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Synthetic development: learning to program multicellular self-organization

Satoshi Toda, Jonathan M. Brunger and Wendell A. Lim

Abstract

Recent advances in genetic engineering technologies have enabled our ability to construct artificial genetic circuits that drive user-defined cellular functions in mammalian systems. In this review, we discuss how we can engineer intercellular communication networks to orchestrate complex multicellular collective behaviors leading to formation of tissues and organoids. This bottom-up engineering approach is called 'synthetic development,' and the field aims to elucidate how genetic programs direct formation of multicellular structures to improve our understanding of the design principles of tissue development. By leveraging this knowledge with the programmability of customized molecular toolkits, synthetic development will provide new capabilities to engineer complex tissues and organoids with desired functions for cell-based regenerative medicine applications.

Addresses

Department of Cellular and Molecular Pharmacology, Howard Hughes Medical Institute, Center for Systems and Synthetic Biology, University of California San Francisco, San Francisco, CA 94158, USA

Corresponding author: Lim, Wendell A (wendell.lim@ucsf.edu)

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Keywords

Morphogenesis, Collective behaviors, Self-organization, Cell-cell communication, Regenerative engineering, Organoid.

Introduction: engineering perspectives on development

All multicellular animals, even a large elephant, emerge from a single fertilized cell that can differentiate into a multitude of diverse cell types that spatially self-organize into three-dimensional tissue architectures. All the genetic circuits necessary to develop complex tissues and organs are written and stored as genomic DNA programs in the single cell. One of the biggest mysteries in biology is how such compact DNA programs can encode algorithms that allow individual cells to build complex macroscale structures by themselves.

To address this question, the development of model organisms has been intensely studied. Over the last century, genetic studies have revealed regulatory networks that play important roles in tissue development. Recently, great advances in optics, imaging, and computational power have enabled dramatic studies of development at extraordinary spatiotemporal resolution. For example, custom light sheet microscopy enables direct observation of mouse development from gastrulation to early organogenesis at the single-cell level [1]. Such imaging technologies allow us to track cell divisions, individual cell fates, and dynamic spatial distribution of cells during development, leading to better understanding of embryo-wide events of development. Another great discovery is organoid culture systems that guide stem cells to develop into mini-organs *in vitro* [2]. Organoid systems allow us to easily access and dissect the organogenesis processes and also have enormous potential in applications for disease models, drug screening, and regenerative medicine [3].

While such elegant studies have produced detailed roadmaps of the events orchestrated during development, we still lack an understanding of the functional coherence of the signaling networks that are activated in individual cells during the collective's morphogenesis into a whole organism. To complement these advances in our ability to observe and map details of development, a different approach is needed to generate a bottom-up understanding of how genetically encoded algorithms can drive multicellular structure formation [4–6]. From this vantage point, researchers take a synthetic biology approach to engineer a cell 'chassis' capable of executing genetically encoded functions but lacking particular programs. User-defined genetic programs are deployed within the cell to attempt to drive morphogenetic behaviors (self-assembly, differentiation, migration, and so on) of the engineered cells. Using genetically encoded molecular tools to manipulate cell behaviors, we can try to write artificial programs that determine development of new higher order structures. Progress in the field of synthetic biology has allowed us to encode distinct morphogenetic modules [7]. By hierarchically assembling distinct modules to forward engineer behaviors, synthetic morphogenesis may mature along a trajectory reminiscent of the emergence of biological phenomena

in evolution [8,9]. While the current toolkit allows us to define how cells assemble, perhaps in the future, we will have improved control over specifying cell fate divergence and, ultimately, whole-organ assembly. Such synthetic systems would provide new insights into developmental biology, physiology, and disease.

