Synthetic Morphogenesis: introducing IEEE journal readers to programming living mammalian cells to make structures

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***Abstract*—Synthetic morphogenesis is a new engineering discipline, in which cells are genetically engineered to make designed shapes and structures. At least in this early phase of the field, devices tend to make use of natural shape-generating processes that operate in embryonic development, but invoke them artificially at times and in orders of a technologist's choosing. This requires construction of genetic control, sequencing and feedback systems that have close parallels to electronic design, which is one reason the field may be of interest to readers of IEEE journals. The other reason is that synthetic morphogenesis allows the construction of two-way interfaces, especially opto-genetic and opto-electronic, between the living and the electronic, allowing unprecedented information flow and control between the two types of 'machine'. This review introduces synthetic morphogenesis, illustrates what has been achieved, drawing parallels wherever possible between biology and electronics, and looks forward to likely next steps and challenges to be overcome.**

***Index Terms*— Synthetic biology, Circuits and systems, Biomedical engineering, Construction, Electrooptics**

I. Introduction: the scope and purpose of this article

This review is about a field of biology that has strong connections to electronics and computer science. Some of these connections are abstract, and concern the field's ways of thinking, designing, and analyzing. Other connections are cybernetic, placing the growth and development of living tissues under electronic control. In the future, there may also be literal, wired connections, electronics and living cells coming together in hybrid 'cyborg' machines, to replace damaged body parts or to perform useful tasks in biofuel manufacture, organic power generation or waste recycling. The article will outline what may be an unfamiliar field to the core IEEE community, will review progress so far, and will highlight opportunities for engineer-biologist collaborations to make rapid advances. The technical language has been chosen to be as close as possible to that of engineers, but biologists' equivalent terms have been placed in parenthesis to help readers understand the research papers cited, most of which use biological nomenclature. Some figures will also be presented two ways, to match the customs of electronic and biologic engineering. There is also a glossary of terms that may not be familiar to the core readership of this journal.

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II. The idea of engineering biology

For most of its history, biology has been an analytic science, its researchers studying in ever more depth and detail the living systems that have evolved naturally on our planet. The dawn of electrical science was similarly analytic: early investigators studied lightning, lodestones and rubbed amber, but electrical science started to advance really quickly when synthetic techniques were added. When engineer-scientists began to make artificial components and circuits, they advanced not just technology, but also basic knowledge. Maxwell's fundamental ideas of electromagnetism, for example, were verified by Herz' transmitter-receive apparatus [1], and measuring devices engineered using new-found knowledge revealed new features of matter, such as semiconductivity [2], or of the world as a whole (e.g. the ionosphere [3]). Now, in the 21st century, tools for manipulating biology have developed to a point that makes it possible to take a synthetic approach to biology too, and to use mixtures of natural and artificial components to build designed living machines. This is being done for the twin objectives of solving societal problems (difficult chemical syntheses, biofuel production, waste recycling [4]) and of accelerating the rate of scientific discovery, much as analytical approaches did in electronics and chemistry over a century ago.

There are many fields and applications within synthetic biology (reviewed in [5]), a general term that covers any aspect of building new biological devices (as distinct from analysing natural ones, which has been the traditional occupation of biologists). Some sub-fields are computational, for example programming living systems to perform logic functions (reviewed in [6]). Some are chemical, altering metabolisms of cells to make valuable compounds or to control diseases such as diabetes or gout [7-9]. This paper will focus on applications that are essentially architectural: modifying cells so that they make multicellular tissues with defined shapes or spatial properties. Because biologists use 'morphology' for 'shape' (wrongly, really, as morphology strictly means '*study* of shape'), the field has been called

variously ‘synthetic morphology’ [10] or ‘synthetic morphogenesis’ [11-12], morphogenesis being the development of shape. There are many reasons for trying to do this, ranging from the need to test theories about how biological shapes naturally form, to the desire to build custom body parts to replace ones that are malformed or injured [13], to making interface tissues between body and machine. Although the gooey, wet world of synthetic tissues may seem a world away from the core material of IEEE, at a deep level the deep principles of biologic and electronic algorithms, networks, systems behavior, feedback and control are essentially similar. There is obvious potential for expertise from electronic engineering to be applied usefully to synthetic morphogenesis. Less obviously, it may be that useful discoveries and ways of working might pass the other way, from biology to electronics. This has already happened with genetic algorithms and neural networks, both used in machine learning. The fields may therefore converge in unexpected ways.

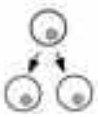
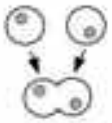
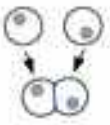
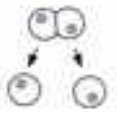
III. A BRIEF OVERVIEW OF NATURAL TISSUE DEVELOPMENT

Synthetic morphogenesis is inspired by the natural processes that build a body, mostly during embryonic and fetal life, with a few examples acquired only after puberty. The rich and complex anatomy of an adult human rests on the shapes and mechanical properties of the person's component tissues. These each consist of collectives of specific cells arranged in specific ways, together with some extracellular ‘packing’ material made by the cells themselves. In tissues such as brain, cells dominate in terms of volume, in tissues such as bone, extracellular material dominates, while most other tissues lie somewhere in between.

The shapes of tissues arise primarily through the activities of their constituent cells [14]. Careful analysis of cells in embryos suggests that individual cells have a relatively modest repertoire of shape-generating (morphogenetic) behaviors, and that differences in the choice, order, timing and extent of these is responsible for different body parts, and indeed different animals, having different structures [10, 15]. The possibility of creating vast variety from only a few basic mechanisms is of course a commonplace in engineering too: from the earliest days of consumer electronics, the number of different electronic appliances vastly exceeded the number of individual resistors, capacitors and tubes from which they were made. Even now, in the era of VLSI ICs custom-designed for different goods, the same applies to the rather limited range of devices fabricated within the ICs themselves. The key to diversity of outcome lies less in diversity of components than in the range of options about how they can be connected together.

The basic repertoire of behaviors is summarized in Table 1, which includes a brief definition of each biological term. Seen at the level of an individual cells, it may not be obvious how these behaviors relate to morphogenesis at a tissue scale, but the next few paragraphs will outline the connections between cell-scale behavior and tissue-scale effects.

Cell proliferation can make a tissue larger, as noted in the table, but it can also be used to change the shapes of tissues. The plane in which a mother cell divides is seldom random. [16]. If cells in a single-layered sheet in the x-y plane arrange their direction of division so that the daughter cells are produced along a line parallel with the z axis, the proliferation will have transformed the single layered sheet into a two layer one, and so on (Fig 1: skin is many cell layers deep thanks to this mechanism). If, on the other hand, the daughters are produced along the x axis, then when all cells have divided the tissue will have doubled its length along that axis while remaining the same size along the y axis: it will have changed shape.

Behavior	**Brief explanation**	**Illustration**
Proliferation	A cell divides into two daughter cells, each of which usually grows to be the size of the original cell.	
Elective cell death	Cells activate a ‘suicide program’ that actively kills them without activating any alarm signals that accompany other ways of dying (for example, under viral attack).	
Cell fusion	Two or more cells come together and join their membranes so that their contents are now in one communal volume.	
Cell-cell adhesion	Cells stick together, something obviously critical to our bodies being solid rather than completely liquid.	
De-adhesion	The removal of adhesive systems, often accompanied by cell motility to create a positive separation between cells.	
Cell motility	Cell movement, usually in the sense of migration. Cells move a lot in development (your face comes from the back of your head).	

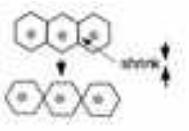
Selective boundary shrinkage	A cell shrinks the length of parts of its boundaries while conserving overall volume (so other boundaries expand).	

Table 1. Elementary morphogenetic behaviors.

Having the probability of a cell proliferating along the y axis determined by a function of the cell's position along the x axis can create more elaborate transformations (Fig 2). Such probability functions are by no means fanciful – a growth-promoting hormone produced by another cell type at the x=0 boundary, and diffusing to make a concentration gradient across our cells of interest, is one example of a natural mechanism that embryos use (reviewed by [17]). Where such a diffusing molecule controls the production of shape

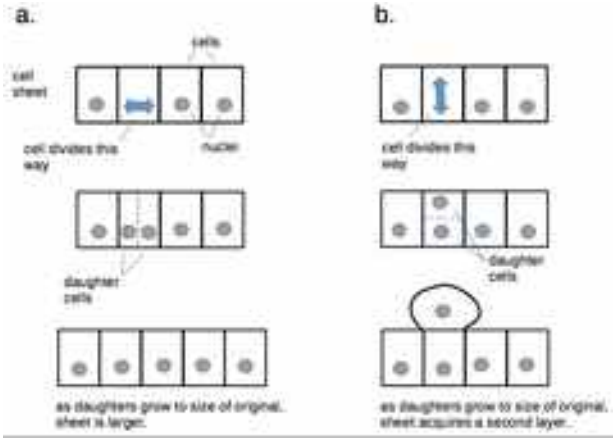


Fig. 1. Different directions of cell division can alter tissue shape in different ways. In both cases, the diagram depicts a view of part of a cell sheet, seen from the plane of the sheet.

(morphogenesis), embryologists call it a morphogen [18]. Other types of biologist, who encounter the same molecule doing other things in the adult, will call it something other than a morphogen. This can be confusing to the uninitiated, but no more than one engineer referring to a BC108 transistor as 'the oscillator' and another engineer working later in the circuit referring to another BC108 transistor as 'the preamplifier'. In both cases, the names refer not to what something is, but what it does.

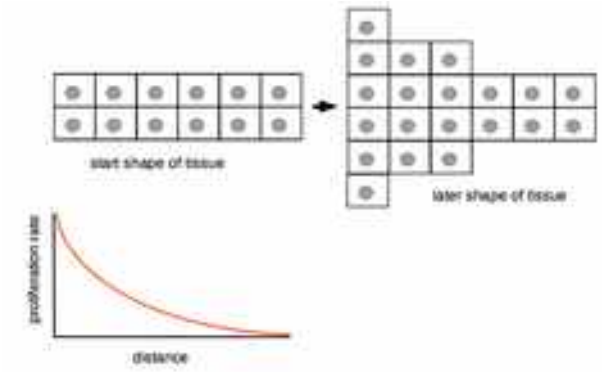


Fig. 2. Having proliferation rate depending on position along one axis (for example, because of a graded concentration in a growth hormone) can transform the shape of a tissue. Cells are shown as blocks for simplicity: in reality they will distort to create overall smooth edges to the tissue.

