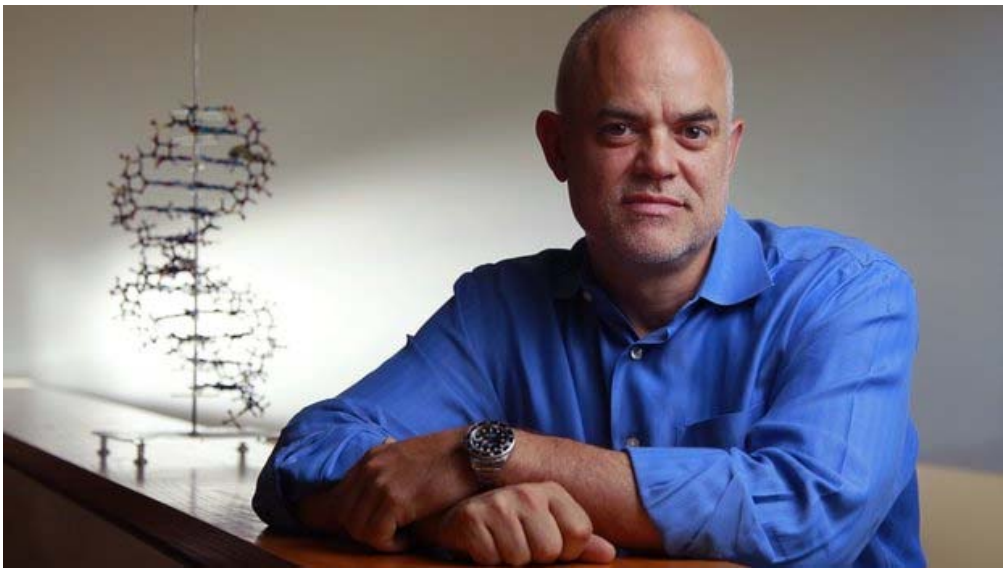
Life engineered with expanded DNA code

By Bradley J. Fikes (/staff/bradley-fikes/) 10 a.m. May 7, 2014 Updated 4:24 p.m.



Floyd Romesberg and colleagues from Scripps Research have engineered a bacterium that uses a six-letter genetic code, including two artificial letters that function alongside the four natural ones. *— K.C. Alfred*

Achieving a first in synthetic life, two artificial letters of the DNA alphabet (http://www.eurekalert.org/emb_releases/2014-05/sri-scf050614.php) have been added to the genetic code of a replicating bacterium, functioning alongside the four natural ones life has used for billions of years.

The feat by a team led by Floyd Romesberg (http://www.scripps.edu/romesberg/) and Denis Malyshev (http://www.researchgate.net/profile/Denis_Malyshev2) of The Scripps Research Institute greatly increases DNA's information storage capacity. This enables production of a vastly expanded number of proteins inside living cells, potentially useful for drugs, vaccines and even nanomaterials, they said.

The study (http://www.nature.com/nature/journal/vaop/ncurrent/full/nature13314.html), "A semi-synthetic organism with an expanded genetic alphabet," was published in the journal Nature (http://www.nature.com/nature/journal/vaop/ncurrent/full/nature13314.html). Romesberg was the senior author, Malyshev was the first author.

A new San Diego biotech company, Synthorx (http://www.synthorx.com/), has licensed the technology from TSRI to exploit those possibilities. Avalon Ventures and Correlation Ventures have invested an undisclosed amount into the company.

The two unnatural letters, d5SICSTP and dNaMTP, or X and Y for short, pair with each other along the DNA's double helix. In addition to the two natural pairs -- adenine with thymine and cytosine with guanine -- X and Y form a third base pair (http://ghr.nlm.nih.gov/glossary=basepair).

"We're not messing up what it normally does," Romesberg said. "It really is a third base pair. If nature had a third letter, it would integrate seamlessly with the other two. And that was our goal."

And to surmount a final challenge nobody has yet solved, engineering the cell to make the unnatural base pair, the team devised a clever workaround. The trick incidentally prevents the engineered bacterium from growing in nature. That safety feature should reassure fears that the bacterium might escape and cause havoc, Romesberg said.

When DNA replicates, the double helix unzips, leaving two single helixes. Enzymes called DNA polymerases add the complementary letters to the helixes, assembling two double helix strands. The enzymes match A with T, C with G. And, in the partly synthetic E. coli Romesberg's team engineered, X pairs with Y.

Romesberg and colleagues have shown in the lab that these unnatural letters integrate well with the natural letters. But getting them to work in a living creature had never been done before. Extensive tinkering with the bacterium was needed to make it work. And to get over a final hurdle, the team employed a clever trick.

