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Abstract

Bioengineering can address many important needs, from transformative biomedicine to environmental remediation. In addition to practical applications, the construction of new living systems will increase our understanding of biology and nurture emerging intersections between biological and computational sciences. In this Review, we discuss the transition from cell-level synthetic biology to multicellular synthetic morphology. Importantly, we highlight experimental embryology studies, including organoids and xenobots, that go beyond the familiar, default outcomes of embryogenesis, revealing the plasticity, interoperability, and problem-solving capacities of life. In addition to traditional bottom-up engineering of genes and proteins, design strategies can be pursued based on modelling cell collectives as agential materials, with their own goals, agendas and powers of problem-solving. Such an agential bioengineering approach could transform developmental biology, regenerative medicine and robotics, building on frameworks that, include active, computational, and agential matter.

[H1] Introduction

Many problems in biomedicine, from birth defects and traumatic injury, to aging and degenerative disease, could be addressed by answering the fundamental interdisciplinary question of how groups of cells cooperate to build specific anatomical structures. Understanding natural morphogenesis, (the creation or development of anatomical shape), which is, in effect, navigation in morphospace, is key to designing

interventions that induce cells to build, repair or remodel complex structures on demand. In addition, synthetic morphologies [1], that is, cells genetically engineered to generate a bespoke biological structure, could be designed to produce bio-robotic platforms for applications in industry, the environment, and exploration.

Beyond enabling the design of useful living machines [2], synthetic bioengineering allows the examination of the behaviour of living material in new configurations. Pushed beyond their normal evolutionary niches, cells and tissues reveal aspects of plasticity, robustness and problem-solving in transcriptional, metabolic, physiological and anatomical spaces [3]. The existence of these latent, off-script, hard-to-predict behaviours has important implications for evolution and for unconventional computing, in addition to biomedicine; for example, an anatomical compiler could translate an arbitrary anatomical specification into a set of stimuli and/or genetic constructs that can then be applied to cells to coax them to build this anatomy. However, despite tremendous progress in molecular genetics and cell biology, anatomical compilers have not yet been realized. Although we can often identify the sequence of events and mechanisms that cause a natural morphogenetic event, predicting morphogenesis from distinct starting conditions remains challenging, unless the outcome with wild-type cells is known, and the starting conditions reflect only a trivial change, for example, knockout of a specific gene. Even the morphological result of wild-type cells can vary; for example, combining the epithelial progenitor of one organ with the mesenchyme of another organ can result in a chimeric anatomy approximately following that of the source of the mesenchyme [4], the epithelium [5], or in an anatomy different from either progenitors [6]. Uncertainties multiply when more than one genotype is involved; for example, although the genomes of the frog and the axolotl are known, we cannot predict whether a frogolotl (a hybrid embryo (larva) with cells from a frog and an axolotl) will have legs, and whether those legs would consist of just axolotl cells or also include frog cells. Similarly, although planarian stem cell regulation pathways have been well studied at high resolution, we cannot predict whether planarian flatworms containing a mix of cells from worm species with different head shapes will regenerate a specific shape or will never cease remodelling because neither set of cells is satisfied with the current shape relative to their species-specific target morphology.

How cell behaviour is driven by, and enables, collective decision-making at the level of tissues and organs remains largely elusive, limiting progress in regenerative medicine, bio-robotics, and the synthesis of information science with evolutionary biology. Furthermore, it is not well understood what parts of the genome are most important to the phenotype of self-organizing systems; for example, planarian flatworms, which are the most highly regenerative species with extremely robust anatomical fidelity, can be mixoploid, with different chromosome complements among their body cells, owing to millions of years of somatic inheritance and reproduction by whole-organism fission [7].

In many ways, synthetic morphogenesis is now where computer engineering was in the 1940s, where changes to system behaviour had to be made at the level of rewiring hardware; similarly, changes to morphology are often thought to have to be engineered by constructing custom genetic devices. In this Review, we describe a roadmap for synthetic morphogenesis based on exploiting fundamental properties of cell collectives that have enabled evolution. The aim is to engineer and control robust, adaptive multicellular morphology by taking advantage of the innate control structures of living forms; their innate computational and behavioural properties, problem-solving abilities, homeostatic agendas, and abilities to navigate diverse spaces. This approach to reprogramming biology can complement bottom-up strategies focused on genomic editing and protein pathways.

[H1] Morphogenetic mechanisms

Morphogenetic mechanisms are more goal-seeking than blueprint-following. Morphogenesis, during embryogenesis, regeneration or remodelling, relies but is not restricted to one possible outcome or one possible route to achieving an outcome. The range of possible futures available to a given embryonic cell is acknowledged in the regulative development of embryos, in which signals from outside the cell act with internal factors to regulate fate [8]. By contrast, in mosaic embryos, internal factors inherited from the asymmetric egg are thought to control fate; however, even in *Caenorhabditis elegans*, the archetypal 'mosaic' model organism, there is regulative development in specific places, such as the vulva [9]. Beyond choices of outcome, there are choices of route to the same anatomical outcome, which may have been overshadowed by the focus on a few standard developmental model systems, in which regulation is expressed as a range of cell fates and not as a range of routes. In addition, morphogenesis is often seen as an open-loop, emergent result of local interactions; here, open-loop refers to a type of control, in which the result of a process is not used to modulate the input to the process. The open-loop view is challenged by numerous examples of anatomical homeostasis – the ability of cell groups to achieve or restore specific outcomes despite drastic changes in circumstances; for example, the recreation of correct salamander limbs (or deer antlers) despite massive damage and appendage loss; or the remodelling of tadpoles with scrambled craniofacial organs into largely normal frogs [10]. In this case, tissues can migrate until achieving the correct morphology, even if they start in the wrong position. Impressively, living systems can accommodate changes to their fundamental parts while keeping their function; for example, tadpoles with eyes moved to their tails can see [11], and newts with substantially enlarged cells can have regularly-sized kidney tubules, [12,13] by reducing the number of cells that make each tubule. fewer. These examples indicate that the genome does not specify a blueprint of movement and location, but rather encodes systems with anatomical and

functional goals that can actively minimize errors to reach or restore the target morphology [14].

Life is surprisingly plastic and able to adapt to novel (evolutionarily un-expected) circumstances, as demonstrated by chimeric biological materials - viable cells fused to other living and non-living material substrates [15] [16]. Here, we discuss how anatomical homeostasis [17,18] and the ability of biological systems to deal with external and internal novelty [19,20] represent an exciting opportunity for the field of morphogenic engineering. Specifically, we argue for engineering along a continuum: in addition to approaches using passive, active or computational materials, we discuss strategies related to agential materials (Box 1) – cells, tissues and molecular networks with homeostatic and other agendas. Importantly, we do not use 'agency', 'goal' and 'agenda' as mere metaphors, but as the most appropriate (experimentally efficacious) words to describe what cells are about.

Agential materials, that is, materials with agency, have a goal and an ability to monitor their environments and select behaviours to achieve that goal (FIG. 1). Using agential materials will enable engineers to work in simpler, more predictable spaces of possible signals, stimuli, and rewards. Assigning a degree of agency for a given system is in effect an engineering claim; that is, establishing techniques and formalisms appropriate to that level of agency to predict and control the system. Computational models and tools can be developed to construct or alter the action space, in which cellular collectives make decisions, providing high-level prediction and control to complement efforts focused on altering the nano-machinery of cells. Offloading computational complexity onto modules that not only perform functions but also accomplish their local goals despite changes in composition and environment was key to natural evolution. We argue that exploiting this principle will be a step change in biological engineering. Control of growth and form can be achieved by re-specifying homeostatic set points, and by exploiting multi-scale competency and biology's inherent problem-solving capacities, while error correction and healing come at no extra effort.

