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MEETING REVIEW

Engineering life in synthetic systems

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ABSTRACT

The second EMBO-EMBL Symposium 'Synthetic Morphogenesis: From Gene Circuits to Tissue Architecture' was held virtually in March 2021, with participants from all over the world joining from the comfort of their sofas to discuss synthetic morphogenesis at large. Leading scientists from a range of disciplines, including developmental biology, physics, chemistry and computer science, covered a gamut of topics from the principles of cell and tissue organization, patterning and gene regulatory networks, to synthetic approaches for exploring evolutionary and developmental biology principles. Here, we describe some of the high points.

KEY WORDS: Developmental biology, Gene regulatory networks, Morphogenesis, Patterning, Synthetic systems

Introduction

Morphogenesis [from the Greek words μορφο (form/shape) and γένεσις (generation)] encompasses the set of processes by which tissues, organs and organisms acquire distinctive shapes (Slack, 1991). Throughout biology, form is intimately connected with function; acquisition of specific shapes is indeed necessary for tissues and organs to work properly (Lu and Werb, 2008). How this occurs is a fundamental question in biology with important implications in disease and regenerative medicine.

During embryonic development, morphogenetic events require precise spatial coordination of a repertoire of cellular behaviours, such as proliferation, polarized growth, oriented cell division, directional collective migration, differentiation and cell death (Gilmour et al., 2017). These processes are controlled in space and time by specific gene expression programs and gene regulatory networks (GRNs) (Davidson and Erwin, 2006). In addition to genetic and cellular inputs, mechanics play a fundamental role in shape and form acquisition during development. Although the mechanisms underpinning individual cell behaviour have started to be elucidated, we still know surprisingly little about how the cells cooperate together in building structures. Which mechanisms connect gene regulation, cellular effectors and tissue-scale mechanics? In addition, what is the hierarchical organization among 'morphogenetic effector modules' and how the same repertoire can be reused in different contexts *in vivo*?

Synthetic approaches that mimic embryonic development are now being used to study morphogenesis *ex vivo* and to answer these open questions (Gritti et al., 2021). Such bottom-up approaches, including organoids or synthetic stem cell-based embryos, offer the possibility to tease apart the different 'building blocks' and de-construct morphogenetic events, which is not possible *in vivo*. Moreover, these model systems are based on the inherent self-organization capacity of stem cells, providing an entry point to elucidate the principles associated with self-organized morphogenetic events. Finally, in addition to mimicking *in vivo* morphogenetic processes, recent advances in synthetic biology and genome engineering have opened up the possibility of directing morphogenesis towards novel ends and programing alternative biological shape and pattern-forming systems (Gritti et al., 2021).

State-of-the art approaches and future challenges for this nascent field, so-called synthetic morphogenesis, were the focus of this EMBO-EMBL Symposium organized by Justin Crocker (EMBL Heidelberg, Germany), Stefano De Renzis (EMBL Heidelberg, Germany), Dagmar Iber (ETH Zurich, Switzerland), Dora Tang (Max Planck Institute of Molecular Cell Biology and Genetics, Germany) and Vikas Trivedi (EMBL Barcelona, Spain). The meeting was extremely diverse and pushed beyond the boundaries of how synthetic approaches can be used not only as a toolbox for understanding development, but also for exploring the origin of life. A keyword to describe this meeting is 'multiscale': speakers discussed work that spans different scales from molecules to multicellular systems, such as embryos or tissues.

In each of the six sessions, the organizers brought together speakers from different fields combining theoretical and experimental approaches. Such a well-balanced mix served the purpose to learn each other's languages, stimulate discussion and reduce the separation between developmental biologists, chemists, physicists and engineers. Even though networking remains challenging in a virtual setting, this meeting showed that it is possible to still capture some elements of in-person events; e.g. through the 'meet the speakers session', and to enjoy a really valuable few days sharing ideas and listening to exciting science. The range of work presented can be grouped into the five following themes.

