**Cell cycle and developmental control of cortical excitability in Xenopus laevis**

Zachary Swider, Ani Michaud, Marcin Leda, Jennifer Landino, Andrew Goryachev, and William Bement

*Corresponding author(s): William Bement, University of Wisconsin-Madison*

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*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.)
1st Editorial Decision

RE: Manuscript #E22-01-0025
TITLE: "Cell cycle and developmental control of cortical excitability in Xenopus laevis"

Dear Prof. Bement:

As you'll see below, both reviewers find you paper fit for publication in MBoC, though they provide several suggestions. Please attend to the reviewer comments as you see fit, but do pay particular attention to Reviewer #2 comments about the Z plane, which may change over time.

I'm glad your paper will find a home in MBoC!

Sincerely,

John Wallingford
Monitoring Editor
Molecular Biology of the Cell

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Dear Prof. Bement,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) 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.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

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.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL):
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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

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Reviewer #1 (Remarks to the Author):

See attached.

Reviewer #2 (Remarks to the Author):

The authors present a detailed analysis of temporal and spatial changes of F-actin abundance in the cell cortex of living frog embryos by tracking changes in F-actin abundance through fluorescence localization of the F-actin binding domain of utrophin. Periodic changes of these intensities and their spatial representation in waves suggest these changes indicate the excitable nature of the F-actin cell cortex. Changes are quantified through power spectrum analysis to obtain both amplitude and frequency over the cell cycle and progressive cleavage stages where cell volumes and apical areas are reduced with every division. While the authors had previously identified traveling and spiral waves this manuscript correlates oscillatory kinematics with cell cycle regulation. F-actin exhibits strong oscillatory behavior after egg activation and during interphase in the cell cycle. Excitability itself appears coupled to cell cycle events both in meiosis and later mitotic cycles during cleavage as kinematics are quenched as cells transit from mitotic entry through anaphase. The figures demonstrating these changes are excellent and highlight oscillatory behavior using striking kymographs together with quantitatively determined frequency and amplitudes. The strength of the Xenopus model is evident as F-actin can be visualized across very large territories of apical cell cortex and correlated to microtubule abundance and cortical contraction waves. Cell cycle regulation is inferred by observations of cortex movements and cytoskeletal localization. Cycloheximide treatment demonstrates that cycling abundance does not depend on new protein synthesis but the mitotic-associated quenching events are dependent. The analysis continues to investigate changes in patterns of episodic F-actin as cell size deceases through Xenopus reductive cleavages, through MZT and up to gastrulation, and including a single late neurula stage. Additional analysis of actin during cytokinesis in the cleavage furrow is also included.

The authors take great care in making the descriptions concise and presenting the results in a well written manner.

Some questions:

The authors use the term 'robust' where it has not been demonstrated. Robust generally refers to stability after perturbation, such as the spiral waves in a B-Z reaction, but I do not see the authors demonstrate robustness here. Waveforms are not challenged in a way that restorative mechanisms would be exposed.

Interesting that oscillations and traveling waves do not persist to St 20. The authors suggest changes in regulators of actin assembly and disassembly, however, the net F-actin and the partitioning of mobile and immobile sets are likely quite different in the early oocyte to late neurula. Such changes are obvious in Drosophila and C. elegans and might suggest that the F-actin substrates for waves might be very different across these stages. As the mobile fraction diminishes the detection of these dynamics may be lost in the higher F-actin signal of the immobile network.

The method describing the wave area is ambiguous. What does the absolute area, in micrometers^2 refer to? The region used for segmenting high F-actin intensities might be arbitrary so the area might be better represented as a percentage.

The conclusions drawn about assembly and disassembly are highly dependent on the model they adopted earlier in the analysis. The image analysis pipeline interprets increased intensity as increased assembly and decreased intensity as disassembly. However, they might have taken two alternative approaches. What if assembly was constant and disassembly variable, or the reverse? The authors may want to review their conclusions to reflect alternative interpretations of intensity changes.

