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Synthetic developmental biology: build and control multicellular systems

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Synthetic biology offers a bottom–up engineering approach that intends to understand complex systems via design-build-test cycles. Embryonic development comprises complex processes that originate at the level of gene regulatory networks in a cell and emerge into collective cellular behaviors with multicellular forms and functions. Here, we review synthetic biology approaches to development that involve building de novo developmental trajectories or engineering control in stem cell-derived multicellular systems. The field of synthetic developmental biology is rapidly growing with the help of recent advances in artificial gene circuits, self-organizing organoids, and controllable tissue microenvironments. The outcome will be a blueprint to decode principles of morphogenesis and to create programmable organoids with novel designs or improved functions.

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Introduction

A single zygote is capable of generating the whole organism through a process of cellular differentiation, proliferation, inter-cellular communication, and self-organization. Substantial level of information that encodes this process is hardwired in the cellular DNA and is presented in the form of complex gene regulatory networks (GRNs). Decoding how a single cell is progressively programmed to generate a complex tissue, distinct organs or the final organism with high degree of robustness and spatiotemporal control has been the holy grail question in developmental biology. In contrast, synthetic biology aims at designing and building biological systems. Novel artificial proteins, signaling pathways, GRNs, organelles, and even cells have been created or redesigned so far. Now, in the emerging field of 'synthetic developmental biology', researchers start combining developmental biology with synthetic biology: they build and control developmental processes to better understand them or to improve their functionality. Although the methods and purposes of synthetic developmental biology are versatile, here we classify them into three major categories:

1 Reconstituting developmental mechanisms to test our current knowledge.
2 Recapitulating self-organizing morphogenetic processes to study them *in vitro*.
3 Controlling developmental trajectories of stem cells to create multicellular systems with improved functions.

Reconstituting developmental mechanisms through building artificial gene circuits

During embryonic development, cells interact with each other, self-organizing into complex tissue architectures. To better understand the complex processes of multicellular development, one approach is to recreate or reconstitute the essence of developmental mechanisms as artificially as possible [1–5]. Such a reconstitution can test the sufficiency of current understanding of the mechanism of interest as well as lead to unexpected discoveries. As making an artificial cell is still at early stages, the most popular strategy for reconstituting a developmental mechanism is to create an artificial gene circuit that implements cell–cell communication in a cell line that does not have an equivalent endogenous cell communication system.

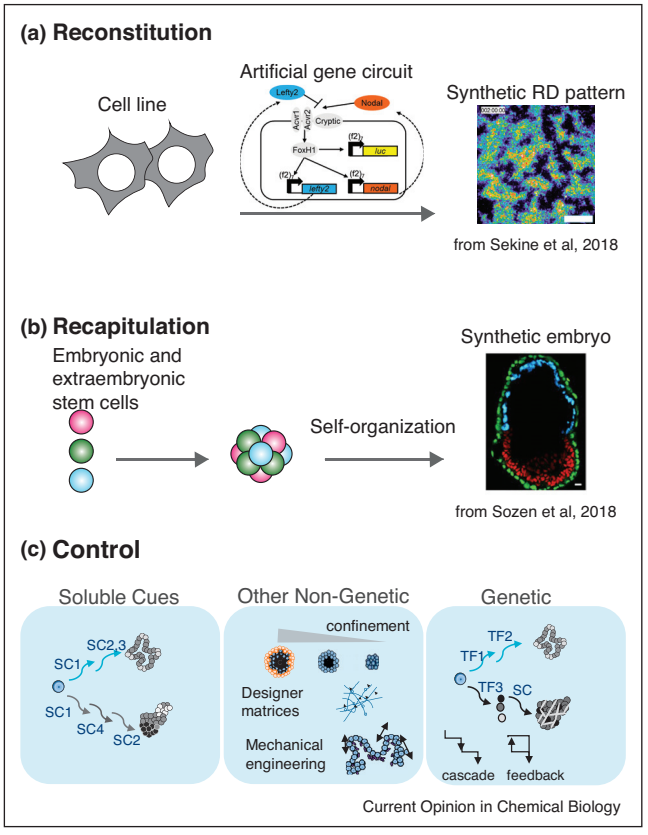
A leading example is synthetic gene circuits for self-organized patterning (well summarized in a recent review [6]). Pattern formation is one of the most important principles of development, and the reaction-diffusion (RD) system [7–9] originally proposed by Alan Turing is considered to underlie a number of developmental periodic patterns, such as animal skin patterns and digits in the limb [10–12]. In 2011, a synthetic RD gene circuit that regulates motility of the engineered bacteria through diffusion of the small molecule AHL was created, giving rise to a clear stripe pattern of the bacterial density [13•]. While this work was a milestone, two challenges remained: The classic RD system for pattern formation is based on the interaction of two morphogens with different diffusivity, the slowly diffusing activator and

