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Deoxyribozyme

Deoxyribozymes, also called DNA enzymes, DNAzymes, or catalytic DNA, are DNA oligonucleotides that are capable of performing a specific chemical reaction via catalytic function which can be use as amplifying labels for the development of optical or electronic sensors.

From: Nano-Inspired Biosensors for Protein Assay with Clinical Applications, 2019

Related terms:

Combination Therapy, Aptamer, Cleft, Nucleic Acid, Ligation, DNA, Oligonucleotide, Messenger RNA, Ribozyme, Small Interfering RNA

Antisense Nucleic Acids: Clinical Applications

Georg Aue, Alan M. Gewirtz, in Encyclopedia of Cancer (Second Edition), 2002

II.E DNAzymes

DNAzymes are essentially RNA-cleaving DNA enzymes. The structure of a DNAzyme consists of a catalytic domain of 15 deoxynucleotides and two substrate recognition domains of 8 nucleotides each (Fig. 2). The substrate recognition domains contain the necessary nucleotide sequence for specific binding to the RNA substrate. By composing the appropriate recognition domain sequences, it is possible to create DNAzymes that are able to cleave any RNA that contains a purine–pyrimidine junction. DNAzymes have two advantages over ribozymes: stability secondary to containing DNA instead of RNA and an inexpensive cost of production. However, DNAzymes must be proven to be able to achieve stable, efficient intracellular concentrations without toxicity and to perform targeted RNA cleavage. They must also have the ability to target the cell and cellular compartment of interest, as well as the ability to hybridize with the mRNA target *in vivo*.

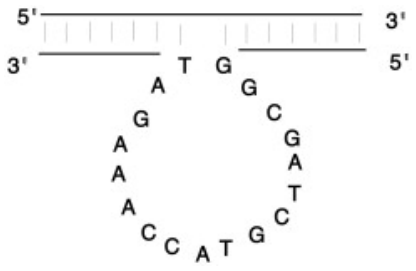

Fig. 2. DNAzyme Adapted from [4].

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Biophysical, Chemical, and Functional Probes of RNA Structure, Interactions and Folding: Part B

Scott K. Silverman, Dana A. Baum, in Methods in Enzymology, 2009

1 Introduction

Deoxyribozymes (also called DNA enzymes or DNAzymes) are specific sequences of DNA that have catalytic activity. All currently known deoxyribozymes have been identified by *in vitro* selection from large random-sequence DNA pools (Joyce, 2004; Silverman, 2009). The catalytic range of DNA encompasses both oligonucleotide and nonoligonucleotide substrates (Baum and Silverman, 2008; Silverman, 2008). This report focuses on deoxyribozymes that are useful for reactions of RNA substrates, especially to assist studies of RNA structure, folding, and catalysis.

For anyone who wishes to use a deoxyribozyme as a practical RNA cleavage or ligation catalyst by following the procedures described in this chapter, we recommend that a positive control experiment should be performed in parallel, using an RNA substrate that is known to be tolerated well by an analogous deoxyribozyme. For any nucleic acid enzyme that binds to its oligonucleotide substrate via extensive Watson–Crick interactions, it is impossible in practice to validate catalytic activity with all possible substrate sequences. Therefore, rates and yields with any particular substrate can vary from the values reported for other substrates. If the "experimental" sample fails to show the desired reactivity, then the results with the positive control will distinguish specific failure of the particular deoxyribozyme–substrate combination from a more general problem with the overall application of deoxyribozymes (e.g., a problem with buffers, metal ions, oligonucleotide purification, and so on).

