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MEETING REVIEW

Creating to understand – developmental biology meets engineering in Paris

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ABSTRACT

In November 2016, developmental biologists, synthetic biologists and engineers gathered in Paris for a meeting called 'Engineering the embryo'. The participants shared an interest in exploring how synthetic systems can reveal new principles of embryonic development, and how the *in vitro* manipulation and modeling of development using stem cells can be used to integrate ideas and expertise from physics, developmental biology and tissue engineering. As we review here, the conference pinpointed some of the challenges arising at the intersection of these fields, along with great enthusiasm for finding new approaches and collaborations.

Introduction

Recent advances in *in vitro* stem cell organization have demonstrated the potential for obtaining new knowledge by re-creating embryonic tissues and organs outside of their context in the developing embryo. However, the systematic creation, manipulation and deciphering of such synthetic systems requires knowledge not only of stem cell biology and synthetic biology, but also of development and biophysics. The 'Engineering the Embryo' meeting, which was held in Paris in November 2016, thus came at an important time. Its organizers, Matthias Lutolf (EPFL, Switzerland), Alfonso Martinez-Arias (University of Cambridge, UK), Francois Schweisguth (Institut Pasteur, France) and Shahragim Tajbakhsh (Institut Pasteur, France), recognized that these fields closely intertwine and can benefit from dialog and exchange of ideas. Thus, the aim of the meeting was to bring together the knowledge and ideas of complementary fields and to formulate current challenges.

Designed control: insights from physics and engineering

The opening talk by Arthur Lander (University of California, Irvine, USA) was an excellent example of how engineering can be bridged to, and be useful in, developmental biology. Lander pointed out that engineering provides a wealth of knowledge on system properties necessary for carrying out specific tasks, and that this can be applied to understanding natural systems. Elaborating on principles of control theory, he focused on how integral feedback control provides robustness to tissue growth. Such control could occur, for example, via mechanical feedback (e.g. Shraiman, 2005) or via the control of lineage progression, in which cells at later steps in the lineage can signal back to earlier cell populations through secreted factors (Lander et al., 2009). Lander also discussed the trade-offs that such strategies may have, for example the range of tissue sizes that can be sensed or the sensitivity to noise in the rate of cell loss (Buzi et al., 2015). Overall, the presented examples illustrated how an engineering theoretical framework can be used to systematically generate testable predictions and understand control during development.

Continuing the theme of applied theoretical approaches, Peter Zandstra (University of Toronto, Canada) discussed the application of computational modeling to understanding the establishment of patterns in synthetic systems. He focused on simulations of the gene regulatory rules governing early pluripotent stem cell commitment (Onishi et al., 2014), as well as on the interplay between chemical and physical factors in the establishment of patterns *in vitro* (Peerani et al., 2007), including those recently described by Warmflash et al. (Etoc et al., 2016; Warmflash et al., 2014). Crucially, Zandstra outlined the ambitious and challenging questions that lie ahead. For example, can we turn simulations and data into discovery and design? How can we interface synthetic and endogenous control (as recently started by Qiao et al., 2014)? Can the spatial and temporal scales observed in development be aligned with those in simulations? If the goal of regenerative medicine is to have practical applicability in real systems, these become important issues that need to be addressed.

Regulatory circuits in cells and embryos

Following the opening talks motivated by engineering, several presentations started from a point of view of *in vivo* embryonic development and applied principles from physics and *in silico* simulations to these problems. James Sharpe (Centre for Genomic Regulation, Barcelona, Spain) focuses on understanding the growth and patterning of the vertebrate limb. Recent work in his group established the existence of a Turing-like mechanism operating in digit patterning (Raspopovic et al., 2014), which radically changed pre-existing views. This research has benefited not only from physics and mathematical simulations, but also from *ex vivo* assays in which cells could be dissociated and re-aggregated, demonstrating the emergence of pattern and constraining the underlying mechanisms. Thus, it was a slightly surprising turn when Sharpe declared that "there is more to life than physics". He emphasized that the details of the physical components are not important and illustrated this with 'swarm robotics' (Jansson et al., 2015 preprint): miniature robots can follow the same rules as limb cells and self-organize in similar patterns. This comparison reflects the current approach to studying developmental systems, which still relies on reducing them to tractable modules. However, Sharpe argued that this is not enough. Much like understanding a language is not simply about knowing a collection of phrases, it is the connections and feedbacks between modules that we need to focus on if we are to understand the 'language' of development.

