SYNTHETIC BIOLOGY

Living shapes engineered

Synthetic genetic circuits can induce cells to form simple 3D structures reminiscent of those generated during early embryonic development. This advance will help engineers build tissues that have desirable structures.

JESSE TORDOFF & RON WEISS

The structures of living organisms have properties that any engineer might hope to recreate. They can self-heal, grow and adapt, and they can have an astonishing range of material properties, from the strength of bone to the lightweight flexibility of an insect wing. To make these structures, a fertilized egg follows a developmental program — a set of instructions for cell behaviour encoded in its DNA. If we could understand and control the development of biological shapes, then we could harness the properties of living structures to build better organs *in vitro* and to generate designer materials that could mimic some of the abilities of living organisms. Writing in *Science,* Toda *et al.*[1] present a method for creating synthetic, designable developmental instructions, paving the way for researchers to engineer customizable biological shapes.

It has been proposed that all that is needed to make the diverse structures of the animal kingdom is a small set of fundamental tools — about ten shape-changing operations, including cell death, adhesion and movement[2]. To decide which of these actions to use, cells can communicate with each other to establish their relative positions.

Toda *et al.* used an engineered cell-communication system called synNotch[3] to mirror this biological set-up. SynNotch is adapted from Delta–Notch signalling — a signalling pathway found in nature, in which cells that have membrane-spanning Notch receptors sense Delta proteins on the surface of neighbouring cells. An intracellular effector domain is cleaved from Notch following ligand binding, and moves to the nucleus to regulate gene expression. In synNotch, the natural core of the Notch protein is used, but the ligand that is sensed and the effector domain that responds are customizable. In this way, it is possible to create multiple channels of modifiable cell–cell communication. With the appropriate choice of ligand and effector, the system can act independently of native Delta–Notch signalling to drive cell behaviour in customizable ways.

The authors engineered the cells so that the synNotch sensors regulated the expression of genes that encode cadherin proteins, which have long been known for their ability to create spatial organization in tissues. Cadherin proteins mediate cell–cell adhesion, and so are essential for holding cells together and creating tissue boundaries during development[4]. Much like oil and water, cell populations that have different patterns or levels of cadherins can sort themselves into separate groups after being mixed together, and can self-assemble into a range of structures *in vitro*[5,6].

To create a synthetic program to guide shape formation, Toda *et al.* built several genetic circuits composed of different synNotch sensors that, when activated by a neighbouring cell, drive the expression not only of different levels or types of cadherin, but also of different ligands to bind to other sensors. In addition, each sensor drives the expression of a gene that encodes a fluorescent protein (green, red or blue), so changes in cell organization can be easily visualized. The authors mixed together cell populations harbouring these different circuits and allowed them to communicate and move freely. They found that engineered communication between the cells led to cadherin-driven cell rearrangement, which in turn led to different cell–cell interactions, producing cycles of communication and shape change (Fig. 1).

Toda and colleagues observed remarkably complex cell behaviours. Cells self-organized to generate 3D structures, including a bullseye pattern and a sphere surrounded by multiple smaller nodes of different colours. The researchers could design instructions to produce specific structures, such as asymmetric forms — a key part of embryonic development. Furthermore, they showed that a structure of nested spheres could regenerate after being cut in two, as is often the case for self-organized tissues in living organisms.

The researchers next built a circuit to generate differential gene expression in a population of cells that was initially identical — a

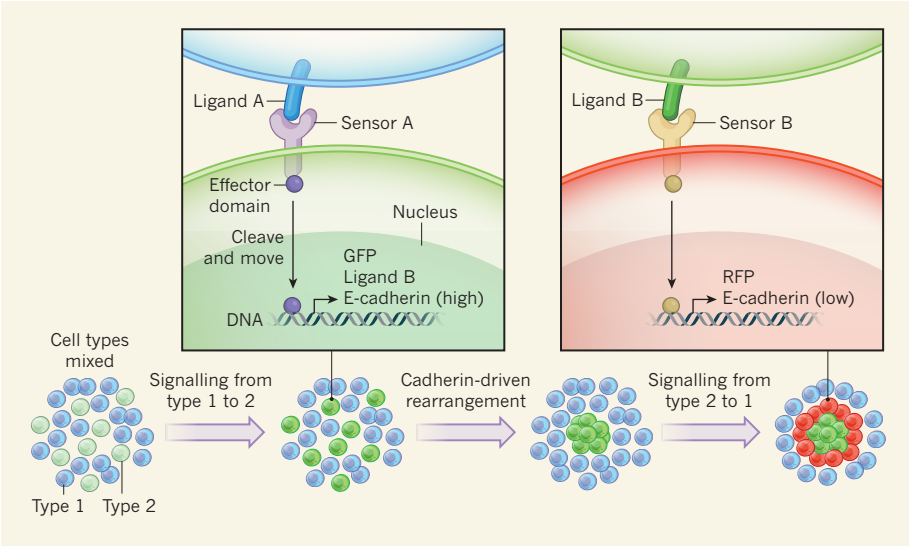

Figure 1 | Synthetic genetic circuits generate self-assembling structures. Toda *et al.*[1] have built circuits that, when expressed in different combinations in a population of cells, caused the cells to self-organize into various structures. In this example, the authors used two circuits. Initially, one group of cells (labelled type 1) expressed a blue fluorescent protein, and the other (type 2) did not fluoresce. When the populations were mixed, a ligand (designated A) produced by type 1 cells activated a receptor (sensor A) on type 2 cells, leading to cleavage of an intracellular effector domain from the sensor. This domain moved to the nucleus to trigger the expression of genes encoding ligand B, the adhesion protein E-cadherin and the protein GFP, which made the cells fluoresce green. E-cadherin caused type 2 cells to adhere to one another, rearranging the cell population. Ligand B then signalled to nearby type 1 cells, activating a different sensor (B). This led to the expression of a protein (RFP) that caused red fluorescence and low levels of E-cadherins. Because low E-cadherin production made the cells somewhat adhesive, they formed a second ring of cells. In this way, the circuits produced cycles of cell–cell communication and self-organization.

