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2′,5′-Linked DNA Is a Template for Polymerase-Directed DNA Synthesis

Surajit Sinha, Paul H. Kim, and Christopher Switzer*

Department of Chemistry, University of California-Riverside, Riverside, California 92521

Received March 4, 2003; E-mail: switzer@mail.ucr.edu

Genomes are composed of nucleic acids bearing 3′,5′-phosphodiester-linked sugars. The 2′,5′-phosphodiester linkage is a fundamental alternative to the natural linkage (Figure 1) and is the predominant product of most nontemplate, nonenzymatic oligomerizations of nucleotide monomers.[1] This fact suggests a chemical bias for the formation of 2′,5′-phosphodiester bonds, an important consideration in the context of molecular evolution on Earth or elsewhere.

Although 2′,5′-linked RNA occurs naturally,[2] it is evidently not used to encode genetic information. Nevertheless, the capacity of the 2′,5′-phosphodiester linkage to serve as a genetic material has been probed in laboratory experiments. Initial studies examined both self-pairing and pairing with natural nucleic acids. These studies showed that while 2′,5′-linked DNA and RNA form double and triple helices with themselves,[3] their interactions with natural nucleic acids are asymmetric—pairing is seen with 3′,5′-linked RNA but not DNA.[3g,4] Important differences in structure between 2′,5′- and 3′,5′-linked DNA helices were revealed by NMR,[3c] and some of these may account for the apparent inability of 2′,5′-linked DNA to form stable helices with natural DNA.

While the ability to form a stable structure (such as a helix) is likely a necessary condition for nonenzymatic information transfer,[5] in enzymatic reactions this precondition may be expected to be lifted in cases where the enzyme can serve as a "template for the template" and make up for structural deficiencies that would be fatal to its abiotic counterpart. In support of this suggestion, we demonstrate that 2′,5′-linked DNA is a template for enzymatic synthesis of natural DNA with several DNA polymerases and reverse transcriptases directing the incorporation of all four natural nucleotides with fidelity.

Initial investigation of the template properties of 2′,5′-linked DNA was conducted on a U′4 template in the presence of different metal ions with two polymerases as shown in Figure 2. Divalent metal ions are required for polymerase activity, generally Mg^{2+}. In the past other divalent ions have been shown to substitute for magnesium, leading to increased nucleotide incorporation error rates[6] and the acceptance of nonstandard substrates by polymerases.[7] Owing to the anticipated nonstandard orientation of atoms 2′,5′-linked DNA would present to polymerases, the use of nonstandard divalent ions was seen as a ready means of altering active-site structure in a way that it might adapt to its new substrate. Of the two polymerases screened initially, two catalyzed the formation of significant amounts of product, Klenow-exo- and HIV reverse transcriptase (RT), and weak product formation was seen for a third, MMLV RT (data not shown). Comparing activity of Klenow-exo- and HIV RT in the different metal ions, it may be seen that the highest activity occurred with Mg^{2+} and Mn^{2+}. With the latter metal ion and Klenow-exo-, full-length product ($n+4$) is produced nearly exclusively (Figure 2, left panel, lane 4).

With activity of the 2′,5′-linked DNA template established for two polymerases, and Mn^{2+} found to be optimal in both cases, the ability of a mixed-sequence template to be copied by a wider variety
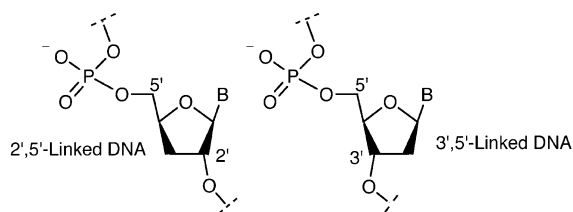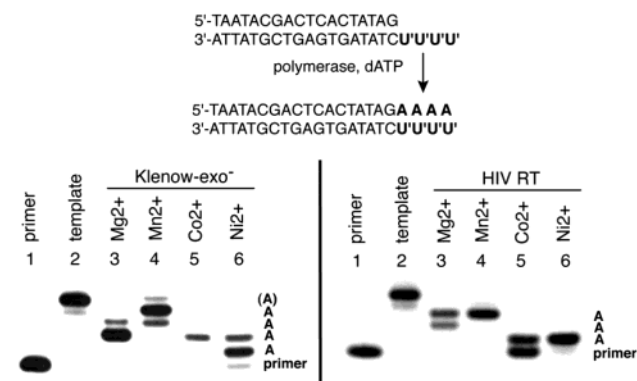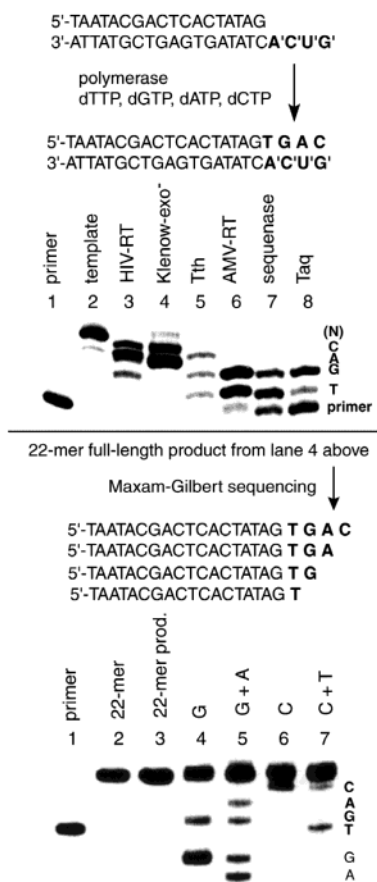

Figure 1.