Picking up on this theme were presentations from Andy Oates (Francis Crick Institute, London, UK) and Ani Kicheva (IST Austria),

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both of whom reflected on what has become a trend in developmental biology in recent years, of drifting towards more integrative approaches, across cell and tissue scales. Kicheva presented work in progress on the patterning of the spinal cord by opposing morphogen gradients. Previous studies have focused on the establishment of pattern either in the ventral or in the dorsal part of the spinal cord, but new quantitative data from mouse embryos is allowing this question to be addressed at the level of the whole organ (Kicheva et al., 2014). Together with *ex vivo* assays in which explants of neural tissue are cultured in defined morphogen concentrations, these data have provided insight into how cells respond to simultaneous exposure to multiple morphogen signals. This led to the proposal that cells use a mechanism, called maximum likelihood decoding, to interpret gradients and minimize patterning errors.

Andy Oates presented compelling new data on the mechanisms underlying the establishment of periodicity in vertebrate segmentation. A long-standing question in the field has been whether the oscillatory behavior of progenitors in the presomitic mesoderm (PSM; the tissue that gives rise to somites) is cell intrinsic or requires extrinsic factors, for example rhythmic signals from neighboring cells. The ability to dissociate and culture individual cells of the zebrafish PSM has provided the answer (Webb et al., 2016): isolated individual cells can oscillate in culture. These data suggest that cell-cell communication and signaling gradients are not required to produce noisy oscillations, although these factors might be key for the precision of pattern and ensuring that cells keep a regular period. Future work in the Oates lab aims to investigate the nature of the pacemaker circuit and the control of oscillation dynamics.

Similar to other developmental biologists at the meeting, a biophysical theoretical framework for studying development has been central to Oates' research. The success of this approach has relied on technological advances in quantitative and dynamic imaging of developing embryos (Oates et al., 2009). The recent advances in engineering of micropatterns, fluidic systems and stem cell technologies are further strengthening the developmental biology toolbox. Indeed, Oates' talk was a good example of how *in vitro* assays of developmental systems offer new opportunities for experimentally probing these systems and addressing questions that are hard to tackle *in vivo*.

The use of *in vitro* experiments to understand development cannot be discussed without mentioning the name Austin Smith (Wellcome Trust-MRC Stem Cell Institute, Cambridge, UK). Smith's research perhaps best exemplifies the inherent link between stem cell and developmental biology. In his talk, he discussed how iterative comparison between *in vivo* embryonic development and *in vitro* capture of stem cells resulted in a concept of naive and primed forms of pluripotency. He then pointed out that the two-day developmental time progression between naive and primed epiblast in mouse, as well as the regionalization of cells as they are specified, support the view that pluripotent cells transit through a distinct 'formative' phase, rather than existing in a condition of reversible metastable pluripotency (Smith, A., 2017). This developmental sequence is masked at the population level in heterogeneous embryonic stem cell cultures, but may be revealed by analysis of distinct subpopulations of cells during the time course of exit from naive pluripotency (Kalkan and Smith, 2014; Smith, 2017).

In vitro models of development and disease

Some of the most remarkable studies in the last decade, following the steps of seminal work from Yoshiki Sasai's group (Eiraku et al., 2008, 2011) and as presented by Mototsugu Eiraku (Riken CBD, Kobe, Japan), have revealed the intrinsic capacities of embryonic and somatic stem cells to organize into 3D structures – so-called organoids – that recapitulate aspects of development. Sasai rightly noted that this intrinsic capacity to self-pattern offers new possibilities to manipulate development systematically *in vitro*, to test its limits beyond the natural ones (Sasai et al., 2012). This approach sheds new light on *in vivo* development and provides avenues of research for regenerative medicine. However, defining experimental strategies to induce, control or scale-up these cultures, and form full organs or organisms is challenging. Here, biologists and engineers shared their successful strategies to predict and increase reproducibility and purity in the production and organization of cell types.

