

Identification of Sequence-Selective Tyrosine Kinase Deoxyribozymes

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Abstract Deoxyribozymes (DNA enzymes) have been developed for a growing variety of chemical reactions, including with peptide substrates. We recently described the first tyrosine kinase deoxyribozymes, which lacked the ability to discriminate among peptide substrates on the basis of the amino acids surrounding the tyrosine residue. Those deoxyribozymes were identified by *in vitro* selection using a DNA-anchored peptide substrate in which the residues neighboring tyrosine were all alanine. Here, we performed *in vitro* selection for tyrosine kinase activity using three peptide substrates in which the neighboring residues included a variety of side chains. For one of these three peptides, we found numerous deoxyribozymes that discriminate strongly in favor of phosphorylating tyrosine when the surrounding residues are specifically those used in the selection process. Three different short peptide sequence motifs of 2–4 amino acids were required for catalysis by three unique deoxyribozymes. For a second peptide substrate, the selection process led to one deoxyribozyme which exhibits partial discrimination among peptide sequences. These findings establish the feasibility of identifying DNA enzymes that catalyze sequence-selective tyrosine phosphorylation, which suggests the downstream practical utility of such deoxyribozymes. More broadly, this outcome reinforces the conclusion that nucleic acid catalysts can discriminate

among peptide substrates in the context of biochemically relevant reactions.

Keywords Deoxyribozyme · *In vitro* selection · Peptide · Phosphorylation · Kinase · Post-translational modification

Introduction

Natural proteins are post-translationally modified in many ways at specific amino acid residues (Shahbazian and Grunstein 2007; Tiganis and Bennett 2007; Shi 2009; Tarrant and Cole 2009; Moremen et al. 2012; Moore and Gozani 2014). Using artificial catalysts to introduce covalent modifications into proteins with high peptide sequence selectivity is valuable for many biochemical investigations but is a significant challenge. Small-molecule reagents and catalysts for protein modification have been developed, but typically any accessible instance of the relevant side chain is modified without regard for its sequence context (Stephanopoulos and Francis 2011). The natural protein enzymes that catalyze protein modification can be subjected to directed evolution (Renata et al. 2015; Roiban and Reetz 2015) for higher rate constant and other improved practical characteristics (Currin et al. 2015), but achieving sequence selectivity that is not already present in the natural enzyme is often difficult (Yoo et al. 2012). Therefore, creating entirely new biomolecular catalysts for site-specific protein modification is an important objective.

Deoxyribozymes are catalytic DNA sequences that have been identified for various chemical reactions, including modification of amino acid side chains in peptide substrates (Silverman 2015). We previously reported the first tyrosine kinase deoxyribozymes (Walsh et al. 2013), which do not discriminate among peptide substrates on the basis of the

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amino acid identities near the reactive tyrosine residue. Tyrosine kinase deoxyribozymes capable of such discrimination will provide an efficient means for site-specific phosphorylation of peptides and proteins. In this study, we performed *in vitro* selection using three different peptide substrates that have a variety of amino acids near the reactive tyrosine, rather than only the alanine residues that were present in our previous selection experiments. For one of three peptides, the resulting deoxyribozymes were specific for tyrosine phosphorylation within that particular peptide sequence, but a different peptide motif was required by each deoxyribozyme. For a second peptide substrate used during selection, we observed partial discrimination among peptide sequences by the one resulting deoxyribozyme. These findings demonstrate that tyrosine kinase DNA enzymes can have the ability to differentiate among peptide substrates on the basis of their sequences.

Materials and Methods

All citations to “as described” refer to our previous report (Walsh et al. 2013). Oligonucleotides, peptides, and DNA-anchored peptide conjugates were synthesized and purified as described. The CADPYDQS and CMTGY-VAT peptide sequences correspond to amino acids 320–326 and 179–185, respectively, from the MAPK14/p38 α protein (Raignaud et al. 1995; Salvador et al. 2005), and the CKVIYDFI peptide sequence corresponds to amino acids 253–259 from the WASP protein (Yokoyama et al. 2005); each artificial *N*-terminal Cys was appended to allow conjugation. *In vitro* selection and cloning of individual deoxyribozymes was performed as described, except with an antibody-based capture step described below. Deoxyribozymes were assayed *in trans* as described, using incubation conditions of 70 mM HEPES, pH 7.5, 1 mM ZnCl₂, 20 mM MnCl₂, 40 mM MgCl₂, and 150 mM NaCl at 37 °C. For assays with the DNA-anchored CKVIYDFI substrate, in addition to 0.25 pmol of the 5′-³²P-radiolabeled substrate in each 20 μ L assay, also included were 20 pmol of an unrelated 5′-phosphorylated DNA oligonucleotide and 1 nmol of free peptide, to suppress loss of signal that we presume is due to nonspecific adherence of the substrate to the plastic microcentrifuge tube walls.

