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EMERGING APPLICATIONS OF RIBOSWITCHES IN CHEMICAL BIOLOGY

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Abstract

Living systems use RNA sequences known as riboswitches to detect the concentrations of small-molecule metabolites within cells, and to regulate the expression of genes that produce these metabolites. Like their natural counterparts, synthetic riboswitches also regulate gene expression in response to small molecules, however, because synthetic riboswitches can be engineered to respond to non-endogenous small molecules, they are powerful tools for chemical and synthetic biologists interested in understanding and reprogramming cellular behavior. In this review, we present an overview of natural riboswitches, highlight recent studies toward developing synthetic riboswitches, and provide an overview of emerging applications of these RNA switches in chemical biology.

The central dogma of molecular biology formulated by Crick was very simple. DNA was transcribed to mRNA, which was translated into proteins. However, it has become clear that biology is a bit more complicated. While the role of DNA as an information repository and the role of proteins as building blocks of the cell have remained largely intact, the role of RNA as a transient intermediate on the way from DNA to protein has been revised significantly.(1) We now know that RNA sequences are essential for the function of some enzymes,(2) can perform self-cleavage,(3) and can regulate gene expression either in cis(4,5) or in trans.(6) Because RNA sequences can carry out diverse tasks and are amenable to engineering both in vitro and in vivo, they are particularly attractive for controlling cell behavior.

RATIONALE FOR REPROGRAMMING CELLS USING RNA

A major goal of synthetic biology is to program bacteria to autonomously perform a variety of tasks.(7) To operate autonomously, cells need to sense and respond to changes in their environment, evaluate their performance, and modify their behavior to accomplish the assigned tasks. Because most cellular behavior can be regulated at the genetic level, cells can be programmed to perform new duties by providing them with instructions in the form of new genetic material. By encoding genetic instructions that are executed by the cell only when it achieves a certain metabolic state, or when it detects a specific exogenous ligand, it is possible to program a cell to perform a desired task when it encounters specified conditions.

Traditionally, genetic engineering efforts have focused on introducing recombinant DNA into cells to direct the heterologous expression of proteins. However, for many applications in synthetic biology, RNA provides a powerful alternative to using proteins to regulate cell behavior. In addition to storing information, RNA sequences can fold into complex 3-dimensional structures that are capable of binding ligands and catalyzing chemical reactions, much in the way that proteins do. RNA sequences that can bind ligands are known as aptamers.

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Aptamers are particularly useful tools for reprogramming cellular behavior because in addition to recognizing ligands, appropriately placed aptamers can also regulate gene expression in a variety of organisms, and through a variety of mechanisms.(7–14) Such RNA switches, known as riboswitches, are ideal tools for modulating bacterial behavior because, as we will see, it is possible to create riboswitches that recognize new ligands, and to introduce these genetic switches into cells by well established techniques. Bacterial behavior can be controlled by using the riboswitch to directly regulate the expression of a single gene, or by incorporating the switch as a control element within a more elaborate genetic circuit. We begin this review by presenting an overview of naturally occurring riboswitches, move on to discuss the state-of-the-art in synthetic riboswitch engineering, and conclude by reviewing current and prospective applications of ligand-sensing RNA switches in chemical biology.

METABOLITE-SENSING CAN BE ACHIEVED WITH RNA

The elucidation of the *cis*-acting regulatory mechanism of the *trp* operon during the late 1970s demonstrated that the structure of an mRNA molecule may attenuate gene expression.(15) However, it was not yet apparent that gene expression could be regulated by a small-molecule binding directly to an mRNA. Over the past decade, it has become increasingly evident that small-molecules bind to mRNA molecules to regulate a variety of biochemical pathways across all domains of life through a mechanism known as riboswitch control.(16) Almost exclusively found in eubacteria, riboswitches are typically located in the 5′-untranslated regions (5′-UTRs) of metabolic genes, where they regulate expression of the genes in the pathway in response to the concentration of a specific metabolite. Notably, riboswitches perform their work without the need for protein cofactors.(17) Indeed, searches for riboswitches began because previous searches for protein mediators of several metabolite-regulated biochemical pathways proved fruitless.(18,19) As an example, the discovery of an FMN riboswitch in *Bacillus subtilis* in 2002 ended a decade-long search for a putative repressor protein that might use FMN as a cofactor.(4)

To date, at least a dozen riboswitch classes have since been discovered;(16) they respond to metabolites with diverse structures, such as flavin mononucleotide (FMN), thiamine pyrophosphate (TPP), adenosylcobalamin (AdoCbl), *S*-adenosylmethionine (SAM), *S*-adenosylhomocysteine (SAH), cyclic di-GMP, adenine, guanine, glycine, and lysine (Figure 1).(20–29) Each of these classes has been found, or is predicted to exist, within the genomes of many species of bacteria, leading some to postulate that riboswitches may be remnants of ancient metabolic ribozymes,(30) although others have speculated that the wide distribution of riboswitches is the result of horizontal gene transfer.(31) Regardless of their evolutionary origins, modern-day riboswitches are highly conserved genetic control elements that maintain metabolic homeostasis within cells.

