the purely biological realm – highlighting that morphogenetic principles may be universal enough to use in human engineering. Buzz Baum's (University College London, UK) simulated development demonstrated a remarkable result: evolving rules for cell behavior that matched only the criterion of ceasing growth at some point (having a developmental endpoint) is apparently sufficient to produce a regenerative system that self-repairs after damage (despite no selection for this specific functionality). This is a good example of how *in silico* models that are not based on specific quantitative datasets can suggest hypotheses about fundamental open questions in this field: for example, do regenerative mechanisms evolve as a separate capacity in some lineages or could they be an inevitable side effect of more basic morphogenetic rules?

Synthetic developmental biology

Mustafa Khammash (ETH Zurich, Switzerland) described a biomolecular synthetic circuit that, when expressed in living cells, implements integral feedback – a strategy that is widely used in engineering. He also demonstrated optogenetic feedback control of single cell behavior and intercellular communication, which can give rise to complex dynamic patterns among cell collectives. Marta Shahbazi (University of Cambridge, UK) studied cell-cell

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communication in the early human embryo, in which implantation causes a massive morphological change. She performed single cell RNA-seq of individual human embryos and characterized the signaling activity of the hypoblast. Based on these findings, she developed a 3D matrix stem cell co-culture to explore the crosstalk between embryonic and extra-embryonic stem cells. Further attempts to capture *in vitro* features of mammalian embryos were illustrated by presentations on blastoids by Nicolas Rivron (Hubrecht Institute and MERLN, Netherlands) and gastruloids (multicellular contraptions resembling different stages of early mammalian development) by Alfonso Martinez-Arias (University of Cambridge, UK). Meanwhile, Ron Weiss showed attempts to engineer directed differentiation. These talks highlighted how we can, and should, use the differences that currently exist between *in vitro* and *in vivo* systems to gain insights into how embryos build themselves.

Engineering

Wendell Lim presented a set of orthogonal molecular components that serve as a toolkit for programming the formation of tissue-level phenomena. By exploiting and re-engineering juxtacrine/paracrine signaling and adhesion proteins, he demonstrated the formation of several tissue-level patterning events that serve as the beginning of synthetic morphology. Benoit Sorre (CNRS Paris Diderot University, France) demonstrated *in vitro* recapitulation of early mammalian embryonic patterning by confining mouse embryonic stem cells to circular disks using micropatterning, showing how geometric confinement is sufficient to trigger self-organization and symmetry breaking in the colony at the tissue level, and also highlighting differences between mouse and human embryos. Zev Gartner (University of California San Francisco, USA) presented data on robust self-organization in the breast and gut, emphasizing how perturbations in these programs underlie diseases such as cancer. Milica Radisic showed organ-on-a-chip technology for drug discovery and testing. Using a variety of improvements in the materials (with microholes for endothelial cell sprouting) and combinations of electrical and chemical stimulation, she made cardiac tissues from induced pluripotent stem cells (iPSCs) and, crucially, 10% non-myocytes to get heteropolar (atrial versus ventricular) tissues to determine real-time force and calcium signaling mechanisms. These are being scaled up for screening personalized (patient iPSC) organoids to predict high blood pressure and to understand long-standing biological questions, such as why is metastasis to the heart so rare? Justin Crocker's (EMBL Heidelberg, Germany) work is beginning to uncover a 'grammar' in how DNA sequences drive three-dimensional patterns of gene expression, using minimal enhancers and engineered transcription factors to build synthetic enhancers from scratch and control the probability of expression and stripe formation. His pipeline of automated fixation/staining and automated image analysis was especially impressive, enabling high throughput screening of transcriptional pattern outcomes driven by instances of this DNA grammar and their relative positions.