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URL: https://www.sciencedirect.com/science/article/pii/S0076687909690054

Signal Amplification

Chang Feng, Xiaoli Zhu, in
Nano-Inspired Biosensors for Protein Assay with Clinical Applications, 2019

12.3.2 DNAzyme

Deoxyribozymes, also called DNA enzymes, DNAzymes, or catalytic DNA, are DNA oligonucleotides that are capable of performing a specific chemical reaction via catalytic function which can be use as amplifying labels for the development of optical or electronic sensors. Compared with protein enzymes and RNA ribozymes, DNAzyme has the advantages of better thermal stability, cost-efficiency, and constructability. The most commonly used DNAzyme in biosensors is hemin/G-quadruplex DNAzyme which was first proposed by Sen et al. in 2001 (Travascio et al., 2001). In this molecule, the complexation of hemin with a guanine-rich single-stranded nucleic acid possesses a G-quadruplex structure that catalyzes the oxidation of 2,2'-azino-bis(3-ethylbenzothiozoline)-6-sulfonic acid ($ABTS^{2-}$) by H_2O_2 to form the respective colored radical product, $ABTS^{-}$. In addition, the hemin/G-quadruplex structure can also catalyze the oxidation of luminol by H_2O_2 and the generation of chemiluminescence (Fig. 12.9; Willner et al., 2008). Therefore, this DNAzyme was used for the colorimetric or chemiluminescent detection of nucleic acids, or to follow the activity of telomerase, a versatile marker for cancer cells. For instance, a new binding-induced and label-free colorimetric method for protein detection has been developed on the basis of an autonomous assembly of hemin/G-quadruplex DNAzyme amplification strategy by Zou et al. (Wu et al., 2015). In the presence of target protein, a stable DNA–protein complex is formed when two proximity probes bind to the protein simultaneously. Then the complex triggers an autonomous cross-opening of the two functional hairpin structures, resulting in the formation of numerous hemin/G-quadruplex DNAzymes. The generated DNAzymes catalyze the oxidation of colorless 2,2'-

azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{2-}$) to the green-colored $ABTS^{2-}$ with the presence of H_2O_2, thus producing the amplified colorimetric detection of target (Fig. 12.10). This method does not require any chemical modification of DNA, which makes it technically label-free and cost-effective.

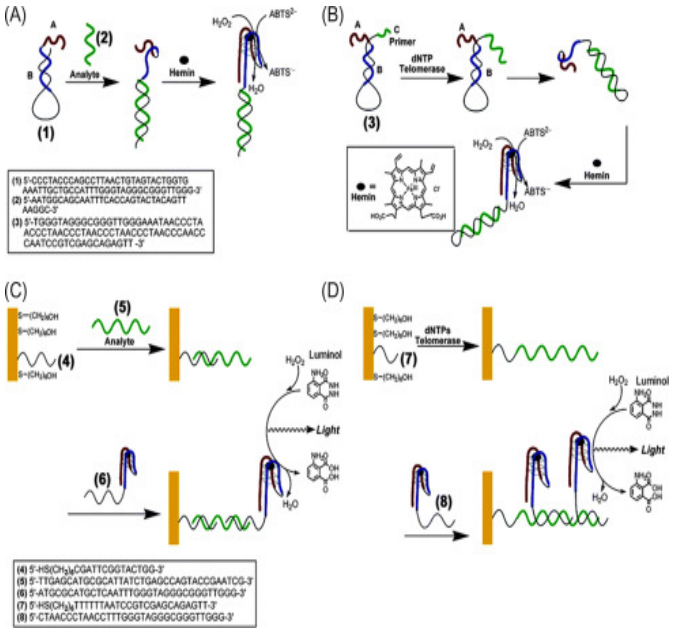

Figure 12.9. (A) Analysis of a DNA by a hairpin that generates the horseradish peroxidase-mimicking DNAzyme. (B) Detection of telomerase activity by a hairpin structure that yields the horseradish peroxidase-mimicking DNAzyme. (C) and (D) The chemiluminescence analysis of DNA or telomerase activity on surfaces by the use of nucleic acid-functionalized DNAzyme units as labels.

Reprinted from Willner, I., Shlyahovsky, B., Zayats, M., Willner, B. 2008. DNAzymes for sensing, nanobiotechnology and logic gate applications. Chem. Soc. Rev., 37(6), 1153–1165.

