In the study by Wang *et al.*, AAV8 was found to be more effective than other serotypes at transducing multiple organs and disseminating throughout the body when injected intravenously. Persistent systemic transduction was achieved with this vector in both skeletal and cardiac muscles after a single intravenous injection in neonates. Moreover, additional pharmacological intervention was not required. Global muscular transduction was dramatic in neonates; in these animals the vector persisted in muscle but was lost from rapidly dividing liver cells. It is of interest to note that in the same study AAV1 capsids were superior to other serotypes when directly injected into muscle tissue, but were not as effective when delivered by the intravascular route (**Fig. 1**).

Wang *et al.* also demonstrated the potential clinical applications of vectors packaged in the new AAV serotype by showing effective delivery in hamsters of δ-sarcoglycan, a component of the dystrophin-glycoprotein complex. Mutations in this gene cause cardiomyopathy and skeletal muscle dystrophy. Improved histopathology of heart and muscle was achieved in this hamster model of muscular dystrophy.

The authors suggest that facile crossing of the blood vessel wall is responsible for the impressive images of whole-body transduction achieved with AAV8 (**Fig. 1**). However, the mechanism by which AAV8 accomplishes global gene delivery is still unclear. Very little is known about AAV8, its natural tropism, the cellular receptors it uses and the means of cell entry that it exploits. The superior transduction of AAV8 may result from its affinity for cell surface molecules, its pathway for intracellular trafficking or uncoating of the vector genome.

Future studies using labeled virus particles to track the AAV8 particles will allow assessment of its biodistribution and survival in the blood system. It should be noted that along with the widespread gene delivery obtained with AAV8 came undesirable transduction in cells where it is not required, such as liver cells and gonadal tissue. Incorporation of transcriptional elements restricted to muscle-specific expression might improve the safety profile of AAV8 vectors for clinical applications.

Recent results with new AAV serotypes demonstrate that alterations in the viral capsid can affect the outcome of local or systemic gene delivery. Obviously, the key question is how well results from rodent models translate to larger animals and ultimately to humans. One might also question the relevance of results with neonatal animals to human clinical applications, particularly for the treatment of heart disease, which mainly affects older individuals.

Despite encouraging data from preclinical studies using direct intramuscular injection of an AAV2 vector for production of Factor IX, a clinical trial in hemophilia B patients required an impractical number of injections to achieve widespread transduction[10]. The chemical and genetic approaches described by Gregorevic *et al.*[5] and Wang *et al.*, may help overcome the barriers to efficient transduction in humans. The gene therapy field awaits data on the efficiency and safety of AAV8 in larger animals and humans. Low levels of neutralizing antibodies to AAV8 in human serum[6] may result in higher transduction efficiencies and be advantageous for readministration.

Vectors for gene therapy should be tailored to the disease being treated. Molecular treatments of cardiac disease must be highly focused to one organ, with minimal extra-organ tropism and cytotoxicity. For example, an acute myocardial infarction may be best treated by direct injection to the specific site in the damaged ventricle. In contrast, muscular dystrophies that result in lifelong impairment to virtually every skeletal muscle in the body may be best treated using a gene therapy vector that transduces a large target site and provides persistent transgene expression. Vectors based on AAV1 may thus be ideal for focused targeting by intramyocardial injection[3], whereas the broad muscular tropism of AAV8 may be more appropriate for global muscular diseases. Explorations into the tropism of AAV serotypes and capsid modifications are still in their infancy, and the potential of this field of research is just beginning to be revealed.

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Plug-and-play with RNA

Farren J Isaacs & James J Collins

Ligand-responsive riboregulators enable extracellular control of post-transcriptional gene expression.

Most computer users, particularly those not technically inclined, are thankful for the recent advent of 'plug-and-play' devices that enable them to expand the functionality of their computer without the help of tech support. Molecular biologists would similarly benefit from a diverse library of easy-to-use biological components that expand their capability to probe and control the inner workings of a cell. In this issue, Bayer and Smolke[1] address this need by providing a novel framework for creating and using small molecule–binding RNAs to turn genes on and off in a 'plug-and-play' fashion.