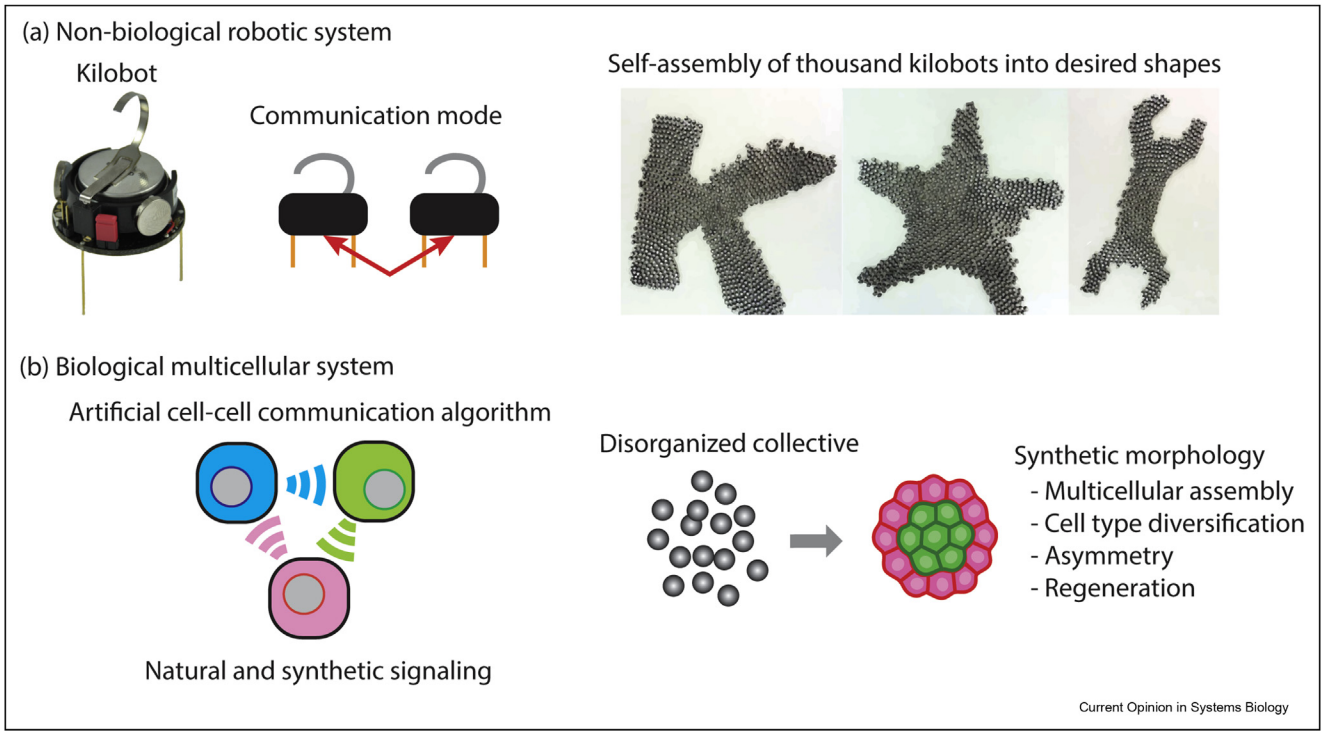
Lessons from collective systems

Development is a microscale multicellular collective behavior where diverse cells cooperate to generate complex tissues and organs. Such collective behaviors are not unique to the processes of development; rather, they are easily found in nature. Examples include macroscale animal collective behaviors such as flock formation of birds and sociality of ants. These collective behaviors can arise without any central control, instead generated by local interactions among individual animals [10]. From them, we can learn how we can code multicellular collective behaviors from the bottom up. Computational studies of swarm behaviors can show what kind of interactions between individuals can give rise to dynamic shapes [11–13]. In addition, recent advances in robotics illustrate the complexity that can be achieved by orchestrating the behaviors of relatively simple components in a larger collective. For example, Kilobots are engineered robotic swarms composed of one thousand small robots (Figure 1a [14]). The robots communicate by infrared light pulses and travel via vibration motors. Each individual unit is autonomous, yet through programmable algorithms outlining basic organization principles, this ensemble of unreliable individuals can self-assemble into two-dimensional shapes robust to variability. Here is a lesson on how we can code multicellular collective behaviors from the bottom up: first we need to define channels by which the individuals communicate and interact with one another; second, we can test algorithms that control how well the collective can attain a desired, steady-state assembly. In a similar way, we can encode the rules guiding cooperative, local, and large-scale cell–cell interactions that enable the assembly of tissues and organs from individual cells (Figure 1b). In the case of animal development, cells within an organism are programmed with the same code but implement divergent, spatially and temporally gated morphogenetic routines.