The authors report that F-actin is not moving within the XY imaging plane but what of motion in the Z-plane, toward and away from the apical cortex. Recent work by the Paluch lab (Quang et al, 2021, Nat Comms) suggests myosin II translocates along this same axis. Additionally, I am concerned that cortical thickness is changing during these stages, early cortex may be quite thick and easily found with 0.5 um intervals but may thin at later stages to 0.2 um or less. 0.5 um stacks may not be sufficient to capture, or rule out such movements. Perhaps higher resolution stacks could be acquired and compared across the cell cycle and as cleavage progresses.

Minor issues:

Please report the "room temperature" and confirm that this was held stable over the time-course of the experiments. Von Dassow et al (2014, PLoS One) showed F-actin dynamics are especially sensitive to temperature and likely to alter the frequency of the measurements.
Fig 1B -- The presentation of the "color-encoded-phase" image is shown as a triangle below still image but the boundaries are not so clear. Perhaps contrast can be increased in the LUT and certainly dividing lines should be added like those in Fig 3B'.

Fig 2B' -- Please include the absolute F-actin intensity over this time course? It would appear that the average intensity decreases as the amplitude increase. This might suggest an increasing level of episodic disassembly.

Fig 3A -- The red tick marks indicating the times of still frames are very small and nearly indiscernible. Larger tick marks like in 1B would be better.
Dear John,

Please find enclosed our revised manuscript titled:

“Cell cycle and developmental control of cortical excitability in *Xenopus laevis*”

by Zachary Swider, Ani Michaud, Marcin Leda, Jennifer Landino, Andrew Goryachev, and me, for consideration as an article by *Molecular Biology of the Cell*. We have taken into consideration all of the comments and suggestions of the reviewers and made changes to the text and figures as described below.

**Reviewer 1**

*Together, this manuscript provides a quantitative description of a poorly characterized phenomenon of cortical excitability and links it to cell cycle progression.*

*The manuscript is of high quality, especially the initial part describing the dependence of wave dynamics on cell cycle progression. The following aspects, the relation of wave properties and cell size and cleavage furrow vs. non-furrow cortical excitability, are less developed. Nevertheless, the manuscript provides novel insights into the regulation of cytokinesis by cortical excitability.*

*We thank the reviewer for their kind words and encouragement.*

*Minor comments:*

*The authors propose that changes in wave behavior reveal remodeling of F-actin, specifically the actin assembly and disassembly rate. Given the surface contraction waves, could cortex compression and decompression be responsible for observed F-actin dynamics?*

*The reviewer raises a valid point, namely, could relaxation of the cell cortex contribute to observation that F-actin waves are sparsely distributed upon mitotic entry, and could contraction of the cell cortex contribute to the observation that F-actin waves are tightly packed midway through mitosis?*  

*In principle, relaxation and contraction could indeed contribute to these observations. However, we argue that the contribution is likely minimal as we do not see the expected outward or inward flow of F-actin waves upon relaxation and contraction respectively. Moreover, our net F-actin assembly and net F-actin disassembly account for this possibility; if a given wave simply moved in space over time with no change in size and/or intensity the net assembly and disassembly measurements would return equal amounts of assembly and disassembly. However, instead we measure differential increases and decreases between net assembly and net disassembly. Finally, we continue to observe the reported changes in F-actin dynamics in*

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cells where enough developmental time has passed after egg activation that the surface contraction wave become undetectable (Hara et al., 1980).

Reviewer 2

The figures demonstrating these changes are excellent and highlight oscillatory behavior using striking kymographs together with quantitatively determined frequency and amplitudes.

The authors take great care in making the descriptions concise and presenting the results in a well written manner.

We thank this reviewer for their kind words.

Some questions:
The authors use the term 'robust' where it has not been demonstrated. Robust generally refers to stability after perturbation, such as the spiral waves in a B-Z reaction, but I do not see the authors demonstrate robustness here. Waveforms are not challenged in a way that restorative mechanisms would be exposed.

