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This month: RNA nanodevices that compute (Yin), self-organizing skin follicles (Shyer), cancer drivers identified by network hitting time (Sahinalp), single-cell microfluidics breaks the Poisson barrier (Abate).

Ribocomputing: RNA Computes in Living Cells

Alexander A. Green, Arizona State University; Jongmin Kim and Peng Yin, Harvard University

Principles

Synthetic biological circuits have wide-ranging uses in reprogramming and rewiring organisms for transformative technological applications. Despite many advances, challenges remain for scaling up these networks due to the limited number of high-performance biological parts and the difficulties that arise when applying these parts in the crowded intracellular environment. To address these issues, we developed a strategy for constructing RNA-only nanodevices that compute complex logic expressions in *E. coli* by exploiting the predictable and designable RNA base-pairing rules (Green et al., Nature *548*, 117–121). These ribocomputing devices function at the post-transcriptional level; use interacting networks of *in silico*-designed RNAs; and condense circuit sensing, computation, and signal transduction tasks into a single multi-functional transcript.

We demonstrated that ribocomputing devices can evaluate two-input AND and OR operations with output signal modulation of up to 900-fold, and we scaled up the circuits to compute six-input OR and four-input AND operations. Moreover, we carried out a 12-input disjunctive normal form expression that combined five ORs, five ANDs, and two NOTs, which represented the most complex of its kind performed in a living cell.

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What's Next?

We are now interested in moving the ribocomputing devices into other cellular hosts as well as employing them to interpret the endogenous transcriptional responses of cells subjected to different stimuli. Beyond cellular applications, these systems could be used to make more effective low-cost diagnostic tests.

Morphological Alchemy: How the Skin Gets Its Follicles

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Principles

As an embryonic organ matures into its differentiated morphology, it must coordinate the activation of appropriate gene-expression programs with changes in tissue architecture. In the avian skin, such a morphological transformation occurs during follicle initiation, when a uniform bilayer develops spaced aggregates of progenitor cells with an activated follicle gene-expression program. Despite decades of study, the initiating trigger for follicles, often assumed to be of molecular nature, has remained elusive.

In stark contrast to molecularly driven models, we find that follicle initiation is the result of self-organizing cellular processes (Shyer et al., Science, published online July 13, 2017. http://dx.doi.org/10.1126/science.aai7868). Importantly, there is no molecular pre-patterning event that "instructs" the emergence of follicle structure. Instead, cellular contractility is responsible for spontaneously reshaping dermal progenitors into follicle aggregates. This physical impulse also triggers a mechanosensitive protein, β-catenin, which then goes on to activate the follicle gene-expression program in aggregated cells. In this context, β-catenin serves as both the "receptor" and effector of mechanical impulse, acting as the critical link between physical/mechanical input and molecular output.

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What's Next?

While focused on the avian skin, this study illustrates the power of viewing organogenesis as a cellular process propelled by mechanical forces. We plan to develop this perspective further by using it to frame morphological changes in other organs as well as across evolutionary timescales.

Identifying Rare or Private Cancer Drivers

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Principles

Knowledge of biomolecular alterations driving cancer is critical for precision therapeutics. We recently developed HIT'nDRIVE (Shrestha et al., Genome Research, published online July 18, 2017, http://dx.doi.org/10.1101/gr.221218.117), a combinatorial algorithm that measures the potential impact of genomic aberrations to expression of other genes that are in close proximity in a gene/protein-interaction network and that prioritizes those aberrations with the highest impact as drivers.

HIT'nDRIVE formulates the driver prioritization problem as a "random-walk facility location" problem, which differs from the standard facility location problem by its use of "hitting time," the expected number of hops to reach a "target" gene from a "source" gene, as a distance measure in an interaction network. HIT'nDRIVE uses "inverse" hitting time as a measure of influence of a source gene over a target gene to identify the subset of sequence-wise altered/source genes whose overall influence over expression altered/target genes is maximum possible. We demonstrate that drivers predicted by HIT'nDRIVE seed network modules/pathways that can effectively classify cancer phenotypes and sub-types as well as predict drug efficacy and survival time.

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What's Next?

HIT'nDRIVE may help clinicians contextualize massive omics data in therapeutic decision making, enabling precision oncology. Application of HIT'nDRIVE to predict drug efficacy in clinical trials may improve the response rate to targeted therapies. HIT'nDRIVE may also be utilized to identify causal genes in other complex diseases facing problems analogous to cancer.

Cell Systems Call

Breaking the Poisson barrier: A Microfluidic Robot for Single-Cell Analysis

Russell H. Cole, California Institute for Quantitative Biosciences and University of California, San Francisco; Zev J. Gartner, University of California, San Francisco and Chan Zuckerberg Biohub; Adam R. Abate, California Institute for Quantitative Biosciences, University of California, San Francisco, and Chan Zuckerberg Biohub

Principles

Complexity is an inherent property of biological systems, but characterizing it requires huge numbers of measurements across many conditions. Microfluidics have the potential to accelerate the study of biological complexity but often afford limited control over reaction conditions.

We developed Printed Droplet Microfluidics, a new approach that allows construction of intricately defined picoliter reactions with complete control. We use a microfluidic robot to read and dispense droplets containing media, chemicals, and single cells, at kilohertz rates with complete determinism (Cole et al., PNAS, published online July 31, 2017. http://dx.doi.org/10.1073/pnas.1704020114). This allows construction of tens of thousands of reactions containing defined combinations of chemicals and cells.

Traditional microfluidics randomly loads cells and are a limited by Poisson statistics…[our approach] dispenses cells deterministically, enabling new combinatorial experiments and multiplexing of different forms of single-cell analysis.

What's Next?

Printed droplet microfluidics is an advance in microfluidics akin to the integrated circuit in computing. Workflows previously requiring custom devices and specialized skill are reduced to scripting, performed by a universal hardware platform. Moreover, while traditional microfluidics randomly loads cells and are limited by Poisson statistics, Printed Droplet Microfluidics dispenses cells deterministically, enabling new combinatorial experiments and multiplexing of different forms of single-cell analysis. The ability to dispense cells with micron-scale precision may enable bottom-up synthesis of tissue architectures by 3D printing.