DDT Vol. 7, No. 5 (Suppl.), 2002

Archemix: drug discovery innovation based on evolutionary nucleic acid technology platforms

David Epstein, Charles Wilson and Marty Stanton

Archemix, develops aptamers and riboreporters™ (allosteric ribozymes that couple recognition to detection) for therapeutics and drug discovery. Aptamers and riboreporters™ recognize drugs, metabolites and proteins, and function in multiple formats, including chip, solution and cellular settings. Using novel nucleic acid platforms, Archemix builds mechanism-based drug discovery programs around virtually any target.

David Epstein*
Charles Wilson
and Marty Stanton
Archemix
1 Hampshire Street
Cambridge, MA 02139
USA
tel: +1 617 621 7700
fax: +1 617 621 9300
*e-mail: depstein@archemix.com

▼ Does a drug lead enter the cell or diseased tissue? Does it hit its target? And does it do so to cause the predicted biological effect? These fundamental questions, faced by many in the pharmaceutical industry, are the current drivers for Archemix's technological innovation. Thousands of under-validated targets have been identified using limited genomic data, and these targets have been partially characterized through HTS efforts. Identifying good chemical leads and simultaneously linking these leads to quality drug targets remains a significant hurdle, one which is best accomplished using mechanism-based drug screening.

Over the past decade, mechanism-based drug discovery has led to the development of novel therapeutics with far fewer side effects and greater target specificity. Key breakthroughs in this area include the new kinase and protease inhibitors developed as treatments for cancer and human immunodeficiency virus (HIV) infection [1]. The current problem is that the effectiveness of mechanism-based screening is limited by information on the drug target itself. Traditionally, mechanism-based approaches require some information on the role of the target or its biochemical pathway in disease. Hence, it could be argued that for the promises of genomic-based drug discovery to be realized, the pharmaceutical industry needs additional technological advances that afford detailed mechanistic information on both the biological target and drug lead.

Archemix's riboreporter™ and aptamer technology is being developed expressly to provide these mechanistic insights. Proprietary *in vitro* evolution techniques make it possible to engineer nucleic acid-based sensors with high specificity, detecting, for example, post-translational modifications to a target, or ligand-induced conformational changes. The fact that these sensors can be configured to report in live cells means that this information about a therapeutic target can be acquired in an *in vivo* setting with direct relevance to disease biology. Backed by over 200 patents, the Archemix technology platform can be used at each stage of drug discovery and development to obtain information on both compound efficacy and mechanism.

Target-activated ribozymes as protein and metabolite-specific biosensors

Archemix's drug discovery sensors are based on key scientific insights from two of its academic founders. Working independently, the laboratories of Andrew Ellington (University of Texas, Austin, TX, USA) and Ronald Breaker (Yale University, New Haven, CT, USA) have shown