the rapidly diffusing inhibitor [7–9], instead of one small molecule that regulates cell motility. Secondly, a synthetic RD pattern has not been achieved in mammalian cells. Quite recently, the two challenges have been addressed. Karig *et al.* created another pattern forming gene circuit in bacteria that comprises two diffusible small molecules, 3OC12HSL (the slow activator) and C4HSL (the rapid inhibitor), displaying a spot pattern in an initially homogeneous bacterial lawn [14••]. Although the parameter condition for RD pattern formation, so called Turing instability, is hard to achieve experimentally, the authors have shown that the condition can be relaxed by considering the stochasticity of the activator–inhibitor dynamics, and proposed that their synthetic bacterial pattern is a 'stochastic Turing pattern'. Sekine *et al.* successfully created a mammalian synthetic RD pattern by using two diffusible ligand proteins, Nodal and Lefty [15••]. The reconstituted Nodal–Lefty signalling in a mammalian cell line showed differential diffusivity of Nodal (the slow activator) and Lefty (the rapid inhibitor), giving rise to a spot pattern in an isogenic mammalian cell population (Figure 1a).

Another way for self-organized patterning relies on direct interaction of adjacent cells rather than diffusible molecules. Delta-Notch signaling is activated upon direct cell interaction, and lateral inhibition, the mutual inhibition of Delta-Notch signaling between adjacent cells, has been known to underlie the formation of salt-and-pepper patterns of two different cell-types in several organs, including the inner ear, lung, and intestine [16,17]. To reconstitute this patterning mechanism, a synthetic lateral inhibition circuit was created in a mammalian cell line, and it spontaneously gave rise to two different cell-types [18•]. Direct cell interaction coupled with differential cell adhesion can lead to cell rearrangement and sorting: the difference in adhesion strength between different cell-types causes phase separation of the cells [19]. Such a differential adhesion-mediated cell sorting was implemented by overexpressing different Cadherins in two cell populations and then mixing them [20•]. Furthermore, Toda *et al.* recently combined the lateral inhibition and cell sorting mechanisms to create a multilayered three dimensional (3D) pattern in a programmed manner [21••]. A synthetic Notch receptor (synNotch) [22] was used to harness Delta-Notch signaling to Cadherin expressions. Combining different modules of developmental mechanisms like this will be the key to reconstitute more complex morphogenesis in the future.

Another popular theme in synthetic biology is oscillations and their intercellular synchronization [23–25]. Oscillations also play an important role in developmental biology: the timing of body segments formation in vertebrates is regulated by the synchronized oscillatory gene expression called the segmentation clock [26,27]. Synthetic segmentation clock-like oscillations based on a delayed negative feedback have been reconstituted in mammalian cells [28•,29], and thus the next challenge will be to synchronize the reconstituted segmentation clocks among the engineered cell. Other interesting examples of reconstituting developmental mechanisms include the *in vitro* transcription–translation network that mimics the sequential expression of Drosophila gap genes [30•] as well as the synthetic morphogen-sensing system reconstituted in a mammalian cell line to test different network architectures [31••]. These reconstitutions have not only proven the sufficiency of current morphogenesis theories but also provided novel discoveries regarding the design

Figure 1



(a) An artificial gene circuit that reconstitutes a reaction-diffusion (RD) system of Nodal–Lefty signaling in mammalian cells gives rise to a spatial pattern of Nodal-reporter positive regions and negative regions. Modified from Sekine R *et al.*, Nat Commun, 9, 2018.
(b) A mixture of mouse embryonic stem cells (ESCs), trophoblast stem cells (TSCs), and extra-embryonic endoderm (XEN) cells can self-organize into an embryo-like structure, offering a unique opportunity to study early developmental processes *in vitro*. Picture from Sozen B, Nat Cell Biol, 20, 2018.
(c) Establishing control during *ex vivo* tissue development from stem cells can employ variety of non-genetic or genetic strategies. These methods span from simple addition of soluble cues at different times to designer matrices and mechanical manipulations. Engineering gene circuits using transcription factors provide foundation for genetic control in complex tissues. SC, Soluble Cue; TF, Transcription Factor.

principles of the gene network architectures and dynamics.

