Evolva Breeds Small Molecule Drugs Au Naturel

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Drugs were traditionally derived from natural molecules. Over half of the new drugs approved in the past 20 years can trace their structural origins to sources as disparate as poisonous sea snails, soil fungus, and tree bark. In recent years, pharmaceutical companies turned their backs on natural products because of the difficulty of rapidly isolating and synthesizing molecules from natural sources on a large scale. However, a number of drugs and drug intermediates including artemisinin, capsaicin, arachidonate, and amyrin can be made using genetic techniques coupled to chemistry.

According to David Newman, D.Phil., Chief, Natural Products Branch, National Cancer Institute (NCI), the advent of genomic techniques in the late 1970s changed the scale of drug discovery. Pharmaceutical companies invested heavily in combinatorial chemistry libraries and high throughput screening. "Companies were looking for billion dollar drugs," said Newman. "Natural products just didn't hack it at the time due to time constraints." While combinatorial chemistry excels in optimizing structures, only one drug, Nexvar, resulted de novo from this method. New compounds that mimicked natural products were developed through alternate methods, such as diversity oriented synthesis. According to Newman, only one pharma in the US retains a natural products division, although the US arm of a Japanese company uses natural products as structure leads. Smaller biotechs are retrenching on new research once initial compounds enter clinical trials, as costs are substantial and VCs want fast return on investment.

Meanwhile, research is uncovering cryptic clusters of genes and genetic switches that constitute more detailed blueprints for metabolic natural product synthesis. "Over the last five years, it has been obvious that microbes actually have in their genomes incredibly more compounds than have ever been seen," said Newman.

Reality Show in a Dish

A niche player, Swiss company Evolva (http://www.evolva.com) is using an intriguing genetic chemistry strategy to discover novel small molecule drugs as well as to produce difficult-to-synthesize compounds from natural sources. Inside Evolva's main laboratory in Basel, an international team of scientists subjects yeast cells to an evolutionary sweepstakes based on survival. The researchers start with a desired set of targets and use *S. cerevisiae* as a work-horse organism.

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They synthesize artificial chromosomes from interesting genes isolated from a variety of organisms. "Usually we take a biochemical pathway of yeast optimized for a molecule and then cross it with a cDNA library, a collection of expressed coding regions of a genome," said Stan Goldman, Ph.D, Director of Technology at Evolva subsidiary Genetic Chemistry.

Evolva scientists then mix the genes and ligate them, constructing artificial chromosomes from the concatemerized genes. The chromosomes are combined to make new biosynthetic pathways. The scientists insert the artificial chromosomes into the haploid yeast as a first step in making the library and then breed the yeast together to make a complete library. The yeast has to produce or optimize the desired compound to survive. If the yeast cells die, the molecule is booted off the island, so to speak. The winning molecules then go on to the next iteration. Evolva scientists can build a protein-protein assay into the yeast. They can also incubate yeast in the presence of bacteria, mammalian cells, and get assays for secretion and activity in another complex system.

According to Goldman, it is possible to miss certain molecules in classic discovery efforts if they exist in a cell in a concentration undetectable by conventional analytic techniques, have a short lifetime, or only appear at some specific point in an organism's lifecycle. "If you take a set of genes that express a molecule and put in a heterologous host, you can have more control," said Goldman. Since Evolva randomly combines genes, it can get everything the genes produce, not just the end product. Instead of doing the synthesis in a test tube, the yeast cell synthesizes the molecule.

According to Pascal Longchamp, Ph.D, Vice President, Business Development at Evolva, pharma companies are particularly interested in diverse scaffolds. "The Evolva platform mixes genes in novel ways, combining, for example, genes from sponges and the human brain," Longchamp said. Evolva's patent applications cover a veritable Noah's ark of expressible nucleotide sequences ranging from humans to crocodiles and butterflies, with archaeobacteria and coffee thrown in.

Evolva uses concepts equivalent to the directed-evolution technologies used for research. These techniques are also used by a number of companies such as Maxygen (http://www.maxygen.com), which develops protein drugs, and its spinoff Codexis (http://www.codexis.com), which develops biocatalysts to optimize enzymes to produce antibiotics, plastics, detergents, and biofuels. However, Evolva uses varied gene sequences to produce multiple small-molecule backbones and evolve them.

Doing What Others Couldn't

Evolva's commercial compounds are a broad-spectrum antifungal compound that is expected to enter the clinic at the end of the year and an oral thromboxane receptor and thromboxane synthase inhibitor for proteinuric kidney disease and prevention of platelet aggregation (aimed

at the approximately 20% of kidney disease patients who don't respond to Plavix or aspirin), which is now in phase I. Evolva's broad-spectrum antifungal (MIC-$_{90}$ versus Candida ~1ng/ml) has a novel mechanism of action and overcomes resistant fungal strains. The thromboxane compound is potent (IC_{50} ~0.8 nM), fast acting, and reversible, thus improving its safety profile.