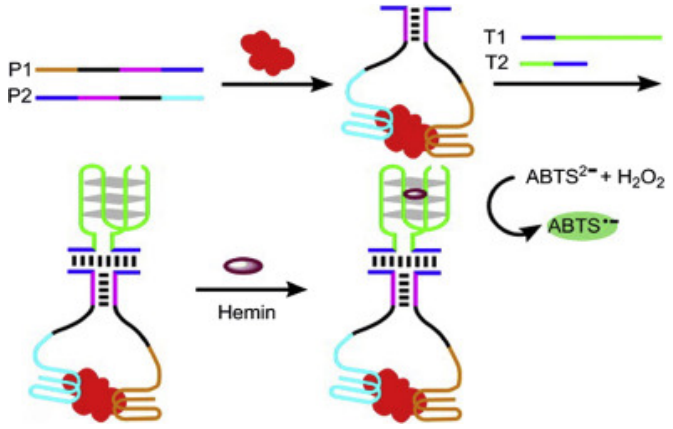

Figure 12.10. Schematic illustration of binding-induced colorimetric assay for thrombin detection without amplification.

Reprinted from Wu, H., Zhang, K., Liu, Y., Wang, H., Wu, J., Zhu, F., et al., 2015. Binding-induced and label-free colorimetric method for protein detection based on autonomous assembly of hemin/G-quadruplex DNAzyme amplification strategy. Biosens. Bioelectron. 64, 572–578.

Furthermore, DNAzymes exhibited nucleic acid cleavage activities in the presence of added cofactors, which have been used for the specific scission of DNA sequences. For example, nucleic acid sequences that specifically bind Pb^{2+}, Mg^{2+},

or Cu^{2+} ions, UO^{2+} or histidine could yield supramolecular coiled structures that cleave specific DNA sequences (Fig. 12.11). Utilizing these properties, predesigned nucleic acid sequences were tethered to the DNAzyme structures to yield functional units for the amplified detection of the cofactors and for target DNA/RNA, or alternatively protein. For example, by adopting a well-designed DNAzyme, Li et al. proposed an integrated strategy to comprehensively analyze tumor-associated membrane proteins (TMPs), which achieved in situ imaging and amplified detection of TMPs in a single system and was capable of providing both the locational and the quantitative information of TMPs (Fig. 12.12; Chen et al., 2018). In comparison with some conventional methods, this DNAzyme-based method possesses the advantages of two functions in one integrated system (two-in-one): high sensitivity and nondestructivity. Therefore, the concept of dual-labeling in this work also expands its application and provides a reference for the research on DNAzyme.

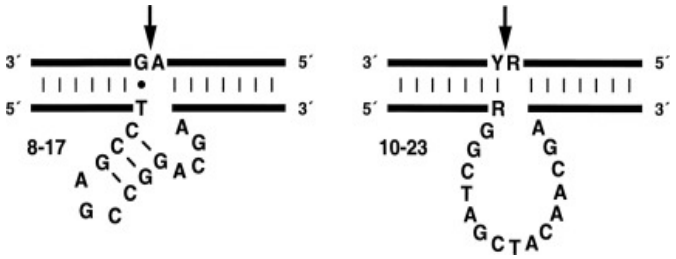

Figure 12.11. Composition of the DNAzyme catalytic motifs. The DNA enzyme (bottom strand) binds the RNA substrate (top strand) through Watson–Crick pairing. Cleavage occurs at the position indicated by the arrow. R 5 A or G; Y 5 U or C.

Reprinted from Santoro, S.W., Joyce, G.F., 1997. A general purpose RNA-cleaving DNA enzyme. Proc. Natl. Acad. Sci. U. S. A. 94, 4262–4266.