The resurgence in RNA biology has highlighted the important and diverse regulatory roles RNA assumes in the cell. Among them is the critical responsibility of RNA in mediating gene expression. For example, small RNAs that act as regulators include microRNAs in eukaryotes[2] and riboregulators in prokaryotes[3]. These rely on sequence-specific binding to control post-transcriptional gene expression. Other sets of functional RNAs affect gene expression through catalytic or regulatory activity. For example, recently discovered riboswitches contain structured nucleotide pockets, or aptamer domain sites, in the 5′UTRs of mRNAs that bind to small molecules or ligands, regulating downstream gene expression in bacteria[4]. In another prokaryotic study, a natural ribozyme was identified that cleaves mRNA in the presence of a specific metabolic product, thereby repressing gene expression[5].

Molecular engineers are now extracting well-characterized features from natural networks and exploiting RNA's versatility to construct designer systems that perform increasingly complex functions *in vivo*. Two recent papers exploit different facets of functional RNAs—one through the construction of a *cis*/*trans* riboregu-

Farren J. Isaacs is at the Lipper Center for Computational Genetics and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA and James J. Collins is at the Center for BioDynamics and Department of Biomedical Engineering, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA.
e-mail: jcollins@bu.edu

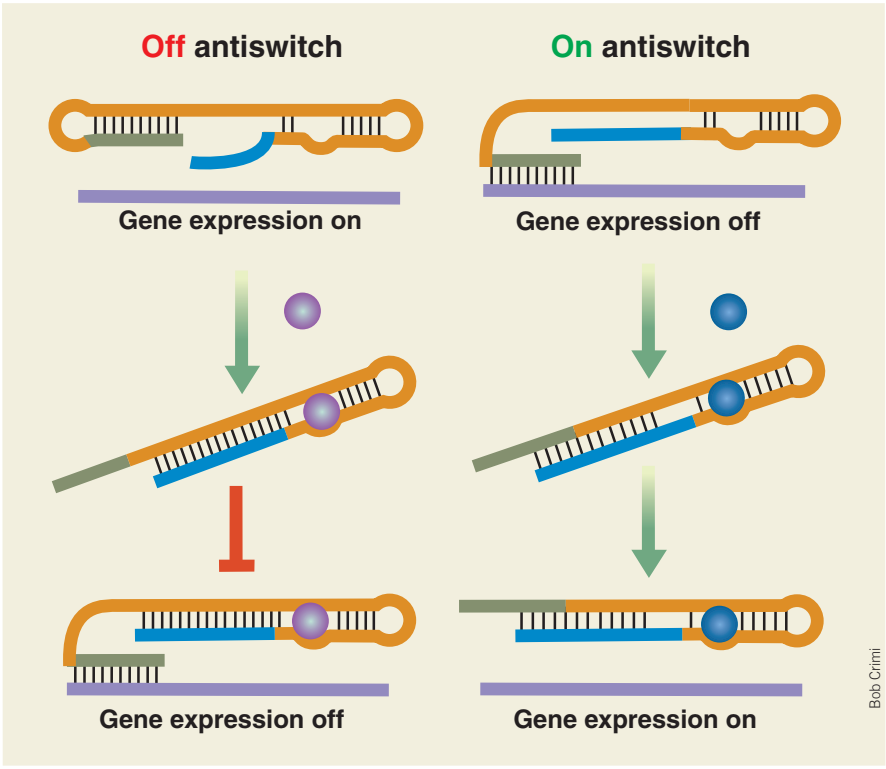

Figure 1 Engineered RNA switch system. This switch is a non-coding RNA (orange) comprising an antisense domain (green) that targets the 5′-end of an mRNA (purple) and an aptamer domain that binds a small molecule. In the presence of the small molecule, a conformational change that alters the antisense domain occurs. For the 'off antiswitch,' the antisense domain is sequestered in a stem-loop. Upon binding of the ligand, the antisense domain is exposed and represses target gene expression. For the 'on antiswitch,' the antisense domain is free to bind to its target mRNA, suppressing gene expression. In the presence of its target ligand, a conformational change prevents this interaction, releasing the 'on antiswitch' and permitting target gene expression.

