Non-intertwined strands of plasmid DNA contradicts the Watson and Crick model of DNA structure [version 2; peer review: 1 approved, 1 approved with reservations, 1 not approved]

Pawan Kumar

Dr. B. R. Ambedkar Institute Rotary Cancer Hospital, All India Institute of Medical Sciences, New Delhi, Delhi, 110029, India

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 NaOH to pUC19 and 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. Higher electrophoretic mobility DNA (HmDNA) in NaOH-denatured DNA 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 HmDNA from NaOH-denatured pUC19 was subjected to renaturing conditions, it formed native pUC19 plasmid, which was digestible with HindIII and induced ampicillin resistance in Escherichia coli cells. 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.

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Author roles: Kumar P: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

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https://doi.org/10.12688/f1000research.18134.1
**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 1953. 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. 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 community.

In the present study, W/C model of DNA structure is 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 a 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 previously. 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 M) 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 CCTGCAATGTTC-3') and reverse primer (5'-AAGGCCCTTGCTT 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 in the same order] to amplify target sequence in A2780 cell line genomic DNA. PCR product was analyzed on 1% agarose gel.

**Preparation of single-stranded circular DNA**

Single-stranded circular DNA was prepared from pCMV6-AN-HA. Plasmid was co-digested with bottom (Nt.BbvCI) or top (Nt.BbvCI) strand-specific nicking endonucleases (New England Biolabs, catalog no. R0631 and R0632) along with exonuclease III (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 bands were 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-alpha 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 alkali. 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 previously.
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 (Exo I) and alkaline phosphatase (AP), which would digest single-stranded linear DNA, digested neither of pUC19 or HmDNA (Figure 1e). 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 (Figure 1f). Representative data of 2–3 independent experiments are shown.

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 M 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).
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.v1

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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7. Kumar P: Formation and characterization of higher electrophoretic mobility DNA (HmDNA) in NaOH-denatured plasmid. figshare. Figure. 2020. http://www.doi.org/10.6084/m9.figshare.12949280.v1
Open Peer Review

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Reviewer Report 15 October 2020

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Dmytro M Hovorun

Department of Molecular and Quantum Biophysics, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine

I recommend this paper for indexing in the presented form and also with author fruitful investigations in this direction.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Molecular Biophysics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 01 August 2019

https://doi.org/10.5256/f1000research.19832.r51293

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Rebecca Schulman

Chemical and Biomolecular Engineering and Computer Science, Johns Hopkins University, Baltimore, MA, USA

Yi Li
John Hopkins University, Baltimore, MA, USA

Plasmid DNA molecules have been determined by researchers to be circular, double stranded helical structures. In this work, however, the author claims plasmid DNA molecules follow a non-helical model, supported by experimental results that showed alkali-denatured plasmid DNA is not intertwined but separated. The author further showed separated single stranded plasmid DNA renatured to form native plasmid DNA at neutral pH that could transform E. coli bacteria.

This article challenges the helical DNA model of plasmid DNA accepted by the mainstream scientific community. However, the claim that plasmid DNA is not intertwined cannot be supported by the experimental results presented and the logic in the article is flawed.

The agarose gel electrophoresis was the only method used to show separation of plasmid DNA strands. Figures in the article showed that alkali-denature plasmid DNA resulted in a band of higher mobility, which could only be digested by S1 nuclease that degraded single stranded DNA. However, the appearance of a higher-mobility band could not prove that the two single strands of were separated and not intertwined. It was totally possible that the denatured DNA strands did not form helices but still had a part intertwined to each other to keep them from separating. Such intertwined DNA could also be digested by S1 nuclease but not Hind III or exonuclease. Furthermore, since the two strands might not be separated, they could easily re-hybridize to form native plasmid DNA in the neutralized pH. Thus, the author’s claim that the two strands of the plasmid were separated was untenable.

The helical structure of plasmid DNA has been supported by many studies, some of which also used denatured plasmid DNA and presented results directed contradicted to the claims of this article. JiaYu et al. showed alkali-denatured plasmid could either remain supercoiled double-stranded or became single-stranded but not separated with atomic force microscopy, both of which showed increased mobility in agarose gel. Lifeng Yan and Hiroshi Iwasaki showed similar structures with AFM after thermal denaturation. The phenomenon that the two strands of plasmid DNA could not be separated under denaturing conditions has been demonstrated with electron microscopy and other techniques decades ago. The author should comment on those papers in this article when proposing a contradicting model.