Riboswitches are composed of an aptamer domain, which binds the metabolite with high affinity and specificity, and an expression platform, which couples binding to a change in gene expression.(32) Riboswitches can operate by a variety of mechanisms to regulate the expression of a gene or set of genes in a metabolic pathway (Figure 2). Riboswitches have been discovered in diverse bacterial species,(20,25–27,33–35) a fungus,(36) and a plant.(37) Computational searches also indicate that riboswitches exist in archaea, suggesting that they are present in all kingdoms of life.(16) Barrick and Breaker provide an excellent overview of the distribution and mechanisms of confirmed and putative natural riboswitches.(16)

Riboswitch-mediated changes in gene expression can occur either transcriptionally or translationally. The expression platform for a riboswitch that acts during transcription typically involves the ligand-dependent formation of an intrinsic terminator or anti-terminator structure. In contrast, riboswitches that operate at a translational level most often do so by masking or

revealing the Shine-Dalgarno (SD) sequence (also known as the ribosome binding site; RBS) in a ligand-dependent fashion. When the Shine-Dalgarno sequence is revealed, the mRNA can bind to the ribosome and permit translation; masking the SD sequence represses translation. Transcriptionally regulated riboswitches are most often found in Gram-positive bacteria, while translationally regulated riboswitches are more common in Gram-negative bacteria.(16) While it is not yet clear why Gram-negative and Gram-positive bacteria appear to favor different riboswitch mechanisms, it is apparent that both expression platforms are functional across eubacteria. This flexibility is particularly attractive as researchers have become increasingly interested in developing synthetic riboswitches that function in diverse bacterial species.

SELECTIONS FOR SYNTHETIC APTAMERS

While the riboswitch mechanism was conclusively demonstrated in a natural system for the first time in 2002,(22) the idea that RNA could bind small molecules was already firmly established.(38,39) In 1990, Ellington and Szostak reported an *in vitro* selection protocol to isolate RNA aptamers that could bind specific dyes.(38) In the same year, Tuerk and Gold independently reported a similar method to isolate protein-binding RNA sequences from large pools of randomized RNA sequences.(39) What is remarkable about these studies is that creation of an RNA aptamer did not require a pre-existing RNA scaffold. Compared to most protein engineering efforts, where an existing protein is subjected to computational design (40) or directed evolution methods,(41) RNA aptamers can be generated *de novo* by subjecting large pools of randomized oligonucleotides (~10^{14} unique sequences) to an *in vitro* selection process known as SELEX (Systematic Evolution of Ligands by EXponential enrichment).(39) Selections for RNA aptamers using SELEX require two alternating steps of partitioning and amplifying the desired sequences (Figure 3).

To select aptamers, the starting pool of randomized RNA sequences is typically passed through a column that is covalently coupled to the desired target ligand. After the column is washed to remove unbound RNAs, the free ligand is used to competitively elute potential aptamers. This pool of initial binders is enriched by reverse transcription and PCR amplification, and the process is repeated to select for aptamers that bind the ligand with increased affinity. To select for aptamers that bind the ligand with high specificity, RNAs that bind to the column can be washed with structurally related ligands. After competitive elution, aptamers that remain bound to the column are expected to bind the desired ligand tightly, while discriminating against structurally related molecules. Typically, high-affinity RNA aptamers can be obtained with 10–15 rounds of in vitro selection.

As the tremendous potential of RNA aptamers became apparent, further studies were initiated to select for sequences that could bind new ligands tightly and with high specificity.(42) To enable more efficient identification of aptamers, many efforts have focused upon improving the SELEX strategy and have been reviewed extensively elsewhere.(43) As an alternative to SELEX-based selection strategies, allosteric selections based upon the directed evolution of ligand-dependent self-cleaving ribozymes have also enabled the isolation of RNA aptamers.(44,45) Although allosteric selection can be particularly advantageous when it is undesirable or challenging to couple the target ligand to a solid support, this *in vitro* approach has not consistently led to the isolation of allosteric ribozymes that function well within the intracellular milieu.(46) The idea of selecting aptamers that recognize ligands free in solution, as opposed to bound to a solid support, has been pursued widely recently.(43,47) In addition to obviating the need for synthesizing a solid-supported ligand, performing selections in the solution phase (ideally under conditions that mimic the environment in which the aptamer will be used), satisfies the maxim “you get what you screen for”.(48)

Many efforts toward more efficient aptamer selections have focused on using electrophoresis strategies to separate unbound RNAs from the desired aptamer complexes, and there is some evidence that this partitioning reduces the number of amplification cycles needed to discover aptamers.(49) However, while electrophoresis-based approaches show promise for identifying aptamers that bind protein or peptide targets, they have not (yet) proven generally effective for identifying aptamers that recognize small molecules.(49–51) This may be due, in part, to relatively small changes in charge and molecular weight upon ligand binding. In addition to classical separation technologies, a recent effort has implemented a magnetic-bead based selection on a chip-based microfluidic platform. While this technique may enable rapid and automatable selections for small-molecule binding aptamers, it does not overcome the disadvantage of having to couple the small-molecule to a solid support.(52)

