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Gene expression control

Harnessing RNA switches

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As reported in a recent issue of *Nature*,[1] Richard Mulligan and co-workers have created a ribozyme-based genetic control element and demonstrated its ability to control a reporter gene in virus-transfected mammals. This is an exciting advance in the quest to create designer genetic control elements, as it provides a striking example of how engineered RNAs can be harnessed to control gene expression in higher organisms. Although the RNA control element developed in this remarkably ambitious study might not find wide used in future gene therapy treatments in its current form (Figure 1a), this drug-controlled ribozyme does provide a much-needed prototype for those who seek to engineer the ultimate designer gene regulation systems (Figure 1b).

Some genes are expressed with little or no modulation over the life of a cell, while the expression of others must be tightly controlled in order for the cell to respond to numerous physical and chemical changes. This issue is exceedingly important for those who wish to create gene therapy treatments, as the imprecise regulation of the gene delivered could cause just as much harm as potential good. Ideally, a specific signaling agent would control each therapeutic gene. Such agents could be externally delivered drugs, natural compounds or, most preferably, factors that are indicative of the need for the gene product.

With the availability of well-established control systems based on protein factors,[2–4] why should we care about harnessing RNA for this purpose? As with most molecular tools, there are drawbacks to each system that can best be addressed by having a greater variety of choices. For protein-based systems, these choices are currently limited. To overcome this, researchers could simply co-opt more natural systems that rely on protein factors. However, this does not resolve other problems that are inherent when proteins are involved. To use protein-based genetic elements, the genes that encode the required protein factors must be transfected along with the therapeutic gene. Furthermore, the delivery of additional foreign proteins to mammals increases the likelihood that adverse immune responses will materialize.

To circumvent these problems, Mulligan and co-workers chose to construct entirely new gene control elements from relatively nonimmunogenic RNAs. RNA genetic switches have the additional advantage that, if crafted appropriately, they could be efficiently inserted into genes of interest with far less transgenic DNA. The authors reasoned that self-cleaving ribozymes, the most common form of natural RNA catalysts, could be used to repress gene expression in a controlled manner. If intact ribozymes are inserted within an mRNA, then the action of the ribozyme is expected to cleave the message and render it inactive. Controlling gene expression is then reduced to a matter of using anti-ribozyme compounds to control ribozyme function.

Unfortunately, this simple strategy is ordinarily doomed to fail, as it has in several laboratories over the years. Finding an appropriate self-cleaving ribozyme, and a place to embed it within the mRNA so that ribozyme activity results in substantial reduction of gene expression, is expected to be difficult, as Mulligan's team has now confirmed. Of more than 100 natural and engineered self-cleaving ribozymes tested, only two ribozymes (both of the hammerhead class[5,6]) caused more than a 10% reduction in gene expression. A number of issues likely conspire to make the success rate less than 2%. In particular, ribozymes need to act fast enough *in vivo* to cleave nearly all mRNA constructs before translation progresses, and they need to be situated such that alternate RNA folding does not preclude ribozyme function.

The most active ribozyme identified comes from *Schistosoma mansoni*, a species of parasitic blood fluke. The ribozyme is distinctive in that it carries stem loop and bulge structures adjacent to the catalytic core of the hammerhead ribozyme that are

