BRIEF REPORT

REVISED **RETRACTED: Non-intertwined strands of plasmid DNA contradict the Watson and Crick model of DNA structure [version 3; peer review: 1 approved, 1 approved with reservations, 1 not approved]**

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Retraction

At the request of the author, the article titled *"Non-intertwined strands of plasmid DNA contradict the Watson and Crick model of DNA structure"* [version 3; peer review: 1 approved, 1 approved with reservations, 1 not approved]. F1000Research 2020, 8:356 (<https://doi.org/10.12688/f1000research.18134.3>) has been retracted from F1000Research. Previous versions of this article published on F1000Research have also been retracted (<https://doi.org/10.12688/f1000research.18134.1>; <https://doi.org/10.12688/f1000research.18134.2>).

Since publication, Dr Pawan Kumar has performed additional experiments on the structure of a DNA molecule and was not able to generate further results that support this article. As such, they are no longer confident in the conclusions drawn in this article, and wish to retract. They apologise for any inconvenience caused.

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Retracted

Abstract

According to Watson and Crick (W/C) model of DNA structure, a DNA molecule consists of two antiparallel polynucleotide chains, intertwined with each other. Although W/C model is accepted widely, some researchers have raised questions against it and proposed alternative structures for DNA. In the present study, we examined W/C model using plasmid DNA. It was hypothesized that two strands of plasmid will remain intertwined (and not separate from each other) under denaturing conditions if it follows W/C model. To test this,

plasmid DNA was denatured using sodium hydroxide (NaOH) and analyzed by agarose gel electrophoresis. It was observed that addition of 0.5 N NaOH to pUC19 or pBR322 plasmids resulted in a new form of DNA having higher electrophoretic mobility in agarose gel. Higher electrophoretic mobility DNA (HmDNA) in NaOH-denatured pUC19 was digestible with S1 nuclease, but not with HindIII and 'exonuclease I + alkaline phosphatase'. These results demonstrated that HmDNA is single-stranded circular DNA, formed due to separation of two strands of NaOH-denatured plasmid. Single-stranded and circular nature of HmDNA was corroborated by its comparable electrophoretic mobility with purified top and bottom strands of plasmid DNA. Next, we examined whether HmDNA can re-anneal into the native plasmid. Interestingly, when subjected to renaturing conditions, HmDNA from NaOH-denatured pUC19 re-annealed to form native pUC19 plasmid, which was digestible with HindIII and induced ampicillin resistance in *Escherichia coli*. These findings demonstrated the reversible separation of two strands of plasmid DNA and contradicted the W/C model of DNA structure.

Keywords

DNA structure, Watson and Crick model, plasmid denaturation, single-stranded circular DNA, non-helical structure, side-by-side model.

Associated Retraction

Kumar P » RETRACTED: Non-intertwined strands of plasmid DNA contradict the Watson and Crick model of DNA structure [version 3; peer review: 1 approved, 1 approved with reservations, 1 not approved], F1000Research 2022, 11:1209 (<https://doi.org/10.12688/f1000research.127733.1>)

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REVISED Amendments from Version 2

There is no major difference between this and the previous version of the article except for the correction of some typos and Figure 1e.

Any further responses from the reviewers can be found at the end of the article

Abbreviations

DNA, Deoxyribonucleic acid

NaOH, Sodium hydroxide

HCl, Hydrochloric acid

HmDNA, Higher electrophoretic mobility DNA.

Introduction

DNA is the genetic material of all organisms, with the exception of some viruses. The currently accepted model of DNA structure was proposed by James Watson and Francis Crick in 1953¹. According to this model, a DNA molecule consists of two antiparallel polynucleotide chains, intertwined with each other. Although the Watson and Crick (W/C) model is accepted widely, some researchers have raised questions against it and proposed alternative models for DNA structure^{2,3}. Among these, Rodley's model which envisaged side-by-side positioning of two strands in a DNA molecule, has generated significant interest in the scientific community².

In the present study, W/C model of DNA structure was examined with the help of plasmid DNA. It was hypothesized that two strands of a plasmid will remain intertwined and not separate into single-stranded circular DNA molecules under denaturing conditions, if it follows the W/C model. To test this, pUC19 and pBR322 plasmids were denatured using sodium hydroxide (NaOH) and analyzed by gel electrophoresis. Interestingly, addition of NaOH to pUC19 and pBR322 plasmids resulted in new form of DNA showing higher electrophoretic mobility in agarose gel. DNA corresponding to higher electrophoretic mobility band of pUC19 was found to be single-stranded and circular, suggesting the separation of two strands of plasmid DNA. Under suitable conditions, higher electrophoretic mobility DNA (HmDNA) re-annealed to form native pUC19 plasmid. These results demonstrated the reversible separation of plasmid DNA strands and contradicted the W/C model of DNA structure.

