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Previews

Aptamer Rivalry

A controllable, intracellular RNA aptamer could be an invaluable therapeutic or discovery reagent. A report published in this journal shows that aptamer behavior can be regulated by a small molecule, paving the way for the development of more sophisticated ligand-regulated aptamers.

Since 1990, when the now familiar technology of in vitro selection, SELEX (systematic evolution of ligands by expontential enrichment), was first reported in the scientific literature, there has been a sense of excitement about the vast potential of this methodology. In its early stages, SELEX was used primarily to select novel, antibody-like affinity reagents made from RNA and DNA (aptamers) as well as new RNA and DNA enzymes (ribozymes and DNAzymes). Astonishingly, some of these enzymes had catalytic activities not found in living systems. These initial success stories sparked the tantalizing idea that the ever-growing list of aptamers and nucleic acid enzymes may eventually be used within living cells and organisms both in therapeutics and as tools for dissecting fundamental cellular processes. Finally, in 2002, we now find ourselves firmly in that anticipated realm. Several potentially therapeutic ribozymes are currently being tested in clinical trials (reviewed in [1]), and the dream of using both aptamers and ribozymes to probe cellular pathways is now a reality.

A key milestone on the path toward developing clinically useful RNA and DNA was the finding that it is possible to express aptamers and ribozymes within cells at sufficiently high levels to influence their phenotype. This was first demonstrated by Werstuck and Green in 1998 [2], who showed that two different RNA aptamers that specifically bound an antibiotic (either Kanamycin A or Tobramycin) could be robustly expressed in *E. coli* cells, conferring specific antibiotic resistance to those cells. Further, the authors showed that incorporation of an antibiotic-specific aptamer into the 5′ untranslated region of a messenger RNA resulted in repression of translation of that message in a specific antibiotic-dependent manner. Since that groundbreaking report, RNA aptamers targeted against various proteins have been expressed in different cells (with appropriate expression vectors dispatching the aptamer RNA to different cellular compartments and locations). Using this approach, aptamer-generated phenotypes have been observed in diverse eukaryotic cell types as well as in transformed *Drosophila melanogaster*. The proteins that have been targeted by this method are wide ranging and include RNA polymerase II in *S. cerevisiae*, the RNA splicing factor B52 in *Drosophila*, the transcriptional regulator NFκB expressed in yeast, and a domain of the β2 subunit of human integrin (reviewed in [3]). In this regard, it is notable that a thrombin binding DNA aptamer has been found to confer significant anticoagulant properties in an animal model [4], and a chemically modified RNA aptamer for vascular endothelial growth factor (VEGF) has been shown to inhibit the growth of new blood vessels in a rat model [5].

Many of the above-mentioned experiments have made use of methods to constitutively express aptamers within cells to allow stable phenotypes to be manifested. However, it has been recognized that this technology may prove especially useful if it were possible to turn the intracellular aptamer on and off on demand. This would be a powerful tool to study the developmental programs of different cells and organisms. One possible approach toward such flexibility might be to regulate the production of the aptamer by placing its expression under the control of an inducible promoter. An alternative approach would be to express the aptamer intracellularly at high levels but in a quiescent state, from which it could then be activated or rescued on demand. A paper by Vuyisich and Beal published in this issue of *Chemistry & Biology* [6] reports on a prototypical scheme for precisely this latter mechanism.

The goal set out by Vuyisich and Beal was to select for aptamers that not only bound a target protein tightly and specifically (thus inhibiting its cellular function) but which *also* bound specifically to a small molecule ligand. The protein chosen by Vuyisich and Beal for their proof-of-concept experiment was a DNA repair enzyme, formamidopyridine glycosylate (Fpg), and the ligand the common antibiotic neomycin. These two compounds were chosen because they both naturally have low-level affinities for binding nucleic acids. The selection procedure itself was both simple and elegant. First, folded RNA molecules from a random-sequence RNA library were selected for their ability to bind Fpg protein that had been immobilized to a column. The next, innovative, step, eluting the bound RNA with a solution containing neomycin, ensured that RNAs were selected that bound Fpg in the absence of neomycin but released Fpg (effectively activating the protein) in the presence of the antibiotic. Analysis of RNA clones obtained following many iterations of this selection cycle and footprinting experiments carried out on individual clones revealed physically overlapping aptamer elements for binding Fpg and binding neomycin. This provides a physical basis for understanding the ability of neomycin to disrupt the aptamer-Fpg interaction. Fundamentally, competition between the overlapping aptamer elements for binding their respective ligand decides the functional state of the RNA molecule. Interestingly, Vuyisich and Beal emphasize that future development of this technology will utilize not only known cytotoxic agents such as neomycin but also "neutral" or "unobtrusive" small molecule ligands, which ideally would enjoy both high permeability into cells and minimum intrinsic interaction with cellular processes and pathways.