Figure 2. Denaturing 20% PAGE assay of template activity for 2′,5′-linked-3′-dU4 (U′4) with various polymerases and divalent ions. All substrate oligomers were prepared on an ABI 394 synthesizer. Reactions were carried out with 5′-^{32}P-labeled primer under standard conditions for a given polymerase with the exception of divalent metal ions, the amount of enzyme, and incubation time. Nonstandard divalent ions were used at 1.0 mM for reverse transcriptases and 1.5 mM for DNA polymerases.[6] Additional conditions were: Klenow-exo- 2 h, 5 units of enzyme; HIV RT 2 h, 5 units; MMLV RT 2 h, 15 units.

of polymerases was next investigated. Figure 3 (top panel) shows the activity of six polymerases with the mixed-sequence 2′,5′-linked DNA template A′C′U′G′. While every polymerase directed the incorporation of at least two product nucleotides, only three polymerases incorporated three or more nucleotides, Tth, HIV RT, and Klenow-exo-. The highest activity was observed with Klenow-exo- and HIV RT, similar to the U′4 template, where up to four nucleotides were incorporated to provide full-length product (the major bands in lanes 3 and 4 are $n+3$ and $n+4$).

A critical question to be answered in light of the observed polymerase activity on 2′,5′-linked DNA templates concerns the fidelity of nucleotide incorporation into the product strand. To address this question, the full-length 22-mer product from the Klenow-exo-catalyzed copying of the A′C′U′G′ 2′,5′-linked DNA template (Figure 3, top panel, first dark band from the top of lane 4) was Maxam–Gilbert sequenced. The results of the sequencing experiment are shown in the lower panel of Figure 3. As is evident from examination of lanes 4–7 of the sequencing experiment, no misincorporation is observed—the nonstandard template faithfully directs the incorporation of cognate bases according to canonical pairing rules. An identical sequencing experiment was also

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Figure 3. Denaturing 20% PAGE assay of template activity for 2′,5′-linked-3′-dACUG (A′C′U′G′) with various polymerases in the presence of Mn^{2+}, top panel, and Maxam—Gilbert sequencing of the full-length 22-mer product strand produced by Klenow-exo- (lane 4 of top panel), bottom panel. Lane 2 of the bottom panel contains authentic machine-synthesized 22-mer product; lane 3 contains full-length product from lane 4 of the top panel. Manganese ions were used as the divalent metal (in all cases except Tth which employed a standard Mg^{2+}/Mn^{2+} mixture for this enzyme) at concentrations of 1.0 mM for reverse transcriptases and 1.5 mM for DNA polymerases.[6] Additional conditions were: HIV RT 2 h, 10 units of enzyme; Klenow, same as Figure 2; Tth, 6 h, 10 units; AMV RT, 2 h 10 units; sequenase and Taq 30 min, 10 units.

conducted on the product 1-nt short of full length (21-mer) from the Klenow-exo- catalyzed copying of the A′C′U′G′ 2′,5′-linked DNA template (Figure 3, top panel, second dark band from the top of lane 4), and the correct product sequence was again observed (see Supporting Information).

Limitations on the extent to which a 2′,5′-linked DNA template may be copied were investigated on a template consisting of U′₇, employing the same sequence for the primer region found for substrates in Figures 2 and 3. The primer was extended by 5-nts maximally on the U′₇ template using Klenow-exo-.

Nucleic acid polymerases are primarily responsible for maintaining the integrity of genetic material and provide fidelity equal to $\sim10^3$ in the absence of an exonuclease. Despite their high fidelity, polymerases can be flexible in terms of their substrates. Nonstandard bases,[8] base pairs,[9] and backbones[7,10] all may be accommodated. We suggest that the ability of polymerases to accept 2′,5′-linked DNA as a template lies in their ability to serve as a template themselves and, in so doing, to cause the nonstandard DNA to conform to elements of native DNA structure. This mechanism also provides an explanation for observed limits of activity[11]—when deformation energy of the 2′,5′-linked DNA exceeds compensating binding energy to the polymerase, the nonstandard DNA is released.

Since Klenow enzyme is known to bind duplex DNA via the phosphate backbone,[12] it is further tempting to suggest that phosphates are not optimally positioned within the chimeric DNA domain, although we are unable to rule out other possible effects at this time.

In summary, the enzymatic transcription of 2′,5′-linked DNA into natural DNA occurs despite the inability of these two oligonucleotide systems to form a double helix in solution.[13] This observation suggests it may be possible to evolve efficient enzymes from extant polymerases.[14,15] Such enzymes would enable the fitness of 2′,5′-linked DNA to be assessed at a new level.[16]

Supporting Information Available: Figure depicting autoradiogram for Maxam—Gilbert sequencing of the 21-mer product and a Table summarizing melting temperature data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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