[H1] Morphogenetic engineering

Morphogenetic engineering has mainly been performed in the context of synthetic biology thus far, based on the bottom-up design of genetic devices (Box 2) to invoke specific morphogenetic behaviours and a target morphology under an open-loop or closed-loop control. Low-level mechanisms are well understood in synthetic biology; however, prediction of precise high-level outcomes remains challenging. By contrast, surgeons typically work from the top down, wounding and apposing tissues to create a target morphology, which may or may not reflect the natural tissue. In this case, high-level outcomes can be better predicted. In addition, the aims of a clinical trial need to be

defined and made available before the trial begins, and predictions can therefore be relied on to be genuine, and not invented post-hoc. Between these extremes, tissue engineers and constructors of organoids usually tend to use multi-level approaches.

Synthetic morphogenesis of multicellular systems by synthetic biology was first suggested in 2008 [1]. A library of genetic devices [21,22] was then constructed to induce human cells to exhibit the ten basic morphogenetic behaviours of normal development (TABLE 1). In addition, genetic systems were created to drive spontaneous pattern formation in initially unpatterned two- and three-dimensional fields of cells [23-26] (FIG. 2). Pattern formation can be connected with morphogenetic effectors to design simple architectures. For example, cells can be engineered to self-organize into a pattern of islands of two different types. Programmed cell death of one type of cellular island then leaves behind a sieve-like sheet of cells[27] (FIG. 2). However, synthetic biological genetic devices have not yet been able to drive cells to make anatomically complex structures, such as organs.

In surgery, tissues are typically first cut and then re-connected by sutures, relying on wound healing mechanisms to generate a coherent tissue over time, with the aim to recreate a healthy tissue. However, it is also possible to connect tissues that are normally not connected to each other; for example, following the excision of a section of cancerous colon tissue, the end of the healthy gut is typically connected to a deliberately made new hole in the abdominal wall (colostomy). This non-evolved connection can be made because tissue excision activates a wound-healing and -sealing response that has evolved in one tissue, but can also work between almost any pair of apposed tissues. This healing response remains active until the open wound is sealed. Such goal-directed tissue behaviour can be applied without considering molecular genetic details. Surgeons do not design agential materials, but they are working with them, relying on the tissues to seal gaps and re-vascularize.

[H1] Engineering with agential materials

Engineering typically starts with assuming, designing, and assembling specific parts. The key question for an engineering design is what can the parts be depended upon to do on their own – what degree of micromanagement is necessary. Parts may be passive structural components, active matter [28,29], computational, or – in the case of cells – agential (FIG. 3), and a morphogenetic goal is reached owing to the spectrum of their competencies, based on different circumstances, natural or modified pattern memories, inputs, and forces. In the case of cells, these competencies cannot be deduced from the genome, even with knowledge of epigenetic modifications, but must be discovered empirically. For example, predicting the functional properties of a protein based on the encoding gene remains challenging. Artificial intelligence (AI) has

improved the prediction of protein structure and protein-protein interactions; however, drug development still relies on empirical testing, for both efficacy and toxicity, rather than on predictions of interactions. At the organism level, deduction is even more challenging. For example, the anatomy and physiology of an organism cannot be predicted based on its genome sequence. The best guess would be to ignore first principles, and apply comparative genomics to place the organism within a phylogenetic tree and deduct its appearance from its neighbours. This particularly applies for chimeric constructs based on cells (and genomes) from multiple sources.

Understanding competencies from empirical investigation provides a powerful class of interventions complementing the traditional bottom-up approach; for example, the learning capacity of cells can be exploited by using precisely-formulated patterns of stimuli to train cells and tissues [30]. Even simple gene-regulatory networks may be capable of different kinds of learning, including associative conditioning on past chemical stimuli [25-28] , suggesting that pathways could be stably modified through specific patterns of repeated stimuli (training). This approach does neither require bottom-up rewiring of the transcriptional network hardware, nor knowledge of the entire network. Cells (neural and non-neural) and tissues can modify their transcriptional or morphogenetic responses as a function of prior experience (for example, chemical, bioelectrical or biomechanical stimulation), implying that specific responses can be engineered on ontogenic and not just evolutionary time scales [14,31,32]. The computational (including probabilistic anticipation and learning [33-35]) capacities of cellular material can be exploited by strategies that alter cell behaviour ‘in the software’ by temporally controlling aspects of their microenvironment to induce desired cell- or tissue-level behaviours.

Acknowledging agency in the material replaces familiar concepts of command and control with that of collaboration, and replaces the need for micromanagement with the opportunity to let the system take care of most of the details. A concept familiar at the level of whole organisms. Shepherds don’t manoeuver a sheepdog by micromanaging its muscles; instead, the dog gets a clear goal, and moves its muscles accordingly. We argue that the same is true for cells. Importantly, agential materials extend and smooth the space of possibilities, reducing the effort needed by engineers. To follow our analogy, sheep could not be rounded up by micromanaging the muscles of a dog, even if an interface could be established to exert muscle-by-muscle control in the dog, because we do not understand enough about animal locomotion or sheep psychology to 'compile' a desired arrangement of sheep into a detailed program of sheepdog muscle contractions. For such a complex task, the agential approach would be needed. Similarly, we do not understand enough about morphogenesis to 'compile' a desired complex arrangement of cells into a detailed program of gene and protein interactions. The agential approach is not just a stop-gap while we learn more about morphogenetic

mechanisms, but an engineering approach that makes best use of the inherent properties of living cells and tissues.

[H1] Agential bioengineering

In addition to surgery, the agential approach is commonly applied to modulate the behaviour of growing plants by manipulating their environments. For example, living bridges can been grown by training tree roots or branches to grow in certain directions. Similarly, goal-seeking behaviour of cells and tissues can be exploited for the production of distinct cellular structures, including organoids and synthetic 'embryonic organizers', (FIG. 4) by altering the electric fields and currents that influence developing tissues and by controlling their microenvironment (FIG. 5).

[H2] Organoids

The construction of organoids is based on using cells as agents with their own agendas [36]. Initiated by the observation that disaggregated sponge cells re-aggregate to construct a new, anatomically-correct sponge [37], organoids are typically created by controlled culturing of disorganized stem cells capable of making tissues of a specific organ. Stem cells can be obtained from an embryo, by differentiation of pluripotent cells, or from adult stem cells. For example, random mixing of various types of stem cells responsible for making kidney tissues leads to the development of an aggregate, in which the cells differentiate and organize themselves into an arrangement of kidney tubules and supportive stroma. The resulting kidney organoid is similar to the cortex of a natural developing kidney [38] (FIG. 4). The innate kidney-building agenda of these stem cells is strong enough to overcome the disruption of randomized relative positions. This stem cell-based reconstruction of kidney tissue anatomy is driven by reasonably-well understood cell signalling, feedback, differentiation, adhesion and thermodynamic sorting mechanisms that underlie the ability of the cellular collective to adaptively traverse the morphospace of tissue-level configurations [3,39]. However, although stem cells can reproduce the micro-anatomy of the organ, they do not recapitulate large-scale features, such as arrangement of tubules around a urine-collecting duct tree that drains via a ureter.

Natural development is influenced by symmetry-breaking in the embryo, which does not occur in an organoid. Such symmetry-breaking can be implemented in kidney organoids by imposing concentration gradients of signalling molecules to mimic gradients that occur in the embryo. Addition of gradients results in the formation of the large-scale, arranged-on-a-tree-with-ureter, organotypic organization [40] (FIG. 4). Therefore, the true innate agenda of these stem cells is not to make a kidney, but only its micro-structures; indeed, agents do not need to have the final outcome as their goal. Instead, they work towards a 'local' goal that, under the right circumstances, will produce the final outcome.

By applying spatiotemporally textured environments, in this case, signalling environments, the natural agendas of the agents can be exploited to produce large-scale and complex structures. Importantly, evolution may operate in a similar way; that is, instead of altering agendas of cells, structures can be modified by modulating their environment or external signalling to implement behaviour-shaping in morphospace.

