Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1816–1820

# Dda helicase unwinds a DNA–PNA chimeric substrate: Evidence for an inchworm mechanism

Travis L. Spurling, Robert L. Eoff and Kevin D. Raney*

*Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA*

Received 12 November 2005; revised 2 January 2006; accepted 5 January 2006
Available online 24 January 2006

**Abstract**—Helicases are ubiquitous enzymes involved in all aspects of DNA metabolism including replication, repair, recombination, and transcription. The mechanism of the bacteriophage T4 Dda helicase was investigated by preparing a DNA–PNA chimeric substrate. Surprisingly, Dda was able to unwind a substrate containing 12 PNA moieties in the loading strand of the enzyme. We suggest a mechanism whereby the Dda helicase contains two distinct DNA binding domains which allow an inchworm mechanism for translocation. A single step of the enzyme is sufficient to unwind the DNA–PNA chimera because several base pairs melt spontaneously due to thermal fraying. Hence, Dda helicase can unwind the substrate without actually translocating along the PNA.


Helicases are enzymes that unwind duplex DNA to provide ssDNA intermediates during DNA replication, recombination, and repair.[1,2] An inchworm mechanism has been suggested for some helicases whereby two DNA binding sites are contained on the enzyme.[3,4] The inchworm mechanism requires that one binding site maintain contact with the DNA while the other binding site moves along the lattice in a process driven by ATP binding and hydrolysis. The mechanism of Dda helicase from Bacteriophage T4 was investigated in this study. Dda can function as a monomeric helicase during unwinding of short oligonucleotide substrates, suggesting that an inchworm mechanism might apply to this enzyme.[5,6]

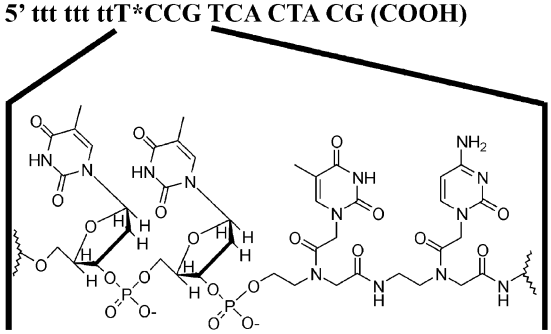
In vitro, most helicases must initiate unwinding by first binding to a region of ssDNA. These enzymes translocate unidirectionally into the duplex region resulting in separation of the two strands. The strand on which the helicase translocates is referred to as the loading strand, whereas the complementary strand is called the displaced strand. The mechanism for unwinding by Dda was previously investigated by preparing a substrate in which the displaced DNA strand was substituted with a strand of PNA, forming a DNA–PNA hybrid. Standard PNAs are achiral, electrostatically neutral, chemically and biologically stable, and synthetically compatible with standard solid-phase chemistry protocols.[7,8] PNAs have demonstrated sequence-specific, efficient in vitro inhibition of telomerase activity,[9] HIV replication,[10] bacteria multiplication,[11] ribosomal RNA function,[12] eukaryotic RNA translation,[13] and DNA polymerase activity.[14] Dda helicase was able to unwind the DNA–PNA hybrid substrate at rates that were similar to that of a normal DNA substrate.[15] Subsequent studies indicated that Dda does not bind tightly to PNA.[16] These results support a mechanism whereby Dda unwinds the DNA–PNA hybrid substrate by translocating along the loading strand of DNA and stripping away the complementary PNA strand through steric interactions that force apart the duplex; that is, a snowplow or wirestripper mechanism.[17,18] More recently, a DNA–PNA–DNA chimera was prepared as a substrate for Dda.[19] Monomeric Dda was unable to unwind this substrate, indicating that a single PNA moiety in the loading strand is able to block the monomeric form of the helicase.

