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Aptamers Meet Allostery

Engineered RNAs have demonstrated remarkable properties of molecular recognition and allosteric function. Liu and colleagues now report the isolation and in vivo function of a ligand-dependent RNA-based transcription factor that opens wide the door for allosterically controllable aptamers.

RNA is a highly versatile biopolymer capable of exhibiting fundamental biochemical properties once believed to be unique to the realm of protein factors and enzymes. The numerous and varied activities that cellular RNAs fulfill as catalysts or regulators of biological processes have shattered the view of RNA as a simple biological intermediary. Moreover, engineered RNAs have served to further expand the repertoire of biochemical capabilities ascribable to RNA and have offered unique insights to RNA's inherent potential for catalysis [1], molecular recognition and discrimination [2], and allosteric function [3]. Such engineering efforts are made possible by RNA's unique tractability to both rational design and combinatorial selection techniques [1], the latter of which is facilitated by the dualistic character of RNA as an informational and functional molecule. RNA is thus regarded as an attractive biopolymer for tailoring novel molecular therapeutic agents and biotechnological tools.

In this issue of *Chemistry & Biology*, Liu and colleagues report the successful exploitation of RNA's molecular recognition and allosteric capabilities in the creation of an RNA-based transcriptional activator that is facilely modulated by an effector compound in yeast [4]. The transcriptional activator functionality is derivative of a previously isolated RNA aptamer that binds an unidentified host factor and activates reporter gene expression when localized to the promoter region of DNA [5]. By integrating a second RNA aptamer domain that binds tetramethylrosamine (TMR) [6], Liu and coworkers sought to modulate the function of the adjacent transcriptional activator through conformational changes in aptamer structure arising from TMR interaction, and have succeeded in generating the first biologically active allosteric aptamer (Figure 1A).

Such integration of functional RNA domains has previously been achieved in the generation of allosteric RNA catalysts by joining aptamer and ribozyme domains [7]. The union of ligand binding and catalytic functions through rational design strategies has proven to be moderately successful. Such judicious integration of functional domains typically relies on a phenomenon of RNA-ligand interaction termed adaptive binding [2], in which ligand binding stabilizes local RNA structure. By replacing a critical element of a catalyst's secondary structure with an aptamer domain, ligand-induced structural stabilization and ribozyme activation has been demonstrated [8]. However, this design strategy can be significantly augmented with combinatorial strategies, in which nucleotide positions in the region conjoining functional domains are randomized, and individuals are selected on the basis of optimal allosteric performance [9]. In this manner, allosteric nucleic acid catalysts that are either activated or inhibited by ligand binding have been isolated.

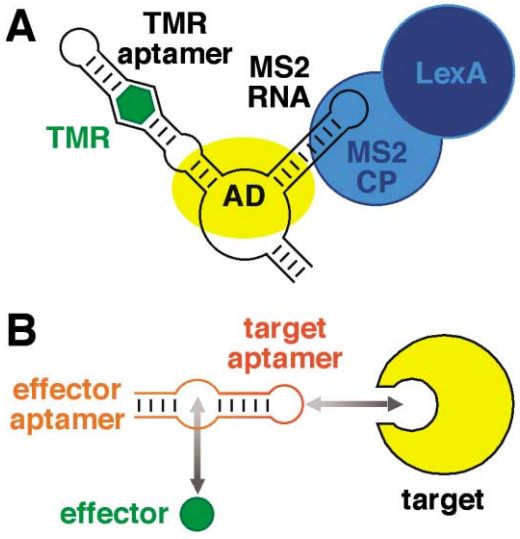

Figure 1. Allosteric Aptamers

(A) TMR-dependent transcriptional activator isolated by Liu and coworkers [4]. An RNA aptamer that functions as a transcriptional activation domain (AD) in yeast by binding an unidentified host factor is integrated with the TMR aptamer in such a manner that TMR binding promotes formation and function of the activation domain. The RNA is localized to the promoter of a reporter gene through the respective RNA and DNA binding activities of an MS2 coat protein (MS2 CP)-LexA fusion protein.

(B) General scheme for allosteric aptamer function. Integration of effector and target aptamer domains as interdependent or mutually exclusive functional domains might achieve effector activation or inhibition of target aptamer function, respectively.

Equipped with knowledge regarding the structural features and requirements of both the TMR aptamer and the RNA-based transcriptional activator, Liu and coworkers applied the principles of allosteric nucleic acid design and selection to isolate TMR-dependent transcriptional activators from a relatively small combinatorial library in which a segment linking the aptamer domains was randomized. TMR-dependent transcriptional activators were isolated based on their ability to activate *HIS3* expression in yeast and confer TMR-dependent growth in the absence of histidine. The stringency of the selection was aided using 3-aminotriazole (3-AT), an inhibitor of HIS3 activity, to isolate the most potent TMR-dependent transcriptional activators. Of four isolates obtained, the most potent transcriptional activator confers absolute TMR-dependent growth in the absence of histidine and presence of 3-AT. β-galactosidase assays demonstrate that the same isolate provides a 10-fold TMR-dependent increase in expression and functions in a dose-dependent manner, thereby establishing the general function of the TMR-dependent transcriptional activator as an artificial genetic regulatory switch. Sequence analysis of the isolates obtained reveals a general strategy for TMR-dependent function in which the region joining aptamer domains consists of a weakened stem element that is likely stabilized by TMR binding to form the active structure of the adjacent transcriptional activator. Mutational analyses validate both TMR-dependent function and mechanism, as point mutations designed to obviate TMR binding indeed disrupt transcriptional activation altogether, while a point mutation that stabilizes the conjoining segment promotes TMR-independent transcriptional activation.

Liu and colleagues' accomplishment adds a layer of sophistication upon previous efforts that have applied aptamers toward genetic regulation. Such efforts have achieved ligand-dependent inhibition of gene expression by incorporating aptamers within the 5′ untranslated regions (UTRs) of eukaryotic mRNAs, where ligand binding interferes with translation initiation [10, 11]. The present work, however, provides a *trans*-mechanism of transcriptional control rather than *cis*-mechanism of post-transcriptional regulation; a feature that might be more generally applicable toward modulating target gene expression, given that such ligand-dependent transcriptional activators can be localized to specific promoters. Additionally, the present work parallels recently discovered mechanisms of natural genetic regulation by riboswitches [12]. These naturally occurring metabolite binding aptamers generally reside in the 5′-UTRs of prokaryotic mRNAs and regulate gene expression through metabolite-induced conformational changes in RNA structure that affect transcriptional termination or translation initiation. The work of Liu and colleagues represents a significant contribution to the limited number of demonstrations that similarly refined mechanisms of RNA allostery can both be achieved with wholly engineered species and brought to bear on cellular processes [13, 14].

Importantly, the work presented by Liu and coworkers suggests a general scheme for developing allosterically controllable aptamers. In principle, allosteric aptamer function has previously been observed for a DNA aptamer that exhibits mutually exclusive binding of small molecule ligands between coupled domains [15] and by an allosteric ribozyme that exhibits cooperativity in ligand binding between coupled RNA aptamer domains [16]. However, the present work demonstrates more adaptable methodology for integrating two aptamer domains and isolating either interdependent or mutually exclusive aptamer operations (Figure 1B). In this manner, either effector activation or inhibition of target aptamer function might be used to modulate target molecule activity. Overlaying such allosteric regulation upon aptamers that inhibit target protein function could provide controllable therapeutic agents that are either activated or inactivated at will or fine-tuned to achieve a desired outcome. More practically, such allosteric aptamers could be useful biotechnological tools for dissecting intracellular protein function.

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