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Aptamers and Small Molecules Play Tug of War

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Recently, aptamers began to emerge as therapeutics. In this issue of *Chemistry & Biology*, Famulok and colleagues [1] present a twist on this concept, using an enzyme-inhibiting aptamer as a tool to screen for small molecules that block HIV replication.

For almost two decades now nucleic acid receptors, commonly known as aptamers, have been isolated from large random-sequence libraries. These molecules have been designed to bind a wide range of interesting targets and have been applied in many different ways in research areas such as molecular sensing and affinity purification. Aptamers have also established their potential to provide therapeutic benefits, with one aptamer drug already on the market and others currently in clinical trials. In this issue of *Chemistry & Biology*, Famulok and colleagues have cleverly combined the ability of an engineered ribozyme to act as a sensor with an aptamer originally isolated for its therapeutic potential [1]. The result of this approach was the isolation of a novel small-molecule inhibitor for HIV reverse transcriptase (HIV-RT) that binds an unexploited site on this highly targeted protein.

The authors developed a high-throughput assay to search for HIV-RT inhibitors based on a rationally designed allosteric hammerhead ribozyme (shown schematically in Figure 1). They combined the RNA-cleaving ribozyme with an anti-HIV-RT aptamer. The aptamer binding to its target changes the conformation of the integrated RNA molecule and results in the inactivation of the ribozyme. In this assay, established in a previous publication by Famulok's group [2], the ribozyme is in an inactive state when aptamer is bound to its protein target (Figure 1B). If the small molecule binding to the protein displaces the aptamer, the ribozyme will become activated. The activation of the ribozyme results in the cleavage of the substrate RNA labeled with a fluorophore/quencher pair, which releases the fluorophore from quenching and generates a measurable signal (Figures 1A and 1C).

Using functional nucleic acids as sensor elements for screening is a relatively new area that has begun to see good success. Aptamers can be modified to produce many different types of signals such as electrochemical, acoustic, and piezoelectric [3]. However, fluorescence has become the method of choice for high-throughput approaches. In 2006, Elowe and colleagues isolated inhibitors of adenosine deaminase in a screen of more than 40,000 small molecules by using a "structure switching" aptamer assay that produced a fluorescent signal in response to changing adenosine concentrations [4]. Also in 2006, the Famulok group published an aptamer displacement assay that relied on fluorescence polarization to screen for inhibitors of a cytohesin protein. These inhibitors were then used to help elucidate the role of cytohesin proteins in insulin signaling and diabetes [5].

While these methods relied on conformational changes in the aptamer domain to generate a signal, enzymatic approaches using ribozymes have also been employed. In 2004, Archemix Corporation published an assay that was suitable for screening for inhibitors of protein kinases [6]. Researchers seeking molecules that interact with bacterial RNA gene regulation elements, known as riboswitches, have also looked at designing assays for such systems. It is thought that the ability of a small molecule to disrupt the normal function of a riboswitch could result in the misregulation of essential genes and thus such a molecule may function as an antibiotic. This idea was demonstrated earlier this year using analogs of lysine that bind the aptamer domain of lysine-responsive riboswitches [7]. In 2006 the Breaker group, a forerunner in the field of riboswitches, and the Famulok group, a forerunner in the field of ribozyme-based screening, each developed a high-throughput assay for activators of the glmS riboswitch [8, 9]. While the assay from Breaker's group used a fluorescent resonance energy transfer (FRET) mechanism [8], the Famulok group made use of fluorescence polarization [9].

The Famulok group has been developing ribozyme-based screens since 2001 when they published an assay for identifying inhibitors for the hammerhead ribozyme [10]. Their transition to allosteric ribozyme systems has greatly expanded the arsenal of targets that can be screened. Self-cleaving ribozymes have been converted, using both rational design and selection processes, into allosteric systems that are responsive to many different regulating molecules [11]. The engineering and optimization of aptamer displacement assays is therefore quite practical and broadly applicable. The successful example in the article presented here [1] will warrant further attempts to take advantage of this strategy.