Cell proliferation can also create complex forms without itself being organized in a complicated way. Where two adhering tissues have different rates of proliferation, mechanical stresses drive curvature (think of the bimetallic strip in a thermostat). In long, thin systems such as the developing avian gut, this effect can produce elaborate 3-dimensional loops [19] (Fig 3).

Elective cell death is a natural feature of development (more than half of the cells you made as an embryo had died before you were born). It is used for many purposes [20]. One is error control – in the embryo and in the adult, cells depend on survival signals from their 'intended' neighbors and, if they are in the wrong place and do not receive the correct signals, they die [21]. Another is the elimination of temporary structures, which the body uses like scaffolding on a building site: necessary for construction but in the way of the final product. Almost all humans form the 'plumbing' of both male and female reproductive systems but eliminate one set to leave them with a classically male or classically female body [22] (rare people keep both, or eliminate both, to create bodies that do not fit a male-female binary classification [23]). Another important use for elective cell death is in balancing populations of cells. The nervous system, for example, vastly over-produces the nerve cells that serve muscles in the limbs, and then eliminates any that failed to wire up properly or that make duplicate connections.

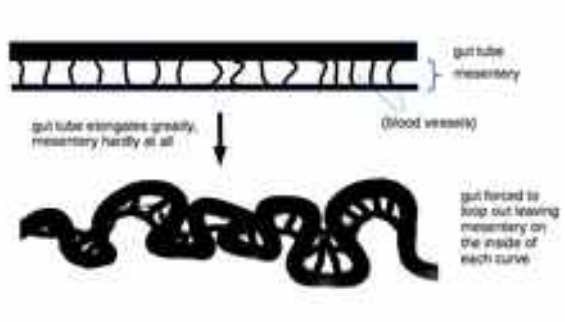


Fig. 3. Production of elaborate loops during interesting development, by differences in growth between two attached tissues, the gut tube itself and the mesentery that provides it with blood and other 'support services'.

Fusion of cells can make giant metabolism-sharing assemblies such as those of skeletal muscle or the placenta. In mammals, it is a one-way process, though animals such as the fruit fly have evolved mechanisms to divide cells with many nuclei up into individuals again [24]. Fusion is a rare event in development, but is included in this discussion because engineering very large cells this way may solve a lot of resource allocation and communication problems in some designed systems.

Adhesion of cells is needed for structures to be stable at all, but the use of different adhesion systems by different cells, or even different amounts of the same adhesion system, can allow one type of cell to make clump that is separate from its

surroundings [25]; the cells that form bone separate from soft tissues this way for example. Cell migration is critical for animals, particularly in ‘wiring up’ the nervous system, and it typically follows cues secreted by other cells to attract or repel. The ability of cells to shorten specific boundaries while leaving others to expand sounds niche, but is really important to create three-dimensional shape. When cells in sheets do it, they cause sheets to curve or roll up into tubes (the spinal cord, which is a tube, forms this way: Fig 4a) [26, 27]. Alternatively, by altering different combinations of boundary lengths, cells in a sheet can exchange neighbors and cause the sheet’s shape to change in 2-dimensions (Fig 4b) [28].

Given that this relatively modest list of behaviors seems to be responsible for the formation of most of our anatomy, it follows that, if we could ‘program’ cells to undergo one or more of these behaviors at times or in orders of our choosing, we ought to have the technology to make ‘designer anatomies’.

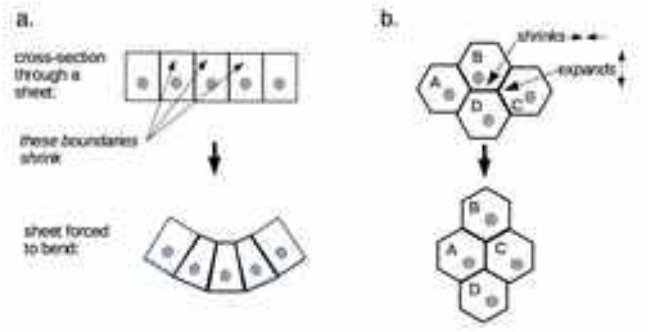


Fig. 4. How selective boundary shrinkage results in morphogenesis. (a) When the boundaries on one side of a cell sheet shrink, the cells are forced into keystone shapes and the sheet is forced to bend into the 3rd dimension. (b) When boundaries parallel to one axis of a plane (the x axis here) shrink while others are allowed to expand to accommodate cell volume (y axis here), the shape of a cell sheet changes from, in this case, squat and wide to tall and thin. Though only 4 cells are shown, this works with any number (one can tile a plane with these shapes).

This review restricts itself to making synthetic 'tissues' by engineering new genetic systems into cells. It will not concern itself with techniques that depend on bringing natural cells together outside the body, where they can spontaneously (re)create tissue-like 'organoids'. Readers interested in these techniques are referred to [29].

IV. Pattern formation

The phrase 'at time or in orders of our choosing' raises the question of how natural embryos manage to evoke specific morphogenetic mechanisms in specific sets of cells at specific times. The general answer lies in the processes of pattern formation, the creation of differences in a field of initially identical cells, and pattern elaboration, the creation of finer patterns from coarse ones. Sometimes patterns are made plainly visible by causing cells to make different pigments (the stripes on a zebra, for example): usually, they are not visible directly but their existence is suspected due to patterned cell behavior. Invisible patterns can often be made visible by staining a tissue for the activity of genes expressed in one phase of the pattern (e.g. stripes, spots) but not the other (e.g. background). Patterning can be spatial, as in the stripes of a zebra, or temporal, as in the cell division cycle. Many patterns are both spatial and temporal, as when the progenitors of vertebrae form in a head-tail sequence at regular intervals in the development of vertebrates such as ourselves. For some organisms, such as 'higher' animals, patterning is a largely internally controlled affair whereas for others, notably plants and fungi, environmental influences such as gravity and light are important cues [30,31].

Once patterning formation has made initially similar cells different, pattern elaboration can rapidly add more details. If, for example, one phase of a pattern (think of a black zebra stripe) produces a diffusible short-lived signaling molecule, this will create a concentration gradient of that molecule in the surrounding background. Responsive cells near the black stripe will receive enough of the molecule to be activated, for example to produce an orange pigment, while those further away will not. Thus a two-phase pattern of colors has now become a three-phase one. This type of process can then repeat. There is reasonable evidence that exactly this type of system operates in living embryos, though a plethora of components and the fact that many things are usually happening at once makes analysis difficult: some influences are still inferred to exist from their effects, rather than proved as physical realities (as was electric current before the discovery of the electron).

It is important to note that, while patterning is important to determine which cells exhibit a morphogenetic behavior at a given time, the complexity of the ultimate form does not have to be reflected in the pattern that evokes it. A clear demonstration of this is given by the avian gut already described (Fig 3) [19], in which a very simple pattern of differential growth, with one of two side-by-side connected tissues growing faster than the other, results in mechanical strain that is relieved by deformation of the tissue into a complicated set of loops. These loops were not present in any biological pre-pattern; they simply emerged from mechanics.

V. Synthetic morphogenesis

A. Components and modules for synthetic morphogenesis

Though there are dangers in extending any analogy too far, many synthetic biologists find it helpful to borrow proven concepts and ways of working from electronic engineering, given its record of success [32,33]. One important tool is hierarchical design, in which an overall problem is broken down into modular functions with clearly specified performance and input and output standards. This allows different teams to work separately on specific modules, where necessary breaking these down into sub-modules before dropping to the level of individual components.

In the world of synthetic biology, 'components' are mainly proteins and DNA. Cells make proteins according to

specifications laid down in the language of DNA so, while the function of a synthetic biological device might be described in terms of proteins and DNA, in reality engineers construct only the DNA and include on it the instructions to make the proteins. There are several reasons for this way of working. One is that proteins are extremely difficult to make chemically, but making DNA with a specified sequence of bases that will cause the cell to make the protein is comparatively easy. Another is that proteins are relatively short-lived, but DNA, copied faithfully by cells as they divide, effectively lasts forever and scales automatically with the cell population.

The part of a DNA sequence that encodes a protein is the 'gene'. There is nothing chemically different about the DNA in a gene from the DNA outside it – the definition of 'gene' is purely functional (think of the paper tape of an antique computer, carrying instructions and the data on which they will operate: the 'program' and 'data' sections of the tape are defined purely by the information they carry and the context in which it operates, and the paper is the same throughout). Next to the gene are regions of DNA that are recognized by certain proteins, the presence of which can cause the gene to be expressed ('read', to make the protein it specifies) or to prevent it from being expressed. These systems are summarized graphically using standard symbols, analogous to those used in circuit schematics: the most common are shown in Fig 5.

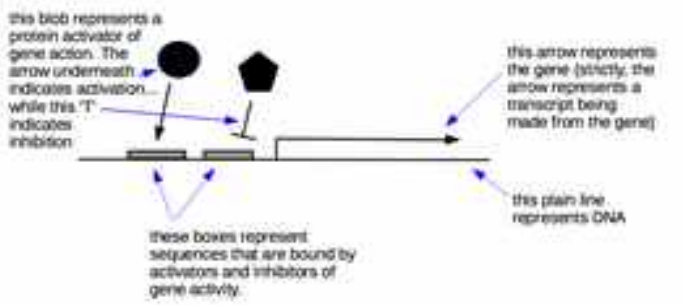


Fig. 5. Common symbols used in schematics of genetic systems. These are symbols typically used by biologists in general. There is an alternative schematic system, SBOL (https://sbolstandard.org/visual-glyphs/), used by some synthetic biologists but it is not used here, because it is very 'busy' with small symbols and makes diagrams difficult to read.

Different gene-controlling proteins bind to different DNA sequences. The system can therefore operate in an approximation of Boolean logic, a gene being expressed, for example, if protein A OR protein B is present AND protein C is NOT present (Fig 6). The Boolean abstraction is a useful aid to thought but it is important to remember that, even if a gene being transcribed or not at a given moment is effectively 'digital', the binding of each protein to its DNA sequence at that moment is governed by probability functions that depend on the concentration of the protein and on its own chemical properties. DNA 'switches' are therefore much noisier than logic gates. Fortunately, morphogenetic events are slow enough (hours) that the noise in the control systems generally averages out as a relatively smooth analogue response in amount of behavior displayed. But sometimes the noise matters. Synthetic biologists have devised approximate equivalents of Schmidt triggers to deal with it in much the same way that electronics engineers worked out how to do years ago [34] (Fig 7). It should be noted that the proteins that switch genes on or off are themselves the products of genes, so the system operates as a complex network rich in feedback.

