DYNAMIC CONTROL OF GENE EXPRESSION USING RNA REGULATORS

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INTRODUCTION

Regulation of gene expression in the cell is achieved in part through a complex interplay between RNA regulatory elements. RNAs regulate every level of gene expression, from mRNA riboswitches that allow transcripts to self-regulate, to microRNAs that control levels of mRNA in the cell, to ribozymes that process the tRNAs needed for translation. Although some RNA-based methods for dynamic control of gene expression have been extensively studied, like RNA interference, many natural RNA regulatory mechanisms have not been fully exploited for their synthetic biology applications.

While many current methods of targeted gene regulation employ engineered proteins, namely transcription factors, RNA has several properties that make it attractive for dynamic control of the genome.[1] First, RNA regulators are small, which decreases cellular burden and simplifies optimization of devices for systems of interest. Second, RNA regulatory devices can ostensibly be engineered to bind and respond to any ligand of interest, allowing greater flexibility in design. Finally, RNA devices have faster signal propagation times than proteins, allowing for a more rapid and dynamic response to target stimuli.

DESIGN OF SYNTHETIC RNA CONTROLLERS

The simplest type of RNA regulator present in the cell is a riboswitch. These regulatory devices are coded directly into mRNA, allowing the transcript to regulate its own translation. A typical riboswitch contains both an aptamer—a ligand-binding domain—and an expression platform that regulates translation. The expression platform is often a loop that sequesters the ribosomal binding site (RBS), preventing ribosome binding. In this manner, translation is halted until the riboswitch aptamer binds its cognate ligand, inducing a conformational change that frees the RBS. The SELEX method, a directed evolution method for developing aptamers, has expanded the breadth of riboswitches able to bind novel ligands.[2] Once new ligand-binding aptamers are identified with SELEX, they can be linked to virtually any riboswitch expression platform to create synthetic riboswitches that respond to binding a target molecule or metabolite.

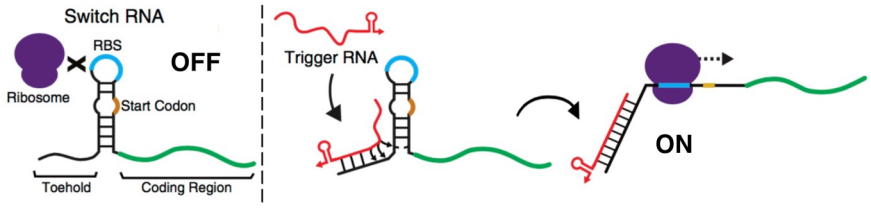

Figure 1. Activation mechanism for toehold riboregulators by a trigger RNA provided *in trans*. Figure adapted from Chappell et al.[4]

One alternative to laborious optimization of aptamers lies in utilizing synthetic devices called riboregulators. Like riboswitches, riboregulators are regions of mRNA that form a loop, sequestering the RBS. However, unlike riboswitches, this expression platform is activated *in trans* by a small, synthetic trigger RNA, which invades the loop and frees the RBS. In 2014, Green and coworkers expanded on this method by sequestering most of the regulatory region of the mRNA in the loop, leaving only a small "toehold" region to be recognized by the trigger RNA (Fig. 1).[3] The specific base-pairing interactions of the regulatory region and the trigger RNA allowed for the generation of 168 orthogonal riboregulator constructs.[4] This is a distinct advantage over engineered riboswitches, because it obviates the time spent optimizing aptamer binding sites and is entirely orthogonal to native cellular regulatory mechanisms.

Unfortunately, the methods used to regulate prokaryotic genes are insufficient for regulating eukaryotic genes, because eukaryotic genes do not possess an RBS. One alternative method uses synthetic aptazymes, which are ligand-binding aptamers grafted to self-cleaving ribozymes. Furthermore, work by Ogawa in 2012 allows for regulation of mRNA transcripts through an aptamer-linked internal ribosome entry site, in a manner analogous to engineering of riboswitches in prokaryotes.[5]

Other recently developed methods for regulating expression are able to reprogram translational machinery to control genes in more ways than simple on/off responses. A recent 2016 study by Anzalone and coworkers uses –1 programmed ribosomal frameshifting paired to ligand-binding aptamers.[6] Frameshifting allows multiple distinct transcripts to be synthesized from one genomic region. By grafting an aptamer into these systems, Anzalone and coworkers were able to precisely control the relative output of two separate transcripts by modulating available metabolite levels. Another method by Ogawa pairs aptamer ligand binding to the formation of a loop that induces ribosomal shunting, or jumping of the ribosome from one sequence to a downstream sequence (Fig. 2).[7] Even CRISPR systems can be employed as RNA controllers by employing a catalytically dead Cas9 protein, which can bind DNA sequences targeted by a guide RNA and repress transcription.[4] In all, these methods expand the repertoire of controls available to researchers, regardless of the regulatory mechanism employed by a gene of interest.

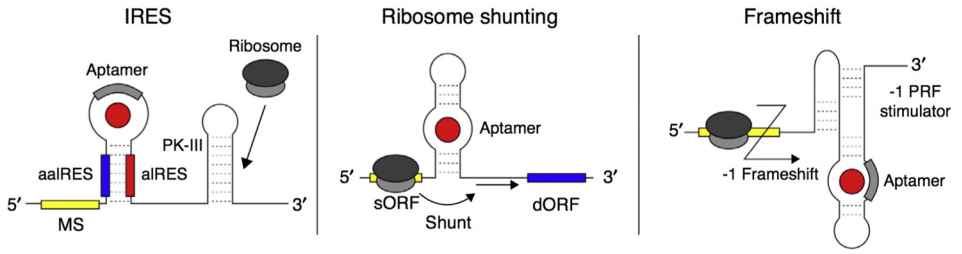

Figure 2. Three separate methods for linking aptamer ligand binding (ligand shown in red) to eukaryotic gene regulation. Figure adapted from Jang et al.[1]

APPLICATIONS AND CHALLENGES OF RNA CONTROLLERS

Most of the above studies looked only at simple on/off control of single genes, as a proof-of-concept. However, RNA controllers have been used in several more complex synthetic biology applications. First, many studies have used aptamer ligand binding as an indicator or selection tool for specific metabolite production in bacteria. For example, metabolite-binding aptamers have been linked to reporter genes like GFP to allow screening populations of cells by cell sorting. Riboswitches have also been installed upstream of selection markers to intrinsically link cell survival to the production of a molecule of interest.[1]

RNA controllers have also been used in constructing genetic circuits and in metabolic engineering. The modularity of RNA-controlled platforms allows construction of genetic circuits not only with basic on/off switches, but also with NOT and NOR gates that invert signal propagation or respond to multiple metabolites at once.[4] The orthogonality of methods that use complementary RNA sequences to activate gene expression allows RNA-only genetic circuits to have many components without triggering unwanted pathways in the cell. Furthermore, the ability to downregulate a gene's expression temporally is invaluable in metabolic engineering, where production of unwanted byproducts remains a constant challenge.

OUTLOOK AND CHALLENGES

Although significant improvements in understanding RNA structure and regulation has helped bring RNA controllers into the same range of dynamic control as transcription factors, RNA-based methods still have drawbacks. First, the use of riboswitch systems is limited by the small collection of established ligand-binding aptamers, and little research is being done to expand the range of ligands these systems can bind. In addition, before RNA controls can be universally applied to eukaryotic gene regulation, more basic research must be done to discover other means by which mRNA transcripts are regulated in the cell.[1] Nevertheless, if these limitations are addressed, then RNA controllers could represent a parallel—and often superior—method to protein-based controls for regulating gene expression.

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