Review COMMONS

JCB

Elimination of nurse cell nuclei that shuttle into oocytes during oogenesis

Zehra Ali-Murthy, Richard Fetter, Wanpeng Wang, Bin Yang, Loic Royer, and Thomas Kornberg

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Revision 0
Review #1
1. How much time do you estimate the authors will
need to complete the suggested revisions:
Estimated time to Complete Revisions (Required)
(Decision Recommendation)
Less than 1 month
2. Evidence, reproducibility and clarity:
Evidence, reproducibility and clarity (Required)
Summary:
In the Drosophila ovary, nurse cells support the growing oocyte and undergo programmed cell death at the completion of oogenesis. This phenomenon is conserved in other insects and other organisms. The authors here describe the remarkable finding that two nurse cells are eliminated by a separate mechanism, displaying an early fusion with the oocyte and disappearing. They provide a detailed microscopic analysis of this process.
Major comments:
The key conclusions are convincing. By using specific culture techniques, they increased the frequency of egg chambers at the stage of nurse cell fusion and provide documentation of many instances of nurse cell fusion with the oocyte. Other conclusions such as the loss of the plasma membrane between the nurse cells and the oocyte are well-supported by the images. However, the effects on fertility in Figure 10 are correlative, and this conclusion should be downplayed ("Nurse cell nuclear elimination is essential for oocyte maturation"). While the correlation is intriguing, the conclusions are over-stated. For example, there could be spontaneous defects that lead to both more nurse cell nuclei and the dorsal appendage morphological changes so the lack of nurse cell fusion may not be causative. Some of the egg chambers look dumpless suggesting the defects go beyond spacing of dorsal appendages (Fig. 10G, J right side).
There are some experiments that could strengthen the conclusions. They report doing some ex vivo imaging and more studies along those lines would be a terrific addition. For example, if they used the nuclear GFP, they could provide time lapse imaging that shows the entrance of the nucleus and subsequent dissipation of the GFP. Another (optional) line of experimentation that could strengthen the paper is to identify a mutant that disrupts the process. It is strange to read a Drosophila paper without a single mutant, making it a descriptive paper.
The data are well-described and quantified appropriately.
Minor comments:
I find the title and abstract very understated. The authors have found a novel form of cell elimination. I would suggest changing the title and abstract to emphasize that this is a form of cell death or elimination,

rather than simply fusion.
In the discussion there should be some speculation as to forms of cell death it is similar to such as entosis or erythrocyte nuclear extrusion.
I find the "reversible" part confusing. It seems that the membranes/ring canals are reversible but not the nuclei so I suggest removing reversible from the title.
More recent references on NC elimination should be used in the introduction as there are some inaccurate statements by relying on old papers.
3. Significance:
Significance (Required)
This is a highly significant paper to the field. Drosophila oogenesis has been well-studied for decades, and this unusual nurse cell elimination has not been noticed. It is a very important contribution, and might make it to textbooks!
Interest will go well beyond the Drosophila field, and will be noted by cell death researchers. It may also be important for mammalian reproduction.
I am a Drosophila researcher with interests in cell death and the ovary. I do not have much expertise in the cytoskeleton.

Review #2
1. How much time do you estimate the authors will need to complete the suggested revisions:
Estimated time to Complete Revisions (Required)
(Decision Recommendation)
Less than 1 month
2. Evidence, reproducibility and clarity:
Evidence, reproducibility and clarity (Required)
Ali-Murthy et al. report the unexpected finding that during Drosophila oogenesis two nurse cell nuclei enter the oocyte at stage 10B. Both nuclei disappear later by an unknown mechanism. Analysis of cell positioning and cell linage indicates that nurse cells that extrude their nuclei into the oocyte are not randomly selected but determined by a genetic program. The nuclei of the two nurse cells do not enter the oocyte through the ring canals but through a channel, which might be formed by fusion of the plasmamembranes of the oocyte and the nurse cells. The authors address the question if this process is essential for oogenesis by correlating the numbers of follicles, in which this process fails with follicles and embryos showing abnormal morphologies. Based on these correlations they conclude that follicles with defective nurse cell nuclei extrusion do not proceed properly through the last steps of oogenesis and are eventually not fertilised.
The observation that a channel is formed between the oocyte and two nurse cells through which nurse cell nuclei enter the oocyte is an exciting

finding for labs working on Drosophila oogenesis. Moreover, it is an important contribution to the fields of cell and developmental biology, since it opens the possibility that similar mechanisms exist in other animals. Although the data surprise in their clarity, experiences from our laboratory support the entry of nurse cell nuclei into the oocyte, which we observed in a genome scale oogenesis screen repeatedly. The shown data are of high quality and well support the conclusions that are made by the authors. I would like to make a few comments which might help to further improve the manuscript.

- I cannot find a clear statement how often nurse cell nuclei enter the oocyte at stage 10B and how variable this is between different preparations and genotypes. The authors report 134 oocytes with ectopic nuclei (p. 6) but they do not say how many follicles they analysed in total to come to this number. Moreover, they write that they observed ectopic nuclei not only in wild type flies but also in many other genotypes. Can they say anything about the variability between different fly stocks? Such information would be valuable to estimate the chances of success for a screen for genes controlling this nuclear extrusion.
- The authors propose that the oocyte plasmamembrane fuses with the plasmamembrane of the nurse cells that form the channel with the oocyte (Fig. 7). They also detect ring canals deep inside the oocyte (Fig. 4A), which suggests that the fused plasmamembrane penetrates deep inside the oocyte. If these ring canals are indeed anchored by a fused plasmamembrane they should be anchored by only two membranes, while the ring canals of the other nurse cells (which do not form a channel) should be anchored by four plasmamembranes (two from the oocyte and two from the nurse cells). Is it possible to address this question with the present EM pictures?
- The conclusion that those follicles which fail to extrude nurse cell nuclei into the oocyte do not develop properly and are not fertilised is only based on correlations and thus not totally convincing. The authors mention in the discussion that the fusion generated channel brings the border cells in close proximity to the nucleus of the oocyte (p. 18). Border cells give rise to the micophyle, which is important for sperm entry. Thus, it is possible that a defect in border cell positioning, which is caused by a failure to extrude nurse cell nuclei leads to a defective microphyle, which then prevents fertilisation. It would further strengthen the authors conclusion if they could demonstrate impaired micropyle development in the follicles that are suggested to originated from egg chambers, which failed to extrude nurse cell nuclei.

Minor points:

- Please revise the second sentence in the second paragraph of the introduction (p. 3): "Four consecutive mitotic division..."
- It sounds very mysterious when the authors mention a "special feeding regime" (p. 5). The description of this regime indicates that it concerns

simply the age of the females, which are a few days younger.
- The manuscript refers to Fig. 2F-H (p. 7), which is not present in the current version.
3. Significance:
Significance (Required)
The observation that a channel is formed between the oocyte and two nurse cells through which nurse cell nuclei enter the oocyte is an exciting finding for labs working on Drosophila oogenesis.
Moreover, it is an important contribution to the fields of cell and developmental biology, since it opens the possibility that similar mechanisms exist in other animals.

Review #3
1. How much time do you estimate the authors will need to complete the suggested revisions:
Estimated time to Complete Revisions (Required)
(Decision Recommendation)
Between 1 and 3 months
2. Evidence, reproducibility and clarity:
Evidence, reproducibility and clarity (Required)
Summary:
The fruit fly egg is derived from a structure called an egg chamber, which contains a cluster of germ cells surrounded by a layer of somatic cells. Within the germ cell cluster, the oocyte is connected to supporting nurse cells through intercellular bridges, or ring canals. The predominant model in the field is that through stage 10, there is slow transfer of materials from the nurse cells to the oocyte; then, at stage 11, there is a rapid transfer from the nurse cells to the oocyte during a process termed "dumping." Using a combination of confocal and electron microscopy, the authors describe a novel aspect of the late stages of oogenesis in which one or more of the nurse cells closest to the oocyte transiently fuse with the oocyte, allowing the nurse cell nucleus to enter through a large channel. Once in the oocyte, the nurse cell nucleus (or nuclei) are degraded. The authors have observed this early fusion event in 37 wild type and mutant D. melanogaster lines as well as in three additional species (D. simulans, D. hydei, and D. virilism). Correlations between the number of abnormal stage 11-14 egg chambers (which do not show evidence of this fusion event) and the number of eggs that do not hatch led the authors to conclude that this cell fusion and nuclear transfer is essential for oocyte maturation and embryonic viability.
Major Comments
• The description of this novel cell fusion event is fairly convincing. The authors have demonstrated that this event occurs in both wild type and mutant backgrounds in D. melanogaster, as well as in wild type strains of

three additional species.
• The identification of the specific nurse cells that underwent this fusion event was very interesting and fairly convincing, suggesting that there are previously
unappreciated differences between the nurse cells.
• The conclusion that this fusion event was necessary for oocyte maturation was the least convincing aspect of the paper. The data that supported this conclusion were all correlative (the relative number of "abnormal" late stage egg chambers was similar to the relative number of "abnormal" mature eggs), so it seemed a stretch to establish a causative relationship between these observations. Without extensive long-term live imaging of egg chambers ex vivo, it would be challenging to confirm that the cell fusion event is necessary for oocyte maturation. Therefore, without additional data, I think that qualifying some of these stronger conclusions that were made in the discussion would be appropriate.
Additional Experiments or Analysis
• The authors propose that typically one or two nurse cells transfer their nuclei to the oocyte; the entering nuclei are then degraded, although the mechanism by which they are cleared is not known. It could be interesting to measure nurse cell nuclear size and DNA condensation to see if the authors could capture an intermediate stage where the nuclei are smaller in size or the DNA is more condensed that could suggest a mechanism by which they are eliminated.
o If the authors already have these images, this analysis would likely be fairly straightforward to perform without additional resources (other than time).
• The authors propose that the membrane channel does not fully close after the nurse cell nuclei are transferred, but they could not be certain about this. Perhaps expressing a membrane-tethered fluorescent marker (such as mCD8-GFP/RFP) in the germline would allow them to confirm the persistence of this channel through stage 11. This fluorescent marker may even allow the authors to better capture different stages of the fusion event in which the channel is more or less open.
o This experiment would require a genetic cross and time imaging egg chambers of various stages (from 9-10b or 11), but likely would not require significant investment of additional resources other than time.
• I also wonder whether the channel that remains is larger if two nuclei have entered the oocyte, or whether there might be a threshold channel size that is able to be tolerated. The authors could measure the channel size in stage 11 (or 12) egg chambers where either one or two nuclei had entered the oocyte.
o If the authors already have these images, this analysis would likely be fairly straightforward to perform without additional resources (other than time).

