Cell and tissue morphology determine actin-dependent nuclear migration mechanisms in neuroepithelia

Iskra Yanakieva, Anna Erzberger, Marija Matejcic, Carl Modes, and Caren Norden

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Dear Dr. Norden,

Thank you for submitting your manuscript entitled "Tissue shape determines actin-dependent nuclear migration mechanisms in neuroepithelia". The manuscript was assessed by three expert reviewers, whose comments are appended to this letter.

As you will see, all of the reviewers are excited about the novelty and potential of this work. Two of them, however, have requested significant experimental extensions of the work (e.g., comment 2 of Reviewer 1 and the suggestions of Reviewer 3). Some of these requests seem fairly open-ended. All reviewers also found the model presented in Fig. 6A to be too speculative. One way forward would be for you to revise the manuscript into the shorter "Report" format (guidelines below), addressing the detailed concerns about the data already present in the manuscript, without substantially expanding the story.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:
Text limits: Character count for a Report is < 20,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Reports may have up to 5 main text figures. To avoid delays in production, figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, http://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

***IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.***

Supplemental information: There are strict limits on the allowable amount of supplemental data. Reports may have up to 3 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.
Our typical timeframe for revisions is three months; if submitted within this timeframe, novelty will not be reassessed at the final decision. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Mark Peifer
Monitoring Editor
JCB

Rebecca Alvania
Executive Editor
JCB

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Reviewer #1 (Comments to the Authors (Required)):

This study uses fixed- and live-cell imaging to investigate the mechanisms facilitating apical nuclear migration during G2 and M phases in developing zebrafish neuroepithelia. Previous work has illuminated the mechanism of microtubule-dependent nuclear migration; however, this study is unique in that it examines actomyosin-mediated mechanisms of nuclear migration in cells maintaining their in vivo tissue organization. This study examines these mechanisms in zebrafish hindbrain and retina, and the findings may provide insight into additional mechanisms for investigation in different tissues and organisms where nuclear migration is less well understood. The use of live-cell imaging in a suitable model system is a clear strength of this study. The technique provides insights that could not be acquired via other methods. Overall, this study presents interesting findings and demonstrates differences in behavior of migrating nuclei in hindbrain and retinal neuroepithelia that suggest the mechanism of nuclear migration may differ in these tissues. The authors present 3 features that may be relevant to the differences in nuclear migration between hindbrain and retina (nuclear deformation, actomyosin regulation, and cell shape). However, the connections between these three features are not well established and the mechanistic implications of these features are not investigated in depth in this study. As they stand, there appear to be three separate conclusions. This study would benefit from focusing on one of these features and further developing its mechanistic contribution to actomyosin-mediated nuclear migration. Specific points that need to be addressed are:

Major points
1) Interpretation of Figure 2E is hindered by the absence of any measurement of nuclear stiffness. Alternative explanations, such as interactions of lamins with cytoskeletal elements, exist for the impaired nuclear migration observed with lamin A/C overexpression. Nuclear deformability cannot be
determined to be critical for nuclear migration without a direct measure of nuclear stiffness.

2) The conclusions from Figure 2C are not consistent with the data represented in the figure. The authors state "..., both retinal and hindbrain nuclei exhibited dramatic shape changes before recovering ellipsoidal shape." This conclusion suggests both tissues demonstrated a similar phenotype; however, the retinal nucleus appears to do undergo a more drastic elongation and fills the space where laser ablation occurs. This suggests nuclear shape and position in retina is cell non-autonomous and tissue-dependent. This study's strength is in investigating the role of tissue context in nuclear migration, so this observation warrants further exploration. Additionally, this figure shows only one nucleus per tissue. Quantification and statistical analysis of multiple observations would be required.

3) As the authors use Figure S2A to support the conclusion that nuclear migration in both hindbrain and retina is actomyosin-dependent, this figure should include representative images of both hindbrain and retina. Additionally, inhibitors of actin polymerization and actin turnover should be included in this figure, as opposed to Figure S3A.

4) In Figure 5I, straight and curved regions of the midbrain-hindbrain border demonstrate similar actin organization as hindbrain and retina, respectively. However, the authors do not provide evidence that nuclear migration is influenced by the same actomyosin regulation in these cells as hindbrain and retina. The link between actomyosin regulation and cell shape could be made stronger by repeating the Rho-ROCK and MLCK/formin inhibition experiments from Figure 4 in the MHBS and MHBC cells.

