Evolution in a test tube: harnessing Darwin's theory to design new molecules.

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Harnessing Darwin's theory to design new molecules

Charles Darwin set off on a voyage in 1831 to study the exotic plants and animals of faraway places. Five years later, he returned with the discovery of a bittersweet truth about life: To leave a lasting legacy on Earth, an individual must wage a brute battle for survival and produce numerous off-spring.

It's a contest driven largely by chance. Some members of a population are gifted with special abilities for resisting predators and attracting mates, for example; others are born with handicaps. Ultimately, the ever-changing environment--climate, food sources, and predators -- calls the shots by defining the winners and shaping, over many generations, the appearance and behavior of a species.

This survival-of-the-fittest scenario takes place even at the level of molecules. On primordial Earth, chemicals with slight individual variations must have replicated themselves and competed with one another, scientists believe. The successful ones gave rise to the complex biological molecules that serve living organisms today.

That's evolution. And ever since Darwin elucidated the idea, scientists have marveled at this process that creates such a variety of animals, cell types, and molecules, each with its own highly specialized talents.

In recent decades, researchers have hoped to harness the power of evolution to make new kinds of drugs and catalysts. Says Gerald F. Joyce, a chemist at Scripps Research Institute in La Jolla, Calif., "People have been saying for a while, 'Wouldn't it be nice to evolve our own molecules, as nature does?'"

That dream is beginning to come true, now that scientists have developed the technological tools for staging molecular evolution in the lab. Already, researchers at about a dozen companies are exploring evolution in a test tube, also known as directed molecular evolution. A fresh approach to drug design using this technique portends a new era in biotechnology, they assert.

Test-tube evolution aims to mimic nature, although the scientist also has a hand in the process. Researchers begin with a single molecule, selected for its potential to do some useful chemical task. They make millions or billions of copies of the molecule, each with a slightly varied structure. Then they launch a talent search by making members of this "population" compete at a task, such as binding to another molecule. They discard those that fail and replicate those that perform well.

By repeating this process again and again, each time selecting the best in that generation, scientists could evolve a molecule exquisitely adapted to do exactly what they want.

The power of this approach to molecular design has researchers quite excited. "The potential in this is what nature has been able to do with evolution over the past 4 billion year," says Joyce, "and that's pretty remarkable."

Chemists see test-tube evolution as a way to accelerate greatly the glacial pace of drug discovery. The process enables them to rapidly test the therapeutic value of trillions of subtly different molecules, most of which don't even exist in nature. With this technique, it takes weeks to find a winning molecule that might take decades for a chemist to synthesize from scratch or millions of years for nature to evolve. With accelerated evolution on their side, drug developers might even stay ahead of microbes that rapidly develop strategies of resistance, Joyce suggests.

In theory, test-tube evolution could enable researchers to mold any kind of molecule to do just about anything. That's the idea that struck Larry Gold and Craig Tuerk of Nexagen, Inc., in Boulder, Colo., during one of those "Aha!" experiences that occurred, naturally, in front of a blackboard.

"There was this magic moment that happens once in a lifetime if you're lucky," recalls Gold. "We saw something remarkable, a wonderful idea."

The two excitedly grappled for a single piece of chalk, he says, as they tried to scribble down their thoughts on the blackboard. What finally emerged was a method for bringing molecular evolution into the lab. They described the protocol, called SELEX, in the Aug. 3, 1990 SCIENCE.

To mimic evolution, chemists must be able to do three things: replicate molecules, introduce random mutations into them, and devise a fitness test for picking out molecules with a certain desirable trait. Researchers have little difficulty with the last step -- testing and selecting molecules. And in the mid-1980s, a new technique called the polymerase chain reaction (PCR) made it possible to generate copies of oligonucleotides, the chains of nucleotides that make up RNA and DNA. introducing individual variations posed the final obstacle: PCR simply copied

molecules too accurately. Gold and Tuerk incorporated randomness in the way that the nucleotide units link together and then combined all of these techniques in SELEX.

"The real power of this is in making vast libraries of molecules," says Gold, whose company is now using test-tube evolution to search for new drugs.

Researchers don't yet have the tools to apply test-tube evolution to molecules of all kinds. So far, only oligonucleotides can be replicated, so the technique remains limited to RNA and DNA. However, scientists are now evolving RNAs and DNAs-the molecules that carry life's genetic blueprint--to take on new roles as drugs.

"The old biotech era used proteins. The new era will use nucleotides, the primary molecules of life, which historically haven't been explored in drug discovery," asserts Michael Riordan, president of Gilead Sciences, Inc., in Foster City, Calif. Drugs made from RNA and DNA may have an advantage over protein-based drugs, he says, because they may be less likely to trigger an immune response.

Scientists at Gilead were among the first to embrace test-tube evolution and run with it. Because DNA naturally sticks to various proteins inside the nucleus of a cell, they reasoned that they could create a new kind of DNA that would bind to other protein targets as well. They decided to try using test-tube evolution to make a DNA that would bind to, and thereby inactivate, a blood-clotting protein called thrombin.

In the Feb. 6, 1992 NATURE they described the results of this experiment, which yielded a potential anticoagulant drug. The researchers are now testing their evolved-DNA product in animals including monkeys. So far, the molecule appears to work as a potent anticoagulant, they report in the June 16 BLOOD.

The Gilead scientists first created a huge molecular library consisting of more than a billion slightly different pieces of DNA. Then they sloshed this population of molecules over a surface studded with billions of thrombin molecules. Applying the principle of survival of the fittest, the researchers saved only the DNAs that stuck to the thrombin; the remaining 99.9 percent were rinsed away. After reproducing the selected molecules into a new generation and repeating the whole process five times, the team had the one DNA molecule in 10 trillion that bound most tightly to thrombin.