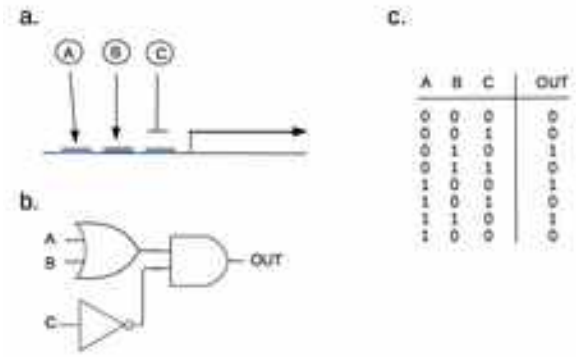


Fig. 6. Boolean logic mediated by gene control. (a) genetic diagram for a system in which OUT = (A OR B) AND (NOT C); (b) electronic version of the same logic operation; (c) truth table for the systems.

So, to take stock… designers of modules generally design a system (examples of which will be presented later), all components of which can be specified in a designed DNA sequence that can be added to the genome of a host cell. One real advantage of biological engineering is that one really does only have to engineer one cell successfully: give it some food and some time, and it will copy itself as many times as needed. As with computer code, almost all of the work is in making the first working version, and churning out copies is trivial.

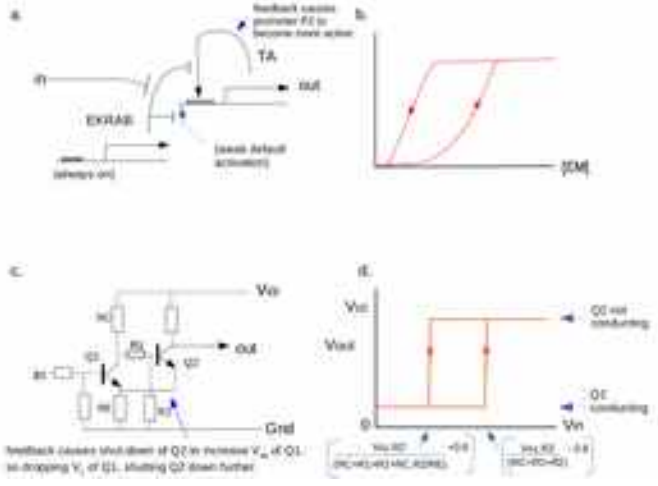


Fig. 7. Using hysteresis to make making firm decisions in the face of noisy inputs. (a) depicts the layout and performance of the hysteretic circuit of Kramer and Fussenegger [35], driven by en input signal of the molecule 'EM'. TA is a transcriptional activator and EKRAB is a transcriptional repressor, both being proteins engineered by combining parts of natural molecules. The placing of the inhibition symbols indicates functional inhibition and does not

imply direct molecular interaction. (b) depicts the real-world performance of this system. (c) depicts a classic electronic solution to the same problem, a transistor version of Otto Schmidt's 'trigger' circuit which happens to have been inspired by Schmidt's study of a biological system (nerve conduction). The 0.6V in the equations for threshold assumes standard silicon transistors, and it is assumed that readers of this journal can understand the schematic without further explanation. (d) depicts the theoretical performance of this circuit, and practice is usually very close to this.

Modules can be at any level of abstraction, but the lowest level of module would typically correspond to a function of a few connected components (the level of, say, an oscillator in electronics) and higher modules would be assemblies of these lower modules (eg a transverter).

For synthetic morphogenesis, low-level modules might include

- Modules for de novo pattern formation (in space or time)
- Modules for pattern elaboration
- Modules for evoking specific morphogenetic behaviors in cells
- Feedback modules (for error control and for detecting a task has been completed)
- Sequencer modules, for systems in which several stages happen in succession.

The extent to which all of these functions should be realized biologically, or to which some might be placed in electronic systems that can communicate with the living cells, is a matter of choice. It may be that hybrid (‘cyborg’) systems will be a valuable intermediate for testing and optimization even when an entirely biological system is the ultimate aim. This approach will be discussed later in this article.

Before leaving the topic of modules and components, it is important to give a warning. In electronics, interactions between components are generally well controlled by their limited physical connections (eg PCB tracks), and there is no reason not to use identical components in different modules within a system. In biology, while interactions between gene-controlling elements and their genes are 'hard-wired' by proximity on the same strand of DNA, interactions between proteins and between those proteins and DNA are not spatially restrained, at least within the same cell. Any protein component can encounter any other protein and any piece of DNA as it diffuses randomly in 3-dimensional space. The ability of components ability to interact during such an encounter is controlled only by their chemical natures. As with badly shielded RF circuits in electronics, modules can interact in ways they were never designed to. Worse, if protein X is a component of module A and a component of module B, there is nothing to isolate one of its activities from the other, and the modules are bound to interact. Thus it is critical to avoid combinations of modules that use the same proteins and this means, that even where modular designs are being used, design teams involved cannot work in isolation but must always know which components have already been used by another module’s team. Also, as mentioned earlier, the cell is far from being an inert 'chassis'. Synthetic modules compete for resources with each other and with the natural systems of the cell, and the scope for unintended interactions when one resource-intensive process steals raw materials needed by another one is very real. At least at this stage of its development, synthetic biology is difficult!

B. *Progress so far: patterning modules*

Confining this discussion to genuinely multicellular animal and plant systems (ie excluding populations of unicellular bacteria), the first synthetic biological patterning systems appeared in the mid 2010s. One approach to patterning built on a series of observations made by Malcolm Steinberg from the 1960s onwards, that animal cells with different types or different quantitative stickiness (affinity) of cell-cell adhesion would spontaneously sort out from one another [36 - 38]. This was initially explained by the thermodynamics of phase separation, the system being in its lower energy state when high-affinity adhesive molecules are not 'wasted' by not binding to an appropriate partner: there is now reason to believe that active cell behaviors also contribute [39]. This sorting behavior was characterized in small aggregates of cells, but computer modeling in the author’s lab suggested that, in larger systems, initial phase separation would starve each phase of potential new recruits, and patterns of patches or islands (depending on cell ratio) would be relatively stable long-term (Fig 8a). We therefore engineered human cells so that one population would express, on a drug-mediated 'command', one homophilic cell adhesion molecule and the other would express a different one [36]. In the absence of the drug, 2- or 3-dimensional mixtures of the cells remained random but, in the presence of the drug, the cells sorted in both 2- and 3-dimensional culture systems, to produce stripes or patches (Fig 8b, c). This was true pattern formation, in the sense that it was de novo and required no existing cues.

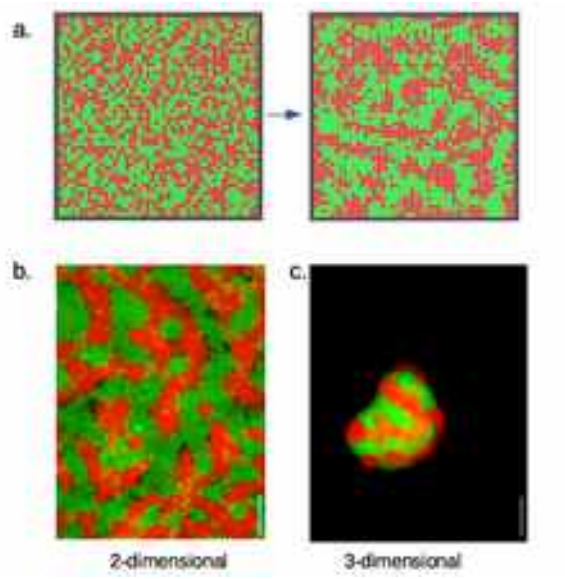


Fig. 8. Patterning by phase separation. (a) shows a grid-based simulation of the effect of having a random mix of red and green cells in a 2-D sheet, then activating homotypic (red-red, and green-green) adhesion systems in the cells and allowing them to move to minimize free energy. (b-c) show patch patterns

made by a real synthetic biological system engineered according to this principle, in 2- and 3-dimensional culture. The images are from the data set from experiments described in our paper [40].

Two years later, Toda and colleagues added synthetic cell-to-cell signaling to an adhesion-based system to generate patterns de novo from a single cell type, rather than from a mixture, as was used above [41]. Both the signaling and signal-receiving ('receptor') proteins were embedded in cell membranes, and the orientation and relative physical inflexibility of these molecules meant that a cell could signal to a contacting neighbor, but not to itself. Indeed, there is evidence that the presence of the signaling molecule on one cell may somewhat inhibit the receptors on the same cell, though the mechanism for this is not well understood. Toda and colleagues engineered cells (Fig 9) so that receiving the signal from a neighbor would activate genes encoding a cell-cell adhesion molecule and genes encoding a green fluorescent protein, the latter playing no role in the sorting itself but being an easily read 'reporter', akin to a status LED on an electronic device. Receiving a signal would also inhibit the otherwise default-on activity of the gene coding for the signaling molecule itself, which was engineered to fluoresce red (again, to tell the experimenters which cells were making it). When it was detecting no signal from a neighbor, a cell would therefore make the red signaling molecule but would make make no adhesion molecules and no green fluorescent protein. When it was detecting a strong signal from its neighbor, a cell would make the adhesion molecule and turn green, but would make no red signal. Two apposed cells initially in the red state would therefore behave as a bistable latch, each trying to tell the other to be green, but doing so less and less strongly the more green it itself became. Equality would be unstable, but a red-green couplet entirely so. The adhesion molecules that accompanied the green state would cause green cells that encountered one another, in the general churn of the a cell aggregate, to adhere, eventually creating a clump of green cells surrounded by red.

a zero

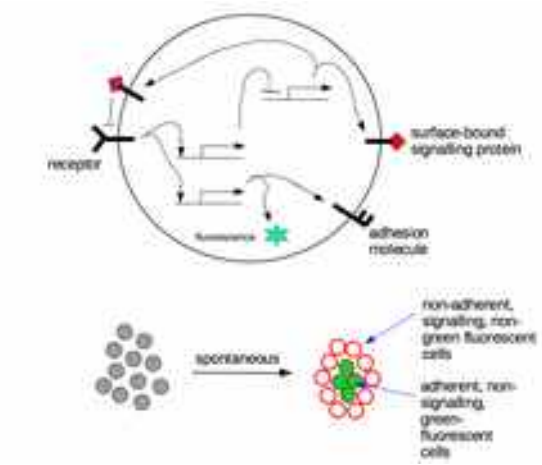


Fig. 9. The genetic device of Toda et al. [41]. If a cell receives a signal from the surface bound protein on a neighbor, it activates its adhesion molecules and green fluorescence, and it inactivates its own expression of the surface-bound signal (and thus reduces its ability to signal to others). The adhesion molecules cause these cells to cluster. Expressing strong levels of signaling protein, on the other hand, reduces the sensitivity of the receptor, stabilizing the signaling state. The cells therefore spontaneously divide into zones of adherent, non-signaling green cells surrounded by non-adhering, non-green but actively signaling cells.