process that mimics cell differentiation. To do this, they designed synNotch circuits to emulate one feature of the native Delta–Notch system known as lateral inhibition, in which Notch, when activated by Delta from a neighbouring cell, inhibits the expression of Delta in the receiving cell. This signalling produces a chequerboard pattern of two distinct cell populations, one expressing Notch, the other Delta, from an initially uniform population.

In the authors' lateral-inhibition circuit, one of these cell populations produced a green fluorescent protein, the other red. In addition, the two effector domains also promoted the production of different levels of the protein E-cadherin. In this way, the group was able to generate a structure that had rings of colour starting from a single uniform cell population.

With this work, Toda *et al.* have shown how we can design developmental programs to make new living shapes. Of course, there are limits to this approach. The authors' biggest structures are only a few hundred micrometres across, and adhesion-driven self-organization alone is unlikely to generate structures of the size or complexity of organs. But advances in other types of synthetic-biology shape control could help to fill in some of the gaps. For instance, cells have been generated that can be artificially polarized such that asymmetric cell–cell contacts can be made[7], and synthetic circuits have been designed to modify the behaviour of bacteria so that, across a whole population, arrangements are formed that resemble Turing patterns[8]. These patterns — such as stripes, spirals or the spots on a giraffe — arise during development as a result of biological signalling programs.

In the future, the toolkit established by Toda *et al.* could be expanded to generate short- and long-distance cell–cell communication alongside a synthetic system that controls all of the shape-changing operations involved in making biological structures. This could eventually give engineers total control when designing shapes that have some of the properties of living multicellular organisms. Such a development would be a huge advance. Not only could we map the rules of developmental biology by establishing the limits and constraints of shape-changing biological operations, but we could also grow replacement organs and make adaptive living materials — for example, buildings that could construct and heal themselves. ■

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PALAEOCLIMATE

Hosing the North Pacific Ocean

Climate anomalies punctuated the last ice age, characterized by the discharge of icebergs that released fresh water into the North Atlantic Ocean. It now emerges that fresh water also sometimes flooded the North Pacific. SEE LETTER P.241

KAUSTUBH THIRUMALAI

Abrupt cold snaps known as Heinrich events occurred during past ice ages[1]. These millennial-scale periods of colder climate were associated with massive influxes of fresh water to the North Atlantic Ocean. The influxes were caused by discharge of icebergs from the Laurentide Ice Sheet — an immense sheet of ice that covered most of central and eastern North America during glacial epochs (Fig. 1). Studies of such events are of great interest because they could help to indicate whether rapid reorganizations of ocean circulation might occur in the future, and how they might affect climate[2]. On page 241, Maier *et al.*[3] investigate how Heinrich events affected the North Pacific region during the last glaciation (roughly 115,000 to 12,000 years ago), and the influence of the Cordilleran Ice Sheet, North America's western counterpart of the Laurentide Ice Sheet. They report links between changes in ocean circulation in the North Atlantic and melting of the Cordilleran Ice Sheet.

There is abundant evidence that fleets of icebergs episodically surged into the North Atlantic during the last glaciation. Heinrich events were initially identified from the coarse, ice-rafted detritus that forms layers in marine sediments[1]. Numerous palaeoclimate records have since been obtained showing that ocean cooling and freshening (freshwater influx) occurred across the North Atlantic during Heinrich events[4]. The subsequent alteration of the Atlantic Ocean's circulation weakened heat transport between the hemispheres, and is hypothesized to have induced global temperature and precipitation anomalies through both atmospheric and oceanic pathways[5].

It has been more challenging to find evidence that meltwater from the break-up of the Cordilleran Ice Sheet freshened the North Pacific Ocean. Near-coastal sediments in the northeast Pacific reveal that large abundances of freshwater biota were transported to the region by glacial-era meltwater[6], and glacial debris has been uncovered in the region that can be associated with some Heinrich events[7]. By contrast, studies[8] of planktic foraminifera (microscopic plankton that have shells made from calcium carbonate) preserved in sediments from the North Pacific indicate that no changes in salinity occurred during North Atlantic Heinrich events, muddying the picture of how the Cordilleran Ice Sheet affected ocean dynamics and climate during these events.

Maier *et al.* now report a study of marine diatoms — single-celled plankton that have

MAP SOURCE: ILLINOIS STATE GEOLOGICAL SURVEY

Figure 1 | Ancient ice sheets. During the last ice age, North America was covered by a complex of ice sheets, including the Laurentide Ice Sheet over the centre and east, and the Cordilleran Ice Sheet across the west; this map shows the maximum extent of the ice. Maier *et al.*[3] analysed oxygen isotopes in the remains of organisms called diatoms trapped in sediments taken from the North Pacific Ocean (the star indicates the location of the sediment core studied). The changing ratio of isotopes in different layers of sediment reflects changes in the salinity of the sea water in which the diatoms lived. The isotopic measurements reveal that fresh water inundated the North Pacific during certain Heinrich events — millennial-scale periods during which the climate was anomalously cold. The authors conclude that the fresh water came from melting of the Cordilleran Ice Sheet.