TECHVIEW: MOLECULAR BIOLOGY

Making Catalytic DNAs

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Nature has had more than 3 billion years to perfect the many thousands of enzymes that are found in living cells. Yet this long and unforgiving process of evolution has given rise to enzymes based on only two molecular formats—protein and RNA. But, in its quest for catalytic perfection, evolution may not have fully exploited all available molecular formats for enzyme construction. Recently, enzyme engineers have created DNA molecules called deoxyribozymes that have enzyme-like properties. Given the importance of DNA to the cell, these engineered DNA enzymes could have considerable practical use.

In opting to build biocatalysts from protein and RNA, nature has made two excellent choices. Proteins exploit the different chemistries offered by their constituent 20 amino acids to form an incredible array of diverse structures and precisely configured active sites. The awesome catalytic potential of proteins is exemplified by enzymes, such as orotidine 5′-phosphate decarboxylase, which converts substrate to product with a rate enhancement of 17 orders of magnitude over the corresponding uncatalyzed rate (*1*). RNA also can bring its lesser chemical repertoire to bear on catalysis by forming surprisingly intricate three-dimensional structures (*2*–*4*). Although RNA's job in modern biocatalysis might be a holdout from life's early evolutionary history (*5*), catalytic RNAs (ribozymes) are certainly capable of generating impressive rate enhancements. For example, group I ribozymes catalyze RNA splicing with a rate enhancement of ~13 orders of magnitude. Only eight distinct classes of naturally occurring RNA biocatalysts have been identified so far, yet nature is extraordinarily trusting of RNA's catalytic prowess—the ribosome, the complex RNA-protein factory that guides protein synthesis in all cells, has an RNA enzyme at its core (*6*, *7*).

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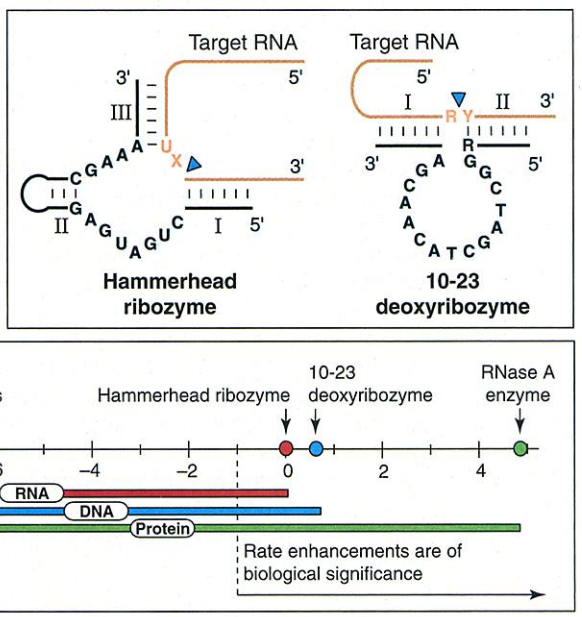
It could be inferred that the absence of natural catalytic DNA is a sign that protein and RNA are somehow exclusively suited to be enzymes. From a chemical viewpoint, however, the lack of DNA enzymes seems to be a simple oversight on the part of evolution (*8*). The most significant difference between DNA and its catalytically powerful RNA cousin is the absence of the 2′ oxygen at each nucleotide. Does the lack of this atom render DNA incapable of forming complex tertiary structures? On the contrary, by using various combinatorial selection strategies, enzyme engineers have created ~100 classes of deoxyribozymes that catalyze nearly a dozen different types of reaction including RNA cleavage and DNA modification.



Comparing DNA, RNA, and protein. (**Top**) Structures of the natural hammerhead ribozyme (RNA enzyme) and the engineered 10-23 deoxyribozyme (DNA enzyme). The arrowheads identify the site of enzyme-catalyzed RNA cleavage. (**Bottom**) A comparison of the catalytic power of three RNA-cleaving enzymes that operate by an identical transesterification mechanism. The plot depicts the logarithms of the rate constants (min^{-1}) (*14*) for each enzyme. Bars depict the rate enhancements that are generated by RNA, DNA, and protein enzymes over the rate constant for the spontaneous cleavage of RNA.