We thank the reviewer for raising this point; in the context of this paper we used ‘robust’ as a synonym for ‘clearly detectable’ (i.e., high amplitude and widely spaced waves). However, this was a mistake, given the more generally applied meaning of the word in biology, as the reviewer (politely) points out. We have therefore replaced all relevant references to waves as being robust with more appropriate adjectives. These changes have been underlined and can be found on pages 8 (instance 1: robust -> clear, instance 2: deleted), 9 (instance1: robust -> useful, instance 2: robust -> obvious), 12 (robust -> obvious), Video 1 Legend (robust -> clear)

Interesting that oscillations and traveling waves do not persist to St 20. The authors suggest changes in regulators of actin assembly and disassembly; however, the net F-actin and the partitioning of mobile and immobile sets are likely quite different in the early oocyte to late neurula. Such changes are obvious in Drosophila and C. elegans and might suggest that the F-actin substrates for waves might be very different across these stages. As the mobile fraction diminishes the detection of these dynamics may be lost in the higher F-actin signal of the immobile network.

The reviewer raises an interesting point - that the reported lack of F-actin waves in later developmental changes could be due to an increase in stable F-actin which masks any underlying dynamic structures. While there is certainly a notable increase in stable F-actin (illustrated in Supplemental Figure 6), we nonetheless expect that any underlying dynamic structures would contribute to the overall fluorescence in a given space. Thus, computational subtraction of stable structures (as in Figure 6) should reveal the underlying dynamics. Such processing failed to reveal any additional dynamics and we therefore suspect that F-actin waves are either absent or present at undetectably low level relative to their properties earlier in development. To better reflect this rationale, modified the sentence reporting this finding (changes in bold).

We easily identified F-actin waves in embryos as late as stage 9, but in later stage embryos (~stage 20) F-actin waves were undetectable against the background of stable F-actin (Supplemental Figure 6A, A').
The method describing the wave area is ambiguous. What does the absolute area, in micrometers$^2$ refer to? The region used for segmenting high F-actin intensities might be arbitrary so the area might be better represented as a percentage.

We thank the reviewer for raising this concern and agree that the explanation can be made clearer. We specifically did not describe area as a percentage because the property we were attempting to describe was not the fraction of the cortex occupied by waves but rather how well defined ‘individual’ waves were. The identification of individual waves is indeed somewhat arbitrary as the waves are not discrete structures, but rather an interconnected web of F-actin. Thus, we reasoned that if wave structures are well defined and interconnected, we should on average measure large thresholded ‘particles’ (interconnected thresholded regions), while if wave structures were less well defined, we should on average measure smaller thresholded ‘particles’. We have modified the corresponding paragraph (bold text) to make this distinction clearer:

First, we used cycloheximide to arrest cells in interphase at different stages of development to standardize wave behavior between cells and qualitatively assessed F-actin wave properties at different stages of development (Figure 5A). It appeared that individual F-actin waves became poorly defined and less inter-connected during later stages of development. We quantified this change by measuring the two-dimensional wave area of individual waves in cycloheximide arrested cells by blindly thresholding UtrCH fluorescence (see methods) and measuring the resulting area of thresholded particles (area of interconnected thresholded pixels) with the rational that wave structures which were well defined and interconnected would produce large areas of continuous thresholding while wave structures which were poorly defined would produce relatively smaller areas of continuous thresholding.

The conclusions drawn about assembly and disassembly are highly dependent on the model they adopted earlier in the analysis. The image analysis pipeline interprets increased intensity as increased assembly and decreased intensity as disassembly. However, they might have taken two alternative approaches. What if assembly was constant and disassembly variable, or the reverse? The authors may want to review their conclusions to reflect alternative interpretations of intensity changes.

The reviewer is correct that our analysis interprets increased signal over the measurement period as an increase in net F-actin assembly. We tried to make it clear in the original text that this technique is not capable of differentiating between the relative contributions of assembly and disassembly to the signal from a single pixel and for this reason underlined the word net to emphasize its importance. Obviously, this was not sufficiently clear. We have therefore modified the paragraph (bold text) describing this approach in an attempt to further clarify this point:

Because the waves are insensitive to myosin-2 inhibition (Bement et al., 2015), and are thus not likely to reflect motor-mediated transport, we reasoned that if an individual pixel value increased over time that this reflected net F-actin assembly in that region and that if the pixel value decreased that this reflected net F-actin disassembly in that region. While this approach cannot differentiate the contributions of assembly and disassembly towards the signal from a single pixel, by summing the net assembly and net disassembly measurements in all regions of the field of view, we can differentiate the contributions of each to the total signal in the field of view. The sum of these pixel-wise measurements revealed that entry into mitosis
is marked by a decrease in net F-actin assembly and a simultaneous increase in net F-actin disassembly (Figure 3B’’; Video 7).