 PII: S1359-6446(02)02170-0

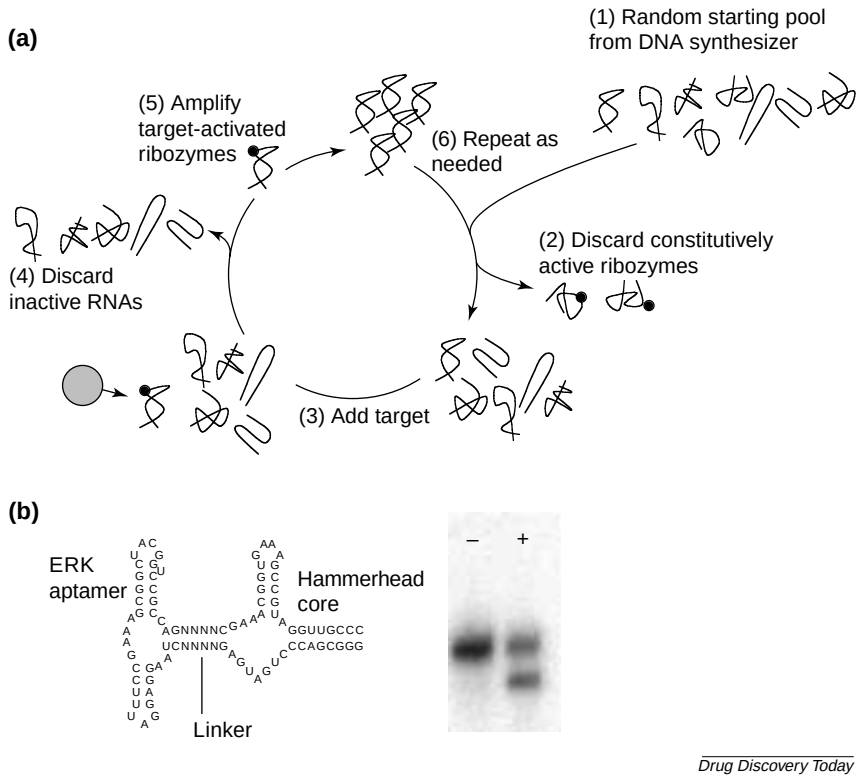

Figure 1. *In vitro* evolution of riboreporters™. **(a)** Evolution of a riboreporter starts with the synthesis of a ribozyme sequence on a DNA synthesizer. Random nucleotides are incorporated, generating pools of approximately 10^{16} molecules. Most molecules in this pool are non-functional, but a handful will respond to a given target and be useful as biosensors. Sorting among the billions of species to find the desired molecules starts from the complex sequence pool. Riboreporters™ are isolated by an iterative process: in addition to the target-activated ribozymes that one desires, the starting pool is usually dominated by either constitutively active or completely inactive ribozymes. The selection process removes both types of contaminants. In a following amplification stage, thousands of copies of the surviving sequences are generated to enable the next round of selection. During amplification, random mutations can be introduced into the copied molecules – this 'genetic noise' enables functional riboreporters™ to evolve continuously and become even better adapted as target-activated enzymes. The entire experiment reduces the pool complexity from 10^{16} down to <100. **(b)** Results from an *in vitro* selection experiment for a riboreporter activated by the protein kinase, ERK2.

that target-activated ribozymes can be created through the application of simple design rules or by the process of *in vitro* evolution (Fig. 1). These target-activated ribozymes can be broken down into functionally separable domains: control domains responsible for target recognition, and catalytic domains responsible for chemistry [2]. Target-activation occurs through many different mechanisms, but often the two domains are coupled by their joint ability to fold in the presence of a target effector. Interaction of the control domain with the target drives a conformational change that is transmitted to the catalytic domain thereby enabling catalysis to proceed [3]. In their initial demonstrations of the technology, Breaker and Ellington showed that targets ranging from ions to proteins could be used to trigger catalysis by both self-cleaving ribozymes and self-ligating ribozymes. Extending this technology, Archemix has incorporated additional catalytic platforms and scaled up the *in vitro* evolution process such that sensors can be created in parallel against dozens of targets.

Riboreporter™ HTS applications

Using an entirely *in vitro* method (Fig. 1), ribozyme libraries are evolved to produce individual sensor sequences well-suited to specific drug discovery applications. Once identified, straightforward modifications to the riboreporters™ convert them into optical signaling molecules that can be monitored with standard commercial detectors, such as microplate readers, confocal scanners and cell sorters (Fig. 2). Fluorophore- and/or quencher-derivatized ribozymes activated by adenosine triphosphate (ATP), cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) [4,5], have been configured to function in multiplexed fluorescence assays [6,7], and are being used to evaluate drug leads that modulate cyclic nucleotide levels. A powerful aspect of the small molecule-directed riboreporter™-based technology is its ability to rapidly yield non-radioactive biochemical assays and HTS formats specific for virtually any enzyme product, with the possible exception of polyanionic molecules.

Target identification and validation using protein-dependent riboreporters™

Using *in vitro* evolution, Ellington and colleagues have created RNA ligases that efficiently join an oligonucleotide substrate to their ends [5]. With these ribozymes as a starting point for further evolution, it has been possible to engineer novel ligases whose joining activity is triggered only in the presence of specific protein targets [8]. Such ribozymes are activated 50,000–100,000-fold by their targets and demonstrate remarkable specificity. Following this lead, Archemix is currently engineering new mitogen-activated protein (MAP) kinase-activated ribozymes that are controlled by low

