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Breaking Up Is Easy To Do (If You're a DNA Enzyme that Cleaves RNA)

In this issue of *Chemistry & Biology*, Cruz et al. use in vitro selection to select deoxyribozymes that collectively cleave almost any RNA dinucleotide junction [6]. More remarkable is the finding that the new enzymes are related to the 8-17 deoxyribozyme that cleaves AG dinucleotide junctions.

About a decade ago, the first artificial DNA enzyme (deoxyribozyme) was reported by Breaker and Joyce in this journal [1]. The intervening years have seen significant progress in developing DNA as a catalyst for many reactions [2]. The RNA backbone is a common cleavage target for DNA enzymes, and the 10-23 and 8-17 deoxyribozymes [3] are now standard tools for in vitro RNA cleavage [4] and for efforts at in vivo mRNA degradation [5]. Now, Cruz et al. have identified RNA-cleaving deoxyribozymes that collectively cleave a wide range of RNA sequence junctions [6]. In an efficient parallel approach using random DNA pools, they applied in vitro selection to pursue DNA enzymes that cut all sixteen possible NrN junctions, where the single RNA linkage was embedded within an otherwise all-DNA oligonucleotide substrate. For 14 out of the 16 possible NrN junctions, active Mn^{2+}-dependent DNA enzymes were identified (one enzyme for each junction). Surprisingly, sequencing reveals that almost all of the new DNA enzymes are clearly related to the 8-17 deoxyribozyme, which was previously reported in several independent studies [3, 7–9].

The new experiments demonstrate that the 8-17 motif is highly favored for RNA cleavage in DNA sequence space. One intriguing facet of the new study is that despite the repeated identification of 8-17-like sequences, the 10-23 deoxyribozyme motif was not observed. This motif was originally reported at the same time as 8-17 [3], and it is an excellent ribonuclease in its own right [10]. Therefore, its absence is striking. Smaller motifs have a competitive advantage during selection; for example, the relatively small hammerhead ribozyme is found preferentially over other possible ribozymes in experiments starting with random RNA pools [11]. However, the 8-17 and 10-23 deoxyribozymes are about equal in size: 13 and 15 nt in the "enzyme region," respectively. So, why was the 10-23 not found at all? While one always worries about selection artifacts such as contamination, it is likely that 10-23 simply has more sequence requirements than does 8-17 and thus is represented at a lower frequency in random DNA pools. Indeed, the 10-23 is nearly intolerant to nucleotide substitution in its enzyme region [10], whereas only four nucleotides are invariant in the collection of 8-17-like enzymes described in the new report [6]. This difference in mutability is apparently sufficient to give 8-17 an insurmountable competitive advantage over 10-23 and other possible ribonucleases when random DNA pools are used to initiate a selection experiment.

All of the new 8-17-like DNA enzymes share significant sequence and secondary structure elements. Furthermore, only four nucleotides are strictly conserved across the set of enzymes; the remaining nine or so nucleotides may have various identities. Based on these data, Cruz et al. offer a modular structural proposal for the RNA cleavage activities (Figure 1). In this model, some DNA nucleotides of a generic 8-17-like deoxyribozyme belong to the catalytic core, while others fine-tune the structure for individual dinucleotide (NrN) specificity without participating directly in catalysis. The nucleobases of the substrate's NrN junction are speculated to stack with a conserved nucleotide in the catalytic core. This structural model is consistent with the experimentally favored cleavage of junctions that are flanked by two purines, which stack better than pyrimidines. In addition, the model is consistent with the clear competitive advantage of 8-17-like sequences during selection. In particular, a compact catalytic core may imply that only modest sequence changes elsewhere within the DNA are sufficient to meet the varied structural demands imposed by different nucleoside combinations that surround the scissile phosphodiester bond.