The availability of high-quality aptamers to relevant proteins is the key to this approach becoming broadly applicable. Aptamers have already been isolated and characterized for many attractive therapeutic targets [12]. For example, thrombin-binding aptamers with impressive in vitro

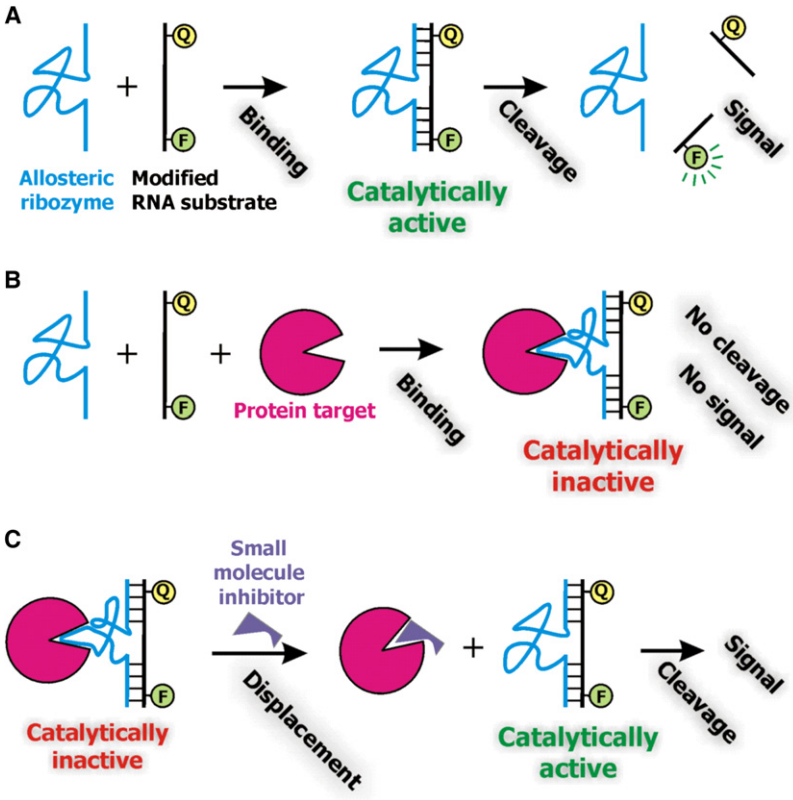

Figure 1. An Inhibitor Screen via a Displacement Strategy Involving an Allosteric Ribozyme, a Protein Target, and a Small Molecule

(A) An allosteric ribozyme containing a protein-binding aptamer element is capable of cleaving a fluorogenic substrate labeled with a pair of fluorophore (F) and quencher (Q), generating a fluorescent signal. (B) The formation of the aptamer-protein complex inactivates the ribozyme, shutting down its signaling capability. (C) Introduction of a potential small-molecule inhibitor that can compete with the aptamer for protein binding will lead to the displacement of the aptamer from the same binding site, restoring the signal-producing activity of the ribozyme.

characteristics have been examined clinically for their potential as drugs to prevent blood clotting. However, the drawback preventing their widespread use is their short lifetime in vivo [12]. Issues of limited bioavailability and inadequate lifetime are recurring problems with aptamer therapeutics that cannot always be overcome by chemical modifications. In the case of thrombin, and others like it, the idea presented here offers an excellent way to use these high-quality aptamers to uncover small molecules that have more desirable drug-like properties.

The small-molecule inhibitors of HIV-RT discovered using the allosteric ribozyme screen [1], in particular SY-3E4, are especially interesting because of the site they target. It had previously been shown that the aptamer used in the screen binds the primer-template binding site of HIV-RT [13]. Using electrophoretic mobility shift assays (EMSA), it was revealed that SY-3E4 also targeted this same site [1]. Clinically used inhibitors of HIV-RT are largely nucleoside or nucleotides aimed at mimicking the building blocks used by HIV-RT to produce its DNA product. No inhibitors currently on the market have been shown to function through blocking the primer-template binding site. Therefore, the molecules isolated in this screen potentially represent a new class of inhibitors. Famulok and coworkers illustrated the importance of this claim by demonstrating that their inhibitors, which disrupted HIV replication in cell culture, were also effective against multidrug-resistant strains of HIV [1]. It was previously shown that mutations that overcame inhibition of aptamers that target this site on HIV-RT led to a replication-deficient virus [13]. This finding makes the inhibitors like SY-3E4 exciting as potential HIV therapeutics.

