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Disintegration of cruciform and G-quadruplex structures during the course of helicase-dependent amplification (HDA)

Dawei Li [a,b,*,†], Bei Lv [b,†], Hao Zhang [b], Jasmine Yiqin Lee [b], Tianhu Li [b,*]

[a] Key Lab of Forest Genetics and Biotechnology, Nanjing Forestry University, 159 Longpan Road, Nanjing 210037, China
[b] Division of Chemistry and Biological Chemistry, Nanyang Technological University, 21 Nanyang Link, 637371 Singapore, Singapore

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ABSTRACT

Unlike chemical damages on DNA, physical alterations of B-form of DNA occur commonly in organisms that serve as signals for specified cellular events. Although the modes of action for repairing of chemically damaged DNA have been well studied nowadays, the repairing mechanisms for physically altered DNA structures have not yet been understood. Our current in vitro studies show that both breakdown of stable non-B DNA structures and resumption of canonical B-conformation of DNA can take place during the courses of isothermal helicase-dependent amplification (HDA). The pathway that makes the non-B DNA structures repairable is presumably the relieving of the accumulated torsional stress that was caused by the positive supercoiling. Our new findings suggest that living organisms might have evolved this distinct and economical pathway for repairing their physically altered DNA structures.

DNA damages refer commonly to chemical modifications of DNA structures in the prokaryotic and eukaryotic cells that make the target DNA molecules incapable of spontaneously resuming their original B conformations.[1–3] In response to the attack of cellular DNA by endogenous metabolites and exogenous causes, all organisms have evolved delicate DNA repairing mechanisms that are able to detect DNA damages, to activate the productions of correlated proteins, and to further repair their damaged DNA.[4–6] Besides these well-recognized chemical damages to DNA, physical alterations of canonical B-form of DNA (e.g., formations of G-quadruplex and cruciform) occur prevalently in organisms that serve as signals for specified cellular events.[7,8] Similar to chemically damaged DNA, many of the non-B DNA structures are incapable of resuming their original Watson–Crick base pairings in a spontaneous manner under the physiological conditions owing to their high thermodynamically stabilities.[9,10] In theory, these stable non-B DNA structures must be disintegrated immediately after their service as cellular signals completes in order to avoid the obstruction of subsequent cellular events. The pathways and driving forces for disintegrating stable non-B DNA structures in vitro and in vivo have, however, not yet been well understood up until now.

Unlike chemical damages on DNA in which covalent bonds are either broken down or newly formed (e.g., UV-mediated dimerization of pyrimidines),[11] there is no alteration of covalent bonds in the formation of non-B structures from canonical B-conformations of DNA. Consequently, resumption of Watson–Crick base pairing from physically altered DNA structures through utilizing the mechanisms for repairing chemically damaged DNA may not be an economical choice for organisms. Particularly for the cases of DNA cruciform structures in which both opposite strands take shape of non-B DNA structures,[12,13] there is no intact single strand left to serve as a template if these physically altered DNA structures had presumably undergone 'single-stranded DNA repairing mechanisms'.[14,15]

In both prokaryotic and eukaryotic cells, helicases are known as motor proteins which can move directionally along a nucleic acid phosphodiester backbone and separate two annealed nucleic acid strands.[16,17] With the special abilities of unwinding DNA double strands, helicase in the replication process unwinds the DNA duplex where it binds and further moves forward at the replication fork that it has created. During this course of action, a positive supercoiling can be formed within the sequence ahead of the DNA replication fork.[18,19] As a result, this part of DNA backbone is forced to rotate, which will lead to the formation of a torsional stress in the whole DNA circle as shown in Figure 1A. Helicase-dependent amplification (HDA), on the other hand, is an in vitro isothermal DNA replication technology, which utilizes a DNA helicase to generate single-stranded templates for primer

* Corresponding authors. Tel.: +65 6513 7364; fax: +65 6791 1961.
E-mail addresses: lida0006@e.ntu.edu.sg (D. Li), thli@ntu.edu.sg (T. Li).
† These two authors contributed equally to this work.

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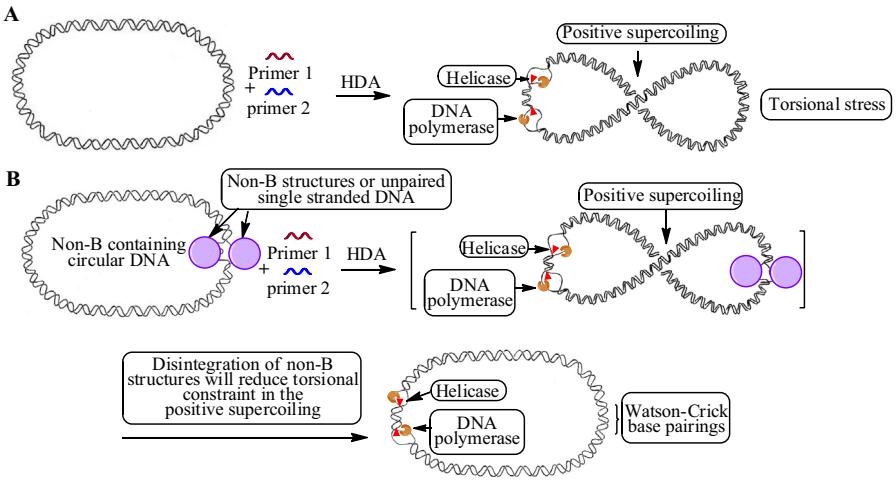