While fundamentally new partitioning approaches may lead to more efficient aptamer selections, there are still many opportunities to improve current selection methods. For example, an automated buffer optimization strategy can identify an ideal buffer identity, buffer concentration, mono- and divalent salt concentrations, and pH before proceeding with selections for aptamers that bind a given target.(53) Additionally, a recent study aimed to improve the selection efficacy of traditional SELEX by decreasing the amplification bias introduced by the conventional PCR amplification step.(54) This approach, termed SELEX-T, replaces most of the PCR amplification cycles with T7 RNA polymerase amplification, and may be an important improvement for raising aptamers to both protein targets and small-molecule targets, alike. In our opinion, advances in selection technology are critical for maximizing the impact of aptamers and riboswitches in chemical biology.

Among the small-molecule binding aptamers discovered to date, perhaps the best-known example is the theophylline-binding aptamer, which was isolated by SELEX in 1994.(55) This aptamer binds theophylline tightly (K_D = 320 nM) and with high specificity: The aptamer binds caffeine (which differs by an additional methyl group) 10,000-fold less tightly. The specificity of the RNA aptamer was 10-fold better than the performance of available antibodies, and the binding-affinity was 100-fold better than had been achieved with any previously identified small-molecule binding aptamer.(55) Furthermore, theophylline is an inexpensive FDA approved drug, whose activity and toxicity profiles have been characterized in a number of biological systems, making it a particularly attractive ligand choice for use in cells. Because of these desirable properties, many studies have drawn upon the theophylline aptamer to develop synthetic riboswitches, with the ultimate goal of pursuing more advanced applications in chemical biology.

FROM APTAMER TO RIBOSWITCH

Before riboswitches were identified in living systems, several groups anticipated that synthetic aptamers could be incorporated into mRNAs to regulate gene expression in living cells. Previous studies had shown that highly structured regions in the 5′-UTR of mRNAs could cause marked reductions in gene expression in both eukaryotic and prokaryotic cells.(56–58) Guided by these studies, Werstuck and Green incorporated a small-molecule binding RNA into the 5′-UTR of a gene, and showed that ligand-inducible gene expression could be accomplished in yeast.(14) Further studies showed that small-molecule binding aptamers could be incorporated within the 5′-UTR of genes in a variety of organisms to generate synthetic riboswitches that could respond to theophylline,(8,12,59) antibiotics,(60) or dyes.(9,61) Since these initial observations, there has been increasing interest in the development of high-throughput screens or selections to isolate synthetic riboswitches that respond to a variety of new ligands.(11,62, 63)

High-throughput screens and selections

In 2004, Desai and Gallivan reported the first example of a synthetic riboswitch that functioned in *E. coli*, while also providing proof-of-principle that a genetic screen could be used to isolate a rare functional riboswitch from a large pool of nonfunctional sequences.(8) In contrast to prior studies, in which ligand binding to the aptamer increased structure in the 5′-UTR of an mRNA and reduced translational efficiency,(10,14,58,60) this study showed that ligand binding could result in an ~8-fold increase in gene expression. Although it was apparent that the spacing between the aptamer sequence and ribosome binding site (RBS) was an important determinant of switching activity, the mechanism by which this riboswitch functioned was not immediately clear.

To investigate the mechanism of switching in *E. coli*, Lynch et al. developed a high-throughput screen to isolate riboswitches from libraries in which the region separating the aptamer and the RBS was substituted with 4–8 randomized nucleotides.(64) With the help of a colony picking robot and an automated liquid handling system, they identified new riboswitch variants that could activate β-galactosidase expression by up to 36-fold in *E. coli*. More importantly, these studies demonstrated that these riboswitches functioned by sequestering the RBS in the ligand-free state and revealing the RBS in the theophylline-bound conformation. These results provided impetus for the development of a screen based upon fluorescence-activated cell sorting (FACS) to identify synthetic riboswitches from even larger genetic libraries (12 randomized bases; 4^{12} = 16,777,216 sequences). While this library was not exhaustively screened, the FACS-assay identified theophylline-sensitive synthetic riboswitches that feature very low background expression levels, and activate protein translation up to 96-fold in *E. coli*. (65) In contrast, a recent FACS-based effort to identify riboswitches that function at the transcriptional level featured more modest (~8-fold) activation ratios.(66) It is possible that transcriptional riboswitches may be more challenging to obtain, or perhaps the sequence space of this library was not optimally designed. Additional rounds of FACS using more diverse genetic libraries may identify synthetic riboswitches with larger activation ratios.

