Engineering RNA Devices for Gene Regulation, Biosensing, and Higher-Order Cellular Information Processing

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Abstract

The proper regulation of gene expression is critical to many biological processes occurring in the cell. It is becoming increasingly apparent that post-transcriptional processing pathways play significant roles in regulating the expression of various genes in both prokaryotic and eukaryotic organisms, where they direct a variety of complex cellular functions. A striking example of a biological communication and control system directing sophisticated gene expression regulation through precise molecular recognition is the class of RNA regulatory elements, called riboswitches, comprised of distinct sensor (ligand-binding) and actuator (gene-regulatory) functions that control gene expression in response to changing levels of specific target ligand concentrations.

Inspired by these natural examples, numerous synthetic riboswitch systems have been developed and have made profound contribution to the field of riboswitch engineering. However, these early examples of synthetic riboswitches pose one or more challenges, such as portability of the switch design across different cellular systems and modularity and programmability of the components comprising the switch molecule. Therefore, we set out to develop a modular and extensible RNA-based gene-regulatory platform that will provide a

framework for the reliable design and construction of gene regulatory systems that can control the expression of specific target genes in response to effector molecules of interest. The platform is called the "ribozyme switch" and composed of distinct functional components, which are modularly coupled and functionally independent of each other. Through this platform, ribozyme switch devices that enable up- or down-regulation of target gene expression were developed. Design modularity and response programmability of the switch platform were also demonstrated. We also exhibited the versatility of the platform in implementing application-specific control systems for small molecule-mediated regulation of cell growth and non-invasive *in vivo* sensing of metabolite production.

Through the ribozyme switch platform, we further constructed higher-order RNA devices that enable complex cellular information processing operations, including logic control (AND, NOR, and NAND gates), advanced computation (bandpass filter and signal shift in the output swing), and cooperativity (signal gain). Finally, we extended the small ribozyme switch platform responsive to small molecules to a different class of ligand molecules, proteins, by developing protein-responsive gene regulators and cellular biosensors. In addition to engineering RNA devices for programming cellular function, we also developed a high-throughput method for functional characterization of small molecule-binding RNA aptamers, which enables robust, accurate, and rapid characterization of such RNA aptamers. This method can be very useful as we (and others) develop RNA aptamers for small molecules of specific interest, which can be subsequently integrated into the ribozyme switch platform as sensing elements for specific applications. Together, these research developments hold synergistic values for the reliable construction of 'designer' gene-regulatory systems for various biotechnological and medical applications.

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