Figure 1. Schematic illustration of the topological relationships during the course of DNA replication in vitro within a circular DNA. (A) Formation of positive supercoiling caused by helicase-dependent amplification (HDA). (B) Our envisioned 'repairing' mechanisms of non-B DNA structures by positive supercoiling affiliated with DNA replication.

hybridization and subsequent primer extension by a DNA polymerase.[20,21] We accordingly choose HDA as a modeling to investigate whether the transformation of non-B structures occurs in this course of DNA replication in vitro. It has been reported that positive supercoiling is overwound[22,23] and some non-B structures can be repaired by positive supercoiling affiliated with nucleosome formation.[24] We therefore speculate that breakdown of non-B structure and resumption of the original canonical B conformation of DNA should be able to relieve the torsional stress accumulated in the backbone of a positively supercoiled DNA, thus leading to the non-B DNA disintegration. Figure 1B depicts our anticipated actions of positive supercoiling on a non-B DNA structure when these two types of structures co-exist in the same duplex DNA strands.

Among many non-B DNA structures, cruciforms play an important role in various biological processes including replication, regulation of gene expression, nucleosome structure and recombination.[12,25–27] It is believed that the formations of cruciform structures occur at or near replication origins of some eukaryotic cells and serve as recognition signals for DNA replication.[28] We accordingly chose cruciforms as the first example of non-B DNA structures in the beginning of our investigation. It has been well established that a segment of any genetic DNA must be considered as a circle when its topological property is evaluated.[29] Therefore, a cruciform-containing circular DNA (DNA 1 in Fig. S1) was subsequently designed as the initial DNA substrate to examine whether such a widespread non-B structure could be transformed into its original B-form conformation. The cruciform in DNA 1 possesses 56 base pairs and 3 bases in each of its stems and loop regions, respectively (Table S3). The size of the cruciform in DNA 1 was designed to be large enough for us to verify its presence using AFM examination. The synthetic route toward DNA 1 was shown in Figure S1. The inverted repeated sequences were introduced into target DNA molecules through using two primers (Primer 1 and 2 see Table S1) in the initial stage of Polymerase Chain Reactions (PCR) on a particular designed DNA template of X4511E. This PCR amplification gave a linear DNA (DNA S1, Lane 1 in Fig. S1B) which contains 1294 base pairs in length and holds two separate inverted segments at its two terminuses. Through using our previous reported methods,[30,31] a circular DNA (DNA S3, Lane 3 and 4 in Fig. S1B) was constructed and the formation of a circular backbone in DNA S3 was accordingly confirmed using AFM examination[32] (Fig. S1C). It is clear in the past that the cruciform structures can be formed in a negative supercoiled circular DNA which promotes breathing effect in the double helix.[33,34] We accordingly introduced negative supercoils into DNA circles through using DNA gyrase (DNA S4, Lane 1 in Fig. S1D). The resulting DNA samples were incubated in 60 mM NaCl, a condition that benefits the formation of cruciform structures.[35,36] The formation of cruciform was authenticated using gel electrophoresis (DNA 1, Lane 2 in Fig. S1D) and AFM examination (Fig. S1E).

Figure 2A depicts our designed route for examining the possible breakdown of cruciform structures during the course of helicase-dependent amplification (HDA) while Figure 2B–E summarize the experimental evidences confirming that such a process takes place. As helicases are able to unwind duplex DNA enzymatically, we tested whether the entire HDA reaction could be carried out at 37 °C using a plasmid DNA (X4511) as template (see Experimental section for detail experimental procedures[21]). As shown in Figure 2B, a band with almost the same mobility shift as 100 base pairs marker can be observed, which indicated that the HDA reaction occurred and a linear DNA with 104 base pairs was amplified as our initially designed (Lane 1 in Figure 2B, Table S5). At this experimental stage, we decided to use our newly synthesized DNA 1 as the template to investigate whether the isothermal HDA reaction can be conducted within this non-B structure containing mini-plasmid DNA (DNA 1). Our results show that a DNA product with the length of around 100 base pairs can be detected, which indicated that the isothermal HDA reaction also occurred when using the non-B-containing substrate as DNA template (Lane 2 in Figure 2B). Our proposed reaction scheme is shown in Figure 2A. Two strands of duplex DNA are separated by DNA helicases and coated by single-stranded DNA (ssDNA)-binding proteins, which facilitate the hybridization of two sequence-specific primers with each border of the target DNA. At the same time, DNA polymerases extend the primers annealed to the templates to produce a dsDNA. Because the partial separation of duplex segments occurred in this course of action, positive supercoiling can be generated within the closed DNA circles (Structure 1 in Figure 2A) in order to satisfy the 'DNA Topological Conservation Law'.[37,38] It has been well studied that the backbone of the positively supercoiled segment is highly overwound. We therefore speculated that disintegration of cruciform structures and restoration of its original B conformation could reduce the torsional constraint (Structure 2 in Figure 2A). Moreover, it has been well studied that the disintegration of a cruciform will lead to a