Title:	**The Biology and Polymer Physics Underlying Large Scale Chromosome Organization**
Authors:	**Shelley Sazer, Helmut Schiessel**
Article Type:	Review

Monitoring Editor	Mark Field
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Decision and Reviews

Dear Dr. Sazer,

Please accept my apologies for the delay in getting a decision to you about your review '"Nuclear Physics": The Intersection of Chromosome Organization and Polymer Physics' for Traffic. I have now obtained comments from two experts in the field and these have been appended below. I share the enthusiasm of the referees for the high caliber of this review and agree with them that this will be of interest to the readers of Traffic. The referees have made a number of suggestions for revisions to correct, clarify and extend the current discussion that I ask you to address.

Although I cannot accept your manuscript for publication at this point, I believe that you will be able to address the referees' concerns and I look forward to receiving your revised manuscript. To expedite handling when you resubmit please be sure to include a response outlining how you have addressed each of the referees' concerns. I will make every effort possible to return a decision about the revised review much more quickly.

Sincerely,

Mark Field, Ph.D.
Guest Editor

Referee's Comments to the Authors

Referee: 1

Comments to the Author
This is a very nicely written review of a very hot topic. Specifically, authors review conceptual development, along with underlying experimental and theoretical investigations, of the three dimensional organization of genetic material in the interphase eucaryotic cell nuclei. The work is the result of a collaboration between physicist and biologist, which nicely reflects on the nature of the subject. Authors explain both physics and biology concepts with great pedagogical clarity. It is fairly clear that each of them was keeping the other from sliding into a professional jargon and technicalities, which resulted in a very readable text for a wide audience of interested scientists. I have no hesitation to enthusiastically recommend publication, except I would like to make a few recommendations to edit and improve presentation in several places. In no particular order:

(1) The work is titled "Nuclear physics", in quotation marks. I also frequently use this joke in my talks. However, I invite authors to consider where their article will go by automatic computer algorithms based on words...

(2) Page 3, left column, top paragraph: "... In contrast to the focus of physicists ... biologists (broadly defined here to include cellular, evolutionary, and molecular biologists)" Should then physicists to be also broadly defined to include molecular, chemical, statistical, theoretical, biological etc? Otherwise it is a bit strange: if the goal is to build a bridge between a physicist's and a biologist's view of chromatin, then emphasizing the breadth of the discipline of biology is out of place -- unless authors want to say something about perception of their subject by, say, evolutionary versus molecular biologists (which does not seem to be the case), or unless authors want to exclude, say, population or some other biologists...

(3) Page 3, right column, paragraph before section 1. Reference to historical context is repeated 3(!) times. Why?

(4) Page 4, right column, third paragraph from the top: what exactly is "submicroscopic" variations of the orientations? (The same word also used elsewhere in the manuscript). Makes no sense to me.

(5) In the same paragraph: "...mean distance averages to zero". No, it does not. End-to-end vector does average to zero, but not distance. Besides, mean distance does not have to be averaged, it is already mean.

(6) Page 5, right column, middle: "... another random walk but with a considerably smaller slope". I think here authors slipped into technical

jargon which they obviously (and quite successfully) avoid in most other places. Even apart from a naive reader who would think that "slope of the walk" refers to a walker going up- or down-hill, do authors mean a sub-diffusive motion? Do they want to call it still a random walk?

(7) Page 8, left column, very top: "... collapse of the simulation"? What do authors want to say?

(8) Same page, begining of section 3.2: "One way ... can become entangled... by reptation" Is there another way? I think it is a very fundamental result from polymer physics, which perhaps authors may want to explain or to mention, is that this is the ONLY way!

(9) Page 9, right column: on the subject and eu- and hetero-cromatin, in addition to Refs. 73 and 76, I recommend to include references to the works by J.Smrek and K.Kremer (PRL 2017) as well as D.Osmanovic and Y.Rabin (Soft Matter 2017).

(10) Page 10, chapter 5. What exactly is meant by loops? Suppose a single cell HiC revealed contact between loci A and B; does it mean, at the moment of cross-linking there was a loop of the segment between A and B? Is that what is meant by loops? Then 1/g behavior of contact probability can be re-stated as 1/g distribution of loop sizes, etc. It seems that authors have in mind some other definition of loops. What is it? Does it refer to some sort of stability in time? AB deserves the name of a loop if A remains in close proximity of B for a long time? How long should this time be?