Why DNA Can Be an Enzyme

The absence of an enslaved nucleophile (the 2′-hydroxyl group) at each phosphodiester linkage makes DNA ~100,000-fold more stable than RNA under physiological conditions (*9*). Similarly, DNA phosphodiester bonds are 100-fold more resistant to hydrolytic degradation than the peptide bonds of proteins (*10*). This stability, coupled with the complementary character of nucleotides, makes DNA an ideal molecule for information storage and transfer. Yet it is precisely these characteristics, the ones that are most attractive for building the "ideal" genetic molecule, that must be conquered if DNAs are to be turned into powerful enzymes. Completely base-paired structures such as DNAs cannot form intricate active sites, and the chemical stability of DNAs hinders their ability to catalyze self-modifying reactions.

DNA can overcome the structurally stifling effects of its typical duplex structure simply by abandoning its complementary strand. Single-stranded DNA can form a great diversity of structures by exploiting more exotic secondary and tertiary interactions including nonstandard base pairs, stabilizing hairpin loops, internal bulges, multistem junctions, pseudoknots, and four-stranded G-quartet structures (*8*). Furthermore, single-stranded DNA is surprisingly adept at forming intricately structured aptamers that selectively bind ligands (*11*). DNA aptamers have been engineered to bind a variety of proteins and numerous small molecules such as nucleotides and amino acids. Once bound, these ligands can even be co-opted by DNA to serve as enzymatic cofactors. For example, HD deoxyribozymes, engineered to cleave RNA, co-opt a bound histidine residue as a general base catalyst to promote the necessary phosphoester transfer reaction, thus behaving much like a ribonuclease enzyme made of protein (*12*). Like RNA and protein catalysts, deoxyribozymes can exploit metal-ion cofactors to expand their limited chemical diversity.

Engineering New Deoxyribozymes

Unless nature harbors some undiscovered reservoir of catalytic DNAs, each new example has to be constructed in the laboratory. DNAs that cleave messenger RNAs (mRNAs) inside cells are at the top of the wish list because by destroying mRNAs they shut off gene expression and so may be valuable therapeutically.

By informal poll, the number of distinct structural classes of RNA-cleaving

deoxyribozymes stands in excess of 20. Each of these enzymes has been created by in vitro selection (*13*), a process that relies on the probability that some DNA molecules in a random-sequence population will fold and cleave an adjoining RNA linkage. However, this combinatorial strategy has its limitations. Difficult reactions that might demand large and highly refined catalytic domains are likely to occur only rarely in the random-sequence population. For example, there are more than 10^{48} different sequence variants of a DNA that is 80 nucleotides long. If a hypothetical deoxyribozyme could only be active by carrying a strictly conserved domain of 80 nucleotides, then we would need to screen the equivalent of about 10,000 Earth masses of random-sequence DNA to have a reasonable chance of encountering the active molecule.

Fortunately, DNA has sufficient potential for diverse structure formation to yield numerous complex structures from population sizes as small as a few trillion members. In addition, both DNA and RNA can form efficient active sites that catalyze RNA transesterification with surprisingly few nucleotides. The "10-23" deoxyribozyme (see figure, previous page) requires only 15 nucleotides to form a catalytic core that is ranked among the most effective nucleic acid enzymes of its kind, even when compared with natural self-cleaving ribozymes (*14*). Both 10-23 and the hammerhead ribozyme produce a rate enhancement of ~10 million–fold over the spontaneous rate of RNA cleavage, whereas the protein enzyme ribonuclease A is several orders of magnitude more effective. If the chemical challenge is too great for DNA, then engineers can play their trump card. Greater catalytic power can be conferred upon DNA simply by grafting more powerful and diverse chemistries directly onto its nucleotides (*15*, *16*).