[H2] Synthetic embryonic organizers

Embryonic organizers, identified almost 100 years ago by Spemann and Mangold, are localized signalling centres in the developing embryo that trigger events in the rest of the embryo and provide spatial information for their patterning. Synthetic biological techniques have been applied to construct cells that modify an environment, in which wild-type stem cells follow their natural agendas [41], effectively acting as an embryonic organizer. For example, a human kidney cell line can be engineered to have two new properties: P-cadherin-mediated adhesion and production of a Wnt signalling molecule. Mixed with embryoid bodies (aggregates of embryonic stem cells), the cell line forms a small compact nodule at the side of the embryoid body owing to its adhesive properties, and creates a gradient of Wnt signalling (FIG. 4). This gradient breaks the symmetry of the system, organizing stem cell differentiation into a predictable large-scale gradient of cell types. Usually, embryoid bodies follow less-predictable patterns of differentiation, based on stochastic Wnt expression followed by positive feedback [42]. Synthetic organizers have also been designed that require manual embedding in their host tissues instead of self-organization. Such a system may be a first step towards connecting bottom-up-style synthetic biology with agent-centered biological engineering.

[H2] Pattern control through bioelectricity

In addition to concentration gradients of signalling molecules, the actions of agential materials are mediated by endogenous bioelectricity [43,44]. For example, the bioelectric networks of the nervous system allow cells to work together towards large-scale, robust problem-solving in 3D behavioral space. Indeed, electrical networks for coordination memory, and decision-making circuits evolved early; for example, bacterial biofilms and microbes already utilize the basic components that were later co-opted by nascent nervous systems [45-48]. In developing or regenerating multicellular organisms, pre-neural bioelectrical networks coordinate cellular behaviour to demarcate developmental compartments and to set the shape and locations of organ-level structures [49], as well as to regulate some cell-level properties, such as differentiation, proliferation and migration [50]. The bioelectric circuits provide spatial and temporal integration over a local second-messenger transduction machinery (including calcium fluxes and serotonin signalling) [51], which feed into changes of transcription and cell behaviour that alter the anatomical configuration (movement in morphospace [3]). These same mechanisms used to navigate morphological and physiological spaces were evolutionarily pivoted (and sped up) to solve problems in 3D space (for example, control of muscle movement).

Ion channels and gap junctions form a powerful interface between cells and electricity, which can be manipulated by drugs, mutations and optogenetic tools to modify the activity of cellular collectives. This interface has been exploited by evolution to control modules, such as the formation of the face [52,53] and alignment of major body axes [54], by microbiota to control morphology in their metazoan hosts [55], and by bioengineers to repair defects of the brain [56] and induce appendage regeneration [57]. Bioelectric interventions illustrate the power of top-down control. A simple bioelectric trigger state can be induced by misexpression of an ion channel to reproduce the spatial pattern of electric potential that normally specifies the location of the eyes in other locations in a frog embryo (FIG. 5a)[58] need to include provisions for establishing the correct number of cells, because the bioelectrically-modified cells automatically recruit their wild-type neighbours at numbers sufficient to make a normal lens (FIG. 5c). Such innate competency (to achieve the goal of making a correctly-shaped and -scaled organ, regardless of location) can be exploited by bioengineering strategies that use bioelectric inputs to trigger appropriate subroutines at a high level, without micromanaging subcellular events.

In this bioelectronic patterning experiment, the change of bioelectric prepattern is a trigger. The bioelectric state is relatively simple and cannot contain all the information needed to specify a complex eye. Moreover, cells do not only obey the 'subroutine call' of making an eye, they also recruit wild-type neighbours as needed (FIG. 4C), without requiring exogenous channel proteins, to make structures of the correct size. This bioelectric state was shown to be movable to reproduce an entire complex organ (the eye) at any location. Importantly, the related competencies of the nervous system ensure that those eyes can see [11,59]. Higher-level triggers do not even have to specify what to build. For example, a bioelectric state induced by a sodium ionophore encodes a 'build the appropriate organ for this location', which correctly triggers formation of a tail [57] or leg [60] depending on the context.

Setpoint information for anatomical homeostasis can also be re-written in agential bioengineering. For example, planaria are simple flatworms that can regenerate in a way that even small fragments of a body can regenerate the entire worm, including a new head. The ability of planarian fragments to rebuild the correct number, shape and size of their head is set by a bioelectric pre-pattern [54,61]. This cell voltage pattern can be transiently manipulated using drugs (or RNAi) that target ion channels and/or gap junctions to permanently re-set the number of heads that genetically wild-type planaria regenerate after damage[62]. Without any further manipulation, these animals will continue to regenerate their new target morphology, if they are cut or spontaneously reproduce by fission. The new heads do not require additional signals to set their size, which is not the case if heads are induced by micromanaging traditional intracellular

signals [63]. Therefore, a permanent line of two-headed animals can be created without requiring any alteration to the genome, illustrating morphogenetic control through editing the bioelectric pattern memory which serves as the setpoint of the homeostatic process[64,65]. Toolkits of plasmids and techniques (such as tiered pharmacological screens using ion channel drugs and voltage dye imaging protocols) allow the control of bioelectrically-mediated morphogenesis[66,67], including with human cells[68]. Because voltage states serve as informational messages to complex interpretation machinery, brief bioelectric modulation of endogenous states (not requiring continuous micromanagement) can induce long-term changes in collective cell behaviour [69]; for example, one-hour treatment with sodium ionophore triggers a multi-week cascade of tail regeneration [57].

Finally, a model, in which normal development involves cells forming networks that replace their single-state goals (maximal proliferation and migratory exploration) with a collective-level morphogenetic goal [70], suggests an alternative strategy for treating tumours. Instead of killing cancer cells, or trying to repair underlying molecular pathways, cells could be forced to reconnect to the bioelectric network that mediates cooperation towards large-scale morphogenetic goals. In a frog model, human oncogenes (that normally cause tumours) can be over-ridden through misexpression or optogenetic activation of ion channels to prevent their normal depolarization and gap junctional uncoupling [71,72]. This intervention does not need to functionally address the mechanistic details of cell cycle checkpoints, abnormal gene expression, physiological remodelling, or any other hallmarks of cancer. Similarly, physiological stimulation to over-ride protein-level defects have been shown in brain patterning; here, a dominant Notch mutation can be rescued by activation of a specific ion channel, selected by a computational model that designed the strategy [73]. These examples illustrate a strategy known from computer programming; states can be achieved through transient inputs that exploit the causal architecture of a complex system, without modification of the hardware.

[H2] Xenobots

Evolution and cell-based bioengineering face the same challenge; the base material is not a blank slate, but harbours evolutionarily-derived competencies and preferences, because somatic cells used to be free-living organisms. Development typically produces consistent outcomes; however, chimeric and bioengineering reveal additional, non-obvious capacities of cells and cell groups. For example, the biorobotics platform xenobots [74] (FIG. 6), which are multicellular assemblies created from cells from *Xenopus* embryos. In contrast to soft robots actuated by externally pacing muscle cells directly seeded onto scaffolds [75,76], xenobots take advantage of the innate capabilities and emergent morphological and behavioural goals of *Xenopus* cells. Here, instead of reprogramming using genetic circuits or nanomaterials, this platform is based on

morphological computation and self-assembly of cells into a coherent motile proto-body, guided by an evolutionary search algorithm [74].

