DNA Content Contributes to Nuclear Size Control in *Xenopus laevis*

Hiroko Heijo, Sora Shimogama, Shuichi Nakano, Anna Miyata, Yasuhiro Iwao, and Yuki Hara

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**Review Timeline:**
- Submission Date: 2020-02-13
- Editorial Decision: 2020-03-27
- Revision Received: 2020-08-28
- Accepted: 2020-09-20

*Editor-in-Chief: Matthew Welch*

**Transaction Report:**

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)
Dear Dr. Hara:

As you will see from their comments, the reviewer's comments span a fairly wide spectrum. While one referee (#3) is largely positive and only raises minor issues, the other two have more significant concerns that need to be addressed thoroughly before we can consider the manuscript any further. Specifically, both of these referees question various aspects of your model. In particular point 1 by referee 1 and points 2 and 3 by referee 2 should be taken into account in your interpretation of the data, although no specific experiments are requested. Referee 1 also questions your interpretation that changes in genome content regulate import; if you wish to make this claim additional experiments are required as suggested by the referee. The one experiment that should be included is the one listed by referee 3 in point 1.

If you are willing and able to fully address these issues, we would be glad to look at a revised version of this manuscript.

Sincerely,

Tom Misteli
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Hara,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

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Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However,
special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

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Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
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Reviewer #1 (Remarks to the Author):

The authors use cell-free Xenopus egg extracts and embryos to demonstrate a strong positive correlation between nuclear expansion and DNA content. They altered DNA amount per nucleus by interfering with DNA replication or by using different sources of sperm chromatin and showed that nuclear membranes accumulate to similar levels independent of DNA content. DNA density correlated better with nuclear volume than with surface area, and treatments that altered chromatin compaction or fragmentation also altered nuclear expansion dynamics. Nuclear import monitored with GFP-NLS also correlated with DNA content and chromatin condensation status. The authors propose that the physical properties of chromatin regulate nuclear expansion.

Overall the data are interesting and well quantified, and the results will be of interest to the MBoC readership. The presentation requires some work, however. The manuscript is difficult to read and should be edited by a native English speaker. Also, in some instances the data could have different interpretations. The following points should be considered:

1) Figure 5: Since DNA density correlates better with nuclear volume rather than surface area, the authors conclude "that chromatin throughout the nucleoplasm rather than chromatin only on the nuclear membrane alone possibly contribute to nuclear expansion..." (Page 11, lines 19-21). However, these results could be interpreted differently. Since the effect of genome size on nuclear expansion kinetics is masked when genome size is normalized to nuclear volume (but maintained when normalized to nuclear surface area), perhaps it is the chromatin interactions with the nuclear envelope that actually determine nuclear expansion rates. This interpretation is more consistent with the authors' model that the forces that chromatin exerts on the nuclear envelope actually contributes to the expansion of the nucleus.
2) Figure 8: The increase in nuclear import rates with DNA content is not surprising, since it could be due to differences in nuclear surface area available for transport. It seems unlikely that "changes in nuclear expansion dynamics by DNA content and chromatin structures are coordinated with the changes in nuclear activity" (page 14). Further experiments would need to be designed to test whether or not changes in genome content regulate import of specific factors (such as lamins) that then directly regulate nuclear size. "coordinated" should be changed to "correlated".

3) The discussion seems a bit long and overly speculative. For example, "Chromatin modulates the magnitude of forces to expand the nucleus from inside" (page 15) is an inappropriate subheading given that no force measurements were made in this study. Similarly, "Chromatin modulates the protein import into the nucleus" (page 17) is also not supported by the data presented.

Reviewer #2 (Remarks to the Author):

Nuclear size is thought to be largely independent of its DNA content. For this reason, the study by Heijo et al is interesting, as it takes a closer look at the relationship between DNA content and nuclear size. For full disclosure, I reviewed an earlier version of this manuscript that was submitted elsewhere.

Using Xenopus laevis and tropicalis DNA and blastomeres, the authors show that both in vitro and in vivo, there is a correlation between the amount of DNA and the rate of nuclear expansion and maximal nuclear size. This is not surprising for the in vivo situation, as these cells likely produce more relevant material. However, in vitro using Xenopus extracts, the differences in nuclear expansion have to be attributed to something associated with the DNA (a caveat to that is comment 1 below). Interestingly, the rate of nuclear import is greater with laevis DNA than with tropicalis, perhaps explaining the higher rate of nuclear expansion. The basis for this difference was not explored. However, in vivo the authors note that there are "differences in initial nuclear size just after the completion of nuclear formation at telophase or early interphase between diploid and haploid blastomeres" (page 19, line 28), which could account for the overall greater size. Moreover, there are several technical issues that put the authors' conclusions into question, as outlined below.

Major comments:
1. The measurements reported here are based on fixed samples mounted on glass slides, which, as I previously noted, likely cause compression. Indeed, when the authors examine nuclear cross section without compression (Figure S1F, which, by the way, is not referred to in the main text), the rate of increase in nuclear cross section is greatly diminished; it is not clear to me that under these conditions, which are more physiological, the authors would have been able to see a difference in nuclear size under the various DNA amount manipulations. In fact, it is formally possible that the amount of DNA affects the "spreading" of the nucleus when it is compressed between two panes of glass rather than nuclear size per se. The accuracy of the measurements is key to this study, and Figure S1F puts this into doubt. The authors don't need to do every experiment using live imaging; all they need to do is mount the fixed nuclei from Figure 1D on a surface that does not cause compression (e.g. agar pads).

