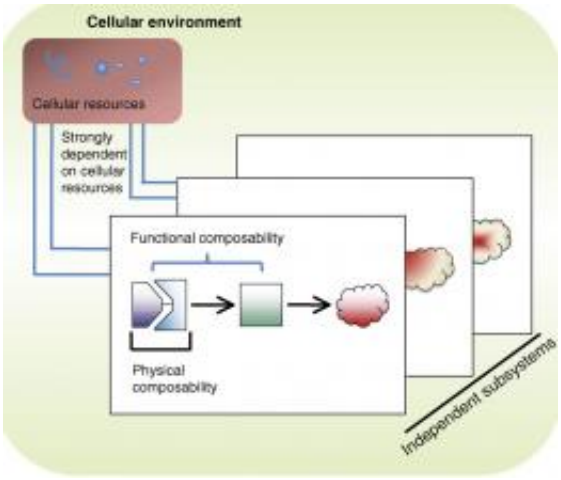
Biological circuits for synthetic biology

May 26 2011, by Lynn Yarris



Synthetic biologists want to design biological circuits from scalable families of interconnecting parts. Multiple independent biological circuits (subsystems) composed of interconnected parts (lines) would share the same resources inside a single cellular environment. Credit: Courtesy of Adam Arkin group

(PhysOrg.com) -- "If you don't like the news, go out and make some of your own." ... Wes "Scoop" Nisker

Taking a page from the book of San Francisco radio legend Scoop Nisker, biologists who find themselves dissatisfied with the microbes nature has provided are going out and making some of their own. Members of the fast-growing "synthetic biology" research community are designing and constructing novel organisms and biologically-inspired systems - or redesigning existing organisms and systems - to solve problems that natural systems cannot. The range of potential applications

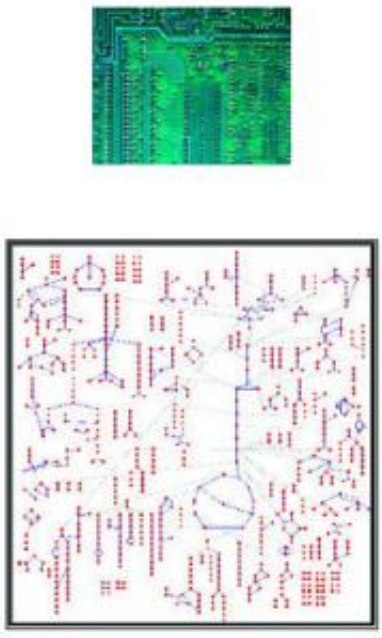
for synthetic biological systems runs broad and deep, and includes such profoundly important ventures as the microbial-based production of advanced biofuels and inexpensive versions of critical therapeutic drugs.

Synthetic biology, however, is still a relatively new scientific field plagued with the trial and error inefficiencies that hamper most technologies in their early stages of development. To help address these problems, synthetic biologists aim to create biological circuits that can be used for the safer and more efficient construction of increasingly complex functions in microorganisms. A central component of such circuits is RNA, the multipurpose workhorse molecule of biology.

"A widespread natural ability to sense small molecules and regulate genes has made the RNA molecule an important tool for synthetic biology in applications as diverse as environmental sensing and metabolic engineering," says Adam Arkin, a computational biologist with the U.S. Department of Energy (DOE)'s Lawrence Berkeley National Laboratory (Berkeley Lab), where he serves as director of the Physical Biosciences Division. Arkin is also a professor at the University of California (UC) Berkeley where he directs the Synthetic Biology Institute, a partnership between UC Berkeley and Berkeley Lab.

In his multiple capacities, Arkin is leading a major effort to use RNA molecules for the engineering of programmable genetic networks. In recent years, scientists have learned that the behavior of cells is often governed by multiple different genes working together in networked teams that are regulated through RNA-based mechanisms. Synthetic biologists have been using RNA regulatory mechanisms to program genetic networks in cells to achieve specific results. However, to date these programming efforts have required proteins to propagate RNA regulatory signals. This can pose problems because one of the primary goals of synthetic biology is to create families of standard genetic parts that can be combined to create biological circuits with behaviors that are

to some extent predictable. Proteins can be difficult to design and predict. They also add a layer of complexity to biological circuits that can delay and slow the dynamics of the circuit's responses.



