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NEWS AND VIEWS

Small hairpin RNA as a small molecule sensor

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Life has perfected the art of connecting the myriad molecular components in a live cell through regulatory links, transforming a cell from a mere protein shake into the most complex physical system known to man. Research in synthetic biology has strived, among other things, to recreate this complexity to a modest degree. This requires building new regulatory links and networks according to pre-designed blueprints to generate new biological functions, augment cells with new capabilities and perhaps fix malfunctions in the cell's own programs. As of today, we are still far from being able to take an abstract specification of a network or function and build its working implementation. In a series of reports, including the recent article published in *Molecular Systems Biology* by Beisel *et al* (2008), researchers have made a significant step towards this goal. They describe rationally designed molecular switches that can, in principle, use an arbitrary small molecule to affect the amount of an arbitrary messenger RNA (mRNA) via the RNA interference (RNAi) pathway. These switches significantly expand the repertoire of tools available to synthetic biologists, and they will undoubtedly benefit basic biological studies and biomedicine.

For about a decade now, researchers in synthetic biology have been co-opting a variety of regulatory modalities into the 'biological parts' toolbox (Baker *et al*, 2006). Ideally, we want these parts to be amenable to rational design, and be scalable and programmable. The former will let us establish regulatory links at will, such as 'high concentration of protein A should activate protein B'. The latter will enable multiplying these links and defining their mutual relation. For example, we may require that protein A, microRNA B and small molecule C should be present in sufficiently high concentrations to activate protein X. How exactly this can be achieved using different regulatory mechanisms is outside the scope of this essay, but it suffices to say that this constitutes perhaps the biggest challenge to synthetic biology.

The focus in the series of papers covered here is on the regulatory process called RNAi. Discovered about a decade ago (Fire *et al*, 1998), this pathway functions in eukaryotes, including plants, worms, insects and mammals. There are different flavors of RNAi in different species, but they share a number of unique features that make them a valuable addition to the 'biological parts' collection. The RNAi pathway can be utilized to downregulate any mRNA by addition to cells of a small double stranded RNA molecule about 20 bp long, called small interfering RNA (siRNA). An siRNA molecule interacts with the RNAi enzymatic machinery (a constitutively active housekeeping module) and undergoes a series of transformations that eventually lead to rapid degradation of, or inhibition of translation from, the target mRNA. The nucleotide sequence of the siRNA and its cousin, the small hairpin RNA (shRNA), depends solely on the mRNA they target. Because of the linear nature of a nucleic acid, establishing this negative regulatory link is much more amenable to rational design (Reynolds *et al*, 2004) than, say, protein–protein or protein–DNA interactions. It has also been recently shown that RNAi is scalable and programmable, and it can form a basis for large-scale Boolean regulatory networks (Rinaudo *et al*, 2007). However, siRNAs or shRNAs are intrinsically synthetic objects and they cannot be used to establish regulatory links between unrelated molecular components.

The published results show how to make a passive shRNA into a sensor for a small molecule, and transduce the small molecule concentration into the level of the target mRNA. The key discovery that made this advance possible came more than a decade ago with the invention of RNA aptamer technology. Aptamers are RNA oligomers that selectively bind other molecules; they can usually be selected in a few rounds of *in vitro* selection and amplification from random libraries (Ellington and Szostak, 1990). This discovery also spurred a tangential effort aimed at the creation of additional 'riboswitches' that encompass a variety of RNA regulators based on ribozymes, RNA secondary structures and others (for review, see Davidson and Ellington, 2007) but not, until recently, RNAi. An *et al* (2006) incorporated an RNA aptamer that binds a small molecule theophylline into the loop portion of a shRNA. It turned out that adding theophylline to the modified shRNA impaired the necessary step in shRNA processing—its cleavage by the enzyme Dicer. In the absence of the small molecule, the hairpin was processed properly. Therefore, the concentration of the small molecule was inversely correlated