The antibody-based capture step was performed as follows, using 2 mL low-retention microcentrifuge tubes treated by allowing to stand overnight with 2 mL of 5 % (w/v) poly(ethylene glycol) (PEG, average molecular weight 3350, Aldrich cat. no. P3640) and rinsing with 3 \times 1 mL of water. All centrifugation steps were performed at 1000 $\times g$ for 1 min. After the selection step (37 °C, 14 h), the sample was precipitated with ethanol

and redissolved in a PEG-treated tube to 10 μ L containing 25 mM Tris, pH 7.2, 150 mM NaCl, 2 mM EDTA, 0.01 % Triton X-100, 50 pmol DNA oligonucleotide (AAC)₂₀ [as a sacrificial oligo to suppress adhesion of DNA pool molecules], 2 μ g pTyr antibody (Thermo Scientific Pierce PY20, cat. no. MA1-82787). To this sample was added 25 μ L of protein A agarose suspension (Thermo Scientific Pierce, cat. no. 20333), previously buffer-exchanged to water by centrifugation and washing (3 \times 500 μ L), where protein A binds to the constant Fc region of the PY20 antibody. The 35 μ L sample was shaken on a vortexer at lowest setting for 1 h at room temperature, transferred to a spin column, centrifuged, and washed with 3 \times 50 μ L of 25 mM Tris, pH 7.2, and 150 mM NaCl; the washes were combined into a single wash fraction. At this point, catalytic DNA pool sequences that phosphorylated their attached peptide are bound to the agarose-immobilized antibody, whereas other DNA sequences have been washed away. The bound sequences were centrifugally eluted with 3 \times 50 μ L of 8 M urea, which were combined into a single elution fraction (each portion of urea solution was allowed to stand in the spin column for 5 min at room temperature before centrifugation). Scintillation counting of the wash and elution fractions was used to quantify selection activity. The elution fraction was precipitated with ethanol and taken onward to the PCR step of the *in vitro* selection procedure.

To prevent enrichment of noncatalytic DNA sequences capable of binding either the pTyr antibody or the protein A agarose, an additional preclear step was included in every third round beginning with round 1. After the ligation step and prior to the selection step, the preclear step was performed using the same procedure as the capture step, except the wash fraction was taken onward to the selection step. When parallel selection experiments were performed omitting all preclear steps, no kinase activity was observed.

Results

In Vitro Selection of Tyrosine Kinase Deoxyribozymes by Anti-pTyr Antibody Binding

We sought tyrosine kinase deoxyribozymes that transfer the γ -phosphoryl group of a 5′-triphosphorylated RNA donor oligonucleotide (5′-pppRNA) to the tyrosine hydroxyl group within a peptide substrate, forming phosphotyrosine (Tyr \rightarrow pTyr; Fig. 1a). For this purpose, rather than use our previous PAGE-shift strategy (Walsh et al. 2013), we developed a new selection approach in which the key “capture” step used an immobilized anti-pTyr antibody to separate the catalytically active DNA sequences in each