Minor Comments (more general)

• It was sometimes unclear how many times a particular observation was made. For example:

o The authors mentioned differences in antibody staining of the nuclear envelope protein when the nucleus was in the nurse cell versus the oocyte, but they did not include any indication of the frequency of this observation.

o It was not clear how many times they observed entering nuclei in the different D. melanogaster lines (and other species) that they looked at.

o On pg. 6 the authors mention that they always counted 15 nurse cell nuclei in stages 2-10A, but there was no indication of how many egg chambers they looked at or how many different lines or species they analyzed.

o On pg. 8, the authors claim that cell 2 enters first. How often was this the case? Are there any examples where NC nucleus 5 entered but not NC nucleus 2?

• Scale bars are not present on many of the image panels

• Because "Reversible" was in the title, I was expecting to read more about this aspect of the process in the discussion. I am also not sure if this fusion should be considered reversible since the channel does remain after the nuclei have been transferred.

• The electron microscopy images are a bit difficult to interpret; including a cartoon showing what the authors believe is occurring would be helpful.

o It could also be useful to include a final model or cartoon showing the progression of this event during the late stages of oogenesis

• It is challenging to see the magenta and red staining in the same panel. Perhaps one of those colors could be changed to allow the reader to more easily visualize both stains.

• It would be helpful to be consistent in the color choices for stains throughout the paper or at least within a figure. Fig. 5 is an example where the same stain is represented by different colors within one figure.

Specific Comments on Figures and text:

• It was a little unclear what is being quantified in Fig. 1B. The legend indicates that the late stage 10B counts are of egg chambers with nurse cell nuclei in the oocyte. Does that mean that they never observed late stage 10B egg chambers without an entering nurse cell nucleus?

• Related to Fig. 2E, does the #15 nurse cell always get pulled towards the posterior? This was not commented on, and it didn't initially make sense that this would be the nurse cell that would rearrange since it isn't connected to nurse cells #2 or #5 (which are the ones that participate in the fusion).

• On pg. 7, the authors refer to Fig. 2F-H', but these panels are not present in the figure. The legend also refers to (F,G) cartoons, but F and

G are not present in the figure itself.
• On pg. 7, it should be clarified that when they are discussing the number of nuclei in stage 11 egg chambers, this data is shown in Fig. 3E. The legend for this figure should indicate the sample size for this data set, and in the pie chart, it would be helpful to add "nurse cells" after the numbers so that the reader does not assume these numbers are the counts.
• In Fig. 3, it would be useful to use the same nuclear numbering system to make it more obvious which nuclei are entering the oocyte (or are missing from the nurse cell cluster). Perhaps a text box could be added to indicate the total number of nurse cell nuclei present in the cluster.
• Fig. 4B - why is nucleus #12 not visible by any stain in the panel?
• Fig. 4G - HtsRC and phalloidin should be shown in 2 different colors (they are both in red)
o This panel does not have the arrows that were indicated in the legend
• Fig. 4F - it is hard to see the discontinuity in the stain with the "NC" labels on top. Perhaps a cartoon next to these panels could illustrate the arrangement of the nuclei, and a linescan could be used to show the relative intensity of the stain across those regions.
• On pg. 8, the authors refer to specific ring canals that do not seem to change in position, but these are not labeled in Fig. 4A (which is what is referenced). It also seems inconsistent with their image in Fig. 2 showing that NC 15 seems to move from the anterior to the posterior at stage 12. This should be commented on
• Fig. 5D did not have any error bars on the graph. The sample size was indicated as n=80. Was this 80 per stage or 80 total?
o Could the authors separate out the data in this graph to show the measurements of the relative size of the oocyte in stage 10 egg chambers with 15 nurse cell nuclei, 14 nurse cell nuclei, and 13 nurse cell nuclei to show that there was no change in the relative oocyte size when NC nuclei enter (compared to when they do not).
• Is Fig. 5E' staining really cadherin? It seems like the bright staining at the ring canals is not observed in other egg chambers stained with cadherin.
• In Fig. 9, would it be possible to count the number of nurse cell nuclei remaining in these egg chambers?
• On pg. 13, the authors refer to the data in Fig. 3G, but this should be Fig. 3E (for the counts of nurse cell nuclei in stage 11 egg chambers)
• The "optimized" protocol for increasing the number of late stage 10b egg chambers was not clear. Were they transferring flies to fresh bottles every day? Were they using wet or dry yeast?
3. Significance:
Significance (Required)
• This work represents a conceptual advance in the field of oogenesis. The egg chamber is a well-studied model of oogenesis, so this is an important

observation that could significantly impact our understanding of the later steps that are involved in material transfer from the nurse cells to the oocyte, which are less well-studied that other aspects of the process. Few studies have been performed to characterize the process of "dumping."
• Because germ cells often develop while connected to other germ cells or somatic cells, this study would be of interest to those studying gametogenesis (and specifically oogenesis) in many models systems, not just the fly. There are a lot of interesting questions that could be asked regarding the mechanisms that would regulate this type of programmed cell fusion event, so I could envision it being of general interest to those interested in many areas of cell or developmental biology.
• My area of expertise is in the egg chamber and oogenesis.

Review #4
1. How much time do you estimate the authors will need to complete the suggested revisions:
Estimated time to Complete Revisions (Required) (Decision Recommendation)
Less than 1 month
2. Evidence, reproducibility and clarity:
Evidence, reproducibility and clarity (Required)
The authors of this study endeavored to characterize a process occurring during oogenesis in Drosophila melanogaster. Each oocyte develops in an egg chamber consisting of clonally related germline cells, surrounded by somatic follicle cells. Among the 16 germline cells, one develops into the oocyte, and the remaining 15 germline cells develop as nurse cells, all cytoplasmically interconnected by ring canals. Stages of oogenesis are defined for 14 stages of oogenesis.
The authors observed that starting in Stage 10B, the polyploid nuclei from two nurse cells directly adjacent to the oocyte, extrude into the oocyte cytoplasm. The authors sought to characterize this process and determine whether this phenomenon, which others have assumed is an artifact, is actually a normal process of oogenesis.
Key findings:
1. Ectopic nuclei are specific to Stage 10B.
Method: Ovaries were fixed immediately after removal from the female before dissection of the ovary. Ovaries were then carefully dissected to minimize damage. They used immunofluorescence to visualize nuclei.
Finding: Up to Stage 10A, there were no ectopic nurse cell nuclei in the oocyte cytoplasm. Late Stage 10B oocytes typically had ectopic nuclei in the ooplasm, and Stages 11 and 12 did not have ectopic nuclei in the ooplasm but the majority of the Stage 11 egg chambers they evaluated had thirteen nucleated and 2 enucleated nurse cells.
2. Establish identities of specific nurse cells.

Method: Confocal fluorescence imaging. The identities o f particular nurse cells were established by the number of ring canals associated with each nurse cell and the association of the ring canals with neighboring nurse cells and the oocyte.
Finding: In all of the late Stage 10B egg chambers with migrating nuclei, the nuclei were from nurse cells 2 and 5.
Conclusion: The authors attribute the lack of nurse cell nuclei in the ooplasm and enucleated nurse cells adjacent to the oocyte in Stage 11 and later to rapid dissolution of the ectopic nuclei observed during Stage 10B.
3. Gap opens at nurse cell/oocyte interface and ring canals from nurse cells 2 and 5 relocate into oocyte in Stage 10B.
Method: Confocal fluorescence imaging and measurements of ring canals and nurse cell nuclei
Finding: At Stage 9, E-cadherin and phalloidin are continuous across the nurse cell - oocyte interface, and the ring canals are located at the interface. In Stage 10, there are gaps in E-cad and phalloidin, and the ring canals are located posterior to the nurse cell - oocyte border. The authors point out that the ring canals are too small for the large nurse cells to migrate through, and do not appear to associate with the nurse cell nuclei. In Stage 11, the ring canals relocate to the interface.
Conclusion: The authors suggest that this is consistent with a gap, but do not rule out the possibility that immunofluorescent confocal imaging is not sensitive enough to detect whether there is a gap at the interface of nurse cells and oocyte.
4. Alternative method of detecting a gap
Method: high resolution with electron microscopy.
Finding: The EM images show an open channel in the plasma membranes at the nurse cell/oocyte interface and a nurse cell nucleus spanning the channel.
Conclusion: Plasma membrane does not separate the oocyte and nurse cells at Stage 10B, and that nurse cell nuclei enter through this channel.
5. The plasma membranes of oocyte and entering nurse cell fuse.
Method: high resolution using electron microscopy
Finding: The plasma membranes of oocyte and entering nurse cell fuse, creating a continuous plasma membrane of oocyte with the entering nurse cell.
Conclusion: Images are consistent with the idea that the plasma membranes of oocyte and nurse cells fuse to create a channel to join the nurse cell and oocyte and connect the nurse cell and oocyte cytoplasm.
6. Egg chambers with 15 nurse cells display morphological defects
Method: Confocal fluorescence imaging
Findings: Stage 12 egg chambers with 15 nurse cells have defects in dorsal appendage morphology and greater inter-appendage distance than Stage 12 egg chambers with less than 15 nurse cells. Stage 14 egg chambers do not have nurse cells or nurse cell nuclei but the frequency of Stage 14 embryos

