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Nonenzymatic Oligomerization of RNA by TNA Templates

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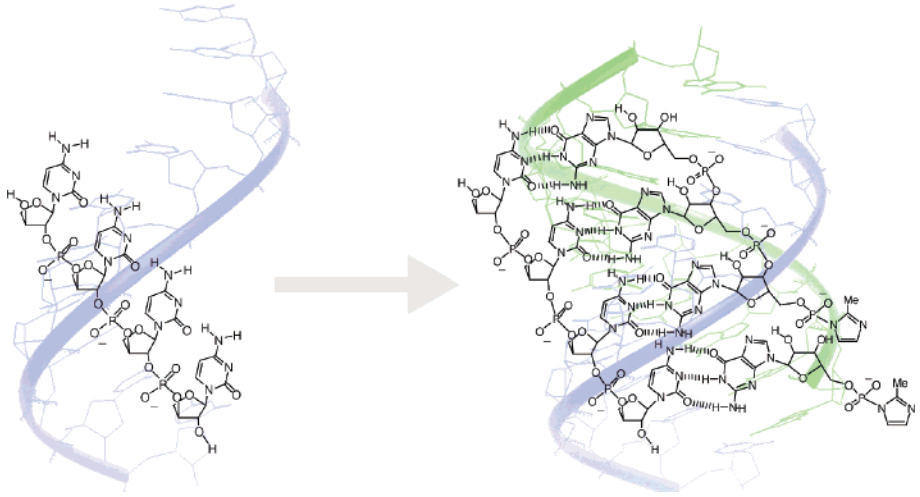
ABSTRACT



Cytosine TNA promotes nonenzymatic, template-directed oligomerization of complementary activated rGMP, leading to selective and efficient formation of RNA products. This process models "genetic takeover" of a pre-RNA by RNA.

Accurate transfer of genetic information is critical for the survival of an organism. In a prebiotic environment, however, the sophisticated enzymes that facilitate replication of modern nucleic acids would have been absent. As a result, prebiotic informational polymers need to access other, nonenzymatic, modes of replication. Yet, to date, RNA monomer synthesis via prebiotic model reactions has proven elusive, although several milestones have been attained in the area of ribose synthesis and stability.[1] Thus, a pre-RNA may have aided the transition from prebiotic materials to the RNA world.[2]

(L)-α-Threose nucleic acid,[3] TNA, is a variant of natural nucleic acids wherein ribose is replaced by a four-carbon sugar (Figure 1). TNA has the capacity to form stable duplex

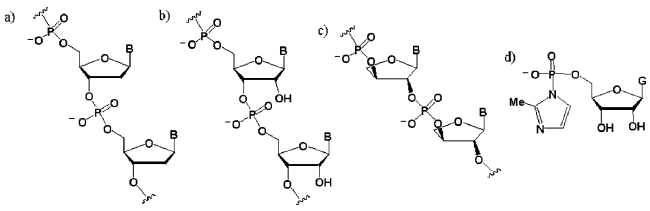


Figure 1. Structures of (a) DNA, (b) RNA, (c) TNA, and (d) 2-MeImpG.

structures with both DNA and RNA.[3] Further, threose is formed during condensation of simple aldehydes in prebiotic model reactions leading to ribose and allose.[1c,4] These observations raise the possibility that TNA may have been a precursor to RNA during molecular evolution.[2,5] In this work, we address the fitness of TNA by exploring its ability

(1) (a) Ricardo, A.; Carrigan, M. A.; Olcott, A. N.; Benner, S. A. *Science* **2004**, *303*, 196. (b) Springsteen, G.; Joyce, G. F. *J. Am. Chem. Soc.* **2004**, *126*, 9578. (c) Müller, D.; Pitsch, S.; Kittaka, A.; Wagner, E.; Wintner, C. E.; Eschenmoser, A. *Helv. Chim. Acta* **1990**, *73*, 1410.

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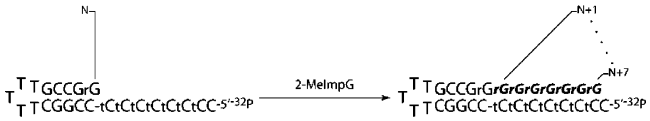
to serve as a template for nonenzymatic transfer of genetic information.[6,7] Specifically, we report successful nonenzymatic oligomerization of RNA on a TNA template (Scheme 1), a process that models genetic takeover[8] of a pre-RNA by RNA.

Scheme 1. Nonenzymatic, Template-Directed Oligomerization Reaction Conducted with Templates **1**−**3**



Template **3** from Figure 2 shown; tC = TNA-C, rG = ribo-G, all other residues = DNA.