lator system in prokaryotes that enables precise control of gene expression via highly specific RNA interactions[6], and another through the development of a ribozyme-based gene regulation system in mammals that responds to the presence of exogenous small molecules[7]. The work by Bayer and Smolke expands the catalog of designer RNA molecules and provides a new system that is essentially a hybrid between a riboregulator and a riboswitch. Their design combines sequence-specific riboregulator domains with ligand motifs that, together, direct the functionality of their RNA-based system.

Bayer and Smolke's novel set of *trans*-acting RNA switches was designed to function in the single-cell eukaryote, *Saccharomyces cerevisiae*. These noncodings RNAs comprise an antisense domain that binds a target mRNA and an aptamer domain that recognizes specific ligand molecules. Upon binding of specific ligands at the aptamer domain, the antiswitch undergoes a conformational change that affects the ability of the antisense domain to bind the target mRNA and modulate its translation. Importantly, the authors show that switching only occurs in the presence of specific ligands, which, along with the ability to readily change the antisense sequence, provides a modular blueprint for creating customized ligand-responsive riboregulators.

The authors detail the design and construction of two types of ligand-responsive riboregulators—an 'off antiswitch' and an 'on antiswitch.' In the absence of ligand, the 'off antiswitch' has a sequestered antisense domain, blocking its ability to repress the target mRNA transcript (**Fig. 1**). Once specific binding of ligand (e.g., theophylline) occurs at the aptamer domain, the 'off' switch undergoes a conformational change, exposing the antisense domain that represses target gene expression (**Fig. 1**).

The authors also designed an 'on antiswitch' where, in the absence of the ligand theophylline, an antisense domain is free to bind a target mRNA (**Fig. 1**). Upon theophylline binding at the aptamer domain, the 'on' switch undergoes an allosteric transition that causes the antisense domain to form a stem-loop, rendering it inactive. Consequently, target gene expression is upregulated (**Fig. 1**).

Interestingly, these engineered RNA switches exhibit sharp transitions between gene expression states over small changes in ligand concentration. As suggested by the authors, this 'digital' switch-like response may originate from competition between the aptamer domain and antisense domain, vying for the same binding site on the antiswitch molecule.

Bayer and Smolke also introduced three important rational modifications to further characterize their 'off antiswitches.' First, they built additional variants of the switch by strategically introducing point mutations. As expected, they found that variants with less complementarity within the antisense domain exhibited lower switching thresholds as a function of ligand concentration, and vice versa. Second, the authors replaced the aptamer domain for theophylline with one for tetracycline to show that switch responses exhibit similar behavior with a different ligand. Third, the authors combined different antisense domains with the previously described theophylline and tetracycline aptamer domains to show that two switches in the same cell can respond independently to different small molecules, each regulating a different mRNA target.

Together, the rational design of 'on' and 'off' antiswitches provides a general framework for realizing the combinatorial power of antisense- and aptamer-based switches. This sets the stage for customized switches that could be designed to either repress or activate the expression of any target gene(s) in response to select, membrane-permeable small molecules in the environment. By assembling various combinations of antisense and aptamer domains, the prospect of creating a library of 'plug-and-play' RNA switches to probe and reprogram regulatory networks[8] is well within reach.

Approximately 15 years ago, scientists developed powerful directed-evolution technologies for nucleic acids that were based on random mutagenesis and *in vitro* selection[9,10]. Now is the time to apply these technologies[9,10] *in vivo* to the RNA systems bioengineers have constructed through rational design[1,6,7]. Such efforts could increase the number, diversity and sophistication of RNA-based switches, expanding the toolbox of molecular biologists.

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