The Toda system [41] used a type of positive feedback, with added negative influences, that is common in latch circuits. And it made use of the 'analogue', probabilistic nature of activating and inhibitory connections (expression of one molecule of signal on a cell will not completely deafen its receptors to signals from other cells) to allow cells to be influenced by neighbors before their internal systems drove them into a latched state.

Positive feedback is also a feature of one of the earliest mechanisms proposed for embryonic patterning, the reaction-diffusion scheme of Alan Turing (he of the Turing machine, and fundamental studies of computability). Turing's original proposal [42] was 'gene-free' and focused on two abstract molecules that diffused in a manner unconstrained by barriers such as cell membranes. One, the activator, diffused only slowly and it catalyzed its own production from a freely available precursor. It also catalyzed the production of the other molecule, a fast-diffusing inhibitor. The inhibitor inhibited the activity of the activator. With suitable parameters for synthesis, diffusion and destruction, the partial differential equations describing the model predict the formation of 'waves' of pattern, peaks of activation being flanked by areas of deep inhibition thanks to the inhibitor diffusing from activation areas. With different parameters, many well-known examples of animal colors pattern can be created in simulation (for example, see [43]). In 2018, Sekine and colleagues published a system inspired by this idea, using the short-range signaling molecule, Nodal, and the long-range signaling molecule, Lefty (both play roles in natural mammalian development, the latter in setting up the left-right polarity of the body, hence the name) [44]. The engineered cultured human cells with extra genetic elements to create the gene network shown in Fig 10. This resulted in patterns of activated cells, that the authors did not claim to be Turing patterns but a closely related phenomenon with more stability than Turing patterns, which they called 'solitary patterns'.

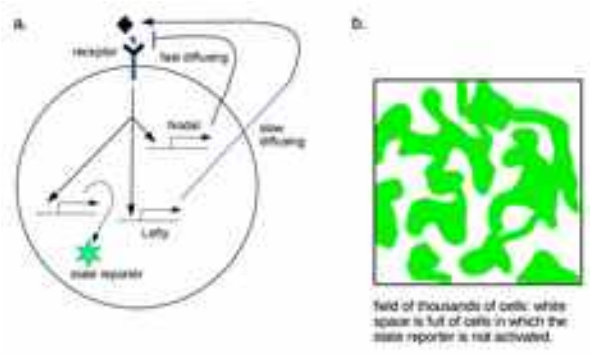


Fig.10.The diffusion-mediated patterning system of Sekine et al. [44]. (a) depicts the synthetic biological mechanism, in which slow-diffusing Lefty

forms a positive feedback loop, antagonized by the fast-diffusing Nodal that Lefty also causes to be expressed. (b) is a sketch from data in the Sekine paper, showing the types of pattern produced in 2-dimensional cultures of thousands of these cells.

C. Progress so far: patterning elaboration

At least one (semi-)synthetic pattern elaboration system has now been constructed [45]. It couples de novo patterning by phase separation with elaboration driven by a diffusible signaling molecule. The phase separation is again between two types of cell. The first is a human cell type engineered to express one of the cell-cell adhesion molecules (Cdh3) described in the phase separation system described above, and also now engineered to secrete a diffusible signaling molecule of the Wnt family. The other cell type is mouse embryonic stem (ES) cells, which represent the cells of a very early embryo and can make any mouse tissue. These cells naturally make the cell-cell adhesion molecule Cdh1. Cdh1-bearing cells stick to other Cdh1-bearing cells strongly, but only weakly to Cdh3-bearing cells. Cdh3-bearing cells, on the other hand, stick strongly to their own kind but only weakly to Cdh1-bearing cells. When added to a suspension of clusters of the mouse embryonic stem cells, the Chd3-bearing engineered human cells form tight balls stuck to the outside of the mouse clusters (Figure 11). These balls secrete their Wnt protein, and this causes nearby cells in the mouse embryonic stem cell clump to switch on genes typical of early mesoderm, the part of an embryo that gives rise to most of our connective tissue (bones, muscles, tendons etc) and a few other things. The rest of the ES cells make other things. Thus the synthetic biological human cells organize themselves into a clump on the edge of their 'targets' as a primary pattern, and their secretions then pattern the internal development inside their target clusters of mouse cells. This is an example of pattern elaboration, and also a demonstration of how synthetic biological devices can be used to control the behavior of entirely natural cells.

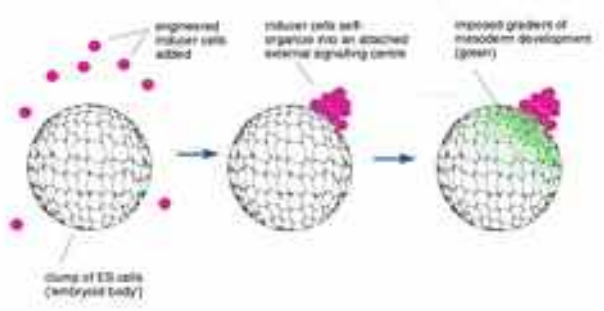


Fig. 11. The pattern elaboration system of Glykofrydis et al. [45]. For the synthetic part, human cells were engineered to express the adhesion molecule Cdh3, the signaling molecule Wnt3a, and a red fluorescing protein to facilitate identification. The Cdh3 drove them to form an adhesive group stuck to but not entering a clump of wild-type (not-engineered) embryonic stem (ES) cells. The production of Wnt3a imposed an overall order on ES cell development, mesoderm forming not randomly but close to the point of attachment of the engineered cells.

Creating signaling gradients on 2D surfaces presents a particular problem, because of the risk that the diffusing molecule is just lost to fluid above. In living embryos, this seems to be solved by cells expressing molecules that can diffuse freely in the plane of their cell membranes and that bing the signaling molecule reversibly (and with only moderate affinity). The signaling molecule is therefore mainly tethered to the membranes and effectively diffuses in 2D, with only small amounts lost [46]. Feedback systems are used in natural embryos' gradient responses, to improve robustness [47].

The use of membrane-tethered molecules works in a similar way, to solve the problem of loss by diffusion, when only neighbours need to receive a signal. A very recent preprint shoes the value of this approach to 2D pattern elaboration of the type described in 3D in Fig 11 [48]

It should be noted that a numbers of patterning systems have also been constructed in bacteria. The short generation times of these organisms means that they are frequently used as the first test-beds for ideas that later appear in mammalian systems, but they lie beyond the scope of this article. Examples of bacterial patterning can be found in [49, 50].

D. Progress so far: morphogenetic modules

Activity in a number of labs has produced a set of morphogenetic modules, active in mammalian cells, to drive one specific type of morphogenetic behavior. Cachat and colleagues published, in 2014, a set of modules to control proliferation, elective cell death, adhesion, fusion and locomotion, and demonstrated each of them in cultured human cells [51]. The adhesion modules were the basis of the patterning-by-phase-separation system described in the section above. To these has been added a system that causes a cell to contract a specific region of its borders, as long as light of a suitable wavelength is present [52].

Each of these systems works by using a 'master regulator' of the behavior, identified either from a natural developing embryo or, in some cases, from a virus that happens to drive that behavior very well (fusion is an example of this). The master regulators are proteins, and are made from genes introduced as part of the DNA-based synthetic device. In most cases, on-off control is exercised at the level of gene activity, and is therefore slow: the time taken to go from gene activation to a finished protein is of the order of an hour in mammalian cells, and the time taken to return to the 'off' state depends on the longevity of the protein, which can be up to days. These long delays are generally tolerable in the context of tissue development because even the natural form of this is slow (it takes nine months to make a baby). The border-shortening module works in a completely different, and highly ingenious way. It is based on a natural protein, Shroom. Natural Shroom binds to actin protein filaments at the upper (apical) borders of cells in a sheet and its other end binds to an enzyme, ROCK [53]. ROCK then activates a version of the filament-contracting enzyme, myosin, that is responsible for muscle action. Martínez Ara and colleagues engineered a highly modified version of Shroom, which now consisted of two separate components, one with the filament-binding activity and the other with the ROCK-binding activity [52].

Each part was extended, simply by adding extra genetic code in the gene encoding it, to include an element from the plant proteins iLID and SspB, that have the property of binding together in the presence of blue light. In the dark, the two halves of 'Optoshroom' were independent, and therefore failed to recruit ROCK to the filaments. In the light, the two halves of Optoshroom clicked together (Figure 12), and ROCK was therefore recruited to the region of the filaments and this part of the cell contracted. Once the light was removed, Optoshroom fell apart into its constituents. This system was fast (seconds to minutes) and control by light opens up possibilities for electronic control of morphogenetic systems (see later).

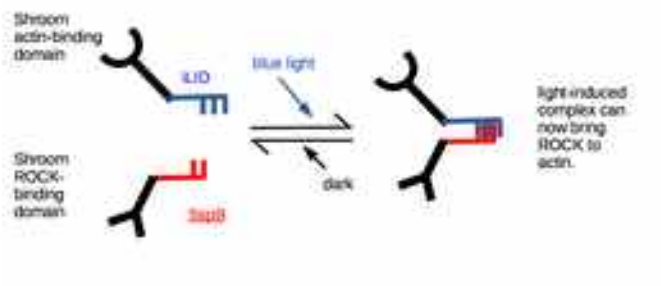


Fig. 12. The OptoShroom system of Martínez Ara et al. [52]. The gene sequence encoding natural Shroom is divided into two sections, one encoding the actin-binding domain and the other encoding the ROCK-binding domain. Each of these is genes is then extended with sequences derived from plant genes, iLID and SspB. The result is two genes, each of which specifies a hybrid protein consisting of one of the Shroom domains and a domain from the light-activated plant proteins. In the presence of light, the plant-derived parts bind together, bringing the two halves of Shroom together also and allowing them to cross-link actin and ROCK.

