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Innovations

Archemix

Nucleic Acid Platforms

It all started 4 billion years ago: According to one of the founders of Archemix, a 35-person drug discovery, biosensors, and biotherapeutics company in Cambridge, MA, the company partially owes its creation to events that may have been crucial for the origin of life itself.

"There is a controversial theory of how life began and progressed called the 'RNA World Theory,'" says Ron Breaker, Associate Professor in the Department of Molecular, Cellular, and Developmental Biology at Yale University and an Archemix cofounder. "It holds that at one time, before the invention of protein and DNA, RNA molecules served both as genetic information and as catalysts," he says. Breaker and colleagues at Yale set out to test one of the key aspects of the RNA World Theory. That is, if RNA gave rise to the current cellular environment, it would have had to catalyze many reactions. It would have had to be able to sense both its environment and how much material it was making and turn things on and off at the right time.

"We used in vitro evolution to create entirely new RNA molecules—RNA enzymes that would cut themselves only when we add a certain compound," says Breaker. What they created were RNA molecules that catalyzed a reaction when they were bound to an effector. "It's really a simple molecular switch," he concludes, referring to compounds also commonly known as allosteric ribozymes, or "RiboReporters" in Archemix parlance. He and others, including Andrew Ellington at the University of Texas at Austin, worked on this research for 2–3 years. They made several different molecular switches. "Eventually, we theorized that if we can make these in the lab today and nature made them billions of years ago, maybe we can make some use out of it in the commercial setting," says Breaker. "We were asking a very arcane academic question, and out came this information that looks to have significant value." It is this work that led to Archemix's founding in May 2001.

Two Companies in One

Archemix is a drug discovery and development company focusing on two enabling technologies. "When we formed the company, the idea was to take these RiboReporters, build a unique platform for drug discovery, and then develop partnerships using those as tools to help other companies with their drug discovery programs" says Martin Stanton, president of Archemix. "We also intended to put our own small-molecule discovery program into place." But the research agenda grew sharply in October 2001 when Archemix purchased intellectual property for aptamer technology from Gilead Sciences (Foster City, CA). Aptamers are modified oligonucleotides that bind molecular targets, especially proteins. They have potential for therapeutic and detection purposes. "Our focus is now two-fold: RiboReporters for drug discovery and aptamer therapeutics," says Stanton. Both receive equal time among Archemix's 15 Ph.D.'s.

"We refer to our focus as an evolved nucleic acid platform because both programs essentially utilize in vitro selection of nucleic acids to derive molecules—either as therapeutic aptamers or as RiboReporters," says David Epstein, senior director of research and development at Archemix. The goal: to identify therapeutically relevant systems and targets and build fully integrated assays around those targets.

"Imagine these things like gene chips that are smart oligonucleotides," says Ron Breaker, Archemix cofounder, of his company's RiboReporters. "They not only bind to their unique target, but then they report the fact that they've bound by doing a chemical reaction".

RiboReporters: Bind, Fold, and Snip

"RiboReporters are ribozymes modified to turn on in the presence of an analyte but are completely off in the absence of an effector molecule," says Epstein. They are detection molecules with an active site as well as a separate site that binds an effector. When a target binds the allosteric activation domain engineered into a RiboReporter, it changes the speed at which the active site works. Once bound, RiboReporters quickly cut in a particular position and thus form two compounds. "They bind to target, fold up, and then cleave," summarizes Breaker. "We can measure how much RNA has been cleaved and equate that with how much target we've put into the solution." The rate at which these RNAs cleave correlates with the amount of target in the mixture. "Imagine these things like gene chips," suggests Epstein, "that are smart oligonucleotides in that they not only bind to their unique target, but then they report the fact they've bound by doing a chemical reaction."

Archemix researchers are exploiting the ability of RiboReporter technology to work in solution, on microarrays, and inside cells and cell lysates with the goal of turning them into the next generation of biodetection molecules to replace antibodies. "You can't do that with an antibody," says Epstein referring to the primary competitive technology for RiboReporters. Comparing the two technologies, Epstein says, "In contrast with an antibody system that always requires three components, with a RiboReporter you're working with a single molecule cap-

ture-and-detection system." He continues, "I think a lot of people have overlooked nucleic acids as a powerful detection system and focused on antibodies for routine detection. They didn't think of what they could do if they had a catalytic platform, not just a binding platform."

Archemix got its start with ribozymes in May 2001 when it licensed self-cleaving ribozyme technology from Ribozyme Pharmaceuticals, Inc. in Boulder, CO. RPI retains its focus on ribozymes for therapeutic purposes. "Researchers at the University of Texas and Yale showed they could make ribozymes that switch on and off by the presence of an analyte," says Stanton. Archemix then licensed that technology, plus detection and other ribozyme platforms, from Stanton's previous employer, Brandeis University, to create the RiboReporter technology.

Detection methods are a current focus of the RiboReporter effort. One can detect the presence of an effector by watching the release of fluorescence by a "molecular beacon"—an RNA molecule with a fluorescent group on its 5′ end and a quenching group on its 3′ end. If that RNA forms a hairpin, then the fluorescent group and quencher are next to each other and there is no or little fluorescence. If the ribozyme comes in and splits that molecule, the solution glows. According to Breaker, "In the future, it would be great to have a ribozyme immobilized on a surface or in solution that can be detected by direct electronic means." If the ribozyme binds, it creates an electronic signal confirming the presence of the desired effector.