Xenobots result if nascent skin cells are removed from a *Xenopus laevis* (frog) embryo, dissociated, and allowed to reboot their multicellularity. Instead of dying, dispersing, or forming a monolayer, the cells self-assemble overnight into a spherical construct that is self-motile[77]. The cilia (normally used to distribute mucus along the surface of the animal) are repurposed to enable the proto-organisms to spontaneously move in straight or curved paths, spontaneously turn around, navigate mazes and other structures, and exhibit collective behaviour (their proto-cognitive capacities, in terms of learning, remain unknown). Xenobots also regenerate after damage (to their xenobot shape), and, although they do not contain neurons, they exhibit extensive calcium signalling dynamics that resemble signalling during neural decoding in brains. Remarkably, although xenobots are incapable of reproducing like frogs, they can nevertheless replicate. Xenobots (as individuals and in groups of xenobots) can collect loose skin cells in their environment into piles, which then mature to the next generation of xenobots. The newly-formed xenobots can then repeat this replication process. Although not exhibiting strong heredity, this is nevertheless a (kinematic) self-replication process, in von Neumann's sense of a machine that assembles copies of itself from materials in its environment [78].

Such spontaneous competencies could be merged with synthetic biology circuits and stimulation (bioelectric, biochemical and biomechanical) to increase the repertoire of morphological, behavioural, and metabolic capabilities and perhaps discover new ones; for example, guided self-assembly towards desired shapes; programming behaviour towards specific movement (for example, to explore and return to a specific location); augmenting computational capacity through circuits that can store memories of sensory experiences or execute logic functions [79-81]; and adding metabolic or biochemical pathways that sequester or modify molecules in the microenvironment.

[H2] Key implications of xenobots

The xenobot example illustrates several key concepts. First, xenobots can replicate because cells are an agential material with the inherent capability to assemble into a coherent new organism. Thus, assembling capability does not have to be created de novo. Moreover, xenobots can make the next generation of xenobots for the exact same reason. Once the material (cells) are pushed into a pile, morphogenesis takes over. Second, xenobots are essentially engineered by subtracting constraints (that is, by removing cells), rather than by adding traits to skin cells. The power of subtraction in interrogating the internal agendas of cells has also been demonstrated for embryonic stem cells. Embryonic stem cells from different species make very similar structures in vitro, that is, a 'ground state', whereas, in vivo, they form different structures owing to

species-specific mechanical and geometric boundary conditions [82]. Third, the example of xenobots illustrates how the reliability of wild-type development obscures plasticity and capabilities of cell collectives. The default activity of skin cells is typically thought to be the formation of a 2D layer on the outside of the organism; however, the xenobot example demonstrates that their baseline preference (in the normal, default environment) is to make an active xenobot. Therefore, skin cells are only forced into the skin phenotype by instructive interactions with their neighbouring cells. To control outcomes, evolution modulates the signals given to an active material by shaping behaviour or by guiding self-assembly. This concept also has practical implications; for example, concerning the debate whether the baseline state of cancer cells is quiescence or proliferation, which strongly impacts biomedical strategies. Fourth, the xenobot example supports the idea that biorobots do not have to be created by micromanaging all capabilities of passive components. Instead, the microenvironment of biorobotic components could be modulated to guide their innate behaviours toward desired goals.

Many of the capabilities of xenobots, such as kinematic self-replication, were not predicted in advance. The wild-type genome of *Xenopus* offers no obvious guidance that would have enabled this prediction, nor does its natural life cycle. Xenobots are not part of the frog's life cycle, and thus, the frog genome has not been naturally selected for all the qualities that enable their skin cells to form xenobots. Therefore, explaining the origin of the structural and functional properties of xenobots relies on a better understanding of two ideas. The first is a variation of an idea put forward by Stuart Kaufman, who modelled networks of genes and expressed the state of overall expression in a cell as a point in a multidimensional state space (one dimension for each gene). For typical gene networks, this space contains attractors, that is, points in the state space to which a network would naturally proceed [83]. Each attractor is surrounded by a basin of attraction in the state space (comparable to the drainage basin of a river). Importantly, in these model genomes, attractors exist that do not occur in normal development. Xenobots suggest the existence of analogous 'not-natural attractors' in morphospace (behaviour space) [84,85]. A better understanding of how these arise, and their relationship to attractors in normal development, as well as 'generic' laws to define the space of possibilities[86-88], will be important to explain the origin of xenobots [72-74] and many other possible constructs that remain to be discovered. Second, it needs to be understood how evolution creates genomes that do not encode solutions to specific environments, but rather seed the production of highly-competent 'machines' that can solve numerous new problems [89].

[H2] Evolved and designed systems

A major consequence of synthetic morphology is the dissolution of artificial boundaries between categories, forcing us to sharpen terminology. Machine versus organism, evolved versus designed, life vs. robotics, and many other distinctions based on prior limitations of technology and imagination should give way to a continuum of chimeric approaches, and a focus on strategies that achieve best prediction and control of a system. Conceptual frameworks, such as guided self-assembly and a spectrum of agency [90,91] (FIG. 3), can provide a scaffold with which to address the aforementioned open questions and achieve new capabilities.

To cross the simulation-to-reality gap in engineering, cues can be taken from evolution [92], which crosses this gap not by relying heavily on prior experience (which, like simulations, often fails to predict the future), but by exploiting problem-solving competencies of modules at different scales. By taking advantage of agential materials, an entirely new class of machines can be built, that is, living systems with desirable structure and function, exploiting and enhancing the basal intelligence of life at all scales.

[H1] Challenges, implications and impact

A transition to collaborating with agential materials will be challenging. Bottom-up construction by synthetic biology creates at least an illusion of predictability (although most platforms need extensive optimization). The inherent agendas and capabilities of living materials at different scales are often neglected in bioengineering. Similar as in the xenobot case, whose capabilities had to be discovered empirically, agential properties of cells will need to be revealed, at least until patterns and proto-cognitive capacities will be recognized. However, working with agential materials may also lead to novel failure modes; for example, 'robot cancer', in which competent components pursue agendas of their own that may not align with those of the system as a whole. However, such a defect has not yet been identified, because engineering does not yet exploit biology’s multiscale competency architecture. Intellectual property considerations may also be challenging. Patenting a designed genetic construct is more straightforward than patenting an approach to collaborating with the agendas of wild-type cells. However, such challenges must be faced, in particular, in medicine, in which genetic manipulation is not only limited in terms of achieving complex organ-level outcomes, but also related to safety issues. Alternatively, robotics and autonomous engineered systems offer many conceptual tools and results to assist the biological sciences in working with complex emergent systems [93-95].

Evolution creates problem-solving machines, and not just agents fit for a specific environment, through multi-scale competency and cooperation or competition of subunits within and across scales in an organism. Living systems are robust and often

thrive in unknown scenarios, because evolutionary search does not over-train on historical priors; most organisms are not hardwired for specific dynamics, but have to solve problems dynamically, on-the-fly. Normal embryogenesis, not just in chimeric or bioengineered contexts, requires cell collectives to establish and scale computational boundaries, make decisions, and deploy navigation policies in anatomical and physiological spaces. This view suggests a deep invariant across human design and biological evolution – that of the hacker. Not in the pejorative sense of misusing a system (relative to some absolute 'proper' usage), but in the fundamental sense of approaching each environment with a beginner's mind, having to discover and internally model the boundary between themselves and the outside world, and identify relevant sensory inputs and effective actions in behavioural space [96]. Cells, microbes (bacteria and viruses), host-altering parasites, engineers, and even evolution itself are all hackers in facing the same problem; that is, identifying the most causally-potent, efficient control knobs to manage a complex system. In nature, this often takes the form of behaviour-shaping, not micromanagement, offering bioengineers an efficient new path to regenerative medicine and synthetic morphology. Drawing from the extensive toolkits of behaviour science, cybernetics, and basal cognition, desired complex outcomes can be achieved by rational cooperation with the collective intelligence of life.

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Acknowledgements
We thank Julia Poirier for assistance with manuscript preparation. M.L. gratefully acknowledges support via grant 62212 from the John Templeton Foundation and grant TWCF0606 of the Templeton World Charity Foundation. J.D. gratefully acknowledges support via The European Commission (grant CyGenTig), the Biotechnology and Biological Sciences Research Council (grant BB/M018040/1) and the Medical Research Council (grant MR/R026483/1). The opinions expressed in this publication are those of the authors and do not necessarily reflect the views of the funders.