Evolva was spun out of US company Phytera in 2004. In addition to Genetic Chemistry in Palo Alto, Evolva also has divisions in India and Denmark. The 70 person company was funded with USD $34 million by seven venture capital companies. The company mainly pays its R&D costs through approximately $80 million in US biodefense contracts.

"The most impressive part was they had developed a new approach to do what other companies had tried to do, which is to use metabolic pathways from various types of cells from invertebrate plants, vertebrates, to develop new compounds based on scaffolds thought to be promising in drug development by using the etymology of these pathways to link together these genes," said scientific advisor Dr. Richard Ulevitch, who was involved in initial diligence for Aravis Ventures, an investor. "They were able to express these genes in yeast. In some cases, the yeast itself would be part of the high throughput screening for the compound because they could also put a biological readout or signal into the yeast. A company like Kosan [Biosciences] tried to do this with polyketides and had some degree of success. It is just another approach, different from combinatorial chemistry that provides you with a high throughput mechanism to screen for leads by using derived structures that could be produced by other methods."

It's Harder to Make a Five-Legged Dog

Willem (Pim) Stemmer, Ph.D, a scientific advisor to Evolva, was the technical founder of Maxygen. Stemmer is now CEO of Amunix (http://www.amunix.com), which makes nonimmunogenic proteins with an improved half-life.

According to Stemmer, many natural sources contain molecules that can't be made via chemical libraries. For example, the NCI found a number of compounds in sponges and other rare organisms that were active against cancer, but it was unable to manufacture them. "Evolva's approach doesn't suffer from that because manufacturability and activity are linked in its process," said Stemmer. "Quality and quantity of the drug coevolve."

According to Stemmer, Kosan Biosciences initially focused on creating a plethora of complex polyketides but in the end had to give up, largely because the host organism, *Streptomyces*, grows slowly and is much more difficult to work with than yeast.

"The closer you stick to natural pathways, the higher the quality of the result," said Stemmer. "The shuffling of existing chemical pathways is an efficient way to recreate the chemical diversity of the past. The chemical diversity that existed over the past billion years can be recreated simply by repeated recombination of the existing pathways." According to Stemmer, it is more efficient to recapture evolutionary history, for example, to modify the size or coat color of a dog, than to do something completely novel, like making a dog with five legs. "The remarkable thing about breeding is that it is very conservative; you can create diversity by juggling millions of mutations, without paying a functional price," observed Stemmer. He cautions that while gene shuffling techniques are "very powerful," they can be dangerous if willfully abused.

In the Meantime, Breeding in the Black

Evolva is working on a USD $28 million DTRA contract with Functional Genetics (http://www.functional-genetics.com), a Maryland-based company that develops broad spectrum antivirals for civilian and biodefense applications. Evolva is developing molecules for an Ebola therapeutic, but the targets may also be relevant for HIV and influenza. This involves an inhibitor for TSG101, a human protein that is common to many viral pathways and ubiquitous in a variety of cells. When a cell is infected with Ebola or HIV, the viruses "hijack" TSG101, reprogramming it to the cell surface where TSG101 plays a critical role in virus budding, allowing the virus to escape from the cell.

"Evolva came to our attention for an interesting capability in gene shuffling," said Michael Goldblatt, CEO of Functional Genetics. "We have, as a result, with Evolva, come up with two strong leads and are developing more." According to Goldblatt, one of the leads gives animals 100% protection from Ebola infection, the other substantially lower. "We are in the process of doing pharmacophores around the chemistry of these molecules," said Goldblatt. "They model well in disrupting protein-protein interactions." According to Goldblatt, Evolva's ability to do gene shuffling and determine in one step in vivo what targets are toxic to cells produced fewer false leads.

Evolva also has two preclinical compounds in a USD $26.8 million DTRA program developing immunomodulators for infectious disease and pandemics. According to Neil Goldsmith, Evolva CEO, one of its preclinical compounds, EV-009-1440, provides 50% protection against lethal bacterial infections (in mice), while another is comparable to Tamiflu in its activity against influenza and potentially synergistic in its effects with Tamiflu.

Evolva subsidiary Genetic Chemistry has a third USD $22.8 million contract from the ARO to develop antibacterials for Gram-negative bacteria. "They have a unique ability to mix genetic pathways across species," said Dr. Jennifer J. Becker, Ph.D., Chemical Sciences Division, US Army Research Office. "It could potentially lead to a wider variety of targets. It could lead to small molecules that may not be possible using traditional synthetic chemistry techniques."

Military scientists say little about ongoing biodefense projects, and so far Evolva is closemouthed about its IP, publishing few details. "It is interesting, and I'm pro anything that can produce novel or previously undetected natural products," said David Newman. But can Evolva convince big pharma to go back to natural products on a large scale? Only if the company can prove it can develop its novel small molecules cost effectively. Newman feels that European and perhaps Japanese big pharmas could be swayed, but probably not pharmas in the current US business climate. "Wyeth is the only big pharma group left," Newman said.

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