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Scribble and E-cadherin cooperate to control symmetric daughter cell positioning by multiple mechanisms

Anchi S. Chann, Ye Chen, Tanja Kinwel, Patrick Humbert and Sarah M. Russell
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MS TITLE: A Scribble-E-cadherin complex controls symmetric daughter cell positioning by multiple mechanisms

AUTHORS: Anchi S Chann, Ye Chen, Tanja Kinwel, Patrick Humbert, and Sarah M Russell
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave favourable reports but raised some critical points that will require amendments to your manuscript. I hope that you will be able to carry these out because I would like to be able to accept your paper. I f you can address the referees' comments to my satisfcation in revised manuscript, there will be no need for further review.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

In their paper, Chann and colleagues characterise the molecular composition of retraction fibres (RFs) in single mitotic MCF10A cells and identify the presence of the cell-cell adhesion molecule E-cadherin and the polarity proteins Scribble and Dlg in these structures. This is an intriguing

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observation as these proteins, which are known to modulate spindle positioning relative to cell/cell adhesions in epithelial monolayers, have not previously been shown to be involved in substrate adhesion in individual mitotic cells. The authors then show that a reduction in these proteins from the cortex/RFs results in asymmetrical post-mitotic respreading and an associated deviation of mitotic spindle orientation from the substrate. Consistent with playing a role in spindle orientation, Scribble and E-cadherin depletion also reduces the localisation NuMA to the mitotic spindle poles and cortex, and LGN depletion results in a similar spindle misorientation phenotype. The authors therefore propose a pathway in which substrate cues are communicated through E-cadherin/Scribble in order to recruit the spindle positioning machinery.

In the second part of the paper, the authors observe a relocation of Scribble, E-cadherin and Dlg to the developing junction between the nascent daughter cells. They find that Ecad and Dlg at the post-mitotic junction is dependent on Scribble, and that Scribble KD reduces the width of the new junction. This is consistent with the known role of scribble in cell junction formation.

Overall this is a well presented manuscript, which reports a previously unrecognised role for the cell-cell adhesion molecules Scribble and E-cadherin in structures that mediate substrate adhesion and influence spindle orientation in individual mitotic cells. The statistical analysis is appropriate and the conclusions are largely justified by the data presented (with a few exceptions detailed below). This work would be of interest to cell biologists studying the fundamentals of division, but it is not clear how far these observations are likely to extend to other cell types or model systems.

Comments for the author

Major points

- The authors show that mitotic retraction fibres in single MCF10A cells are enriched in E-cadherin and Scribble but lack integrins. This is in contrast to previous studies (Dix et al. 2018, Lock et al. 2018), which observed integrins basally in mitotic cells. The authors do not fully discuss this difference. They suggest that it may be because cells are cultured on uncoated plastic, however it seems just as likely that it is because these cells are epithelial, unlike the cells used in previous studies. The authors need to discuss this further. Have the authors tried culturing the cells on fibronectin or other ECM for example? Does this change things? Also, integrin-based adhesions are very hard to see in adherent mitotic cells. Given this, it is worth testing whether integrins are required for mitotic adhesion to the substrate in these cells. This seems likely, since Cadherin RNAi cells still adhere to the substrate.

- The authors have not shown that E-Cadherin and Scribble act along metaphase retraction fibres. It seems more likely that they act to organise the cortex where the fibres meet the cell body. This could explain why Scribble and E-Cad don't co-localise well in fibres, although they do in the cell body (Fig 2A). Also, there is no enrichment of E-Cadherin at fibre tips. In addition, NUMA is lost from the cortex in siScribble cells - implying a role for Scribble at the cortex (e.g. Fig4b). This should be discussed and care should be taken not to imply that the data show that Cadherin participates directly in cell-substrate adhesion.

- siScribble has profound effects. It leads to loss of cortical NuMA, Dlg and Cadherin. Therefore, it could act via a number of pathways. To make it clearer how the system works, it would be good to know how E-Cad siRNA and E-Dlg siRNA affect Scribble localisation and NuMA localisation.

- It would be good to know how NuMA RNAi affects cell-cell adhesion at telophase as a control for the effects of spindle misalignment on this process.