Cell–cell communication links: toolkit of natural and orthogonal channels for engineering collective self-organization

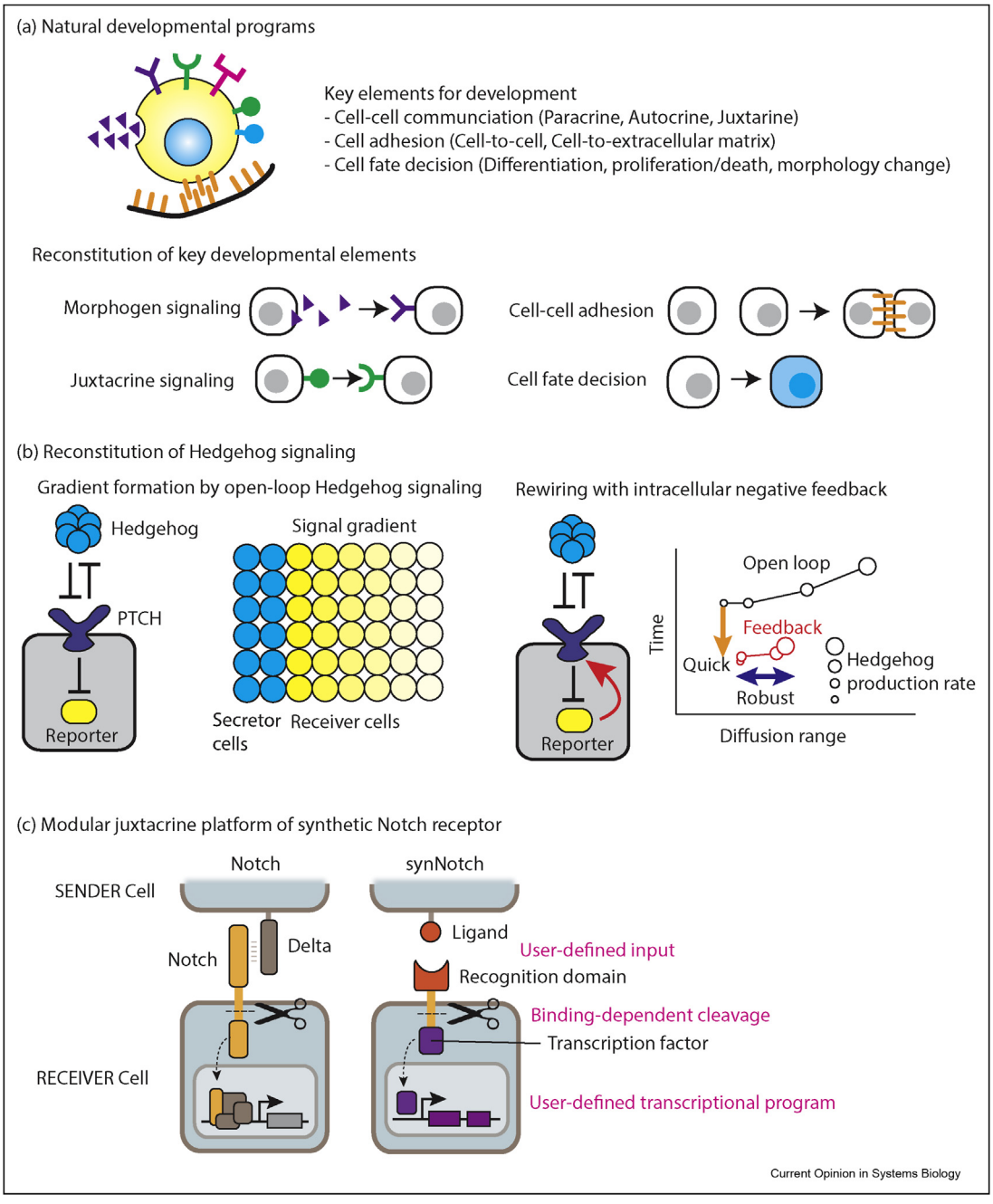
While a vast number of specific biological routines are executed during development, the mode of transmission

Figure 1



Forward engineering of collective systems. (a) Robotic swarms with artificial communication algorithms. Kilobots can communicate with neighbors by reflecting infrared light off the table below to decide how they move according to a user-designed communication algorithm. Here are examples of collective emergent patterns with a swarm of 1024 Kilobots (adapted from Rubenstein et al., 2014). **(b)** Engineering of cell–cell interactions to drive multicellular self-organization. Applying the idea of Kilobot study to the microscale cell–cell communication, we test how artificial algorithms of cell–cell interactions can program disorganized cells to behave collectively and self-organize into particular structures. The processes of synthetic self-organization could include key features common with natural developmental systems such as self-assembly, cell type diversification, symmetry breaking, and regeneration.

