Dynamic genome engineering in living cells

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supplied by upwelling water masses at 2.7 million years ago. Either of these scenarios points to enhanced stratification and sea ice coverage after this time (*9*, *10*). Glacial sediments, diatom-based proxies, and lipid biomarker–based proxies in the ANDRILL record also indicate a prolonged cooling and changing stratification of the surface waters at the Antarctic margin beginning 3.3 million years ago (*8*). This capping of the Southern Ocean may have led to numerous other possible feedbacks that could have contributed to Late Pliocene cooling. For example, reduced ventilation of the deep waters around Antarctica may have caused more atmospheric CO_2 to be sequestered in the abyssal ocean. Indeed, paleorecords indicate that atmospheric CO_2 levels fell from above 400 ppm to below 280 ppm in the Late Pliocene, with the sharpest decline near 2.7 million years ago (*11*)—a threshold considered key for the triggering of the Northern Hemisphere glaciation (*12*). Also, a steepened pole-to-equator temperature gradient resulting from the Antarctic cooling may have led to a contraction of the Southern Hemisphere subtropical gyres, reducing heat and salt exchange between the major ocean basins by surface ocean currents (*8*).

Other suggested mechanisms for the Late Pliocene cooling involve wholesale shifts in Northern Hemisphere oceanic and atmospheric circulation patterns driven by tectonic events, or low-latitude processes such as the termination of hypothesized permanent El Niño conditions in the Pacific. Although tectonic events may indeed be the ultimate driver for the onset of Northern Hemisphere ice ages, it is becoming increasingly clear that a major cooling step occurred in the Antarctic before 2.7 million years ago. Given the importance of the Southern Ocean for the carbon cycle and the redistribution of heat around the planet, mechanisms to help explain the cooling of the Antarctic in the broader context of Late Pliocene climate should be explored further. ■

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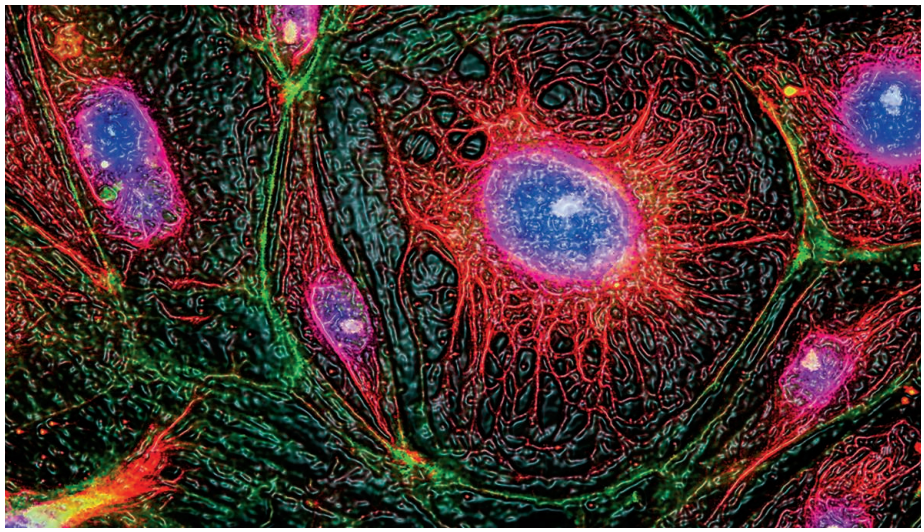
IMAGE: FRANCE-BIOTECH



Synthetic circuits program living cells to remember their past.

SYNTHETIC BIOLOGY

Dynamic genome engineering in living cells

Engineered gene expression systems provide cells with a molecular memory of their past

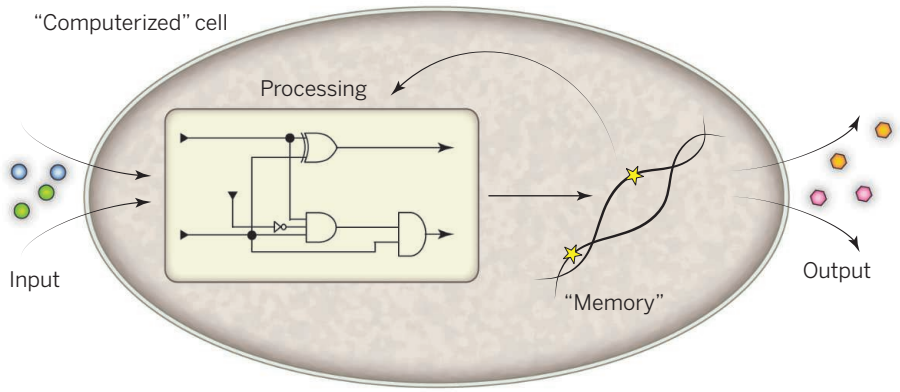
By **Simon Ausländer** *and* **Martin Fussenegger**

Living cells continuously measure, process, and store cellular and environmental information in response to specific signals. Bioengineers are now starting to use these systems to build customized genetic regulatory circuits that can control targeted biological processes. They are also using them to develop new regulatory modes and novel biochemical pathways (*1*). In combination with new genome-editing tools (*2*), this technology holds great promise for the development of biomedical and biotechnological applications of specially engineered "designer" cells. On page 825, Farzadfard and Lu (*3*) move us closer to this goal by constructing a cellular memory device that is based on a conditional gene-editing platform. In this way they have gained access to the enormous storage capacity of genomic DNA to record analog information.