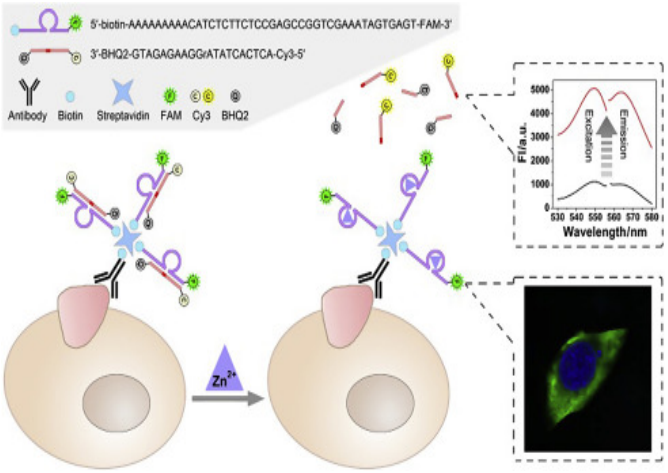

Figure 12.12. Scheme of the DNAzyme-based nondestructive analysis of tumor-associated membrane protein integrating imaging and amplified detection.

Reprinted from Chen, X., Zhao, J., Chen, T., Gao, T., Zhu, X., Li, G., 2018. Nondestructive analysis of tumor-associated membrane protein integrating imaging and amplified detection in situ based on dual-labeled DNAzyme. Theranostics 8, 1075–1083.

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Advances in the design of new types of inhaled medicines

Werngard Czechtizky, ... Rhona J. Cox, in Progress in Medicinal Chemistry, 2022

5.6.4 DNAzymes

DNAzymes are antisense *DNA* molecules that are designed to cleave a specific mRNA at purine-pyrimidine junctions thus down-regulating gene expression. A rare example of an inhaled molecule in this class is *hgd40* (formulated drug substance known as *SB-010*), an immunomodulatory GATA 3 transcription factor inhibitor, also referred to as a GATA-3-specific DNAzyme [283]. Inhaled dosing of radioactively labelled compound in preclinical species shows localisation of hgd40 in the lungs and bladder following intratracheal dosing, indicating excretion through the kidneys, and compound was detected in the lungs up to seven days after dosing. Interestingly, complex delivery systems appear not to be required and the naked DNA can be delivered in a simple formulation. SB-010 is currently in Phase 2 clinical trials for the potential treatment of asthma and COPD, although progress seems to have been slow. Initial results in patients with COPD suggest that SB-010 reduces sputum eosinophilia [284], but further studies are needed to demonstrate clinical efficacy. In patients with asthma, both early and late asthmatic responses after allergen challenge were observed [285].

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Rare-Earth Element Biochemistry: Characterization and Applications of Lanthanide-Binding Biomolecules

Po-Jung Jimmy Huang, Juewen Liu, in Methods in Enzymology, 2021

5.2 In vitro selection

To select DNAzymes, a high concentration of target metal ion is typically used in the early rounds of selection. However, adding too much polyvalent metal ions especially lanthanides could have an adverse effect. Excess metal ions can non-specifically fold DNA and render it catalytic inactive (Vazin, Huang, Matuszek, & Liu, 2015). To address this issue, incremental addition of metal ions is recommended. For instance, 2 μL of 1.34 mM of Ce^{4+} were added every 20 min into 10 μL of the library solution over a total of 60 min in the first three rounds of our Ce^{4+} selection (Table 2). As the selection progresses and the cleavage yield is increased, the used Ce^{4+} salt can be decreased and the incubation time can be shortened. For trivalent lanthanides, they tend to interact with DNA even more strongly, and they were typically added at low micromolar concentrations (e.g. between 10 and 100 μM). After this incubation, a small fraction of the library might be cleaved due to its unique sequence, although nonspecific cleavage might also occur. The cleavage reaction is then quenched with an equal volume (16 μL) of 1 × blue loading dye containing 8 M urea and separated with 10% dPAGE. A typical gel micrograph is shown in Fig. 3B. The lane on the left contains three DNA strands serving as a ladder (see the next paragraph for ladder construction). For the lane on the right, the bright band on top is the uncleaved library, and the band at the bottom is the cleaved short fragment containing the FAM-label. There is an invisible band in the yellow box (due to a lack of fluorophore label) corresponding to the longer cleaved fragment (91mer), which contains the active library sequence to be harvested. The cleaved product (91mer) is excised from the gel with a new razor blade based on the ladder position and extracted with buffer C. Following the same C18 desalting/purification procedure described above, the extracted DNA can be amplified by PCR and used for subsequent library generation (see Section 5.3).