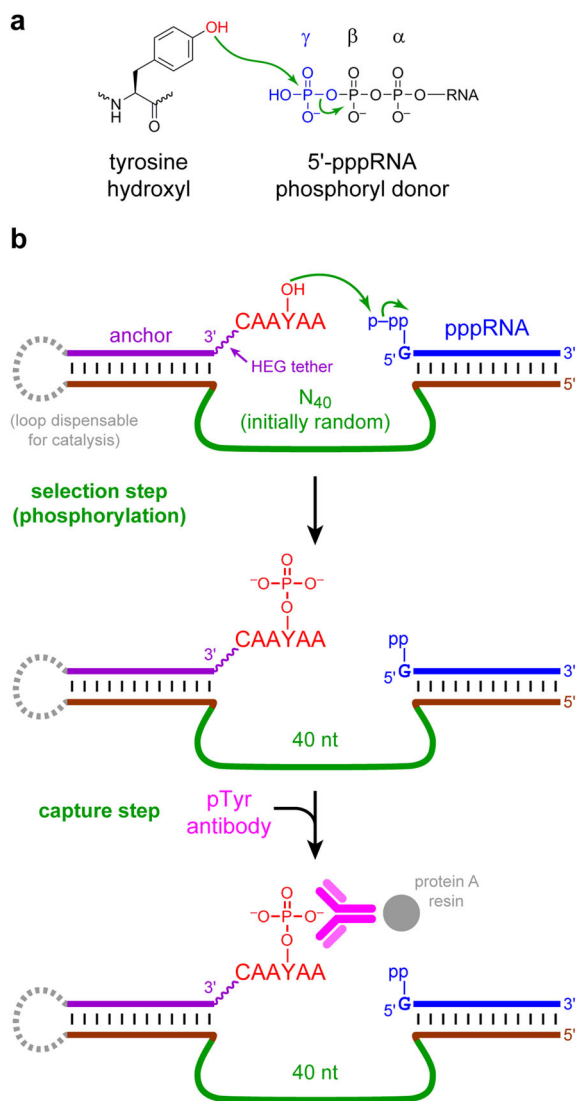


Fig. 1 **a** Tyrosine phosphorylation using 5'-pppRNA as the phosphoryl donor. **b** Deoxyribozyme selection strategy using an anti-pTyrosine antibody and a model peptide. After the capture step, DNA sequences are amplified by PCR and ligated to the DNA-anchored peptide substrate. Not depicted is a preclear step immediately prior to the selection step in every third round (see “Materials and Methods” section)

selection round (Fig. 1b). The antibody-based approach shortens the selection procedure and allows more samples to be processed in parallel.

Three tyrosine kinase selection experiments were performed, each using one of three octapeptides CADPYDQS, CMTGYVAT, or CKVIYDFI, each of which was derived from a natural pTyrosine-containing protein sequence (see “Materials and Methods” section). These more complex peptides were used here in place of the simple hexapeptide CAAYAA that we used previously (Walsh et al. 2013). Each octapeptide was anchored as a disulfide via its artificial *N*-terminal Cys residue through a hexa(ethylene

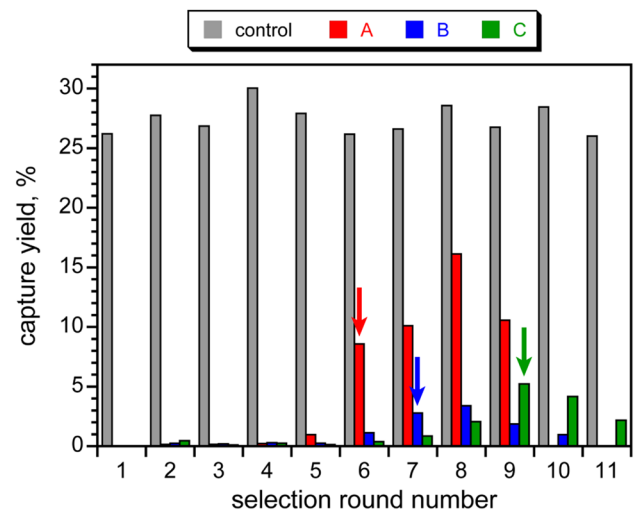


Fig. 2 Progression of the in vitro selection experiments. Control refers to a capture reaction performed each round using the CADPY^pDQS peptide ($Y^P = p\text{Tyrosine}$). A, B, and C refer to the N₄₀ CADPYDQS, N₈₀ CADPYDQS, and N₄₀ CMTGYVAT selections, which were halted after round 9, 10, and 11, respectively. Arrows denote the rounds at which individual deoxyribozymes were cloned from these three selections

glycol) (HEG) tether to a DNA anchor oligonucleotide, which interacted with a fixed-sequence DNA segment by Watson–Crick base pairs. To enable the selection process, the DNA anchor was additionally connected by a covalent loop to the deoxyribozyme pool, but this loop was found to be dispensable for catalysis by all individual deoxyribozymes. The 5'-pppRNA phosphoryl donor oligonucleotide interacted with the second fixed-sequence DNA segment by Watson–Crick base pairs. The length of the initially random region is an important variable in selection experiments (Velez et al. 2012). Our previous tyrosine kinase deoxyribozyme selections used N₃₀, N₄₀, and N₅₀ random regions, i.e., 30, 40, or 50 consecutive random nucleotides. Here, we used N₄₀ and longer N₈₀ random regions with each of the three octapeptides, for a total of six selection experiments.

