Evolution not revolution

By Lev Osherovich, Senior Writer

An international team has engineered enzymes capable of replicating nucleic acid polymers that are made of non-natural nucleotides.¹ Despite general media reports that the findings are a breakthrough on the way to artificial life, the practical utility of the technology is in generating new types of aptamers.

Aptamers are small nucleic acid oligomers selected for high-affinity binding to specific protein or nucleic acid targets. To find an aptamer that hits a given target, researchers perform a recursive *in vitro* selection procedure called systematic evolution of ligands by exponential enrichment (SELEX). In SELEX, aptamers that bind their targets are purified and replicated by polymerases, and then the cycle is repeated. After many such cycles, only the highest-affinity aptamers remain.

Although SELEX has been very good at generating potent aptamers, the molecules are seldom stable *in vivo* because they are recognized by host antiviral defenses and are rapidly degraded.

To improve the biological stability of aptamers, researchers have previously made aptamers with non-natural sugar backbones that are resistant to degradation. However, the polymerases used in the SELEX procedure do not readily recognize these non-natural nucleotides. As a result, manipulating and refining non-natural aptamers has been difficult.

Now, a team led by Philipp Holliger, group leader in the Laboratory for Molecular Biology at the **Medical Research Council (MRC)**, has bridged the gap between SELEX and degradation-proof aptamers. The group engineered a set of DNA polymerases and reverse transcriptases that can copy short oligonucleotides made from non-natural nucleotides.

Because aptamers made with these new polymerases are non-natural and thus less likely to be recognized by the body's host defenses, they could have high *in vivo* stability.

"Natural nucleic acids don't have much stability, so you can find aptamers with some utility *in vitro* but you can rarely use them for medicine," said Vitor Pinheiro, investigator scientist at MRC and the lead author of a report in *Science* describing the new enzymes. "You can now synthesize and replicate synthetic nucleic acids" that might be more suitable as therapeutics.

Further refinement of the enzymes also could yield high-potency

aptamers with new structural features resulting from their non-natural sugar backbones.

Dispensing with DNA

The MRC team began by conducting an *in vitro* evolution procedure to identify mutant DNA polymerases capable of copying DNA into an RNA-like polymer containing either of two non-natural nucleotides. The team performed a similar procedure to obtain a mutant reverse transcriptase capable of copying this non-natural RNA-like molecule back into DNA.

The researchers used these two mutant enzymes as starting points for further *in vitro* evolution experiments, yielding a toolkit of engineered polymerases and reverse transcriptases that could copy and reverse transcribe aptamers made from any of six non-natural nucleotides.

As a proof of principle, the group conducted a SELEX-like procedure with the new enzymes to identify non-natural nucleic acid aptamers that bound to the HIV transactivation response (TAR) element and to hen egg lysozyme, a model protein.

Pinheiro said other groups have made aptamers with non-natural nucleotides,² but his team's engineered enzymes improve on those methods by eliminating the need for a DNA template.

"There have been previous synthetic aptamer systems where it was possible to copy genetic information, but this required the continuing attachment of the DNA," said Pinheiro.

Getting rid of the DNA template streamlines the aptamer selection procedure and makes it possible to identify conformationally new aptamers that are not confined by the helical structure of DNA, he noted.

The MRC team's findings are patented and available for licensing.

Toward better aptamers

The popular press characterized the *Science* report as the creation of artificial life, but in fact the findings are the latest incremental advance in a long line of research on non-natural nucleic acid polymers.

Although the technology could in principle be used to create artificial genes, the utility of non-natural genetic polymers is hobbled by the poor efficiency of the engineered polymerases and the lack of compatible cellular machinery to support survival of the artificial genes outside of a test tube.

Instead, Pinheiro and companies in the nucleic acid space agree that the technology is best suited for making new types of aptamers.

Arthur Levin, EVP of R&D at **miRagen Therapeutics Inc.**, said Pinheiro and Holliger's enzymes "have obvious utility for SELEX-derived therapeutics. Using these modified nucleotides could greatly expand what kinds of molecules one could pull out" of aptamer screens.

miRagen is developing locked nucleic acid (LNA) antisense therapeutics against microRNAs involved in cardiovascular disease.

Levin cautioned that the mutations that allow the MRC team's engineered polymerases to use non-natural nucleotides appear to cripple

"Natural nucleic acids don't have much stability, so you can find aptamers with some utility *in vitro* but you can rarely use them for medicine."

**— Vitor Pinheiro,
Medical Research Council**

the enzymes, leading to a high error rate and to premature termination of replication. As a result, aptamers made by the new enzymes tend to be short and heterogeneous, potentially limiting their therapeutic utility.

Pinheiro agreed that using the enzymes for therapeutic aptamer discovery will require improving their accuracy and staying power.

Larry Gold, chairman, CEO and founder of aptamer diagnostics company **SomaLogic Inc.**, said the potency of the non-natural aptamers needs to be improved.

“They showed that they can do SELEX with non-natural molecules with not very good affinity” for their targets, said Gold. “They are a long way away from good aptamers.”

Gold co-invented SELEX in 1990 (ref. 3) and founded NeXagen Inc. in 1991 to commercialize the procedure. In 1995, NeXagen and Vestar Inc. merged to become NeXstar Inc., which **Gilead Sciences Inc.** acquired in 1999. Gilead exclusively licensed SELEX to SomaLogic for diagnostic use in 1999 and to **Archemix Corp.** for therapeutic use in 2001.

Archemix is being liquidated.

Gold thinks a better way to create new aptamers is to chemically modify the bases of nucleotides rather than the sugars. He cited a 2010 study from his own team at SomaLogic that used aptamers with normal sugars but modified pyrimidine bases to find protein biomarkers of chronic kidney disease.⁴

“Futzing with pyrimidines is naturally acceptable to many polymerases, more so than adapting them to modified sugars,” said Gold.

Pinheiro thinks the biggest draw of non-natural aptamers will likely be increased bioavailability compared with conventional aptamers. He noted that several of the non-natural nucleotides used by his team “have been reported to be quite stable *in vivo*.”

Testing non-natural aptamers in cell culture and animal models of disease is thus a logical next step. Indeed, Pinheiro plans to screen for non-natural aptamers to treat hematological malignancies.

Osherovich, L. *SciBX* 5(18); doi:10.1038/scibx.2012.458

Published online May 3, 2012

REFERENCES

1. Pinheiro, V.B. *et al. Science*; published online April 20, 2012; doi:10.1126/science.1217622
Contact: Philipp Holliger, Medical Research Council, Cambridge, U.K.
e-mail: ph1@mrc-lmb.cam.ac.uk
2. Yu, H. *et al. Nat. Chem. Biol.* **4**, 183–187 (2012)
3. Tuerk, C. & Gold, L. *Science* **249**, 505–510 (1990)
4. Gold, L. *et al. PLoS ONE* **5**, e15004; published online Dec. 7, 2010; doi:10.1371/journal.pone.0015004

COMPANIES AND INSTITUTIONS MENTIONED

Archemix Corp., Cambridge, Mass.
Gilead Sciences Inc. (NASDAQ:GILD), Foster City, Calif.
Medical Research Council, Cambridge, U.K.
miRagen Therapeutics Inc., Boulder, Colo.
SomaLogic Inc., Boulder, Colo.