Methods

Plasmid isolation, denaturation and agarose gel electrophoresis

pUC19, pBR322 and pCMV6-AN-HA plasmids were isolated from *E. coli* strains [cultured in Luria Bertani broth (HiMedia, catalog no. M1245) in a shaking incubator at 37°C] by alkaline-lysis method as described previously⁴. For denaturation, approximately 5 µl plasmid DNA (concentration, ~1.0 µg/µl) was added with 5 µl NaOH solution of indicated concentration.

Agarose gel (1%) was prepared in TAE buffer and DNA was visualized using ethidium bromide.

Digestion of plasmid DNA and polymerase chain reaction

For enzymatic digestion, NaOH in denatured pUC19 solution was neutralized using 5 µl HCl (concentration, 0.5 N) and plasmid was incubated with HindIII (SibEnzyme, catalog no. E073), S1 nuclease (Promega Corporation, catalog E576A) or exonuclease I and alkaline phosphatase (Thermo Scientific, catalog no. EN0581 and EF0651, respectively) at room temperature for 30 min. Samples were immediately run on 1% agarose gel. A mix of forward primer (5'-CTGCTTT CCTGCATGTTT-3') and reverse primer (5'-AAGCCCTTGCTT CTTATACT-3') (experimental control) was also digested with exonuclease I and alkaline phosphatase. Digested and undigested primers were used in polymerase chain reaction [initial denaturation, 95°C/3 min followed by 30 cycles of (i) 95°C/30 sec, (ii) 55°C/30 sec, (iii) 72°C/30 sec in the same order] to amplify target sequence in A2880 cell line genomic DNA. PCR product was analyzed on 1% agarose gel.

Preparation of individual strands of pCMV6-AN-HA

Individual (bottom and top) strands of pCMV6-AN-HA were prepared by co-digesting the plasmid with bottom (Nb.BbvCI) or top (Nt.BbvCI) strand-specific nicking endonucleases (New England Biolabs, catalog no. R0631 and R0632) and exonuclease I (New England Biolabs, catalog no. M0206S) at 37°C for 30 min. Digested plasmid was analyzed by gel electrophoresis.

DNA extraction from agarose gel

HmDNA band was cut with the help of a clean knife. DNA was purified using an extraction kit, as suggested by manufacturer (FairBiotech, catalog no. DE0100). Briefly, gel was dissolved in DE buffer and passed through a column. After washing with buffers, DNA was eluted in 50 µl nuclease free water.

Bacterial transformation

Transformation of *E. coli* strain DH5-α with gel-extracted plasmid DNA was carried out by heat-shock method (42°C for 30 sec) using water bath. Transformed and non-transformed bacteria were spread on ampicillin-nutrient agar plates supplemented with 25 µl X-Gal (Himedia, catalog no. MB0690). Plates were kept overnight in a 37°C incubator.

Results and discussion

Two strands of a DNA molecule are held together by non-covalent interactions, which can be disrupted by alkali⁵. In the present study, approximately 5 µg of plasmid DNA was denatured using NaOH solution of increasing concentration. It was observed that addition of 0.5 N NaOH to pUC19 resulted in a new form of DNA having higher electrophoretic mobility in agarose gel (Figure 1a). Similar results were obtained with pBR322 added with 0.5 N NaOH (Figure 1b). In keeping with these findings, formation of higher electrophoretic mobility DNA (HmDNA) in NaOH-denatured pBR322 has also been reported previously⁶.

Figure 1. Formation of higher electrophoretic mobility DNA (HmDNA) in NaOH-denatured plasmid and its characterization using DNA modifying enzymes. Approximately 5 μ l plasmid DNA was added with an equal volume of NaOH solution of indicated concentration and after ~5 min, run on 1% agarose gel. A distinct band of HmDNA (marked by arrows) was observed in pUC19 (a) and pBR322 (b) plasmids added with 0.5 N NaOH. For enzymatic digestion, NaOH in denatured plasmid was neutralized using 0.5 N HCl followed by incubation with different enzymes for 30 min. Native pUC19, but not HmDNA was digested by HindIII, which acts on double-stranded DNA (c). S1 nuclease, which digests single-stranded DNA, degraded HmDNA but not native pUC19 plasmid (d). Exonuclease I (Exo I) and alkaline phosphatase (AP), which would eliminate single-stranded linear DNA, digested neither of native pUC19 or HmDNA (e). Electrophoretic mobility of top and bottom strands of pCMV6-AN-HA was compared with that of its HmDNA. Both HmDNA and purified strands of pCMV6-AN-HA exhibited comparable migration in agarose gel (f). Representative data of 2–3 independent experiments are shown.