- In the figures, the authors switch between observing single cells (mostly in figures 1-6) and cells growing in a confluent monolayer (figures 7-8 & S1A, S3, S6). What role does cell/cell adhesion play in the context of spindle angle orientation? Does the spindle angle still alter in confluent cells following scribble knockdown as would be expected? The authors need to be clear about how cell confluency was controlled and show whether it affects observed phenotypes.

- In figure 7, the authors show that scribble knockdown prevents E-cad accumulation at new cell/cell junctions following mitosis but doesn't affect interphase junctions. Isn't this somewhat

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surprising given the known role of scribble in junction formation and stabilisation? (Navarro et al. 2005, Yates et al. 2013 and others)? How do the authors account for this mitosis-specific phenotype? Similarly, do they think that E-cadherin is important for substrate attachment in interphase? It's not clear from the model how they propose the interphase to mitosis transition to work (and how this would differ between single cells and cells with existing neighbours as discussed above)

Minor points

- There are too many figures with a small amount of data in each. I would suggest merging figures 6 -9 into a maximum of 2 figures
- The cell margin and therefore what is segmented is not clear in the brightfield images of Figs 1b, 3b, d,. Highlighting the cell area would emphasise the difference between daughter cells.
- The quantification in Fig 1b – how is the 'end of spreading' defined? There is a significant difference between the control daughter cells. I didn't find this graph/representation of the data clear.
- Fig S2b – is this mislabelled? What is being shown in the +Ig grey scale image? Should it be HECD-1?
- Fig 4b – This spindle looks very strange, like a monopolar spindle. Is this a representative example of spindle defects resulting from reduction of NuMA?
- Fig 8a –does the Ecadherin signal disappear with scribble KD in interphase junctions, yet was retained in interphase junctions in Fig7a?

<u>Reviewer 2</u>

Advance summary and potential significance to field

In this manuscript, the authors suggest that a Scribble/E-cadherin complex regulates spindle orientation in single MCF10A cells and as a result daughter cell placement. The authors suggest that Scribble stabilises E-Cadherin at the cell cortex and at the retraction fibres during mitosis and is required for NuMA recruitment to the cortex. During telophase, Scribble relocates to the nascent cell-cell junction and promotes the formation of a new E-Cadherin based adhesion between the daughter cells. The main findings are quite similar to the study by Wang et al who also showed that loss of e-cadherin leads to SO defects in prostate epithelial cells both in vivo but also in an epithelial cell line.
Although this manuscript does contain some interesting findings, these are not sufficiently developed and many of the author's conclusions are not supported by the provided data. As such, the manuscript is not suitable for publication at JCS in its current form.

Comments for the author

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Although this manuscript does contain some interesting findings, these are not sufficiently developed and many of the author's conclusions are not supported by the provided data. As such, the manuscript is not suitable for publication at JCS in its current form.

<u>Major points</u>

1. The manuscript title "A Scribble-E-cadherin complex controls symmetric daughter cell positioning by multiple mechanisms" does not reflect the findings and is not supported by the data. There is no evidence that Scribble and E-cadherin form a complex or that they interact presented in this manuscript! The authors need to change the title to reflect their findings or provide biochemical evidence for the existence of a complex.

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2. The initial finding is that E-Cadherin is necessary for SO in single MCF10A cells. A role for cadherins in SO of single cells attached to the ECM would be quite interesting; however, this is not convincingly demonstrated by the authors. I am unable to rationally explain such a requirement, especially when one considers the author's data, which suggest that the cell does not only require cadherin expression but cadherin ligation, which is presumably what the inhibitory antibody (HECD-1) blocks. Given the above it would be critical for the authors to convincingly prove their point, yet in the first experiment using the inhibitory antibody HECD-1 no quantification is given for the SO defects elicited (Figure S2ii). Additionally, the XZ projection showing the antibody treated cell (Figure S2ii) suggests that the selected cell is detached. It would be important for authors to show more cells! Same with the one image provided in Figure 1c of an XZ projection after E-Cadherin siRNA. This image also suggests that the cell is detached (control is flat basally and cortical actin enrichment is absent basally as expected for an attached cell, while the KD cell is round basally with basal cortical actin enrichment, just like the antibody treated cell shown in Figure S2ii). As such, the main premise of the paper is undermined by the authors data, which suggest that their treatments somehow lead to poor cell attachment or detachment during mitosis which of course would lead to spindle misorientation with respect to the substrate due to loss of the spatial cues responsible for SO on planar substrates. Unfortunately, the same is true in Scribble KD exps (Figure 3a) where again the control cell is flat basally and cortical actin enrichment is absent in this region, while the KD cell is round basally with cortical actin enrichment. Based on the above, the authors need to convincingly show that the SO defects which form the basis of this manuscript do not stem from the detachment of cells from the substrate during mitosis.