Figure 2



Natural and orthogonal cell–cell communication links. (a) Reconstitution approach against complex natural cell–cell communication systems. Stem cells express key molecular elements for tissue development such as cell–cell communication, cell adhesion, and cell fate specification. Reconstruction of specific elements in cell culture systems allows isolation of the signaling pathway, quantitative analysis and perturbation, and rewiring new signaling circuits. **(b)** Reconstitution of Hedgehog signaling pathway. Blue and yellow cells represent Hedgehog-secreting cells and receiver cells, respectively, and plated in a distinct region. The emergent varying opacity of the yellow signal corresponds to the gradient of reporter activity (left). In this reconstitution system, a synthetic intracellular negative feedback loop has been tested for gradient formation with a variety of Hedgehog production rate. The feedback loop made the gradient formation quicker to reach steady state and more robust against the amount of Hedgehog production (right) (adapted form Li, Markson et al., 2018). **(c)** Modular juxtacrine platform of synthetic Notch receptor. While native Notch receptor binds to Delta to drive natural gene expression programs, synNotch receptor can recognize user-defined ligand input and output expression of user-defined target genes (adapted from Morsut, Roybal et al., 2016).

of cell–cell signals is critical for morphogenetic programs (Figure 2a). Autocrine and paracrine communication with secreted morphogens occurs within the framework of multiple network architectures, including positive feedback, negative feedback, and feedforward loops [15]. The output of such functions depends on numerous factors, including the relative abundance and diffusion range of soluble morphogens and their antagonists. In turn, the interaction of morphogens with the extracellular matrix (ECM) or cell surfaces can impact signal transmission [16]. As such, morphogens supply positional information and/or participate in reaction–diffusion systems that govern critical developmental events [17–20].

Information flow in development also occurs via juxtacrine signaling. Cell-substrate interactions governed by focal adhesions or cell–cell interactions mediated by adherens junctions transduce biophysical microenvironmental features into biochemical signaling cascades [21–24], whereas gap junctions facilitate bioelectric signal transmission [25]. The Notch receptor represents a highly conserved signaling platform capable of transforming cell–cell contact into gene expression with regulatory features of signal strength and directionality [26]. These modes of communication allow cells to respond to the dynamic changes of the structure and composition of their microenvironments and play important roles in establishing cell polarity, guiding cell migration, and determining cell fate and function.

We can develop a quantitative framework to better understand the molecular programs active during morphogenesis through synthetic reconstruction of minimal signaling modules (Figure 2a). The signal propagation of the diffusible morphogen Hedgehog was reconstituted in a mammalian system (Figure 2b [27]). Engineered Hedgehog-secreting cells were cultured with engineered Hedgehog-responsive cells in adjacent, contiguous regions. Hedgehog diffused within the cell layer to form a graded pattern of signaling activity. Systematic perturbation of signaling pathways revealed that the intracellular negative feedback regulation results in a shortened time to establish a steady gradient and improved robustness of gradient formation against changes in the Hedgehog amount.

The Notch signaling system is also well studied in reconstituted systems. On binding to membrane tethered ligand (Delta or Jagged family proteins), mechanical strain results in exposure of protease cleavage sites on the transmembrane and intracellular domains of Notch. Protease cleavage results in liberation of the Notch intracellular domain, which translocates to the nucleus to modulate target gene transcription ([28], Figure 2c). Reconstitution of the Notch-Delta signaling pathway enables quantitative analysis of important morphogenetic features of Notch signaling—*trans*-activation and *cis*-inhibition. These synthetic approaches have revealed that *cis*-interactions between Notch and Delta generate an ultrasensitive switch of the cell status between sender cells (high Delta/low Notch) and receiver cells (high Notch/low Delta) [29]. The reconstitution experiments have also shown that the Notch receptor can discriminate between distinct ligands, which trigger either pulsatile or sustained activation to generate distinct gene expression programs [30].

To establish novel cell–cell communication channels that control customized cell sensing and response pathways, we have developed a synthetic Notch (synNotch) receptor ([31], Figure 2d). The synNotch receptor is composed of an extracellular recognition domain (e.g. single chain nanobody) that also contains a transmembrane domain incorporating the native Notch-core regulatory domain, including protease cleavage sites. Within the synNotch platform, the intracellular domain is an artificial transcription factor that can drive expression of target genes from cognate promoters (e.g. the tetracycline-responsive element or upstream activation system). Therefore, when it recognizes its cognate ligand on a neighboring cell, the synNotch receptor undergoes cleavage at the transmembrane region, leading to the release of the intracellular transcription effector domain to enter into the nucleus and modulate transgene expression. Thus, we can design synthetic cell–cell communication programs with user-defined ligand input and gene expression output without crosstalk to native signaling pathways.