For each selection experiment, the key tyrosine phosphorylation step was performed in 70 mM HEPES, pH 7.5, 1 mM ZnCl₂, 20 mM MnCl₂, 40 mM MgCl₂, and 150 mM NaCl at 37 °C for 14 h. Three of the six selection experiments (CMTGYVAT with N₈₀ and CKVIYDFI with either N₄₀ or N₈₀) showed no phosphorylation activity by round 11 and were discontinued. In contrast, the other three selections all showed phosphorylation activity (Fig. 2). From each of these three selections, individual deoxyribozymes were cloned and studied in more detail. All deoxyribozymes were assayed in trans, i.e., with the DNA-anchored peptide substrate not covalently attached to the deoxyribozyme (loop at far left of Fig. 1b absent).

Table 1 Sequences of the tyrosine kinase deoxyribozymes

Deoxyribozyme	Sequence
TyrKinA1	GTGAACGGCACAATATTAATATTTGCGACATCTGGAAGGC
TyrKinA2	GTACCAATTGAGGAGGCGGGCTCGTCACGAAATAGTGACG
TyrKinA3	GTGGGCGACGATACCAAGGTCAGGACCCTGGTAGAGCCGC
TyrKinB1	GGTGGCATAACCAGATCCGGTGCCACCAGGATGGGTTC CGAGTGAATAAGACAGTAGGCTACCACAGAAACGAGACG
TyrKinB2	GTGGAAGCGCACGTTCCACCGCAAATGCCCCAGATCCCCCA GATATCGTAGCCGTGGATGGTGAACAAGCGTGTCAAGTA
TyrKinC1	TGGGCGAAGTAAGCTTCTCAGGGGTGCACTGCACCGGTTT

Only the initially random (N_{40} or N_{80}) DNA region is shown, written in the 5'-to-3' direction. For all TyrKinA and TyrKinB deoxyribozymes, the fixed-sequence 5'-segment was CGAAGTATAAACCTGTTC, and the fixed-sequence 3'-segment was ATATTTGTATCCAGGA. For the TyrKinC1 deoxyribozyme, the fixed-sequence 5'-segment was CGAAATAGATTATCATTC, and the fixed-sequence 3'-segment was ATAATTAGTAACCTGA

Peptide Sequence Selectivities of Individual Tyrosine Kinase Deoxyribozymes

Three unique N_{40} and two unique N_{80} tyrosine kinase deoxyribozymes were identified using the CADPYDQS peptide substrate and designated TyrKinA1–3 and TyrKinB1–2, respectively (Table 1). Secondary structure predictions [mfold (Zuker 2003)] for each deoxyribozyme indicated 4–8 (N_{40}) or 10–18 (N_{80}) energetically similar structures (not shown). We have not attempted to distinguish or validate any of these structures experimentally, considering that doing so would provide only limited information about DNA tertiary structure, catalytic mechanism, and peptide sequence selectivity. MALDI mass spectrometry confirmed phosphorylation by each deoxyribozyme (Table 2), and k_{obs} values ranged from 0.05 to 0.11 h^{-1} (Fig. 3). All five deoxyribozymes were assayed with the three different peptide selection substrates, in all cases revealing phosphorylation activity only with the CADPYDQS substrate used during selection.

The peptide sequence dependence was dissected in greater detail for all five deoxyribozymes by testing mutants of the substrate (Fig. 4). As expected for tyrosine phosphorylation, mutation of tyrosine to phenylalanine abolished all kinase activity. In addition, mutation of tyrosine to serine also abolished activity, establishing that these deoxyribozymes are specifically tyrosine kinases. We then systematically and individually replaced with alanine all non-alanine amino acids surrounding the tyrosine (i.e., each of the five residues CADPYDQS, where Y becomes phosphorylated), seeking to identify the peptide sequence motif required for kinase activity. Interestingly, for the three highest-yielding deoxyribozymes, three different peptide sequence motifs were identified. In particular, for TyrKinA1, TyrKinA2, and TyrKinB1, the respective motifs were DPYD, DPY, and YD (more comprehensive substrate mutations were not evaluated). For the two remaining

deoxyribozymes, TyrKinA3 and TyrKinB2, motifs could not be assigned with confidence due to the lower yields.

In parallel to the above experiments, one unique N_{40} tyrosine kinase deoxyribozyme designated TyrKinC1 was found with the CMTGYVAT substrate (Tables 1, 2). This DNA enzyme discriminates poorly between the CMTGYVAT and CADPYDQS substrates (k_{obs} 0.089 h^{-1} and 79 % yield at 48 h vs. k_{obs} 0.059 h^{-1} and 60 % yield at 48 h, respectively), whereas activity was much lower with the CKVIYDFI substrate (k_{obs} 0.043 h^{-1} and only 11 % yield at 48 h; data not shown). This outcome indicates that TyrKinC1 has partial selectivity with regard to peptide sequence, although some generality is observed.