Vuyisich and Beal's demonstration of a ligand-regu-

lated aptamer is a logical progression from parallel innovations in the selection and, occasionally, rational design of ligand-regulated ribozymes and DNAzymes, a number of which have been described in the literature over the past few years. The first convincingly allosteric ribozyme (a hammerhead ribozyme whose activity was modulated by the binding of the small molecule adenosine) was generated by rational design by Tang and Breaker [7]. Subsequently, however, many different small molecule ligand-regulated variants of both naturally occurring and in vitro selected ribozymes and DNAzymes have been described and are discussed in the recent review by Breaker [8]. The challenge for researchers producing both ligand-activated ribozymes and ligand-activated aptamers is now to demonstrate that the level of control that can be exercised with these promising reagents in vitro will also be reflected in intracellular environments.

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Selected Reading

1. Sullenger, B.A., and Gilboa, E. (2002). Nature *418*, 252–258.
2. Werstuck, G., and Green, M.R. (1998). Science *282*, 296–298.
3. Famulok, M., Blind, M., and Mayer, G. (2001). Chem. Biol. *8*, 931–939.
4. Griffin, L.C., Tidmarsh, G.F., Bock, L.C., Toole, J.J., and Leung, L.L. (1993). Blood *81*, 3271–3276.
5. Ruckman, J., Green, L.S., Beeson, J., Waugh, S., Gillette, W.L., Henninger, D.D., Claesson-Welsh, L., and Janjic, N. (1998). J. Biol. Chem. *273*, 20556–20567.
6. Vuyisich, M., and Beal, P.A. (2002). Chem. Biol. *9*, this issue, 907–913.
7. Tang, J., and Breaker, R.R. (1997). Chem. Biol. *4*, 453–459.
8. Breaker, R.R. (2002). Curr. Opin. Biotechnol. *13*, 31–39.

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Discovering Antimalarials: a New Strategy

Recent discoveries have uncovered some key processes that occur in the food vacuole of the malarial parasite. Consequently, new families of potential antimalarials that inhibit HRP-2, a hitherto unexplored drug target, were identified using a novel screening method.

Malaria is one of the world's leading killer infectious diseases. Although almost a third of the Earth's population is considered to be at risk from this disease, about 90% of infections and deaths occur in Africa [1], contributing significantly to underdevelopment and poverty on this continent [2]. The reasons for the current severity of the malaria problem are multifaceted, but among them is the occurrence of drug-resistant strains of parasite. Most notable in this regard is parasite resistance to chloroquine, which is now almost universal. In the past, this drug, having several excellent properties, became the mainstay of treatment and was a key component of malaria control strategies. In particular, chloroquine was highly effective against the parasite, had very few adverse side effects, was safe for use in pregnancy and young children, and was very cheap [3]. Its loss has been a major setback [4]. The challenge now is to find new compounds with antimalarial activity that can be developed into drugs that are cheap enough for use in poor third world countries.

A positive development over the last decade has been the considerable increase in our understanding of processes occurring within the parasite that are relevant to the mode of action of current antimalarials and which provide targets or potential targets for new antimalarial compounds. The causative agents of malaria are protozoal parasites of the genus *Plasmodium* (with *P. falciparum* the cause of fatal cases) [5]. They have a complex life cycle involving liver and blood stages in the human host, where asexual reproduction occurs, and a stage in the vector mosquito (*Anopheles* genus), where sexual reproduction occurs [5]. Symptoms and pathology are associated entirely with the blood stage, during which the parasite is located within the red blood cell of the host [5]. During this part of the life cycle, the parasite ingests hemoglobin into a specialized acidic compartment called a food vacuole. The hemoglobin is proteolytically digested into small peptides that ultimately supply the parasite with amino acids [6] (although intriguingly, these nutrients appear to be oversupplied [7]). Proteolysis is carried out by four aspartic proteases, namely plasmepsins I, II, and IV, and histo-aspartic protease (HAP) [8], three cysteine proteases (falcipains) [9], and a zinc protease (falcilysin) [10]. All of these represent potential targets for antimalarials (see Figure) and are currently the subject of intense investigation. Digestion of hemoglobin releases heme [iron(II)protoporphyrin IX, Fe(II)PPIX] into the food vacuole, where it is oxidized to hematin [H_2O-Fe(III)PPIX] [6]. Heme is another possible drug target and has been implicated in the mode of action of endoperoxide antimalarials, such as artemesinin, which have been proposed to form radical adducts with heme that act against the parasite [11]. Hematin is believed to be the target of chloroquine and other quinoline antimalarials, and there is evidence suggesting that these drugs act by preventing the detoxification of hematin (see Figure) [12], essentially all of which is normally converted to a very insoluble microcrystalline dimer of Fe(III)PPIX called hemozoin (or malaria pigment) [13]. Chemically, hemozoin is identical in composition [14] and structure [15] to β-hematin, a synthetic product that can easily be prepared from a solution of hematin.

The mechanism of hemozoin formation in the parasite