Competing Interests
The authors declare no competing interests.

Author contributions
Both authors contributed equally to all aspects of this paper.

Key Points
- Synthetic bioengineering allows the construction of new arrangements of living material
- Synthetic morphology aims at creating an 'anatomical compiler' that writes DNA instructions based on a specific design goal

- Bottom-up bioengineering approaches are limited by knowledge gaps in developmental biology, thus relying on the micromanagement of passive materials
- Cells and tissues are effectively manipulated as agential materials, by targeting their pattern memory and homeostatic capabilities
- Extending bottom-up approaches by adding empirically- and computationally-characterized agential materials (cells and tissues) will greatly improve the rational creation and repair of complex morphologies

Table 1. The ten basic morphogenetic mechanisms in anatomical development [97]

Mechanism	Proximate effect	Examples in vertebrate morphogenesis
Proliferation	Cells increase in number	Growth of tissues. If one of two linked tissues grows more than the other, the result is bending (for example, avian gut folds)
Elective cell death	Cells die	Population balancing; elimination of temporary structures (webs between fingers disappear)
Cell fusion	Cells connect to share a common cytoplasm	Syncytial cells, such as the myotubes of muscles, form this way.
Locomotion	Cells move, often under guidance	The peripheral nervous system is formed of neurons, whose progenitors left the dorsal part of the spinal cord (neural crest) and migrated through the body
Aggregation	Cells stick together to make a compact mass	Common mechanism in morphogenesis; for example, the first sign of bone formation in limbs is aggregation of limb mesenchyme at the place, where bones will be located
Adhesion-mediated sorting	Cells change position, so that cells of similar type crowd together and minimize contact with other types	Thought to be a major basis of tissue stability and for correcting navigational errors
Mesenchyme-to-epithelium transition	Mesenchyme aggregates and makes epithelial sheets	Epithelia of the excretory system form this way
Apical/basal constriction	Epithelial cells become wedge-shaped	Sheets are forced to curve and fold; for example, the dorsal ectoderm folds inward to make the neural tube
Intercalation	Cells contract cell-cell contact boundaries in one direction, while expanding at 90 degrees to that direction	Cell masses thin in one direction and lengthen in the orthogonal direction (thereby changing neighbours). The entire body elongates this way
Epithelium-to-mesenchyme transition	Epithelia give rise to separate, mesenchymal cells	The mesodermal layer of the body is formed this way at gastrulation

Figure captions

Note - these are the figures from the accepted MS but the journal will rework many of them with professional graphic artists.

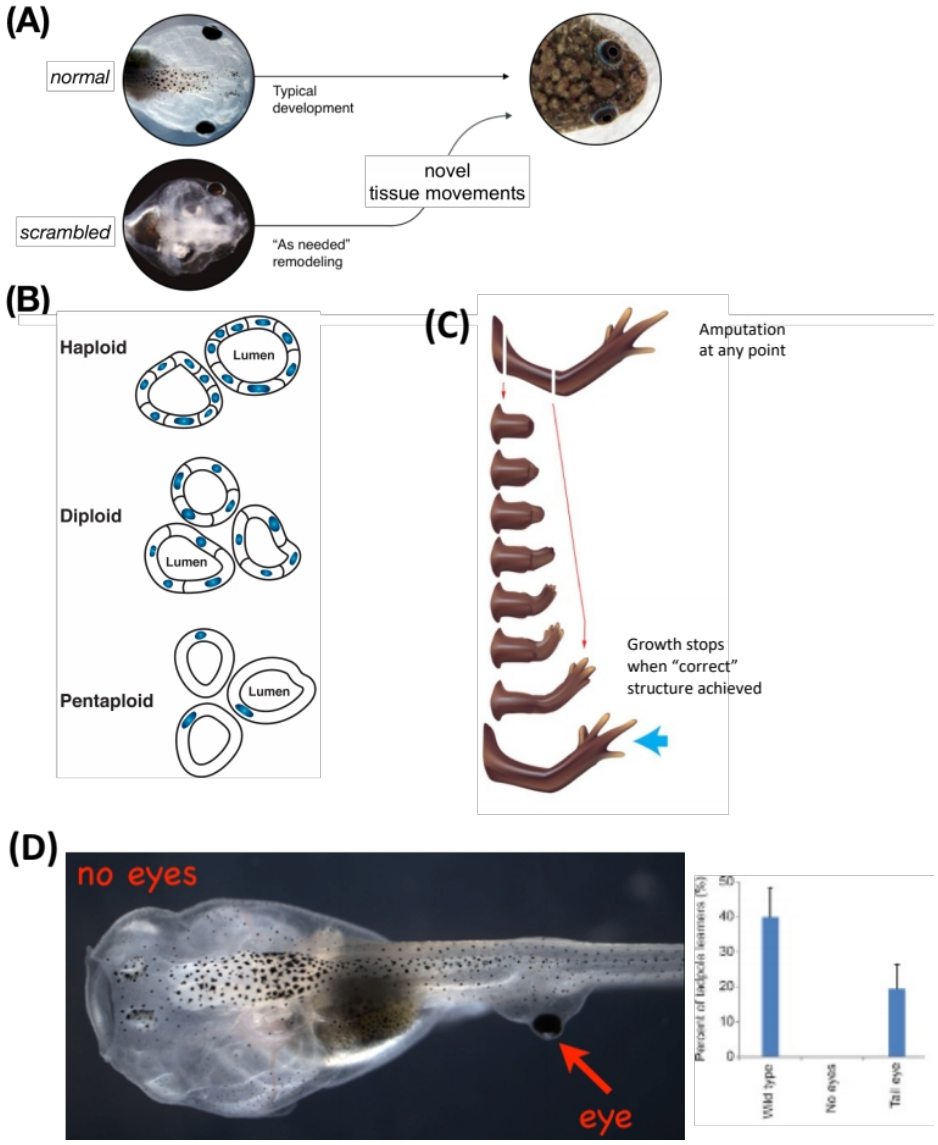


Figure 1. Competencies of cellular collective intelligence. A) Genomes specify the micro-level hardware in cells. Gene-regulatory networks (GRNs) give rise to proteins, which enable local cell behaviours, from which large-scale anatomy arises. In morphogenesis, two kinds of feedback loops exist: feed-forward (open-loop) emergent complexity and anatomical allostasis (stability through prediction and anticipation). These feedback loops enable corrective action by biophysical and biochemical signals that trigger cell growth and tissue remodelling to reduce error relative to a target morphology. (B) Following amputation of salamander limbs, the correct amount of active proliferation and differentiation allow the recreation of a new limb; remarkably, the

system stops growing and remodelling once it reaches the anatomical setpoint. (C) The size of amphibian kidney tubules is maintained even if the size of the cells that constitute the tubules is altered by changes of ploidy. Cells can employ new molecular mechanisms (for example, cytoskeletal bending of one cell instead of cell-cell communication) to achieve the same anatomical target morphology [12,13]. The genomically-specified cellular hardware sets parameters that describe positions and trajectories through morphospace [98,99], such as that formed by axes defining possible planarian head shapes [100] The computational machinery within cell collectives enables living systems to navigate morphospace to reach the anatomical goal configuration. For example, cells can reconstruct a frog head even when their initial positions are scrambled, through new motion paths of organs, such as eyes, mouth, and nostrils [10]. (E) The goal configuration (G) is reached despite diverse starting positions (s1-s4) and local minima (LM). Panels A,B reproduced after Jeremy Guay of Peregrine Creative, from [91]. Panel C reproduced from [12]. Panel D reproduced from [100]. Panel E reproduced from [3].