Inducing the polarized patterning of organoids in a controlled way is likely to be a prominent trend in the field in the next few years. It extends an overall effort to establish conditions for reproducible morphogenesis of organoids that can allow more developmentally advanced stages of organogenesis to be recapitulated *in vitro*. Branching morphogenesis is an aspect of this that was touched upon by Jason Spence (University of Michigan, USA) in the context of lung organoids and by Anne Grapin-Botton (DanStem, Copenhagen, Denmark) in the context of pancreas organoids. The lung organoids derived from human stem cells resemble fetal lung tissue, but remain immature in culture (Dye et al., 2015). Spence reported that growing these organoids in synthetic microporous scaffolds enhances their ability to mature and survive when transplanted into mice (Dye et al., 2016), but is in itself insufficient for branching. Identifying conditions for the establishment of branching in these organoids is an ongoing challenge and the culture of embryonic lung explants might provide useful hints in that direction.

Grapin-Botton demonstrated how dispersed progenitors from embryonic day 10.5 mouse pancreatic bud can be expanded into 3D epithelial structures containing both endocrine and exocrine cells (Greggio et al., 2013, 2014). When they reach a certain size, the pancreatic organoids form ductal trees and branches and, in that sense, appear to be more similar to lung tissue than previously thought. Grapin-Botton also discussed the factors needed for the efficient formation and growth of these organoids. Careful monitoring of specific combinations and ratios of progenitors within microwells by live imaging revealed that heterogeneity in the levels of Notch signaling is essential. These data highlight the advantages of *in vitro* systems in allowing systematic assessment of parameters such as the number and ratios of specific cell types, which cannot be easily manipulated *in vivo*. Importantly, this work provides a foundation for the development of human pancreatic organoids and cellular therapy for diseases such as diabetes.

The use of synthetic systems to model human development was further exemplified by Ali Brivanlou (The Rockefeller University, New York, USA). In collaboration with Eric Siggia, his team reported the generation of self-organized patterns of human embryonic stem cells on micropatterned substrates, which are reminiscent of the germ layer patterns of gastrulating embryos (Warmflash et al., 2014). Brivanlou presented a recently published study (Etoc et al., 2016) unraveling the mechanism of self-organization in this system. Besides the differences in subcellular localization of TGFβ receptors at different positions within the colony, an important aspect is a reaction-diffusion system created by bone morphogenetic protein (BMP) and its inhibitor Noggin. Brivanlou emphasized that BMP induces the production of Noggin in human cells, but not in mouse, reminding us that we cannot always extrapolate the knowledge of mouse development to human systems and underscoring the advantages of *in vitro* human models.

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Synthetic models of human systems have strong medical relevance, which was made clear in several talks. As one of the first stem cell systems recapitulating development, the optic cup presented by Eiraku has gone a long way and is currently being used for transplantation in primate models of retinal degeneration. Also with a clear goal in regenerative medicine, Guo-Li Ming (Johns Hopkins University, Baltimore, USA) presented an efficient method for producing brain organoids using a two-step procedure, which involves the differentiation of human induced pluripotent stem cell (iPSC)-derived embryoid bodies into neuroepithelium of forebrain identity in Matrigel, followed by 3D suspension culture. When combined with a custom-engineered spinning bioreactor, this protocol achieved a more stereotypical, better defined and reproducible type of brain organoid (Qian et al., 2016) that builds on previous protocols (Lancaster et al., 2013). Ming and colleagues have also used such brain organoids to validate candidate Food and Drug Administration (FDA)-approved drugs identified in high-throughput screens, and discovered molecules that inhibit Zika virus infection or suppress infection-induced caspase-3 activity in different neural cells (Xu et al., 2016). The use of organoids for disease modeling was further discussed by Meritxell Huch (Gurdon Institute, Cambridge, UK). During her postdoctoral time, Huch used adult stem cells to grow organoids from liver ducts (Huch et al., 2013). This technology can be transferred to human cells and shows that, similar to other organoid systems, the liver organoid system can be used to model monogenic diseases such as alpha-1-antitrypsin deficiency and holds great promise to model other diseases and reveal novel and personalized therapeutic strategies.

From these examples, it becomes clear that synthetic systems require the use of new technologies, developed by engineers. Such tools presented during the meeting include: micropatterned substrates (Warmflash et al., 2014) and microwell arrays (Rivron et al., 2012b) for the spatial constraint of a small number of cells in 2D and 3D, respectively; high-throughput screening (Vrij et al., 2016) to test large numbers of combinations of growth factors or drugs; high-content imaging and machine/deep learning to extract predictive features from images (Jones et al., 2009); and bioreactors to improve diffusivity and homogeneity in larger tissues (Qian et al., 2016). These platforms are crucial to increase control, and reproducibility, and to explore these systems systematically.