E. Progress so far: actual synthetic morphogenesis

Proving that morphogenetic modules work in simple test systems is one thing, but creating actual morphogenesis in the sense of tissues with a definite shape is a taller order, and there are so far relatively few examples. As a proof-of-concept, we engineered an elective cell death module, activated by the drug tamoxifen, into cells of the patterning-by-phase separation system, so that it was present only in cells of the 'patches' and not the background [54]. First, patterning was induced by the master control drug (doxycycline) used to activate the genes of that patterning module. Then, when the pattern had been made, the tamoxifen was applied to the system and cells of the patches killed themselves, to leave a sieve-like network of holes (Figure 13). There was no

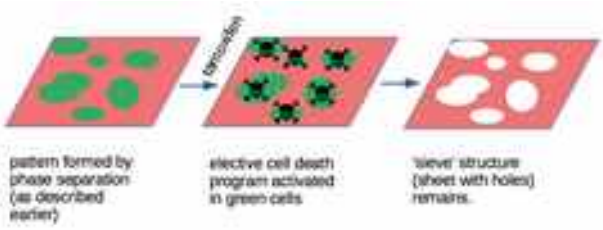


Fig. 13. Transformation of a synthetic biological pattern into morphology by using tamoxifen to induce a second synthetic biological module, in this case for elective cell death, into one phase of the cells (the action of tamoxifen in this engineered system has no connection to its action in a cancer patient; here, synthetic biologists are just using it as a convenient input signal).

particular end-use for such a 'tissue', but its production did demonstrate the idea of coupling patterning modules to morphogenetic modules to create a simple example of biological form.

The Optoshroom-driven boundary shortening module has been used to generate 3-dimensional form from a 2-dimensional cell sheet. Here a 2-dimensional sheet of Optoshroom-carrying cells growing on a flexible surface was subject to all-over illumination, and in response it curved up into the third dimension (Figure 14) [52]. In principle, evoking this type of response locally, and perhaps in a sequence that bends the tissue around different axes in sequence, could create three-dimensional forms using principles analogous to origami.

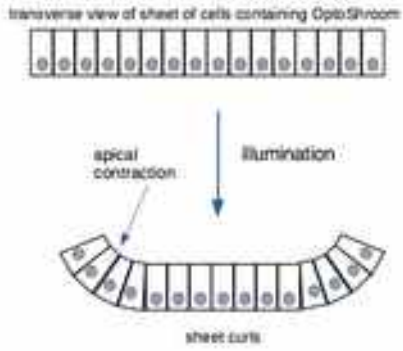


Fig. 14. The use of optically activated Shroom to curl a two-dimensional sheet into three dimensions. This sketch is based on the ideas and work in [52].

F. Progress so far: external control

At this very early stage of synthetic morphogenesis, whatever control needed has generally been exerted from the outside, by experimenters. This has been both to keep the complexity down so that the project is achievable, and to allow relatively rapid exploration of parameters. The most common method of control is to 'borrow' environmental sensing systems from bacteria [55]. Bacteria have a common gene control motif in which a gene is next to the binding site for a protein that represses expression of genes. Since that protein is always present, the gene is usually off. But the protein can also bind a specific environmental small molecule, for example a specific problem molecule such as an antibiotic or a toxic metal ion and, when it does bind this, it falls off the DNA, allowing the gene to switch on, or in other cases it remains on the DNA and is and activator of gene expression when the toxin binds it. The gene will produce proteins that deal with the problem, for example by destroying the antibiotic or pumping the metal out of the cell. It is a simple and rapid system for mounting an emergency response to a chemical threat [56]. Fortunately, if the genes for these repressing proteins are transferred to mammalian cells, they still work. One can put genes driving a

synthetic morphogenesis behavior under the control of a binding site of the repressor protein that binds the antibiotics tetracycline or doxycycline, say, and, with the gene encoding that protein also active in the cell, the synthetic morphogenesis can now be switched on by adding docycycline to a cell culture (Figure 15).

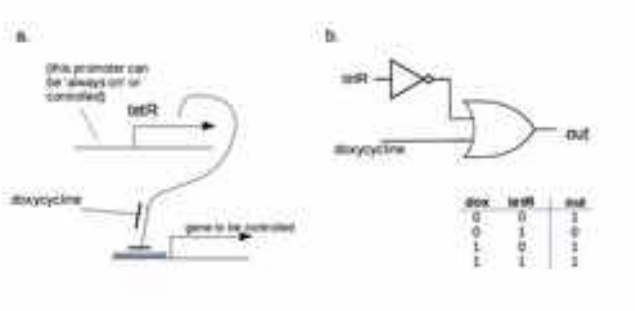


Fig. 15. T he use of antibiotics to control gene expression in a cell. (a) the genetic system, in this case using the tetR transcriptional repressor and the antibiotic doxycycline, which binds to tetR and inhibits its repression of gene activity downstream of tetR-binding DNA sequences. There are many other systems that work similarly. (b) depicts the same logic in logic gate form, with a truth table: in the table, 'dox' stands for doxycyline.

This type of chemical control is very simple to implement and is useful for testing modules on their own and, if different examples are used in the same system, sequential control can be achieved. This type of control was, for example, the basis of the patterning-then-morphogenesis in Figure 13 [54]. But it has the disadvantage that it operates everywhere at the same time. It is therefore more useful for a permissive purpose ('do what you are programmed to do now') than an instructive one ('do this here, but not there'). Light activation has the advantage that it can be directed to specific places, even different places within a cell if this is required [57]. Plants have a variety of light-activated proteins that can be adapted for gene control [58], and the OptoShroom technique already described illustrates one method for achieving this. Another is to use light-controlled binding of proteins containing those plant elements to make a deliberately divided gene-activating protein intact again, and therefore functional as long as light of the correct wavelength is present. Some systems can even be toggled on with one wavelength and off with another [59]. We have done this to control a morphogenetic module for elective cell death so that light prevents cells from killing themselves in response to a hormonal signal (Figure 16) [60]. In principle, using light in this way might allow different parts of a morphogenetic system to be given different instructions, particularly as different examples of these plant proteins respond to different wavelengths.

Direct electrical control can also be done for electrically responsive cells such as neurons and muscles, but these cell types are not much used for morphogenesis because they tend to have very fast and reversible responses, such as muscle contraction, rather than switching on genes to drive production of permanent shapes. Also, physically connecting wires into cells is a lot more trouble than shining light at them.

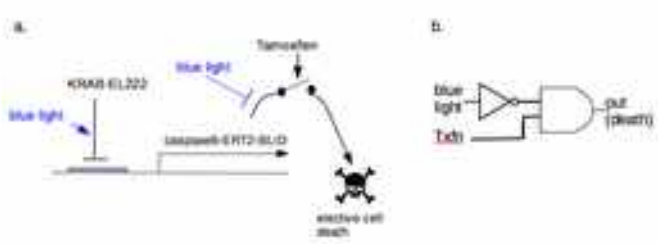


Fig. 16. The optically controlled cell death system of Baaske et al. [45]. (a) depicts the biological system. Blue light allows the KRAB-EL222 protein to inhibit expression of the caspase8-ERT2-BLID death effector, and it also interacts directly with the BLID domain of this effector to trigger destruction of the entire protein. This is required to destroy any protein already made in the dark. Tamoxifen is required for the death protein to function (this extra input was included so that cells could be maintained routinely in dark incubators without dying). In the presence of tamoxifen, the cells carrying this construct die unless blue light is present to save them. (b) shows a gate-based diagram of the logic. Txfn stands for tamoxifen.

External control is very useful for testing purposes. At our current state of knowledge, biological systems are far less predictable then electronic ones, so testing is critically important at all levels from basic components to modules to complete assemblies [61,62]. Electronic engineers entering synthetic biology for the first time (generally to a very warm welcome!) often view their new biological colleagues as absolutely paranoid that their cells are out to make fools of them. After a few weeks, they tend to discover for themselves why, and after a few months, have adopted the paranoid habits themselves, although they will now call them not 'paranoid' but ‘cautious’. The most basic test of modules will verify that an effector module has no effect when it is switched off, and that it has the intended effect and no other apparent effects when it is switched on. More subtle tests might characterize of the on- and off-slopes of the response with respect to time, and the stability of the response in the plateau (fully-on) phase. Where test inputs can be coupled easily to electronics, for example using the optogenetic systems outlined in the previous section, more sophisticated, contextual testing of modules can be done. If module A is intended to be a subcomponent of a complete synthetic biological system S that also contains modules B, C, D..., a computer code can model the inputs to A that will be expected from B,C,D... and any outputs from A to the other modules, and simulate the actions of these additional modules so that A is presented with inputs as if the other modules are really there. This allows engineers to test that module A should operate with the other modules in exactly the way expected. Performing this kind of test for each module would allow debugging to be done module-by-module, which is much easier than it would be in the context of the complete interacting system S. A concrete example of this approach is provided by Perkins et al. [63], who used a combination of synthetic biology and computer simulation of unbuilt systems, with values from the model fed to the living cells by the medium of light, to drive a checkerboard-type patterning system.

External control may be a permanent feature of a system. Light may also be the basis of permanent control and feedback interfaces between medical devices and bodies, and response to the body's own signaling systems may be used to control morphogenesis automatically. For example, a synthetic tissue (an artificial pancreas, for example, to control type I diabetes) may grow as long as the body carries stress signals caused by relative lack of the activity that the tissue should perform, but then stop growing and just maintain itself when the stress has been resolved. High blood urea could be regarded as such a stress signal for a synthetic biological kidney, for example, and cause it to grow large enough to resolve the problem. Real examples of synthetic biological insulin-producing cells have been built, that use blood sugar as a regulator in this way, though in this case to control a hormone useful in diabetes rather than to control growth [8].