Table 2. Ce^{4+} selection conditions and progress based on our work in Huang, Lin, et al. (2014).

Round #	Final [Ce^{4+}] (μM)	Incubation time (min)	Cleavage (%)
1	500	3 × 20	1.1
2	500	3 × 20	7.4
3	500	3 × 20	12.7
4	500	3 × 14	34.4
5	50	40	53.5
6	50	40	56.2

As shown in the top half of Fig. 3A, the initial library (for round 1 selection) constructed from the ligation has a fluorophore label at the 3′-end, while the subsequent library generated from PCR has a fluorophore label at the 5′-end (see the bottom half of Fig. 3A). Since the round 1 library statistically would contain very few active DNAzyme sequences, no fluorescence can be observed for the desired band even though a FAM label is on the DNA. For the subsequent rounds, the cleaved library containing fragment does not have a fluorophore and thus is invisible on the gel. To achieve an accurate gel excision, a precise ladder is needed. This initial library can be hydrolyzed by base and used as a 91mer ladder. To produce a hydrolyzed library with a 3′-FAM label, 10 μL of the ligated library (107mer) is mixed with 3.4 μL of 1 M NaOH at 90 °C for 2 min. The reaction is then neutralized with 1.9 μL of 3 M sodium acetate (pH 5.2) and further diluted with 5 mM HEPES (pH 7.5). For every round of selection, 10 pmol each of the full-length library (107mer for initial round and 119mer for subsequent rounds), the hydrolyzed library (91mer), and FAM-labeled P3 primer (30mer) are mixed and used as a ladder.

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RNA-Based Technologies

David P. Clark, Nanette J. Pazdernik, in Biotechnology (Second Edition), 2016

Allosteric Deoxyribozymes Catalyze Specific Reactions

Because RNA may display catalytic properties, researchers investigated whether DNA can do the same. Although no natural DNA enzymes are known, DNA nonetheless can catalyze various reactions in a manner analogous to RNA-based ribozymes. Indeed, *in vitro* selection has been used to create a variety of artificial deoxyribozymes or DNAzymes that catalyze various reactions. Most DNAzymes catalyze reactions involved in processing RNA or DNA because they are easiest for SELEX type schemes to select. Examples include RNA cleavage, DNA cleavage, DNA depurination, RNA ligation, DNA phosphorylation, and thymine dimer cleavage.

One of the most interesting DNAzymes can split thymine dimers caused by UV radiation of DNA. Different organisms have various mechanisms to deal with these dimers. For example, excision repair removes the damaged strand and replaces it with new DNA. Another mechanism involves photolyase enzymes, which recognize and repair thymine dimers when activated by blue light. To isolate a DNA sequence to perform the photolyase reaction, scientists carried out *in vitro* selection on a pool of random DNA oligonucleotide sequences. The random sequences were first linked to a substrate that consisted of two DNA oligonucleotides joined via a thymine dimer. If a random DNA oligonucleotide split the thymine dimer after exposure to blue light, then the overall length of the DNA construct would be smaller. The smaller species were isolated by gel electrophoresis. This experiment was successful, and a specific DNAzyme (UV1C) that could catalyze a photolyase reaction was identified (Fig. 5.34).

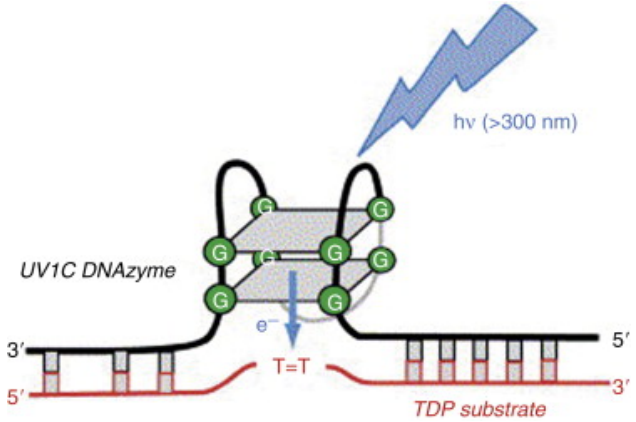

FIGURE 5.34. Deoxyribozyme That Repairs Thymine Dimers

A model for the deoxyribozyme UV1C–substrate complex. Light energy is absorbed by the guanine quadruplex. The thymine dimer is thought to lie close to the guanine cluster within the folded deoxyribozyme. This allows electron flow from the excited guanines to the thymine dimer.