Evolutionary processes at the origin of biological complexity

Lessons learned from the evolutionary history of morphogenic processes have important implications for building novel morphogenic systems. Douglas Erwin (Smithsonian Institution, Washington DC, USA) kicked off the meeting with an inspiring talk on the emergence of novelty in biology. He focused on evolutionary diversification and proposed a conceptual framework to explain novelty and innovation: novelty corresponds to the genetic and developmental mechanisms that generate new characters, while innovation refers to the processes involved in the ecological and evolutionary success of a clade (Erwin, 2021). Thus, genomic and developmental novelties may arise well before they are used in morphological or phenotypic novelty. Moreover, he presented evidence suggesting that extensive gene co-option is at the origin of characteristic bilaterian features, including appendages, gut formation and segmentation (Erwin, 2020). Co-option was also discussed by Isabelle Peter (California Institute of Technology, Pasadena, CA, USA) in the context of evolutionary changes in

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GRNs underpinning cell fate specification and body plan organization. The GRNs controlling the specification of endodermal and mesodermal cell fates in the sea urchin embryo are almost completely solved, providing a unique model for investigation. She showed that GRN evolution can occur by rewiring pre-existing regulatory circuits and reconnecting them in a different way, while the GRN components (e.g. transcription factors) remain the same even between distant sea urchin species (Erkenbrack et al., 2018). To tackle the problem on a larger scale, her lab performed an *in situ* mapping of all transcription factors in the sea urchin genome at five developmental stages and defined their differential combinatorial expression states. Their findings will shed further light on how major novelties can arise in development and in evolution. Another example of how to use evolution to gain insight into the emergence of novelty in biology was presented by Viola Noeske (Lemke group, Heidelberg University, Germany). In her fascinating talk, she discussed how cell function and cell height are linked moving down the scale from tissue- to cell-level organization. By comparing two different fly species, Noeske and her colleagues identified a newly emerged Rho/F-actin regulator, which controls epithelial cell lengthening during evolution. Such crucial innovation is responsible for an increased barrier function of the epithelium.

How do natural patterns arise during development and how do these underlying mechanisms contribute to pattern evolution? Marie Manceau (Collège de France, Paris, France) studied natural variation in feather patterns by comparing different bird species, including chick and penguin. The Manceau team showed that the regularity of feather patterning depends on early cell shape anisotropy, which optimises cell motility and enables the precise localization of primordia formation. These results suggest a cellular mechanism through which self-organization is constrained, ensuring species-specific pattern fidelity (Curantz et al., 2021 preprint). A similar balance should be aimed in tissue-engineering approaches, combining initial self-organization to induce diversity with mechanisms to ensure stability and reproducibility.

Using *in vitro* reconstitution to study self-organization in biology

Reconstitution biology aims to build complex cellular and tissue structures *in vitro* from the bottom-up by using a minimal set of ingredients. Over the past decade, Petra Schwille (Max Planck Institute of Biochemistry, Martinsried, Germany) and colleagues have studied Min protein dynamics in a reconstituted system. Min proteins bind to and react on a supported lipid bilayer *in vitro*, generating self-organized spontaneous planar surface waves (Loose et al., 2008). Schwille argued that the MinDE protein system is not only a platform for studying pattern formation, but also has additional hidden functions. She showed how membrane-bound cargos can be sorted on the basis of their effective size (Ramm et al., 2021), providing an example of how synthetic systems can be engineered at will to achieve different functions.

With the advent of microfluidic technologies, it is now possible to generate synthetic lipidic compartments containing the desired components to recapitulate cellular processes *in vitro*. Wilhelm Huck (Radboud University, Nijmegen, The Netherlands) studied how the kinetics of cell-free gene expression is affected by macromolecular crowding in such lipid-based compartments, referred to as cytomimetic protocells. Huck showed that macromolecular crowding induces differential effects on transcription and translation kinetics, which in turn can lead to a switch from reaction-to-diffusion control that depends on the sizes of the macromolecules involved (Vibhute et al., 2020). Finally, cell-free systems also allow us to visualize and explore biological processes, which would otherwise be inaccessible *in vivo*. Jan Brugués (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany) reconstituted the process of single-molecule DNA loop extrusion in *Xenopus* egg extracts and found out that two different loop extrusion proteins (cohesin and condensin) extrude DNA loops in a cell cycle-dependent manner (Golfier et al., 2020). Additionally, he also showed that pioneer transcription factors, such as FoxA1, can mediate DNA condensation through capillary forces (Quail et al., 2020 preprint).