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
No

**Are sufficient details of methods and analysis provided to allow replication by others?**
No

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Partly

**Are the conclusions drawn adequately supported by the results?**
No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** DNA nanotechnology, self-assembly, soft materials

We confirm that we have read this submission and believe that we have an appropriate level of expertise to state that we do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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Author Response 21 Oct 2020

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

I am thankful to Prof. Rebecca Schulman and Dr. Yi Li for in-depth review of this manuscript and sincerely appreciate their insightful comments. I have revised the manuscript as per these comments. I sincerely believe that the reviewers will find the revised manuscript suitable for their approval. The point-by-point response to the reviewer's comments is given below.

**Comment:** Plasmid DNA molecules have been determined by researchers to be circular, double stranded helical structures. In this work, however, the author claims plasmid DNA molecules follow a non-helical model, supported by experimental results that showed alkali-denatured plasmid DNA is not intertwined but separated. The author further showed separated single stranded plasmid DNA renatured to form native plasmid DNA at neutral pH that could transform E. coli bacteria.

This article challenges the helical DNA model of plasmid DNA accepted by the mainstream scientific community. However, the claim that plasmid DNA is not intertwined cannot be supported by the experimental results presented and the logic in the article is flawed.

The agarose gel electrophoresis was the only method used to show separation of plasmid DNA strands. Figures in the article showed that alkali-denature plasmid DNA resulted in a band of higher mobility, which could only be digested by S1 nuclease that degraded single stranded DNA. However, the appearance of a higher-mobility band could not prove that the two single strands of were separated and not intertwined. It was totally possible that the
denatured DNA strands did not form helices but still had a part intertwined to each other to keep them from separating. Such intertwined DNA could also be digested by S1 nuclease but not Hind III or exonuclease. Furthermore, since the two strands might not be separated, they could easily re-hybridize to form native plasmid DNA in the neutralized pH. Thus, the author’s claim that the two strands of the plasmid were separated was untenable.

The helical structure of plasmid DNA has been supported by many studies, some of which also used denatured plasmid DNA and presented results directed contradicted to the claims of this article. JiaYu et al. showed alkali-denatured plasmid could either remain supercoiled double-stranded or became single-stranded but not separated with atomic force microscopy, both of which showed increased mobility in agarose gel. Lifeng Yan and Hiroshi Iwasaki showed similar structures with AFM after thermal denaturation. The phenomenon that the two strands of plasmid DNA could not be separated under denaturing conditions has been demonstrated with electron microscopy and other techniques decades ago. The author should comment on those papers in this article when proposing a contradicting model.

Response: I appreciate the reviewer’s point regarding the possibility that the denatured DNA strands had a part intertwined with each other to keep them from separating. To rule out this, higher electrophoretic mobility DNA (HmDNA) in NaOH-denatured plasmid was compared with the individual strands of plasmid DNA for its electrophoretic migration in the gel. Interestingly, HmDNA and the individual (top and bottom) strands of pCMV6-AN-HA exhibited comparable electrophoretic mobility. These results corroborated the single-stranded and circular nature of HmDNA and have been incorporated in the revised manuscript (figure 1f).

Further, the reviewers have cited some studies to counter the results presented in this manuscript. However, I wish to highlight that in Jia Yu and colleague’s study, the authors had extracted alkali-denatured plasmid from gel before examining it by atomic force microscopy [1]. It is noteworthy that during gel-extraction procedure, alkali-denatured DNA reanneals into double-stranded DNA molecule (please see figure 2b in our manuscript). In keeping with this, no significant difference is seen between ‘form I’ and ‘form IV’ DNA in Jia Yu’s study except that the ‘form IV’ DNA image is slightly blurred (please see fig. 2).

In Lifeng Yan’s study, the authors denatured plasmid DNA by heating its solution [2]. However, it should be noted that heating is not a very efficient method to denature plasmid DNA. Xiaofang Wang et al. have shown that heating DNA fragments at 95°C for up to 30 min did not denature the DNA fragment [3]. Similarly, Stroop et al. demonstrated that heating pBR322 in boiling water for up to 300 seconds led to degradation, but not the denaturation of plasmid DNA [4]. It is also noteworthy that Yan et al. did not confirm the denaturation of plasmid DNA before observing it by Atomic force microscopy.