As an alternative to these genetic screens, Yokobayashi and coworkers developed a genetic selection for synthetic riboswitches based on expression of the TetA protein.(67) Cells that express TetA are resistant to tetracycline but are sensitive to $NiCl_2$, and cells lacking TetA are sensitive to tetracycline but are resistant to $NiCl_2$. Such a selection allows gross control over dual phenotypes (live or dead), but may not allow the graded control afforded by a genetic screen. Nevertheless, Nomura and Yokobayashi were able to assay 75,000 unique clones to identify TPP riboswitches that activate, rather than repress, gene expression in *E. coli*. Moreover, they identified a riboswitch that could activate gene expression 11-fold in the presence of TPP, completely reversing the activity of the parent riboswitch, which represses gene expression 9-fold in the presence of the ligand.(67)

Finally, our group reported a high throughput selection for synthetic riboswitches based on ligand-dependent changes in cell motility.(68) This simple screen not only isolated several riboswitches that activate gene expression in the presence of ligand, but also simultaneously optimized the riboswitches for dynamic ranges that are conducive to cell migration on semi-solid agar. Because ligand-dependent changes in cell motility are a desirable phenotype for a variety of applications, including bioremediation and cell targeting, we anticipate that motility selections may provide a foundation for a variety of new applications in chemical and synthetic biology.

EMERGING APPLICATIONS OF RIBOSWITCHES

Riboswitches as tools for regulated gene expression

Ligand-inducible expression systems are important genetic tools for common laboratory organisms such as *E. coli* and *B. subtilis*. However, many of these inducers (such as IPTG) are too expensive to be useful on the industrial scale. Natural riboswitches that are activated by amino acids may therefore represent an affordable alternative for such applications. Toward this goal, a tandem glycine riboswitch from *B. subtilis* was used for glycine-inducible production of β-galactosidase in *B. subtilis* cells.(69) Although this system provided only 6-fold induction when glycine was added, it may be worthwhile to revisit this general strategy if riboswitches with more ideal induction parameters can be developed.

In a recent report, Jin et al. proposed that ligand-sensitive riboswitches may be useful genetic tools for the production of conditional hypermorphic mutants, particularly in cases where null mutants are lethal.(70) To test this hypothesis, a theophylline-sensitive synthetic riboswitch was designed and used to regulate the chromosomal copy of an essential *E. coli* gene that is known to modulate motility (*csrA*). The creation of a *csrA-lacZ* fusion at this chromosomal position verified that expression levels were very low in the absence of theophylline, but approached wild type expression levels in the presence of theophylline. The authors further showed that the cells were non-motile in the absence of the inducer, but regained their motility phenotype in the presence of theophylline. These experiments fortuitously revealed that CsrA is a negative regulator of autoaggregation in *E. coli*. The ability of a synthetic riboswitch to permit reversible and tunable ligand-dependent gene expression of a protein over its native expression range suggests that synthetic riboswitches may find broad use in studying microbial genetics.

Riboswitches as antimicrobial targets

RNA is a primary target of many antibacterial compounds. While it has been known for quite some time that ribosomal RNA is an important antimicrobial target, it was recently discovered that some antibiotics whose mechanisms of actions were previously unknown may act, in part, by targeting riboswitches.(71) Following up on observations that some roseoflavin-resistant strains of *B. subtilis* featured mutations within an FMN aptamer sequence, Lee et al. demonstrated that this naturally-occurring antimicrobial binds the FMN riboswitch as a major target.(72)

Additionally, it has been shown that two lysine analogs that repress the growth of some Gram-positive bacteria ($_{L}$-aminoethylcysteine and $_{DL}$-4-oxalysine) also bind the *lysC* riboswitch of *B. subtilis*.(73) Although the primary antimicrobial mechanism of these lysine analogs may not involve riboswitch-binding,(74) Breaker and coworkers were inspired by this discovery and asked if they could identify new antimicrobials that target natural riboswitches.(75) With the goal of identifying an antimicrobial compound that could specifically repress bacterial purine metabolism, they tested a panel of 16 guanine analogs for the ability to bind the *B. subtilis* guanine riboswitch.(75) By pairing *in vitro* in-line probing assays with *in vivo* growth inhibition and reporter gene expression assays, they identified a specific analog that may inhibit *B. subtilis* growth by the intended mechanism. Although *B. subtilis* is not pathogenic, these advancements serve as a guide for future efforts to screen for riboswitch-binding antimicrobial agents in pathogenic bacteria.

Riboswitches as Boolean logic gates

While most natural riboswitches consist of a single aptamer domain and an expression platform, some natural riboswitches are more complex.(76) Some riboswitches, such as the tandem TPP riboswitch(77) from *B. anthracis* and the tandem glycine riboswitch(28) from *B. subtilis*, have

two aptamers that work together to produce a more digital response than can be achieved by riboswitches that employ only a single aptamer. Other complex riboswitches, such as the *metE* tandem riboswitches (SAM and AdoCbl) from *B. clausii*, function independently of one another to constitute a two-input Boolean NOR logic gate.(76) In this system, high concentrations of SAM repress the *metE* operon, as well as other related operons, in a widespread effort to prevent further SAM biosynthesis. Additionally, AdoCbl independently represses the *metE* operon because this cofactor enables MetH to synthesize methionine more efficiently than MetE. Thus, high concentrations of either SAM or AdoCbl cause transcriptional termination of *metE* RNA. The net result is a NOR logic gate, whereby MetE is produced at high levels only when both SAM and AdoCbl are present at low concentrations. These complex riboswitches have inspired others to develop synthetic logic gates to reprogram cell behavior and to engineer metabolic pathways.