5) The model presented in Figure 6A has several caveats. First, the model suggests myosin is located only in the cell cytoplasm and is absent from the cortex. Figure S2B does not show a clear absence of myosin from the cell periphery. Additionally, this model does not explain the impairment of nuclear migration by blebbistatin seen in Figure S2A. The model suggests myosin is acting as a crosslinker of parallel actin filaments. This is not how myosin II is known to bundle actin filaments and the mechanism of action of blebbistatin would not be expected to interfere with this function.

Minor points
1) The authors should address the difference in starting position of hindbrain nuclei in Figure 2B,B'.
2) Absence of Fmn3 in hindbrain should be represented, as is done for retina in Figure 4H, as further support for a formin-independent mechanism in these cells.
3) Figure 2E should not use binary "yes" or "no" classifications. The authors' should formulate a definition of successful apical migration and quantify the percentage of observations that satisfy that definition per condition. Alternatively, the distance migrated could be quantified and presented.

Reviewer #2 (Comments to the Authors (Required)):

There is a rich literature of mechanisms of interkinetic nuclear migration in neuronal epithelial tissues in development. There are many contradictions in the field, with different groups arguing for different mechanisms to move nuclei to the apical position before cell division. The literature can't even agree on whether this is a microtubule or actin-mediated process. I've always suspected the simplest answer is that there isn't a universal mechanism for nuclear migration in neuroepithelial cells, and that everyone is probably right because different organisms and different tissues will use a different combination of mechanisms.

The strength of this manuscript is that it directly compares the mechanisms and kinetics between multiple different nuclear migration events in pseudostratified neuroepithelia in different tissues in the same animal at the same time of development. I believe this is the first careful comparison of apical nuclear movements in G2 in two tissues at the same time of development of the same animal. This manuscript focuses nuclear movements to the apical surface at G2 in zebrafish
Surprisingly and interestingly, the stiffness of the nucleus does not seem to play a role, as these cells don't even express lamin A/C.

There are a surprising number of differences in the nuclear migration of these two tissues. The differences are very well characterized in this manuscript. The key difference seems to be that the hindbrain has a flat tissue while the retina is curved. This leads to completely different mechanisms in the two tissues. The findings are nicely confirmed by looking at additional curved and flat epithelia in the mid hindbrain. They find that nuclei move faster in the flat tissues vs the curved tissues. Through careful quantitative imaging of actin, they show that a formin-based actin cloud is especially important in the retina, but not the midbrain. In contrast, ROCK is required in the hindbrain, but not retina (nicely shown by both drugs and dominant negative constructs). These different actin networks push differently on retina and hindbrain nuclei, shown by imaging nuclear deformability. These mechanisms should be of broad interest to a wide variety of cell biologists.

Overall, the figures were beautiful, and the manuscript was well written and easy to follow. At the end of the paper, they present a mathematical model describing how the formin-based actin networks could push nuclei forward. I do not have expertise to evaluate this, but it was interesting to read.

I have a few minor comments that could improve the paper:

1. I find the model in Figure 6A to be a little too speculative. Perhaps the manuscript could be strengthened by its removal, while keeping its discussion.

2. I am not convinced by the data presented in Figure 3C. I think the goal of 3E is to convince me they are correlated, but the sample size is small, and I am not convinced.

3. Do you have data showing that overexpression of the dominant negative FmnI3deltaC disrupted the actin cloud seen in figure 3?

4. The data in Table 2 are important, but hard to follow. Perhaps moving some of them to graphs would make it easier to follow? Also, it would be better to show +/- 95% CI instead of SEM. This would allow you to remove the long list of difficult to read p-values in the Table 2 legend. In general, I find 95% CI more useful.

Reviewer #3 (Comments to the Authors (Required)):

Yanakieva et al. compared the mechanism of the actomyosin-dependent apical movement of the nucleus of neuroepithelial cells between the developing zebrafish retina and hindbrain. Imaging, quantification, and experiments are carefully done, and very interesting results are abundantly shown. While the authors' discovery that retinal neuroepithelial cells use basal cytoplasmic actomyosin to push the nucleus apically is novel and important, this reviewer finds several points that should be clarified and improved.

1. Is the "tissue shape" really the key factor?
For example, can (1) certain underlying regional differences (retina vs. hindbrain, even though the two different portions in the midbrain-hindbrain boundary [MHB] region showed two different, i.e., a retina-like and a HB-like, patterns) and/or (2) stage differences (18 hpf for HB, 24 hpf for retina) be excluded? What will happen to formin distribution or nuclear migration if a retinal neuroepithelium is forced to be more "straight" and a HB neuroepithelium is "curved". Surgical manipulations to externally push HB neuroepithelia to artificially bend/curve would be fruitful. Molecular manipulations on the basal lamina and/or the basal surface of neuroepithelia would be a means to change the curvature. Even in a single retinal neuroepithelium, curvature would be different between the periphery (near the ciliary margin) and the center. And, the concavity of the basal surface seems to change during development. These physiologically provided in vivo variances should be more extensively utilized.