In other studies too, the authors had denatured plasmid DNA by heating DNA solution. However, the word limit of the present article prohibits discussion of these studies. Nevertheless, the new data in the revised manuscript confirm the argument that two strands of plasmid DNA are not intertwined with each other and therefore can be reversibly separated into single-stranded circular DNA molecules.
References:
1. Yu, J., et al., Visualization of alkali-denatured supercoiled plasmid DNA by atomic force microscopy. Biochem Biophys Res Commun, 2008. 374(3): p. 415-8.
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Competing Interests: No competing interests to disclose.

Reviewer Report 01 August 2019
https://doi.org/10.5256/f1000research.19832.r51294

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Bharathwaj Sathyamoorthy
Indian Institute of Science Education and Research, Bhopal, India

Abhishek Cukkemane
Indian Institute of Science Education and Research, Bhopal, India

Dr. Pawan Kumar has performed simple yet and interesting experiments trying to understand a fundamental assumption in the field of DNA biophysics. However, the following are the questions I would like to post to the author for further clarification.

1. A common methodology that is employed during plasmid preparation is denaturation by NaOH followed by renaturation with acetic acid. The experiments performed in this article seem to be reproducing the same and is on expected lines. The reference of a basic textbook such as TA Brown's Gene Cloning should be provided.

2. Enzymatic digestions performed in this study are done in the presence of NaOH in a number of cases, where the pH of the solution would be ~13 going by the concentrations given by the author. At this pH will the enzymes HindIII, S1 nuclease and Exo1+AP function at all? Since, the bands observed in these gels in the presence of NaOH is used as an observation to support the hypothesis of the study. This is a concern that must be addressed by the author.

3. In Fig 1d, the author claims that S1 nuclease digests single stranded DNA in the right most lane (7th from left, 1st lane ladder) while double-stranded remains intact. Going by this
argument, why hasn't the enzyme chewed up the NaOH denatured DNA (hmP19) band in the 6th lane?

4. Additional point is that, the gel isn't capable of providing the information of what is the nature of the hmP19 band. It could very well be that the tertiary structure of the puC19 is altered in the presence of NaOH with Watson-Crick pairs remaining to a significant extent forming something like a super-supercoiled state of DNA. The assumption that hmP19 is a single stranded state seems a little far fetched with only gel based assays.

5. One thing that I am unable to wrap around is that, if the two single strands are indeed not paired by Watson-Crick rules, then what is the author suggestion regarding the re-annealed DNA? How are they bound together? In similar line, it would help if the author gives some plausible models on what could hold the strands together that might pave way to more experiments.

Minor comments:
- Please keep the units of concentration constant across the manuscript (some places use molar while other places use normal).
- If Greek letters could be input, I would strongly recommend have them placed in the right places (for instance, DH5alpha)

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Nucleic acid structure and dynamics, Solution-state NMR spectroscopy

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.
Pawan Kumar, All India Institute of Medical Sciences, New Delhi, India

I am thankful to Dr. Bharathwaj Sathyamoorthy and Dr. Abhishek Cukkemane for reviewing this manuscript and appreciate their insightful comments. I have revised the manuscript as per these comments. I sincerely believe that the reviewers will find the revised manuscript suitable for their approval. The point-by-point response to the reviewer's comments is given below.

Comment: A common methodology that is employed during plasmid preparation is denaturation by NaOH followed by renaturation with acetic acid. The experiments performed in this article seem to be reproducing the same and is on expected lines. The reference of a basic textbook such as TA Brown's Gene Cloning should be provided.
Response: It is to be brought to the reviewer's kind attention that a previous article, wherein plasmid DNA was isolated by the alkaline lysis method has been cited in the manuscript.

Comment: Enzymatic digestions performed in this study are done in the presence of NaOH in a number of cases, where the pH of the solution would be ~13 going by the concentrations given by the author. At this pH will the enzymes HindIII, S1 nuclease and Exo1+AP function at all? Since, the bands observed in these gels in the presence of NaOH is used as an observation to support the hypothesis of the study. This is a concern that must be addressed by the author.
Response: My apology for this confusion. Indeed, the enzymes used in this study would not work in the presence of 0.5 N NaOH (as also observed in our results, lane 5 in each of figure 1c-e). Therefore, for enzymatic digestion, NaOH was first neutralized using HCl (lane 3, pUC19+NaOH+HCl and lane 6, pUC19+NaOH+HCl followed by enzymatic digestion). The same is also stated in the methods section.

