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Current Opinion in Biomedical Engineering

Scaling computation and memory in living cells

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Abstract

The semiconductor revolution that began in the 20th century has transformed society. Key to this revolution has been the integrated circuit, which enabled exponential scaling of computing devices using silicon-based transistors over many decades. Analogously, decreasing costs in DNA sequencing and synthesis, along with the development of robust genetic circuits, are enabling a "biocomputing revolution". First-generation gene circuits largely relied on assembling various transcriptional regulatory elements to execute digital and analog computing functions in living cells. Basic design rules and computational tools have since been derived so that such circuits can be scaled in order to implement complex computations. In the past five years, great strides have been made in expanding the biological programming toolkit to include recombinase- and CRISPR–based gene circuits that execute complex cellular logic and memory. Recent advances have enabled increasingly dense computing and memory circuits to function in living cells while expanding the application of these circuits from bacteria to eukaryotes, including human cells, for a wide range of uses.

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Current Opinion in Biomedical Engineering 2017, **4**:143–151

This review comes from a themed issue on **Synthetic Biology and Biomedical Engineering**

Edited by **Charlie A. Gersbach**

Received 4 August 2017, revised 30 September 2017, accepted 10 October 2017

https://doi.org/10.1016/j.cobme.2017.10.003

Keywords

Recombinase-based gene circuits, CRISPR-based gene circuits, Cellular computing.

Abbreviations

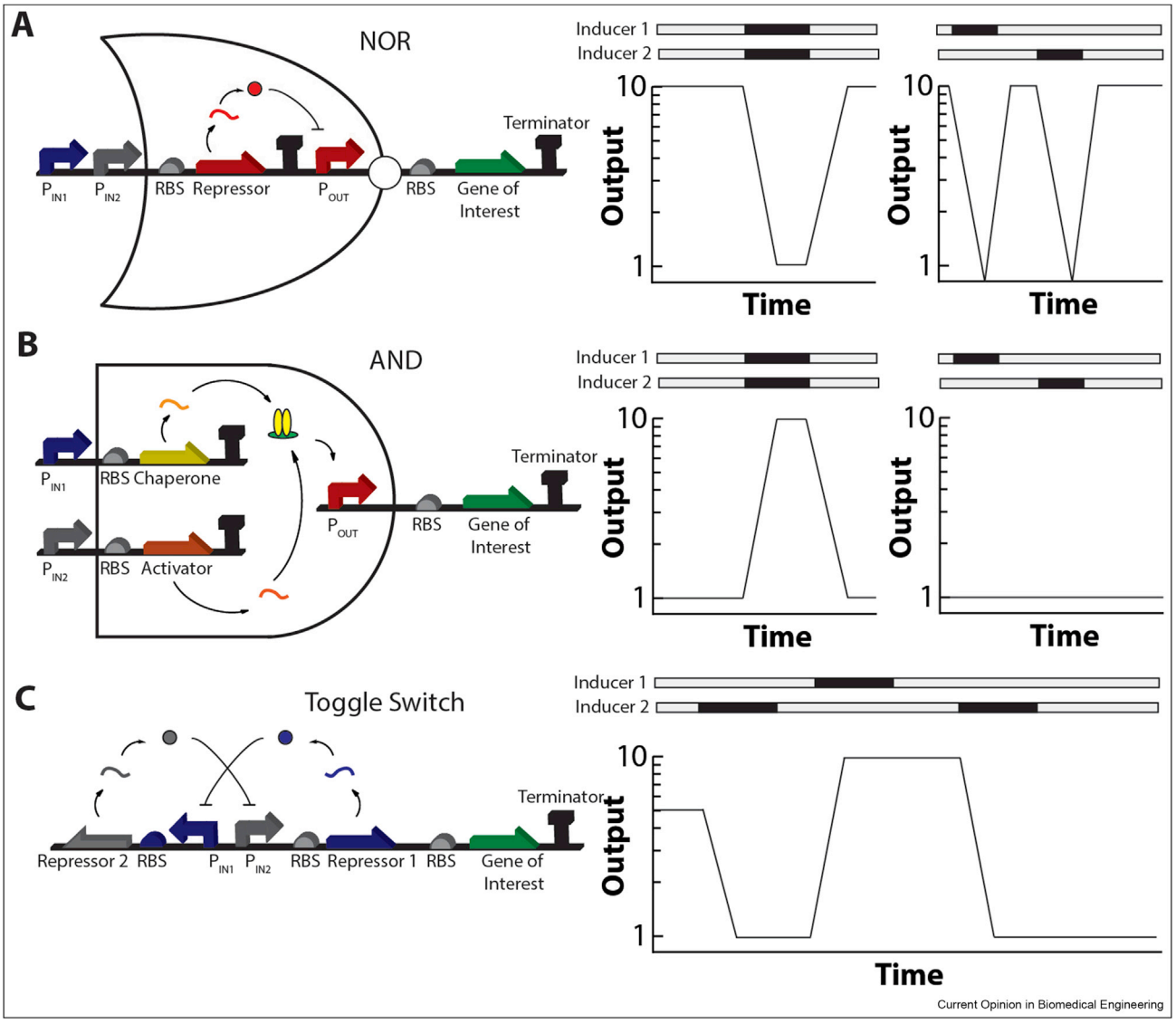
ADC, analog-to-digital converter; att, attachment sites; BLADE, Boolean logic and arithmetic through DNA excision; Cas, CRISPR-associated nuclease; CRISPR, clustered regularly interspaced short palindromic repeats; DNA, deoxyribonucleic acid; FISSEQ, fluorescence *in situ* sequencing; GESTALT, Genome Editing of Synthetic Target Arrays for Lineage Tracing; GFP, green fluorescent protein; gRNA, guide RNA; hgRNA, homing guide RNA; indels, insertion-deletion mutations; LPS, lipopolysaccharide; LSTP, large serine-type phage; mSCRIBE, mammalian synthetic cellular recorders integrating biological events; NHEJ, non-homologous end joining; PAM, protospacer adjacent motif; RAD, rewritable recombinase addressable data; RNA, ribonucleic acid; RSM, recombinase-based state machine; RT, reverse transcriptase; SCRIBE, synthetic cellular recorders integrating biological events; ssDNA, single stranded deoxyribonucleic acid; stgRNA, self-targeting guide RNA; TALEN, transcription activator-like effector nuclease; TNFα, tumor necrosis factor–α; ZNF, zinc finger nuclease.

