In Search for Alternative Genetic Material

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Recent results suggest that modifications to natural nucleic acid bases, as well as the backbone, could be accommodated in natural systems. Philipp Holliger and Steven Benner, two scientists studying modified nucleic acids systems, describe their recent work and its potential implications for astrobiology.

Recently, a team led by Philipp Holliger of the UK Medical Research Council's Laboratory of Molecular Biology discovered a number of enzymes that operate using backbones that differ from the standard DNA and RNA backbones. Here, they discuss their potential relevance to astrobiological systems.

The search for extraterrestrial life (and indeed the search for its origin on Earth) is framed by the biology we know. Since all life on Earth depends on DNA and RNA, these two nucleic acids would seem to be tell tale molecules in the search for life. But are they the only molecular solutions for heredity?

Indeed, a good argument can be made in favour of their uniqueness. DNA and RNA display a range of physical and chemical properties that render them exceptionally well suited to store genetic information. While alternative nucleic acid chemistries and the design of synthetic nucleic acid analogues (termed *xeno*-nucleic acids (XNAs), Figure 1) have been methodically explored, it had remained unclear how many (if any) XNAs would have the potential to serve as genetic materials.

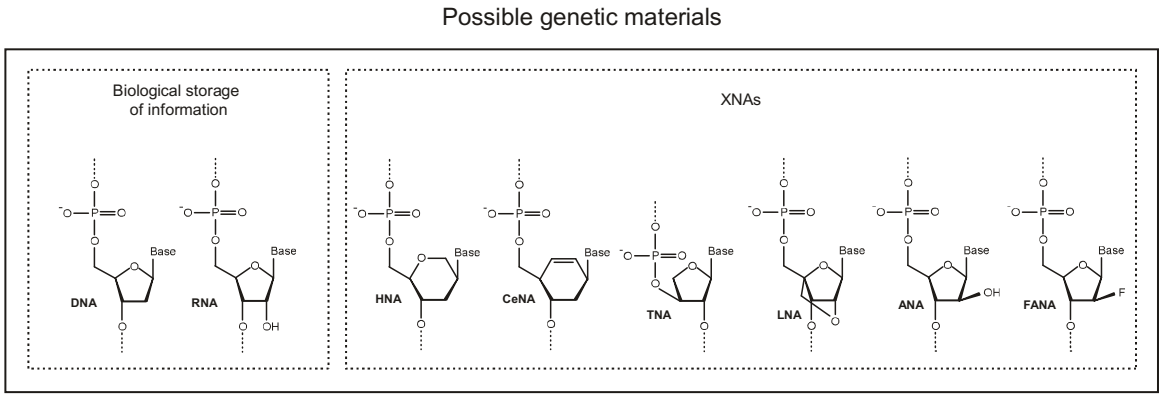

Figure 1. Structures of canonical nucleic acids, DNA and RNA (left) and XNAs (right) with modified backbone structures.

A key hurdle to the exploration of the genetic potential of such XNAs is the means of replication – DNA and RNA rely heavily on enzymes, called polymerases, for their replication. Natural polymerases are exquisitely specific for their cognate nucleic acid (DNA or RNA) and respective building blocks and generally cannot synthesise or replicate XNAs.

Harnessing the power of directed evolution and protein design, we discovered polymerases that could synthesise (DNA → XNA) six different XNAs and others that could reverse transcribe (XNA → DNA) them back into DNA. We showed that genetic information could be propagated in a cycle akin to retroviral replication by transferring it from DNA to any of those XNAs, as well as recovering the information from any of those XNAs back into DNA. The accuracy of the information transfer varied between the different nucleic acids, due to both nucleic acid and polymerase contributions, but the process was good enough for meaningful information to be stored in all XNAs.