From Chinnapen DJ, Sen D (2007). Towards elucidation of the mechanism of UV1C, a deoxyribozyme with photolyase activity. *J Mol Biol* **365,** 1326–1336.

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Deoxyribozymes are DNA sequences that catalyze an enzymatic reaction. All are artificial.

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Polymers in Biology and Medicine

D.M. Perrin, in Polymer Science: A Comprehensive Reference, 2012

9.02.7 M^{2+}-Independent RNA-Cleaving DNAs

Whereas Mg^{2+}-dependent DNAzyme catalytic rate constants reached >1 min^{-1}, Mg^{2+} concentrations needed to attain these rates were on the order of 10–50 mM, which far exceed the *in vivo* concentrations (~0.5 mM) found in the cell.[84] Therefore, a M^{2+}-independent DNAzyme would be desirable. For example, the HH ribozyme was found to perform detectable M^{2+}-independent catalysis in the presence of a high concentration of monovalent cation (e.g., Na^+, Li^+, or K^+) present to promote proper folding.[41] As noted, this finding provided important evidence that the chemical functionality of the HH ribozyme is sufficient for M^{2+}-independent catalysis while the role of the divalent M^{2+} is restricted to folding a catalytically competent M^{2+}-independent species.

Efficient cleavage (k_{cat} ≥ 0.1 min^{-1}) was observed twice under nonphysiological conditions: (1) at 5 mM histidine in 1 M NaCl/KCl[92] and (2) at pH 3–5,[57] where adenine and cytosine competently play the roles of general acid–base catalysis.[93,94] The exogenous histamine cofactor provided both an amine and imidazole and derived inspiration from the M^{2+}-free mechanism of RNases A that employs the amino acids histidine and lysine. Attempts at finding activity under more physiological conditions involved selection for M^{2+}-independent cleavage at pH ~7 in 0.25–1 M monovalent cations (Na^+ or K^+).

Seeking to avoid M^{2+} dependence altogether, Faulhammer and Famulok[95] attempted to select for a histidine-dependent self-cleaving DNAzyme with minimal dependence on divalent metal cations. Unfortunately, the isolated DNAzyme was discovered to be histidine independent and instead was completely dependent on either Mg^{2+} or Ca^{2+}. Three subsequent selections were used to assess the dependence of DNAzymes on divalent metal cations and one of the selections

incorporated the use of a positively charged cofactor spermine.[96] The second selection was carried out using Mg^{2+} and the third selection used no cofactors or divalent metal ions. All three led to the isolation of minimally active DNAzymes. In 1997, Geyer and Sen[97] selected an M^{2+}-independent DNAzyme, DzG3, and further evolved this sequence into DzNa8, both of which were minimally active in the absence of M^{2+}. In a third case, Carrigan *et al.*[98] identified an M^{2+}-independent DNAzyme that was capable of two turnovers in ~100 h. The selection of these M^{2+}-independent DNAzymes is concrete evidence that DNA, like the HH ribozyme, bears sufficient functionality for catalysis that does not absolutely necessitate a M^{2+}. Nevertheless, the substantially lower activity in the absence of Mg^{2+} demonstrates two important points: (1) unmodified nucleic acid catalysts are inherently limited in the absence of M^{2+} and (2) combinatorial selection has so far failed to identify a reasonably active Mg^{2+}-independent catalyst. If robust activity actually exists, one is left with the rather unsatisfying consolation that it must exist in very rare sequences that reside in the outer reaches of sequence space such that they have escaped discovery in three different laboratories. With this evidence, the development of M^{2+}-independent DNAzymes turned toward the enhancement of catalytic rate by chemical means (*vide infra*).