HmDNA in NaOH-denatured pUC19 was characterized using DNA modifying enzymes. HindIII, which acts on double-stranded DNA, digested pUC19 plasmid but not HmDNA (Figure 1c). S1 nuclease, which digests single-stranded DNA, degraded HmDNA but not pUC19 plasmid (Figure 1d). Exonuclease I and alkaline phosphatase, which would digest single-stranded linear DNA, digested neither of pUC19 or HmDNA (Figure 1e) but a mix of primers (experimental control). Digested primers did not form product in polymerase chain reaction (Supplementary Figure 1). To rule out the possibility that HmDNA might be denatured but unevenly intertwined plasmid DNA, its electrophoretic mobility was compared with those of individual strands of plasmid. Interestingly, top and bottom strands of pCMV6-AN-HA and its HmDNA exhibited comparable electrophoretic mobilities in agarose gel (Figure 1f).

These results showed that HmDNA is single-stranded and circular DNA formed with the separation of two strands in NaOH-denatured plasmid.

Next, we examined whether HmDNA from NaOH-denatured pUC19 can reanneal to form the native plasmid. Interestingly, it was observed that neutralization of NaOH in denatured plasmid with 0.25 N HCl resulted in the appearance of pUC19 plasmid (Figure 2a). Similar results were obtained with HmDNA extracted from agarose gel. Gel-extracted HmDNA re-annealed to form pUC19, which exhibited electrophoretic mobility comparable with that of the native plasmid (Figure 2b). Further, we observed that similar to the native plasmid, pUC19 formed by gel-extracted HmDNA was degraded by HindIII but not by S1 nuclease (Figure 2c).

Figure 2. Formation of native plasmid by higher electrophoretic mobility DNA (HmDNA) and its characterization. pUC19 plasmid was denatured using 0.5 NaOH as described above. After 15 min, NaOH in denatured pUC19 was neutralized using hydrochloric acid (HCl) solution of indicated concentration and plasmid DNA was run on agarose gel. Native pUC19 DNA reappeared in denatured plasmid DNA solution added with 0.25 N HCl (**a**). HmDNA was extracted from agarose gel and approximately 1–3 µg of it was rerun on 1% agarose gel. Gel-extracted HmDNA re-annealed to form pUC19, with its electrophoretic mobility comparable with that of the native plasmid (**b**). Gel-extracted HmDNA from NaOH-denatured pUC19 was incubated with HindIII and S1 nuclease. pUC19 plasmid formed by HmDNA was degraded by HindIII but not by S1 nuclease (**c**). *E. coli* strain DH5-α was transformed with gel-extracted pUC19 HmDNA by heat-shock method. HmDNA-transformed bacteria formed colonies on ampicillin-X-Gal-nutrient agar plates (**d**). No colonies were formed by non-transformed bacteria (**e**). Representative data of 2–3 independent experiments are shown.

Functionality of pUC19 formed by gel-extracted HmDNA was demonstrated by its ability to induce bacterial transformation. HmDNA-transformed *E. coli* acquired ampicillin resistance and formed colonies on ampicillin-nutrient agar plates (Figure 2d). No colonies were formed by non-transformed bacteria (Figure 2e). These results showed that pUC19 plasmid formed by re-annealing of HmDNA is structurally and functionally similar to native plasmid DNA.

Concludingly, reversible separation of two strands of plasmid DNA into single-stranded circular molecules shows that DNA strands are not intertwined with each other. These findings contradict the W/C model of DNA structure and provide evidence for the side-by-side structure of DNA.

Data availability

Underlying data

Figshare: Formation and characterization of higher electrophoretic mobility DNA (HmDNA) in NaOH-denatured plasmid. <https://doi.org/10.6084/m9.figshare.12949280.v17>.

This project contains the following underlying data:

- Underlying data for figure 1a
- Underlying data for figure 1b
- Underlying data for figure 1c
- Underlying data for figure 1d
- Underlying data for figure 1e
- Underlying data for figure 1f
- Underlying data for figure 2a
- Underlying data for figure 2b
- Underlying data for figure 2c
- Underlying data for figure 2d-e
- Underlying data for supplementary figure 1

Data are available under the terms of the [Creative Commons Attribution 4.0 International license \(CC-BY 4.0\)](#).

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RETRACTED

Open Peer Review

Current Peer Review Status: **RETRACTED**

Comments on this article

Version 3

Author Response 04 Jan 2021

Pawan Kumar, All India Institute of Medical Sciences, New Delhi, India

Dr. Kevin Lewis and Dr. You Cheng Xu have commented on a previous version of this article that higher electrophoretic mobility DNA (HmDNA) described herein could be a denatured form of plasmid with interlocked or concatenated strands. To rule this out, we purified top and bottom strands of pCMV6-AN-HA and compared them with pCMV6-AN-HA HmDNA for their electrophoretic properties. Interestingly, purified plasmid DNA strands and the HmDNA exhibited comparable mobility in the gel and formed bands adjacent to each other (see figure 1f). These results showed that HmDNA represents single-stranded circular DNA, which is not intertwined/concatenated with its complementary strand.