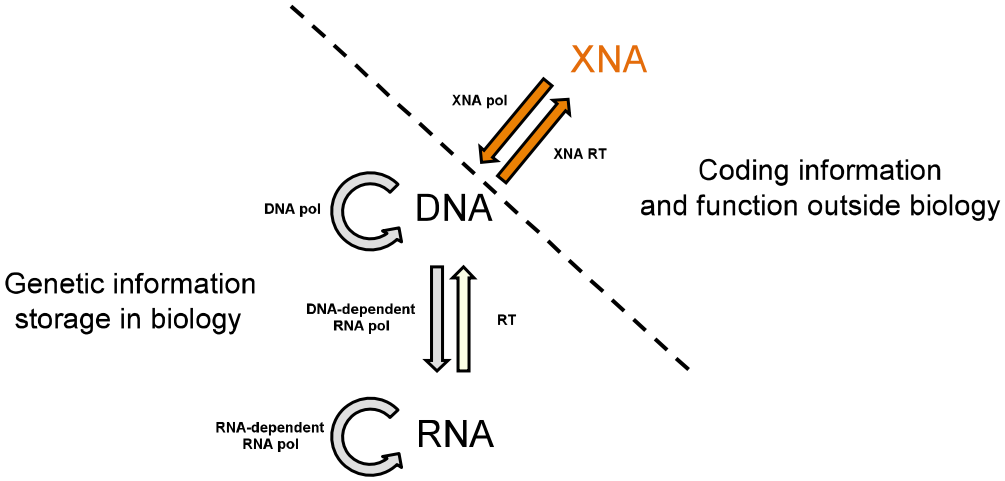

Figure 2. Schematic of information storage inside biological systems using DNA and RNA (below, black and white) and outside existing biological systems using XNA (above, orange)

For HNA, one of the XNAs investigated in which the canonical ribofuranose sugar ring of DNA and RNA is replaced by a six-membered anhydrohexitol ring, we showed that this genetic material and associated polymerases form a genetic system robust enough that it can be used to select functional HNA molecules. We isolated HNA molecules of precise sequences that can bind model targets tightly and specifically.

By developing new genetic materials and demonstrating their functionality, we have shown that there is no overwhelming functional imperative limiting genetic information storage to DNA and RNA – at least at this basic level, all six XNAs (and presumably a wide range of others) can serve as genetic materials. This means that although DNA and RNA are the genetic molecules for life on Earth, they may not be the first or indeed the only molecular solutions for genetic information storage in the cosmos (Figure 2).

Depending on the prevalent conditions and available chemistries, abiogenesis may take different routes on different planets. As we search for non-terran life, we should incorporate our expanding knowledge on the possible chemistry of genetic materials, considering any polymers capable of information storage and replication as potentially viable genetic systems.

However, unless we discover extraterrestrial life within our own solar system, it is unlikely that we will be ever able to scrutinize the molecular makeup of its genetic system. Nevertheless, synthetic biology can provide a way to explore the parameters of the chemical space compatible with genetic information storage, replication and evolution. Such

“synthetic genetics” cannot tell us what other life forms there are but should help us define what they could be.

Steven Benner has prepared numerous DNA and RNA derivatives with modified backbones, as well as nucleobases. Here, he describes the role of noncanonical biopolymers in potential astrobiological systems, as well as their utility in modern biology and medicine.

The search for non-terran life, models for the origins of terran life, and even our concept about what life fundamentally is, are all constrained by the view that a linear polymer is needed to support the heredity presumed to be essential for Darwinian evolution, Darwinian evolution is, in turn, thought to be the only mechanism by which the natural tendency of organic matter to devolve to "tar" might be constrained to generate molecular systems that have attributes that we value in life. Accordingly, we can ask: What kinds of other polymers, other than standard RNA and DNA (collectively xNA), can support heredity?

Absent an actual encounter with a non-terran life form, the most useful approaches to answering such questions come from a chemistry laboratory. There, "synthetic biologist" might make and study alternative biopolymers, to see which support molecular recognition essential to heredity, and which do not.

However, even with the best synthetic chemistry, this research must choose a manageable number of alternative polymers to examine; their numbers are far too large for any comprehensive examination. Here, a research paradigm might start with the standard xNA that we know, and then take small structural steps away. The bigger is the step perhaps the more likely is the failure. Or perhaps not. Either way, the "universe" of possible genetic biopolymers will be adumbrated.

This heuristic has been followed now for a quarter century, and we have learned much. For example, early efforts to take small steps away from the natural backbone structure provided success and failure, both instructive. Eckstein, for example, showed that replacing the phosphate units by thiophosphates did not destroy molecular recognition needed for heredity. Replacing the standard phosphates by dithiophosphates, however, moved into a structure space that displays molecular pathologies not auspicious for genetics.

Likewise, removing the repeating charge on the xNA backbone created problems. The most successful example, from Nielsen, Egholm, and their coworkers, was the uncharged "peptide nucleic acid" backbone (PNA). PNAs maintained rule-based molecular recognition as long as they remained soluble, short (generally less than 20 nucleobases), and G-poor. Molecular recognition in other analogs lacking a negative charge, such as our own dimethylene sulfones, was preserved only so long as the strands were shorter [1].

These efforts show the importance of the repeating charge in a Darwinian biopolymer. It ensures water solubility, for sure. But it also constrains folding, something that templating molecules should not do. In xNA, the repeating backbone charge also directs strand-strand interactions to the Watson-Crick (not Hoogsteen) edges of the nucleobases, a requirement for the rules "A pairs with T and G pairs with C". Most importantly, the repeating charge so

dominates the properties of the biopolymer that it can replace nucleobases without dramatically changing its biophysics. This is an essential feature for a mutable evolving biopolymer, and a feature rare in any other chemical system. This has led to a component of a universal "theory of the gene": In water, a genetic molecule must have a repeating charge (positive or negative) [2].