with misshapen dorsal appendages and greater inter-appendage distance is the same as the frequency of Stages 11-13 egg chambers with 15 nurse cell nuclei.
Conclusion: The authors suggest that the abnormal Stages 11-13 and Stage 14 egg chambers come from the same population because the frequencies are the same.
7. The similar frequencies also suggest that the Stage 14 egg chambers are laid.
Method: Analyze proportions of hatched and unhatched, and dorsal appendage defects in the unhatched eggs.
Findings: About 10% of WT embryos do not hatch. Of those, about 50% had normal morphology and developed (indicated by internal organs) but did not hatch. The other 50% had abnormal morphology and did not develop internal organs, suggesting that they had not been fertilized. The proportion of infertile, laid eggs with abnormal morphology is the same as the proportion of Stage 14 oocytes with abnormal morphology, and the morphology is similar.
Conclusion: The authors concluded that the abnormal laid eggs, being in the same proportion as Stage 14 oocytes with dorsal appendage defects, most likely originate as the abnormal Stage 14 oocytes and they were unfertilized.
Major comments:
1.You state that in Stage 9 the staining across the oocyte-nurse cell interface is continuous but the image in 4E has 3 upper quadrants that are stained with E-cad whereas the lower quandrant is dark. Please explain.
2.The unhatched eggs with dorsal appendage defects did not develop internal organs. You concluded that they were not fertilized. Could they have been fertilized but subsequently did not develop further?
Taking these findings together, and considering the different angles and methodologies the authors used for their analysis and from which they drew their conclusions, the results provide convincing evidence that two specific nurse cell nuclei migrate and are engulfed by the oocyte. The evidence suggests that this is a key feature of oogenesis in Drosophila melanogaster and not an artifact. The evidence further suggests that this process is required for embryonic development to proceed.
The authors clearly stated when any of their conclusions were speculative or preliminary.
Minor comments:
1.Page 3 second paragraph, bottom ("Although the more posterior...") and top of page 4, correct the redundant sentence.
2.Page 4, last paragraph, second line, change "prior to the dumping phase and prior the..." to "prior to the dumping phase and prior to the..."
3.At the bottom of page 7, last line, there is a reference to Fig. 2F-H'. Figure 2 has only panels A-E.

4.Top of page 11, first line, change sentence to "...cytoplasm of the oocyte and nurse cells..."
5.Page 11, last paragraph, third line: the figure references Fig. 1E. It appears it should reference Fig. 1D.
6.Page 12, second to last line in last paragraph. The figure reference appears to be incorrect for 5D-F'.
7.Page 13, second paragraph, first line states: "Counts of stage 11 egg chambers in Figure 3G..." but Figure 3G is labeled as Stage 12.
8.Page 13, last paragraph discusses abnormalities in Stage 14 and refers to Fig. 10E and G, but the legend for E says the graph represents Stages 12 and 13.
9.Figure 10 legend, second line: change "...stage 13 egg chambers marked with..." to "...stage 13 (C,D) egg chambers marked with..."
10.Figure 2 legend, third line refers to Figure 2, F,G. The sentence, "(F,G) Cartoons of stage 10B egg chambers with identities of nuclei marked" should be deleted. The next sentence appears to be the correct one.
11.In Figure 4C, it would be helpful to state the orientation of the egg chamber and point out the anterior face of the oocyte with an arrow.
12.Figure 4 legend, last line, it says, "arrows indicate regions of low phalloiding staining. I don't see any arrows.
13.Figure 4: are C, D, and E the same egg chamber?
14.Figure 6 legend, second line, change "and α-cadherin" to "and α-cadherin (red)".
15.Figure 6B: The pore is hard to see in B. Consider indicating it with an arrow. The images in B-F appear to be not entirely axial or dorsal. Consider stating this in a legend or including a small cartoon indicating the orientation.
16.In Figure 4D-E, is the dark area the pore? It looks larger than what has been depicted in other images. Can you explain?
17.Figure 6, Supplement 1: What are the asterisks for in C and D?
3. Significance:
Significance (Required)
This study advances the field of Drosophila oogenesis because it provides convincing evidence of a new and unexpected feature of this process. Ectopic nurse cell nuclei in the oocyte have been observed in studies cited by the authors, but the meaning of this phenotype has not been characterized, or it has been dismissed as an artifact. Here, the authors used several alternative methods to show that this process is not artifactual, and is required for oocyte maturation; egg chambers that do not execute this program are infertile. The authors suggest that the fact that the enucleated nurse cells are always the same two nurse cells is an indication that nurse cells do not all execute the same developmental program. This is consistent with a study showing that a Drosophila Imaginal disc growth factor (Idgf2) is is expressed transcriptionally in two nurse

cells adjacent to the oocyte (Zimmerman et al., 2013) but not in the other nurse cells.

Fusion of nurse cells with an oocyte is a phenomenon that has been observed in other organisms, including mammals (authors cite (Alexandrova et al., 2005; Miller et al., 2000;Lei and Spradling, 2016) so this study may have broader significance for the field of oogenesis.

The authors speculate that the function of fusion of the nurse cell/oocyte plasma membranes and nurse cell migration into the oocyte serves to provide a wide gap to allow efficient transfer of materials from the nurse cells.

They further speculate that the position of the border cells next to the gap might have significance for a possible role for border cells in the enucleation program, or alternatively for the cell fusion/enucleation program in bringing the germinal vesicle and border cells into close proximity.

This study will be of great interest to the Drosophila oogenesis community, and more generally, to developmental biologists focusing on oogenesis in other species.

My field of expertise:

My field of expertise includes 17 years of experience in Drosophila research in the areas of morphological development and genetics, with 8 years of focus in the area of oogenesis.

REFEREE'S CROSS-COMMENTING

I agree with all of the reviewers that the conclusions concerning fertilization and oocyte maturation are correlative and speculative.

Reviewer 1's suggestion for a live imaging experiment showing entrance of a nurse cell nucleus into the oocyte and it's subsequent dissipation would strengthen this paper.

I agree that numbers of egg chambers looked at for each figure/experiment was not always clear. These numbers should be stated in the figure legend or made more clear in the text.

I agree with Reviewer 1's comment that the nurse cell migration being necessary for fertility and oocyte maturation is correlative and overstated. The authors should downplay this conclusion and offer alternative explanations.

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

Summary:

In the Drosophila ovary, nurse cells support the growing oocyte and undergo programmed cell death at the completion of oogenesis. This phenomenon is conserved in other insects and other organisms. The authors here describe the remarkable finding that two nurse cells are eliminated by a separate mechanism, displaying an early fusion with the oocyte and disappearing. They provide a detailed microscopic analysis of this process.

Major comments:

The key conclusions are convincing. By using specific culture techniques, they increased the frequency of egg chambers at the stage of nurse cell fusion and provide documentation of many instances of nurse cell fusion with the oocyte. Other conclusions such as the loss of the plasma membrane between the nurse cells and the oocyte are well-supported by the images. However, the effects on fertility in Figure 10 are correlative, and this conclusion should be downplayed ("Nurse cell nuclear elimination is essential for oocyte maturation"). While the correlation is intriguing, the conclusions are over-stated. For example, there could be spontaneous defects that lead to both more nurse cell nuclei and the dorsal appendage morphological changes so the lack of nurse cell fusion may not be causative. Some of the egg chambers look dumpless suggesting the defects go beyond spacing of dorsal appendages (Fig. 10G, J right side).

There are some experiments that could strengthen the conclusions. They report doing some ex vivo imaging and more studies along those lines would be a terrific addition. For example, if they used the nuclear GFP, they could provide time lapse imaging that shows the entrance of the nucleus and subsequent dissipation of the GFP. Another (optional) line of experimentation that could strengthen the paper is to identify a mutant that disrupts the process. It is strange to read a Drosophila paper without a single mutant, making it a descriptive paper.

The data are well-described and quantified appropriately.

Minor comments:

I find the title and abstract very understated. The authors have found a novel form of cell elimination. I would suggest changing the title and abstract to emphasize that this is a form of cell death or elimination, rather than simply fusion.
Title and Abstract modified

In the discussion there should be some speculation as to forms of cell death it is similar to such as entosis or erythrocyte nuclear extrusion.

Paragraph noting entosis and erythroblast enucleation added.

I find the "reversible" part confusing. It seems that the membranes/ring canals are reversible but not the nuclei so I suggest removing reversible from the title.
Modified

More recent references on NC elimination should be used in the introduction as there are some inaccurate statements by relying on old papers.
Reference to Spradling review added; please advise which additional references might be relevant.

Reviewer #1 (Significance (Required)):

This is a highly significant paper to the field. Drosophila oogenesis has been well-studied for decades, and this unusual nurse cell elimination has not been noticed. It is a very important contribution, and might make it to textbooks!

Interest will go well beyond the Drosophila field, and will be noted by cell death researchers. It may also be important for mammalian reproduction.

I am a Drosophila researcher with interests in cell death and the ovary. I do not have much expertise in the cytoskeleton.

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

Ali-Murthy et al. report the unexpected finding that during Drosophila oogenesis two nurse cell nuclei enter the oocyte at stage 10B. Both nuclei disappear later by an unknown mechanism. Analysis of cell positioning and cell linage indicates that nurse cells that extrude their nuclei into the oocyte are not randomly selected but determined by a genetic program. The nuclei of the two nurse cells do not enter the oocyte through the ring canals but through a channel, which might be formed by fusion of the plasma membranes of the oocyte and the nurse cells. The authors address the question if this process is essential for oogenesis by correlating the numbers of follicles, in which this process fails with follicles and embryos showing abnormal morphologies. Based on these correlations they conclude that follicles with defective nurse cell nuclei extrusion do not proceed properly through the last steps of oogenesis and are eventually not fertilised.

The observation that a channel is formed between the oocyte and two nurse cells through which nurse cell nuclei enter the oocyte is an exciting finding for labs working on Drosophila oogenesis. Moreover, it is an important contribution to the fields of cell and developmental biology, since it opens the possibility that similar mechanisms exist in other animals. Although the data surprise in their clarity, experiences from our laboratory support the entry of nurse cell nuclei into the oocyte, which we observed in a genome scale oogenesis screen repeatedly. The

shown data are of high quality and well support the conclusions that are made by the authors. I would like to make a few comments which might help to further improve the manuscript.