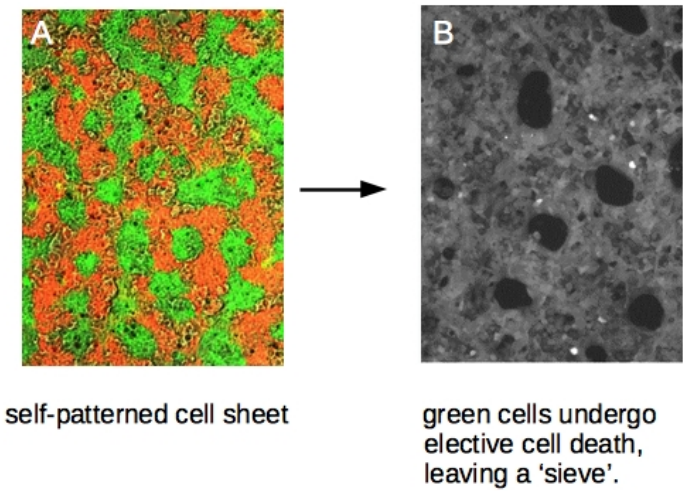


Figure 2. A synthetic biological construct confers inducible homophilic adhesion to two cell types, which leads to spontaneous pattern formation by adhesion-mediated phase separation. Adding a module for inducible apoptosis in one of the cell lines, leads to elimination of one pattern, resulting in a sieve-like sheet morphology [27].

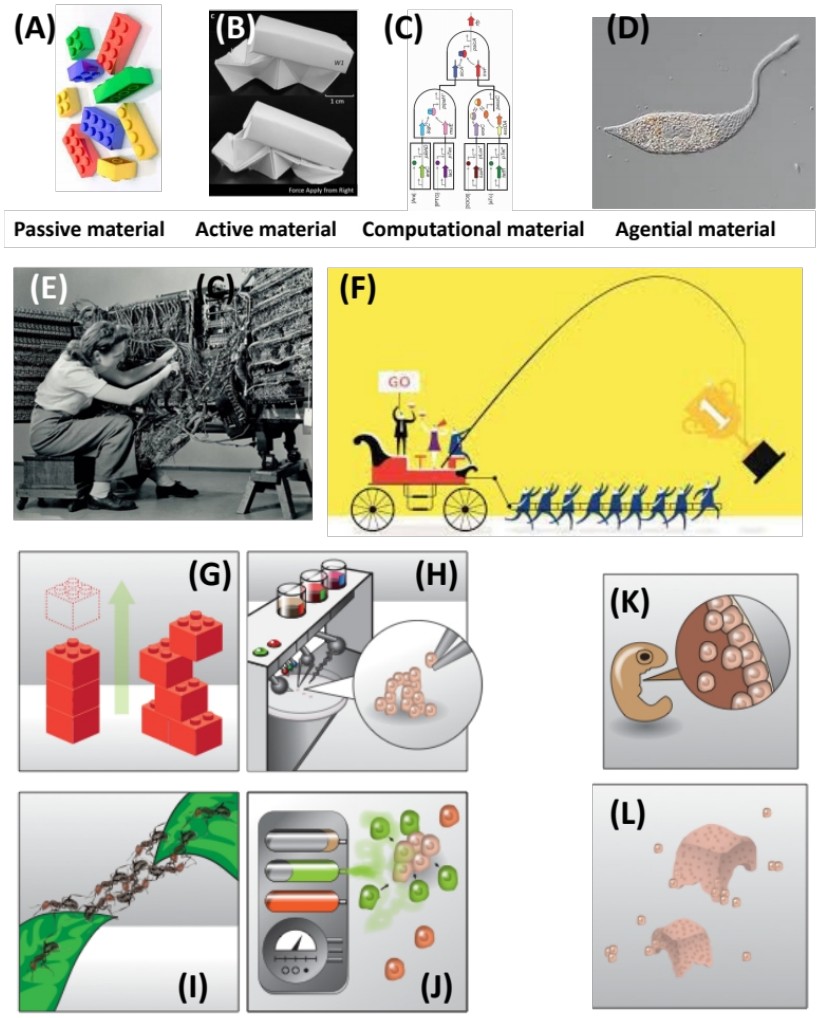


Figure 3. Optimal engineering strategies depend on the agency level of the material. (A) Passive materials typically keep their shape or conduct energy, and therefore, require micromanagement and implementation of features. (B) Active matter, for example, origami, and (C) computational materials, such as, springs and transistors, can autonomously change their properties or process information, enabling more complex, semi-autonomous dynamic systems. (D) Agential materials, such as, cells (*Lacrymaria* protozoan) were once independent organisms and have a long evolutionary history of competencies and behaviours. The behavioural complexity of agential materials offers different degrees of control over macro- and microscale properties. (E) Similarly, precursors of modern computers had to be programmed at the hardware level, but can now be controlled with stimuli and signals. (F) Engineering with agential materials allows simple, tractable rewards, punishments and signals. (G) Instead of micro-specifying the structure of a system, for example, by (H) micro-positioning cells, (I) bioengineering can apply evolutionarily-inspired strategies (such as, ant bridges) by manipulating the competencies of components. (J) Setpoints of homeostasis loops can

be modulated by signals. (K) For example, xenobots demonstrate that the default behaviour of skin cells (forming a 2D layer) is a consequence of instructions from other cells and the environment. (L) In the absence of such signals, skin cells form a motile, spherical construct with diverse new functions.

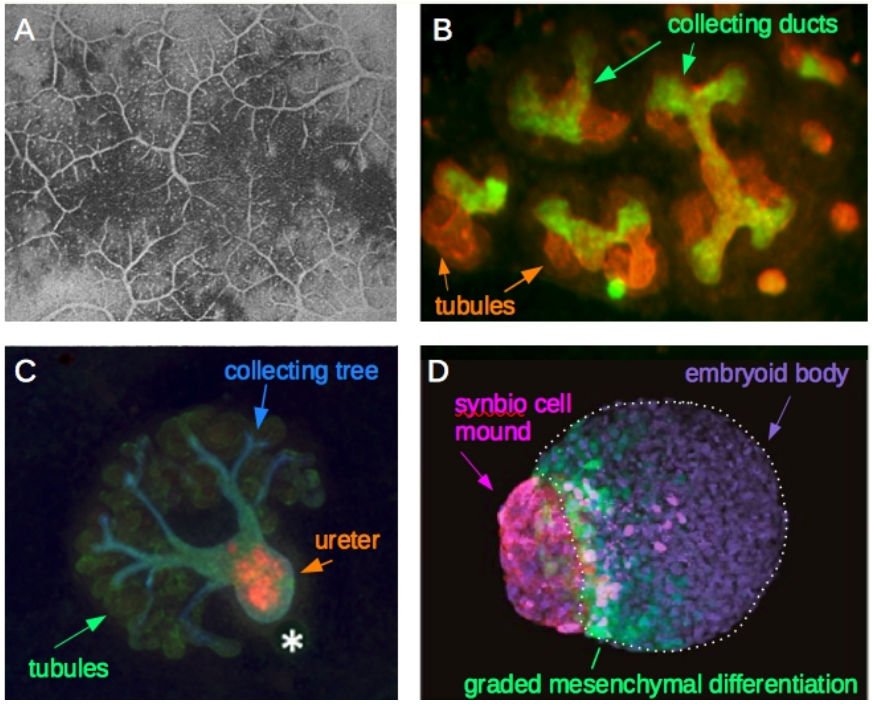


Figure 4. Agential bioengineering. Agential bioengineering is based on modifications of the environment to manipulate the goal-seeking behaviour of cells to new goals. For example, root bridges are made by guiding roots; bonsai trees can apply unusual constraints; organoids are made by growing stem cells in environments favouring differentiation and, in some cases, organ-scale patterning. Stem cell differentiation can also be patterned by synthetic biological 'organizers' that control time and location of differentiation.

