configuration, or stereochemistry, but the steroids preserved in sedimentary rocks are typically mixtures that include more reduced and thermodynamically stable forms, as a result of chemical transformations during burial and subsequent heating. By contrast, the *Dickinsonia* cholesteroids mostly have the same 5β(H) stereochemistry. The only known pathway to this steroid, informally termed coprostane, is via the steroid coprostanol, which is produced in the gut of higher mammals (*10*–*12*). Coprostane is thought to be unstable on geological time scales (*13*). Bobrovskiy *et al.* attribute the presence of these unusual steroids to reduction of *Dickinsonia* cholesterol by bacteria during the original decomposition of the animal. Yet, coprostanes are absent in much younger, exceptionally preserved animal fossils, where the dominant steranes are 5α(H)-cholestanes (*14*). The association of unusual steroids associated with *Dickinsonia* suggests that it may have had a distinct metabolic physiology (see the photo).

Molecular clock evidence suggests that animals originated before 720 million years ago, although the pattern of their divergence during the Cryogenian (720 million to 635 million years ago) and Ediacaran (635 million to 541 million years ago) remains unresolved (*15*). Because molecular clock estimates and morphological characters from fossils offer limited resolution, our best hope for unraveling the early history of animals and the affinities of the Ediacara biota lies with identification of biomarkers that allow us to differentiate specific metazoan clades, particularly among the bilaterians. Further refining the phylogenetic signals from biomarkers may also help to resolve the early history of animals during the Cryogenian and early Ediacaran. Moreover, the fossil-specific biomarker approach taken by Bobrovskiy *et al.* promises to yield many new insights into the fossilization processes that led to soft-tissue preservation across the animal kingdom and throughout geological time. ■

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10.1126/science.aau9710

SYNTHETIC BIOLOGY

Programming cells and tissues

New toolkits of biological parts allow powerful cell programming by synthetic biologists

By **David S. Glass** *and* **Uri Alon**

The field of synthetic biology envisions designing genetic circuits to program cells and tissues. These circuits will enable cells to detect disease states and act to remedy them, direct cells to produce useful substances and materials, and even allow cells to self-assemble into new, user-defined tissues (*1*). Starting from circuits comprised of a few components (*2*, *3*), registries of biological parts have been curated, and increasingly larger circuits have been engineered in cells (*4*), but the size and capabilities of these circuits have been limited. A key challenge to engineering larger systems is composability: the ability to connect any two parts and achieve predictable behavior. On page 1252 of this issue, Gao *et al.* (*5*) describe a composable protein-based system for building circuits, and on page 1217, Andrews *et al.* (*6*) describe a sequential logic system with many states. Recently, Toda *et al.* (*7*) used synthetic cell-cell signaling to drive differentiation and adhesion to form prototype tissues. These studies demonstrate that careful attention to composability can expand synthetic biology beyond its traditional limits.

"...the future is bright for the engineering of new capabilities in biomedicine..."

Composability is a relatively subtle concept, and it is important to keep in mind several related—but distinct—concepts. Orthogonality in synthetic biology refers to parts that do not interfere or that minimally interfere with one another. This amounts to a lack of cross-talk between the parts. For example, two transcription factors are orthogonal if they do not regulate each other's promoters. Modularity refers to a system that can be divided into subsystems, each with a defined function. These concepts can apply at multiple levels—within a protein, in circuits, and in multicellular systems (see the figure).

Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel. Email: urialon@weizmann.ac.il

Composability is a more stringent criterion than either orthogonality or modularity. Parts that are composable are modular units that have matching inputs and outputs and are designed so that any two parts can be connected to each other and yield predictable behavior. Standardization of parts and their interfaces is one way to develop such "plug-and-play" capability (*1*). Even electronic circuits, often considered easy to engineer compared to biological ones, are not necessarily composable, and combining parts can fail if input and output impedances are not designed appropriately; a similar concept in biological circuits has been termed retroactivity (*8*). Natural systems show modularity and orthogonality to a first approximation but do not automatically provide parts that are composable enough for engineering purposes. Thus, synthetic biologists must carefully engineer natural parts to gain composability.

One important goal in synthetic biology has been to bring circuit design from the level of gene regulation, which takes hours owing to the slow process of making proteins, to the level of protein-protein interactions, which can occur within seconds to minutes. Protein circuits are not only faster but also provide powerful capabilities, including interfacing directly with cellular pathways and operating at distinct subcellular sites (*9*). The problem in producing protein-based circuits has been the lack of a toolkit of composable proteins that can regulate one another in arbitrary and predictable ways.

Gao *et al.* solved this issue using molecular scissors called viral proteases (*10*). They engineered these proteases to specifically regulate each other in a programmable way using binding domains, degradation tags, and orthogonal cleavage sites to define sites of protease activity. Because of the attention paid to composability, the authors were able to produce a variety of two-input Boolean logic gates encoded strictly at the protein level. They also formed more complex circuits, including a pulse generator, which benefited from the rapidity of protein-protein interactions. They even used the protease system to implement circuits that selectively kill cells harboring mutant and

hyperactive proteins that drive cancer, all at the protein level and all encoded on a single RNA molecule. This capability expands the possibility of using synthetic circuits as therapeutics, because a protein circuit could be delivered to cells as RNA without any need to edit the genome.