- I cannot find a clear statement how often nurse cell nuclei enter the oocyte at stage 10B and how variable this is between different preparations and genotypes. The authors report 134 oocytes with ectopic nuclei (p. 6) but they do not say how many follicles they analysed in total to come to this number. Moreover, they write that they observed ectopic nuclei not only in wild type flies but also in many other genotypes. Can they say anything about the variability between different fly stocks? Such information would be valuable to estimate the chances of success for a screen for genes controlling this nuclear extrusion.
Figure 3E provides the best measure of frequency of nurse cell entrance/elimination - 89% for the genotype we used for this experiment. Nuclear extrusion is a reproducible fixture of late stage 10B egg chambers and although we have not tabulated frequencies in other genotypes, we did not note any variation in frequency that would have suggested that such (very labor intensive) analyses are warranted or would be informative. All of these experiments were carried out with well-fed, 3-7 day old females The observations we describe in Fig 11 suggest that the frequency varies with age of the female so any screen for genes that affect the process will need to account for this variation. 10B follicles represent a small fraction of any ovary prep and we did not count other stage follicles in our screens, but it must number in the thousands.

- The authors propose that the oocyte plasmamembrane fuses with the plasmamembrane of the nurse cells that form the channel with the oocyte (Fig. 7). They also detect ring canals deep inside the oocyte (Fig. 4A), which suggests that the fused plasmamembrane penetrates deep inside the oocyte. If these ring canals are indeed anchored by a fused plasmamembrane they should be anchored by only two membranes, while the ring canals of the other nurse cells (which do not form a channel) should be anchored by four plasmamembranes (two from the oocyte and two from the nurse cells). Is it possible to address this question with the present EM pictures?
The presence of ring canals in the ooplasm apparently unlinked to plasmamembrane was unexpected and we do not understand its structural implications. We looked for but did not succeed in attempts to identify the ring canals in EM sections, and the time that Rick Fetter has for these studies is unfortunately limited.

- The conclusion that those follicles which fail to extrude nurse cell nuclei into the oocyte do not develop properly and are not fertilised is only based on correlations and thus not totally convincing. The authors mention in the discussion that the fusion generated channel brings the border cells in close proximity to the nucleus of the oocyte (p. 18). Border cells give rise to the micophyle, which is important for sperm entry. Thus, it is possible that a defect in border cell positioning, which is caused by a failure to extrude nurse cell nuclei leads to a defective microphyle, which then prevents fertilisation. It would further strengthen the authors conclusion if they could demonstrate impaired micropyle development in the follicles that are suggested to originated from egg chambers, which failed to extrude nurse cell nuclei.
We show (Fig. 11) that follicles that are morphologically abnormal at stage 12-13 also have abnormal nurse cells – they number 15 not 13, and they are more disperse than normal. The

similar percentage of stage 14 oocytes that have a comparable morphology is indeed a correlation, but one that we consider strong. We agree that the link to morphologically abnormal and unfertilized embryos is also indirect, but again the similarities in morphology and percentages is highly suggestive. We have modified the text to evaluate these correlations with "suggestions" rather than "conclusions". We agree with the reviewer that there is a possible link between border cell functions and nuclear extrusion, and we have pursued this extensively with molecular studies and studies of various genotypes that affect border cells. We prefer to leave descriptions of this subject to a separate manuscript that is now in preparation.

Minor points:

- Please revise the second sentence in the second paragraph of the introduction (p. 3): "Four consecutive mitotic division..."
Done
- It sounds very mysterious when the authors mention a "special feeding regime" (p. 5). The description of this regime indicates that it concerns simply the age of the females, which are a few days younger.
Done
- The manuscript refers to Fig. 2F-H (p. 7), which is not present in the current version.
Done

Reviewer #2 (Significance (Required)):
The observation that a channel is formed between the oocyte and two nurse cells through which nurse cell nuclei enter the oocyte is an exciting finding for labs working on Drosophila oogenesis.

Moreover, it is an important contribution to the fields of cell and developmental biology, since it opens the possibility that similar mechanisms exist in other animals.

Reviewer #3 (Evidence, reproducibility and clarity (Required)):

Summary:

The fruit fly egg is derived from a structure called an egg chamber, which contains a cluster of germ cells surrounded by a layer of somatic cells. Within the germ cell cluster, the oocyte is connected to supporting nurse cells through intercellular bridges, or ring canals. The predominant model in the field is that through stage 10, there is slow transfer of materials from the nurse cells to the oocyte; then, at stage 11, there is a rapid transfer from the nurse cells to the oocyte during a process termed "dumping." Using a combination of confocal and electron

microscopy, the authors describe a novel aspect of the late stages of oogenesis in which one or more of the nurse cells closest to the oocyte transiently fuse with the oocyte, allowing the nurse cell nucleus to enter through a large channel. Once in the oocyte, the nurse cell nucleus (or nuclei) are degraded. The authors have observed this early fusion event in 37 wild type and mutant D. melanogaster lines as well as in three additional species (D. simulans, D. hydei, and D. virilism). Correlations between the number of abnormal stage 11-14 egg chambers (which do not show evidence of this fusion event) and the number of eggs that do not hatch led the authors to conclude that this cell fusion and nuclear transfer is essential for oocyte maturation and embryonic viability.

Major Comments

• The description of this novel cell fusion event is fairly convincing. The authors have demonstrated that this event occurs in both wild type and mutant backgrounds in D. melanogaster, as well as in wild type strains of three additional species.

• The identification of the specific nurse cells that underwent this fusion event was very interesting and fairly convincing, suggesting that there are previously unappreciated differences between the nurse cells.

• The conclusion that this fusion event was necessary for oocyte maturation was the least convincing aspect of the paper. The data that supported this conclusion were all correlative (the relative number of "abnormal" late stage egg chambers was similar to the relative number of "abnormal" mature eggs), so it seemed a stretch to establish a causative relationship between these observations. Without extensive long-term live imaging of egg chambers ex vivo, it would be challenging to confirm that the cell fusion event is necessary for oocyte maturation. Therefore, without additional data, I think that qualifying some of these stronger conclusions that were made in the discussion would be appropriate.

Additional Experiments or Analysis

• The authors propose that typically one or two nurse cells transfer their nuclei to the oocyte; the entering nuclei are then degraded, although the mechanism by which they are cleared is not known. It could be interesting to measure nurse cell nuclear size and DNA condensation to see if the authors could capture an intermediate stage where the nuclei are smaller in size or the DNA is more condensed that could suggest a mechanism by which they are eliminated.
As shown in Fig. 9D, the entering nuclei expand and the DAPI staining becomes diffuse. The images shown in the various panels (Figs. 1,3,4,5,7,9) are typical. Live imaging analysis depicted in the new Fig. 4 revealed that the process of nuclear extrusion and elimination is completed in a 7-17 minute time frame.

o If the authors already have these images, this analysis would likely be fairly straightforward to perform without additional resources (other than time).

• The authors propose that the membrane channel does not fully close after the nurse cell nuclei are transferred, but they could not be certain about this. Perhaps expressing a membrane-tethered fluorescent marker (such as mCD8-GFP/RFP) in the germline would allow them to confirm the persistence of this channel through stage 11. This fluorescent marker may even allow the authors to better capture different stages of the fusion event in which the channel is more or less open.
We have used various markers including membrane-tethered GFP, membrane-tethered Cherry, phalloidin, and cadherin in extensive efforts to better characterize the state of the plasma membrane throughout this process. Although the plasma membranes of the nurse cells and oocyte are clearly distinct in more lateral regions at stage 11, the geometry of the oocyte:nurse cell interface is complex in the region of interest and the images are unfortunately not definitive.

o This experiment would require a genetic cross and time imaging egg chambers of various stages (from 9-10b or 11), but likely would not require significant investment of additional resources other than time.

• I also wonder whether the channel that remains is larger if two nuclei have entered the oocyte, or whether there might be a threshold channel size that is able to be tolerated. The authors could measure the channel size in stage 11 (or 12) egg chambers where either one or two nuclei had entered the oocyte.
This is an interesting idea but not one that our data can address.

o If the authors already have these images, this analysis would likely be fairly straightforward to perform without additional resources (other than time).

Minor Comments (more general)

• It was sometimes unclear how many times a particular observation was made. For example:

o The authors mentioned differences in antibody staining of the nuclear envelope protein when the nucleus was in the nurse cell versus the oocyte, but they did not include any indication of the frequency of this observation.
Numbers now included in text

o It was not clear how many times they observed entering nuclei in the different D. melanogaster lines (and other species) that they looked at.
Numbers added to legend and Methods

o On pg. 6 the authors mention that they always counted 15 nurse cell nuclei in stages 2-10A, but there was no indication of how many egg chambers they looked at or how many different lines or species they analyzed.

Text has been modified

o On pg. 8, the authors claim that cell 2 enters first. How often was this the case? Are there any examples where NC nucleus 5 entered but not NC nucleus 2?
Numbers included in revised text

• Scale bars are not present on many of the image panels
Scale bars added

• Because "Reversible" was in the title, I was expecting to read more about this aspect of the process in the discussion. I am also not sure if this fusion should be considered reversible since the channel does remain after the nuclei have been transferred.
Title changed

• The electron microscopy images are a bit difficult to interpret; including a cartoon showing what the authors believe is occurring would be helpful.
Cartoons and summary diagram added

o It could also be useful to include a final model or cartoon showing the progression of this event during the late stages of oogenesis
Cartoons and summary diagram added

• It is challenging to see the magenta and red staining in the same panel. Perhaps one of those colors could be changed to allow the reader to more easily visualize both stains.
Colors modified

• It would be helpful to be consistent in the color choices for stains throughout the paper or at least within a figure. Fig. 5 is an example where the same stain is represented by different colors within one figure.
Colors modified

Specific Comments on Figures and text:

• It was a little unclear what is being quantified in Fig. 1B. The legend indicates that the late stage 10B counts are of egg chambers with nurse cell nuclei in the oocyte. Does that mean that they never observed late stage 10B egg chambers without an entering nurse cell nucleus?
Numbers and frequencies given in revised text on pg 6.

• Related to Fig. 2E, does the #15 nurse cell always get pulled towards the posterior? This was not commented on, and it didn't initially make sense that this would be the nurse cell that would rearrange since it isn't connected to nurse cells #2 or #5 (which are the ones that participate in the fusion).
Whereas the location of each nurse cell is not precisely determined, the relative laying is reproducible as described in the text. The arrangement in Fig 2E is rare and perhaps therefore

misleading. The modified Fig 2 has been simplified.