Shortly before the first riboswitch was reported, Jose et al. recognized that the catalytic benefits gained by cooperative binding in allosteric proteins might be harnessed in the RNA realm by constructing binary allosteric ribozymes.(78) In an impressive demonstration of modular rational design, these researchers engineered a binary ribozyme that would self-cleave only in the presence of two effectors (theophylline and FMN). *In vitro* studies demonstrated that this binary ribozyme responds in a digital fashion, exhibiting little cleavage in the presence of a single effector, but providing a ~300-fold rate enhancement when both effectors are present. (78)

Building upon these earlier efforts, Win and Smolke recently engineered several versions of binary ribozymes to obtain genetic logic gates that operate by Boolean logic.(79) By incorporating the previously reported theophylline and tetracycline aptamers in various positions relative to a self-cleaving ribozyme, the researchers sought to construct AND, NOR, NAND, and OR genetic gates that might function within living cells. When tested in yeast, however, it was found that each of these allosteric ribozymes exhibited less than 3-fold modulation of reporter gene expression when the appropriate ligands were present.(79) The modest performance of these ribozymes in cells compared to their *in vitro* counterparts (78) might be improved by subjecting the ribozymes to an *in vivo* selection.(46)

Wieland et al. recently performed *in vivo* selections for allosteric ribozymes featuring improved modulation of reporter gene expression in bacteria,(80,81) suggesting that this strategy might be extended to optimize binary riboswitches for *in vivo* applications. With a similar strategy in mind, Yokobayashi and coworkers used their TetA dual genetic selection system to perform *in vivo* selections for complex riboswitches, which function in *E. coli* as AND or NAND Boolean logic gates.(11) The AND gates exhibited particularly good properties, as gene expression remained low unless both theophylline and thiamine were present, which then enabled up to 18-fold induction of gene expression *in vivo*. These impressive results suggest that dual genetic selection may be a useful approach for creating Boolean logic gates in living cells.

Riboswitch-based control of bacterial behavior

Because riboswitches are versatile tools for controlling gene expression, they can be used to reprogram a variety of bacterial behaviors. Bacterial chemotaxis has been studied extensively and is well understood at the genetic level. The ability to modulate bacterial motility in response to arbitrary chemical signals would provide new tools for bioremediation and drug delivery. We hypothesized that the *E. coli* chemotaxis system could be reprogrammed by placing a key chemotaxis signaling protein (*cheZ*) under the control of a theophylline-sensitive riboswitch. (82) Reprogrammed cells would then migrate up gradients of this ligand and autonomously localize to regions of high theophylline concentration, which is a behavior that cannot be accomplished by the natural *E. coli* chemotaxis system. To test this hypothesis, theophylline

or caffeine was pipetted in a T-shape pattern on the surface of a semi-solid media plate. Reprogrammed cells were then spotted at the bottom of the theophylline path. As shown in Figure 4, the population of reprogrammed cells migrated up the first portion of the theophylline path, and then made a right turn to continue following this ligand, without migrating off the path. This precise localization to the ligand represents a sharp contrast to the behavior of wild type *E. coli* (which do not stop moving, and thus cannot localize to a chemical signal), and such behavior may prove useful for targeting cells.

Future Prospects

Just as natural riboswitches can regulate gene expression in response to small-molecule ligands during transcription or translation, synthetic riboswitches can be engineered to repress or activate gene expression in a ligand-dependent fashion. To maintain the optimal concentrations of critical cellular metabolites, natural riboswitches generally bind ligands to regulate the expression of metabolic genes. In contrast, synthetic riboswitches can be employed to regulate the expression of any gene (or genetic circuit) in response to any non-toxic molecule that is capable of being bound by RNA. This feature should enable RNA switches to play an increasingly important role as chemical biologists seek to modulate many types of cellular behavior in response to a broad range of chemical signals. We anticipate that as selection strategies improve, synthetic riboswitches will be easier to obtain, and that they will become a standard tool for the chemical biologist.

Glossary

RNA aptamer	An RNA sequence that binds a ligand with high affinity and specificity.
Riboswitch	An RNA consisting of an aptamer and an expression platform that controls gene expression in a ligand-dependent fashion, without the need for protein cofactors.
5′-UTR	The 5′-untranslated region of a messenger RNA (mRNA).
Ribozyme riboswitch	An RNA that performs self-cleavage in a ligand-dependent fashion.
SELEX	“Systematic Evolution of Ligands by EXponential Enrichment”; an in vitro selection process to discover aptamers.
Chemotaxis	The process by which an organism directs its motion in response to a chemical cue.
NAND logic gate	An AND function with an inverted output. Output is low only when all inputs are high.
NOR logic gate	An OR function with an inverted output. Output is high only when all inputs are low.