Three hairpin templates with varying numbers of TNA residues were synthesized (Figure 2, left of panel) on an ABI-394 oligonucleotide synthesizer and purified by 20% denaturing PAGE.[9] Hairpin templates enable low concentrations of template/primers to be used without interference of off-template reactions.[6f] All three TNA-bearing templates were found to promote guanosine 5′-phosphoro-2-methylimidazole (2-MeImpG) oligomerization. As Figure 2 shows, oligomerization products are apparent after 3 days of incubation and continue to form up to day 10. The rates of oligomerization in Figure 2 depend on TNA content. Whereas oligomerization on template **1** (one TNA residue) appears to be all but complete after 3−5 days, templates **2** (two TNA residues) and **3** (seven contiguous TNA residues) require 5−10 days to achieve maximum oligomerization. Past work on hairpins appended with $(rC)_7$ and $(dC)_5$ templates showed the half-lives for disappearance of the templates to be 0.83[7d] and 3.0 h,[6f] respectively, for the oligomerization of 2-MeImpG. By plotting the disappearance of templates **1**, **2**, and **3** when incubated in the presence of 2-MeImpG, we estimate the half-lives as 14, 37, and 53 h, respectively.

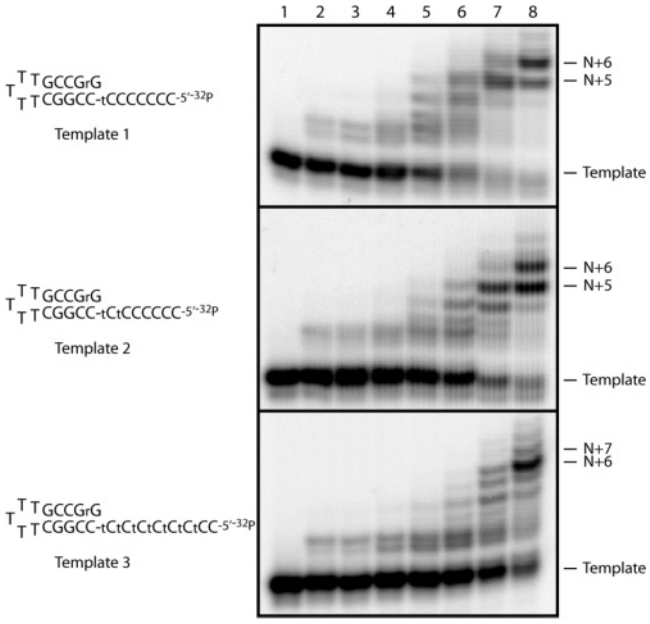


Figure 2. Time course study of nonenzymatic oligomerization reactions on ^{32}P-end-labeled TNA bearing templates **1**−**3** (tC = TNA-C, rG = ribo-G, all other residues = DNA). The extent of oligomerization was assessed at 30 min, 1 h, 4 h, 12 h, 1 day, 3 days, and 10 days for each template (lanes 2−8, respectively). Lane 1 is the template only. Oligomerization conditions were: 1.2 M NaCl, 200 mM 2,6-lutidine·HCl pH 8.0, 0.5 μM template, 100 mM 2-MeImpG and 200 mM $MgCl_2$, 5 °C. The oligomerization procedure from refs 6f and 7d,h was followed.

Oligomerization products may contain 2′,5′- phosphodiester linkages, 3′,5′-linkages, or a combination of the two. To determine the types of linkages present in our system, the reaction products from all three templates were treated with RNase T1.[6f,7d,h] The RNase T1 enzyme cleaves oligoribonucleotides specifically at the 3′-side of a 3′,5′- linked guanosine residue while leaving 2′,5′-linkages untouched. Figure 3 shows that RNase T1 treatment of oligomerization products from all three templates resulted in the cleavage of the first linkage formed to give the template plus a 3′-phosphate residue (comparison of lanes 1 and 4). Thus, the

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(8) Cairns-Smith, A. G. *Genetic Takeover and the Mineral Origins of Life*; Cambridge University Press: Cambridge, 1982.

(9) *N*4-Benzoyl-1-{2′-*O*-[(2-cyanoethoxy)(diisopropylamino)-phosphino]-3′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-α-L-threofuranosyl}-cytosine was synthesized according to the reported procedure.[3b] Templates were synthesized on an ABI 394 DNA synthesizer using standard protocols. Guanosine 5′-phosphoro-2-methyl-imidazolide was prepared from guanosine 5′-monophosphate according to the reported procedure: Joyce, G. F.; Inoue, T.; Orgel, L. E. *J. Mol. Biol.* **1984**, *176*, 279.

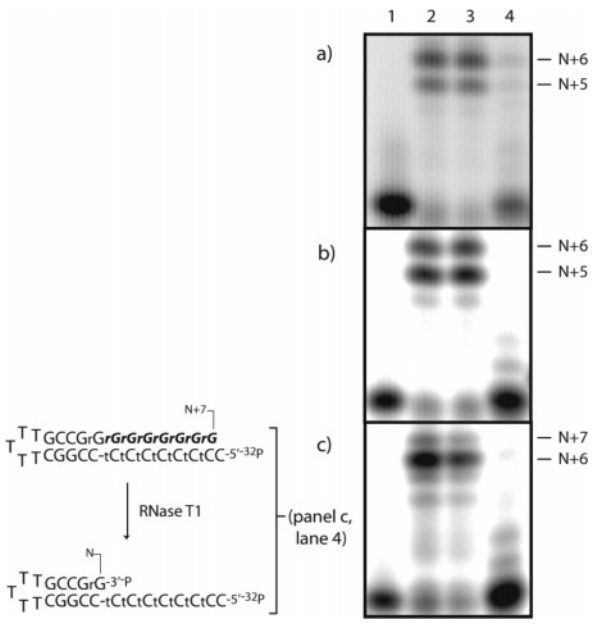

Figure 3. RNase T1 digests of oligomerization products from templates **1**, **2**, and **3** (panels a, b, and c, respectively). Lanes contain: template only (lane 1), oligomerization products only (lane 2), oligomerization products and buffer with no enzyme (lane 3), and oligomerization products, buffer, and RNase T1 (lane 4).

first phosphodiester bond formed during oligomerization on all three templates is 3′,5′-linked.

