ACS SyntheticBiology

Editorial

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RNA Synthetic Biology: From the Test Tube to Cells and Back Again

As a synthetic biologist who's academic home has always been in a chemistry department, I think it is fitting that the American Chemical Society launched a journal dedicated to synthetic biology. Chemists have embraced synthetic approaches for nearly two centuries because synthesis often produces something useful, and when coupled with analysis and theory, it can teach us something about how the world works.[1]

The theme of this issue of *ACS Synthetic Biology* is RNA Synthetic Biology. RNA biologists have been using synthetic approaches to study and manipulate biological systems for over 50 years. Studies of synthetic RNA systems in the test tube have not only presaged significant discoveries in the natural world, but have also provided the foundation for the work described in this issue. I'll highlight a few examples here.

In the 1960s, Sol Spiegelman and colleagues identified viral-derived RNA-dependent RNA polymerases (replicases) that could copy an existing RNA template. Spiegelman did not study these replicases in a cell, but in a test tube, where the molecules would be "liberated" from the requirements of carrying out a viral life cycle.[2] He asked: "What will happen to the RNA molecules if the only demand made on them is the Biblical injunction, *multiply*, with the biological proviso that they do so as rapidly as possible?" These studies showed that under selective pressure to replicate quickly, an RNA genome shed 83% of its sequence. In addition to demonstrating that a smaller genome replicated faster (and launching scores of experimental molecular evolution studies), Spiegelman observed: "It should not escape the attention of the reader that the situation described places at our disposal a completely novel method for the resolution of a variety of interesting problems. Potentially, other selective stresses can be imposed on the system to generate RNA entities which exaggerate other molecular features."

Spiegelman's advice was prescient, and just over two decades later, Tuerk and Gold,[3] and independently, Ellington and Szostak[4] put a different kind of selective stress on pools of RNA molecules. Instead of selecting for fast replication, Tuerk and Gold selected for RNA sequences that bound a protein with high affinity, while Ellington and Szostak selected for RNAs that bound small molecules. Through iterative cycles of selection and amplification (today known as SELEX or *in vitro* selection), it became possible to isolate RNA sequences known as aptamers that bind other molecules tightly and selectively. What is more, the discovery of aptamers in the lab presaged (and arguably hastened) the discovery of riboswitches, which are naturally occurring RNAs that use aptamers to regulate gene expression in a ligand-dependent fashion.[5]

The articles in this special issue of *ACS Synthetic Biology* draw on this rich history of RNA synthetic biology. Klauser *et al.* present a small-molecule dependent conditional gene expression system that works in yeast. In their study, they graft aptamers that bind to either theophylline or the antibiotic neomycin to a hammerhead ribozyme (an RNA that cleaves itself) in an attempt to regulate the ribozyme in a ligand-dependent fashion. One of the challenges to creating allosteric switches is finding the appropriate "communication module" that couples the ligand-binding event to a change in ribozyme activity. In the late 1990s, Breaker and colleagues showed that optimizing the communication module could be performed using selection in a test tube.[6] Here, the authors perform positive and negative selections in live yeast cells, where ligand-dependent cleavage of the ribozyme leads to a conditionally beneficial, or conditionally toxic phenotype based on activation of a transcription factor. Using this strategy, the authors were able to screen libraries of $\sim 10^5$ members to discover ribozymes that activate gene expression ~25-fold in the presence of neomycin.

Beilstein *et al.* use a rational, rather than selection-based approach to engineer tetracycline-dependent hammerhead ribozymes that function in mammalian cells. In these experiments, the synthetic ribozymes are cloned in the 3′-untranslated region of a gene. In the absence of tetracycline, the mRNA cleaves itself, removing the 3′-poly-A tail, which leads to fast degradation; when tetracycline is present, RNA cleavage is inhibited, leading to increased expression of the protein coded by the mRNA (GFP). Using this system, the authors show clear tetracycline-dependent increases in gene expression in HeLa cells with a minimal level of background expression in the absence of the ligand. What is more, gene expression is induced at levels of tetracycline that do not appear to affect the cells themselves, which suggests the possibility to use these systems to conditionally control gene expression in animal models.

The final article of this issue by Takahashi *et al.* reminds us again of the utility of test tube experiments in synthetic biology. In this article, the authors show how *in vitro* transcription/translation experiments (TX-TL) can be used to rapidly prototype biological circuit designs. Experiments carried out in cells often take significant amounts of time to perform: the DNA that encodes the constructs has to be cloned into an appropriate vector for delivery into the cell, and the cells need to be transformed and grow to an appropriate density to carry out the desired experiments. These steps lengthen the design-build-test-learn cycle for developing genetic devices. In this paper, Takahashi *et al.* show that experiments in the test tube have the potential to reduce the time needed to screen systems for use in cells (which require greater time and effort to study). Moreover, the experiments are readily accessible to the nonexpert. I had the privilege of spending several days at the Cold Spring Harbor Laboratory Course in Synthetic Biology as these experiments were getting off the ground and can attest that the students (now coauthors) went from pipetman boot camp to producing results in very short order.

The results presented in this issue of *ACS Synthetic Biology* show that the field of RNA Synthetic Biology is vibrant. However, it is worth noting that the field still relies on a relatively small set of aptamers that were discovered in the 20th

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century. I hope that the field continues to develop effective methods to select aptamers that bind small molecules, which will help expand the RNA Synthetic Biology toolkit.

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Notes

Views expressed in this editorial are those of the author and not necessarily the views of the ACS.

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