Very recently, synthetic adhesion toolkits have been developed in bacterial systems [32]. In addition to natural adhesion molecules, these orthogonal cell–cell adhesion toolkits would increase our composability to create more complex multicellular assemblies.

In short, to study natural cell–cell interactions, we can use native linkages in different ways by isolating each signaling pathway in reconstituted systems. We can also design orthogonal links that recapitulate natural links, allowing more straightforward engineering without crosstalk with or interference from natural signaling pathways.

Creating organizational subroutines

By uncovering the molecular underpinnings of cell signaling logic in development, we can begin to compile new code to implement artificial signaling networks in synthetic development. We can ask how synthetic systems can be built to execute certain essential morphogenetic subroutines: what are the minimal components required to program multicellular assemblies? how is asymmetry established within an ensemble of cells? how do progenitor cells interpret signals to diverge into appropriate fates? how can multicellular systems

regenerate on injury? how do cells build specific structures such as sheets, tubes, and patterns? Here, we elaborate the significant achievements that have been made in developing predictive models of pattern formation and in the programmable assembly of multicellular structures.

In recent years, there has been significant progress in *in vitro* developmental systems that model embryogenesis [33]. For example, coculture of three cell types—mouse embryonic stem cells, trophectoderm stem cells, and extraembryonic endoderm cells—leads to the spontaneous self-assembly of embryo-like structures [34,35]. These structures undergo anterior–posterior patterning and exhibit features of epithelial–mesenchymal transition along with specification of mesoderm and definitive endoderm—hallmarks of the key spatiotemporal events of gastrulation (Figure 3a). Simpler systems composed of only mouse embryonic stem cells stimulated for 24 h with a Wnt agonist leads to self-organization of 'gastruloids,' which establish the three major body axes [36,37]. Pluripotent stem cells can also be cultured in two-dimensional micropatterns to recapitulate germ layer formation. This is achieved by activating specific signaling pathways or regulating cortical tension and cell–cell adhesion [38,39]. These achievements enable the ability to study highly complex gene regulatory circuits involved in development and morphogenesis. Although minimal requirements to form embryo-like patterns and structures remain to be studied, the technology development for the spatial organization and differentiation of embryonic cells will lead to understanding of the molecular mechanisms underlying embryogenesis and will facilitate the engineering of biomimetic artificial tissues useful for regenerative medicine.

Using reconstituted cell–cell signaling systems, we can design and build artificial signaling circuits to regulate cell fate. The lateral inhibition circuit is a natural bistable circuit that induces cell-type bifurcation [40]. Using the Notch-Delta system, the lateral inhibition circuit has been reconstructed in CHO cells, which normally do not laterally inhibit one another [41]. The Notch-based lateral inhibition circuit showed spontaneous bifurcation into Notch-active/Delta-negative and Notch-inactive/Delta-positive cell populations to form a checker board pattern (Figure 3b). It was also shown that the ratio of the two cell populations could be tuned by modulating the degree of cell–cell adhesion and by incorporating positive feedback regulation of Notch in the circuit architecture.

Using the synNotch system, we have engineered multicellular self-organizational programs in which cell–cell signaling networks control three transcriptional outputs: cell adhesion molecules to control spatial cell sorting [42–44], fluorescent proteins to indicate cell fate divergence, and new synNotch ligands to produce layered signaling cascades (Figure 3c [45]). We demonstrated that a multistep signaling cascade that generates varying degrees of cell adhesion yielded the stepwise formation of a three-layer structure from a random mixture of two interacting cell populations. By introducing different adhesion molecules that induce cellular phase separation, we also generated diverse asymmetric structures. These synthetic self-organizing processes reproduced key hallmarks of natural developmental systems: cell type divergence, symmetry breaking, and regeneration on injury [45]. These results show the flexibility and power of the modular synthetic system to program self-organizing structures. In addition to differential adhesion, many other mechanisms have been proposed for symmetry breaking during embryogenesis, and these remain to be studied in reconstituted or synthetic systems [46].