The scientists inscribed the unnatural letters into plasmids (http://askabiologist.asu.edu/plasmids) and introduced into the bacterium. The plasmids -- and the unnatural DNA inside them -- faithfully replicated along with the natural ones as the bacterium grows. The

natural genetic code builds proteins with just 20 amino acids; the expanded code can use up to 172 amino acids, Romesberg said.

Adding new letters to DNA in a functioning organism was widely considered impossible, making research grants very hard to get, said fellow synthetic biology researcher Steven A. Benner (http://www.ffame.org/sbenner.php), not involved in the study.

Federal agencies that had refused to fund the research may now reconsider, said Benner, of the Foundation for Applied Molecular Evolution in Gainesville, Fla

"That's why Floyd's paper is so friggin' important," Benner said. "What you think is impossible or possible depends on how you were trained. It's a cultural statement. Floyd is not violating any of the laws of physics. He's not saying that he's found a neutrino that travels faster than the speed of light (http://news.sciencemag.org/2012/02/breaking-news-error-undoes-faster-light-neutrino-results)."

There's still a long way to go before the semi-synthetic cells make proteins. Only one base pair was introduced per bacterium, providing proof of concept, but not producing anything commercially useful. Synthorx is already at work on this task.

Ethics considered

Concerns that the feat amounts to playing with God is a "nonissue", because the scientists did their work carefully, said bioethics expert Art Caplan.

"It's not relevant here, despite the drama and the promise," said Caplan, a professor of bioethics at NYU Langone Medical Center. "No one is playing God. What we're seeing is very careful and responsible, cautious research. The design of the organism is being handled very carefully. We may get to a time when we have to discuss what kind of organisms do we want to make, but we're not there yet. This is a tool. I'm not worried about alien life-forms taking over downtown Philadelphia or something."

However, whether patents should be allowed for this technology is more questionable, Caplan said, because the bacterium as of yet can't do anything useful.

"This seems more like creating a great tool, and we'll have to see where it goes," he said.

Assuming safety, there's no inherent ethical problem in expanding the genetic code in bacteria, said Michael Kalichman, director of the Center for Ethics in Science and Technology at UC San Diego.

"I think we might have more difficult questions down the line if the researchers were able to produce animals with the additional base pairs," Kalichman said. "At the moment, that seems difficult and out of reach, but much of what is being done in science today seemed out of reach just a few short years ago."

Engineering an animal capable of suffering would pose a moral concern, Kalichman said.

Robert Bohrer, a biotech attorney whose field includes bioethics, also said he didn't see any ethical concerns with this research.

"I think it is very nifty biochemistry that is a very long way from having any practical application, although the eventual practical applications in terms of novel proteins made with novel amino acids could be quite useful," said Bohrer, a professor of law at Cal Western School of Law in San Diego. "I realize that an immediate response to these kinds of developments from some quarters is to decry the research as "playing God" but I think that is an empty charge. 'playing God' is a term that could be applied to virtually any major technological achievements of humans- from the development of cardiac pacemakers to damming streams for hydroelectric power."

"From a bioethics perspective, it is far more useful to ask more focused questions: does it violate any notion of respect for the autonomy of persons?," Bohrer said by email. "Does it do more good than harm? This is the utilitarian perspective - it certainly has the potential to do good, with apparently no harm at all at this point."

"The other issue that must always be addressed for any research of this type is one of laboratory safety and risks to persons in the lab and outside the lab," Bohrer said. "As described, this is very low risk."

Synthetic genomics advances

Genomics pioneer J. Craig Venter led the first successful (https://www.sciencemag.org/content/329/5987/52.abstract) effort to create a self-replicating microbe with entirely synthesized DNA, in 2010. Venter's team even included "watermark" messages in the DNA to prove it was their handiwork. Their feat required extraordinary precision, because the DNA had to be synthesized with virtually 100 percent accuracy. (This week, Venter's Synthetic Genomics in La Jolla announced it would use its precision tools to engineer pig DNA so the animals could provide immune-compatible organs for human transplantation (http://www.utsandiego.com/news/2014/may/07/romesberg-dna-scripps-d5SICSTP/)).

But while the microbe's DNA was chemically synthesized in a lab, the letters themselves were the same four that naturally occur.

Adding new letters to the DNA alphabet presents additional challenges. The organism's metabolism must be modified to make the

unnatural letters. They must not only fit inside the double helix, they must be recognized by the cell's replication machinery and accurately copied. And these unnatural components must not interfere with the myriad of chemical pathways that exist inside even the simplest cell.

Benner, Romesberg and other synthetic life researchers such as Philippe Marlière in France and Ichiro Hirao in Japan have been leap-frogging each other for years in the field of unnatural base pairs. In 2011, Marlière co-led an international team that generated a strain of E. coli that replaced thymine (http://www.sciencedaily.com/releases/2011/06/110628132438.htm) with the synthetic base 5-chlorouracil (c).