From signalling processing to developmental patterning in synthetic systems

Gene regulatory circuits in cells can be engineered to generate complex signalling processes. Ahmad (Mo) Khalil (Boston University, MA, USA) showed how non-linear regulatory circuits can be engineered using synthetic cooperative transcriptional assemblies in yeast. By studying GFP expression induced by transient doxycycline pulses, he showed that cellular networks are able to 'decode' input signals and respond according to the duration and frequency of the pulses (Bashor et al., 2019). In more recent work, Khalil's lab advanced similar approaches to design clinically-driven synthetic gene regulatory programs in human cells for the production of immunotherapeutics (Israni et al., 2021 preprint). Similar concepts were discussed by Mustafa Khammash (ETH-Zürich, Basel, Switzerland), who built gene regulatory circuits using optogenetic transcription factors in yeast. By engineering transcription factors to act as activators or repressors, a variety of synthetic circuits were constructed that respond selectively to different light input signals (Benzinger et al., 2021 preprint). These circuits were then used to demonstrate synthetic spatial patterns using light inputs. Finally, Yolanda Schaerli (University of Lausanne, Switzerland) showed how one can build synthetic oscillators, bistable networks or stripe pattern-forming systems in *E. coli*, using CRISPR interference (CRISPRi) (Santos-Moreno et al., 2020).

Although GRNs can be engineered from the bottom-up in simple systems such as *S. cerevisiae* or *E. coli*, such circuitry becomes increasingly complex in multicellular organisms, especially in the context of development. To what extent is a detailed knowledge of the circuitry necessary to understand cell differentiation and developmental patterning? James Briscoe (Francis Crick Institute, London, UK) presented a mathematical framework to understand cell fate decisions, reconstructing Waddington landscapes using flow cytometry data. Pluripotent mouse embryonic stem cells (mESCs) were exposed to several different conditions and their cell fates were further analysed using flow cytometry. By classifying the different cellular states and using 'catastrophe theory', the dynamic landscape could be reconstructed and the proportions of cell fates under different perturbations could be quantitatively predicted (Saez et al., 2021 preprint). This approach overcomes the issue of degeneracy in biological systems, a process by which different GRNs might lead to the same developmental landscape. Is this also true for Turing and positional information regulatory networks? Can Turing and positional information networks lead to similar patterns? Michael Stumpf (University of Melbourne, Australia) addressed this question by performing an exhaustive analysis of potential Turing (Scholes et al., 2019) and positional information patterns. He showed that both Turing and positional information networks have similar architectures, concluding that they are not as different as often assumed.

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Can we use the basic rules of development and synthetic engineering approaches to create new biological systems? Wendell Lim (University of California, San Francisco, CA, USA), a leader in the field of synthetic biology, started his talk by proposing that synthetic systems not only allow us to rebuild tissues from the bottom-up but also to create new systems, similar to how a unicorn can be made using paper-folding instructions in origami. He showed recent examples of the toolkit for synthetic multicellular development generated in his lab, including Synthetic Notch (synNotch) (Morsut et al., 2016), a synthetic system for engineering cell-cell adhesion (Toda et al., 2018) and a synthetic morphogen system using GFP (Toda et al., 2020). Using a similar approach, Kristina Stapornwongkul (Vincent group, Francis Crick Institute, London, UK) engineered a synthetic GFP morphogen in *Drosophila* wing primordia. She showed that when anti-GFP nanobodies are fused to the Dpp receptors, GFP can replace Dpp to induce patterning *in vivo* (Stapornwongkul et al., 2020). Finally, Leonardo Morsut (University of Southern California, Los Angeles, USA) presented the work carried out by Marco Santorelli, a postdoc in his lab, on how the synNotch system is affected by mechanical cues. He showed that signalling is not affected by the rigidity of the substrate, but rather by cell density. By controlling cell density in space and time he was able to show the emergence of waves of gene expression.