These findings further corroborate the argument that two strands of plasmid DNA can be reversibly separated from each other, provide insight into DNA structure, and contradict the Watson and Crick model.

Competing Interests: No competing interests to disclose.

Version 1

Reader Comment 07 Dec 2019

You Cheng Xu, UT Southwestern Medical Center at Dallas, retired scientist, USA

The author is trying to argue the Watson-Crick Model with his experimental result observed from the faster mobility of denatured plasmids. Unfortunately, his explanation is completely wrong and cannot support his conclusion for four main reasons:

1. The **higher** electrophoretic **m**obility band of plasmids (hmpUC19 or hmpBR322) is actually random coils of the NaOH denatured plasmids composed of a pair of single stranded circular DNAs (ssc DNA). In alkaline solution, the denatured plasmids are paired ssc DNAs; they are probably tightly tangled with each other just without hydrogen bonds. In 0.25 N NaOH, the backbone of the plasmid is stable and keeps intact, however, due to topological rule, the paired ssc DNAs cannot separate from each other; they just tangled with each other. As soon as the paired ssc DNAs entered into agarose during agarose gel electrophoresis (AGE), the

sudden pH change pushes the denatured ssc DNAs renature quickly but not in legitimate way i.e., adopting many inter-strand or intra-strand hydrogen bonds between the AT or GC pairs, causing the formation of tightly tangled entity with electric mobility higher than their supercoiled counterparts. The differences of the denatured plasmid in solution and in agarose gel are not commonly noticed by many scientists. It is reasonable and unquestionable that the NaOH denatured plasmids can renature under suitable conditions as the author has indicated. It does not mean the alkaline denatured complementary ssc DNAs has been completely physically separated.

2. If the phenomenon of higher mobility plasmid is really separated ssc DNAs, as the author supposed to be, they should be separable by AGE. The figure 3 in the report of BBA (Xu, YC. 2008. *Finding of a zero linking number topoisomer*. 2009. B.B.A. 1790, 126-133.) indicated that the two complementary plasmid strands of ssc DNA or ssl DNA of a singly nicked plasmid can all be separated on agarose gel. However, there is no indication that the two ssc DNA of NaOH denatured plasmids can be separated by AGE.
3. If the phenomenon of higher mobility plasmid is really separated ssc DNAs, as the author supposed to be, their electrophoresis behavior should not be affected by the supercoiling of the plasmids. The figure 7 of a paper (Xu, YC, 2011, *Replication Demands an Amendment of the Double Helix*. In book: (Seligmann, H., Ed., *DNA Replication-Current Advances*, InTech, Rijeka, 29-56.) indicated that the mobility of denatured plasmid is closely related to the supercoiling of plasmid, the alkaline denature relaxed plasmids moves much faster than that of their highly supercoiled counterparts.
4. All native plasmids are composed of a set of covalently closed circular DNA (ccc DNA), the author cannot provide a reasonable explanation on how the paired ssc DNAs overcome the topological rule without the help of strand passing ability of topoisomerases.

What the DNA really is? Many different answers can be heard from different observers. Just as a cubic or cylinder shaped iceberg seen by observers cannot jump to the conclusion that bottom of the iceberg is in the same shape.

The DNA structure has been studied by many scientists all over the world for many decades. Although many facts and experimental results contradicted or collided with one of the claims of the Watson-Crick Model, it is still hard to know what DNA really is. Based on the ambidextrous double helix model, it is predicted that in any plasmid there is a zero linking number topoisomer. This double helix conjecture can be proved by experiment. It is not hard to do. (Xu, YC. 2019. Evidence falsifying the double helix model. *Symmetry*, 11, 1445).

Once the double helix conjecture was proven, it would be a shock to the molecular biology because it confirmed the prediction of the ambidextrous model is correct and its meaning can be easily understood by anybody with normal IQ. Therefore, it may induce a "Paradigm shift" and maintain lasting influence on the tertiary structure of DNA. This meme will be transferred generations after generations since knowledge cannot inherit from parents; every student has to learn from the simplest beginning. As textbooks are the main source of their knowledge, it would be guilty if we keep on teaching them the Watson-Crick Model that we know is wrong or questionable.

Competing Interests: No competing interests.

Reader Comment 03 Aug 2019

L. Kevin Lewis, Texas State University, USA

It appears most likely that the fast moving denatured form of the plasmid is composed of interlocked (concatenated) single-stranded DNA circles. The molecules adopt an unusually small conformation that makes them migrate quickly down the gel. W/C base-pairing isn't a factor because the hmP19 DNA is mostly or completely single-stranded.

Competing Interests: No competing interests were disclosed.

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