Introduction

Synthetic biology is a highly interdisciplinary field that focuses on engineering biological systems for a wide range of applications [1]. A major goal in synthetic biology is to perform computation and memory in living cells, thus enabling the programming of "smart" cells that have novel functions [2]. This is achieved through the design and implementation of synthetic gene circuits (Figure 1A and B). Gene circuits are composed of genetic parts that encode RNA, proteins, and gene regulatory elements. These parts direct the temporal and spatial execution of a network of chemical reactions that enable living organisms to dynamically sense, respond, and adapt to their environment. Being able to control and engineer such functions *de novo* would yield transformative applications in the biomedical sciences. However, limitations in the scalability and reusability of genetic parts and the lack of broadly applicable design principles have hindered progress in building high-performance synthetic circuits. The biological parts used to create these circuits often function in a context-dependent manner, thus requiring time-consuming and non-rational optimization strategies.

Now is an exciting time because advances in DNA sequencing, synthesis, and assembly technologies combined with high-throughput experimental approaches are enabling the identification and validation of robust circuit components and topologies. Such approaches are being taken to develop computational programs that can be used to design genetic circuits with much higher success rates than manual strategies [3], [4]. This will eventually lead to the democratization of biological programming technologies, thus enabling experts and

Figure 1



Synthetic gene circuits. Schematic illustrations showing designs for synthetic gene circuits that encode various logic functions and a memory device: **A)** a NOR gate, a genetic circuit that produces an output signal (GFP) only when neither input (chemical inducers) is present [2]; **B)** an AND gate, a genetic circuit that requires both inputs to produce an output signal [2]; **C)** a toggle switch, a genetic memory device that encodes two stable states via two mutually inhibitory transcriptional repressors. In the presence of neither input, 50% of the cells express GFP and 50% are repressed. Gene expression is turned OFF upon exposure to chemical inducer 1 and is turned ON upon exposure to chemical inducer 2. The gene is continually expressed, even in the absence of inducer 1, and is repressed only when cells are exposed to inducer 2 [5].

non-experts alike to realize novel applications much more efficiently.

Memory is a central feature for complex computing, as it enables current behaviors to be dependent on past history as well as current events. Memory is a hallmark of natural living systems, which are able to record events through many different molecular strategies, including transcriptional pathways, epigenetic mechanisms, and protein-based memory. The ability to encode artificial memory in living cells is broadly useful. For example, the stable expression of a protein or metabolic pathway could be triggered transiently with a chemical inducer, thus reducing the cost of a bio-manufacturing process. A cancer cell's history of exposure to various environmental signals could be recorded and then read out at a later time, thus allowing scientists to determine which signals are involved in tumorigenesis. Similarly, factors controlling stem cell differentiation can be recorded and used to develop genetic circuits that enhance tissue and organ regeneration.

One of the first demonstrations of synthetic memory in living cells was the synthetic toggle switch (Figure 1C)