3. The authors claim that E-Cadherin is enriched at the RFs and active integrins are not! Their images however do not reveal any enrichment of E-Cadherin on the RFs compared to the cortex. Had they generated a simple intensity profile this would be clear. In Figure 1a it's evident on the XZ view that most of the E-Cadherin signal is on the cortex, not on the RFs. Every transmembrane protein would be expected to display a similar distribution and be found on the RFs, since they are membrane protrusions; it in no way implies a functional role at the RFs. They also claim that no active integrins are detected (Figure 1a), but they are using 12G10, an antibody against extended open headpiece integrins (fully activated typically ligand bound integrins) which one would not expect to be found on RFs other than perhaps at the basalmost region where the RFs are in contact with the substrate. Had they used a total integrin beta 1 antibody the distribution would be very similar to that of E- Cadherin. If the authors believe that E-Cadherin is selectively enriched at the RFs, they could use a membrane tethered FP and quantify the ratio of the FP to that of E- Cadherin on the cortex vs RFs.

4. The premise that cadherins may be required, or play a role in SO in single cells needs to be further examined in other cell types and using other approaches. Do other epithelial cells require cadherin expression to orient their spindles? Single MCF10A cells presumably act as mesenchymal cells in the absence of tight junctions and adherens junctions forming with neighbouring cells. Do non polar cells, such as HeLa cells, display spindle orientation defects if N-Cadherin is downregulated or blocked? Does expression of E-Cadherin DN constructs such as cadherin constructs lacking the ectodomain which would behave similar to the wild type protein bound with the inhibitory antibody elicit SO defects? How about constructs lacking the cytoplasmic tail? Additionally, the authors should also test other function blocking cadherin antibodies, since some of these have been shown to also affect cell-ECM interactions. These experiments would go a long way towards providing convincing evidence that cadherin function is necessary for SO responses to ECM substrate cues.

5. The link between scribble and e cadherin is tentative at best. For some reason the authors initially carry out a couple of experiments using E-Cadherin inhibition and downregulation and then move on to say Scribble down regulation elicits the same defects and focus on Scribble for the rest of the study. If Scribble elicits SO defects via E-Cadherin downregulation, this can be tested quite easily by overexpression of E-Cadherin in their scribble shRNA cells. Does exogenous E-Cadherin rescue the SO defects?

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6. In an effort to explain how loss of E-Cadherin or Scribble leads to spindle orientation defects, the authors suggest that somehow loss of Scribble prevents association of NuMA with the cell cortex. Without numa, astral microtubules would indeed fail to anchor on the cortex, leading to spindle misorientation. However, the authors a) fail to show what happens to LGN localization in E-Cadherin KD cells or Scribble KD cells?
b) although they show that Dlg fails to associate with the cortex in Scribble KD, they fail to show if Dlg is affected in E-Cadherin KD cells and c) the authors need to also examine if Gai, which typically recruits LGN on the mitotic cortex, still associates with the cortex in E-Cadherin and Scribble KD cells.

7. The authors make a very interesting observation that in the absence of Scribble, E-Cadherin is selectively lost during mitosis but they largely ignore it. The fact that Scribble controls the levels of E-Cadherin is known, since Lohia et al.2012 showed that loss of Scribble enhanced endocytosis of E-Cadherin by weakening the E- Cadherin-p120-catenin interaction, leading to the accumulation of E-Cadherin in the Golgi apparatus (https://doi.org/10.1371/journal.pone.0051130). Given that a drastic decrease of E-Cadherin is detected specifically during mitosis in MCF10A cells, it is possible that Scribble may play a role in the stabilisation of E-Cadherin specifically during mitosis. This would be an important finding worth examining further. It is also possible that Scribble and E-Cadherin form a complex as the authors suggest and this may happen specifically during mitosis. The authors could check if this is the case, as well as the impact of Scribble on E-Cadherin stability, by carrying out experiments in synchronised cells using IP and western blotting.