To summarize, I repeat that the work deserve publication and the above remarks are intended as a help to the authors.
(10) A

Referee: 2

Comments to the Author
The review by Sazer et al discusses the historical as well as most recent experimental findings in how chromosomes are organized and the continuing effort by physicists to understand this organization through the use of polymer models. This is a very well-written review that is easy to understand and comprehensive, bridging the fields of chromosome biology and polymer physics and I really enjoyed reading it. One small criticism is the omission of the big role fluorescence microscopy and in particular recent super-resolution methods have played and continue to play in enhancing our understanding of chromosome organization. It would have been nice to see more discussion on super-resolution microscopy, which can visualize genomic elements that are Kb to Mb scale with very high resolution and in the 3D context of the nucleus (unlike HiC). In particular recent work from Zhuang lab has demonstrated the differential packing of genomic regions having different epigenetic signatures and used models of "sticky polymer immersed in a sea of non-sticky polymers" to explain the exclusion of repressed and active domain. Zhuang lab has also visualized the organization of TADs within chromosomes and it seems appropriate to mention some of these contributions. Finally, I would have liked to see more discussion on future perspectives, unsolved problems and what is next in this field in the Conclusions section.

Author Rebuttal

Referee: 1

Comments to the Author
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Thank you for these comments.

In no particular order:

(1) The work is titled "Nuclear physics", in quotation marks. I also frequently use this joke in my talks. However, I invite authors to consider where their article will go by automatic computer algorithms based on words...

Response:

We agree and deleted the words "Nuclear physics" from the title and changed the title to: "The biology and polymer physics underlying large scale chromosome organization"

(2) Page 3, left column, top paragraph: "... In contrast to the focus of physicists ... biologists (broadly defined here to include cellular, evolutionary, and molecular biologists)" Should then physicists to be also broadly defined to include molecular, chemical, statistical, theoretical, biological etc? Otherwise it is a bit strange: if the goal is to build a bridge between a physicist's and a biologist's view of chromatin, then emphasizing the breadth of the discipline of biology is out of place -- unless authors want to say something about perception of their subject by, say, evolutionary versus molecular biologists (which does not seem to be the case), or unless authors want to exclude, say, population or some other biologists...

Response:

We agree and deleted the phrase "(broadly defined here to include cellular, evolutionary, and molecular biologists)"

(3) Page 3, right column, paragraph before section 1. Reference to historical context is repeated 3(!) times. Why?

Response:

We apologize for this repetitive wording and have revised the text to remove it.

(4) Page 4, right column, third paragraph from the top: what exactly is "submicroscopic" variations of the orientations? (The same word also used elsewhere in the manuscript). Makes no sense to me.

Response:

We agree with the referee and replaced "the submicroscopic variations in the orientations" by "random orientations". Also on page 4 we replaced "submicroscopic details" by "underlying chemical composition".

(5) In the same paragraph: "...mean distance averages to zero". No, it does not. End-to-end vector does average to zero, but not distance. Besides, mean distance does not have to be averaged, it is already mean.

Response:

We totally agree. By trying to avoid jargon, this statement became meaningless. We replaced "...mean distance averages to zero" with "…end-to-end vector averages to zero."

(6) Page 5, right column, middle: "... another random walk but with a considerably smaller slope". I think here authors slipped into technical jargon which they obviously (and quite successfully) avoid in most other places. Even apart from a naive reader who would think that "slope of the walk" refers to a walker going up- or down-hill, do authors mean a sub-diffusive motion? Do they want to call it still a random walk?

Response:

Thank you for this comment - this is indeed too technical and unclear. We replaced "In fact, FISH measurements for longer genomic distances (up to 190 Mb) did not show a true levelling-off but instead a crossover to yet another random walk but with a considerably smaller slope." by "In fact, when plotting the mean squared distances determined from FISH measurements for longer genomic distances (up to 190 Mb) the data did not level off but lay on a straight line with a small slope." This statement makes also clear that this is not related to "sub-diffusive motion" as suggested by the referee.

(7) Page 8, left column, very top: "... collapse of the simulation"? What do authors want to say?

Response:

Indeed "collapse of the simulation" is meaningless. We replaced it by "simulated polymer collapse". To further clarify this point, on the previous page we replaced the text "The authors of Ref. 5 supplied computer simulations to support…" by "The authors of Ref. 5 supplied computer simulations of collapsing polymers to support…"

(8) Same page, begining of section 3.2: "One way ... can become entangled... by reptation" Is there another way? I think it is a very fundamental result from polymer physics, which perhaps authors may want to explain or to mention, is that this is the ONLY way!