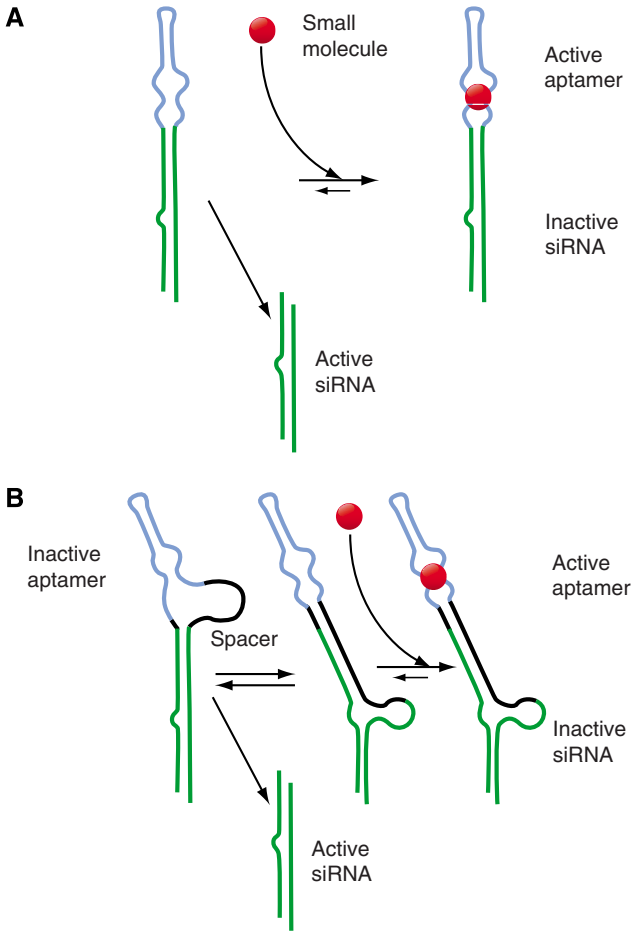

Figure 1 The block diagram of shRNA small molecule sensors. (**A**) Direct fusion of the aptamer to the siRNA motif described by An *et al.* (**B**) A tri-component sensor of Beisel *et al.* An active aptamer is in equilibrium with an active siRNA motif, but is stabilized upon binding of the small molecule.

with the RNAi activity and directly proportional to the concentration of the target mRNA and its corresponding protein (Figure 1A). Of course, this mRNA had to be expressed at sufficiently high level from its gene in the first place—the switch merely reversed the RNAi downregulation, but was unable to activate an otherwise dormant gene. The small RNA and the aptamer portions of the sensor are physically adjacent but separate—An *et al* could replace the small RNA motif without changing the aptamer, and target two different genes, exogenously introduced GFP and DsRed. These studies were further expanded in the more recent publication from the same laboratory that showed regulation of an endogenous mRNA (Tuleuova *et al*, 2008).

Beisel *et al* propose an even more elaborate way to affect the shRNA activity by a small molecule. Their shRNA sensor adopts two different conformations that exist in equilibrium; only one of them presents an active aptamer conformation that can bind the molecule but at the same time is inactive in the RNAi pathway. Another is active in the pathway but not as an aptamer. The binding of the small molecule to the active aptamer 'freezes' the shRNA molecule in an RNAi-inactive state. Unlike the previously described approach, the shRNA sensor comprises three parts: the 'stem', the 'spacer' and the 'aptamer' (Figure 1B). The parts are modular, and the aptamer can be replaced without changing the target, and *vice versa*. The authors developed a thermodynamic model that took these elements into account and predicted the signal response behavior of the sensor. The combination of the modular sensor architecture and the predictive model made it possible to rationally tune the response behavior of the sensor. For example, more efficient siRNA motif would lower the mRNA level in the Off state and improve the dynamic range, whereas stronger small molecule binding to the aptamer motif would lower the switching concentration. The model allowed the authors to rationally improve their switch targeting an exogenous GFP mRNA, reaching an impressive dynamic range of seven-fold between the Off and On states. They also demonstrate a shRNA switch against an endogenous gene, and perform a logic integration of two small molecule inputs.

These works and the research on other riboswitches represent an important thrust that could lead to quantum leap in our ability to design synthetic regulatory networks. It has been noted by multiple authors that RNA is an exceptional substrate for synthetic projects, because its function is based on primary and secondary structures, whereas protein function is crucially dependent on the tertiary folding. Secondary structure, as well as the thermodynamic parameters of RNA–RNA interactions, can be predicted with reasonable precision, making possible the development of accurate models such as that shown by Beisel *et al*. Such models will enormously facilitate forward design of new regulatory elements by bridging the gap between sequence and function. There is still a lot to be done in the area of riboswitches at large, and shRNA switches in particular. For example, the efficiency of regulatory RNAi-based links has to be improved to increase the dynamic range of the switches and afford true scalability. As with other biosensors, there is a need to characterize their performance in individual cells and assess the cell-to-cell reproducibility of the signal response curve. An exciting proof of concept would be a synthetic network where multiple shRNA sensors respond and integrate intracellular levels of endogenous metabolites to report on a metabolic pathway.

Conflict of interest

The author declares that he has no conflict of interest.

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