2. What does the "tissue shape" mean?
If the abovementioned possibilities can be clarified, the authors would need to more deeply describe their discovery. Title needs to be revised appropriately. Since each neuroepithelium has both apical and basal surfaces, simply saying "curved" does not clearly tell the morphology of retinal neuroepithelia. Retinal neuroepithelia "curve" showing a clear concavity at the basal surface (this is contrast to the situation in developing avian/mammalian brain primordia which are concave at the apical surface). The basal concavity specific to the retinal neuroepithelium makes the basal compartment of each neuroepithelial cell (portion basal to the nucleus) more cone-like (triangle) rather than rectangle, as illustrated in Fig. 3B. Is the narrowness and/or possible centripetal compression of the basal compartment important, suitable for, or upstream to the formin-based pushing action of actomyosin that the authors nicely found? Is tissue-level stiffness in the basal neuroepithelium different between retina and HB, and is that important?

3. Intracellular events and situations:
Although the schematic illustration in Fig 6A is very attractive, with a high spatial resolution like at the immunoEM level), it is not fully supported by the results shown by the authors. In MRLC-GFP imaging (Fig S2B), retinal cells' basal compartment is filled with intense signal (supporting the illustrated model), but the accompanied HB case does not show "more directional" apical nuclear migration and it is hard for this reviewer to follow "cytoplasmic basal enrichment only in the retina" (p.9) because quantification was not performed. Also regarding actin, although the authors state that "actin signal in hindbrain cells was mainly localized to cell periphery" (p.9), Fig 3B' show the basal cytoplasmic actin profile around the level from 0.4 to 0.6 (with actin profile at the "periphery" from 0.4 to 0.8). It is clear that the basal cytoplasmic actin profile is weaker in HB cells than in retinal cells. But, too simplified description should be revised. And, if the authors wish to show a model like in Fig. 6A, comparatively showing events in HB cells would be helpful. If possible, comparative EM analysis for actin distribution would be appreciated. Were imaging data collection using GFP-UtrophinCH or Fmn3-GFP performed only for G2-phase cells? Are the observed retina-HB differences applied only for G2-phase cells? Is the apical compartment of neuroepithelial cells similar between retina and HB? How the difference in actomyosin mechanism can be linked with the difference in nuclear deformability? Does laminA expression in HB cells also affect their nuclear migration as in LaminA-expressed retinal cells? Is the apical part of retinal neuroepithelium more crowded or stiffer than that of HB neuroepithelium? (if greater deformability is really beneficial for managing crowding as the authors suggested, p.7).
Response to Reviewer comments Yanakieva et al.:

We would like to thank all three reviewers for their constructive and thoughtful comments on the manuscript. We believe that implementing the additional experiments and text changes as outlined below further improved the quality of our study. Thus, we hope that the reviewers agree that the revised manuscript meets the high expectations associated with publishing in the Journal of Cell Biology.

Reviewer #1 (Comments to the Authors (Required)):

This study uses fixed- and live-cell imaging to investigate the mechanisms facilitating apical nuclear migration during G2 and M phases in developing zebrafish neuroepithelia. Previous work has illuminated the mechanism of microtubule-dependent nuclear migration; however, this study is unique in that it examines actomyosin-mediated mechanisms of nuclear migration in cells maintaining their in vivo tissue organization. This study examines these mechanisms in zebrafish hindbrain and retina, and the findings may provide insight into additional mechanisms for investigation in different tissues and organisms where nuclear migration is less well understood. The use of live-cell imaging in a suitable model system is a clear strength of this study. The technique provides insights that could not be acquired via other methods. Overall, this study presents interesting findings and demonstrates differences in behavior of migrating nuclei in hindbrain and retinal neuroepithelia that suggest the mechanism of nuclear migration may differ in these tissues. The authors present 3 features that may be relevant to the differences in nuclear migration between hindbrain and retina (nuclear deformation, actomyosin regulation, and cell shape). However, the connections between these three features are not well established and the mechanistic implications of these features are not investigated in depth in this study. As they stand, there appear to be three separate conclusions. This study would benefit from focusing on one of these features and further developing its mechanistic contribution to actomyosin-mediated nuclear migration.