Discussion

In 2008, we reported the first DNA-catalyzed modification of an amino acid side chain in any context, forming a nucleopeptide linkage between a tyrosine side chain and an RNA oligonucleotide (Pradeepkumar et al. 2008). Since that initial study, we have expanded our efforts with deoxyribozymes to include a variety of side chain modifications (Silverman 2015). However, in all of these experiments, to date, we have previously reported only a single set of deoxyribozymes that discriminate among peptide substrates on the basis of sequence (Chu et al. 2014). Similar to that report, which involved nucleopeptide formation, here, we found that for tyrosine kinase activity, presenting a variety of side chains on the peptide substrate during selection can lead to deoxyribozymes that are peptide sequence selective. Our previous report used a discrete (not DNA-anchored) peptide substrate during selection, whereas here the selection process demanded use of a DNA-anchored peptide to allow identification of the catalytic DNA sequences. The greater preorganization associated with the anchor may place less of a burden on the

Table 2 MALDI mass spectrometry of the phosphorylated peptide products

Deoxyribozyme	[M+H] ⁺ calcd.	[M+H] ⁺ found	Error, % (found – calcd.)
TyrKinA1	7352.2	7352.4	+0.002
TyrKinA2	7352.2	7353.1	+0.009
TyrKinA3	7352.2	7353.0	+0.008
TyrKinB1	7352.2	7351.6	-0.006
TyrKinB2	7352.2	7349.6	-0.026
TyrKinC1	7304.3	7305.0	+0.007

Each DNA-anchored peptide was the sequence used during selection of the corresponding deoxyribozyme. Mass spectrometry was performed as described previously (Walsh et al. 2013), except after 48 h incubation, the crude sample was precipitated with ethanol and desalted using a Bio-Rad Micro Bio-Spin P-6 column before analysis

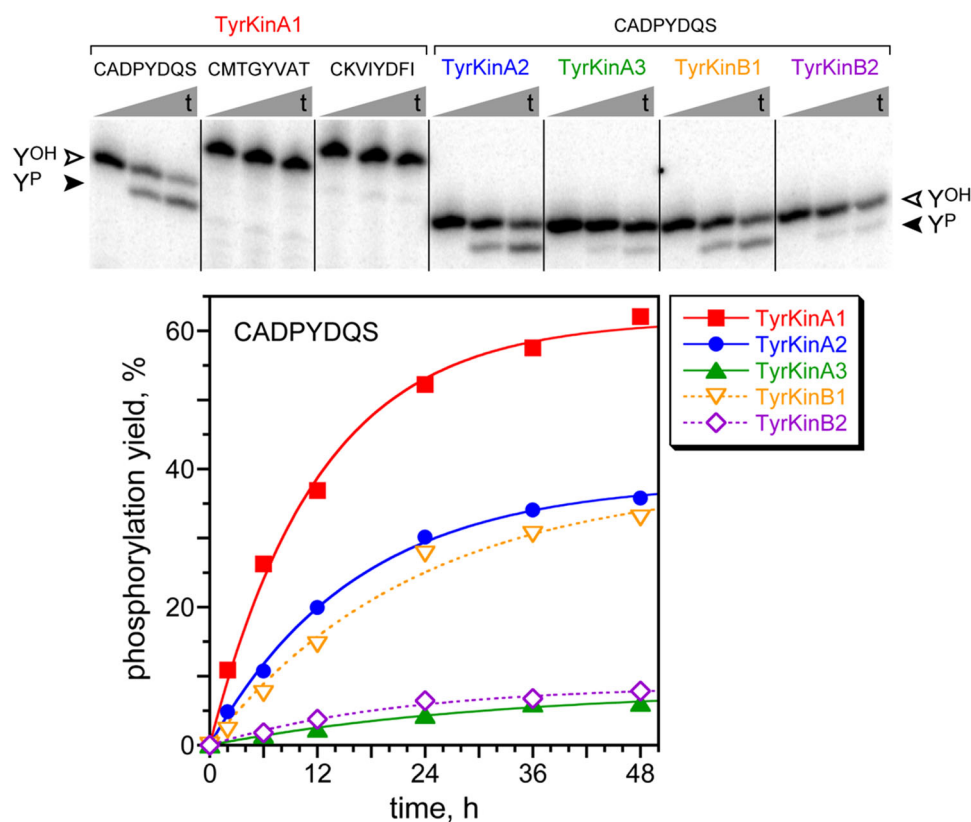


Fig. 3 Activities of sequence-selective tyrosine kinase deoxyribozymes. Each of 15 combinations of deoxyribozyme and DNA-anchored peptide substrate (five deoxyribozymes, three substrates) was evaluated under the incubation conditions used during selection (70 mM HEPES, pH 7.5, 1 mM ZnCl₂, 20 mM MnCl₂, 40 mM MgCl₂, and 150 mM NaCl at 37 °C). One representative PAGE image is shown ($t = 0, 12, 48$ h), including data for the TyrKinA1