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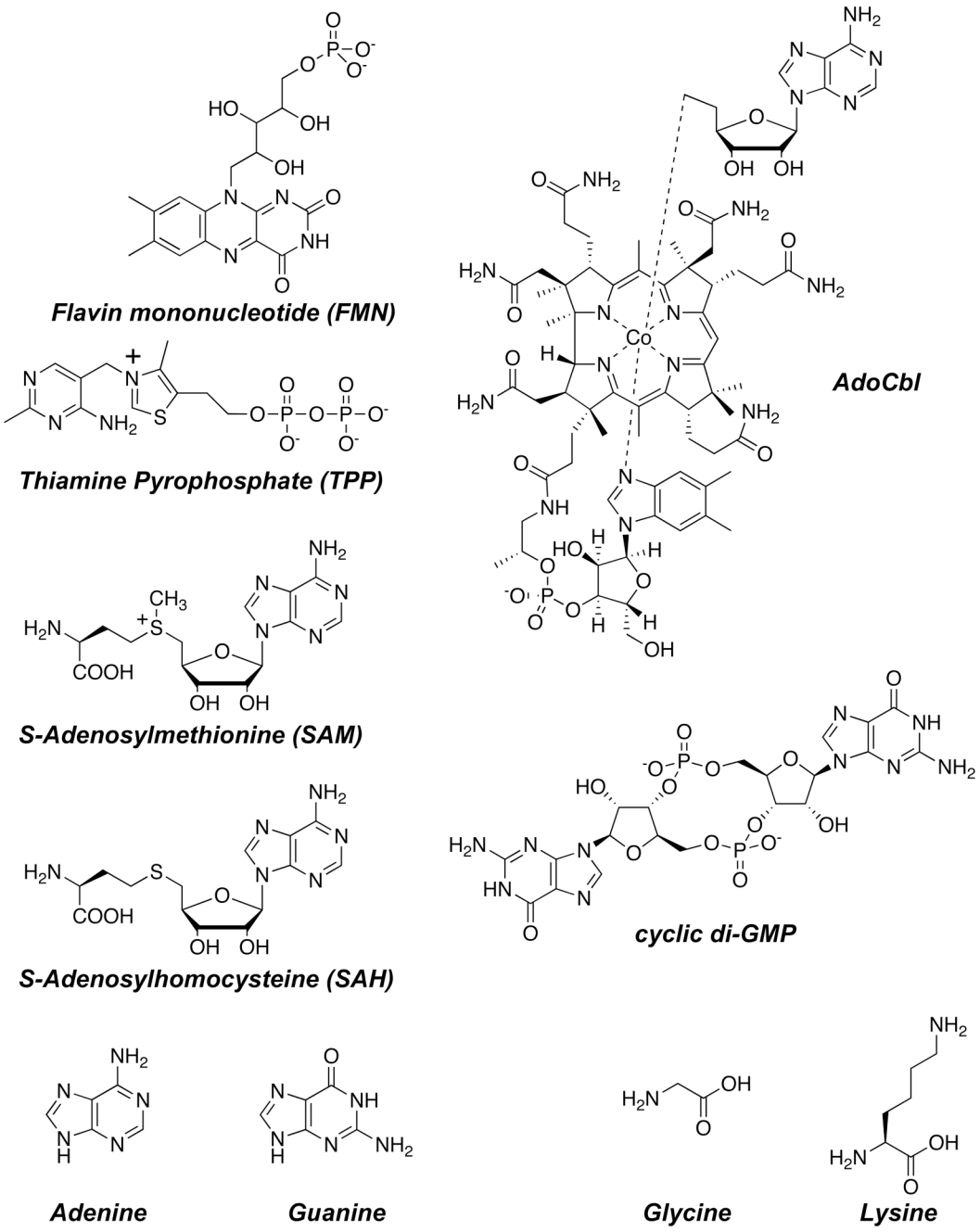


Figure 1.

Examples of metabolites that bind natural riboswitches. These ligands vary substantially in size and structure, yet all are able to control gene expression by binding various RNA sequences in cells.

