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An RNP switch raises a roadblock

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Abstract

Control of gene expression at the mRNA level is used extensively by cells. Now a bio-mimetic strategy yields a synthetic genetic switch in which an RNA-binding protein bound at the translation start site blocks progression of the ribosome.

Cells regulate the expression of genetic information at a variety of stages, including chromatin structure, transcription, splicing, polyadenylation, transport, translation and mRNA turnover. In their quest to manipulate living things and to generate new life forms, synthetic biologists have been tinkering with the machinery responsible for gene regulation at several of these stages. Regulation at the RNA level (after the mRNA has been transcribed, but before it is translated) is attractive because it allows the cell (and the biologist) rapid response to stimuli (on a timescale shorter than it takes to transcribe long eukaryotic mRNAs) as well as spatially restricted gene expression (*e.g.*, by localization of an mRNA in a highly polarized cell). Nature employs genetic switches that are composed of RNA alone as well as RNA-protein complexes (RNPs) to regulate gene expression at the RNA level. On page XYZ of this issue of *Nature Chemical Biology*, Saito *et al.*, describe a recombinant switch that comprises an archaeal protein and its cognate RNA binding site, and demonstrate that this RNP can regulate translation of mRNAs in mammalian cells¹.

Synthetic biologists have had success in emulating natural strategies for gene regulation at the mRNA level. For instance, Suess and colleagues developed a translational repression system that employs an *in vitro* selected RNA domain that binds very tightly ($K_d \sim 0.8$ nM) to the antibiotic tetracycline². These authors demonstrated that this tetracycline aptamer, when introduced into the 5' untranslated region of yeast messenger RNAs (including those of essential genes), causes rapid tetracycline-dependent shutdown of translation *in vivo* (Figure 1a). A system such as this, consisting of an RNA domain that binds selectively to a small molecule in conjunction with its cognate ligand, mimics naturally occurring gene-regulatory mRNA domains called riboswitches (reviewed in ref. ³).

The new translational switches described by Saito *et al.* mimic another type of naturally-occurring regulators. Many RNA-binding proteins (such as ribosomal proteins) and RNA-modifying enzymes (such as aminoacyl-tRNA synthetases) regulate their own expression by binding to a regulatory site near the translation initiation site on their own mRNAs. Thus, for instance, when a particular ribosomal protein is present in excess over free ribosomal RNA, it can bind its own mRNA and shut down its translation, in effect completing a negative feedback loop. In many instances, not only the sequence but also the structure of their mRNA binding site is closely related to that of their non-regulatory site, be it *e.g.* ribosomal RNA or tRNA (reviewed in ref. ⁴).

For their genetic switch, Saito *et al.*, employ a small RNA binding protein from archaea called L7Ae. In organisms from this kingdom, L7Ae is part of several RNPs, including the large ribosomal subunit, the box H/ACA RNP and the box C/D RNP (reviewed in ref. ⁵). In

all cases, L7Ae binds specifically to an RNA structural motif called the K-turn or to a minimized version of thereof, called the K-loop^{6–8}. Saito *et al.* introduce a stem loop derived from a box C/D RNP (which contains a K-turn) at the translation start site of mRNAs, and find that, when L7Ae is provided to an *in vitro* translation system or is expressed *in vivo*, it represses translation of the mRNA by ~10-fold (Figure 1b). In addition to this translation “OFF” switch, Saito *et al.* also devise translation “ON” switches that respond to L7Ae. For these, translation of an mRNA of interest is shut off by using an antisense RNA. This antisense RNA can also fold, on its own, into a structure containing a K-turn. Thus, when L7Ae is present, the protein sequesters the anti-sense RNA, thereby activating translation of the mRNA. Unlike riboswitches and their mimics, these RNP switches require that the regulatory protein be expressed before the switch can be thrown. Thus, these switches have a lag time in their response based on this initial protein synthesis. However, the requirement for translation of the switch component can be an advantage in some scenarios. Saito *et al.* suggest that the small L7Ae protein could be used to tag other proteins of interest. If the cell were to contain a reporter mRNA with a K-turn in the appropriate location, the RNP switch would allow the biologist to monitor the expression levels of those proteins. More generally, this approach can be generalized by employing several different proteins with different RNA-binding specificities. Judicious deployment of such switches in genetic networks could amplify signals through a cascade, function as logic gates to make decisions, etc.

Future work in this direction will surely employ artificial proteins binding to non-natural RNA elements, to provide a higher level of orthogonality (mammalian cells have L7Ae homologs, with subtly different RNA-binding specificities). The two examples of artificial translational switches shown in Figure 1 immediately suggest the possibility of devising small molecule-responsive riboswitches that also respond to the presence of a protein. Such a switch would allow integration of two separate signaling pathways. Predictably, nature has probably been there first: Recent work by Lanfontaine and colleagues suggests that the bacterial class-I S-adenosylmethionine riboswitch may in fact bind to an L7Ae homolog *in vivo*⁹. Bio-mimetic switches of this kind would be able to exploit, synergistically, the structural complexity of RNA, the exquisite specificity of small molecules, and the plasticity and versatility of proteins.

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Figure 1. Bio-mimetic roadblocks to gene expression that function at the mRNA level. **(a)** The *in vitro* evolved tetracycline aptamer bound to its cognate ligand¹⁰ blocks passage of the ribosome. **(b)** Protein L7Ae bound to a K-turn⁸ introduced at the translation start site blocks the ribosome, and shuts off translation of the mRNA¹.