In this report, a DNA–PNA chimera has been utilized as a helicase substrate. We created a substrate that contains 8 nt of ssDNA that serves as a loading site for the helicase. Adjacent to the loading site are 12 PNA moieties which were hybridized to a complementary 12mer of ssDNA to complete the substrate (Fig. 1). We have previously shown that a normal DNA substrate containing 12 bp is readily unwound by Dda.[6]

*Abbreviations*: MMT, monomethoxytrityl; DMT, dimethoxytrityl; PNA, peptide nucleic acid; ss, single stranded; ds, double stranded.
*Keywords*: Helicase; Peptide nucleic acids; PNA; DNA–PNA chimera; Unwinding.
* Corresponding author. Tel.: +1 501 686 5244; e-mail: raneykevind@uams.edu

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.01.013

**A** 5' ttt ttt ttT*CCG TCA CTA CG (COOH)



**B** 20Chimera:12DNA (DNA-PNA chimera substrate)

```
5′ ttt ttt ttT*CCG TCA CTA CG (COOH)
          3′a ggc agt gat gc 5′
```

**30DNA:15DNA (normal DNA substrate)**

```
5′ ttt ttt ttt ttt ttt ctg tcc tgc atg atg 3′
                   3′ gac agg acg tac tac 5′
```

**30DNA:15PNA (DNA-PNA hybrid substrate)**

```
5′ ttt ttt ttt ttt ttt ctg tcc tgc atg atg 3′
                (COOH) GAC AGG ACG TAC TAC (Nh2)
```

**Figure 1.** Substrates designed for investigating the mechanism of Dda helicase. (A) Schematic illustration of the DNA–PNA chimera (uppercase letters = PNA; lowercase letters = DNA). The structure of the PNA linker and a PNA moiety are shown at the interface with the DNA sequence. (B) A substrate for the helicase was prepared by hybridizing the chimera to a complementary strand of DNA to form the chimera:DNA substrate. A well-characterized DNA substrate served as a positive control and a DNA–PNA hybrid was examined to compare the PNA location in the loading strand or in the displaced strand of the helicase substrate.

*Synthesis of PNA monomers.* The DNA–PNA chimera was prepared using PNA monomers containing protecting groups that are compatible with DNA synthesis (Fig. 2).

Synthesis of the PNA backbone was performed by a modified two-step reaction sequence.[20] The reaction between ethylenediamine as solvent and monomethoxytrityl chloride proceeded in 30 min to the desired protected ethylenediamine (Scheme 1A, compound **3**). Refluxing (**3**) with methyl bromoacetate in methylene chloride for 2.5 h followed by flash chromatography gave the desired standard aminoethyl glycine PNA backbone

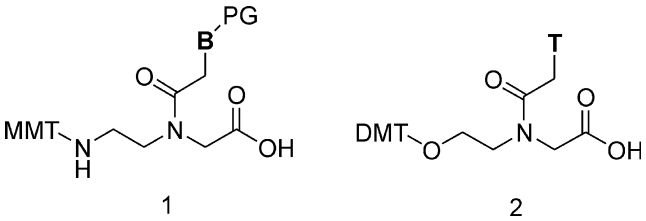
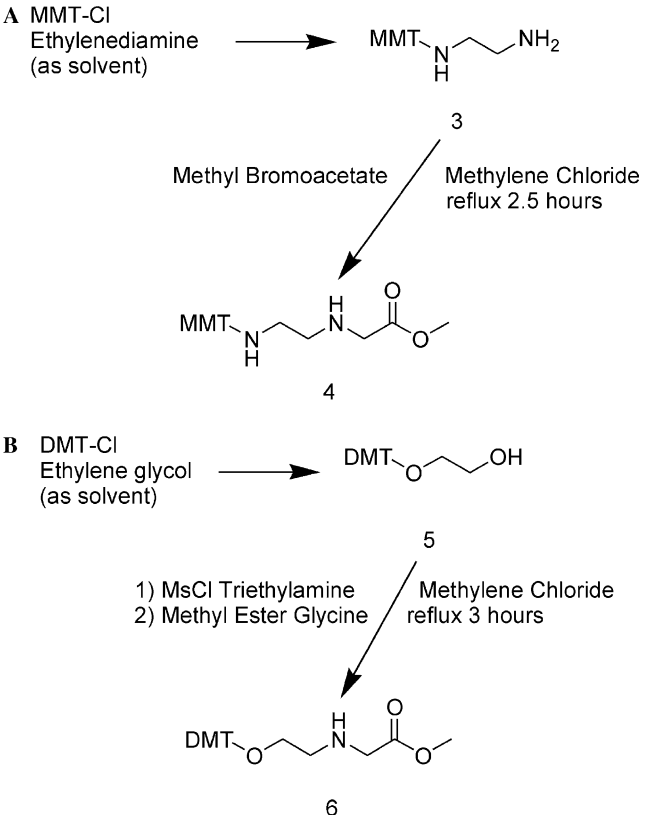


**Figure 2.** PNA monomers (**1**), PNA–DNA linker monomer (**2**).




**Scheme 1.** (A) MMT-protected aminoethyl PNA glycine backbone synthesis. (B) DMT-protected hydroxyl ethyl PNA–DNA linker backbone synthesis.