Recapitulating morphogenesis through self-organization of stem cells: organoids and synthetic embryos

Whereas the works described above aim at reconstituting developmental mechanisms by building artificial gene circuits, another synthetic approach to developmental biology is recapitulating developmental processes *in vitro* through self-organization of stem cells. Recapitulation, unlike reconstitution, does not require prior knowledge of the mechanism itself, and often involves seeking for a suitable culture condition that triggers differentiation and self-organization of stem cells and their progenies by modulating developmentally inspired growth factors stimulation in a 3D culture. Using this strategy several types of stem cell derived self-organizing multicellular systems have been produced [32]. Among them, organoids are 3D stem cell cultures that recapitulate some key structures and functions of organs [33,34]. Wide variety of organoids are now available, including the intestine, brain, kidney, liver, and lung organoids, and they are an ideal platform to study organ formation and to model human diseases.

Similarly, recent studies have succeeded in recapitulating key developmental processes seen in early embryos with 3D cultures of pluripotent stem cells (PSCs). Here, we call such embryo-like structures derived from PSCs as synthetic embryos (well summarized in recent papers [35–37]). An aggregate of mouse embryonic stem cells (ESCs) briefly stimulated with Wnt signaling starts elongating and self-organizes into a gastrulating embryo-like structure, termed gastruloid [38••,39,40•]. Similar symmetry breaking events can be caused with a human ESC colony geometrically confined on micropatterned substrates [41••,42•]. Furthermore, to overcome the challenge that currently available PSCs cannot differentiate into extraembryonic lineages, researchers started combining PSCs with extraembryonic stem cells: Rivron *et al.* demonstrated that mouse ESCs and trophoblast stem cells (TSCs) can self-assemble into a blastocyst-like structure, termed a blastoid [43••]. Sozen *et al.* mixed mouse ESCs, TSCs, and extra-embryonic endoderm (XEN) cells to mimic a gastrula stage embryo more accurately [44••,45•] (Figure 1b). Because synthetic embryos and organoids are formed from stem cells *in vitro*, they are more amenable to imaging and experimental manipulation than *in vivo* embryos.

Recapitulating even later stages of development may be possible: Mouse and human neural tube organoids successfully recapitulated patterning along the dorsal–ventral (DV) axis [46•,47]. The synchronized oscillation of the segmentation clock was recapitulated by using mouse and human PSCs [48•,49,50]. Interestingly, the oscillation period of the recapitulated human segmentation clock was 5–6 hours whereas that of mouse was 2–3 hours [48•,49,50]. Thus, these recapitulated systems offer unique opportunities to study human development and even compare developmental mechanisms of different animal species in the same culture conditions.

Controlling developing tissues in space and time

In every system, control is an important objective. In fact, it is an actual proof of our full understanding of the dynamics of the system, and eventually it is instrumental for improving robustness and performance. *In vitro* practices to develop organoids and synthetic embryos are not an exception. Efforts to establish control during the morphogenetic events of organoids can advance our understanding of principles governing tissue formation, eventually enabling to steer through more accurate cell identity and cellular assemblies that can fulfil desired functions [51]. In longer term, this capacity may provide us with fully synthetic programmable superorganoids that are engineered with novel tasks that were not dictated by the evolution-based design of nature [4,52].

A simple form of control in self-organizing stem cells is reflected in experiments where addition of niche related soluble cues mimics embryonic development and regulates the final phenotype [53]. By controlling the timing, duration, order, and magnitude of exposure to developmental cues, various organoids representing an organ (i.e. lung, intestine) [54,55,56••] or regions of a given organ (i.e. forebrain) were created [57]. However, achieving high degree of robustness, native cellular populations with accurate genetic signatures, and mature developmental stages are often challenging. While the field started by simply exploring stem cell self-organization and addition of soluble cues in culture media, these initial approaches lack key features such as spatial control. Hence, integration of other engineering techniques might be necessary to overcome existing hurdles. We can catalog current efforts in controlling morphogenesis in two classes: non-genetic and genetic control strategies (Figure 1c).