G. Stability

External control systems are easy to arrange, but real biology operates with rich networks of internal control, for several good reasons. One reason is to improve reliability and predictability of system performance. As any electronics engineer knows, the best way to ensure predictable performance of an analogue system, for example an amplifier, is to use a sample of the output to control what proportion of the input signal enters amplification stages. Use of negative feedback of this type has been used to control gain for nearly a century, and for the last fifty years or so it has been common to use operational amplifiers with extremely high, but not accurately predictable, inherent gain and to rely completely on feedback networks to clamp system gain precisely where it is needed. Natural metabolic and genetic pathways use similar approaches, a product at or near the end of the pathway typically interacting directly with an essential component at or near its beginning to 'mop it up' and reduce the amount available for the pathway. Recently, Aoki and colleagues constructed synthetic biological pathways according to this principle, which they called and 'antithetical feedback controller' (Figure 17). They verified in bacteria that it does indeed produce stable and reliable operation, and also showed that feedback based on steric hindrance or a functional equivalent is an essential component of such a system [64].

H. Sequential control

Another type of control, very important in morphogenesis, is sequential control: that is, a system that activates modules in a defined order (for example, 'grow, then fold, then have your edges stick to make a tube'). This can be mediated in several ways, bearing in mind that, in synthetic biology, simplicity of construction can trump precision in operation: think of the early days of radio, when tuned radio frequency (TRF) receivers dominated over superheterodynes, despite the precision and ease of use of the more complicated machines, because of the low component count of the former. One crude

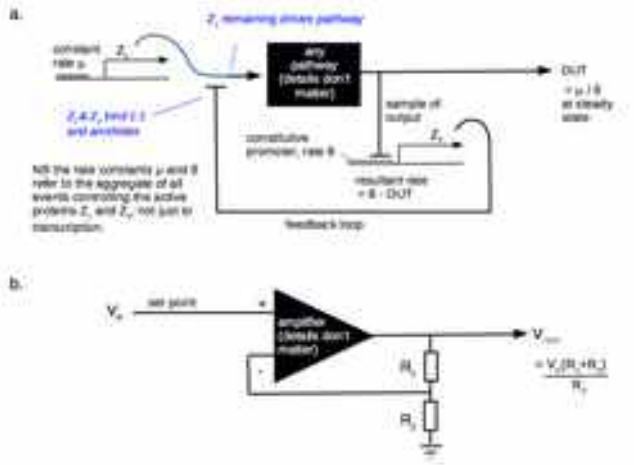


Fig. 17. The antithetical feedback controller of Aoki et al. [64]. (a) shows the basic architecture of the system, which clamps the output at a set level depending on two parameters, however unpredictable are the details of the controlled system. (b) shows, for comparison, a broadly similar system that will be familiar to readers of this journal: the use of negative feedback to control the output of an amplifier, which clamps the output level according to three parameters (input and the values of resistors in a voltage divider). In both cases, care must be taken that components of the feedback loop do not introduce significant phase shifts at any frequency the controlled process can amplify, of there is a risk of oscillation. In electronics, the risk is from stray capacitance, in biology, it is in the long delays between gene control and protein production.

possibility is open-loop control, based solely on timing, each stage being given more than enough time to complete its action before the next begins. Where module action is fast compared to the dynamics of gene expression, such sequential control can be mediated by module 1 including a gene coding for the transcription factor that activates module 2, and that including a gene coding for the transcription factor that activates module 3 and so on. Delays can be added by making some of these stages do nothing but make the transcription factor for the next, introducing a delay or around 20 minutes per stage. They may also include repressors to shut down the stage before (Figure 18).

There are two main problems with time-mediated sequential control: morphogenetic events can be slow compared to genetic ones, making timing difficult, and its open-loop nature means it cannot compensate for unexpected delays. Much more reliability can be achieved by introducing contingency, so that the next stage of the sequence is entered only if the current one completes. This, however, requires a mechanism to detect completion, and in most cases that is far from straightforward to arrange. The next sections consider a few possible ways of achieving this, and are all prospective in the sense that, as far as I know, none has yet been built into a real synthetic biological device.

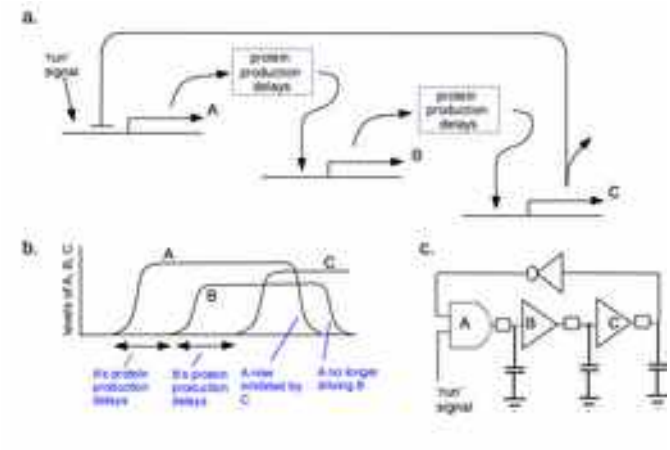


Fig 18. An example of sequential control based on timing alone, each stage triggering the next (as well as performing its own functions, not shown). In this example, the third event shuts down the first, while will have the eventual effect of shutting down all three. (a) depicts a genetic scheme for this, and (b) is a sketch of its behavior: the different plateau concentrations are not significant, and were chosen simply to separate lines in the sketch. (c) illustrates an approximate electronic equivalent, delays arising from the RC networks: high impedance gate inputs are assumed.

I. Detecting the size of a group of cells

The size of a cell population can be altered by morphogenetic effectors that control proliferation or elective cell death [65], so the endpoint of this alteration will require a measurement of size, either absolute or relative to other things. The ease with which the sizes of closely related organisms can change over evolutionary time suggests that, in natural biology, relative size is measured more commonly than absolute: it is unlikely that the parameters of many separate absolute measurements could all mutate at the same time and in the same direction over the short times involved when, for example, dog breeds of very different sizes arise, but relative size measurement would make the sizes of individual body parts adjust automatically as the size of the body changes. Despite this, it will probably be much easier to begin by designing absolute size detection, if for no other reason than it will be easier to test in isolation.

One method for measuring the size of a population is to borrow from the principles of bacterial quorum sensing, something that some bacteria do to change behavior from that of lone agents to cooperative organisms when there are enough of them to modify a local ecosystem. In general, these bacteria secrete a diffusible molecule, and detect the concentrations of that molecule in their immediate environment (reviewed by Abisado et al. [66]). If a bacterium is alone in a liquid, the secreted molecule diffuses rapidly so the concentration at the bacterium making it remains low. If, on the other hand, it is surrounded by other bacteria of the same type, the local concentration rises rapidly for two reasons. One is that the physical presence of other bacterial cells impedes free diffusion, trapping secreted molecules where they are made. The other is that these cells too are producing it, so that the small spaces between the bacteria are being filled by the activities of more than one cell. The bacteria have transcription factors that are activated, directly or indirectly, by the molecule and so change their gene expression in response to it.

Quorum sensing systems can be designed along similar lines to operate in mammalian cells, some using components 'borrowed' from bacteria [67]. The type of quorum-sensing system described above works well enough for bacteria, which are 'trying' to control population-level biochemical behavior but not to make a specific size or shape of colony. For synthetic morphology in mammalian systems, it will not be adequate in its simple form because of edge effects. Even where cells in the centre of a population are receiving high concentrations of the trapped quorum-sensing signal, those on its edge will not be. Any simple system that represses further proliferation based on this single signal would still allow proliferation at its edge. A second, coordination signal is probably therefore needed, which would be produced by cells in the middle that are the first to detect that the quorum-sensing signal has passed its concentration threshold. This second signal, the only direct consequence of the quorum-sensing signal passing its threshold, would also be diffusible but would be acted on by cells even at low concentrations, and would shut off their proliferation (including that of the cells producing it). Thus a decision on group size first made by cells at the centre would be communicated rapidly to neighbors to control the whole group.

In the above paragraph, I glibly wrote of thresholds, but the rising concentration of the quorum-sensing signal will have an analogue nature and, if threshold-type behavior is required, the synthetic genetic systems must be designed to introduce this element. A second requirement is some protection against vacillation where a noise signal is centered at the threshold. Both requirements, a threshold and protection against vacillation, can be met by a hysteresis system based on the well-known Schmidt trigger circuit of electronics . As described earlier in this article, and in Figure 7, a "genetic Schmidt trigger" has been realized by synthetic biologists and behaves in approximately the same way as long as input transitions are slow compared to the response times of circuit elements [35]. This restriction is true for the electronic version too, of course, but whereas the internal response times for the electronic version are typically in the nanosecond range, depending on compromises between speed and current consumption, those of the genetic system are in the range of tens of minutes to hours. For responses to secreted signals cells themselves produce, without any stages of storage for fast release, this is fine, because the rise time of such signals is generally in the scale of hours too, and each round of proliferation takes around a day. It therefore seems feasible to control the size of a cell collective by using secretion of a quorum-sensing molecule, coupled via a Schmidt-trigger to synthesis of a coordination molecule, receipt of which inhibits proliferation (Fig 19).

A completely different mechanism for detecting size relies on a signal being synthesized not by cells of the population in question, but by their neighbors. Consider a population of mammalian cells of one type ('A') bordered on one side by a different cell type 'B', that secretes a diffusible signaling molecule. As long as it has a reasonably short half-life, the molecule will form a concentration gradient, with a high concentration at the B cells falling away across the A cells. The type A cells would be engineered with a receptor-Schmidt trigger-coordination signal as described above [35], but this time with an inverter stage, so that the coordination signal is made only when the concentration at that point in the gradient is below the downward-threshold of the Schmidt trigger. Again, a coordination signal would be required to convey the 'stop proliferating' command to the entire population including those cells higher up the gradient. Gradients of this type can also be used for patterning, different thresholds triggering different morphogenetic activities (see 'patterning' above).

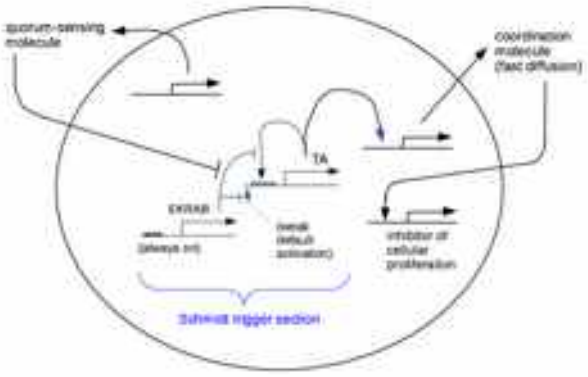


Fig. 19. A possible quorum-sensing system designed to allow cells to proliferate to reach a given colony population, then stop. TA is a transcriptional activator and EKRAB is a transcriptional repressor, both being proteins engineered by combining parts of natural molecules. The electronic version of the Schmidt trigger was shown in Fig 7.