Furthermore, we have modified supplemental video 7 to show both the net assembly and net disassembly measurements unrolling in sync with a plot of these measurements over time. To stay consistent with formatting, we have similarly modified supplemental video 6 and added an additional supplemental video (now supplemental video 8) corresponding to figures 3B’ and 3B’’ respectively. Finally, we have changed the axes labels for the relevant figures and movies to read "Net Assembly" and "Net Disassembly".

The authors report that F-actin is not moving within the XY imaging plane but what of motion in the Z-plane, toward and away from the apical cortex. Recent work by the Paluch lab (Quang et al, 2021, Nat Comms) suggests myosin II translocates along this same axis. Additionally, I am concerned that cortical thickness is changing during these stages, early cortex may be quite thick and easily found with 0.5 um intervals but may thin at later stages to 0.2 um or less. 0.5 um stacks may not be sufficient to capture, or rule out such movements. Perhaps higher resolution stacks could be acquired and compared across the cell cycle and as cleavage progresses.

For all datasets used in this study we chose a z-step size and z range which 1) produced some overlap in successive optical sections and 2) captured the cell cortex in the middle of the z-stack. Thus, while this study did not specifically investigate sub-micron movements in the z-plane, this optical set up rules out the possibility that such movements, if they occurred, could contribute to any significant gain or loss in fluorescence.

Minor issues:
Please report the "room temperature" and confirm that this was held stable over the time-course of the experiments. Von Dassow et al (2014, PLoS One) showed F-actin dynamics are especially sensitive to temperature and likely to alter the frequency of the measurements.

We thank the reviewer for pointing this out - F-actin dynamics, as well as cell cycle dynamics, are indeed sensitive to temperature. We have added a range of expected room temperatures in Celsius to the methods (bold text) and do confirm that the specific temperature, which could vary depending on the day/season, was held study over the course of an imaging experiment:

For all imaging experiments, embryos were maintained in 0.1X MMR at room temperature (20-24˚C), which was maintained over the course of an imaging experiment.

Fig 1B’’ -- The presentation of the "color-encoded-phase" image is shown as a triangle below still image but the boundaries are not so clear. Perhaps contrast can be increased in the LUT and certainly dividing lines should be added like those in 3B'.

We agree that the boundaries are unclear in this figure and have added dividing lines as in 3B’ as suggested.

Fig 2B’ -- Please include the absolute F-actin intensity over this time course? It would appear that the average intensity decreases as the amplitude increase. This might suggest an increasing level of episodic disassembly.

We agree that this would be a worthwhile addition to Figure 2B’ and have added the suggested plot. We have also added the following reference to the figure in the results (in bold):
While mean UtrCH fluorescence steadily decreased following egg activation, quantification of wave amplitude and wave period revealed that the normalized wave amplitude steadily increased over time. Similarly, the wave period initially decreased and then subsequently began to increase at about 20 minutes post-washout of ionomycin (Figure 2B').

And amended the figure legend as follows (in bold):

Plot showing the mean UtrCH signal (in black) mean wave period (in green) and mean normalized wave amplitude (in pink) for the region indicated in B.

Fig 3A -- The red tick marks indicating the times of still frames are very small and nearly indiscernible. Larger tick marks like in 1B would be better.

We have increased the size of the tick marks in both 1B and 3A to ensure that can be clearly visualized.
RE: Manuscript #E22-01-0025R
TITLE: "Cell cycle and developmental control of cortical excitability in Xenopus laevis"

Dear Prof. Bement:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell. Thank you so much for sending us this intriguing submission. Details of the publication process are below.

Sincerely,
John Wallingford
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Bement:

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.

Within approximately four weeks you will receive a PDF page proof of your article.

Your paper is among those chosen by the Editorial Board for Highlights from MBoC. Highlights from MBoC appears in the ASCB Newsletter and highlights the important articles from the most recent issue of MBoC.

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

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