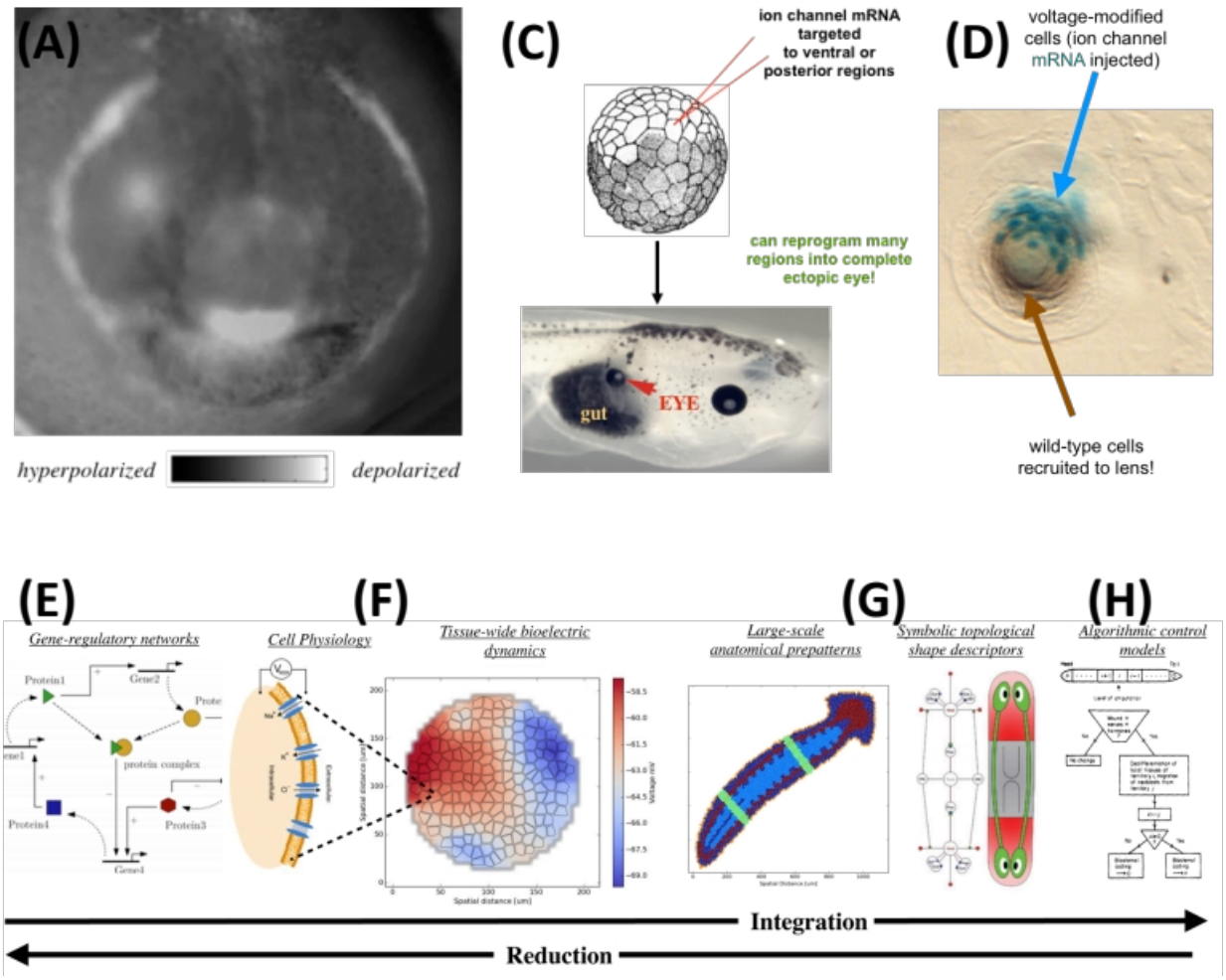


Figure 5. Bioelectric interface for organ-level control. Experimentally replicating the bioelectric characteristics of the site of eye formation (panel A, electric face revealed by voltage-sensitive fluorescent dye [52]) in other parts of the body of frog embryos via microinjection of ion channel mRNA (B) can cause eye formation in ectopic regions, by providing a simple signal that relies on cells to perform morphogenesis and recruit other cells as needed to form the complex organ (C, blue cells, marked with beta-galactosidase, express exogenous channel; the rest of this ectopic lens in a tadpole's tail are made of wild-type cells recruited by the blue ones); from [58]. Work in bioelectric control of morphogenesis shows one way in which computational tools can help bridge the gap between mechanism and goal-directed complexity: by building full-stack, integrative models that show how molecular networks that determine the biophysical hardware (D) result in tissue-level emergent voltage patterns (E) that implement resulting organ- and axis-level decisions, such as the planarian regeneration control circuit (F). Machine learning tools can help convert these into human-understandable meso-scale algorithms which facilitate inference of interventions to make desired system-level anatomical changes [101]. Panel A reproduced from [52]. Panel B top image

reproduced from www.xenbase.org after [102]; Panel B bottom image reproduced from [58]. Panel C reproduced from [91]. Panels D-F reproduced from [14].