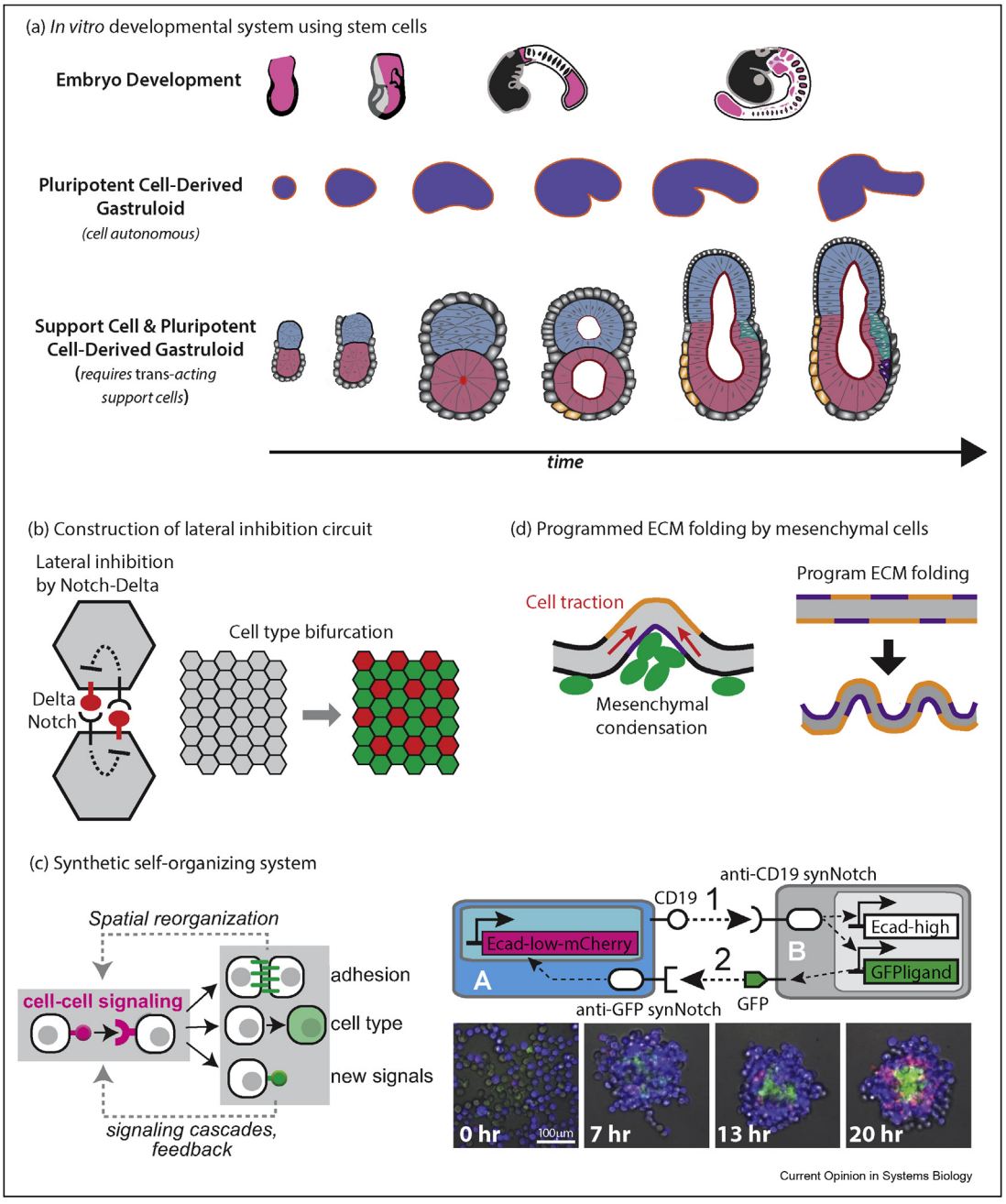
Using bacterial quorum sensing systems, synthetic sensing and secreting platforms can be constructed. Such programs recapitulate pattern formation, including stripe or spot patterns, based on positional information and reaction diffusion [47–49]. Periodic stripe patterns can also be achieved by controlling cell motility coupled with the capability to sense cell density [50]. Synthetic orthogonal morphogen systems and synthetic control of cell density and mobility in mammalian systems remain to be established, but such platforms would represent powerful tools engineer cells capable of autonomously establishing patterns and developing spatially organized structures.