Response:

We reformulated the sentence as follows: "Two linear chromosomes can become entangled in the nucleus if a free chromosome end moves by reptation, as it is following the restricted path of a hollow tube through surrounding chromosome polymers." We deliberately did not formulate this sharper, since in principle one could imagine that the action of topo II could produce entanglements, even though, more typically, the enzyme's function is to resolve entanglements.

(9) Page 9, right column: on the subject and eu- an d hetero-cromatin, in addition to Refs. 73 and 76, I recommend to include references to the works by J.Smrek and K.Kremer (PRL 2017) as well as D.Osmanovic and Y.Rabin (Soft Matter 2017).

Response:

Thank you for pointing out these references. They have been included in the revised manuscript.

(10) Page 10, chapter 5. What exactly is meant by loops? Suppose a single cell HiC revealed contact between loci A and B; does it mean, at the moment of cross-linking there was a loop of the segment between A and B? Is that what is meant by loops? Then 1/g behavior of contact probability can be re-stated as 1/g distribution of loop sizes, etc. It seems that authors have in mind some other definition of loops. What is it? Does it refer to some sort of stability in time? AB deserves the name of a loop if A remains in close proximity of B for a long time? How long should this time be?

Response:

Thank you for asking us to be more specific about this point. With loops we mean structures as defined in the review by Robert Schleif (DNA Looping. Ann Rev Biochem 61:199-233 (1992)): "DNA looping is generated by a protein or complex of proteins that simultaneously binds to two different sites on a DNA molecule." This definition rules out random interactions as they e.g. occur in random configurations of polymer coils. The 1/g behavior, the referee refers to, was explicitly discussed by Lieberman et al. (2009) in the context of polymer models and would thus, according to this definition, reflect the probability of random interactions. Obviously not all contact found in Hi-C are random contact and chapter 5 focuses on contacts that correspond to loops. We now added at the beginning of chapter 5:

"So far we have mainly spoken of contacts between different sections of DNA molecules in general. Here we look more specifically at DNA loops, which are structures that are "generated by a protein or complex of proteins that simultaneously binds to two different sites on a DNA molecule."[86]"

To summarize, I repeat that the work deserve publication and the above remarks are intended as a help to the authors.

Referee: 2

Comments to the Author
The review by Sazer et al discusses the historical as well as most recent experimental findings in how chromosomes are organized and the continuing effort by physicists to understand this organization through the use of polymer models. This is a very well-written review that is easy to understand and comprehensive, bridging the fields of chromosome biology and polymer physics and I really enjoyed reading it.

1. One small criticism is the omission of the big role fluorescence microscopy and in particular recent super- resolution methods have played and continue to play in enhancing our understanding of chromosome organization. It would have been nice to see more discussion on super-resolution microscopy, which can visualize genomic elements that are Kb to Mb scale with very high resolution and in the 3D context of the nucleus (unlike HiC). In particular recent work from Zhuang lab has demonstrated the differential packing of genomic regions having different epigenetic signatures and used models of "sticky polymer immersed in a sea of non-sticky polymers" to explain the exclusion of repressed and active domain. Zhuang lab has also visualized the organization of TADs within chromosomes and it seems appropriate to mention some of these contributions.

Response:
We agree with the reviewer. Although we did discuss optical microscopy approaches to understanding chromosome organization, including FISH to provide the context in which chromosome capture techniques were developed we did not include a discussion of more recent optical approaches. We have added text to discuss super-resolution microscopy approaches and compare them to HiC in the new section on Future Perspectives that also addresses comment #2 from this reviewer.

We have added a new section to the text, entitled Future Perspectivs which will be section 7 of the manuscript and has been inserted just before the Conclusions section which we have renumbered as section 8. The text of this new section is as follows:

7. Future Perspectives

Our understanding of the organization of the nucleus has progressed rapidly over the past ten years, in large part due to the innovative and impactful technological advances we have described in this paper. Continued progress will depend on improved imaging, tools for nucleomics, and modeling, and their application to address a variety of outstanding issues.