In Vivo Biodetection

"What's really exciting about this technology is what we call protein PCR. Utilizing the ligase format where an effector molecule triggers a ligation and that then allows for the formation of a PCR product, we can then detect ligase activity by quantitative PCR," says Epstein. Protein PCR allows real-time protein expression profiling of human cells and tissue. The company has developed the technique to profile gene expression at the protein level as opposed to the mRNA level, which is more common. Epstein says they can differentiate between posttranslationally modified forms of proteins and can track not only the level of a given protein but also its state in real time. "We've shown you can add a drug to a cell and follow the action of that drug on a protein target using RiboReporters in this protein PCR platform." The power is that one can do this inside live cells.

The primary challenges with the RiboReporter technology have been to industrialize the process and to adapt the system to therapeutically relevant targets. "It isn't clear what the potential is for these molecular switches," cautions Breaker. "We know that RNA can fold up and see many different proteins and organic compounds, but can we see everything we want? We don't know yet."

Aptamers: Nucleic Acid Antibodies

Aptamer technology is not new. Created back in 1989 by Larry Gold at the University of Colorado, it moved into NeXstar Pharmaceuticals, which was purchased by Gilead in 1999. NeXstar accomplished about 150 patents in the technology development, methods of selection and generation, and the entire portfolio of what constitutes in vitro selection—known as the SELEX enrichment process. In addition, the company developed methods for serum stabilization and delivery, as well as key patents on biologically relevant targets. It developed about 20 preclinical serum-stabilized candidates, with six completing pharmacokinetic testing and another six shown to be active in various animal models for diseases such as cancer, rheumatoid arthritis, and inflammation.

"We then licensed all this technology into an evolved nucleic acids company, which has ribozymes for drug discovery and now has the power of serum-stabilized aptamers," boasts Epstein of the $17.5 million Gilead deal. Archemix is benefiting from recent advances in aptamer stabilization, including modifications improving delivery and pharmacokinetic and therapeutic properties.

The Competition: Monoclonal Antibodies

Archemix also sees monoclonal antibodies, defined internally as "nucleic acid antibodies," as the primary competition to aptamer therapeutics. "The key advantage with aptamers is their size relative to antibodies," says Epstein. Aptamers range from 10,000 Da if they are not coupled to the delivery agent, polyethylene glycol (PEG), to 50,000 Da when PEGylated. Ranging from 150,000 Da to significantly more, Monoclonal antibodies are much larger. This means aptamers can be delivered subcutaneously, whereas intramuscular injections are required of antibodies. They are less costly to produce—$1,000/gram of aptamer versus $1,000–$10,000/gram of antibody—and are easily generated at the benchtop through in vitro selection. "This allows us to engineer in specificity at the same time that we generate affinity," says Epstein. A desired consequence is avoidance of unintended targets that give rise to side effects and toxicity. "Because the cost is so much lower, we can reengineer or redesign and synthesize and generate an aptamer much the same way one would with a small molecule," says Epstein, "plus they have incredibly long half-lives and are stored lyophilized indefinitely at room temp indefinitely." Just add water and you're ready to go.

Archemix is limiting its aptamer therapeutics portfolio to targets in the extracellular, intravascular compartment; such targets include growth factors and cytokines. "We don't think extravascular is off-limits because of the size," cautions Epstein, "but we're clearly not going to try get into intracellular space." They have yet to firmly select lead aptamer candidates, but Epstein and colleagues are building a development team to help evaluate the current aptamer portfolio as well as the potential for new programs. The portfolio contains therapeutic targets, including Platelet Derived Growth Factor (PDGF), which has already shown some efficacy in end-stage renal disease and restenosis, in which the aptamer blocks binding of PDGF to its receptor and thus prevents cell proliferation, matrix accumulation, and subsequent glomerular damage. Aptamers have also been designed to target other growth factors, selectins, and complement components.

Archemix is one of three companies benefiting from Gilead aptamer licenses. EyeTech Pharmaceuticals (New York, NY) is taking the first aptamer therapeutic, developed at Gil-

ead, into clinical trials. The anti-VEGF drug, EYE001, is now in Phase II/III for age-related macular degeneration. SomaLogic Inc. (Boulder, CO) is developing an aptamer-based in vitro diagnostic aptamer array project capable of simultaneously detecting 10,000 proteins on one chip. "We're not doing that ourselves," says Stanton. Instead, the company intends to focus on low-throughput, high-value targets for therapeutics.

Archemix is now passing along parts of its aptamer technology treasure. In January 2002, the company made a licensing agreement with NOXXON Pharma AG for use of the SELEX process in combination with its own Speigelmer oligonucleotide technology. "We can't possibly derive all of the value from the aptamer technology ourselves," says Stanton, "though we'll certainly take some therapeutic aptamers all the way ourselves." So, if you're in the market for a share of the aptamer patent technology, Archemix is listening.

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