Reviewer 3

Advance summary and potential significance to field

This paper from the Russell lab identifies a role for E-cadherin and Scribble in spindle orientation of MCF10A cells grown on plastic. This study is well-controlled and has beautiful imaging of the cells.

Comments for the author

This paper has already undergone a substantial revision in JCB. I therefore only have minor comments.

Western blots and their quantifications (or alternative method) need to be included to show the extent of E-cadherin knock-down (siRNA-mediated silencing of E-cadherin in Fig. 1), Scribble (siRNA-mediated silencing in Fig. 4,5). I realise that Fig. S3c shows that Scribble is not visible in the knock-down cells (shRNA and siRNA) as observed by immunofluorescence, but only one image is shown.

Line 421: Fig S2a does not show Scribbe depletion

First revision

Author response to reviewers' comments

As you will see, the reviewers gave favourable reports but raised some critical points that will require amendments to your manuscript. I hope that you will be able to carry these out because I would like to be able to accept your paper. I f you can address the referees' comments to my satisfaction in revised manuscript, there will be no need for further review.

Reviewer 1 Advance Summary and Potential Significance to Field...

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In their paper, Chann and colleagues characterise the molecular composition of retraction fibres (RFs) in single mitotic MCF10A cells and identify the presence of the cell-cell adhesion molecule E-cadherin and the polarity proteins Scribble and Dlg in these structures. This is an intriguing observation as these proteins, which are known to modulate spindle positioning relative to cell/cell adhesions in epithelial monolayers, have not previously been shown to be involved in substrate adhesion in individual mitotic cells. The authors then show that a reduction in these proteins from the cortex/RFs results in asymmetrical post-mitotic respreading and an associated deviation of mitotic spindle orientation from the substrate. Consistent with playing a role in spindle orientation, Scribble and E-cadherin depletion also reduces the localisation NuMA to the mitotic spindle poles and cortex, and LGN depletion results in a similar spindle misorientation phenotype. The authors therefore propose a pathway in which substrate cues are communicated through E-cadherin/Scribble in order to recruit the spindle positioning machinery.

In the second part of the paper, the authors observe a relocation of Scribble, E-cadherin and Dlg to the developing junction between the nascent daughter cells. They find that Ecad and Dlg at the post-mitotic junction is dependent on Scribble, and that Scribble KD reduces the width of the new junction. This is consistent with the known role of scribble in cell junction formation.

Overall this is a well presented manuscript, which reports a previously unrecognised role for the cell-cell adhesion molecules Scribble and E-cadherin in structures that mediate substrate adhesion and influence spindle orientation in individual mitotic cells. The statistical analysis is appropriate and the conclusions are largely justified by the data presented (with a few exceptions detailed below). This work would be of interest to cell biologists studying the fundamentals of division, but it is not clear how far these observations are likely to extend to other cell types or model systems.

Reviewer 1 Comments for the Author...

Major points

- *The authors show that mitotic retraction fibres in single MCF10A cells are enriched in E-cadherin and Scribble but lack integrins. This is in contrast to previous studies (Dix et al. 2018, Lock et al. 2018), which observed integrins basally in mitotic cells. The authors do not fully discuss this difference. They suggest that it may be because cells are cultured on uncoated plastic, however it seems just as likely that it is because these cells are epithelial, unlike the cells used in previous studies. The authors need to discuss this further. Have the authors tried culturing the cells on fibronectin or other ECM for example? Does this change things? Also, integrin-based adhesions are very hard to see in adherent mitotic cells. Given this, it is worth testing whether integrins are required for mitotic adhesion to the substrate in these cells. This seems likely, since Cadherin RNAi cells still adhere to the substrate.*

We did not mean to imply that integrins played no role, or that E-cadherin mediated adhesion *per se*. Indeed, as described in the third paragraph of the discussion, we believe that E-cadherin is more likely to mediate spindle orientation in response to a sensing of membrane stiffness or tension. We have now inserted several statements in the results section to make this clearer, and to directly compare with the references suggested by the reviewer. Specifically, we have now made clear in describing Figure 1 that the integrins are low, but not absent, in prometaphase retraction fibres, and that active integrin β1 is upregulated by telophase. We make more explicit in text around that figure that we do not believe E-cadherin mediates adhesion in this context. We directly compare these retraction fibres with those in the two papers mentioned by the reviewer, and one other. For the suggestion about fibronectin, we believe the risk of transformation of MCF10A as mentioned by(Park and Schwarzbauer, 2014) would confound interpretation.