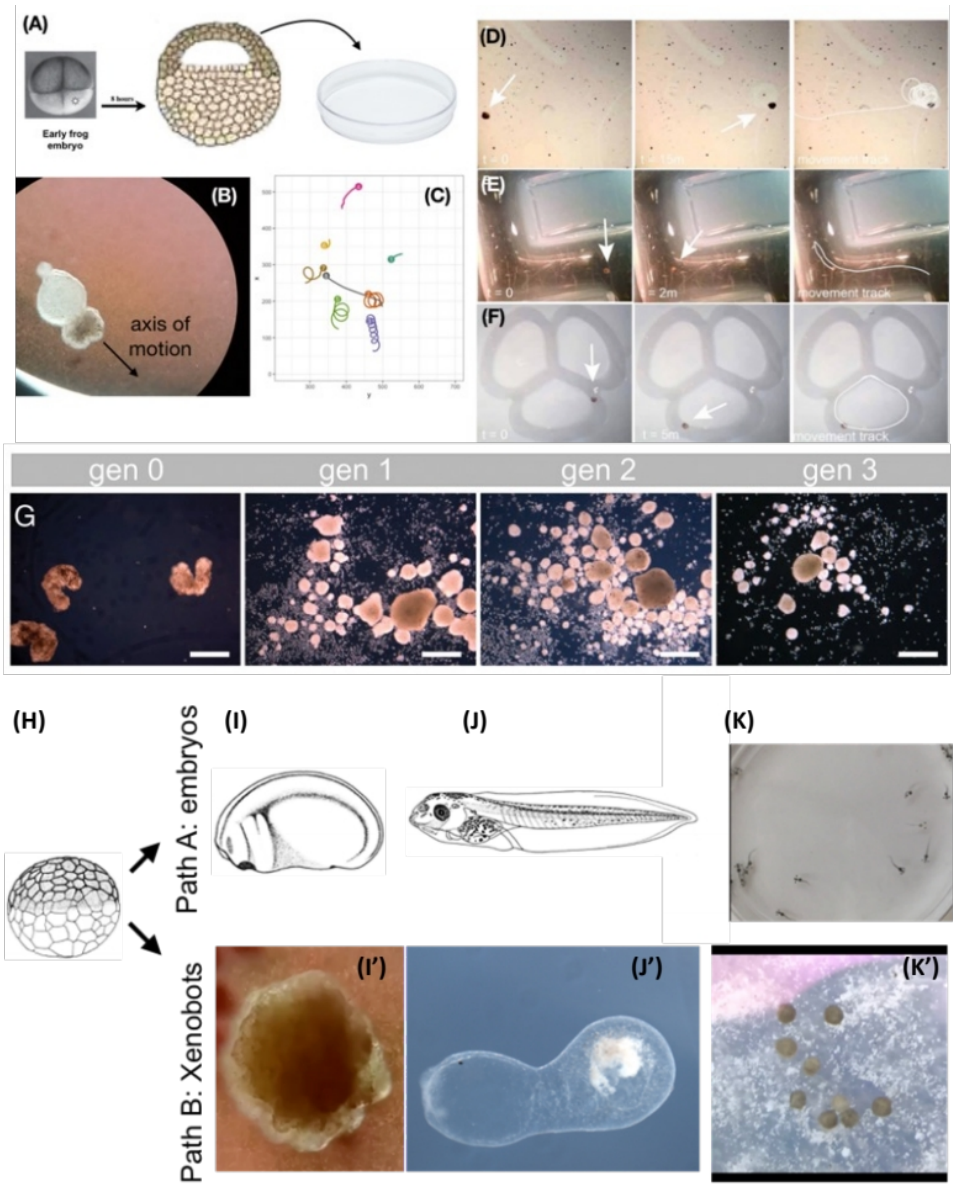


Figure 6. Xenobot platform for cracking the morphogenetic code. (a) Frog embryos at the blastula stage dissociate to release ectoderm (skin) cells, which self-assemble in a petri dish into a spherical, motile biorobot [74,77,103]. (b) These synthetic living proto-organisms move through cilia beating (B) and exhibit diverse kinds of motion separately and in groups (C, showing tracking data for a collection of Xenobots). They exhibit a variety of behaviors, including the ability to circle features in their environment (D), traverse mazes (E) including taking corners without hitting the opposite wall, and navigating a variety of environments (F). Most remarkably, when placed in an environment with loose skin cells, they push the cells into piles, which then mature into the next generation of Xenobots, repeating the cycle (G). Xenobots reveal that the same cellular hardware (Xenopus laevis cells with a wild-type genome, H) can exhibit multiple

diverse paths in morphological and behavioral space, going through the standard developmental sequence (I, J) and exhibiting standard behaviors (tadpole schooling, K), or becoming a Xenobot (I') with a different and novel developmental sequence over weeks (J') resulting in different behavior such as kinematic self-replication (K'). These capabilities reveal an example of evolution producing problem-solving machines with capacity for collective-level robustness in novel configurations which can be exploited in future efforts in guided self-assembly. Panels A-C,I',K,K' reproduced from [20]. Panels D-F,J' reproduced from [77]. Panel G reproduced from [103]. Panels H-J reproduced from www.xenbase.org after [102].

Box 1. The scale of persuadability in engineering

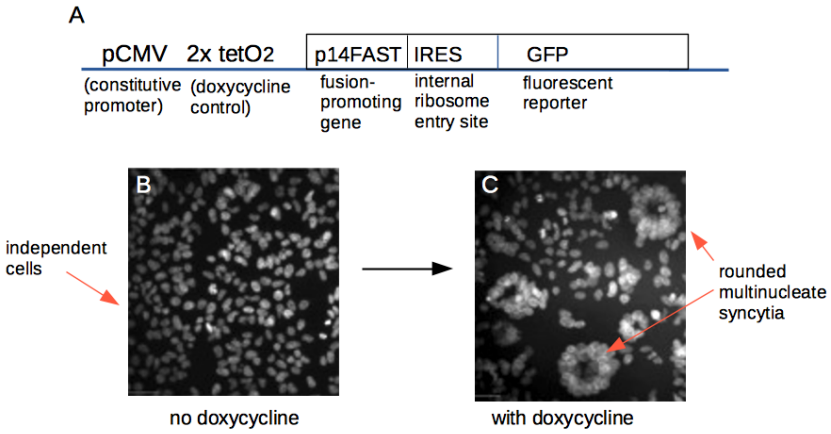
Natural and engineered systems span a continuum with respect to techniques and approaches needed to predict, control, modify and create them. The engineering stance on agency suggests that systems span a spectrum of persuadability. Persuadability refers to the level of micromanagement and the expected degree of sophistication through autonomous behaviour and problem-solving needed to obtain a specific result. For example, lowest-agency systems, such as mechanical clocks, must be physically re-wired at their lowest level, in order for their functionality to be controlled. More sophisticated systems with homeostatic capacity, such as thermostats, can be controlled by editing their setpoint. Such systems can also respond adaptively in real-time to changing circumstances. Notably, this feature can be exploited without detailed knowledge of, or re-wiring of, the functionality of individual components. More agential systems, such as animals, can be trained using conditioned stimulus, unconditioned stimulus and response in associative learning. Training enables more control toward more complex outcomes without requiring micromanagement of the arrangement of information and functional linkages inside the system's cognitive apparatus. This continuous view, in contrast to binary, essentialist perspectives that assume the existence of a bright line between "truly cognitive systems" (D, linguistic, metacognitive agents) and "mere chemistry and physics", is more compatible with the gradual origins of complex information-processing architectures from single-cell origins, on both ontogenic and evolutionary timescales. Moving up the scale of persuadability, the degree of effort (energetic and computational) needed to achieve complex, system-level results is reduced, as is the amount of mechanistic knowledge needed to exert influence. Increasingly more effort is shifted onto the system itself. Brief stimuli and triggers can exploit the native competencies and modular capacities of the system.

Passive materials can only be expected to maintain their physical properties, and can be engineered only by hardware modification. Active and computational materials can perform specific functions that adapt automatically. Tools from control theory and behavioural science are being adapted to improve engineering capacities with these substrates. Cells and tissues, the materials of bioengineers, are agential materials because they perform fixed functions, and are also able to optimize aspects of form and function independently, with different degrees of competency, based on their prior evolutionary history as unicellular organisms [32,104]. Cybernetics [90] offers a useful, scale-free framework, compatible with a very wide range of physicl implementations, with specific milestones across the spectrum of agency (see figure). It is essential to discover where on this spectrum any given system is, by testing hypotheses about its prediction and control (as opposed to philosophical assumptions that favour low-agency explanations a priori, and thus constrain engineering capabilities) [90]. This lens on living systems enables powerful techniques and concepts from other sciences (for example,

computer science and behavioural neuroscience) to be exploited for synthetic bioengineering.

Box 2. The synthetic biology approach to designer morphologies

In synthetic biology, genetic constructs are designed to confer new properties to cells, for example, to produce drugs or biofuels by enzymes, and to construct new devices. This approach differs in aims and scale from gene-manipulating methods developed to investigate cellular mechanisms and functions. In synthetic morphogenesis, genes are introduced to activate specific morphogenetic behaviours (TABLE 1). These genes can be activated by chemical signals or applied light. Alternatively, genes can be designed to respond to signals of other cells, allowing complex feedback.



For example, a drug-triggered cell-fusion module [21] can be engineered by introducing a gene that encodes a cell fusion-promoting protein. The gene, which is derived from a reptile virus, is placed under the control of a promoter responsive to the drug doxycycline. In the absence of doxycycline, human embryonic kidney cells carrying the construct exhibit their typical morphology of separated, moderately motile cells. In the presence of doxycycline, cells that encounter one another fuse to create large, non-motile, multinucleate masses.

More complex systems can be constructed that are independent of external signals. For example, genes can be introduced that are controlled by a receptor that detects a specific surface-bound ligand on a neighbouring cell [23]. Activation of the receptor represses production of the ligand by the receiving cell, and activates production of a cell adhesion molecule, resulting in bifurcation of the cell population into stable, layered structures. Here, an inner core of sticky, non-signalling cells is surrounded by a layer of non-sticky cells that express the signal.

Short Summary
Synthetic morphogenesis is limited by knowledge gaps about the competencies of cells and cell groups. This Review discusses a synthetic bioengineering framework based on empirically-determined properties of cells, including goal-seeking and agential behaviours, which will allow the creation of complex devices that cannot be built using bottom-up approaches.