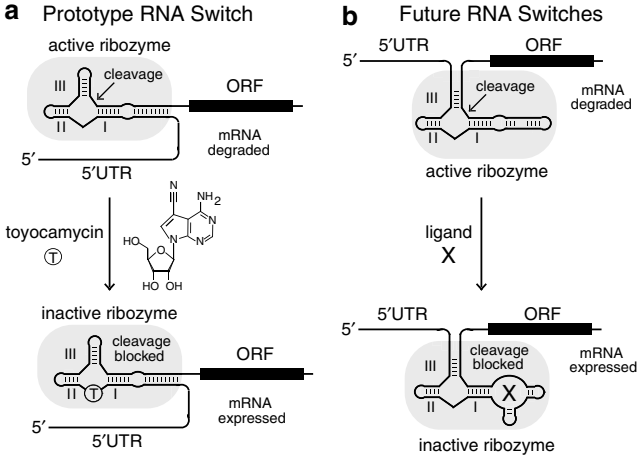

Figure 1 *Engineered RNA switches. (a) Drug-mediated control of gene expression by a hammerhead ribozyme as devised by Mulligan and co-workers. The ribozyme self-cleaves and destabilizes the mRNA, which is linked via stem I of the hammerhead secondary structure. Toyocamycin causes an increase in gene expression by inhibiting ribozyme function, presumably by becoming incorporated into the mRNA during transcription. (b) A proposed future design for RNA gene control elements that takes advantage of the validated hammerhead ribozyme. Fusions between the ribozyme and ligand-binding aptamers yield a diversity of drug- or metabolite-inactivated ribozymes, which permits the construction of user-defined genetic switches.*

known to permit RNA cleavage to proceed at high speed in this[7] and other hammerhead variants,[8,9] even in low intracellular concentrations of divalent magnesium (an essential cofactor for ribozyme function). Disruption of these structures via mutation causes loss of ribozyme-mediated repression of the reporter gene, as does relocation of the ribozyme to certain parts of the mRNA. Ultimately, a ribozyme-reporter fusion provided ~1400-fold dynamic range in gene expression compared to the same construct carrying an inactive ribozyme mutant.

The next challenge facing the authors was to identify compounds that could inactivate the ribozyme, which would increase mRNA stability and induce a corresponding increase in reporter gene expression. Attempts to use antibiotics that are known to inactivate some hammerhead ribozymes failed to trigger gene expression. Ultimately, they turned to a high-throughput screening strategy and assayed more than 50 000 compounds. This screen identified several compounds that caused an increase in expression from the ribozyme-reporter fusion.

The compound examined in greatest detail, toyocamycin (Figure 1a), induces gene expression to near maximum level in cell culture and exhibits dramatic modulation of gene expression in rats that have had the ribozyme-reporter fusion delivered by a retroviral vector system. Toyocamycin is a nucleoside analog[10] that does not seem to inhibit RNA self-cleavage by directly binding to the ribozyme. Perhaps, it becomes integrated into cellular RNAs where it interferes with ribozyme function.

Regardless of the precise mechanism of toyocamycin, herein resides wonderful opportunities for further enhancement of the technology. Much effort in recent years has been directed towards the design of hammerhead ribozyme-based molecular switches.[11–14] A variety of RNA switches have been created[13] that are selectively triggered to self-cleave in response to metal ions such as divalent cobalt, metabolites such as ATP and cyclic AMP, and drug compounds such as theophylline. With a proven ribozyme platform now in hand, the coupling of ligand binding domains to this or related ribozymes should yield a diverse collection of RNA switches. Of course, target compounds for this application should be bioavailable and nontoxic.

Can designer RNA switches truly offer sufficient functional versatility and sophistication to compete with the intricate genetic control systems that use proteins? Without question, RNA has what it takes to selectively bind target compounds and to control gene expression as a result. RNA switch technology might be new to ribozyme engineers, but modern cells have tapped RNA to serve in this capacity for billions of years. Riboswitches[15] are natural versions of RNA switches that have recently been found to exist in all three domains of life.[16] Some riboswitches are surprisingly complex and are entrusted by modern cells to control the expression of essential metabolic processes.[17] Indeed, one riboswitch makes use of a novel class of self-cleaving ribozyme to control gene expression in response to rising concentrations of the metabolite, glucosamine-6-phosphate.[18]

Nature has nicely provided the validation for RNA switch technology. With a sufficiently powerful hammerhead ribozyme in hand, now is the time to make a concerted effort to create a larger collection of designer RNA switches (Figure 1b). A related approach that involves embedding RNA 'aptamers' at sensitive parts of mRNAs would achieve similar goals.[19–24] These ligand-binding RNAs could be used to control genes for basic research purposes, and also could be useful as *in vivo* sensors for natural metabolites. Furthermore, tailor-made RNA switches should empower researchers to deliver gene products using expression constructs with nonintrusive genetic control systems modulated by specific compounds. ■

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