deoxyribozyme with all three peptide substrates and data for the other four deoxyribozymes with the CADPYDQS peptide used during selection. As was observed for TyrKinA1, the other four deoxyribozymes had no activity with the two peptides that were not used during selection. Rate constants (k_{obs} in h^{-1} ; $n = 4$, mean \pm SD): TyrKinA1, 0.11 ± 0.02 ; TyrKinA2, 0.08 ± 0.02 ; TyrKinA3, 0.05 ± 0.02 ; TyrKinB1, 0.05 ± 0.01 ; TyrKinB2, 0.06 ± 0.01

DNA enzyme to interact tightly with the peptide, in turn reducing the opportunity for interactions that would promote sequence selectivity. Therefore, it is especially notable that the current study led to sequence-selective deoxyribozymes.

Considered together, the previous and current findings indicate that using peptide substrates of varied sequence during in vitro selection fosters emergence of sequence-selective DNA enzymes. The observation of three distinct peptide motifs (DPY_D, DPY_Y, and Y_D) for three

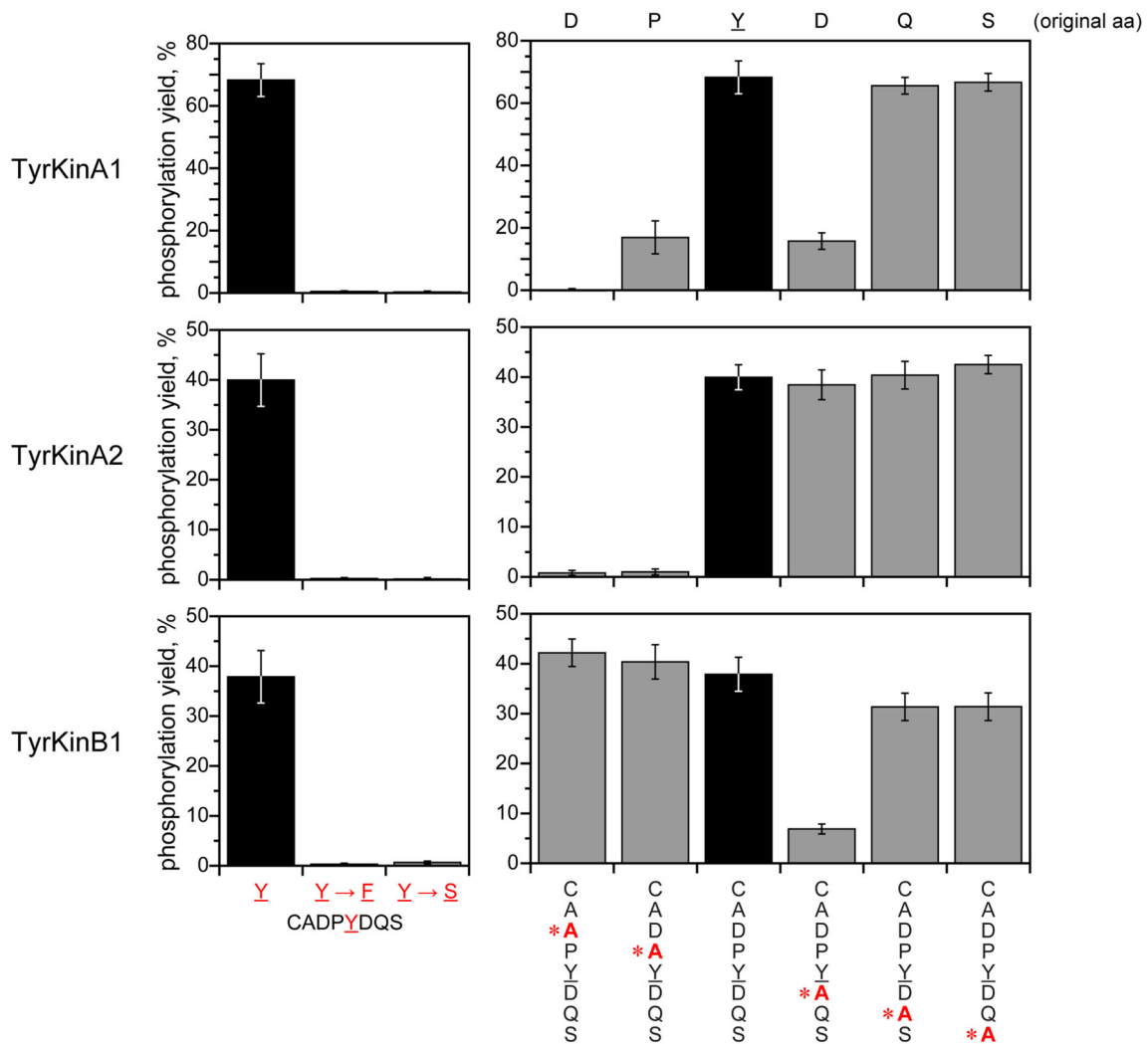


Fig. 4 Substrate mutations to dissect the peptide sequence requirements of the deoxyribozymes. Phosphorylation yields at 48 h are shown for TyrKinA1, TyrKinA2, and TyrKinB1, each with a series of DNA-anchored peptide substrates ($n = 5-8$ for original sequence

peptides and $n = 3$ for mutant peptides, mean \pm SD). Not shown are yields for the remaining two deoxyribozymes, which were $<10\%$ for all tested peptides including the unmutated sequence (Fig. 3)

different sequence-selective tyrosine kinase deoxyribozymes demonstrates experimentally that the DNA enzymes can interact in diverse ways with their peptide substrates. However, the details of these interactions (e.g., involving charge, hydrophobicity, and other side chain characteristics) and how they lead to peptide sequence selectivity is not yet defined by the available data. The short length of the peptide sequence motifs, 2–4 amino acid residues including the tyrosine to be phosphorylated, suggests the practical utility of kinase and other peptide-modifying DNA enzymes. To illustrate this point with a simple calculation, consider arbitrary protein sequences that comprise all 20 amino acids equally represented. A motif of two particular amino acids will be found at every one out of $20^2 = 400$