• On pg. 7, the authors refer to Fig. 2F-H', but these panels are not present in the figure. The legend also refers to (F,G) cartoons, but F and G are not present in the figure itself.
Corrected

• On pg. 7, it should be clarified that when they are discussing the number of nuclei in stage 11 egg chambers, this data is shown in Fig. 3E. The legend for this figure should indicate the sample size for this data set, and in the pie chart, it would be helpful to add "nurse cells" after the numbers so that the reader does not assume these numbers are the counts.
Done

• In Fig. 3, it would be useful to use the same nuclear numbering system to make it more obvious which nuclei are entering the oocyte (or are missing from the nurse cell cluster). Perhaps a text box could be added to indicate the total number of nurse cell nuclei present in the cluster.
Numbering explained in legend

• Fig. 4B - why is nucleus #12 not visible by any stain in the panel?
All the nuclei are not visible in any one rotational image of these 3D optical reconstructions. The particular image was chosen to show the entering NC nuclei and ring canals.

• Fig. 4G - HtsRC and phalloidin should be shown in 2 different colors (they are both in red)
Legend corrected: phalloidin stains both the ring canals and egg chamber periphery

o This panel does not have the arrows that were indicated in the legend
corrected

• Fig. 4F - it is hard to see the discontinuity in the stain with the "NC" labels on top. Perhaps a cartoon next to these panels could illustrate the arrangement of the nuclei, and a linescan could be used to show the relative intensity of the stain across those regions.
Discontinuity outlined in revised panel

• On pg. 8, the authors refer to specific ring canals that do not seem to change in position, but these are not labeled in Fig. 4A (which is what is referenced). It also seems inconsistent with their image in Fig. 2 showing that NC 15 seems to move from the anterior to the posterior at stage 12. This should be commented on
Immobile ring canals indicated in revised panel

• Fig. 5D did not have any error bars on the graph. The sample size was indicated as n=80. Was this 80 per stage or 80 total?
Data included in supplemental table

o Could the authors separate out the data in this graph to show the measurements of the

relative size of the oocyte in stage 10 egg chambers with 15 nurse cell nuclei, 14 nurse cell nuclei, and 13 nurse cell nuclei to show that there was no change in the relative oocyte size when NC nuclei enter (compared to when they do not).
Data included in supplemental table

• Is Fig. 5E' staining really cadherin? It seems like the bright staining at the ring canals is not observed in other egg chambers stained with cadherin.
The staining is indeed for Cadherin which normally stains but not as brightly as HTS antibody or phalloidin

• In Fig. 9, would it be possible to count the number of nurse cell nuclei remaining in these egg chambers?
Unfortunately this is not possible with the sections we have.

• On pg. 13, the authors refer to the data in Fig. 3G, but this should be Fig. 3E (for the counts of nurse cell nuclei in stage 11 egg chambers)
Corrected

• The "optimized" protocol for increasing the number of late stage 10b egg chambers was not clear. Were they transferring flies to fresh bottles every day? Were they using wet or dry yeast?
Clarified in Methods

Reviewer #3 (Significance (Required)):

• This work represents a conceptual advance in the field of oogenesis. The egg chamber is a well-studied model of oogenesis, so this is an important observation that could significantly impact our understanding of the later steps that are involved in material transfer from the nurse cells to the oocyte, which are less well-studied that other aspects of the process. Few studies have been performed to characterize the process of "dumping."

• Because germ cells often develop while connected to other germ cells or somatic cells, this study would be of interest to those studying gametogenesis (and specifically oogenesis) in many models systems, not just the fly. There are a lot of interesting questions that could be asked regarding the mechanisms that would regulate this type of programmed cell fusion event, so I could envision it being of general interest to those interested in many areas of cell or developmental biology.

• My area of expertise is in the egg chamber and oogenesis.

Reviewer #4 (Evidence, reproducibility and clarity (Required)):

The authors of this study endeavored to characterize a process occurring during oogenesis in Drosophila melanogaster. Each oocyte develops in an egg chamber consisting of clonally related germline cells, surrounded by somatic follicle cells. Among the 16 germline cells, one develops into the oocyte, and the remaining 15 germline cells develop as nurse cells, all cytoplasmically interconnected by ring canals. Stages of oogenesis are defined for 14 stages of oogenesis.

The authors observed that starting in Stage 10B, the polyploid nuclei from two nurse cells directly adjacent to the oocyte, extrude into the oocyte cytoplasm. The authors sought to characterize this process and determine whether this phenomenon, which others have assumed is an artifact, is actually a normal process of oogenesis.

Key findings:

1. Ectopic nuclei are specific to Stage 10B.

Method: Ovaries were fixed immediately after removal from the female before dissection of the ovary. Ovaries were then carefully dissected to minimize damage. They used immunofluorescence to visualize nuclei.

Finding: Up to Stage 10A, there were no ectopic nurse cell nuclei in the oocyte cytoplasm. Late Stage 10B oocytes typically had ectopic nuclei in the ooplasm, and Stages 11 and 12 did not have ectopic nuclei in the ooplasm but the majority of the Stage 11 egg chambers they evaluated had thirteen nucleated and 2 enucleated nurse cells.

2. Establish identities of specific nurse cells.

Method: Confocal fluorescence imaging. The identities of particular nurse cells were established by the number of ring canals associated with each nurse cell and the association of the ring canals with neighboring nurse cells and the oocyte.

Finding: In all of the late Stage 10B egg chambers with migrating nuclei, the nuclei were from nurse cells 2 and 5.

Conclusion: The authors attribute the lack of nurse cell nuclei in the ooplasm and enucleated nurse cells adjacent to the oocyte in Stage 11 and later to rapid dissolution of the ectopic nuclei observed during Stage 10B.

3. Gap opens at nurse cell/oocyte interface and ring canals from nurse cells 2 and 5 relocate into oocyte in Stage 10B.

Method: Confocal fluorescence imaging and measurements of ring canals and nurse cell nuclei

Finding: At Stage 9, E-cadherin and phalloidin are continuous across the nurse cell - oocyte interface, and the ring canals are located at the interface. In Stage 10, there are gaps in E-cad

and phalloidin, and the ring canals are located posterior to the nurse cell - oocyte border. The authors point out that the ring canals are too small for the large nurse cells to migrate through, and do not appear to associate with the nurse cell nuclei. In Stage 11, the ring canals relocate to the interface.

Conclusion: The authors suggest that this is consistent with a gap, but do not rule out the possibility that immunofluorescent confocal imaging is not sensitive enough to detect whether there is a gap at the interface of nurse cells and oocyte.

4. Alternative method of detecting a gap

Method: high resolution with electron microscopy.

Finding: The EM images show an open channel in the plasma membranes at the nurse cell/oocyte interface and a nurse cell nucleus spanning the channel.

Conclusion: Plasma membrane does not separate the oocyte and nurse cells at Stage 10B, and that nurse cell nuclei enter through this channel.

5. The plasma membranes of oocyte and entering nurse cell fuse.

Method: high resolution using electron microscopy

Finding: The plasma membranes of oocyte and entering nurse cell fuse, creating a continuous plasma membrane of oocyte with the entering nurse cell.

Conclusion: Images are consistent with the idea that the plasma membranes of oocyte and nurse cells fuse to create a channel to join the nurse cell and oocyte and connect the nurse cell and oocyte cytoplasm.

6. Egg chambers with 15 nurse cells display morphological defects

Method: Confocal fluorescence imaging

Findings: Stage 12 egg chambers with 15 nurse cells have defects in dorsal appendage morphology and greater inter-appendage distance than Stage 12 egg chambers with less than 15 nurse cells. Stage 14 egg chambers do not have nurse cells or nurse cell nuclei but the frequency of Stage 14 embryos with misshapen dorsal appendages and greater inter-appendage distance is the same as the frequency of Stages 11-13 egg chambers with 15 nurse cell nuclei.

Conclusion: The authors suggest that the abnormal Stages 11-13 and Stage 14 egg chambers come from the same population because the frequencies are the same.

7. The similar frequencies also suggest that the Stage 14 egg chambers are laid.

Method: Analyze proportions of hatched and unhatched, and dorsal appendage defects in the unhatched eggs.

Findings: About 10% of WT embryos do not hatch. Of those, about 50% had normal morphology and developed (indicated by internal organs) but did not hatch. The other 50% had abnormal morphology and did not develop internal organs, suggesting that they had not been fertilized. The proportion of infertile, laid eggs with abnormal morphology is the same as the proportion of Stage 14 oocytes with abnormal morphology, and the morphology is similar.

Conclusion: The authors concluded that the abnormal laid eggs, being in the same proportion as Stage 14 oocytes with dorsal appendage defects, most likely originate as the abnormal Stage 14 oocytes and they were unfertilized.

Major comments:

1.You state that in Stage 9 the staining across the oocyte-nurse cell interface is continuous but the image in 4E has 3 upper quadrants that are stained with E-cad whereas the lower quandrant is dark. Please explain.
Better image in revised panel.

2.The unhatched eggs with dorsal appendage defects did not develop internal organs. You concluded that they were not fertilized. Could they have been fertilized but subsequently did not develop further?
Agreed; the only criteria we used was to examine under transmitted light. Text has been revised.

Taking these findings together, and considering the different angles and methodologies the authors used for their analysis and from which they drew their conclusions, the results provide convincing evidence that two specific nurse cell nuclei migrate and are engulfed by the oocyte. The evidence suggests that this is a key feature of oogenesis in Drosophila melanogaster and not an artifact. The evidence further suggests that this process is required for embryonic development to proceed.

The authors clearly stated when any of their conclusions were speculative or preliminary.

Minor comments:

1.Page 3 second paragraph, bottom ("Although the more posterior...") and top of page 4, correct the redundant sentence.
Corrected

2.Page 4, last paragraph, second line, change "prior to the dumping phase and prior the..." to "prior to the dumping phase and prior to the..."