Screening for site-specific inhibitors is of great interest for those developing antiviral treatments. Strategies to develop aptamers that are specific to a binding site on a protein [14] could be translated into screens for small molecules targeting that same site using this aptamer displacement screening strategy. Given the existence of many aptamers for important viral proteins [15], future endeavors might not be long in waiting. The utility of this methodology is clearly not limited to antiviral screening [5], and, in principle, could be employed for any protein or nonprotein target. One such area is the development of antibiotics, where similar resistance problems can evolve, and site-specific screening would be an obvious benefit.

The use of DNA and RNA aptamers and enzymes as sensors for high-throughput small-molecule screening is still a relatively new area of research. However given the protocols in place to isolate such molecules using custom-designed selection methods and the impressive signaling properties they can be made to display, it appears to be a field with a bright future.

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Mending the Bones with Natural Products

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In this issue, Qiu and colleagues [1] demonstrate in vitro and in vivo activity of a triterpene glycoside from black cohosh. The isolated compound acts as a suppressor of osteoclastogenesis, targeting specifically RANKL, a member of the TNF superfamily.

Bone-related diseases, such as Paget's disease, osteoporosis, arthritis, or cancer metastases, affect millions of people worldwide. Bones are constantly remodeled through the synthesis of bone matrix by osteoblasts and the resorption of bone by osteoclasts. Osteoblasts and osteoclasts arise from distinct cell lineages and maturation processes—osteoclasts arise from mesenchymal stem cells while osteoclasts differentiate from hematopoietic monocyte/macrophage precursors [2]. One of the key factors mediating the process of osteoclast formation known as osteoclastogenesis is receptor activator of nuclear factor-kappaB (NF-κB) ligand (RANKL), a member of the tumor necrosis factor (TNF) family that has also been called osteoclast differentiation factor, TNF-related activation-induced cytokine, and osteoprotegerin (OPG) ligand [3].

RANKL, protein expressed on the surface of osteoblastic/stromal cells, is directly involved in the differentiation of monocyte macrophages into osteoclasts [4]. Mice with disruptions in the *RANKL* gene show a lack of osteoclasts, severe osteopetrosis, and defective tooth eruption, indicating that RANKL-induced osteoclastogenesis is mediated through the cell surface receptor RANK [5]. That RANK can mediate osteoclastogenesis was first demonstrated by Hsu and coworkers in 1999 [6]. Further gene-deletion analysis of RANK, RANKL, and TNF receptor-associated factor 6 (TRAF6) showed that these genes are positive regulators of osteoclastogenesis [7], whereas OPG, a decoy receptor for

Table 1. Current Drugs Used for Bone Loss Related Disorders

Drug	Chemical Class	Mechanism of Action	Side Effects
Alendronate/Risedronate/ Ibandronate/Zoledronic Acid	Bisphosphonates	Inhibits osteoclast	GI toxicity, weight loss, bone pain, low calcium levels
Estrogen	Sex steroid	Inhibits osteoclast development	Endometrial cancer, stroke
Raloxifene	Estrogen mimic	Inhibits osteoclast development	Leg cramps, hot flashes
Estren	Estrogen derivative	Inhibits osteoblast apoptosis	Breast cancer
Denosumab	RANKL antibody	Inhibits osteoclast development	Nausea, diarrhea, cramps
Calcitonin	Peptide hormone	Inhibits osteoclasts	Nausea, skin redness, diarrhea
Teriparatide	Peptide	Induces bone formation	Pain, headache, diarrhea
PTHrP	Osteotrophin	Induces bone formation	Nausea, weakness

GI, gastrointestinal; RANKL, receptor activator of NF-κB ligand; PTHrP, parathyroid hormone-related peptide.