After careful consideration, we agree with the reviewer. In the last version of our manuscript we tried to touch upon too many different points which made the general story line a bit convoluted. To address this criticism, we now changed parts of the text to link the different areas of the manuscript more clearly than previously achieved. We also added additional experiments that emphasize the connection between basal actin enrichment and the nuclear deformations and velocity fluctuations that actin causes during apical migration (see below answer to Reviewer 2 point 2). To further clarify the manuscript, we removed most of the Lamin data. The part of the manuscript that investigates nuclear deformations (mainly Figure 2) has now been streamlined and concentrates on nuclear deformations and how these deformations can be used to assess the forces acting on the nuclei in the different neuroepithelia.

Specific points that need to be addressed are:

Major points

1) Interpretation of Figure 2E is hindered by the absence of any measurement of nuclear stiffness. Alternative explanations, such as interactions of lamins with cytoskeletal elements,
exist for the impaired nuclear migration observed with lamin A/C overexpression. Nuclear deformability cannot be determined to be critical for nuclear migration without a direct measure of nuclear stiffness.

We now tuned down all these statements and moved all speculation to the discussion. As mentioned above, we further removed the Lamin A/C overexpression data and focus on nuclear deformability and how it can be used to infer force generation mechanisms.

2) The conclusions from Figure 2C are not consistent with the data represented in the figure. The authors state “... both retinal and hindbrain nuclei exhibited dramatic shape changes before recovering ellipsoidal shape.” This conclusion suggests both tissues demonstrated a similar phenotype; however, the retinal nucleus appears to undergo a more drastic elongation and fills the space where laser ablation occurs. This suggests nuclear shape and position in retina is cell non-autonomous and tissue-dependent. This study’s strength is in investigating the role of tissue context in nuclear migration, so this observation warrants further exploration. Additionally, this figure shows only one nucleus per tissue. Quantification and statistical analysis of multiple observations would be required.

We agree with the reviewer that the laser ablation data was not convincing due to the low n. We previously intended to use this data as a demonstration of the extreme nuclear deformations that can potentially occur. However, we agree that the way we presented this data could be interpreted as pointing to a cell non-autonomous mechanism that defines nuclear shape, which was not our intention. Thus, we removed the data from the manuscript. To assess nuclear deformations and the differences between retinal and hindbrain nuclei more quantitatively we analyzed nuclear deformability in both tissues at different cell cycle stages. This analysis shows that hindbrain and retinal nuclei display comparable variability of aspect ratios in S-phase. The comparable variability of nuclear shape in the absence of the forces that cause the directed movements of nuclei in G2 indicates that they are able to deform to a similar extent. With the onset of G2, as seen in the previous version of the manuscript, the fluctuations of aspect ratio do not change in the hindbrain while we observe persistent and significant increase in the aspect ratios of retinal nuclei. (Figure 2 D and E). We conclude that in the absence of directed movement nuclei in both tissues show a similar ability to deform and that the difference observed between hindbrain and retinal nuclei in G2 shows that they are subjected to different forces during apical migration.

3) As the authors use Figure S2A to support the conclusion that nuclear migration in both hindbrain and retina is actomyosin-dependent, this figure should include representative images of both hindbrain and retina. Additionally, inhibitors of actin polymerization and actin turnover should be included in this figure, as opposed to Figure S3A.

The effect of blebbistatin in the retina was shown already in previous studies (Norden, 2009, Strzyz, 2015...others). Nevertheless, we repeated these experiments and they are consistent with previous findings. This data can now be found in Figure S2C. In addition, the actin turnover data using Latrunculin A and Japlakinolide can now be found in Figure S2D.
4) In Figure 5I, straight and curved regions of the midbrain-hindbrain border demonstrate similar actin organization as hindbrain and retina, respectively. However, the authors do not provide evidence that nuclear migration is influenced by the same actomyosin regulation in these cells as hindbrain and retina. The link between actomyosin regulation and cell shape could be made stronger by repeating the Rho-ROCK and MLCK/formin inhibition experiments from Figure 4 in the MHBS and MHBC cells.

This is a good point raised by the reviewer and we added new experiments to the manuscript. We find that indeed Rho-ROCK inhibition interferes with nuclear migration in the MHBS but not in the MHBC (Figure S4D).

5) The model presented in Figure 6A has several caveats. First, the model suggests myosin is located only in the cell cytoplasm and is absent from the cortex. Figure S2B does not show a clear absence of myosin from the cell periphery.

We agree with the reviewer that the way we presented the model did not state clearly enough that we indeed consider this a theoretical model that currently is supported by our data but that more studies are needed for its verification. At its previous location in Figure 6 readers could have easily thought that this model represents a summary of our experimental findings, which is not necessarily the case. For this reason, we moved the model into Figure 4I together with all other Formin data and state clearly in the Figure legend and the discussion that it is a theoretical model in which not all parameters have been measured yet. We believe that this clarifies it to the readership.