The design of reliable and robust gene circuits faces a number of challenges, including the need for (i) a library of well-characterized gene switches that can be scaled up into complex regulatory circuits; (ii) the stable, long-term propagation of multicircuit networks that ensure correct and dynamic functionality in the cell; and (iii) synthetic memory devices that guarantee sustained storage of the information that is computed by the genetic program. The first synthetic memory devices were based on two distinct small molecule–responsive gene expression units that were configured for mutually exclusive transcription control. These negative feedback loops enable reversible switching of bistable reporter protein expression by a specific trigger compound (*4*, *5*).

More recently, bacteriophage-derived recombinases, which can bind and cut DNA sequences flanked with specific recognition sites, have advanced the design of synthetic on-off circuits (*6*, *7*). Different recombinase-specific sites can be used in combination to control reporter gene expression, thus simplifying circuit design. Moreover, these recombinase-based genetic circuits can perform complex logic functions (AND, OR) and have been shown to record and reset corresponding target gene expression states (*6*). The number of available recombinases, however, limits overall circuit complexity, and the approach is laborious, requiring the integration of each recombinase recognition site at the appropriate DNA target locus.

Farzadfard and Lu neatly sidestep this problem and at the same time harness the entire genomic DNA to store synthetic circuit–computed biological information. Instead of using conventional recombinases



Controlling "computerized" cells. Synthetic gene circuits perform information processing tasks that include three main processes. Environmental and cellular signals (inputs) are detected by biosensors (e.g., trigger-responsive transcription factors) that transmit the information into the gene circuit for interpretation and logic processing. The circuit's architecture determines the executed computation and controls the output response. When coupled to a synthetic memory device, the computed output information can be stored in genomic DNA that in turn can feed again into the circuit to modify the circuit's behavior.

that rely on site-specific recognition, the authors took advantage of the beta recombinase from bacteriophage λ, which uses a single-stranded DNA (ssDNA) oligonucleotide to target complementary DNA and mediate recombination, thereby enabling precise genome editing. While the oligonucleotide template encoding the desired DNA modification is normally exogenously delivered to the cells, the method of Farzadfard and Lu enables the efficient production of ssDNA templates in living bacteria. The approach is based on a bacterial-derived "retron" cassette encoding a reverse transcriptase, an RNA template (msd), and a primer (msr) that together synthesize a single-stranded, covalently linked RNA-DNA molecule (*3*). A major advantage of this tool comes from the authors' finding that the msd sequence can be reprogrammed to include a short variable sequence (about 75 nucleotides) that can target and modify complementary sequences in the genome without disrupting the reverse transcriptase activity of the retron cassette. In an initial experiment, beta recombinase and an engineered retron cassette were coexpressed in bacterial cells to revert deleterious stop codons in a *kanR* resistance gene; the result was increased cell survival when selecting for kanamycin resistance.

Another challenge, however, is to engineer synthetic circuits that operate autonomously in living cells by accurately detecting and responding to input information. Trigger-inducible gene expression systems allow adjustable gene expression in response to specific input levels. For example, in biomedical setups, microencapsulated mammalian cells are expected to autonomously take over essential therapeutic tasks in the patient's body when engineered with closed-loop gene networks. These engineered cells have been shown to control hyperglycemia (*8*) and urate homeostasis (*9*) when plugged into the metabolism of mice. Engineered gene circuits are useful not only for therapeutic applications but also in diagnostics to classify cell types (*10*) or to detect allergic reactions in human blood (*11*).

The work of Farzadfard and Lu exploited a variety of genetic regulatory circuits to drive the expression of the engineered retron as well as beta recombinase. This system was able to provide an analog recording of the magnitude and duration of a treatment by a chemical inducer. The recording was stored in the distributed genomes of a living bacterial cell population and was recoverable over a long time period. Moreover, it is possible to increase the number of engineered retrons to target diverse genomic regions or reset specific, genomically encoded memory information (see the figure). The three functions "write" (beta recombinase), "input" (msd template), and "read" (reporter enzyme) can also be functionally decoupled by putting them under the control of different gene expression units; this strategy results in a three-input AND gate, which highlights the potential of applying this framework to the design of more complex memory and computational circuits (*10*, *12*).

It is the low recombination efficiency of this gene-editing platform that enables the analog recording of input signals in bacterial subpopulations. This setup could be applied in diagnostic settings by using bacterial cells to generate a long-term recording of a set of environmental events that can be subsequently analyzed using DNA sequencing methodologies (as shown in this report) to score recombinant frequencies. Substantially enhancing the recombination efficiency of the gene-editing platform would allow the efficient manipulation of genomes on a single-cell level and would also provide a range of other applications, including digital biocomputational devices.

Recently, another report described an inducible gene-editing platform in mammalian cells, based on the CRISPR/Cas9 system (*13*). In this case, a genomically integrated, inducible Cas9 nuclease was used to introduce DNA strand breaks at specific guide RNA (gRNA)–targeted loci to direct the homologous recombination of an exogenously provided ssDNA template. Retrons have also been shown to be functional in mammalian cells (*14*). The combination of this CRISPR/Cas9-based platform with programmable control of gRNA expression as well as retron-based ssDNA template production could herald a new era of dynamic genome editing in mammalian cells. Coupled with recent advances in gene circuit design, such a system could provide prosthetic cell implants with the capacity to record and display information about disease states in the genome. It could also be used to dynamically program gene circuits to fine-tune tailored therapeutic interventions.

Despite our limited understanding of retrons and the long-term impact of genome engineering in living cells, the extent to which Farzadfard and Lu could combine naturally derived tools to build a new system with novel functionalities is remarkable. These new synthetic biology–inspired engineering approaches will provide us with strategies for combining gene control modalities with genome-editing technologies and will allow the construction of the next generation of "computerized" living cells. ■

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