A general implication of the new report [6] is that it is time for structural biologists to embrace DNA enzymes as they already have ribozymes [12]. DNA enzymes clearly have much to reveal about how nucleic acids can catalyze chemical reactions. However, at present only one deoxyribozyme X-ray crystal structure has been reported: that of 10-23, which crystallized in a catalytically inactive 2:2 enzyme:substrate stoichiometry [13]. Close parallels are now firmly established be-

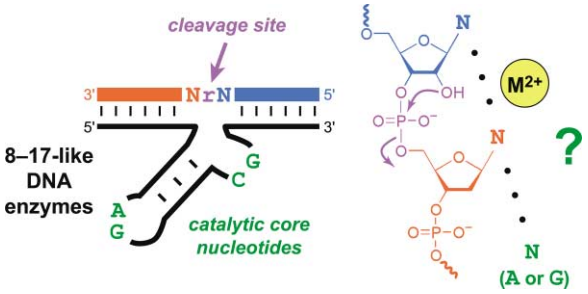

Figure 1. Cleavage of Nearly All Possible RNA Dinucleotide Junctions by a Collection of New 8-17-like DNA Enzymes

Based on the sequences of many new RNA-cleaving deoxyribozymes that resemble the 8-17 DNA enzyme [3], Cruz et al. [6] offer a simple structural proposal to explain their wide substrate applicability. In their model, the conserved catalytic core nucleotides (green) interact with the substrate at the cleavable NrN ribonucleotide junction (purple). The remaining nucleotides of the DNA enzyme (black) facilitate the reaction by fine tuning the structure; thus, most dinucleotide junction sequences are cleavable because they depend on the same catalytic residues. The illustrated role of base stacking (•••) is speculative but consistent with the data. The mechanistic role of the required divalent metal ion (M^{2+}) and the details of the enzyme-substrate interactions are not yet known.

tween enzymes made from RNA and those made from DNA in terms of scope of activity and existence of favored sequences for particular catalytic activities. In addition, for both RNA and DNA enzymes we have specific functional models to test (e.g., Figure 1). Thus, the time is ripe for earnest application of X-ray crystallography and NMR spectroscopy to understand DNA enzyme structure and activity.

Beyond their implications for DNA enzyme function, the new results can be considered in the larger context of nucleic acid enzyme research [14]. With respect to the in vitro activities of (deoxy)ribozymes, we are in a transitional period in the development of this field. Ever-increasing attention is being devoted to applying nucleic acid enzymes as widely as possible for particular tasks such as RNA cleavage, rather than identifying qualitatively "new" activities and immediately moving on to other studies. For example, in addition to RNA cleavage, various labs are using RNA and DNA enzymes to prepare reagents for subsequent biophysical experiments [15], to sense metal ions in solution [16, 17], to sense bioorganic molecules [18], to address chemical reactions of importance for prebiotic RNA world chemistry [19], and to explore reactions related to those of modern biochemistry [20]. I view these developments as encouraging signs that the nucleic acid enzyme field is maturing. Hopefully, the next two decades will reveal even more exciting progress in understanding nucleic acid enzymes—both natural and artificial—and applying them to important theoretical and practical problems.

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Aureolic Acids: Similar Antibiotics with Different Biosynthetic Gene Clusters

In this issue of *Chemistry & Biology*, Méndez and colleagues describe the sequence and organization of the chromomycin gene cluster [20]. Unexpectedly, the arrangement is starkly different from the mithramycin biosynthetic cluster, despite similarity in the individual genes and the near identical structures of the two antibiotic aureolic acids.

The aureolic acids, chromomycin A3, mithramycin, olivomycin, UCH9, and durhamycin A, are a family of aromatic polyketides that share an identical tricyclic core (Figure 1) [1]. The aureolic acids are neoplastic antibiotics that act against gram-positive bacteria and also stop the proliferation of tumor cells [2]. In the presence of Mg^{2+}, these compounds inhibit replication and transcription processes by interacting with G-C-rich regions in the minor groove of DNA [3]. Mithramycin (plicamycin), the only clinically used aureolic acid, is currently in limited use for the treatment of some testicular cancers [4], Paget's Bone Disease [5], and the treatment of hypercalciuria that results from certain tumors [6].

The biosynthetic pathway of mithramycin, a representative member of the aureolic acids, was ultimately elucidated by genetic studies of the producing organism, *Streptomyces argillaceus* [1]. Sequencing of the mithramycin biosynthetic gene cluster indicated that ten acetate units are converted into a 20 carbon chain by the combined action of an acyl carrier protein, a ketosynthase, and a chain length factor in a mechanism that is