(**4**) in moderate yield (41%) for two steps overall (Scheme 1A).

A PNA–DNA linker has also been developed to connect DNA to a PNA strand.[21] In place of the standard aminoethyl glycine backbone, the PNA–DNA linker backbone possesses a hydroxyethyl glycine unit. Synthesis of the PNA–DNA linker backbone requires a third step but only two pots, and the reaction time is only slightly longer. Dimethoxytrityl chloride was added to ethylene glycol as solvent (Scheme 1B). After 30 min, the protected ethylene glycol (**5**) was extracted with ethyl acetate/water. Mesyl chloride with triethylamine was used to convert the alcohol to a good leaving group in less than an hour followed by removal of solvent and addition of triethylamine and methyl ester glycine to the same pot. Refluxing for 3 h gave the desired DMT-protected hydroxyethyl glycine, PNA–DNA linker backbone. Column purification gave the pure backbone (**6**) in good yield (61%) for three steps. The backbones (**4** and **6**) were used to make standard PNA monomers (**1**) and the PNA–DNA linker (**2**) using previously published procedures.[22]

*Solid-phase synthesis of the DNA–PNA chimeric molecules.* The chimeric sequence was designed to serve as mechanistic probe for DNA unwinding by the bacteriophage T4 helicase, Dda. Dda (DNA dependent ATPase) is classified as a superfamily I helicase that has a 5′ to 3′ directional bias for unwinding. Dda binds to the

5′-ssDNA region of the substrate and then translocates into the duplex. We have previously prepared PNA strands by manual synthesis.[23] However, numerous attempts to manually prepare the 20mer DNA–PNA chimera failed. The successful synthesis of the chimera was performed on a modified Expedite 8909 DNA Synthesizer (see Supplementary materials for details). Following synthesis, the chimera was cleaved from the resin and deprotected by treatment with concentrated anhydrous methanolic ammonia. Size-exclusion chromatography and denaturing polyacrylamide gel electrophoresis were then used to remove any truncated species. The identity of the chimera was confirmed by MALDI-TOF mass spectrometry (predicted mass = 5736.7 g/mol; measured $m/z$ = 5737.8 $[M+Na+H]^{1+}$).

*Dda helicase-catalyzed unwinding of the DNA–PNA chimera.* The 20mer chimeric sequence was designed to allow an eight base ssDNA overhang for binding to the helicase and a 12 base duplex after hybridization with a 12 nt complementary strand. The question of whether unwinding could occur when the enzyme encountered the PNA was examined. Three different substrates were compared for DNA unwinding by Dda helicase. In addition to the aforementioned DNA–PNA chimera, a well-characterized, normal DNA substrate was chosen as a positive control (Fig. 1B). The positive control substrate contains a 15 nt ssDNA overhang and 15 bp, and was previously shown to serve as an excellent substrate for Dda helicase.[15] A DNA–PNA hybrid substrate was also examined in which the loading strand contains only DNA while the complementary strand contains PNA (Fig. 1B). For each experiment, the substrate (100 nM) was incubated in unwinding buffer with Dda helicase (1 nM) under steady state conditions (excess substrate). These conditions strongly favor binding of one Dda molecule per substrate. As expected, the normal DNA duplex was readily unwound by the helicase (Fig. 3D, diamonds). We previously reported that the DNA–PNA hybrid serves as a substrate for Dda helicase under conditions in which the enzyme is in excess of the substrate.[15] Excess enzyme conditions favor binding of more than one Dda helicase molecule to the substrate. When substrate is in excess, only one molecule of Dda helicase binds to the substrate, but when the enzyme is in excess, multiple molecules of Dda can bind to the substrate depending on the length of the ssDNA overhang.[19,24] In Figure 3D, we show that the same DNA–PNA hybrid also serves as a substrate under conditions in which the substrate is in great excess over the enzyme, favoring the monomeric form of Dda helicase (Fig. 3D, open squares). Finally, the DNA–PNA chimera was found to serve as a substrate for DNA unwinding by Dda helicase (Fig. 3D, filled circles). The rate of unwinding of the DNA–PNA chimera substrate was very similar to that of the normal DNA substrate and the DNA–PNA hybrid substrate. In light of the previous results that show little or no affinity between Dda helicase and PNA,[16] and previous results indicating that monomeric Dda cannot translocate passed a single PNA moiety,[19] unwinding of the DNA–PNA chimera is most surprising.

