Enzymes allow DNA to swap information with exotic molecules

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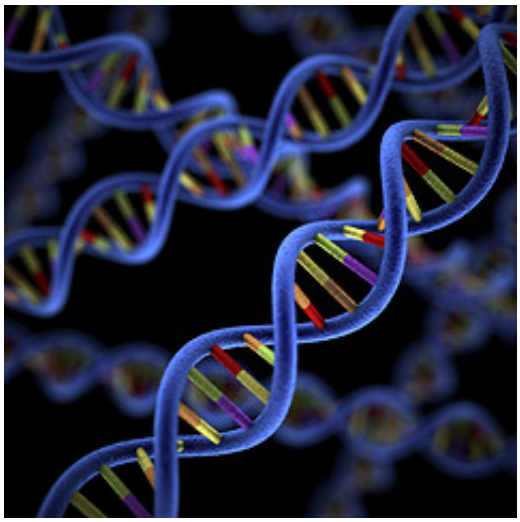

(Phys.org) —The discovery of the Rosetta Stone resolved a longstanding puzzle, permitting the translation of Egyptian hieroglyphs into Ancient Greek. John Chaput, a researcher at Arizona State University's Biodesign Institute has been hunting for a biological Rosetta Stone—an enzyme allowing DNA's 4-letter language to be written into a simpler (and potentially more ancient) molecule that may have existed as a genetic pathway to DNA and RNA in the prebiotic world.

Research results, which recently appeared in the *Journal of the American Chemical Society*, demonstrate that DNA sequences can be transcribed into a molecule known as TNA and reverse transcribed back into DNA, with the aid of commercially available enzymes.

The significance of the research is three-fold:

- It offers tantalizing clues about how DNA and RNA—which encode the building plans for all earthly life—may have arisen from more primitive information-carrying molecules
- Contributes to the field of exobiology—the search for alternative life forms elsewhere in the universe
- Points to possible applications for TNA and other unusual nucleic acid molecules (known as xenonucleic acids or XNAs) in molecular medicine.

In the case of biomedical applications, XNAs may be developed into aptamers—molecular structures that can mimic the properties of naturally occurring polymers, folding into a variety of 3-dimensional forms and binding with selected targets. Aptamers are useful for a range of clinical applications including the development of macromolecular drugs.

"TNA is resistant to nuclease degradation, making it an ideal molecule for many therapeutic and diagnostic applications," Chaput says.

The structural plans for organisms ranging from bacteria to primates (including humans) are encrypted in DNA using an alphabetic code consisting of just A, C, T & G, which represent the 4 nucleic acids. In addition to their information-carrying role, DNA and RNA possess two defining properties: heredity, (which allows them to propagate their genetic sequences to subsequent generations) and evolution, (which allows successive sequences to be modified over time and to respond to selective pressure).

The chemical complexity of DNA has convinced most biologists that it almost certainly did not arise spontaneously from the prebiotic soup existing early in earth's history. According to one hypothesis, the simpler RNA molecule may at one time have held dominion as the sole transmitter of the genetic code. RNA is also capable of acting as an enzyme and may have catalyzed important

chemical reactions leading eventually to the first cellular life.

But RNA is still a complex molecule and the search for a simpler precursor that may have acted as a stepping-stone to the RNA, DNA and protein system that exists today has been intense.

A variety of xenonucleic acids are being explored as candidates for the role of transitional molecule. In the current study, threose nucleic acid or TNA is investigated. Chaput says that establishing TNA as a progenitor of RNA would require demonstrating that TNA can perform functions that would help support a pre-RNA world. Of particular importance, would have been the ability replicate itself in the absence of protein enzymes.

Like DNA, TNA can form double-helices—spiral staircase structures consisting of the 4 nucleotide bases, which make up the ladder-like rungs, and a sugar and phosphorus backbone, which forms the ladder's railing. The sugar portion of this backbone is a defining component of the nucleic acid. DNA uses deoxyribose, RNA uses ribose and TNA uses threose.

Both DNA and RNA have sugars containing five carbon atoms, but TNA's threose sugar contains only four. This enables TNA to assemble from just two identical carbon units, making it far easier to form under the non-biological conditions than RNA or DNA.

Despite TNA's chemical distinctiveness, it is similar enough to DNA and RNA to be able to interact with these familiar molecules and exchange information. In addition to forming helices, TNA segments can bind with complementary DNA and RNA strands through Watson-Crick base pairing, thus making TNA an alternate self-replicating entity. The study of TNA and other artificially-produced genetic polymers is part of a rapidly emerging discipline known as synthetic genetics.

Powerful tools allow for high-throughput production of molecules with specified traits, built from xenonucleic acid molecules like TNA. In the current study, Chaput and his research team demonstrate that certain commercially available enzymes can facilitate the transcription of DNA sequences into TNA and back again into DNA and that the TNA sequences can be induced to evolve under the influence of environmental cues. The process is known as in vitro selection.

To accomplish this, large pools of TNA molecules are produced from DNA templates and then exposed to a particular molecular target. The small fraction of the random-sequence TNA strands structurally capable of binding with the target are extracted and reverse-transcribed back into DNA, then amplified using polymerase chain reaction (PCR).

The process can be repeated, allowing for significant enrichment of the desired aptamer. Indeed, the authors note that a single round of selection in their experiment produced a 380-fold enrichment from an original library of 1014 DNA templates. The authors note that the method is therefore capable of pinpointing and enriching a particular aptamer of predefined function from a staggering 1015 non-functional sequences. (One potential benefit of constructing aptamers from TNA is improved stability—natural enzymes that rapidly break down DNA and RNA do not degrade them.)

Prior to the current study, researchers had been frustrated that only severely abbreviated lengths of DNA could be faithfully transcribed into TNA. The limiting factor in the process was an effective enzyme to guide the accurate transcription of the DNA message. In the case of normal biological transcription, DNA is transcribed into RNA with the help of a specific enzyme known as DNA polymerase. Such naturally occurring polymerases, Chaput points out, are highly specific, and don't work well for DNA to TNA transcription or reverse transcription.

Recent advances in protein engineering however, have produced a new breed of synthetic polymerases. In the current study, one of these—known as Therminator DNA polymerase, faithfully transcribed a 70 nucleotide DNA sequence into TNA, while another, known as SuperScript II (SSII) performed reverse-transcription back into DNA with impressively high fidelity. Sequences of both 3-letter and 4-letter DNA

messages were transcribed and reverse transcribed, both with over 90 percent accuracy.

The research paves the way for more sophisticated manipulation of TNA and other xenonucleic acids and may strengthen the case that TNA or a closely related molecule set the stage for the emergence of RNA and the first earthly life.

Given the enormous potential for this research for the fields of synthetic biology, exobiology and medicine, it is likely that XNAs will be produced in greater abundance by large chemical laboratories. Currently, the synthesis and purification of nucleoside triphosphates needed to form XNA backbones remains a delicate and labor-intensive process.

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