Additionally, this model does not explain the impairment of nuclear migration by blebbistatin seen in Figure S2A. The model suggests myosin is acting as a crosslinker of parallel actin filaments. This is not how myosin II is known to bundle actin filaments and the mechanism of action of blebbistatin would not be expected to interfere with this function.

While the reviewer is correct, that this is not the canonical mode of how blebbistatin is acting in most contexts, it has been previously shown (e.g. Kovacs et al., 2004) that blebbistatin can block myosin in actin-detached states and that this can prevent rigid actomyosin crosslinking. Such mechanism fits with our theoretical model but, as noted before, still needs to be experimentally tested, which is why we now state more rigorously that our model currently is a working model. We further added a sentence in the discussion to clarify that at this point we cannot explicitly exclude a contractility dependent force generation mechanism.

Minor points
6) The authors should address the difference in starting position of hindbrain nuclei in Figure 2B,B'.

Here we were unfortunately confused as no Figure 2B' exists in the manuscript (also no Suppl. 2B') and we therefore do not know what the reviewer refers to. However, the differences in starting position are actually discussed in the manuscript and also addressed in Figure 1D and E. We furthermore added a sentence in the discussion.
suggesting that the differences might be due to the fact that in the retina not the complete apico-basal axis is accessible to nuclei due to the basal actin accumulation.

7) Absence of Fmn3 in hindbrain should be represented, as is done for retina in Figure 4H, as further support for a formin-independent mechanism in these cells.

These experiments have been added and are now shown in Figure 4H.

8) Figure 2E should not use binary "yes" or "no" classifications. The authors’ should formulate a definition of successful apical migration and quantify the percentage of observations that satisfy that definition per condition. Alternatively, the distance migrated could be quantified and presented.

Figure 2E has now been removed from the manuscript, as explained in response to the general comments by the reviewer.

Reviewer #2 (Comments to the Authors (Required)):

There is a rich literature of mechanisms of interkinetic nuclear migration in neuronal epithelial tissues in development. There are many contradictions in the field, with different groups arguing for different mechanisms to move nuclei to the apical position before cell division. The literature can't even agree on whether this is a microtubule or actin-mediated process. I've always suspected the simplest answer is that there isn't a universal mechanism for nuclear migration in neuroepithelial cells, and that everyone is probably right because different organisms and different tissues will use a different combination of mechanisms.

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Surprisingly and interestingly, the stiffness of the nucleus does not seem to play a role, as these cells don't even express lamin A/C.

There are a surprising number of differences in the nuclear migration of these two tissues. The differences are very well characterized in this manuscript. The key difference seems to be that the hindbrain has a flat tissue while the retina is curved. This leads to completely different mechanisms in the two tissues. The findings are nicely confirmed by looking at additional curved and flat epithelia in the mid hindbrain. They find that nuclei move faster in the flat tissues vs the curved tissues. Through careful quantitative imaging of actin, they show that a formin-based actin cloud is especially important in the retina, but not the midbrain. In contrast, ROCK is required in the hindbrain, but not retina (nicely shown by both drugs and dominant negative constructs). These different actin networks push differently on retina and hindbrain nuclei, shown by imaging nuclear deformability. These mechanisms
should be of broad interest to a wide variety of cell biologists.

Overall, the figures were beautiful, and the manuscript was well written and easy to follow. At the end of the paper, they present a mathematical model describing how the formin-based actin networks could push nuclei forward. I do not have expertise to evaluate this, but it was interesting to read.

I have a few minor comments that could improve the paper:

1. I find the model in Figure 6A to be a little too speculative. Perhaps the manuscript could be strengthened by its removal, while keeping its discussion.

As already noted to Reviewer 1 point 5):
We agree with the reviewer that the way we presented the model did not state clearly enough that we indeed consider this a theoretical model that currently is supported by our data but that more studies are needed for its verification. At its previous location in Figure 6 readers could have easily thought that this model represents a summary of our experimental findings which is not necessarily the case. For this reason, we moved the model into Figure 4 together with all other Formin data and state clearly in the Figure legend and the discussion that it is a theoretical model in which not all parameters have been measured yet. We also added a sentence in the discussion to clarify that at this point we cannot explicitly exclude a contractility dependent force generation mechanism. We believe that this clarifies it to the readership.

2. I am not convinced by the data presented in Figure 3C. I think the goal of 3E is to convince me they are correlated, but the sample size is small, and I am not convinced.