7.1 Biological questions

Current techniques raise the possibility of addressing a wide variety of scientific questions that have been intractable in their absence. Outstanding challenges include monitoring chromosome position in three-dimensions and in relation to nuclear landmarks such as the nuclear periphery or nuclear bodies and genome dynamics in living cells as they progress through the cell cycle, undergo development, and respond to external and internal perturbations. Comparative genome-wide maps of chromosome organization in normal and abnormal cells will be informative with regard to the characterization, and perhaps the diagnosis, of a variety disease states, the regulation of gene expression and the rearrangement of chromosomes by recombination and translocation. These and many other topics are the focus of the ongoing National Institutes of Health 4D Nucleome Project[141] (https://commonfund.nih.gov/4dnucleome), the International Nucleome Consortium,[142] and the proposed 4DNucleome Initiative in Europe (https://ec.europa.eu/futurium/en/content/4dnucleome-initiative-europe). It will also be interesting to directly compare imaging and HiC data, for example by mapping the three-dimensional positioning of the A and B compartments defined by HiC and comparing them to the localization of euchromatin and heterochromatin visualized microscopically.

7.2 Nucleome Physical Approaches

The explosion of chromosome capture techniques and their application to a range of cell types carries with it the challenges to comparing data from different sources that may use different strategies. Efforts to validate, standardize, improve and develop new technological, analytical and nucleomics tools and to establish a Data Analysis Center are currently underway as part of the NIH 4D Nucleome Project. Future research includes the development of high-throughput experimental and computational approaches to achieve single cell 4D chromosome capture data, examine higher-order genome structure and develop new methods for crosslinking DNA.

7.3 Genome Imaging Approaches

At the time when chromosome capture and HiC techniques were being developed, FISH analysis was the state of the art for monitoring co-localization of DNA loci at a resolution of several hundred nanometers (nm). Although the optical resolution is limited by the diffraction of light waves, two recent developments now make it possible to overcome this diffraction barrier. The first was the development of new optical instrumentation that increases image resolution to approximately 100 nm and is capable of three-dimensional imaging using optical sectioning. The second was the development of a new class of fluorophores with novel properties that make it possible to resolve the overlapping emissions of neighboring single molecules and achieve subdiffraction limit resolution as low as 10 nm.[143] These techniques have been widely used to study the three-dimensional localization of a variety of proteins in their cellular context.[143] More recently, they have been adapted to allow high-resolution super-resolution imaging of up to 30 genomic loci using short oligonucleotide probes[144] or up to 6 loci using modified CRISPR based systems targeted to the genome by engineered guide RNAs.[145,146] Although both approaches have their drawbacks, they represent significant improvements over traditional FISH analysis yet can still detect only a tiny fraction of the genome-wide contacts seen with HiC.[144,147-150] However, improvements and innovative new approaches are certainly on the horizon. Optical imaging can also capture chromosome dynamics and three-dimensional positioning of loci in live cells, neither of which can be determined using static HiC data from large heterogeneous populations of cells. However, recent HiC analysis of single cells or populations of mouse and yeast cells with known positions in the cell cycle has documented stage-specific differences in chromosome conformation.[151-154] The next challenge in this area is to describe the four-dimensional changes in chromosome structure and dynamics in living cells progressing through an unperturbed cell cycle. All of these efforts will be advanced by the development of new imaging instrumentation and experimental tools that will achieve higher resolution and higher content imaging of live single cells.

7.4 Polymer Physics Approaches

We have pointed out repeatly in this review that discoveries on the structure and dynamics of chromatin at large scales, made possible through new experimental methods, have inspired various new directions in polymer physics. The structure of melts of polymer rings[60-66] or the segregation of polymers at different temperatures[76-78] mentioned earlier are such examples. Some of these new polymer studies are performed specifically to understand experimental findings on chromatin whereas others attempt to come up with general laws that govern such systems and might eventually form new branches in polymer physics. As new experimental data pour in with more and more detailed insights on chromatin structure and dynamics and as information on single cells becomes available, the questions that polymer models need to address will continue to widen the scope of polymer physics in the future, both applied to chromatin and to fundamental physics. In the immediate future, individual polymers or polymer solutions in the presence of energy consuming processes (e.g. the loopy globule[52] or activity based polymer segrxegation[76-78]) certainly provide a wide range of possible questions, as indicated by an increased frequency of publications in this field.

2. Finally, I would have liked to see more discussion on future perspectives, unsolved problems and what is next in this field in the Conclusions section.

Response:
We agree with this suggestion. See Item #1 above with text of the new "Future Perspectives" section.

New references:

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78. Osmanovic D, Rabin Y. Dynamics of active Rouse chains. Soft Matter 2017;13:963–968.
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154. Schalbetter SA, Goloborodko A, Fudenberg G, et al. SMC complexes differentially compact mitotic chromosomes according to genomic context. Nat Cell Biol. 2017;19:1071–1080.