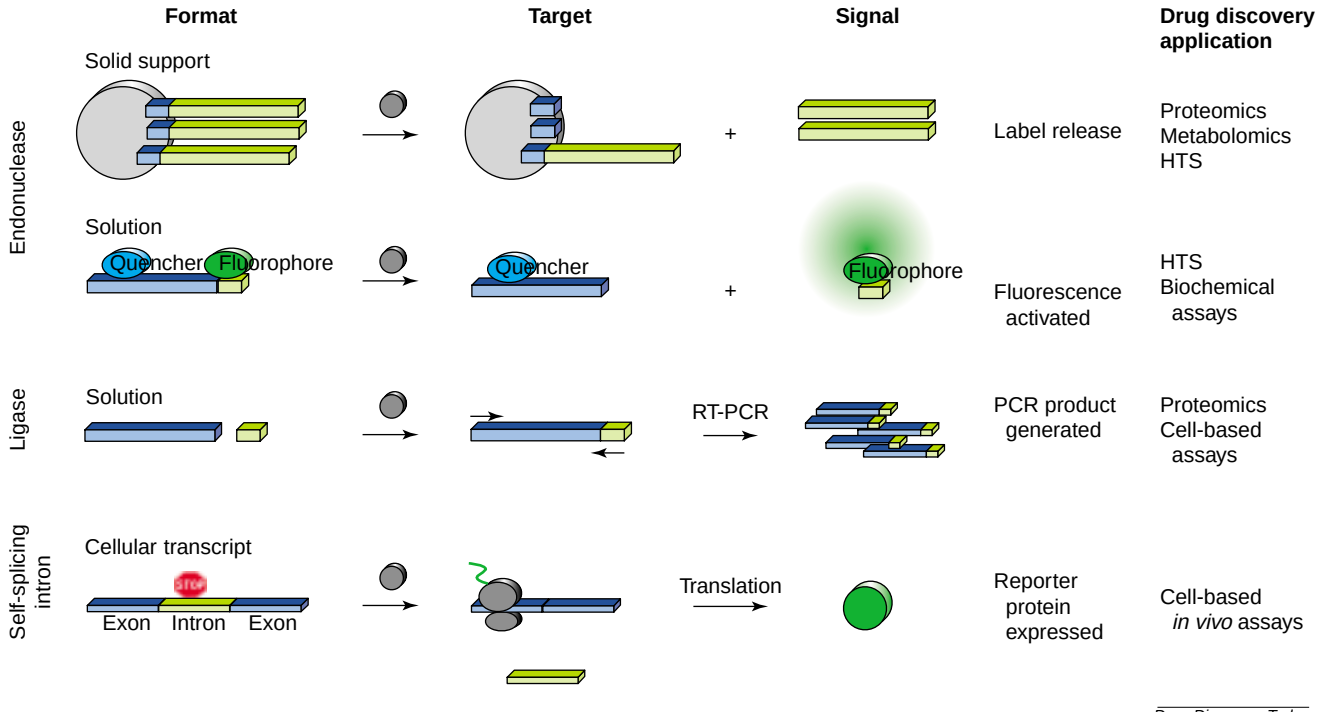

Figure 2. Applications of riboreporters™ in drug discovery. Riboreporter™ technology platforms for drug discovery currently enabled at Archemix include allosterically regulated ribozymes, such as hammerhead nucleases for both chip and solution-based screens, RNA ligases for chip, solution and cell-based screens and self-splicing introns for cell- and animal-based assays.

(<10 nM) concentrations of target and which can distinguish targets on the basis of their phosphorylation state (Fig. 1b). Riboreporters™ sensitive to post-translational modifications or to ligand-induced conformational changes of an enzyme or receptor have enormous potential for applications in protein profiling and in mechanism-based cellular assays.

A major advantage of working with nucleic acids is their demonstrated ability to function while attached to solid surfaces [9]. In contrast to many protein-based capture agents, which quickly lose activity on storage in solid-state form, nucleic acids retain their ability to fold and function following immobilization on both membranes and chips. Starting with both self-cleaving and self-ligating ribozymes, Archemix has enabled solid-phase detection of multiple small molecule and protein analytes in the form of membrane arrays, ribozyme capture chips and ribozyme *in situ* analysis chips. These arrays make it possible to perform rapid and quantitative analysis of the proteomic and/or metabolic content of a sample. Using riboreporter profiling arrays, it will be possible to quickly compare biological samples (such as diseased and normal states) not only on the basis of protein concentration differences but also on the basis of differences in protein functional state (for example, whether signal transduction pathways are activated in one case but not in another).