To improve our cross-correlation analysis, we increased the number of cells in which actin enrichment was analyzed and in all of them the basal actin fluctuations showed similar frequency as the fluctuations of both the instantaneous velocity and the nuclear aspect ratio (Figure 3E). However, as we indeed created only relative measures, we tuned down our interpretations in the results section.

3. Do you have data showing that overexpression of the dominant negative Fmnl3deltaC disrupted the actin cloud seen in figure 3?

We tried this experiment in all possible combinations of markers of actin and Fmnl3 but unfortunately met technical difficulties as the red formin and actin markers were not stable enough to survive the whole course of imaging due to photobleaching.

4. The data in Table 2 are important, but hard to follow. Perhaps moving some of them to graphs would make it easier to follow? Also, it would be better to show +/- 95%CI instead of SEM. This would allow you to remove the long list of difficult to read p-values in the Table 2 legend. In general, I find 95% CI more useful.
We agree that the table alone was hard to interpret and did not do justice to the importance of the data. We now further show the data as graphs where it is relevant and thereby make it more accessible.

Furthermore, we are aware that the use of 95% CI is gaining increasing popularity as a means to avoid the use of P values in the conventional, dichotomous way. However, we don’t utilize P values to decide whether a result refutes or supports a scientific hypothesis, but rather present them to the reader as a reference to the distributions of the pairs of samples, so similarly to the way 95% CI is used. To facilitate reading Table 1 and 2 we added the p values directly to the table, instead of listing all of them in the legend.

After discussing the use of SEM and p values with a statistician we realized that SEM can only be used when the distributions are normal and as we do not claim or test for normality, we now replaced SEM with SD.

Reviewer #3 (Comments to the Authors (Required)):

Yanakieva et al. compared the mechanism of the actomyosin-dependent apical movement of the nucleus of neuroepithelial cells between the developing zebrafish retina and hindbrain. Imaging, quantification, and experiments are carefully done, and very interesting results are abundantly shown. While the authors’ discovery that retinal neuroepithelial cells use basal cytoplasmic actomyosin to push the nucleus apically is novel and important, this reviewer finds several points that should be clarified and improved.

1) Is the "tissue shape" really the key factor?
   For example, can (1) certain underlying regional differences (retina vs. hindbrain, even though the two different portions in the midbrain-hindbrain boundary [MHB] region showed two different, i.e., a retina-like and a HB-like, patterns) and/or (2) stage differences (18 hpf for HB, 24 hpf for retina) be excluded?

   As for (1), as the reviewer correctly states, the region MHBS and MHBC are part of a continuous tissue but nevertheless use different mechanisms according to their morphology. We extended on these findings by showing that Rho-ROCK inhibition interferes with nuclear migration in the MHBS but not MHBC region at the same developmental stage (Figure S4 H).

   As for (2), we believe that stage-specific differences can be excluded as in the MHBC versus MHBS analysis we see different mechanisms in a continuous tissue at the same developmental stage.

   Thus, cell and tissue shape are the defining parameters that dictate nuclear migration mechanism. We now further underlined this point as seen in response to 2).

2) What will happen to formin distribution or nuclear migration if a retinal neuroepithelium is forced to be more "straight" and a HB neuroepithelium is "curved". Surgical manipulations to externally push HB neuroepithelia to artificially bend/curve would be fruitful. Molecular manipulations on the basal lamina and/or the basal surface of neuroepithelia would be a means to change the curvature. Even in a single retinal neuroepithelium, curvature would be
different between the periphery (near the ciliary margin) and the center. And, the concavity of
the basal surface seems to change during development. These physiologically provided in
vivo variances should be more extensively utilized.

We now addressed this point by using a Laminin morpholino which has the effect to flatten
retinal tissue and changes cell morphology to more columnar and thus morphologically
similar to hindbrain cells as shown previously in Sidhaye et al., 2017, eLife. Interestingly, we
observe that when retinal cells show a more columnar morphology similar to hindbrain cells
(Figure 6A,B), in some of these apical nuclear migration can still occur even though the basal
actin cloud is not formed during migration (Figure 6B). These new results further emphasize
the finding that indeed cell and tissue architecture dictate the mode of actin dependent force
generation.