In addition to structure formation based on cell–cell communication, the ability to control interactions between cells and engineered substrates contributes greatly to our ability to understand and program morphogenesis [51]. Mesenchymal cell condensates can induce traction forces and mechanical compaction of the ECM. By controlling spatial patterning of mesenchymal cells, we can program folding of the ECM (Figure 3D [52]). Also, it is shown that mechanical properties of ECM are critical for stem cell proliferation and organoid formation processes [53]. Integrating defined features of ECM or materials in engineered cellular sense and response platforms would allow us to program more complex three-dimensional tissue architectures.

Perspectives

To address fundamental biological questions on how cells generate tissues, organs, and organisms, there are multiple powerful approaches (genetics, microscopy for cell tracking, organoids, and forward engineering of cell signaling networks) that can reveal different features in the developmental system. Convergence of these approaches can lead to better understanding of developmental trajectories and programming of new developmental processes.

Figure 3

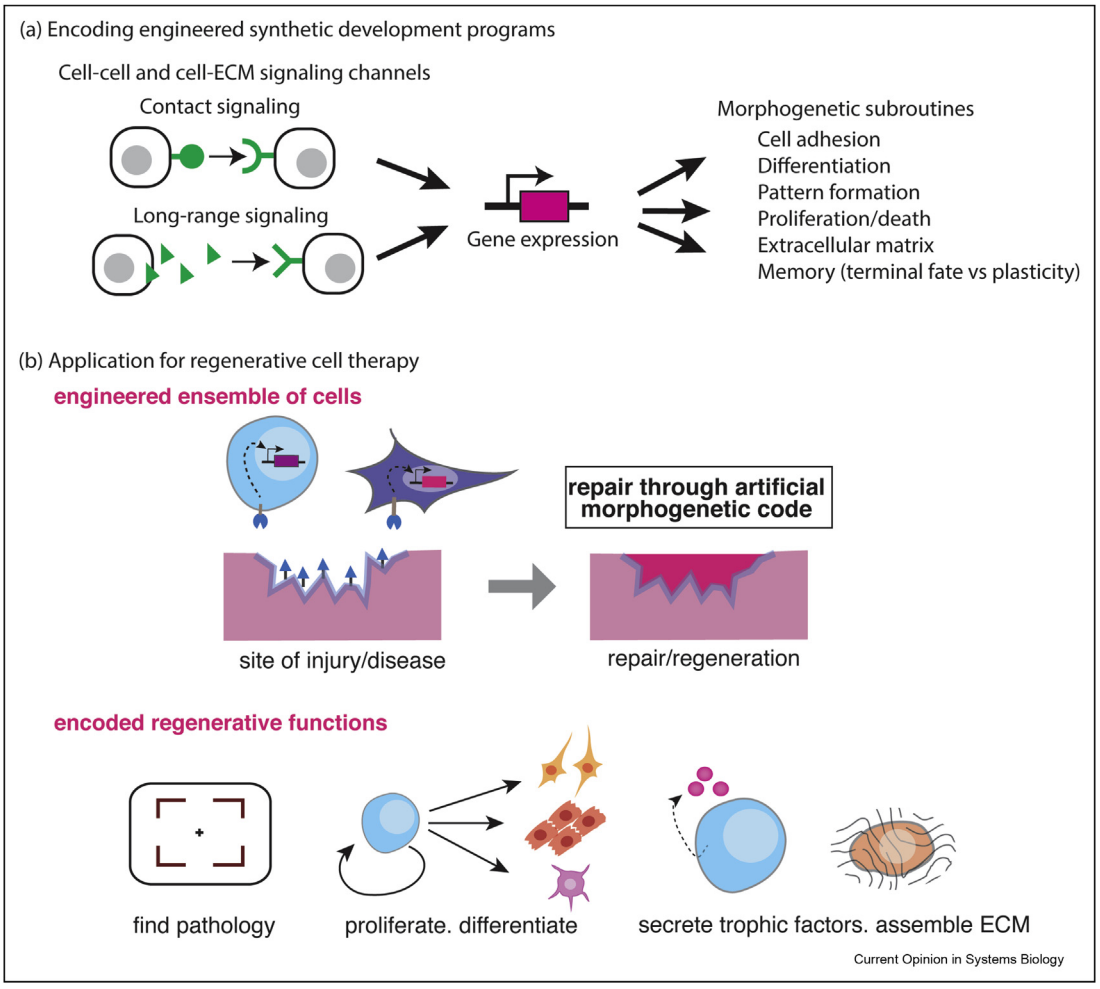


Creation of self-organizing systems. (a) Methods for modeling development in in vitro systems inform our approaches to testing and developing basic artificial signaling routines. Embryonic stem cells display the capacity to self-organize and establish mimetics of the three major body axes after a single pulse of Wnt activation (top and middle, adapted from Beccari et al., 2018). Early coculture with extraembryonic cells allows such in vitro systems to recapitulate the structures formed in natural embryogenesis (bottom, adapted from Sozen et al., 2018). **(b)** Construction of the lateral inhibition circuit using Notch and Delta. The mutual repression circuit of Delta by Notch between neighboring cells has been constructed in a cell line that has almost no endogenous expression of Notch and Delta. Cells spontaneously bifurcated into two cell states to form checker board pattern (adapted from Matsuda et al., 2015). **(c)** Self-organizing multicellular structures programmed with synthetic cell–cell communication. SynNotch-based cell–cell communication induced three types of outputs: cadherin-based adhesion for cell sorting, fluorescent proteins indicating the cell type, and new synNotch ligands for signaling cascades. Induction of cell sorting and new synNotch ligand can subsequently be propagated to generate new cell–cell signaling relationships (left). The design of cell–cell communication that robustly generates three-layer spheroids. First signaling by CD19 ligand induced GFP ligand and Ecadherin (high) to form a green core and blue outer layer (13hr). Delayed second signaling by induced GFP ligand activated Ecadherin (low) and mCherry, inducing the sequential formation of a red middle layer (20hr) (adapted from Toda et al., 2018). **(d)** Programming tissue folding by mesenchymal cells. Mesenchymal condensates can generate traction forces on ECM to compact it and drive curvature at tissue interfaces. The positions of mesenchymal condensates can encode complex curvature profiles, allowing us to program tissue folding (adapted form Hughes et al., 2018).