Corrected

3.At the bottom of page 7, last line, there is a reference to Fig. 2F-H'. Figure 2 has only panels A-E.
Corrected

4.Top of page 11, first line, change sentence to "...cytoplasm of the oocyte and nurse cells..."
Corrected

5.Page 11, last paragraph, third line: the figure references Fig. 1E. It appears it should reference Fig. 1D.
Corrected

6.Page 12, second to last line in last paragraph. The figure reference appears to be incorrect for 5D-F'.
Corrected

7.Page 13, second paragraph, first line states: "Counts of stage 11 egg chambers in Figure 3G..." but Figure 3G is labeled as Stage 12.
Corrected

8.Page 13, last paragraph discusses abnormalities in Stage 14 and refers to Fig. 10E and G, but the legend for E says the graph represents Stages 12 and 13.
Corrected

9.Figure 10 legend, second line: change "...stage 13 egg chambers marked with..." to "...stage 13 (C,D) egg chambers marked with..."
Corrected

10.Figure 2 legend, third line refers to Figure 2, F,G. The sentence, "(F,G) Cartoons of stage 10B egg chambers with identities of nuclei marked" should be deleted. The next sentence appears to be the correct one.
Corrected

11.In Figure 4C, it would be helpful to state the orientation of the egg chamber and point out the anterior face of the oocyte with an arrow.
Corrected

12.Figure 4 legend, last line, it says, "arrows indicate regions of low phalloiding staining. I don't see any arrows.
Corrected

13.Figure 4: are C, D, and E the same egg chamber?
They are different egg chambers; text in legend clarified.

14.Figure 6 legend, second line, change "and α-cadherin" to "and α-cadherin (red)".
Corrected

15.Figure 6B: The pore is hard to see in B. Consider indicating it with an arrow. The images in B-F appear to be not entirely axial or dorsal. Consider stating this in a legend or including a small cartoon indicating the orientation.
Modified

16.In Figure 4D-E, is the dark area the pore? It looks larger than what has been depicted in other images. Can you explain?
Only that these images show discontinuities in cadherin and phalloidin staining

17.Figure 6, Supplement 1: What are the asterisks for in C and D?
Corrected

Reviewer #4 (Significance (Required)):

This study advances the field of Drosophila oogenesis because it provides convincing evidence of a new and unexpected feature of this process. Ectopic nurse cell nuclei in the oocyte have been observed in studies cited by the authors, but the meaning of this phenotype has not been characterized, or it has been dismissed as an artifact. Here, the authors used several alternative methods to show that this process is not artifactual, and is required for oocyte maturation; egg chambers that do not execute this program are infertile. The authors suggest that the fact that the enucleated nurse cells are always the same two nurse cells is an indication that nurse cells do not all execute the same developmental program. This is consistent with a study showing that a Drosophila Imaginal disc growth factor (Idgf2) is expressed transcriptionally in two nurse cells adjacent to the oocyte (Zimmerman et al., 2013) but not in the other nurse cells.

Fusion of nurse cells with an oocyte is a phenomenon that has been observed in other organisms, including mammals (authors cite (Alexandrova et al., 2005; Miller et al., 2000;Lei and Spradling, 2016) so this study may have broader significance for the field of oogenesis.

The authors speculate that the function of fusion of the nurse cell/oocyte plasma membranes and nurse cell migration into the oocyte serves to provide a wide gap to allow efficient transfer of materials from the nurse cells. They further speculate that the position of the border cells next to the gap might have significance for a possible role for border cells in the enucleation program, or alternatively for the cell fusion/enucleation program in bringing the germinal vesicle and border cells into close proximity.

This study will be of great interest to the Drosophila oogenesis community, and more generally, to developmental biologists focusing on oogenesis in other species.

My field of expertise:

My field of expertise includes 17 years of experience in Drosophila research in the areas of morphological development and genetics, with 8 years of focus in the area of oogenesis.

REFEREE'S CROSS-COMMENTING

I agree with all of the reviewers that the conclusions concerning fertilization and oocyte maturation are correlative and speculative.
Reviewer 1's suggestion for a live imaging experiment showing entrance of a nurse cell nucleus into the oocyte and it's subsequent dissipation would strengthen this paper.
Done

I agree that numbers of egg chambers looked at for each figure/experiment was not always clear. These numbers should be stated in the figure legend or made more clear in the text.
Done

I agree with Reviewer 1's comment that the nurse cell migration being necessary for fertility and oocyte maturation is correlative and overstated. The authors should downplay this conclusion and offer alternative explanations.
Done

January 5, 2021

Re: JCB manuscript #202012101T

Dr. Thomas B Kornberg
UCSF
Smith Building
555 Mission Bay South
San Francisco, CA 94143

Dear Dr. Kornberg,

Thank you for submitting your manuscript entitled "Nuclear elimination by two specified germline nurse cells that fuse with the Drosophila oocyte" to JCB. We apologize for the delay in communicating our decision to you. We have discussed the manuscript and reviews editorially. All of the reviewers found your observations novel and surprising. The Drosophila ovary has been a model for numerous aspects of cell biology for decades, and all recognize the importance of this revision of our understanding. While largely descriptive, this novelty is important. We appreciate your findings; however, we do not feel that the revision as submitted is ready for publication in JCB.

If you are willing to further revise the work, we would be open to re-reviewing a revision if you can address the reviews as follows. The reviewers all agree that the conclusions regarding fertility are correlative and should be substantially downplayed. This Figure could go in the Supplement and the text further downplayed as they have suggested. Each also has clear suggestions regarding additional quantification and data clarification -- each of these should be addressed substantively, with details of how you have modified the manuscript in response, rather than simply "explained" in your Response to Reviews. There are several examples including the need to be clearer about numbers of egg chambers looked at for each figure/experiment. We would also like to see you *try* the time-lapse imaging experiment suggested by Reviewer #1, as this was endorsed by the other Reviewers. The idea of a screen to look for alterations in the frequency of this phenotype is beyond the scope of the current manuscript, but as someone who has worked in this field myself, I'd suggest you consider the literature on mutants affecting the cadherin-catenin system and actin regulators, which existing data suggest may increase the frequency of these events. In this regard, perhaps the "fusion" you suggest is, in fact, failure of the nurse cell/oocyte membrane due to the stress of the contractility of dumping-in this case you have identified the response that allows proper oogenesis even in situations where the normal process "fails", an aspect of robustness. After you have done these things, we'd like a full point-by-point response (i.e, one that includes responses to the full reviewer remarks) and plan to send the response and revised manuscript back to the reviewers. Please also highlight all changes in the text of the manuscript. Papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected. Please let us know if you have any questions; we would be happy to discuss the revision further as needed. Please also note that, due to the pandemic, JCB has waived the revision time limit.

Please let us know if you are able to address the major issues outlined above and wish to submit a revised manuscript to JCB. We appreciate that these changes may entail more work than you had planned for this manuscript. If you would prefer not to make these revisions and want to submit the

paper elsewhere, please let us know and we will alert Review Commons so that you may use that platform to contact another affiliate journal again.

If you choose to revise and resubmit your manuscript, please also attend to the following editorial points. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:
Text limits: Character count is < 40,000, 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: Your manuscript may have up to 10 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, https://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. Your manuscript may have up to 5 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.

Regardless of how you choose to proceed, 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. You can contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for thinking of JCB as an appropriate place to publish your work.

Sincerely,

Mark Peifer, PhD
Monitoring Editor, Journal of Cell Biology

Melina Casadio, PhD
Senior Scientific Editor, Journal of Cell Biology

Reviewer #1

I find the title and abstract very understated. The authors have found a novel form of cell elimination. I would suggest changing the title and abstract to emphasize that this is a form of cell death or elimination, rather than simply fusion.

Title and Abstract modified

In the discussion there should be some speculation as to forms of cell death it is similar to such as entosis or erythrocyte nuclear extrusion.

Paragraph noting entosis and erythroblast enucleation added to Discussion.

I find the "reversible" part confusing. It seems that the membranes/ring canals are reversible but not the nuclei so I suggest removing reversible from the title.

Modified and "reversible" deleted

More recent references on NC elimination should be used in the introduction as there are some inaccurate statements by relying on old papers.

Reference to Spradling review added; any suggestions for additional relevant references would be appreciated.

Reviewer #2:

- I cannot find a clear statement how often nurse cell nuclei enter the oocyte at stage 10B and how variable this is between different preparations and genotypes. The authors report 134 oocytes with ectopic nuclei (p. 6) but they do not say how many follicles they analysed in total to come to this number. Moreover, they write that they observed ectopic nuclei not only in wild type flies but also in many other genotypes. Can they say anything about the variability between different fly stocks? Such information would be valuable to estimate the chances of success for a screen for genes controlling this nuclear extrusion.

Unfortunately, we do not have a good way to estimate how many follicles were analyzed because most experiments involved scanning preps for the stagse of interest and frequencies per ovary were measured only for the cases cited in the text. Nuclear extrusion and elimination is a reproducible fixture of late stage 10B egg chambers for all the genotypes we studied for the hundreds of crosses that we analyzed during the more than five years of work this manuscript summarizes, but only two experiments estimated frequencies (an exhausting undertaking). One of these experiments analyzed stage 11 egg chambers (Fig. 3E). Figure 3E provides the best measure we have of frequency of nurse cell entrance/elimination with its determination of the number of nurse cell nuclei in stage 11 egg chambers - 89% for the genotype we used for this

experiment. The other determined the frequency of stage 10B egg chambers with entering nurse cell nuclei in ovaries for three genotypes and is now cited in the text and in Supplemental Table 1. We did not note any significant variability in frequency between any of the genotypes that we examined.

- The authors propose that the oocyte plasmamembrane fuses with the plasmamembrane of the nurse cells that form the channel with the oocyte (Fig. 7). They also detect ring canals deep inside the oocyte (Fig. 4A), which suggests that the fused plasmamembrane penetrates deep inside the oocyte. If these ring canals are indeed anchored by a fused plasmamembrane they should be anchored by only two membranes, while the ring canals of the other nurse cells (which do not form a channel) should be anchored by four plasmamembranes (two from the oocyte and two from the nurse cells). Is it possible to address this question with the present EM pictures?