In 2012, Romesberg and Malyshev led a crucial study (http://www.utsandiego.com/news/2012/Jul/02/unnatural-dna-letters-demonstrate-natural/) that demonstrated their unnatural base pair can function like the natural ones, in the lab.

In 2013, Benner led a study (http://www.pnas.org/content/early/2013/12/26/1311778111.abstract) demonstrating that his group's expanded DNA system, again not in cells, could generate a molecule that binds to breast cancer cells. That molecule is being developed as a drug, Benner said. His system uses up to 12 letters, or six base pairs.

Romesberg gives precedence to Benner.

"Steve's really the founder of the field," Romesberg said. "He published his first papers on unnatural base pairs when I was a first-year graduate student."

Benner is also developing a microbe with an expanded DNA alphabet that uses two unnatural letters, which he calls P and Z. His goal is to get the cells to assemble the letters from simpler precursor molecules supplied to them, an extremely complicated task.

To make each unnatural letter, three specific enzymes must be engineered into the cell. Each enzyme adds a phosphate, a molecule containing phosphorus, to a precursor to form the final precursor to the unnatural letter, which is a trisphosphate molecule. That precursor loses two phosphates as it is attached to the replicating DNA molecue, providing energy for the replication.

Benner has successfully engineered P into the triphosphate precursor but not Z. This is the same formidable challenge Romesberg faced with his unnatural molecules, which are also triphosphates.

"Floyd went around the problem," Benner said.

Finessing the problem

Romesberg's team supplied the E. coli with the completely assembled unnatural letters, and the cell's machinery used them to replicate the unnatural DNA. If the bacterium doesn't get the unnatural letters, it reverts to a wholly natural DNA chemistry since it cannot synthesize them, Romesberg said. This also provides safety in case the bacterium were to escape from the lab.

"I think it's interesting chemistry," Venter said of the approach, also mentioning safety as an important feature. "It's certainly interesting work. We're waiting to see if there's some applications that can help with synthetic genomics. That's one approach that we think might be useful."

Romesberg's approach had its own challenges. The Xs and Ys couldn't get through the cell wall. Romesberg's team got past that obstacle by adding DNA from an algae that codes for a trisphosphate "transporter" protein that ferried the unnatural letters into the cell. They got the gene from Ilka Haferkamp (http://www.bio.uni-kl.de/mitarbeiter/bio-g-i/bio-haferkamp-ilka/), a German researcher who specializes in membrane transport proteins.

But once inside the cell, the unnatural letters were being digested for their phosphate content. The scientists stopped that with some more engineering, and by supplying plenty of phosphate in the form of potassium phosphate.

The final test was whether the cells' DNA polymerases, the enzymes that build copies of DNA from the original one, would recognize the unnatural letters and put them into place. They did. Romesberg had earlier shown that the copying mechanism worked with unnatural letters outside the cell. But confirming it would work inside the cell, and that the cell would replicate, was the final challenge.

"That's the key question," Romesberg said. "If you would have had had a poorly replicated base pair, you could imagine polymerases stalling, having to sit there and wait to divide -- none of that. Once they have all the equipment they need, they appear to replicate DNA and grow without any noticeable effects."

Importantly, the cellular DNA "proofreading" mechanism didn't treat the unnatural letters as errors to be fixed, Romesberg said. It left them alone. Lack of proofreading somewhat impairs the accuracy of replication, but not enough to be a major problem, he said.

The main drawback to the Romesberg team's approach is that these triphosphate molecules are unstable and expensive, Benner said. This limits the commercial usefulness of the approach.

Romesberg said some of the problems discussed in the Nature paper, including some toxicity from the transporter protein, have been much reduced. And less of the protein is needed than first thought.

"So we're producing less of a less toxic protein," Romesberg said.

Despite its limitations, Benner said he was impressed by the cleverness of the Romesberg team's approach.

"He had an idea that I wish that I had had," Benner said.

Like Romesberg, Benner had shown the viability of using his own unnatural DNA letters for such purposes as making the drug that binds to breast cancer cells. That didn't convince skeptics that his proposal to engineer these letters into the DNA of a functioning cell was feasible.

"Of course, the complaint is, well Steve, that's because you control everything outside of the cell," Benner said. "Floyd's paper is extremely important because, in the first place, because he is a bold and daring scientist, who figured out how to get around a difficult problem, this metabolism problem, and to demonstrate without solving the metabolism problem that at least the polymerases will work. And that's why this paper very much deserves to be in Nature."

Other contributors to the paper were Kirandeep Dhami, Thomas Lavergne and Tingjian Chen of TSRI, and Nan Dai, Jeremy M. Foster and Ivan R. Corrêa Jr. of New England Biolabs, Inc.

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