Other studies with different sugars, in our group [2] as well as in the groups of Eschenmoser, Herdewijn, Switzer, and Holliger (most recently, which Chaput, Herdewign, and other collaborators), have adumbrated the limits of alternative sugars. Here, many groups have accepted the additional challenge of recruiting terran enzymes to copy their sugar-modified analogs. While not directly relevant to astrobiology (an alien using an alien genetic biopolymer would have evolved alien enzymes adapted to copy it), recruited terran enzymes are certainly important for any "here-and-now" utility of an alternative genetic system.

Perhaps the best example of "here-and-now" utility has come from efforts to expand the number of nucleobases, "letters" in the genetic "alphabet". Two rules of complementarity stand behind Watson-Crick pairing: size complementarity (large purines pair with small pyrimidines) and hydrogen bonding complementarity (hydrogen bond donors pair with hydrogen bond acceptors). We have shown that by shuffling hydrogen bonding units within the overall Watson-Crick geometry, the number of nucleobases can be increased from four to 12, with the 12 organizing themselves into six mutually exclusive base pairs (Figure 3).

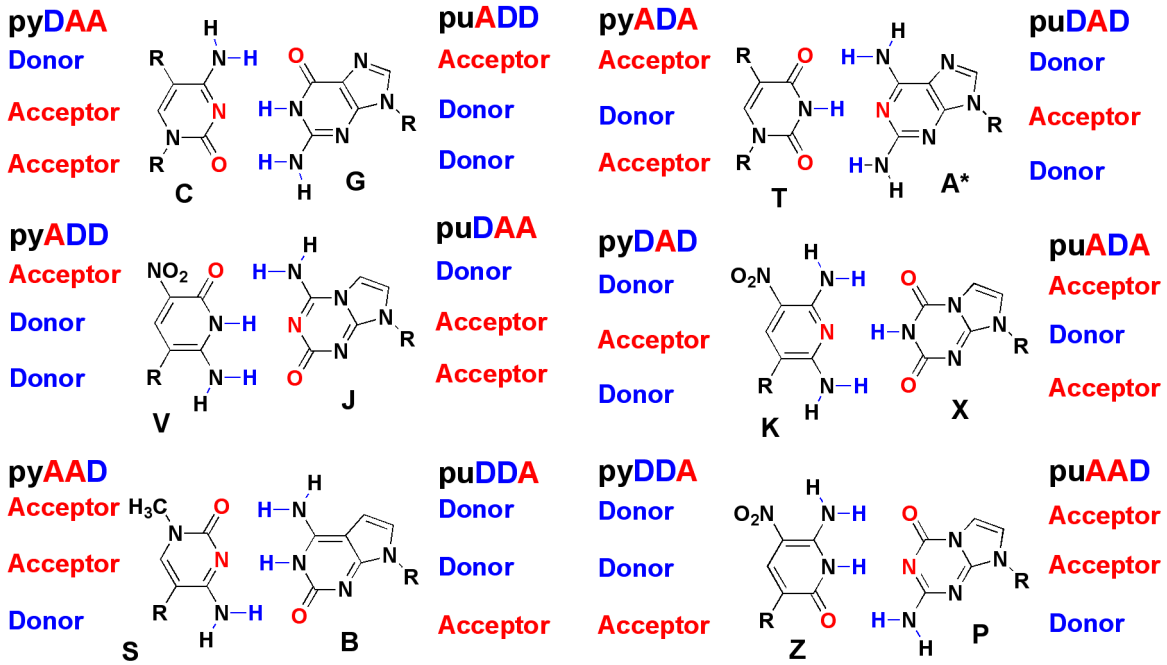

Figure 3. Structures of DNA and xNA base pairs.

Again, we have made only small moves away from standard xNA structures. Accordingly, we have been able to recruit terran polymerases to copy the expanded genetic alphabet. The pairing of the added nucleotides is sufficiently reliable to support diagnostics products with ~$100 million in sales that help personalize the care of some 400,000 patients annually.

Extra letters also helps scientists design nanostructures. Further, the system is fully able to support Darwinian evolution [3].

Has alien life exploited expanded genetic alphabets? Hollywood thinks so (ET had six nucleotides and three base pairs in his genome). Back to reality, as we await our first encounter with alien life to find out, we can still use "alien" genetics in medicine, biotechnology, and nanoscience.

References:

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