Taking riboreporters™ into cells

The fundamental concern in mechanism-based drug discovery is whether the biological effect of a compound correlates with its interactions with a specific target. To facilitate mechanism-based discovery accurately, it is essential to measure the interactions of a drug candidate with its target in a real biological setting. An enabling feature of Archemix's technology is that target-activated ribozymes created *in vitro* can be easily adapted to report on targets *in vivo*. As shown in Fig. 2, one mechanism for creating cell-based ribozyme sensors centers on self-splicing group I introns, which promote their own excision from precursor transcripts to produce mature mRNAs for translation. These ribozymes have been demonstrated to function in a wide range of cellular environments, including in mammalian cell lines [10], and to control the expression of reporter genes. By appending target-activation domains to a group I intron, Archemix scientists have shown that self-splicing (and thus reporter gene expression) can be efficiently regulated by addition of small molecule effectors (Fig. 2). In contrast to competing technologies for cellular analysis, cell-based riboreporter assays can be used for all types of targets. By coupling protein and metabolite effector-responsive ribozymes to reporter genes, Archemix is working to create proprietary mechanism-based cellular assays for drug discovery and development.

Concluding remarks

Archemix is poised to develop widely enabling drug discovery applications based on its proprietary exclusive technology licenses, which derive from over 150 issued or pending patents in the area of ribozyme technology, and over 100 aptamer selection and technology patents. The company's 30 employees are located in the biotechnology hotbed of Cambridge (MA, USA), where they have developed 25,000 ft^2 of laboratory space to facilitate the company's near-term growth objectives. The vision of the company and its founding scientists (David Epstein, Senior Director R&D, formerly of Bayer; Charles Wilson, Vice President Technology, formerly of UC Santa Cruz; and Marty Stanton, President, formerly of Brandeis University) is to be the driver of change as the pharmaceutical industry moves towards mechanism-based analysis of its genomic drug targets. Archemix intends to accomplish this by enabling novel mechanism-based drug screening platforms that extend across all phases of drug discovery.

The company has assembled skilled business and scientific advisory boards, which include industry leaders Jean François Formela (Atlas Ventures); Alex Barkas (Prospect Venture Partners); Michael Pavia (Chief Technical Officer, Millennium Pharmaceuticals) and Nassim Usman (RPI) on the business board. The science board comprises Stephen J. Benkovic (Pennsylvania State University); George Poste (Chief Executive Officer, Health Technology Networks); George M. Whitesides (Harvard University), Peter E. Wright (The Scripps Research Institute) and Lawrence Blatt (RPI). In January 2002, Archemix out-licensed part of its aptamer SELEX technology to Noxxon Pharma for US$5 million. The company is continuing to pursue aggressively additional out-licensing and co-development agreements with other biopharmaceutical partners. In summary, Archemix's technology and business focus is being built from a top-down, quality-driven approach, which will enable the utilization of its unique detection platforms throughout the biopharmaceutical industry.

References

1 Gibbs, J.B. (2000) Mechanism-based target identification and drug discovery in cancer research. *Science* 287, 1969–1973

2 Soukup, G.A. and Breaker, R.R. (1999) Design of allosteric hammerhead ribozymes activated by ligand-induced structure stabilization. *Structure* 7, 783–791

3 Soukup, G.A. and Breaker, R.R. (1999) Engineering precision RNA molecular switches. *Proc. Natl. Acad. Sci. U. S. A.* 96, 3584–3589

4 Koizumi, M. *et al.* (1999) Allosteric selection of ribozymes that respond to the second messengers cGMP and cAMP. *Nat. Struct. Biol.* 6, 1062–1071

5 Robertson, M.P. and Ellington, A.D. (1999) *In vitro* selection of an allosteric ribozyme that transduces analytes to amplicons. *Nat. Biotechnol.* 17, 62–66

6 Hamaguchi, N. *et al.* (2001) Aptamer beacons for the direct detection of proteins. *Anal. Biochem.* 294, 126–131

7 Jenne, A. *et al.* (2001) Rapid identification and characterization of hammerhead-ribozyme inhibitors using fluorescence-based technology. *Nat. Biotechnol.* 19, 56–61

8 Robertson, M.P. and Ellington, A.D. (2001) *In vitro* selection of nucleoprotein enzymes. *Nat. Biotechnol.* 19, 650–655

9 Seetharaman, S. *et al.* (2001) Immobilized RNA switches for the analysis of complex chemical and biological mixtures. *Nat. Biotechnol.* 19, 336–341

10 Hagen, M. and Cech, T.R. (1999) Self-splicing of the tetrahymena intron from mRNA in mammalian cells. *EMBO J.* 18, 6491–6500