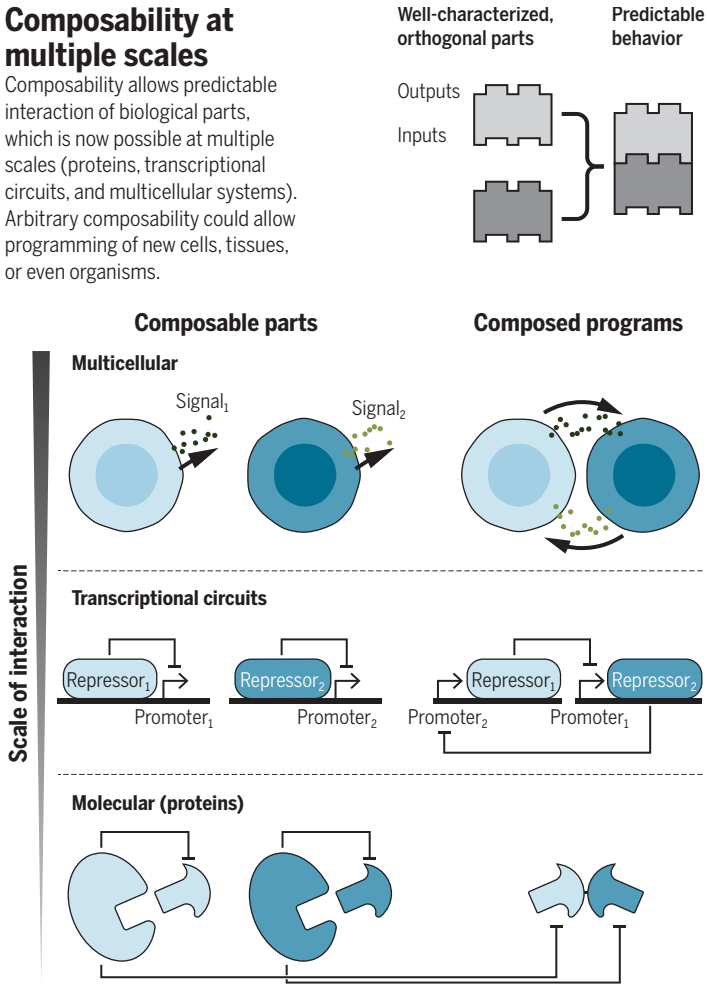
Another goal of synthetic biology has been to produce sequential logic circuits that use memory storage (*11*). Building on their earlier work with combinatorial logic using a composable set of transcription factors (*4*), Andrews *et al.* engineered sequential logic with reversible states, which enabled complex circuits with behavior that depends on memory of past events. This development relied on careful characterization of a library of set-reset latches (toggle switches), each of which is composed of, regulated by, and able to regulate transcription factors. The authors composed these latches together with combinatorial logic circuits into state machines that can remember multiple events, resulting in circuits that can be set to distinct states or locked in place (known as a data latch), as well as cycle between multiple states upon distinct chemical inputs. These versatile circuits could be used to detect, for example, a sequence of "checkpoint" go—no-go events that must occur before a cellular response is appropriate.

Composability can also be applied to the multicellular level, thinking of individual cells as composable units that can be programmed to interact with one another in predictable ways. Synthetic multicellular systems hold promise to aid in tissue regeneration and to program structured living materials capable of responding to the environment (*12*). Key to these abilities are systems for cell-cell signaling, differentiation, and adhesion (*12*). Synthetic signaling (*9*) and differentiation (or memory) (*13*) have been used to produce pattern formation on sheets of cells (*14*). More recently, a genetically encoded system of composable cellular glues (adhesins) was used to program physical cell-cell interactions in bacteria, enabling self-assembly of three-dimensional (3D) patterns at a length scale of individual cells that persist during growth and division (*12*). This capability to engineer precise, 3D patterns could allow efficient separation of chemical intermediates in microbial consortia and enable bottom-up exploration of the evolutionary emergence of multicellularity.

Toda *et al.* programmed tissues to sequentially develop multicellular structures, by means of a composable set of molecular locks and keys (synthetic juxtacrine signaling) that allows neighboring cells to activate each other to express adhesion molecules and to differentiate into new cell types. Sequential steps are self-driven by the timing of adhesion-based cell sorting, a biophysical process that brings cells into direct contact to facilitate signaling. The resultant structures are reminiscent of those that occur during embryo development, with comparable behaviors such as symmetry breaking and regeneration after injury. The regulation of adhesion by synthetic signaling allows the process to be more controlled and versatile than purely adhesion-based structures (*15*), opening up the possibility of engineering artificial development systems for studying in vitro development.

Composability at multiple scales

Composability allows predictable interaction of biological parts, which is now possible at multiple scales (proteins, transcriptional circuits, and multicellular systems). Arbitrary composability could allow programming of new cells, tissues, or even organisms.



In the future, composability is likely to accelerate synthetic biology, especially when circuits at different levels are integrated to achieve much more powerful systems than currently feasible. Memory based on rewriting or excising DNA, if easily composed with flexible, sequential logic (*11*), could allow scalable state machines with long-term stability that do not require active protein expression to maintain memory. Protein-based circuits combined with transcriptional logic could coordinate processes across multiple time scales, with inputs and outputs directly at the clinically relevant protein level. In a multicellular context, coupling signaling and adhesion to sequential logic could allow scalable developmental programs. These integrated processes have natural biological counterparts (for example, animal development and cell signaling networks). Synthetic circuits can shed light on these native systems: One can test in silico simulations by building the circuits within a real biological context but without the unknowns of dissecting a fully native pathway. With the long-standing limits of synthetic biology being crossed by means of composable toolkits for sequential, protein-based, and multicellular systems, the future is bright for the engineering of new capabilities in biomedicine and for gaining deeper biological understanding. ■

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ACKNOWLEDGMENTS

D.S.G. is a member of the Zuckerman Postdoctoral Scholars Program.

10.1126/science.aav2497

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Science, **361** (6408), .
DOI: 10.1126/science.aav2497