2. In Figure 5 the authors calculate "density (D), which is a ratio of the DNA content (C) to the estimated nuclear volume (V)" and based on that draw conclusions on the relationship between
DNA density and nuclear volume vs nuclear surface area. However, in various figures (Figure 2, starting at 120 min, the NE, and Figures 3, 7) the chromatin is largely detached from the nuclear envelope. In fact, in Figure 7, treatment with ICRF193 caused the chromatin to form a highly compact cluster, and yet the nucleus continued to expand. Thus, if the DNA does not fill the nucleus uniformly, the density values and Figure 5 itself (and conclusions drawn thereof) are invalid.

3. The authors suggest that the chromatin applies a force on the NE. However, if this force was directly proportional to the amount of DNA, it would (a) have to act from a distance, as the DNA is not near the NE (see point 2), and (b) nuclear envelope expansion should have stopped as soon as DNA replication was completed. That is not what is observed (Figure 1A and D): dUTP incorporation plateaus at 80 minutes but nuclear expansion continues at a constant rate up to 240 minutes. Thus, something that is not DNA content is likely driving most of nuclear expansion.

4. The Discussion contains extensive speculation on the possible relationship between chromatin and the nuclear import system, as well as the relationship between chromatin properties and membrane availability. None of this was examined experimentally and as it stands it is excessive. The Discussion needs to be rewritten and the highly speculative portions removed.

Minor comments:

5. Please indicate how the boundaries of the nucleus was set in area measurements. In Figure 1A, for example, the DiOC6 marks a rather wide band around the nucleus while in other case there's only a thin margin.

6. Student's t-test is only valid if your data show a normal distribution. Excel might not be able to determine that, but Prism does. If the data do not exhibit a normal distribution please use a non-parametric test.

7. Figure 1D: Based on Figure 1B there is little is any DNA replication at 40 minutes. The authors note that there are less differences, at this time point (presumably compared to later time points) between the different conditions, but why are there differences at all?

8. In figure 4, the authors conclude that there is nuclear membrane accumulation (lines 9, 11 page 10) based on uneven NPC accumulation in a pattern of lines (as in membrane folds or a "wrinkles" in the membrane). However, all these data show are uneven NPC accumulation and the authors cannot refer to these structures as membrane accumulation. In the supplemental material the authors show data based on DiOC6 labeling, but the effect there is either small or non-existent. The idea that smaller nuclei have the same amount of surface area but for a smaller volume (leading to membrane folds) is attractive but the data presented are insufficient to support it. EM analysis would have been better.

9. there are numerous places where the phrasing could be improved. Some are listed below but there are likely to be more:

- Lines 9-11: the sentence "We also found an increase in the amount of membranes on the nuclear surface when DNA content was low and alterations in the nuclear expansion dynamics after converting the chromatin structures" is unclear: an increase relative to what, and converting the chromatin to what?
- Figure legend 1: "visualized against nuclei"; do the authors mean "used to visualize nuclei"?
- The title of Figure 5 "DNA in the nucleoplasm is constant regardless of changes in DNA content"
Reviewer #3 (Remarks to the Author):

Heijo et al., uses Xenopus eggs both in vitro (cell-free extracts) and in vivo to determine how DNA content determines nucleus size. In this novel paper, it is reported that nucleus size is dependent on DNA content as decreasing DNA content through replication inhibition, different genome size (haploid vs diploid and laevis vs tropicalis), digestion, and compaction. Interestingly the density of DNA remains constant per volume and not surface area suggesting chromatin density throughout the nucleus may be the parameter controlling nuclear size. The paper is well written and the conclusions are well supported by the data. I believe this paper will be of general interest to the MBoC community and suggest it be accepted for publication. I do however, have a list suggestion which I think should be added to strengthen the paper.

1) I would suggest using magenta instead of blue for the DNA color because blue is very hard to see on black.
2) The paper speaks of forces in the manuscript but the paper shows a lack of knowledge of the literature. Citations that should be considered are chromatin connections to the periphery (Schreiner et al 2015 Nat Comm), H1 histone (Furusawa et al. 2015 Nat Comm), heterochromatin (Stephens et al. 2017 and 2018 MBoC) determine nuclear mechanics and nucleus morphology. There is also work showing the difference between chromatin and lamin mechanics that could be cited. Overall, a more detailed literature search of chromatin's contribution to nuclear mechanics would aid intro and discussion of chromatin as a force component.
3) The use of linear vs exponential to fit Figure 2 D and E or Figure S2, respectively, was a bit confusing. I would suggest including a sentence stating that there is insufficient data 5-15 pg to determine the true best fit, as a caveat. I do not think you need to collect this data, just acknowledge the fits are preliminary without more data.
4) In figure 2C I would suggest scaling the data the same as 2B.
RE: Manuscript #E20-02-0113R  
TITLE: "DNA Content Contributes to Nuclear Size Control in Xenopus laevis"

Dear Dr. Hara:

Your revised manuscript has now been seen by the original reviewers. They are now both supportive of publication. I am pleased to accept your manuscript for publication in Molecular Biology of the Cell. Thank you for thoroughly revising your manuscript.

Sincerely,
Tom Misteli
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Hara:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org
Reviewer #1 (Remarks to the Author):

The authors have addressed our concerns and the paper is now suitable for publication in MBoC.

Reviewer #2 (Remarks to the Author):

The authors have adequately addressed my main concerns. The conclusions are not as conclusive as the authors make them to be; for example, cell size appears to be just as important, if not more so, for nuclear expansion (see Figure 3C and D). Nonetheless, this is still an important study, as it shows that DNA content has to be taken into account when considering the various factors that contribute to nuclear size.