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DNA and Aspects of Molecular Biology

Dipanakar Sen, in Comprehensive Natural Products Chemistry, 1999

7.17.5.2.1 Nuclease: the catalysis of RNA cleavage

The derivation of these earliest reported DNAzymes was carried out by Breaker and Joyce.[95] The original strategy was to utilize lead ions complexed directly to random DNA sequences to cleave a single ribonucleotide phosphodiester present within the DNA. Lead ions were chosen as potential cofactors because the first pK_a of water molecules coordinated to Pb^{2+} ions is ~7.7. This bound water molecule, substantially deprotonated at physiological pH, is particularly suitable as a general base catalyst for RNA cleavage (prior to the derivation of this DNAzyme, both naturally occurring tRNA molecules, and also artificial "leadzyme" ribozymes,[82–84] had been shown to self-cleave efficiently in the presence of lead).

To obtain lead-dependent, hydrolytic DNAzymes, Breaker and Joyce[76] utilized an ingenious variation on the "direct" selection approach, which was called "catalytic elution." This is shown schematically in Figure 6. Random-sequence, single-stranded DNA molecules were generated which all contained (i) a biotin residue at the 5′-terminus and (ii) a single ribonucleotide residue at a fixed position within the 5′ constant-sequence region. The biotin residue was used to immobilize each DNA molecule to an avidin-containing matrix. The ~10^{14} distinct molecules (containing 50 random nucleotides each) were first allowed to fold up in high salt. 1 mM Pb^{2+} was then added and any self-cleaving DNA molecules within the pool (which thereby detached themselves from the matrix) were collected after a period of incubation. After five rounds of selection and amplification, excellent lead-dependent DNAzymes were isolated and cloned. These had $k_{observed}$ values of ~1 min^{-1}, which represented 10^5-fold rate enhancements over the same hydrolysis in the presence of lead, but uncatalyzed by the DNAzyme.

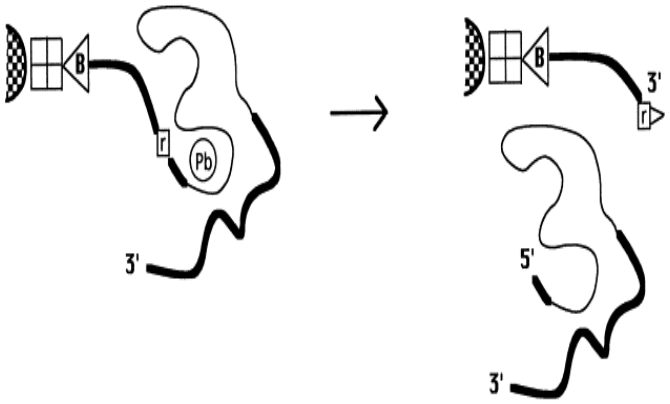

Figure 6. Schematic diagram of the derivation of DNA molecules catalytic for the lead-dependent hydrolysis of an internal ribonucleotide phosphodiester (r). The individual DNA molecules of the library are immobilized as single-strands on to a matrix (via a biotin–avidin linkage). DNA molecules capable of self-cleavage are released from the matrix, and can then be collected and amplified for subsequent rounds of SELEX and, eventually, cloning.

Subsequent to this initial report, Breaker and Joyce[36] used related strategies to generate other phosphodiester-hydrolyzing DNAzymes, which in turn specifically utilized Zn^{2+}, Mn^{2+}, and Mg^{2+} ions as cofactors. A pool of the Mg^{2+}-dependent DNAzymes were improved by a secondary, "focused" selection process; an individual aptamer from this improved pool had a $k_{observed}$ value of 0.02 min^{-1}, which represented a 10^5-fold enhancement over the magnesium-dependent, but DNA-uncatalyzed, rate of self-cleavage of the aptamer.