3) What does the “tissue shape” mean?
If the abovementioned possibilities can be clarified, the authors would need to more deeply
describe their discovery. Titles needs to be revised appropriately.
Since each neuroepithelium has both apical and basal surfaces, simply saying "curved" does
not clearly tell the morphology of retinal neuroepithelia. Retinal neuroepithelia "curve"
showing a clear concavity at the basal surface (this is contrast to the situation in developing
avian/mammalian brain primordia which are concave at the apical surface). The basal
concavity specific to the retinal neuroepithelium makes the basal compartment of each
neuroepithelial cell (portion basal to the nucleus) more cone-like (triangle) rather than
rectangle, as illustrated in Fig. 3B. Is the narrowness and/or possible centripetal compression
of the basal compartment important, suitable for, or upstream to the formin-based pushing
action of actomyosin that the authors nicely found?

We have clarified this throughout the manuscript. We now state that curved in the manuscript
refers to a basally constricted tissue.

4) Is tissue-level stiffness in the basal neuroepithelium different between retina and HB, and
is that important?

While an interesting point, we believe that direct tissue stiffness measurements are outside
the scope of this manuscript. However, we will use such studies as exciting entry points to
follow-up investigations that deal with tissue stiffness and how it affects nuclear migration in
developing neuroepithelia of different shapes.

5) Intracellular events and situations:
Although the schematic illustration in Fig 6A is very attractive, with a high spatial resolution
like at the immunoEM level), it is not fully supported by the results shown by the authors.

As already noted in response to Reviewer 1 and 2:
We agree with the reviewer that the way we presented the model did not state clearly
enough that we indeed consider this a theoretical model that currently is supported by our
data but that more studies are needed for its verification. At its previous location in Figure 6
readers could have easily thought that this model represents a summary of our experimental
findings which is not necessarily the case. For this reason, we moved the model into Figure 4 together with all other Formin data and state clearly in the Figure legend and the discussion that it is a theoretical model in which not all parameters have been measured yet. We also added a sentence in the discussion to clarify that at this point we cannot explicitly exclude a contractility dependent force generation mechanism. We believe that this clarifies it to the readership.

6) In MRLC-GFP imaging (Fig S2B), retinal cells' basal compartment is filled with intense signal (supporting the illustrated model), but the accompanied HB case does not show "more directional" apical nuclear migration and it is hard for this reviewer to follow "cytoplasmic basal enrichment only in the retina" (p.9) because quantification was not performed.

We additionally compared myosin distribution in retina versus hindbrain during G2 and see that, like actin, the myosin profile is flatter in the hindbrain while in the retina we find myosin enrichment in the interior of the cell. These results can now be found in Figure S2 G.

7) Also regarding actin, although the authors state that "actin signal in hindbrain cells was mainly localized to cell periphery" (p.9), Fig 3B' show the basal cytoplasmic actin profile around the level from 0.4 to 0.6 (with actin profile at the "periphery" from 0.4 to 0.8). It is clear that the basal cytoplasmic actin profile is weaker in HB cells than in retinal cells. But, too simplified description should be revised.

We agree with the reviewer and also address this by further measurements also in response to point 9):
More actin analysis data is now added and can be found in Figure 3. We see less cytoplasmic actin in hindbrain cells. While some cytoplasmic actin is seen also in S-phase of retinal cells, the values are much lower than in G2 and show a much higher degree of variation indicating that while actin is present in the basal process, it does not show a lasting accumulation as opposed to in cells in G2.

8) And, if the authors wish to show a model like in Fig. 6A, comparatively showing events in HB cells would be helpful. If possible, comparative EM analysis for actin distribution would be appreciated.

As mentioned above, we moved the model to Figure 4 together with the formin data and clarify also in the discussion that this is a theoretical model that fits the current experimental data but that more experiments need to be performed in future studies.

9) Were imaging data collection using GFP-UtrophinCH or FmnI3-GFP performed only for G2-phase cells?
Are the observed retina-HB differences applied only for G2-phase cells?

This actin data is now added and can be found in Figure 3 and Figure 4. We see little cytoplasmic enrichment in hindbrain cells. While some cytoplasmic actin is seen also in S-phase of retinal cells, the values are much lower than in G2 cells and show a much higher
degree of variation indicating that while actin is present in the basal process, it does not show a lasting accumulation as opposed to in cells in G2.

10) Is the apical compartment of neuroepithelial cells similar between retina and HB? Is the apical part of retinal neuroepithelium more crowded or stiffer than that of HB neuroepithelium? (if greater deformability is really beneficial for managing crowding as the authors suggested, p.7).

As we do not believe that this data adds to the current flow of the manuscript and would possibly distract readers, we add it to this response letter for the reviewer. The answer is that indeed retina and hindbrain have similar numbers of nuclei packed per unit volume. We will follow this point in future investigations.

11) How the difference in actomyosin mechanism can be linked with the difference in nuclear deformability?