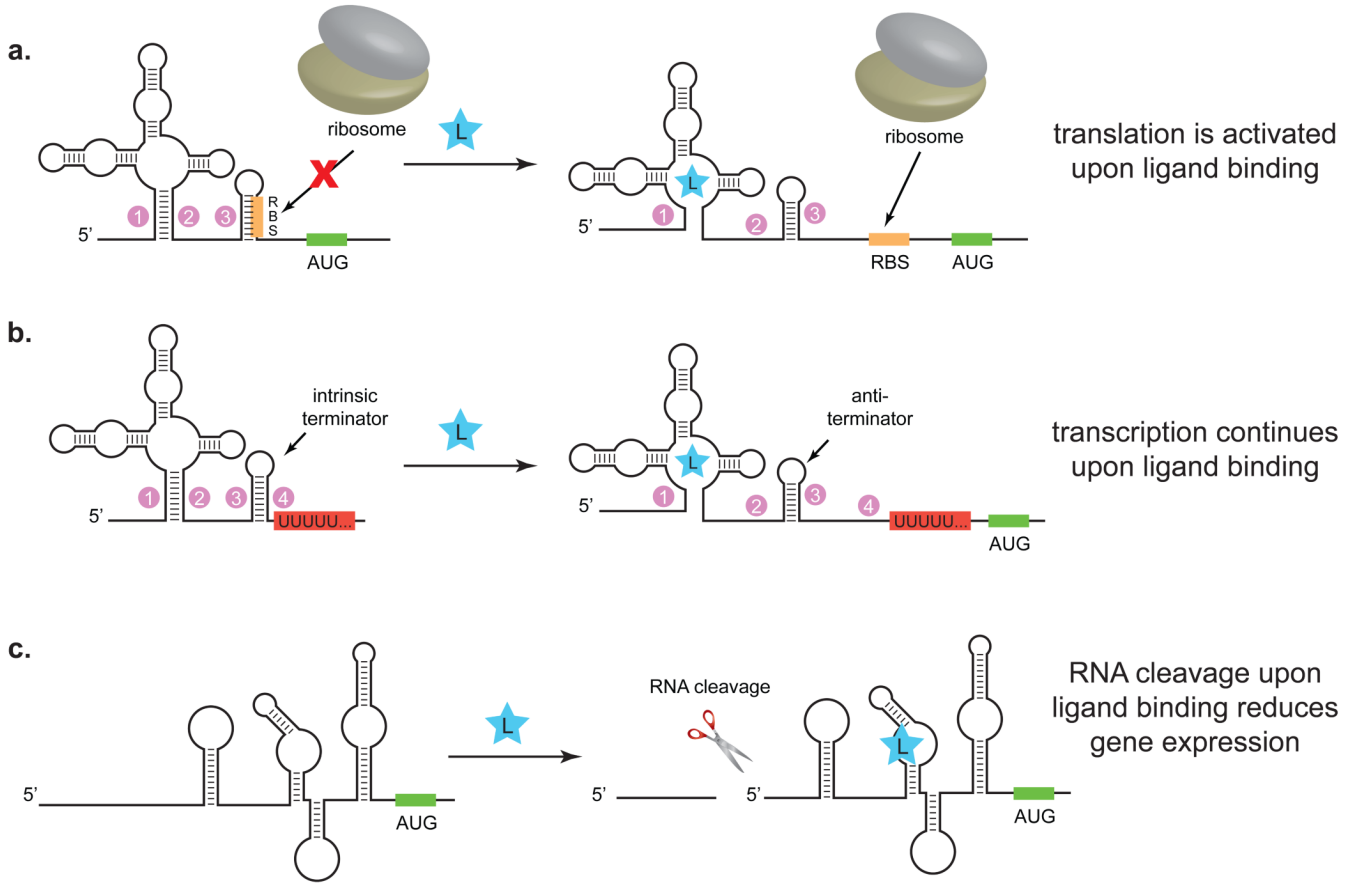


Figure 2.

Examples of riboswitch mechanisms. a) The ribosome binding site (RBS) is sequestered in the absence of ligand. Upon ligand binding, the RNA undergoes a conformational shift, revealing the RBS and enabling translation. b) In the absence of ligand, an intrinsic terminator stops transcription. Ligand binding induces a conformational shift that forms an anti-terminator, enabling expression of the downstream genes. c) The *glmS* riboswitch functions by a ligand-dependent splicing mechanism, whereby protein expression is diminished in the presence of the ligand.