An interesting problem in engineering population control is the risk of 'escape' cells, which mutate their quorum sensing systems and proliferate when they should not, gradually taking over a culture and eventually removing any semblance of control. This problem has been addressed in an interesting pre-print (not yet peer reviewed), which used a signaling system based on a plant hormone to inhibit both cell death and cell proliferation, but with different sensitivities [68]. Very low concentrations allow a part-engineered, part natural cell death pathway to be active. Medium concentrations inhibit death but allow proliferation, and high concentrations inhibit both. If any cell mutates the signaling system to escape hormone-dependent restriction of proliferation, it will also remove its only chance to escape death, and will not therefore be at an advantage. Escape from control is therefore much cell likely (though possible: the death effector might itself mutate. Even complex evolved mechanisms of population control suffer escape mutations, some of which go on to found cancers.

In natural biology, some size control has to operate over very large scales, for example to ensure that your right leg is about the same size as your left. Sometimes, the mechanism for this uses a small-scale correlate of the large-scale item. In the case of the leg, for example, bone growth uses a growth promoting signal that travels through already-formed bone. Briefly, a protein signal called IHH passes from the growing part of the bone, which is deep inside, to the bone's edge. There if stimulates the production of another signaling molecule, PtHRP, that travels back inside to drive proliferation in the growing area [69]. The larger the bone has grown, the less efficient this double-journey is, causing the rate of bone growth to fall according to how much growth has already occurred until it ceases altogether [70]. In other cases, the sensing system is responsive to mechanical signals. If skin is stretched, it will grow along the direction of tension [71], allowing the expansions of pregnancy or obesity to be accommodated automatically, and probably also accounting for the elongated pinnae of people with a fondness for wearing heavy earrings. These natural examples are mentioned here not because there are yet striking synthetic versions, but to indicate the very broad scope of the problem and of natural solutions.

J. *Detecting population ratios*

Useful tissues tend to contain more than one type of cell, specialized for different tasks. For such tissues, measuring the overall size is no guarantee that each cell is adequately represented or that the populations are adequately mixed. One way of promoting this would be to make the proliferation or survival of each cell type require a short-range signal from the other type. If the proliferation of one cell type outstripped the other, even locally, it would run short of the necessary signals and have to wait until the other population caught up. This would not produce absolutely even densities of each type, and can involve oscillation due to overshoots caused by the lag in responses, but would at least ensure a very basic statistical predictability.

Combining detection of overall population size with population ratios is key to anatomical homeostasis. Many body tissues suffer a continual attrition of cells, especially those in areas such as skin and gut that experience sheer stresses against solid objects. That our intestines, for example, stay the same size and shape over decades despite many constituent cells having lives of days or weeks is a clear testament to how strongly feedback systems regulate replacement, either by division of similar cells or, commonly, by division of stem cells that can produce any of a number of cell types needed. In at least some cases, the behaviour of stem cells seems to be controlled by feedback from mature cells, which effectively say 'there are enough of me already' [72, 73]. Their failure to say that leads to production of replacements. IN most systems, the exact dynamics of the feedback (proportional/ integral/ derivative or a combination) are not yet clear, though in fruitflies, at least, there is evidence of integral control [74].

K. Neighbor detection

Detection that a cell has a neighbor somewhere close could be done by the same quorum-sensing systems outlined above, but it is much more common to wish to detect that cells are directly touching. Before considering sensing systems for this, it is important to explain something more about cells in sheets.

Sheets of cells are very important elements of animal anatomy. Obviously the visible skin is a sheet of cells. The linings of our inner passages, from very large ones such as the gut and the great vessels, to smaller ones such as sweat ducts and capillaries, are also sheets of cells, curved round into tubes. The small examples, and also the large ones deep inside the body away from environmental threats, are usually one cell thick. This allows easy transfer of materials from one side to the other, for example so that nutrient molecules can pass from gut to blood or air from lung alveoli to blood. Ones that face the environment or have to stand strong mechanical forces are often many cells thick; skin and vagina are examples and have to stand forces of locomotion and of giving birth, respectively. Cells in sheets are polarized so that they have distinct surfaces: the 'basal' surface faces the body and sticks to other tissues, the 'apical' surface faces the environment or the inside of a tube, and the 'lateral' surfaces stick to neighbors to maintain continuity of the sheet [75]. For cells in a sheet, the concept of touching or not generally refers to the lateral surfaces, and the apical ones would never be expected to touch.

Cell-cell contact can be detected by the type of surface-bound signaling system already described in Fig 9, in which the signal never leaves the surface of a cell so, if it is detected by a receptor in another cell, these cells must be in contact [41]. The example in Fig 9 uses an entirely synthetic signal and receptor system, so will not cross-react with natural cellular signals.

For the purposes of controlling the sequence of synthetic morphogenetic events, though, it would generally be more useful to test not whether any cell has made contact with a target, but whether any free edges still remain. Hole-closing is part of cells' natural repertoire, both in normal development and in wound-healing, and it works by the presence of a free-edge altering the way that cells organize their internal force-generating filaments (the cytoskeleton) [76]. Cells with a lateral surface facing free space respond by proliferating and migrating into that space without letting go of cells behind them [77]. Given time, the overall effect is to repair a hole in a cell sheet. One all-round contact is restored, cells stop proliferating and trying to move, by mechanisms that have been referred to for decades as 'contact inhibition of proliferation and locomotion' The system is complicated, and still not fully understood. It is clear that the natural genes for proliferation need a protein called YAP to be in the cell nucleus to activate transcription factors of the TEAD family, which in turn activate the expression of growth-promoting genes [78, 79]. When cell-cell adhesion proteins stick to similar proteins on neighboring cells, they drive the assembly on the inside of the cell of further proteins. These include signaling molecules that are activated on assembly, and activate enzymes that phosphorylate YAP. Phosphorylated YAP itself becomes part of the protein complex, meaning that it cannot enter the nucleus. Thus cell adhesion sites 'mop up' free YAP, reducing its availability for driving proliferation. When most has been mopped up in this way, proliferation stops [78]. There must be more to find out about this natural system because, for it to work, it seems to me that there must be some system that balances the total amount of YAP in the cell with the total amount that adhesions can mop it up if a cell is completely surrounded. Too little YAP would mean that even cells with one free surface would have none to spare as the other surfaces would hold YAP, and too much would mean that even a surrounded cell would proliferate.

Right now, the easiest way of building edge detection in to synthetic morphogenesis would probably be to use the existing cellular systems, and include a component of the synthetic biological system that is itself activated by YAP. For a system designed to detect when closure is complete before the overall programe advances, the YAP could be arranged to drive the production of a diffusible signal, detection of which would inhibit progression to the next stage. The diffusible nature of the signal would allow each cell to assess the states of it neighbors, effectively putting progression under the control of a Boolean NOR with the YAP of each cell an input to the multi-input of the OR component of the NOR.

L. Detecting tube formation

Detecting that a tube has been formed can make use of the excellent sealing property of cell sheets. In most cases, cell sheets strongly impede the flow of ions, or anything else, across them: trans-epithelial resistance of a typical sheet is around between 50 and 2000 $\Omega cm^{2,}$ depending on the cells involved [80-82]. This strange unit reflects that conventional 'resistivity', as would be measured for a sample of metal, for example, in Ωm, is meaningless for something that can only be one cell 'long' in the direction current flows. The resistance provided by an area of sheet is therefore more useful, and cm^2 are used in preference to m^2 to reflect the scale at which measurements are actually made. Unfortunately, many authors make the error of writing, and journals of publishing, 'Ω/cm^2', which is of course nonsensical: total resistance does not rise as sheet area increases! Cells can secrete substances specifically via their apical surfaces: newly synthesized proteins can carry a structural 'zip code' that is recognized by internal transport machinery of the cell and routed accordingly [83]. This is how, for example, gut cells secrete protein-digesting enzymes apically to destroy food inside the gut, but avoid secreting them basally where they would destroy the body itself.

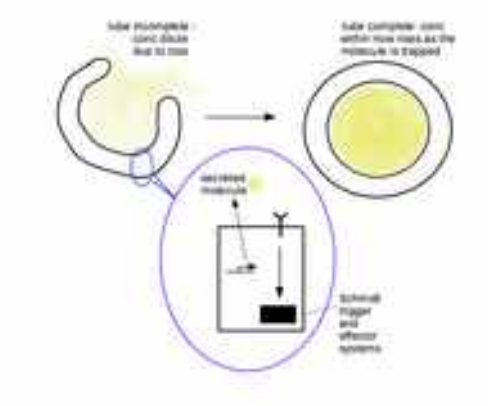


Fig. 20. A possible system for verifying completion of tube formation.

One way of detecting the formation of a patent tube would be to have cells secrete a signaling protein apically, to have receptors for it on their apical surfaces, and to connect the receptors to a Schmidt-trigger-type system as described in the population/ size section above. When the tube is still incomplete, the signaling molecule will be free to diffuse away into the bulk culture medium, or into the general tissues of an organism if the system is operating in a living host. Once the tube is complete, however, the secreted molecule will be trapped inside and its concentration will rise, triggering whatever 'tube complete' response has been engineered downstream of the Schmidt trigger (Fig 20).

For tube-making sheets, this system also confirms that cells have no free lateral surfaces, doing away with the need for the systems described in the previous section. Also, it can operate to detect damage to the tube, at least damage gross enough to allow enough signal to leak away for the off-threshold of the Schmidt trigger to be passed in the downward direction.

The above list of designed to detect completion is by no means exhaustive: it is instead a sample of a few problems and their solutions, intended to show methods of working. The actual problems will vary with the systems being built.