We now added a further set of experiments to clarify this point: we performed laser ablation of a circular region in retina and hindbrain nuclei of different cell cycle stages. We see that only in G2 retinal nuclei does this ablated region become basally deformed, further arguing for a force acting at the basal side of the nucleus in these cells Figure S1 E,F,G.

In addition, please refer to our response to Reviewer 1 point 2): To assess nuclear deformations and the differences between retinal and hindbrain nuclei more quantitatively we analyzed nuclear deformability in both tissues at different cell cycle stages. This analysis shows that hindbrain and retinal nuclei display comparable variability of aspect ratios in S-phase. The comparable variability of nuclear shape in the absence of the forces that cause the directed movements of nuclei in G2 indicates that they are able to deform to a similar extent. With the onset of G2, as seen in the previous version of the manuscript, the fluctuations of aspect ratio do not change in the hindbrain while we observe persistent and significant increase in the aspect ratios of retinal nuclei. (Figure 2 D and E).
We conclude that in the absence of directed movement nuclei in both tissues show a similar ability to deform and that the difference observed between hindbrain and retinal nuclei in G2 shows that they are subjected to different forces during apical migration.

12) Does laminA expression in HB cells also affect their nuclear migration as in LaminA-expressed retinal cells?

We did not add the result of Lamin A expression in the hindbrain as the Lamin A overexpression data was removed from the manuscript. Nevertheless, we did these experiments outside the scope of this study and the answer is: Yes, Lamin A overexpression also hinders nuclear migration in the hindbrain. However, as suggested by Reviewer 1, we will keep this data out of this study to improve clarity and will in the future investigate these issues in more detail independently.
June 19, 2019

RE: JCB Manuscript #201901077R

Dr. Caren Norden  
Max Planck Institute of Cell Biology and Genetics  
Pfotenhauer Strasse 108  
Dresden 01307  
Germany

Dear Caren:

Thank you for submitting your revised manuscript entitled "Cell and tissue morphology determine actin-dependent nuclear migration mechanisms in neuroepithelia". Your manuscript was reviewed by two of the original reviewers. Reviewer 2 did not re-review as they were supportive of publication originally and had only minor revision suggestions. Having assessed the comments from the two reviewers, we would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below). At this point no additional experimentation or major text revisions are needed. In revising the manuscript for final submission, it would be helpful to consider Reviewer 1’s remaining points and find ways to somewhat soften your language in those places. There will be no need for additional external review.

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Sincerely,

Mark Peifer
Monitoring Editor
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Rebecca Alvania
Executive Editor
JCB

Reviewer #1 (Comments to the Authors (Required)):

The authors have made improvements to the manuscript and strengthened their study. First, the reorganization of figures and text changes have provided a more logical flow throughout the study. Figure 2 is improved from the previous version of the study. Removal of the lamin overexpression data has clarified the purpose of this figure. Also, the disruption of nuclear migration in MHBS on ROCK inhibition strengthens the conclusion that the nuclear migration mechanism may be related to cell shape. The new nuclear photobleaching experiment is interesting and does indeed suggest something is pushing upward on the nucleus during G2 in retina, although clear evidence that actin is acting directly on the nucleus is still not presented. The model now presented in Figure 4I still has not addressed issues regarding actin organization and the role of myosin (see major points below). While they are careful to state the current model is theoretical and requires further validation, it would be useful to have further explanation as to why the authors favor this actin pushing mechanism compared to other mechanisms, such as a mechanism that involves cortical contractility, which they state is not excluded in their discussion. In order to continue with the current model, the authors should further explore the role of myosin in their model, provide direct evidence for actin pushing, and further clarify the reasoning for excluding other models.

Major points
1) The actin filament organization presented in Figure 4I is not clearly evident from any of the experimental data. The arrangement of actin into parallel bundles cannot be concluded using the current imaging resolution.
2) Myosin localization basal to the nucleus is still not clear. Both the immunofluorescent staining (Figure 5A',C) and fluorescent tagged MRLC (Figure S2F) show diffuse localization that is hard to draw conclusions from. The quantification of myosin in Figure S2G also does not show a clear difference in localization between retina and hindbrain.

3) The authors should provide evidence that myosin can incorporate into and crosslink parallel (as opposed to the canonical antiparallel) actin fibers as is presented in their model. Also, in this model, actin length is much longer than its persistent length and would require bundling by a strong crosslinker. Myosin II is not known to be able to fulfill this role.

Reviewer #3 (Comments to the Authors (Required)):

The authors addressed all the points very sincerely. Several interesting results like Fig S4H and Fig 6AB (I really send applause) and careful writing improved the work.