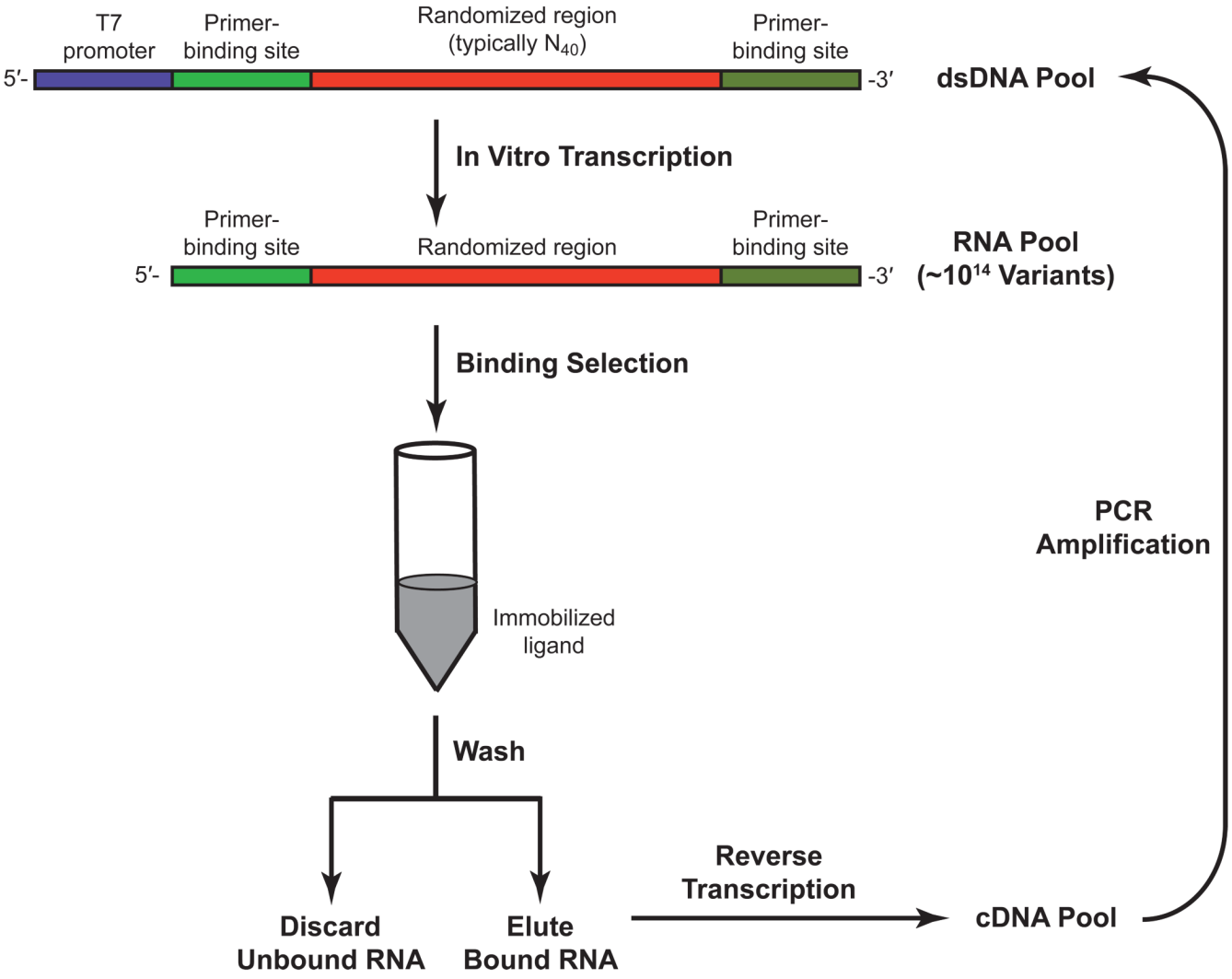
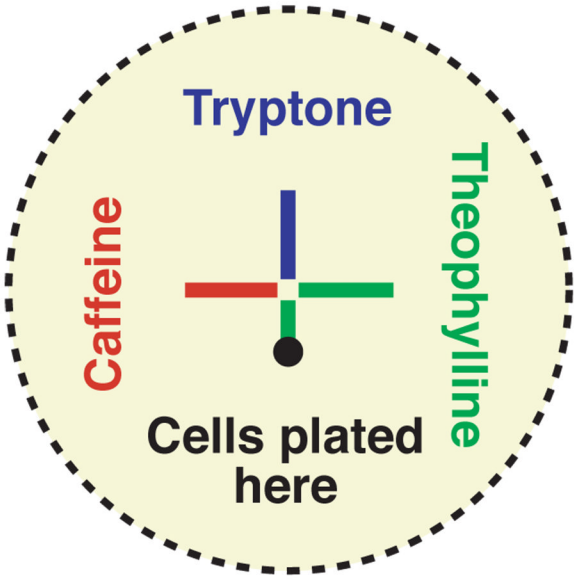


Figure 3.

Aptamer selection scheme. RNA aptamers may be isolated from large pools of randomized sequences using *in vitro* selection. The pool is enriched for sequences that bind an immobilized ligand and are eluted with free ligand. To obtain high-affinity binders, the selected sequences are reverse transcribed, amplified, and subjected to further rounds of selection.



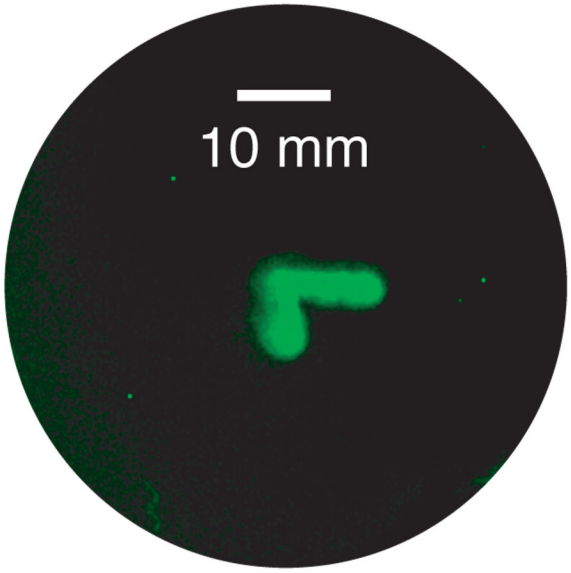


Figure 4.

Controlling cell motility with a synthetic riboswitch. *E. coli* containing the *cheZ* gene under the control of a theophylline-sensitive synthetic riboswitch are plated at the bottom of a path containing theophylline and caffeine on semi-solid agar (the path is diagrammed to the left). Cells migrate exclusively along the theophylline path and disregard the path of caffeine, which is not recognized by the riboswitch.