Non-genetic control starts with manipulation of an input that is extrinsic to the cellular entity known as a part of tissue microenvironments. These inputs can span across multiple scales such as ECM composition, soluble growth factors, physical and mechanical cues as well as bioelectrical properties. Inspired by design and construction of cell-instructive material in tissue engineering, new studies switched matrigel, an animal-derived complex ECM, with chemically defined hydrogels that customized to support tissue-specific organogenesis paths *ex vivo* [46•,58••]. Such an approach is a stepping stone towards designer ECMs in organoids that are tunable, reproducible, and highly dynamic. Bioengineered constructs such as PLGA microfilaments also enabled elongated cerebral

organoids that exhibited higher surface area to volume ratios and subsequently improved neuroectoderm formation [59]. Geometric confinement was employed to control the gastrulation-like behavior and neural rosette emergence as well as patterning of neuroectoderm tissue, highlighting the importance of control over cell density, position, and colony size during stem cell derived multicellular morphogenesis [41••,60,61]. Through usage of a microfluidic stretching device, cell shape and mechanical force have been shown to play a role in BMP signaling that influences self-patterning of the neuroectodermal tissue [61]. Albeit not in the organoids, an exciting study by Hughes *et al.* provided an engineering strategy for tissue folding via controlling mechanical compaction of tissue mesenchyme [62••]. These studies together provide a first wave of non-genetic approaches to better steer self-organization in stem cell derived multicellular systems.

The unique structure of pluripotent gene regulatory network provides a driving force for cascades of transcriptional regulation that steer cells towards different states, cell sorting, higher order organization, pattern establishment, and generation of tissue forms and subsequently functions. Therefore, it is expected that manipulation of gene regulatory network of cells will provide a powerful approach to program tissue morphogenesis in space and time [51]. To this end, recent studies have exploited native transcription factors to steer self-organization of PSCs towards organoids such as thyroid (via overexpression of NKX2.1 and PAX8) [63] and thymus (FOXN1) [64]. Engineering GATA6 heterogeneity in PSCs also resulted in symmetry breaking and the development of three germ layers that self-organized in the form of multicellular fetal liver organoids with adjacent neuroectodermal tissues [65••]. Engineering gene circuits provides the opportunity to control cellular fate in a given cell population using cell type specific promoters [66] or miRNAs [67•] or allows for interfacing cellular fates with microenvironmental contexts [68]. Additionally, optogenetics can provide powerful control over engineered gene circuits to program cell–cell interactions, force transmission, and morphogenesis [69]. Most studies so far employed a single open loop gene circuit that is expressed via an inducible promoter across several cell types. More advanced strategies such as cell type specific promoters, engineering inherent endogenous gene regulatory networks [70,71], and gene circuits with feedback regulations or layered designs [72] can provide a promising avenue to program *in vitro* morphogenesis of organoids. To this end, by coupling synthetic gene cascades and transcription factor-based gene switches, Saxena *et al.* produced a programmable lineage control network that can make stem cells differentiate into homogenous population of beta-like pancreatic cells [73•]. While this effort focused on generation of a single population of cells, similar strategies can be applied in the context of complex multicellular systems such as organoids.

Concluding remarks

One clear future direction of synthetic developmental biology is to engineer more complex, diverse multicellular systems. Towards this goal, combining different modules of gene circuits [21••] and assembling multiple organoids that represent different regions of an organ [74] are promising approaches. As current organoids and synthetic embryos stop growing at a certain size limit, supplying oxygen and nutrients with perfusion will also be crucial [75•]. To make a larger gene circuit, furthermore, automation of circuit design may be useful [76]. There are also several emerging areas to control morphogenesis: for instance, engineering tissue bioelectrical properties has shown a powerful top–down approach to rewire regeneration and tissue formation *in vivo* [77].

Another challenge of synthetic developmental biology is to improve the accuracy and predictability of engineered systems. To achieve this goal, a full understanding of each network components and their dynamics of interaction is essential. Computational modeling should also be useful for quantitative understanding and prediction.

Building and controlling multicellular systems will enable decoding principles of development, producing novel tissues for regenerative therapy, and modeling human diseases for drug discovery. Thus, we expect nothing but excitement of innovation and discovery in the emerging field of synthetic developmental biology.

Conflict of interest statement

Nothing declared.

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