VI. Proof-of-concept machines

In engineering research, it is fairly common to advertise the state of maturity and the potential of a new technology by constructing proof-of-concept machines, the main purpose of which is not to meet a need but to show off the technology and inspire others to do useful things with it. The Wright Flyer carried neither passengers nor cargo and was in the air for a very short time, but it showed that flight was possible and drew many others into the field. The electronics in Sputnik I only transmitted radio beeps effectively saying 'I am here', but this simple machine inspired the space age. It is interesting to speculate on what might be the synthetic morphogenesis equivalents of these machines; devices that grab the imagination without having to do anything of immediate use.

A. Epithelial origami

Epithelia are a type of cell sheet, one cell thick, with the apico-basal polarity described above. They typically grow in two dimensions but they can fold, for example using the Shroom-based system described above [52]. The Japanese art of origami produces complicated 3-dimensional shapes by folding initially 2-dimensional sheets of paper (the paper itself remains 2-dimensional, but occupies 3-dimensional space). In principle, it should be possible to engineer folding into epithelial sheets synthetically, to do this type of thing (Fig 21a). The first proofs of concept may use external specification of the lines along which folding takes place, for example using the Optoshroom system described above [29]. But more interesting would be to engineer patterning systems that will specify these lines of folding, with no need for external inputs beyond a basic, global ‘do it now’ enabling signal. Examples of morphogenesis by local activation of synthetic morphogenetic systems in parts of a cell sheet are shown in figure 21a (local folding) and 21b (local proliferation).

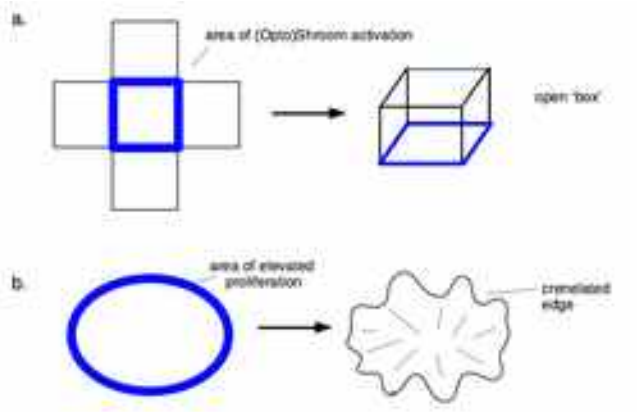


Fig. 21. Examples of ways in which local activation of morphogenetic activities in specific parts of a cell sheet might result in 3-dimensional morphogenesis.

B. Autonomous replicating multicellular systems

Another type of concept device might be a minimalist analogue of a multicellular life-cycle, in which cells express adhesion molecules and grow and as a cohesive colony to a critical size, then switch to a non-adhesive, motile state to disperse, before each founding a new colony. This would be a life-cycle of sorts, including elementary reproduction, but as the cells would be growing in cell culture medium there would be no need for the other complications of real animals, such as needing to have a circulation or a digestive system. Size control could be detected by the quorum-sensing systems described above and drawn in Fig 19, the output being connected not to an inhibitor of proliferation but to a switch that suppresses adhesion and invokes movement. Modules for adhesion and movement already exist [51]. We are currently engineering a 'half-way-house' version of this device, in which the switch between adhesion and dispersal is mediated by light rather than autonomously, with the idea that the device

follows a 'day-night' cycle (albeit one with 'days' around 72h long to allow adequate proliferation in the multicellular phase).

C. Programmable niches

Technologists who wish to exploit human stem cells to build or repair tissues have a problem that the behavior of these stem cells is rather difficult to control in simple culture dishes, and the outcome is never as consistent or reliable as it is when such cells grow in 'niches' surrounded by supportive cells as they do in the body [84, 85]. There are ethical and safety reasons to avoid genetic engineering of human cells intended to enter humans, if possible. The power of synthetic biology might be exploited, though, by engineering cells to make niches that will control the development of wild-type (not engineered) human stem cells. Here pattern formation to make the niche, and sensing and feedback systems to maintain stem cell behavior and to detect and correct departures from what is wanted, might be useful. Our demonstration of a self-assembling signaling centre to control mouse stem cell differentiation, mentioned above [45], might be taken as an earl proof-of-concept but much more subtlety needs to be added for a clinically useful system.

D. Interface tissues

One of the most interesting proofs of concept, from the point of view of IEEE readers, would be the construction of interface structures between electronic circuits and living systems, with a view to eventual application in human bodies. To the best of our current understanding, the natural 'wiring up' of the nervous system works by young neurons producing 'growth cones' that migrate, leaving behind them the axon – the 'living wire' – back to the cell. The growth cones navigate by reading molecular cues on other cells, many of them themselves neurons, and when they meet a cue meaning 'your target', the growth cones stop migrating and instead turn into synapses, which make information-carrying connections to that target cell. Many of the molecules involved in guiding these migrations have been discovered, certainly enough that we ought to be able to engineer neurons to find and connect to targets of our choice within some accessible part of a nervous system. Animals such as worms and insects would be ideal for proof-of-concept, and relatively free of ethical concerns compared to higher animals. If the neurons that were engineered to do this were grown outside the body on a transplantable platform, and if they were also engineered either to 'fire' in response to light (for inputs to the body) or to generate light in response to firing (for outputs from the body), then they would produce a self-wiring optogenetic interface between body and electronic system. One day, a day far away from the crude proofs of concept being discussed here, this type of thing might be used to make artificial limbs or eyes connect fully to the nervous system of a human, to restore function completely.

On the topic of interfaces, it is worth noting that while most of what is known about cell-to-cell communication during morphogenesis is a story of chemical signaling, there is evidence for direct electrical signaling as well. Electrical signaling is not as well understood in morphogenesis - much of the evidence comes from damaging electrical signaling and noting this causes precise defects. Examples have, however, been implicated in various aspects of patterning and growth at multiple scales [86-89]. When electrical control of natural morphogenesis is better understood - and the collaboration of IEEE journal readers with biologists working on these problems might speed this considerably - it may open new areas for synthetic constructs that are particularly easy to interface with computers.

It would be easy to write more about proof-of-concept opportunities, but I would prefer to keep this article grounded in science rather than in science fiction, so will end this section now and move on to one more important point before drawing to a conclusion.

VII. Open vs Closed

The rise of modern-era synthetic biology, around the turn of the century, coincided with a strong and obvious flowering of the Open/ Libre movement in electronic software, then in hardware, then in other areas such as mechanics and even pharmacology. Many leading synthetic biologists shaped the nascent field along Open/ Libre lines, encouraging complete freedom to innovate on platforms already built, and for projects to be 'forked' for different purposes. Part of this was for the same reasons that thee is an Open/ Libre movement in IT (reviewed in [90, 91]). Part of it came from a recognition that life has a unique ethico-philosophical status in the minds of many, including political leaders who legislate limits to allowable research, and in the minds of the public. Much of the opposition to genetic modification of foods, particularly in Europe (where it is still not allowed), arose not because of safety fears but because of a revulsion that any corporation should be able to patent a living thing.

The experience of the genetic modification backlash persuaded some start-up companies in the synthetic biological field to try the open innovation model, which is of course common in academia anyway (at least in biomedicine: surgeons do not patent procedures, for example, they share them). It will be interesting to see, as synthetic morphogenesis matures, whether the open model wins out or whether giant corporations will grow to dominate based on closed models for innovation, or whether we will live with a combination (as with GNU/Linux and Windows in the world of small computers now).

VIII. Conclusion

Synthetic morphogenesis, just an untried idea when it was

suggested 13 years ago, has already matured enough to show that it is definitely possible. Libraries have been made, and primitive demonstration devices have been constructed (by 'primitive' I mean no disrespect to those who built them: Marconi's first radio transmitters were 'primitive', but very important). Constructing them has indicated both that things can be made to work, but also that design and implementation is a lot less straightforward than in modern electronics, with still incompletely understood cells often behaving in unexpected ways.

Electronic engineering has a lot to offer synthetic morphology, not just in terms of devices, but in terms of approach. Electronics engineers have had to face problems to predictive, reliable and now automated design of very highly complex systems, of how to represent them schematically in easy-to-read ways, and also of efficient debugging and analysis. There are many opportunities for engineers for whom IEEE journals are a natural territory, to enter this area of biology and make a positive difference.

It is too early for meaningful commercial products to be made (in the author's opinion), but a next generation of proofs of concept might make the critical bridge between working toys and something that is useful as well as being interesting. At all stages of development, experience with synthetic morphology is likely to continue to enrich our understanding of the natural processes taking place in living embryos, and is therefore a tool for science as well as for future technologies.

GLOSSARY

- **antibiotic** – a now deprecated (but still widely used) name for a molecule that kills bacteria or prevents them multiplying. Because bacteria have evolved receptors that recognize antibiotics, synthetic biologists can co-opt antibiotic and receptor systems to send chemical signals to cells.
- **cell** – the basic unit of animal life (being the smallest component of a body that is 'living' rather than complicated chemistry). We have around 10^{13} cells.
- **embryo** – the early stage of animal development, from egg to when organ primordia form
- **fetus** – the stage of animal development that follows the embryo: a basic body plan set up in the embryo is elaborated and grows.
- **heterophilic** – a molecule is heterophilic if it binds to molecules of a different (specific) kind rather than to its own kind. Contrast with homophilic.
- **homophilic** – a molecule is homophilic if it binds to another of its own kind. Contrast with heterophilic.
- **morphogen** – a diffusible signalling molecule that controls development
- **morphogenesis** – creation of shape (usual during development of an organism)
- **morphology** – shape, study of shape
- **mother cell** – the cell whose division gave rise to two daughter cells. The gendered terminology is just a convention, like ships being 'she', and has nothing to do with biological sex of the cells.
- **motility** – movement
- **promoter** – a section of DNA just upstream of a gene that can bind gene-reading proteins and thus recruit them to read the gene. Typically, the gene is only 'on' when the right combination of proteins is present at the promoter, so it can be viewed as approximating a Boolean switch.
- **quorum sensing** – cells detecting how large a group they are in
- **receptor** – a cellular protein that binds a signalling molecule and initiates some kind of cellular event as a result. Receptors are defined by their function rather than by a common structure (compare with antennas in electrical engineering).
- **steric hindrance** – one molecule binding another and getting in the way of some rival molecule's ability to bind. This is a common control system in biology.
- stress – anything that 'overloads' or damages a cell: stress usually invokes protective responses.
- **tamoxifen** – a drug developed to fight hormone-dependent cancers, that happens to be useable as a signal in synthetic biological systems. Its use here has nothing to do with cancer.

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