Engineering shape and form in synthetic systems

How do mechanical forces and the patterned expressions of genes couple to instruct shape and form during embryogenesis? Several speakers tried to tackle such questions from a physics perspective. Frank Jülicher (Max Planck Institute for the Physics of Complex Systems, Dresden, Germany) reported how the self-organized dynamics of active surfaces can help us understand morphogenetic processes. Using the theory of active fluids in deforming surfaces, he showed by means of a numerical approach how mechanochemical coupling can lead to complex deformations, shape oscillations and directed surface flows (Mietke et al., 2019). During the conference, lumen formation was a recurrent topic as a paradigmatic example of tissue morphogenesis. Virgile Viasnov (National University of Singapore, Singapore) recapitulated lumenogenesis by creating single-cell liver hemi-canaliculi using a synthetic approach. He demonstrated that the mere contact of the cells with the extracellular matrix and an immobilized cadherin layer is sufficient to establish apicobasal polarity (Zhang et al., 2020). Ariadna Marín-Llauradó (IBEC, Barcelona, Spain) from Xavier Trepat's group studied the mechanics of pressurized lumens using spherical epithelial domes of different sizes (Latorre et al., 2018). She showed that both stretching and bending forces are important for dome formation.

Cell and tissue stiffness are crucial in morphogenesis. The fluidity of tissues is controlled by well-known signalling pathways that control rheology both in space and time during embryonic development (Petridou and Heisenberg, 2019). François Fagotto (Montpellier Cell Biology Research Centre, Montpellier, France) explored how the Rho pathway controls cell stiffness during *Xenopus* gastrulation. He demonstrated that the downregulation of Rho kinases (Rock)-dependent actomyosin contractility triggers ectoderm-to-mesoderm transition (Kashkooli et al., 2021). Another paradigmatic example of drastic changes in cellular movements can be found during zebrafish development. By studying cell-cell connectivity dynamics during blastoderm spreading over the yolk, Edouard Hannezo (IST, Vienna, Austria) showed that fish blastoderm undergoes a rigidity phase transition whereby the tissue fluidizes during the doming stage (Petridou et al., 2021). This work represents one of the first *in vivo* examples of a tissue-phase transition in development. How can we study the fluidity of a tissue? A classic technique is to study the fusion dynamics of two multicellular aggregates (Gordon et al., 1972). David Oriola (Trivedi group, EMBL Barcelona, Spain) showed that mESC aggregates display partial, rather than complete, fusion, a phenomenon known as 'arrested coalescence' in soft matter physics. By modelling multicellular aggregates as viscoelastic drops, he was able to infer the mechanical properties of the aggregates from the fusion dynamics (Oriola et al., 2020 preprint). Finally, cells and tissues also adapt and respond to mechanical cues provided by their environment. One example is the growth of axon bundles in response to chemical and mechanical cues during the development of the nervous system. Alain Goriely (OCCAM, University of Oxford, UK) presented a beautiful analogy between durotactic axon guidance and optic ray theory. At the interface between two media of different rigidities, his theory showed how axon bundles can be guided by the equivalent of optical fibres made by regions of different stiffness (Oliveri et al., 2021).

Development in a dish

The close connection between developmental biology and stem cell research paved the way for bottom-up approaches, which recapitulate developmental events outside of an embryo. Stem cell-based synthetic embryos enable to 'reconstitute' early embryonic processes in a controlled fashion, unveiling mechanisms of mammalian development that are difficult to decipher *in vivo*. Additionally, synthetic embryos may be 'reconstructed' by culturing cells in contexts or combinations that diverge from natural embryogenesis. Future efforts in this direction will help to discover novel biological features that can eventually be connected back to actual embryo development. Both 'reconstituting' and 'reconstructing' synthetic embryos or multicellular structures have key implications for tissue engineering and cell-based therapy, as well as modelling human diseases. Speakers at the meeting discussed all these different approaches and related challenges.