Natural developmental systems contain complex codes that enable the programmed assembly of sophisticated tissues and organs [54–56]. To fully understand the critical design features of how to build these structures, we require the ability to rewrite the code. That is, we need to write the code to understand the code. For this, we need to develop a suite of molecular tools that control cell–cell signaling and morphogenetic subroutines. Thus, we can program cell–cell communication codes to drive multicellular collective behaviors (Figure 4a). In this way, we hope to not only reverse engineer development, we hope to drive synthetic development by forward engineering new collective cell behaviors. To do this, the field will capitalize on recent advances and continue to develop more capabilities to understand and control morphogenesis. As our understanding of the molecular mechanisms underlying fate establishment and degrees of plasticity are refined, our ability to repurpose those networks is also improving. In the future, we expect to be able to dynamically encode memory and plasticity into synthetic systems. Moreover, as advanced lineage tracking tools based on CRISPR-Cas9 and next-generation sequencing [57,58] reveal the specific nodes of divergence cells encounter as synthetic structures are assembled, we can engineer improved gene circuits to control fate selection within the parameter space defined by the temporal development of the structure.

Engineering-based approaches are commonly deployed across the basic science disciplines to establish rule-based synthesis of fundamental principles. In turn,

Figure 4



Developing a coding language for synthetic development and its application. (a) Encoding engineered synthetic developmental programs. The cell–cell or cell–ECM signaling channels including contact signaling and long-range signaling control gene expression to induce morphological outputs such as adhesion, differentiation, pattern formation, proliferation, ECM, and fate decision (terminal or plasticity). Using these molecular toolkits, we can mimic natural developmental processes and also test non-natural cell–cell communication networks to build designed tissues. **(b)** Advances in synthetic development can lead to improved cell-based regenerative medicine therapies. Cells programmed to target particular diseases can coordinate repair through a variety of functions that lead to remodeling of a target microenvironment and support of native cells that can participate in repair.

chemistry defines rules for putting together atoms into molecules; molecular biology defines how molecules become cells; now, we are working on rules for how cells can be put together in new relationships. The elucidation of these basic rules can lead to better understanding of sufficiency for tissue development and provide new ways to build synthetic tissues. In addition to synthetic tissues, we can use these tools to program cells to sense specific signals in damaged tissues and follow natural or engineered programs that direct regeneration of complex tissues, such as blood vessels, endocrine organs, or the musculoskeletal tissues, to emerge (Figure 4b). Thus, advances in the synthetic development area represent promising avenues for developing cell-based therapies for regenerative medicine to solve unmet needs for tissue repair and organ transplantation.

Conflict of interest statement

W.A.L. has a financial interest in Gilead Biosciences.

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- •• of outstanding interest

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