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Riboswitch Discovery, Structure and Function

Katarzyna Wawrzyniak-Turek, Claudia Höbartner, in Methods in Enzymology, 2014

5.4 Alternative DNA-catalyzed approaches for site-specific labeling of RNA

Besides catalyzing linear RNA ligations, deoxyribozymes can also activate internal 2′-OH groups of ribonucleotides for reaction with 5′-triphosphates, resulting in the formation of 2′,5′-branched RNA. The binding arms are designed to guide the deoxyribozyme to the desired labeling position by hybridization directly upstream and downstream of the target nucleoside. In an approach termed DNA-catalyzed labeling of RNA, 17-nt-long tagging RNAs were ligated to the RNA of interest (Baum & Silverman, 2007). Recently, a general and versatile, DNA-catalyzed labeling strategy was described that allows ribose-labeled mononucleotides to be site-specifically attached at desired adenosine nucleotides within *in vitro*-transcribed RNA. Fluorescent, spin-labeled, biotinylated, or crosslinker-modified guanosine triphosphates were efficiently installed in up to 160-nt-long functional RNAs (Büttner, Javadi-Zarnaghi, & Höbartner, 2014). Because long, native, or *in vitro*-transcribed RNA can be directly used for this labeling approach, preparation and ligation of RNA fragments can be avoided. So far, adenosines are the only type of internal nucleotides that can be targeted with this strategy, but deoxyribozymes for analogous labeling reactions at other internal nucleotides (guanosines, cytidines, or uridines) are expected from ongoing and future *in vitro* selection experiments. This will further broaden the general applicability of deoxyribozymes for posttranscriptional labeling of RNAs.

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Isotope Labeling of Biomolecules - Labeling Methods

My T. Le, ... T. Kwaku Dayie, in Methods in Enzymology, 2015

11.1 Overview

In this step, 8–17 RNA-cleaving deoxyribozymes (DNAzymes) are used to excise the inserted RNA of interest from the recombinant tRNA-scaffold (Schlosser et al., 2008). These DNAzymes contain catalytic core sequences specific to dinucleotide RNA cleavage sites flanked by two substrate-recognition domains that hybridize with the RNA on either side of the dinucleotide cleavage site. An additional 18-nt synthetic DNA oligonucleotide (5'-GCCCGAACAGGGACTTGAA-3'), complementary to the 3' side of the tRNA acceptor stem and T arm of the tRNA-scaffold, is included to melt the tRNA acceptor stem. This allows the DNAzymes to access their specific dinucleotide cleavage sites. The extent of reaction is analyzed using denaturing PAGE (Fig. 7).

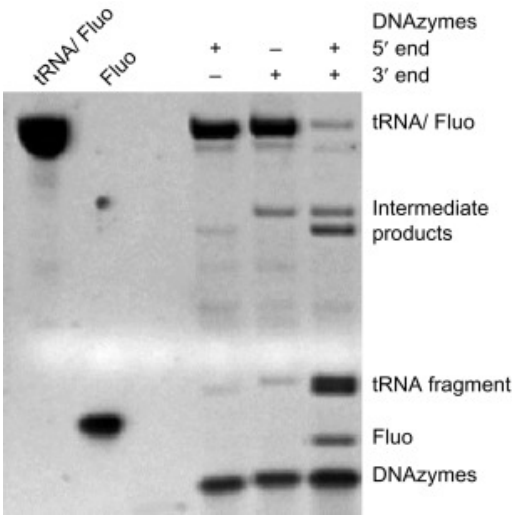

Figure 7. Excision of Fluo from tRNA/Fluo using DNAzymes. Two 8–17 DNAzymes were designed to cleave the 5′ and 3′ ends of the fluoride-binding riboswitch aptamer from tRNA/Fluo. tRNA/Fluo was annealed to either one or both DNAzymes and the 18-nt oligonucleotide by snap-cooling. The mixture was then incubated in DNAzyme buffer for 48 h at RT. The products were analyzed by 10% (w/v) denaturing PAGE.

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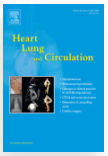

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