Comment: In Fig 1d, the author claims that S1 nuclease digests single stranded DNA in the right most lane (7th from left, 1st lane ladder) while double-stranded remains intact. Going by this argument, why hasn't the enzyme chewed up the NaOH denatured DNA (hmP19) band in the 6th lane?
Response: S1 nuclease did not digest single-stranded DNA in lane 6, figure 1d (lane 5 in the new version of article) because of the presence of NaOH. In lane 7 (lane 6 in the new version of article), NaOH was neutralized using HCl. Therefore, enzyme digested single-stranded DNA in this lane.

Comment: Additional point is that, the gel isn't capable of providing the information of what is the nature of the hmP19 band. It could very well be that the tertiary structure of the puC19 is altered in the presence of NaOH with Watson-Crick pairs remaining to a significant extent forming something like a super-supercoiled state of DNA. The assumption that hmP19 is a single stranded state seems a little far fetched with only gel based assays.
Response: It is indeed a very important point raised by the reviewers. To examine whether hmP19 DNA (HmDNA in the new version) is single-stranded circular DNA, its electrophoretic mobility was compared with that of purified strands of plasmid DNA. It was observed that HmDNA and individual (top and bottom) strands of pCMV6-AN-HA exhibited comparable
mobility in agarose gel. These results corroborate the single-stranded and circular nature of HmDNA and have been incorporated in the revised manuscript (figure 1f).

**Comment:** One thing that I am unable to wrap around is that, if the two single strands are indeed not paired by Watson-Crick rules, then what is the author suggestion regarding the re-annealed DNA? How are they bound together? In similar line, it would help if the author gives some plausible models on what could hold the strands together that might pave way to more experiments.

**Response:** The main objective of the proposed study was to examine whether DNA strands are intertwined with each other as proposed by Watson and Crick. Interestingly, this study shows that two strands of a DNA molecule can be separated reversibly, contradicting the Watson and Crick model of DNA structure. Further, these results provide evidence for the side-by-side model of DNA structure. The same has been stated in the concluding paragraph of the manuscript.

**Competing Interests:** No competing interests to disclose.

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**Reviewer Report 28 May 2019**

https://doi.org/10.5256/f1000research.19832.r46917

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**Dmytro M Hovorun**

Department of Molecular and Quantum Biophysics, Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine

In this manuscript author obtained quite interesting experimental results according the conformational possibilities of DNA. However, in this work no other confirmations, except electrophoretic, are presented according the presented conformation of DNA.

So, author should provide additional arguments obtained by atomic force microscopy, circular dichroism, optical, in particular vibrational spectroscopy.

Only after this submitted manuscript can be accepted for indexing.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

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Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Molecular Biophysics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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Author Response 30 May 2019

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

I am thankful to Prof. Dmytro M Hovorun for timely reviewing my manuscript entitled “Non-intertwined strands of plasmid DNA contradicts the Watson and Crick model of DNA structure” and for his valuable comments. I have carefully gone through Prof. Hovorun’s comments and found them both encouraging and insightful. Although Prof. Hovorun appreciated the findings as quite interesting, one of his main concerns was regarding the usage of only a single technique (gel electrophoresis) in this study. Further, he suggested that additional arguments should have been provided using other techniques such as atomic force microscopy or vibrational spectroscopy. I fully agree that the findings presented in this manuscript have been obtained using electrophoresis as the main readout technique. However, I would also like to draw Prof. Hovorun’s kind attention to the fact that owing to the above reason, this manuscript has been presented as a “Research Note”. According to the journal policy, Research Notes are small, often preliminary studies, descriptions of unexpected and perhaps unexplained observations or lab protocols that can be described in a short report with a few illustrations (figures/tables), or even a single figure.

The findings presented in this manuscript are in contradiction with the Watson and Crick model and are likely to have a huge impact on our understanding of DNA structure. I sincerely appreciate that these findings should be confirmed using other tools/techniques. I would also like to bring it to your notice that I am working in this direction and have obtained the encouraging results. I hope to publish the new findings very soon.
Comments on this article

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 mobility 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 renatured 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 topoisomeras.

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.

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**Reader Comment 03 Aug 2019**

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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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