EVOLUTIONARY CHEMISTRY

Forcing the Evolution of an RNA Enzyme in the Test Tube

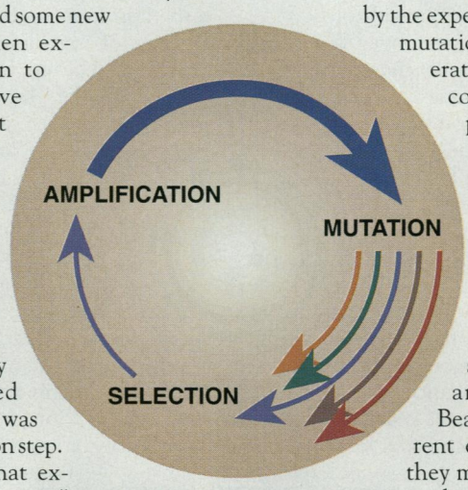
Evolutionary biologists face a fundamental problem: Evolution typically occurs on time scales that dwarf a human lifespan, not to mention the lifespan of a grant. So it's rarely possible to watch evolution as it happens in living organisms. But aided by recent advances in molecular genetics, a handful of research teams are speeding up the clock, using molecules such as RNA and peptides instead of whole organisms to recreate evolution in a test tube.

Now Amber Beaudry and Gerald Joyce of the Scripps Research Institute in La Jolla, California, have brought this in vitro evolution a bit closer to the real thing, by forcing an RNA-cleaving enzyme known as a "ribozyme" to evolve into a DNA-cleaving enzyme in 10 generations in the laboratory (also see page 635). While other researchers have mimicked some aspects of Darwinian evolution, by repeated cycles of reproduction and selection, Beaudry and Joyce's system adds an important new dimension: In each cycle, they introduce new mutations into their evolving population of molecules. Says nucleic acid chemist Leslie Orgel of the Salk Institute, "Jerry's study is the first I know of to combine selection and mutation, which gets much closer to [Darwinian] natural selection than most."

By showing that a ribozyme—an enzyme which is itself made of RNA—can evolve to perform a new chemical activity, the experiment gives comfort to those who believe that early life operated in an "RNA world," where RNA molecules performed all the functions needed for life. And beyond its theoretical implications, the work should be important in the practical world of biotechnology, because this test tube evolution offers another way of coaxing biological molecules to perform new tricks. Indeed, biotech companies are already springing up to exploit the power of evolution in designing new catalysts, enzymes, and drugs. "My guess is this will be the future of biotechnology, because you can adapt substances to any given purpose," says Nobel Prize–winning biochemist Manfred Eigen of the Max Planck Institute for Biophysical Chemistry in Göttingen, himself a leader in this field.

Joyce and Beaudry take as their starting point a well-characterized ribozyme from the single-celled organism *Tetrahymena*. In the natural world, the ribozyme's job is to cut itself out of a larger precursor RNA molecule, leaving behind a mature, ribosomal RNA. Under normal conditions, this molecule doesn't cut DNA at all. But in 1990, in a prelude to the experiments reported in this issue, Joyce and postdoc Debra Robertson identified—through a process of in vitro selection and amplification—a mutant form of the enzyme that can cleave DNA, although only at high temperatures.

To do this, they began with a population of ribozyme molecules into which they had already introduced some new mutations. They then exposed the population to DNA. In the selective step, any mutants that happened to have the ability to cleave DNA were chemically tagged. The tagged ribozymes were copied to cDNAs, which were then transcribed back to RNA. Since many RNAs are produced from one cDNA, this was called the amplification step.



SOURCE: JOYCE, G.F., GENE 82:83-87 (1989)

Following Darwin. Adding a mutation step to the test-tube selection scheme makes it more like natural evolution. (In each cycle several mutants are selected for amplification.)

Joyce described that experiment as "selection," while in the current paper, he and Beaudry use a more ambitious term—"evolution." The difference? In the latest experiments, a round of mutation is included in every cycle of amplification and selection. As in the earlier experiment, tagged ribozymes with the ability to cleave DNA are copied into cDNAs. But now, these cDNAs are themselves both amplified and mutagenized with an error-prone polymerase chain reaction (PCR) that introduces random mutations into some of the molecules. Thus, the population gets an additional shot of variability in each cycle. The result: After 10 molecular generations, the ribozyme's ability to cleave DNA increased by about 100 times, and it worked at physiological temperatures.

This repeated addition of mutation takes Joyce's work a significant step forward, says molecular biologist John Burke of the University of Vermont. "We all develop selection systems, too," he says. "But what Jerry does is select for those molecules that work best in the present pool and mutates them again."

Still, some researchers are less impressed with Joyce's approach, arguing that it is an improvement more in degree than in kind. For example, a group headed by Larry Gold of the University of Colorado has developed another in vitro selection procedure, which identifies RNA molecules that bind to particular proteins. In that method—which is described by its creators as "evolution"—spontaneous mutations arise, although at a relatively low rate, according to codeveloper Craig Tuerk, who now works at a new evolutionary biotech company called Nexagen in Boulder.

But Joyce insists that there is an important theoretical distinction. Because his system is closer to Darwinian evolution, he says, it takes full advantage of evolution's power to optimize "fitness"—which in this case means some useful property defined by the experimenter. Continuous mutation allows an only moderately "fit" molecule to continue to mutate and perhaps find a new, higher peak in what evolutionary biologists call the fitness landscape. "There's no reason to believe that the best answer will be in the first library [of variants]," says Joyce. For example, had he and Beaudry stopped the current experiment too early, they might not have discovered two advantageous mutations that cropped up after the third generation.

This illustrates a key advantage of the evolutionary method: You can get a molecule to do something new without knowing exactly how it's going to do it. That's a marked contrast to rational drug design, which seeks to precision-tune molecules. The evolutionary approach "allows you to be very, very stupid," says evolutionary biochemist Andrew Ellington of Indiana University. "You let the molecule tell you about itself, because it knows more about itself than you do."

Of course, moving from RNA-cleavage to DNA-cleavage is not a giant leap chemically, especially since it was known that some ribozyme mutants had a limited ability to cut DNA. The next goal is to see if Joyce's Darwinian method can come up with a molecule that has a totally new function. For example, Joyce and other scientists, in particular Jack Szostak at Harvard, are hard at work trying to get the *Tetrahymena* ribozyme to evolve the ability to replicate itself. Stay tuned for the latest developments, because if the researchers succeed, they may be the creators of an artificial form of life.

–Elizabeth Culotta