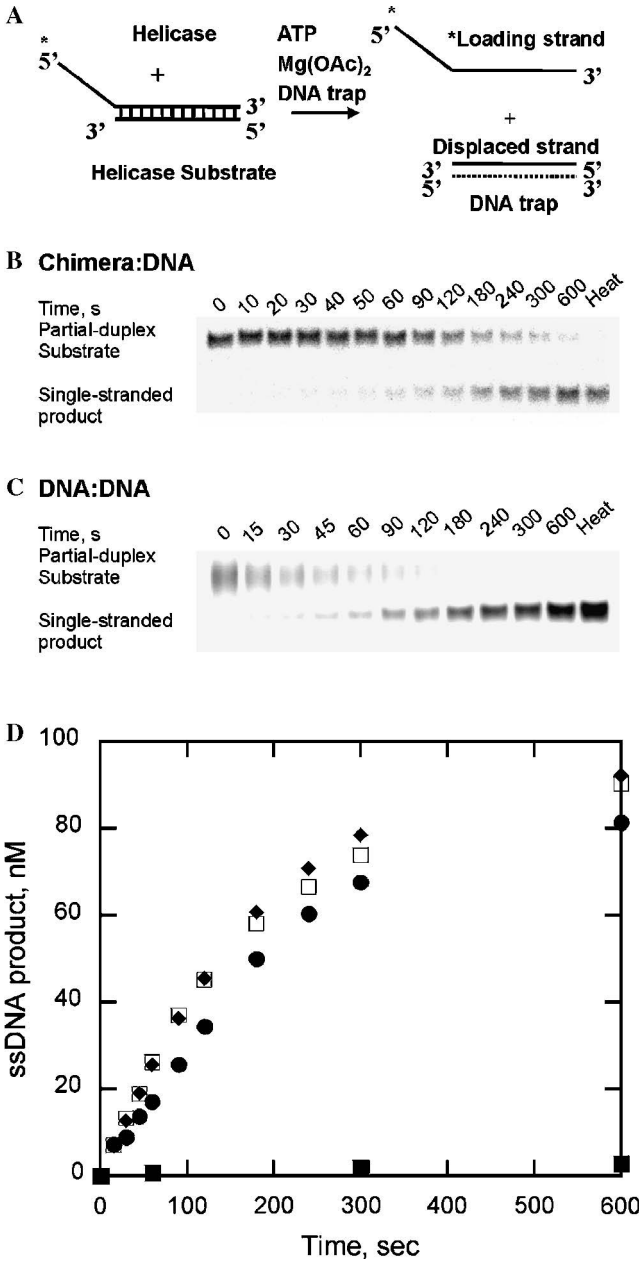



**Figure 3.** Helicase-catalyzed DNA unwinding. (A) Radiolabeled DNA substrate is incubated with helicase followed by initiation of the reaction by addition of ATP, $Mg(OAc)_2$, and DNA trap. The DNA trap prevents re-annealing of the ssDNA products. One hundred nanomolar substrate was incubated with 1 nM Dda helicase and aliquots were quenched at varying times by addition of 400 mM EDTA. (B) Helicase-catalyzed unwinding was measured by electrophoretic separation of ssDNA from dsDNA on a native polyacrylamide gel for the chimera substrate (B) or the normal DNA substrate (C) at increasing times. One lane (heat) in each image was produced by heating an aliquot of the reaction mixture to 95 °C, followed by slow cooling to room temperature. (D) Unwinding of the DNA–PNA chimera in the presence of ATP (filled circles) or the absence of ATP (filled squares) is shown. For comparison, unwinding of a normal DNA substrate (filled diamonds) or a DNA–PNA hybrid substrate (open squares) under identical conditions is shown. The rate of product formation was determined by fitting the early phase of the reaction to a linear function resulting in rates of 0.27 nM $s^{-1}$, 0.34 nM $s^{-1}$, and 0.33 nM $s^{-1}$ for the DNA–PNA chimera, normal DNA substrate, and DNA–PNA hybrid, respectively.