dipeptide groups within these proteins, enabling that degree of selectivity when using deoxyribozymes that require peptide motifs of two amino acids.

Nevertheless, the “rules” remain unclear for when a selection experiment with a particular peptide substrate will lead to sequence selectivity by the resulting DNA enzymes. In this study, three different peptides were used in parallel selection experiments, with three distinct outcomes: tyrosine phosphorylation selectivity for various peptide motifs; partial discrimination among peptide sequences; and no activity at all. In ongoing work, we are exploring in greater detail the relationship between the peptide substrate sequence and the selectivity of the emergent deoxyribozymes. None of the new tyrosine kinase deoxyribozymes was observed to function with a

discrete, non-DNA-anchored peptide substrate (data not shown), and pursuing such function is an ongoing focus.

The findings here can be placed in broader context of other functional nucleic acids. Aptamers are well known to discriminate in binding of peptide and protein substrates on the basis of their sequences (Tuerk and Gold 1990; Brody and Gold 2000; Nimjee et al. 2005; Shi et al. 2007; Cheung et al. 2013; Tan et al. 2013; Walters et al. 2014). In contrast, the ribosome—for which the active site is composed of RNA—operates essentially independently of the identity of the amino acid being attached to the growing peptide chain (Leung et al. 2011). The key difference between these scenarios is that aptamers are artificial sequences identified for their specificity, whereas the ribosome evolved to be a general protein synthesis machine. With that in mind, our current study highlights the principle that selection experiments lead to outcomes that are consistent with the selection design, also known as “you get what you select for” (Joyce 2004). By providing a variety of particular amino acid side chains that have the opportunity to interact with candidate DNA sequences, the resulting deoxyribozymes can—but not necessarily must—interact with those side chains to achieve catalysis. We are following this approach in many ongoing selection experiments that seek a range of DNA-catalyzed reactions of peptide and protein substrates.