We also are fascinated by this, but unfortunately do not have helpful evidence to contribute. We looked for but did not succeed in our attempts to identify the ring canals in EM sections, and are unable to pursue these studies at this time because Rick Fetter is unavailable. Please note that we do not propose that the oocyte-localized ring canals are fused to the plasmamembranes that join the oocyte and nurse cells. Although we do not understand how these ring canals are structured, we also recognize that there is no biophysical understanding how ring canals are normally situated. The presence of ring canals in the ooplasm that are apparently unlinked to plasmamembrane was unexpected and we do not understand its structural implication, but there is a previous report (Warn et al, 1985) of ring canals in the ooplasm that we cite in the text.

- The conclusion that those follicles which fail to extrude nurse cell nuclei into the oocyte do not develop properly and are not fertilised is only based on correlations and thus not totally convincing. The authors mention in the discussion that the fusion generated channel brings the border cells in close proximity to the nucleus of the oocyte (p. 18). Border cells give rise to the micophyle, which is important for sperm entry. Thus, it is possible that a defect in border cell positioning, which is caused by a failure to extrude nurse cell nuclei leads to a defective microphyle, which then prevents fertilisation. It would further strengthen the authors conclusion if they could demonstrate impaired micropyle development in the follicles that are suggested to originated from egg chambers, which failed to extrude nurse cell nuclei.

We share this interest in the micropyle and we examined the anterior structures as best we could, but have not yet been able to link nuclear extrusion/elimination to specific defects in these anterior structures. We agree that the link to morphologically abnormal and unfertilized embryos is indirect, but were persuaded by the similarities in morphology and percentages of early stages in which nuclei can be counted and the later stages when they cannot. To examine this issue further, we have added an experiment that we think makes the link more definitive. We present evidence in the revised manuscript showing that stage 11-12 egg chambers with 15 nurse cell nuclei and abnormal morphology (shape, size, inter-appendage distance) mature to stage 14 with similar abnormal morphology. We hope that this should allay the concerns, and

that the data can now be described as direct and not an "association". Regarding the destiny of these abnormal stage 14 oocytes after egg laying, the similar percentages and morphology of undeveloped eggs does remain an "association", but ask if the reviewers might agree that if the distance between the appendages is in fact set at stage 11,12, then the abnormal distance might be interpreted as an indelible mark that traces the abnormality through development to the egg? Although our thinking is that this is good evidence of lineage and not simply "association", we modified the conclusions and descriptions in the revised text to try to comply with the reviewers – we moved the analysis of non-developing embryos to a Supplemental Figure as per the Editor's suggestion and have deleted text that discusses its implications - but welcome guidance.

Minor points:

- Please revise the second sentence in the second paragraph of the introduction (p. 3): "Four consecutive mitotic division..."
Done

- It sounds very mysterious when the authors mention a "special feeding regime" (p. 5). The description of this regime indicates that it concerns simply the age of the females, which are a few days younger.
Done

- The manuscript refers to Fig. 2F-H (p. 7), which is not present in the current version.
Done

Reviewer #3

Additional Experiments or Analysis

• The authors propose that typically one or two nurse cells transfer their nuclei to the oocyte; the entering nuclei are then degraded, although the mechanism by which they are cleared is not known. It could be interesting to measure nurse cell nuclear size and DNA condensation to see if the authors could capture an intermediate stage where the nuclei are smaller in size or the DNA is more condensed that could suggest a mechanism by which they are eliminated.

As shown in Fig. 9D, the entering nuclei expand and the DAPI staining becomes diffuse - we did not find indication of DNA condensation at any point in the process. The images shown in the various panels (Figs. 1,3,4,5,7,9) are typical. Live imaging analysis depicted in the new Fig. 4 revealed that the process of nuclear extrusion and elimination is completed in a 7-17 minute time frame.

• The authors propose that the membrane channel does not fully close after the nurse cell

nuclei are transferred, but they could not be certain about this. Perhaps expressing a membrane-tethered fluorescent marker (such as mCD8-GFP/RFP) in the germline would allow them to confirm the persistence of this channel through stage 11. This fluorescent marker may even allow the authors to better capture different stages of the fusion event in which the channel is more or less open.

We agree and tried to address this question in several ways. We used markers including membrane-tethered GFP, membrane-tethered Cherry, phalloidin, and cadherin in extensive efforts to better characterize the state of the plasma membrane throughout this process, but unfortunately did not obtain convincing or definitive evidence. Although the plasma membranes of the nurse cells and oocyte are clearly distinct in more lateral regions at stage 11, the geometry of the oocyte:nurse cell interface at stage 11 is complex and could not be clearly resolved in the region of interest, and although structures in the fluorescence images are consistent with the complex assemblage of membranous vesicles that are present in the EM images, we are not confident that they show the structures well enough to justify additional statements..

• I also wonder whether the channel that remains is larger if two nuclei have entered the oocyte, or whether there might be a threshold channel size that is able to be tolerated. The authors could measure the channel size in stage 11 (or 12) egg chambers where either one or two nuclei had entered the oocyte.

This is an interesting idea but unfortunately not one that our data can address.

Minor Comments (more general)

• It was sometimes unclear how many times a particular observation was made. For example:

o The authors mentioned differences in antibody staining of the nuclear envelope protein when the nucleus was in the nurse cell versus the oocyte, but they did not include any indication of the frequency of this observation.
Numbers now included in text

o It was not clear how many times they observed entering nuclei in the different D. melanogaster lines (and other species) that they looked at.
Numbers added to legend and Methods

o On pg. 6 the authors mention that they always counted 15 nurse cell nuclei in stages 2-10A, but there was no indication of how many egg chambers they looked at or how many different lines or species they analyzed.
Text has been modified

o On pg. 8, the authors claim that cell 2 enters first. How often was this the case? Are there any examples where NC nucleus 5 entered but not NC nucleus 2?

Numbers included in revised text

• Scale bars are not present on many of the image panels
Scale bars added

• Because "Reversible" was in the title, I was expecting to read more about this aspect of the process in the discussion. I am also not sure if this fusion should be considered reversible since the channel does remain after the nuclei have been transferred.
Title changed

• The electron microscopy images are a bit difficult to interpret; including a cartoon showing what the authors believe is occurring would be helpful.
Cartoons and summary diagram added

o It could also be useful to include a final model or cartoon showing the progression of this event during the late stages of oogenesis
Cartoons and summary diagram added

• It is challenging to see the magenta and red staining in the same panel. Perhaps one of those colors could be changed to allow the reader to more easily visualize both stains.
Colors modified

• It would be helpful to be consistent in the color choices for stains throughout the paper or at least within a figure. Fig. 5 is an example where the same stain is represented by different colors within one figure.
Colors modified

Specific Comments on Figures and text:

• It was a little unclear what is being quantified in Fig. 1B. The legend indicates that the late stage 10B counts are of egg chambers with nurse cell nuclei in the oocyte. Does that mean that they never observed late stage 10B egg chambers without an entering nurse cell nucleus?
Numbers and frequencies given in revised text on pg 6.

• Related to Fig. 2E, does the #15 nurse cell always get pulled towards the posterior? This was not commented on, and it didn't initially make sense that this would be the nurse cell that would rearrange since it isn't connected to nurse cells #2 or #5 (which are the ones that participate in the fusion).
Whereas the location of each nurse cell is not precisely determined, the relative layering is reproducible as described in the text. The arrangement in Fig 2E is rare and perhaps therefore misleading. The modified Fig 2 has been simplified.

• On pg. 7, the authors refer to Fig. 2F-H', but these panels are not present in the figure. The legend also refers to (F,G) cartoons, but F and G are not present in the figure itself.

Corrected

• On pg. 7, it should be clarified that when they are discussing the number of nuclei in stage 11 egg chambers, this data is shown in Fig. 3E. The legend for this figure should indicate the sample size for this data set, and in the pie chart, it would be helpful to add "nurse cells" after the numbers so that the reader does not assume these numbers are the counts.
Done

• In Fig. 3, it would be useful to use the same nuclear numbering system to make it more obvious which nuclei are entering the oocyte (or are missing from the nurse cell cluster). Perhaps a text box could be added to indicate the total number of nurse cell nuclei present in the cluster.
We agree and the numbering is now explained in legends

• Fig. 4B - why is nucleus #12 not visible by any stain in the panel?
Every nucleus may not be visible in any one rotational section/image of the 3D optical reconstructions (in particular the deeper ones). Although nucleus #12 is visible in other rotations, the particular image was chosen so that the entering NC nuclei and ring canals are clearly visible.

• Fig. 4G - HtsRC and phalloidin should be shown in 2 different colors (they are both in red)
Legend corrected: phalloidin stains both the ring canals and egg chamber periphery

o This panel does not have the arrows that were indicated in the legend
corrected

• Fig. 4F - it is hard to see the discontinuity in the stain with the "NC" labels on top. Perhaps a cartoon next to these panels could illustrate the arrangement of the nuclei, and a linescan could be used to show the relative intensity of the stain across those regions.
Discontinuity outlined in revised panel

• On pg. 8, the authors refer to specific ring canals that do not seem to change in position, but these are not labeled in Fig. 4A (which is what is referenced). It also seems inconsistent with their image in Fig. 2 showing that NC 15 seems to move from the anterior to the posterior at stage 12. This should be commented on
Immobile ring canals are now indicated in revised panel

• Fig. 5D did not have any error bars on the graph. The sample size was indicated as n=80. Was this 80 per stage or 80 total?
Data included in Supplemental Table 2

o Could the authors separate out the data in this graph to show the measurements of the relative size of the oocyte in stage 10 egg chambers with 15 nurse cell nuclei, 14 nurse cell nuclei, and 13 nurse cell nuclei to show that there was no change in the relative oocyte size

when NC nuclei enter (compared to when they do not).
Data included in supplemental table

• Is Fig. 5E' staining really cadherin? It seems like the bright staining at the ring canals is not observed in other egg chambers stained with cadherin.
The staining is indeed for Cadherin which normally stains ring canals, albeit not as brightly as HTS antibody or phalloidin

• In Fig. 9, would it be possible to count the number of nurse cell nuclei remaining in these egg chambers?
Unfortunately this is not possible with the sections we have.