Magdalena Zernicka-Goetz (University of Cambridge, UK; California Institute of Technology, Pasadena, CA, USA) showcased the astonishing progresses made by her lab on building synthetic embryo-like structures from the assembly of mouse embryonic and extra-embryonic stem cells. Such synthetic models have yielded important insights into the developmental mechanisms underlying self-organization, as well as symmetry breaking and axis formation during gastrulation. For example, she presented ongoing work from her lab, which points out a prominent role for cadherin-mediated adhesion in self-organization of embryonic and extra-embryonic stem cells into an embryo. Denis Duboule (Swiss Federal Institute of Technology Lausanne, Switzerland) showed how to make use of a synthetic embryonic system, such as gastruloids, as an analytical platform to address complex questions related to gene regulation. Gastruloids, formed from aggregated mESCs, undergo embryonic axial extension and recapitulate the sequential activation of clustered Hox genes (Beccari et al., 2018). He presented back-to-back *in vivo* mouse genetic experiments and fine molecular dissection in gastruloids performed by Célia Bochaton (a student in his lab) on a long intergenic region in the HoxB cluster. Although 'mESCs cannot be intercrossed', as Duboule stated, they enabled the rapid generation of a wide range of CRISPR-mediated genomic perturbations in a haploid background, which were then characterized in gastruloids. The purpose(s) of using synthetic systems was further discussed by

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Eric Siggia (The Rockefeller University, New York, USA) in the context of modelling human embryonic development. Indeed, synthetic embryo models have helped to shed some light on the mechanisms behind self-organization and axis formation in human embryos. Siggia presented the example of WNT–DKK1 as a key signalling pair that triggers symmetry breaking in a 3D model of human epiblast (Simunovic et al., 2019). New work from Siggia's lab extends that of Simunovic et al. (2019) by wrapping their model epiblasts with a layer of putative extra-embryonic cells, with markers suggesting a mixed population of primitive endoderm and trophoblast. These epiblasts show symmetry breaking that could be initiated by signals from the extra-embryonic layers.

Building from the bottom-up provides the knowledge and tools needed for rationally programming cells to form complex tissues for regenerative medicine. Francesca Spagnoli (King's College London, UK) focused on how to build pancreatic tissue units by assembling the different building blocks (e.g. cellular and non-cellular components). She discussed 3D cell printing approaches for simultaneously printing multiple cell types in patterned structures based on a mouse pancreas image atlas. This is a useful strategy for teasing apart how spatial patterning plays a role in tissue formation and is conceptually interesting to compare with self-organization. A fundamental problem in tissue engineering is the lack of vasculature within synthetic tissues. Takashi Miura (Kyushu University, Japan) addressed this limitation by developing a simple system for generating a perfusable capillary network *in vitro* (Sugihara et al., 2020). Finally, Alejandro Aguilera-Castrejon (Hanna group, Weizmann Institute of Science, Rehovot, Israel) described his recent work on advancing the conditions for *ex vivo* culturing of E5.5 to E11 mouse embryos (Aguilera-Castrejon et al., 2021). The possibility of growing mouse embryos outside the uterus opens up new possibilities to eventually test discoveries made from synthetic approaches in natural embryos.

Concluding remarks

In summary, the meeting perfectly captured the diversity of topics that converge in the field of synthetic morphogenesis. There was a special effort to move the discussion beyond the application of synthetic approaches to recapitulate the embryonic development. The emphasis was rather shifted on 'understanding the origin of life and its regulation', as stated by the meeting organizers in the opening session.

What lies ahead? The next challenge is to use synthetic approaches not only for probing morphogenesis, but also for directing it. Synthetic morphogenic systems allow us to explore configurations and biological functions not seen in evolution. If robust and performant, such novel logic circuits could be scaled up and adapted to multicellular human system models, with the potential to accelerate clinical translation of synthetic systems. Participants came away with a broader vision of this field, empowered by novel tools and fresh ideas, and very much looking forward to the next EMBO meeting on the same topic.

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Competing interests

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