An explanation for how Dda can unwind the DNA–PNA chimera can be provided when considering the proposed mechanisms of unwinding by helicases. A helicase is thought to unwind duplex DNA by melting a given number of base pairs per catalytic cycle. Various mechanisms have been proposed in which a helicase may unwind one base pair per catalytic cycle[25] or more than one base pair per cycle.[26] Regardless of the number of base pairs unwound per catalytic cycle, longer duplex DNA substrates will require more catalytic steps than shorter duplexes. A helicase need not reach the end of a duplex for DNA unwinding to be observed. Other groups have recently reported spontaneous melting of the final 9–11 base pairs of duplex DNA, depending on the particular helicase being studied.[27,28] This means that as the helicase approaches the end of a duplex, the final base pairs melt spontaneously, giving rise to ssDNA products. The DNA–PNA chimera used in this report is only 12 bp in length. Hence, Dda helicase need only unwind 1–3 base pairs in order for the remaining base pairs to melt spontaneously.

A model invoking an inchworm mechanism for Dda and spontaneous melting of the final 9–11 base pairs of the substrate can explain the unwinding of the DNA–PNA chimera. Dda must bind to the ssDNA loading site in order to initiate the first step for unwinding (Fig. 4). The first catalytic step likely requires movement of a sub-domain of the enzyme along the nucleic acid which results in melting of one or more base pairs. After the first 1–3 base pairs are unwound, the remaining bp separate spontaneously due to thermodynamic fraying. Therefore, Dda is able to unwind the substrate without continuously translocating along the PNA.

Interactions between Dda and the PNA need be only transient and could occur through base stacking with aromatic amino acids as has been shown with other helicases.[25,29,30] The resulting model suggests that Dda can overcome a 12-PNA block in the loading strand by melting bases in one step through interactions between the enzymes 3′-binding domain and the nucleobases of PNA, while the 5′-DNA binding domain remains bound to the ssDNA portion of the chimera. The remaining bp then spontaneously separate, consistent with estimates for this minimal dsDNA value found in other helicase studies.[27,28] A previous substrate containing a single PNA moiety was not unwound under pre-steady state conditions. However, this substrate contained 16 bp compared to the 12 bp in the substrate reported here.[19] The 16 bp substrate would not be expected to melt after unwinding of only 1–3 base pairs. Additionally, our results provide evidence that Dda interacts with DNA through at least two distinct DNA binding domains. The presence of two DNA binding domains and the non-specific displacement of the complementary PNA strand support a mechanism in which Dda translocates in a manner analogous to an inchworm and strips away the complementary strand. If this model for spontaneous melting of the final 8 base pairs applies to other helicases, then reported kinetic step sizes that do not account for spontaneous melting might overestimate the actual kinetic step size.

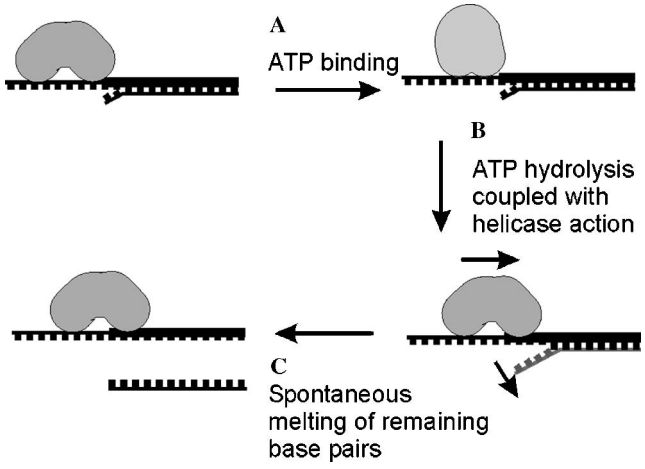



**Figure 4.** Model for Dda-catalyzed unwinding of the 20:12, chimera:DNA substrate. Dda is shown bound to a 5′ DNA–PNA chimera (where PNA is shown as a bold line). ATP binding leads to a conformational change of the enzyme at the ss/ds DNA junction in which the DNA binding domains move relative to each other. ATP hydrolysis, which is coupled to helicase action, leads to unwinding of 1–3 base pairs of duplex due to transient interaction between Dda and the bases of the PNA strand. The initial helicase 'step' displaces enough base pairs to allow the remaining base pairs to spontaneously melt apart.

## Acknowledgments

This investigation was supported by National Institutes of Health Grant R01 GM 059400 (K.D.R.) and by NIH COBRE Grant P20 RR15569 (F. Millet, University of Arkansas). We thank Dr. Tom Goodwin and Hendrix College for use of their NMR spectrometer.

## Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.01.013.

## References and notes

1. Delagoutte, E.; von Hippel, P. H. Q. *Rev. Biophys.* **2002**, *35*, 431.
2. Delagoutte, E.; von Hippel, P. H. Q. *Rev. Biophys.* **2003**, *36*, 1.
3. Soultanas, P.; Wigley, D. B. *Curr. Opin. Struct. Biol.* **2000**, *10*, 124.
4. Yarranton, G. T.; Gefter, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 1658.
5. Morris, P. D.; Tackett, A. J.; Babb, K.; Nanduri, B.; Chick, C.; Scott, J.; Raney, K. D. *J. Biol. Chem.* **2001**, *276*, 19691.
6. Nanduri, B.; Byrd, A. K.; Eoff, R. L.; Tackett, A. J.; Raney, K. D. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14722.
7. Nielsen, P. E.; Egholm, M. *Curr. Issues Mol. Biol.* **1999**, *1*, 89.
8. Norton, J. C.; Piatyszek, M. A.; Wright, W. E.; Shay, J. W.; Corey, D. R. *Nat. Biotechnol.* **1996**, *14*, 615.

9. Harrison, J. G.; Frier, C.; Laurant, R.; Dennis, R.; Raney, K. D.; Balasubramanian, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1273.
10. Koppelhus, U.; Zachar, V.; Nielsen, P. E.; Liu, X.; Eugen-Olsen, J.; Ebbesen, P. *Nucleic Acids Res.* **1997**, *25*, 2167.
11. Good, L.; Awasthi, S. K.; Dryselius, R.; Larsson, O.; Nielsen, P. E. *Nat. Biotechnol.* **2001**, *19*, 360.
12. Good, L. *Cell Mol. Life Sci.* **2003**, *60*, 854.
13. Knudsen, H.; Nielsen, P. E. *Nucleic Acids Res.* **1996**, *24*, 494.
14. Taylor, R. W.; Chinnery, P. F.; Turnbull, D. M.; Lightowlers, R. N. *Nat. Genet.* **1997**, *15*, 212.
15. Tackett, A. J.; Morris, P. D.; Dennis, R.; Goodwin, T. E.; Raney, K. D. *Biochemistry* **2001**, *40*, 543.
16. Tackett, A. J.; Corey, D. R.; Raney, K. D. *Nucleic Acids Res.* **2002**, *30*, 950.
17. von Hippel, P. H. *Nat. Struct. Mol. Biol.* **2004**, *11*, 494.
18. Kawaoka, J.; Jankowsky, E.; Pyle, A. M. *Nat. Struct. Mol. Biol.* **2004**, *11*, 526.
19. Eoff, R. L.; Spurling, T. L.; Raney, K. D. *Biochemistry* **2005**, *44*, 666.
20. Fader, L. D.; Boyd, M.; Tsantrizos, Y. S. *J. Org. Chem.* **2001**, *66*, 3372.
21. Petersen, K. H.; Jensen, D. K.; Egholm, M.; Nielsen, P. E.; Buchardt, O. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1119.
22. Domling, A.; Chi, K. Z.; Barrere, M. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2871.
23. Goodwin, T. E.; Holland, R. D.; Lay, J. O.; Raney, K. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2231.
24. Byrd, A. K.; Raney, K. D. *Nat. Struct. Mol. Biol.* **2004**, *V11*, 531.
25. Velankar, S. S.; Soultanas, P.; Dillingham, M. S.; Subramanya, H. S.; Wigley, D. B. *Cell* **1999**, *97*, 75.
26. Ali, J. A.; Lohman, T. M. *Science* **1997**, *275*, 377.
27. Galletto, R.; Jezewska, M. J.; Bujalowski, W. *J. Mol. Biol.* **2004**, *343*, 83.
28. Levin, M. K.; Wang, Y. H.; Patel, S. S. *J. Biol. Chem.* **2004**, *279*, 26005.
29. Kim, J. W.; Seo, M. Y.; Shelat, A.; Kim, C. S.; Kwon, T. W.; Lu, H. H.; Moustakas, D. T.; Sun, J.; Han, J. H. *J. Virol.* **2003**, *77*, 571.
30. Korolev, S.; Hsieh, J.; Gauss, G. H.; Lohman, T. M.; Waksman, G. *Cell* **1997**, *90*, 635.