Pyrophosphate byproducts may form when 2-MeImpG reacts with the terminal 5′-phosphate instead of the terminal 2′/3′-hydroxyl group of a hairpin template. Pyrophosphate byproducts may be revealed by treating the products from an oligomerization reaction with calf intestinal alkaline phosphatase (CIAP). In the assay, hairpin templates whose 5′-^{32}P group has undergone pyrophosphate bond formation with 2-MeImpG will be protected from CIAP and revealed by subsequent PAGE autoradiographic analysis, whereas those hairpin templates retaining a free 5′-^{32}P group after 10 day incubation will have this group cleaved by CIAP and be invisible to autoradiography. When the oligomerization products from all three templates were treated with CIAP, all product and template bands disappeared leaving very minimal amounts of pyrophosphate byproducts to be observed (data not shown).

Although the above results demonstrate the viability of TNA as a template for nonenzymatic oligomerization, base-pairing fidelity is necessary for the transfer of genetic information. Toward assessing the latter, 2-MeImpA was incubated with template **3** for 10 days under the same conditions as before. Minimal oligomerization was observed, and no formation of full-length product was seen even after a 10 day incubation period (Figure 4, lane 1). 5′-AMP is known to associate via stacking interactions in the absence of a template.[10] The small extent of observed oligomerization

(10) Morcillo, J.; Gallego, E.; Peral, F. *J. Mol. Struct.* **1987**, *157*, 353.

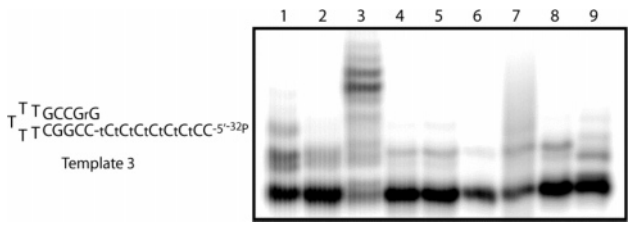

Figure 4. Evaluation of template-directed oligomerization fidelity and divalent ion performance using template **3**. Conditions: 10 day reaction time, 1.2 M NaCl, 200 mM 2,6-lutidine·HCl pH 8.0, 0.5 μM template **3**, 100 mM 2-MeImpA (lane 1), 100 mM 2-MeImpG (lanes 2−9), 5 °C. Divalent metal ion content [200 mM], lanes 1−9: $MgCl_2$, no divalent ions, $MgCl_2$, $MnCl_2$, $CoCl_2$, $Ni(NO_3)_2$, $ZnSO_4$, $HgCl_2$, $Pb(NO_3)_2$.

may be attributed to nucleation of intrinsic 2-MeImpA stacking at the hairpin template 3′-G terminus and subsequent bond formation, possibly enhanced by weak interactions with the template.

Effects of different divalent ions on oligomerization were explored with template **3** incubated in the presence of 2-MeImpG. The results are shown in Figure 4, lanes 2−9. Full-length oligomerization products were only observed when the reaction contained $MgCl_2$. All other divalent ions used essentially led to no oligomerization.

Our results demonstrate that TNA is capable of nonenzymatic, template-directed oligomerization of RNA, a necessary capability of pre-RNA. In past work, PNA has been investigated as a pre-RNA candidate, showing both a capacity for formation from plausible prebiotic chemicals[11] and template-directed RNA oligomerization.[7b,12] Although rates of both PNA[7b,12] and TNA template-directed oligomerization are less than that of DNA or RNA templates, it is notable that the 2-methylimidazole leaving group was optimized for the latter polymers.[13] Nevertheless, the data reported here indicate that TNA rivals PNA in effectively promoting template-directed synthesis of RNA. Further studies exploring nonenzymatic replication with TNA are ongoing.

Acknowledgment. Funding for this work from the National Aeronautics and Space Administration is gratefully acknowledged.

Supporting Information Available: Spectra of all synthetic compounds and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Schmidt, J. G.; Nielsen, P. E.; Orgel, L. E. *Nucl. Acids Res.* **1997**, *25*, 4797.

(13) Other factors appear superficially to favor TNA over DNA as a template for RNA, including A-form structure[3c,7i] and association thermodynamics of mixed base TNA/RNA sequences.[3b] However, TNA homooligomers of the general type used in the present study (e.g., tPyr/rPur) have been shown to have anomalously low duplex stabilities.[3b]