Joyce took test-tube evolution a step farther in an experiment he conducted with Amber A. Beaudry, also at Scripps Research Institute. They were interested in changing the catalytic activity of a "ribozyme"-an enzyme made of RNA. In nature, this ribozyme has the ability to cut itself out of a larger RNA molecule. Joyce and Beaudry wanted a ribozyme that could snip DNA -- something it normally couldn't do. Such a ribozyme could have therapeutic value, says Joyce.

For example, it might be used to chop up the unwanted DNA of an invading virus.

To evolve their ribozyme, they began with one that already had a weak ability to snip DNA. They made a trillion variations of it and then exposed this population of molecules to DNA. Ribozymes that could cleave the DNA picked up a chemical tag in the process. The researchers then selected out these marked molecules and replicated them, creating a new population of DNA snippers.

But unlike the Gilead team, Joyce and Beaudry also randomly incorporated mutations into these ribozymes. This makes the strategy a better approximation of what goes on in nature, the Scripps researchers contend. After 10 generations, they had evolved a ribozyme that was 100 times better than the original ribozyme ancestor at cleaving DNA. It also performed at lower, near-physiological temperatures. Joyce and Beaudry described this work in the July 31, 1992 SCIENCE. Now, after 27 generations, they have a ribozyme that works 100,000 times better than its original ancestor, Joyce told SCIENCE NEWS.

Researchers would like to expand the use of test-tube evolution to proteins, but they're just beginning to find ways to do this. In the June 15 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, for example, two scientists describe how they mimicked evolution to create a protein enzyme that can function in an organic solvent. Normally, enzymes -- which evolved along with organisms -- prefer water. Chemists would like to develop enzymes that can function under a wider range of conditions so that they can use them as catalysts in industrial processes.

Working at the California Institute of Technology in Pasadena, Frances H. Arnold and Keqin Chen (who has since moved to MIT) found an interesting way to tackle test-tube evolution of proteins. They replicated DNA with the same technique used by Gold, Riordan, Beaudry, and Joyce, but this time they chose a specific piece that contained the code for making an enzyme called subtilisin. In essence, they were working with variations on a gene. They implanted each gene version into a host bacterium, cultured the critters, and compared the subtilisins excreted by the bacterial colonies.

Arnold and Chen also developed a clever test to select the enzyme they wanted. In nature, subtilisin breaks apart the chains of amino acids that make up certain proteins. To assess the catalytic activity of the various subtilisin versions the two chemists grew bacterial colonies on protein-rich agar plates and looked to see which colonies developed halos around them -- the telltale sign of active protein degradation. Then they transferred these active colonies to plates containing 60 percent dimethylformamide (DMF), an organic solvent. Finally, they chose the colony that formed the largest halo as the evolutionary winner and replicated its gene to create the next generation.

It worked surprisingly well, Arnold says. She and Chen raised the challenge by increasing the concentration of DMF in the second generation and again in the third generation. They ended up with a subtilisin that was 256 times more active in DMF than the original enzyme. And this result emerged not out of a huge library of molecules, but from just 4,000 colonies screened. "That tells you how flexible nature can be," Arnold notes.

Many chemists instead turn to computer models to help them engineer new enzymes and other molecules - an approach called "rational" design. The computer-aided strategy draws upon researchers' detailed knowledge of molecular shapes and chemical mechanisms. It can take years to come up with the desired molecule through such a painstakingly precise process.

Test-tube evolution, on the other hand, is often described as "irrational" because chemists can design a molecule without fully understanding how it works. Indeed, this method requires only a bit of faith that the proverbial needle can be found in the haystack. As Joyce puts it, "There's power in a mess, so long as you can pluck out the best pieces."

Arnold and Chen found it advantageous to use test-tube evolution because they didn't know much about how solvents inactivate subtilisin. Despite this lack of knowledge, test-tube evolution allowed them to create the enzyme they wanted. In just six weeks they had a new subtilisin that, instead of breaking down proteins, can be coaxed to do the reverse: build long chains of amino acids in DMF solvent, Arnold says. And by studying the mutations that led to this winning enzyme, they can now learn more about its molecular structure.

Not all protein enzymes can be forced to evolve new functions by this route, Arnold adds. With subtilisin, the researchers were lucky to observe a stepwise progression of the protein product toward an enzyme that could function in the hostile environment of organic solvents. They could detect improvements after every round of mutations generated in the genetic code. But what would happen if 10 mutations needed to be in place all at once in order to create a measurable effect in the protein? In that case, researchers wouldn't be able to select the best members to make up the next generation. In fact, they would have nothing to guide them toward their goal, says Arnold.

"[Test-tube evolution] is not a panacea for creating wonderful enzymes," she cautions.

Despite her own success, Arnold doubts the current technique will live up to all of its promises. Oligonucleotides, she believes, will prove limited in their ability to evolve into all that researchers desire. That's because this chemical family, like every other, is defined by a common structural motif and a characteristic set of reactive groups, which determine the talents of its members. "Everybody seems to think that if your ocean is big enough, you can find what you want in it," says Arnold. "But what if the chemistry is not right?"

Until chemists develop the tools to replicate other biological molecules besides RNA and DNA, the full power of test-tube evolution may not be realized. Then the possibilities would mushroom, since there appears to be plenty of room for evolutionary improvements in nature's versatile molecules.

"Evolution," says Joyce, "is the neverending story."