RNA-Cleaving DNA Enzymes as Tools

The quest to harness the newfound catalytic abilities of DNA is perhaps best illustrated by the story of the 10-23 deoxyribozyme. This catalytic DNA was isolated from a random-sequence DNA population on the basis of its ability to efficiently cleave an RNA sequence under simulated physiological conditions (*14*). The 10-23 deoxyribozyme has two very desirable characteristics, catalytic speed and designer-friendly binding arms, which can be exploited to cleave RNA molecules with sequence-specific precision. Unlike most protein nucleases, these deoxyribozymes can be tailored to cleave RNA molecules at defined sites [for example, (*17*)]. Similarly, by specifically cleaving certain mRNAs, the expression of genes inside cells can be altered and disease-causing mRNAs can be destroyed. Recently, 10-23 has been tailored to target the destruction of various mRNAs in cultured cells (*18*–*20*) and even in vivo (*21*). Of course, many of the development challenges that face antisense and ribozyme technologies (such as delivery, biostability, and cellular localization) also confront catalytic DNAs.

More imminent is the development of test-tube applications for RNA-cleaving deoxyribozymes. The chemical stability and "snap-back" structural characteristics of DNA are well suited for certain biotechnology applications. 10-23 has been adapted to create the DzyNA-PCR system that permits the detection and quantitation of nucleic acids by the catalytic action of a deoxyribozyme during thermocycling (*22*). In this process, DNA is made by the polymerase chain reaction (PCR), during which it experiences subsequent high-temperature treatment without chemical damage, then simply folds into its active state and cleaves a molecular beacon to generate a fluorescent report. Another RNA-cleaving deoxyribozyme that selectively uses Pb^{2+} ions for catalysis has been exploited as a biosensor for this highly toxic metal (*23*). Most certainly, other applications will be found where engineered catalytic DNAs will succeed where protein or RNA enzymes would most likely fail.

Modifying DNA with DNA

Destroying RNA is only one facet of DNA's useful catalytic repertoire. Deoxyribozymes have been created that cleave DNA by an oxidative mechanism (*24*) or by depurination (*25*). These deoxyribozymes eliminate a nucleotide from the target DNA chain, and therefore might be valuable in applications where the loss of sequence-encoded information is tolerated or perhaps even desirable.

Alternatively, DNA can catalyze reactions that build more DNA (*26*). To this end, deoxyribozymes have been trained to tap into the chemical energy stored in adenosine 5′-triphosphate, biology's universal energy currency, to catalyze DNA phosphorylation (*27*) and DNA activation by adenylation (*28*). DNAs that energetically charge themselves in this manner are targets for a deoxyribozyme that ligates DNA; these "self-cloning" DNAs that catalyze DNA phosphorylation, DNA adenylation, and DNA ligation are already under development. The prototype self-processing DNAs currently in hand are most likely too slow and structurally mischievous for immediate application, but almost all examples show promise as candidates for further optimization.

The Future of Catalytic DNA

To forecast the catalytic capabilities of DNA, one need only look at the accomplishments of ribozymes. Both polymers already catalyze a similar set of self-modification reactions. If this functional similarity can be extended, then there are many as-yet undiscovered deoxyribozymes that catalyze reactions of considerable interest. Variants of existing deoxyribozymes could be used to create self-labeling DNA probes. It also seems likely that allosteric DNA enzymes could be engineered to serve as precision molecular switches for constructing advanced biosensors or even DNA computing devices. But could simple catalytic DNAs be made to covalently fuse with protein targets inside cells? Could more advanced multidomain deoxyribozymes be made that alter the genetic make-up of a cell? These tasks would appear to be far too challenging for a polymer whose catalytic potential has been rejected by living systems. Yet, the lack of deoxyribozymes in cells may simply be because nature never had any real need to call on them, and so, it is up to enzyme engineers to unveil and put to use DNA's true catalytic potential.

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