Scientists develop first examples of RNA that replicates itself indefinitely

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Now, a pair of Scripps Research Institute scientists has taken a significant step toward answering that question. The scientists have synthesized for the first time RNA enzymes that can replicate themselves without the help of any proteins or other cellular components, and the process proceeds indefinitely.

The work was published on Thursday, January 8, 2008, in *Science Express*, the advanced, online edition of the journal *Science*.

In the modern world, DNA carries the genetic sequence for advanced organisms, while RNA is dependent on DNA for performing its roles such as building proteins. But one prominent theory about the origins of life, called the RNA World model, postulates that because RNA can function as both a gene and an enzyme, RNA might have come before DNA and protein and acted as the ancestral molecule of life. However, the process of copying a genetic molecule, which is considered a basic qualification for life, appears to be exceedingly complex, involving many proteins and other cellular components.

For years, researchers have wondered whether there might be some simpler way to copy RNA, brought about by the RNA itself. Some tentative steps along this road had previously been taken by the Joyce lab and others, but no one could demonstrate that RNA replication could be self-propagating, that is, result in new copies of RNA that also could copy themselves.

In Vitro Evolution

A few years after Tracey Lincoln arrived at Scripps Research from Jamaica to pursue her Ph.D., she began exploring the RNA-only replication concept along with her advisor, Professor Gerald Joyce, M.D., Ph.D., who is also Dean of the Faculty at Scripps Research. Their work began with a method of forced adaptation known as in vitro evolution. The goal was to take one of the RNA enzymes already developed in the lab that could perform the basic chemistry of replication, and improve it to the point that it could drive efficient, perpetual self-replication.

Lincoln synthesized in the laboratory a large population of variants of the RNA enzyme that would be challenged to do the job, and carried out a test-tube evolution procedure to obtain those variants that were most adept at joining together pieces of RNA.

Ultimately, this process enabled the team to isolate an evolved version of the original enzyme that is a very efficient replicator, something that many research groups, including Joyce's, had struggled for years to obtain. The improved enzyme fulfilled the primary goal of being able to undergo perpetual replication. "It kind of blew me away," says Lincoln.

Immortalizing Molecular Information

The replicating system actually involves two enzymes, each composed of two subunits and each functioning as a catalyst that assembles the other. The replication process is cyclic, in that the first enzyme binds the two subunits that comprise the second enzyme and joins them to make a new copy of the second enzyme; while the second enzyme similarly binds and joins the two subunits that comprise the first enzyme. In this way the two enzymes assemble each other — what is termed cross-replication. To make the process proceed indefinitely requires only a small starting amount of the two enzymes and a steady supply of the subunits.

"This is the only case outside biology where molecular information has been immortalized," says Joyce.

Not content to stop there, the researchers generated a variety of enzyme pairs with similar capabilities. They mixed 12 different cross-replicating pairs, together with all of their constituent subunits, and allowed them to compete in a molecular test of survival of the fittest. Most of the time the replicating enzymes would breed true, but on occasion an enzyme would make a mistake by binding one of the subunits from one of the other replicating enzymes. When such "mutations" occurred, the resulting recombinant enzymes also were capable of sustained replication, with the most fit replicators growing in number to dominate the mixture. "To me that's actually the biggest result," says Joyce.

The research shows that the system can sustain molecular information, a form of heritability, and give rise to variations of itself in a way akin to Darwinian evolution. So, says Lincoln, "What we have is non-living, but we've been able to show that it has some life-like properties, and that was extremely interesting."

Knocking on the Door of Life

The group is pursuing potential applications of their discovery in the field of molecular diagnostics, but that work is tied to a research paper currently in review, so the researchers can't yet discuss it.

But the main value of the work, according to Joyce, is at the basic research level. "What we've found could be relevant to how life begins, at that key moment when Darwinian evolution starts." He is quick to point out that, while the self-replicating RNA enzyme systems share certain characteristics of life, they are not themselves a form of life.

The historical origin of life can never be recreated precisely, so without a reliable time machine, one must instead address the related question of whether life could ever be created in a laboratory. This could, of course, shed light on what the beginning of life might have looked like, at least in outline. "We're not trying to play back the tape," says Lincoln of their work, "but it might tell us how you go about starting the process of understanding the emergence of life in the lab."

Joyce says that only when a system is developed in the lab that has the capability of evolving novel functions on its own can it be properly called life. "We're knocking on that door," he says, "But of course we haven't achieved that."

The subunits in the enzymes the team constructed each contain many nucleotides, so they are relatively complex and not something that would have been found floating in the primordial ooze. But, while the building blocks likely would have been simpler, the work does finally show that a simpler form of RNA-based life is at least possible, which should drive further research to explore the RNA World theory of life's origins.

The paper is titled "Self-sustained Replication of an RNA Enzyme," and the work was supported by NASA and the National Institutes of Health, and the Skaggs Institute for Chemical Biology.

Source: Scripps Research Institute