Synthetic circuits

Although exploiting the properties of stem cells has led to new insights, synthetic systems are often at the mercy of cell-intrinsic, noisy processes, which are difficult to manipulate. A rising aspiration is therefore to control these systems in a much more defined way. Synthetic biologist Wendell Lim (University of California, San Francisco, USA) presented genetic tools to design orthogonal biological functions within cells. Among the many tools developed in his lab, he focused on the use of a multicellular communications system based on synthetic Notch molecules (synNotch). These molecules induce gene expression at heterotypic cell contacts by cleaving and releasing an endogenous signaling molecule (e.g. a transcription factor). SynNotch thus generates cascades of user-defined local functions (Morsut et al., 2016) and will undoubtedly be useful to control symmetry breaking and induce patterning, with potential medical applications (Roybal et al., 2016). Notably, synthetic biology can also provide powerful ways to design transcriptional programs (Chavez et al., 2015) or synthetic logic circuits within cells (Green et al., 2014), which will help to manipulate and understand the basic rules of encoding structures during development.

Implementing tissue engineering strategies

Increasing the complexity or scaling-up synthetic systems will necessitate the implementation of strategies previously developed by tissue engineers. Indeed, the interdisciplinary field of tissue engineering has been developing over the last two decades by applying principles from engineering and life sciences to the development of biological substitutes that restore, maintain or improve the function of tissues or organs (Langer and Vacanti, 1993). The field has developed numerous approaches to combine stem cells with biomaterials (e.g. functionalized hydrogels; Gjorevski et al., 2014) and biomimetic environments (e.g. microfluidic-based bioreactors; Andersson and van den Berg, 2004), and thus has a lot to offer. At the meeting, we heard about the advances in this evolving field.

A classical problem in tissue engineering is to form vasculature within tissue implants that can rapidly connect with the host vasculature upon transplantation. Shulamit Levenberg (Technion, Israel) and others have previously shown that this can be achieved by inducing the *in vitro* organization of endothelial cells within engineered muscle (Levenberg et al., 2005), pancreatic (Kaufman-Francis et al., 2012) or bone (Rivron et al., 2012a) tissues. She now showed how cell-induced and externally applied tensile forces pattern the behavior of endothelial networks (Rosenfeld et al., 2016). In addition to its classical transport function, the vasculature also provides cues for the development and patterning of organs (Red-Horse et al., 2007). Levenberg explained how trophic functions of tubular vascular structures produce key signals to cardiomyocytes and pancreatic progenitors (Caspi et al., 2007; Kaufman-Francis et al., 2012). As previously proposed by Yoshiki Sasai (Cyranoski, 2012), vascular networks, possibly arising from embryonic progenitors, might bring key functions to organoids and synthetic embryos.

The influence of biomaterials and drug delivery systems on the development of stem cell-derived tissues is another area in which progress is being made. Todd McDevitt (Gladstone Institutes, San Francisco, USA) is examining the phenotypic effects of biomaterial incorporation in stem cell aggregates. He showed that the aggregates respond to the presence of degradable gelatin microparticles by increasing the activity of matrix metalloproteinases and promoting mesenchymal morphogenesis (Nguyen et al., 2016). The incorporation of microparticles, as performed by McDevitt's lab, opens up the possibility of influencing self-organization from 'within' a growing structure. Given that, so far, *in vitro* developing systems only interact with their environment on their surface, this approach might become important for designing new ways of accessing cells located deep inside organoids.

Finally, in an inspiring talk, Zev Gartner (University of California, San Francisco, USA) exploited the mechanics of developmental programs to design large 3D tissues that fold autonomously. Specifically, he leveraged a quantitative understanding of cell tractions generated in the mesenchyme to drive the autonomous folding of extracellular matrix gels into complex shapes. This approach exemplified how tissue engineering methods can be used to form large systems with predictable macro-scale behavior.

Concluding remarks

The atmosphere of the meeting was one of excitement and enthusiasm, as the promise of collaboration between fields created the feeling that the challenges ahead are difficult but not insurmountable. The tissue engineering field is advancing technically and tools are becoming increasingly available. However, it is clear that to make organoid development more

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robust, reproducible and 'organism-like', synthetic biology and tissue engineering approaches have to build on the knowledge of developmental biology, synthetic biology and physics. In turn, this offers an opportunity for developmental biologists to gain knowledge from synthetic systems, making engineering the embryo a win-win enterprise.

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Competing interests

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