Developmental biology

Darren Gilmour (University of Zurich, Switzerland) addressed resistance of tissues to change, by showing how the lateral line system of zebrafish moves through the embryo by a self-generated local gradient. A trafficking switch underlies the control of a chemokine scavenger to enable robustness, which he called an outsourced adaptation, as other cells help buffer noise for the migrating cells. Anne Grapin-Botton used pancreas organoids to show how cell fate is controlled by the content of the medium, and how cell number and type are intrinsically controlled. She showed a community effect to investigate quorum sensing versus master cell signaling mechanisms, and used network theory to study the architecture of the ductal network. Meritxell Huch's (University of Cambridge, UK) work on liver organoids as models of repair showed how slow turnover of differentiated cells maintains the most regenerative human organ. She studied errors in this process (such as the overproliferation of duct cells), from the signaling that triggers activation to the epigenetic changes required to acquire proliferative states. Xianghua Li (Centre for Genomic Regulation, Spain) explored the plasticity of gene interactions, by studying a minimal transcriptional repressor system to construct hierarchical mechanistic models of how perturbations in gene expression levels affect the functions and interactions of mutant variants.

Cells

Manuel Théry (Hôpital Saint-Louis, France) studied molecular motors, focusing on actin and microtubule self-organization under precise constraints or reconstituted in cell-free *in vitro* systems. He showed how myosins disassemble actin filaments and kinesins damage microtubules, which are then repaired by free tubulin. His emphasis on the energy usage of this system was especially interesting, showing how energy loss forces network turnover, resulting in adaptive repair. Moving to tissue-level organization, Stefano De Renzis used an elegant optogenetic system to understand invagination in *Drosophila*, mapping signaling inputs to shape change. Blue light-activated RhoGEFs, which cause myosin contraction, triggered controlled invagination and demonstrated how protein engineering at subcellular resolution (using a basal anchor for the myosin) can help sculpt anatomical features via precise control of biomechanical forces. Alison McGuigan (University of Toronto, Canada) presented work probing cell fate choice by physical forces. She showed how differentiated lung progenitor cells can be assembled into developmentally relevant tube structures using novel elastomeric materials and pharmacological manipulation of cytoskeleton-dependent stresses. In addition to physical forces, bioelectric signaling in non-neural cells was also represented. Michael Levin (Tufts University, USA) presented how propagation and control of standing patterns of resting potential in frog embryos underlies brain morphogenesis, and how computational modeling can help infer specific interventions to enable repair of brain shape and tadpole behavior under a range of genetic and teratological perturbations. He also showed that frog cells can leverage ion channel activity to break symmetry and self-organize into functional 'biobots' with anatomies and behaviors completely different from the standard frog embryo.

Conclusion

This was, in many ways, a very valuable meeting for this emerging new field (Kicheva and Rivron, 2017). As the field matures, we hope that continued development of tools – powerful simulation platforms for the genetic, physical force and bioelectric dynamics – should be standardized to facilitate the comparison of results across model systems. A 'bioinformatics of shape' should eventually emerge, to do for morphogenesis what BLAST and related tools have done for molecular cell biology.

A crucial area for subsequent research in this space concerns the fact that we really do not understand cells, despite significant progress on DNA dynamics and gene regulation. For example, some single cells (*Acetabularia*, *Caulerpa*, *Stentor* or even mammalian hair cells) exhibit exquisite patterning over a range of scales without the benefit of cell differentiation or cell-cell communication. Such

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single cell organisms use some of the same types of algorithms for spatial control during regeneration as vertebrate limbs (Nelsen et al., 1989), propagate pattern information across generations in physical structures other than DNA (Nelsen et al., 1989) and even execute problem-solving behaviors (Vallverdú et al., 2018). Continued progress in this field will require understanding the physical and computational capabilities of cells, their subcellular components and their multicellular swarms; it is possible that the cell is not a uniquely privileged level of control in biology. Perhaps we have to treat cells in a different way, exploring what they can do and harnessing their physical and computational properties to build tissues, organs and novel synthetic structures. Recent developments in information theory (the quantification, storage and communication of information) applied to biological regulation may enable better convergence of reductionist bottom-up and top-down approaches to understanding growth and form (Hoel et al., 2013; Kauffman and Clayton, 2006; Pezzulo and Levin, 2016).

Major areas for future study, in addition to those described above, include the range of the 'adjacent possible' (Kauffman, 1995) – the range of achievable alternative configurations of living forms that are compatible with genetics and the laws of physics. Exploring life not only as we find it, but life 'as it could be' (Langton, 1995), is an important future direction for the marriage of developmental biology and synthetic engineering that aims to probe areas of the biological design space that are not represented by the natural course of evolution on this planet.

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Competing interests

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