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References

- Brody EN, Gold L (2000) Aptamers as therapeutic and diagnostic agents. *Rev Mol Biotechnol* 74:5–13
- Cheung YW, Kwok J, Law AW, Watt RM, Kotaka M, Tanner JA (2013) Structural basis for discriminatory recognition of *Plasmodium* lactate dehydrogenase by a DNA aptamer. *Proc Natl Acad Sci USA* 110:15967–15972
- Chu C, Wong O, Silverman SK (2014) A generalizable DNA-catalyzed approach to peptide-nucleic acid conjugation. *ChemBioChem* 15:1905–1910
- Currin A, Swainston N, Day PJ, Kell DB (2015) Synthetic biology for the directed evolution of protein biocatalysts: navigating sequence space intelligently. *Chem Soc Rev* 44:1172–1239
- Joyce GF (2004) Directed evolution of nucleic acid enzymes. *Annu Rev Biochem* 73:791–836
- Leung EK, Suslov N, Tuttle N, Sengupta R, Piccirilli JA (2011) The mechanism of peptidyl transfer catalysis by the ribosome. *Annu Rev Biochem* 80:527–555
- Moore KE, Gozani O (2014) An unexpected journey: lysine methylation across the proteome. *Biochim Biophys Acta* 1839:1395–1403
- Moremen KW, Tiemeyer M, Nairn AV (2012) Vertebrate protein glycosylation: diversity, synthesis and function. *Nat Rev Mol Cell Biol* 13:448–462
- Nimjee SM, Rusconi CP, Sullenger BA (2005) Aptamers: an emerging class of therapeutics. *Annu Rev Med* 56:555–583
- Pradeepkumar PI, Höbartner C, Baum DA, Silverman SK (2008) DNA-catalyzed formation of nucleopeptide linkages. *Angew Chem Int Ed* 47:1753–1757
- Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, Davis RJ (1995) Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J Biol Chem* 270:7420–7426
- Renata H, Wang ZJ, Arnold FH (2015) Expanding the enzyme universe: accessing non-natural reactions by mechanism-guided directed evolution. *Angew Chem Int Ed* 54:3351–3367
- Roiban GD, Reetz MT (2015) Expanding the toolbox of organic chemists: directed evolution of P450 monooxygenases as catalysts in regio- and stereoselective oxidative hydroxylation. *Chem Commun* 51:2208–2224
- Salvador JM, Mittelstadt PR, Guszczynski T, Copeland TD, Yamaguchi H, Appella E, Fornace AJ Jr, Ashwell JD (2005) Alternative p38 activation pathway mediated by T cell receptor-proximal tyrosine kinases. *Nat Immunol* 6:390–395
- Shahbazian MD, Grunstein M (2007) Functions of site-specific histone acetylation and deacetylation. *Annu Rev Biochem* 76:75–100
- Shi Y (2009) Serine/threonine phosphatases: mechanism through structure. *Cell* 139:468–484
- Shi H, Fan X, Sevilimedu A, Lis JT (2007) RNA aptamers directed to discrete functional sites on a single protein structural domain. *Proc Natl Acad Sci USA* 104:3742–3746
- Silverman SK (2015) Pursuing DNA catalysts for protein modification. *Acc Chem Res* 48:1369–1379
- Stephanopoulos N, Francis MB (2011) Choosing an effective protein bioconjugation strategy. *Nat Chem Biol* 7:876–884
- Tan W, Donovan MJ, Jiang J (2013) Aptamers from cell-based selection for bioanalytical applications. *Chem Rev* 113:2842–2862
- Tarrant MK, Cole PA (2009) The chemical biology of protein phosphorylation. *Annu Rev Biochem* 78:797–825
- Tiganis T, Bennett AM (2007) Protein tyrosine phosphatase function: the substrate perspective. *Biochem J* 402:1–15
- Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* 249:505–510
- Velez TE, Singh J, Xiao Y, Allen EC, Wong O, Chandra M, Kwon SC, Silverman SK (2012) Systematic evaluation of the dependence of deoxyribozyme catalysis on random region length. *ACS Comb Sci* 14:680–687
- Walsh SM, Sachdeva A, Silverman SK (2013) DNA catalysts with tyrosine kinase activity. *J Am Chem Soc* 135:14928–14931
- Walters RD, McSwiggen DT, Goodrich JA, Kugel JF (2014) Selection and characterization of a DNA aptamer that can discriminate between cJun/cJun and cJun/cFos. *PLoS One* 9:e101015
- Yokoyama N, Loughheed J, Miller WT (2005) Phosphorylation of WASP by the Cdc42-associated kinase ACK1: dual hydroxy-amino acid specificity in a tyrosine kinase. *J Biol Chem* 280:42219–42226
- Yoo TH, Pogson M, Iverson BL, Georgiou G (2012) Directed evolution of highly selective proteases by using a novel FACS-based screen that capitalizes on the p53 regulator MDM2. *ChemBioChem* 13:649–653
- Zuker M (2003) Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* 31:3406–3415