The circuitry of biological cells is not unlike electronic circuitry as shown in this schematic of E. coli metabolic reactions. Synthetic biologists want to create standard genetic parts for the production of their own biological circuits.

"We're now able to eliminate the protein requirement and directly propagate regulatory signals as RNA molecules,"
Arkin says.

Working with their own variations of RNA transcription attenuators - nucleotide sequences that under a specific set of conditions will stop the RNA transcription process - Arkin and his colleagues engineered a system in which these independent attenuators can be configured to

sense RNA input and synthesize RNA output signals. These variant RNA attenuators can also be configured to regulate multiple genes in the same cell and - through the controlled expression of these genes - perform logic operations.

"We have demonstrated the ability to construct with minimal changes orthogonal variants of natural RNA transcription attenuators that function more or less homogeneously in a single regulatory system, and we have shown that the composition of this system is predictable," Arkin says. "This is the first time that the three regulatory features of our system, which are all properties featured in a semiconductor transistor, have been captured in a single biological molecule."

A paper describing this breakthrough appears in the *Proceedings of the National Academy of Sciences* (*PNAS*) under the title Versatile RNA-sensing transcriptional regulators
for engineering genetic networks. Co-authoring the paper with Arkin were first authors Julius Lucks and Lei "Stanley" Qi along with Vivek Mutalik and Denise Wang.

The success of Arkin and his colleagues was based on their making use of an element in the bacterial plasmid (Staphylococcus aureus)known as pT181. The element in pT181 was an antisense RNA-mediated transcription attenuation mechanism that controls the plasmid's copy number. Plasmids are molecules of DNA that serve as a standard tool of synthetic biology for, among other applications, encoding RNA molecules. Antisense RNA consists of non-coding nucleotide sequences that are used to regulate genetic elements and activities, including transcription. Since the plasmid pT181 antisense-RNA-mediated transcription attenuation mechanism works through RNA-to-RNA interactions, Arkin and his colleagues could use it to create attenuator variants that would independently regulate the transcription activity of multiple targets in the same cell - in this case, in Escherichia coli, one of

the most popular bacteria for synthetic biology applications.

"It is very advantageous to have independent regulatory units that control processes such as transcription because the assembly of these units into genetic networks follows a simple rule of composition," Arkin says.

While acknowledging the excellent work done on other RNA-based regulatory mechanisms that can each perform some portion of the control functions required for a genetic network, Arkin believes that the attenuator variants he and his colleagues engineered provide the simplest route to achieving all of the required control functions within a single regulatory mechanism.

"Furthermore," he says, "these previous efforts were fundamentally dependent on molecular interactions through space between two or more regulatory subunits to create a network. Our approach, which relies on the processive transcription process, is more reliable."

Arkin and his colleagues say their results provide synthetic biologists with a versatile new set of RNA-based transcriptional regulators that could change how future genetic networks are designed and constructed. Their engineering strategy for constructing orthogonal variants from natural RNA system should also be applicable to other gene regulatory mechanisms, and should add to the growing synthetic biology repertoire.

"Although RNA has less overall functionality than proteins, its nucleic acid-based polymer physics make mechanisms based on RNA simpler and easier to engineer and evolve," Arkin says. "With our RNA regulatory system and other work in progress, we're on our way to developing the first complete and scalable biological design system. Ultimately, our goal is to create a tool revolution in synthetic biology similar to the revolution that led to the success of major integrated

circuit design and deployment."

More information:

An article by Adam Arkin and Julius Lucks titled “Synthetic Biology’s Hunt for the Genetic Transistor” can be viewed at spectrum.ieee.org/biomedical/d … genetic-transistor/0

A pdf of the PNAS paper by Arkin, et. al., “Versatile RNA-sensing transcriptional regulators for engineering genetic networks” can be viewed here www.pnas.org/content/early/201 … /1015741108.full.pdf

Provided by Lawrence Berkeley National Laboratory