• On pg. 13, the authors refer to the data in Fig. 3G, but this should be Fig. 3E (for the counts of nurse cell nuclei in stage 11 egg chambers)
Corrected

• The "optimized" protocol for increasing the number of late stage 10b egg chambers was not clear. Were they transferring flies to fresh bottles every day? Were they using wet or dry yeast?
Clarified in Methods

Reviewer #4

Major comments:

1.You state that in Stage 9 the staining across the oocyte-nurse cell interface is continuous but the image in 4E has 3 upper quadrants that are stained with E-cad whereas the lower quandrant is dark. Please explain.
Better image now included in revised panel.

2.The unhatched eggs with dorsal appendage defects did not develop internal organs. You concluded that they were not fertilized. Could they have been fertilized but subsequently did not develop further?
Agreed; the only criteria we used was to examine under transmitted light. Text has been revised.

Minor comments:

1.Page 3 second paragraph, bottom ("Although the more posterior...") and top of page 4, correct the redundant sentence.
Corrected

2.Page 4, last paragraph, second line, change "prior to the dumping phase and prior the..." to "prior to the dumping phase and prior to the..."

Corrected

3.At the bottom of page 7, last line, there is a reference to Fig. 2F-H'. Figure 2 has only panels A-E.
Corrected

4.Top of page 11, first line, change sentence to "...cytoplasm of the oocyte and nurse cells..."
Corrected

5.Page 11, last paragraph, third line: the figure references Fig. 1E. It appears it should reference Fig. 1D.
Corrected

6.Page 12, second to last line in last paragraph. The figure reference appears to be incorrect for 5D-F'.
Corrected

7.Page 13, second paragraph, first line states: "Counts of stage 11 egg chambers in Figure 3G..." but Figure 3G is labeled as Stage 12.
Corrected

8.Page 13, last paragraph discusses abnormalities in Stage 14 and refers to Fig. 10E and G, but the legend for E says the graph represents Stages 12 and 13.
Corrected

9.Figure 10 legend, second line: change "...stage 13 egg chambers marked with..." to "...stage 13 (C,D) egg chambers marked with..."
Corrected

10.Figure 2 legend, third line refers to Figure 2, F,G. The sentence, "(F,G) Cartoons of stage 10B egg chambers with identities of nuclei marked" should be deleted. The next sentence appears to be the correct one.
Corrected

11.In Figure 4C, it would be helpful to state the orientation of the egg chamber and point out the anterior face of the oocyte with an arrow.
Corrected

12.Figure 4 legend, last line, it says, "arrows indicate regions of low phalloiding staining. I don't see any arrows.
Corrected

13.Figure 4: are C, D, and E the same egg chamber?
They are different egg chambers; text in legend clarified.

14.Figure 6 legend, second line, change "and α-cadherin" to "and α-cadherin (red)".
Corrected

15.Figure 6B: The pore is hard to see in B. Consider indicating it with an arrow. The images in B-F appear to be not entirely axial or dorsal. Consider stating this in a legend or including a small cartoon indicating the orientation.
Modified

16.In Figure 4D-E, is the dark area the pore? It looks larger than what has been depicted in other images. Can you explain?
We agree that the clear implication is that these dark areas might be indications of the forming/incipient pore, but we do not in fact know how actin distributions relate to the pore in space or time. We agree that limiting our descriptions to simply stating that these images show discontinuities in cadherin and phalloidin staining is conservative, but think that it is appropriate.

17.Figure 6, Supplement 1: What are the asterisks for in C and D?
Corrected

April 6, 2021

RE: JCB Manuscript #202012101R

Dr. Thomas B Kornberg
UCSF
Smith Building
555 Mission Bay South
San Francisco, CA 94143

Dear Tom,

Thank you for submitting your revised manuscript entitled "Nuclear elimination by two specified germline NCs that fuse with the Drosophila oocyte". You will see that the reviewers appreciated your efforts to revise the work for publication in JCB. Please consider the minor textual comments from Reviewer #1 as you prepare your final files. We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below) and pending final changes to address the reviews.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

1) To include the following personal communication in the published ms: "Lastly, Drs. K. McCall and W. Sullivan have independently confirmed the presence of ectopic nuclei in stage 10B oocytes (personal communication)" on page 18, the JCB office needs to receive one email from Dr. McCall and one email from Dr. Sullivan stating that they agree with the inclusion of the above sentence in your manuscript, with the manuscript title. You may also forward emails to us if you have received them yourself from Drs. McCall and Sullivan. Please contact the journal office with any questions.

2) JCB Articles may have up to 10 main figures and 5 supplementary figures, and supplementary figures and legends should be numbered 1-5 (please make sure to check the call-outs in the text also when making edits to figure numbers). Each figure can span up to one entire page without the legend on it, as long as all panels fit on the page. Please rearrange the data so as to meet this limit. For instance, some figure panels could be combined. The legend should appear on a separate page from the figure. Please let us know if you have any questions or concerns.

3) Title: Please consider the following revision suggestions aimed at increasing the accessibility of the work for a broad audience and non-experts.

We editorially aim to avoid acronyms and abbreviations in titles and suggest changes to make the title as broadly accessible and clear as possible. We also typically do not include species in titles because this information becomes clear to the reader as soon as they read the abstract and in the interest of brevity. JCB's preferred style is a concise, clear, and accessible title, in the interest of clarity for a broad and diverse readership.

Title: Elimination of nurse cell nuclei following shuttling into oocytes during oogenesis

4) eTOC summary: A 40-word summary that describes the context and significance of the findings for a general readership should be included on the title page. The statement should be written in the present tense and refer to the work in the third person.
- Please include a summary statement on the title page of the resubmission. It should start with "First author name(s) et al..." to match our preferred style.

5) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Please add scale bars to 1D (Does the same scale bar as 1B apply?); 5 (please clarify if the scale bar in D applies to G G' and E); 6A; figure 7 (all); figure 8; figure 9 (all); figure 10 (all); Figure 4 Supplement 1 (main and magnification); Figure 7, supplement 1 (all); Figure 10 Supplement 1 (all)

6) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends.

7) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions in the text for readers who may not have access to referenced manuscripts.
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- Please include species and source for all antibodies, including secondary, as well as catalog numbers/vendor identifiers if available.
- Sequences should be provided for all oligos: primers, si/shRNA, gRNAs, etc.
- Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
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b. Type, magnification, and numerical aperture of the objective lenses
c. Temperature
d. imaging medium
e. Fluorochromes
f. Camera make and model
g. Acquisition software
h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).

8) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication.
- Please abbreviate the names of journals according to PubMed.

9) A summary paragraph of all supplemental material should appear at the end of the Materials and methods section.
- Please include one brief sentence per item.

10) Author contributions: A separate author contribution section is required following the

Acknowledgments in all research manuscripts. All authors should be mentioned and designated by their full names. We encourage use of the CRediT nomenclature.

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Thank you for this interesting contribution, we look forward to publishing your paper in the Journal of Cell Biology.

Sincerely,

Mark Peifer, PhD
Monitoring Editor, Journal of Cell Biology

Melina Casadio, PhD
Senior Scientific Editor, Journal of Cell Biology

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

The original manuscript was already very interesting and convincing. The addition of new data and the comprehensive scheme make the work even better. The response of the authors to my suggestions is reasonable and appropriate. The new experiment in which the authors follow the development of egg chambers from stage 11 to 14 provides important new evidence that the elimination of the two nurse cell nuclei is required for normal egg chamber maturation.

Before publication, I have four points for the finalisation of the manuscript:

1) The abbreviation "NC" is used throughout the text but never introduced.

2) The abbreviation "NC" should not be used in the title.

3) The authors write "The ooplasm is understood to be constituted mostly of products that the NCs export to the oocyte through ring canals..." (p. 3). This is not correct as also the yolk proteins, which are secreted from the fat body are endocytosed by the oocyte. Thus, the ooplasm contains also products from the fat body, and this is very likely to contribute substantially to the growth of the oocyte between stage 8 and 10. There is an interesting new study by the Luschnig lab showing that the follicular epithelium opens it tricellular junctions from stage 9 to 11 to allow the passage of yolk through the epithelium into the oocyte in a process called "patency". (Transient opening of tricellular vertices controls paracellular transport through the follicle epithelium during Drosophila oogenesis, Jone Isasti-Sanchez, Fenja Münz-Zeise, Stefan Luschnig (bioRxiv). This study is now in press in Developmental Cell. Since this study reports a new cellular event which occurs simultaneously with nuclear elimination, it would be helpful for the reader to cite it.

4) The newly introduced Figure 12 gives a very clear and comprehensive summary of the process of nuclear elimination. However, no reference is given to this Figure in the text. It would be helpful to start the discussion by giving a short summary of the sequence of steps that occur during nuclear elimination by referring to this Figure.

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

Summary:
The fruit fly egg is derived from a structure called an egg chamber, which contains a cluster of germ cells surrounded by a layer of somatic cells. Within the germ cell cluster, the oocyte is connected to supporting nurse cells through intercellular bridges. The predominant model in the field is that through stage 10, there is slow transfer of materials from the nurse cells to the oocyte; then, at stage 11, there is a rapid transfer from the nurse cells to the oocyte during a process termed "dumping." Using a combination of confocal, electron microscopy, and live imaging, the authors describe a novel aspect of the late stages of oogenesis in which one or more of the nurse cells closest to the oocyte transiently fuse with the oocyte, allowing the nurse cell nucleus to enter through a large channel or pore. Once in the oocyte, the nurse cell nucleus/nuclei is/are degraded.

The authors have observed this early fusion event in 37 wild type and mutant D. melanogaster lines as well as in three additional species (D. simulans, D. hydei, and D. virilism). Correlations between the number of abnormal stage 11-14 egg chambers (which do not show evidence of this fusion event) and the number of eggs that do not hatch led the authors to conclude that this cell fusion and nuclear transfer is essential for oocyte maturation and embryonic viability.

The authors have addressed all of the concerns I had on the initial submission, and the main conclusions that they provide are further supported by the additional data that have been added.

I would now support the publication of this work.