- *The authors have not shown that E-Cadherin and Scribble act along metaphase retraction fibres. It seems more likely that they act to organise the cortex where the fibres meet the cell body. This could explain why Scribble and E-Cad don't co-localise well in fibres, although they do in the cell body (Fig 2A). Also, there is no enrichment of E-Cadherin at fibre tips. In addition,*

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NUMA is lost from the cortex in siScribble cells - implying a role for Scribble at the cortex (e.g. Fig4b). This should be discussed and care should be taken not to imply that the data show that Cadherin participates directly in cell-substrate adhesion.
As above, we did not mean to imply that E-Cadherin was required for substrate adhesion, and have altered the text accordingly, as well as changed the text in several places to avoid definitively implicating the retraction fibres (Lines 120-121, 130-132, 147-149, 174-175, and 361-362)

• *siScribble has profound effects. It leads to loss of cortical NuMA, Dlg and Cadherin. Therefore, it could act via a number of pathways. To make it clearer how the system works, it would be good to know how E-Cad siRNA and E-Dlg siRNA affect Scribble localisation and NuMA localisation.*

There is substantial literature on the functional relationship between Scribble and E-cadherin, which is clearly complex and context-dependent (as with Dlg, NuMA and LGN). Given this literature (described in the paper, for example that Ecadherin knockdown disrupts recruitment of Scribble to adherens junction for spindle orientation in the RWPE-1 cell line (Wang 2018a)) we do not feel that the effect of E-cad siRNA on Scribble localisation will add substantively to the story. Similarly with Dlg, where several redundantly acting family members of Dlg would need to be knocked out.

• *It would be good to know how NuMA RNAi affects cell-cell adhesion at telophase as a control for the effects of spindle misalignment on this process.*

NuMA is no longer enriched at the cortex at telophase (we now added an additional image at FigS4A), so NuMA is unlikely to mediate cell-cell adhesion for telophase. And importantly, we don't feel si-NUMA is feasible to test in telophase because spindle disruption (mitotic arrest) will occur upon siNuMA. Given we are not claiming cell adhesion is relevant to these phenomenon as clarified above, we hope you will agree assessing adhesion is not relevant.

• *In the figures, the authors switch between observing single cells (mostly in figures 1-6) and cells growing in a confluent monolayer (figures 7-8 & S1A, S3, S6). What role does cell/cell adhesion play in the context of spindle angle orientation? Does the spindle angle still alter in confluent cells following scribble knockdown as would be expected? The authors need to be clear about how cell confluency was controlled and show whether it affects observed phenotypes.*

It has been shown in many contexts that neighbouring cells rely on E-cadherin for spindle orientation, so we did not formally assess that. We do not believe such experiments would consolidate or confirm any of our findings. We have added more description about the control of confluency at Lines 291, and 487-489 in the Methods section. We also ensured that single or confluent cells were specified in the main text and throughout the figure legends for each experiment.

• *In figure 7, the authors show that scribble knockdown prevents E-cad accumulation at new cell/cell junctions following mitosis but doesn't affect interphase junctions. Isn't this somewhat surprising given the known role of scribble in junction formation and stabilisation? (Navarro et al. 2005, Yates et al. 2013 and others)? How do the authors account for this mitosis-specific phenotype? Similarly, do they think that E-cadherin is important for substrate attachment in interphase? It's not clear from the model how they propose the interphase to mitosis transition to work (and how this would differ between single cells and cells with existing neighbours as discussed above)*

We have expanded the text in results and discussion to accommodate these issues (lines 203-205, 391-398). We didn't mean to suggest that the interphase junction of MCF10A was not affected, and have added clarity at Lines 310-313, and changed the y-axis label of statistics at Fig6D to 'Ecad intensity at NMIIb-enriched junction'.

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Minor points

- *There are too many figures with a small amount of data in each. I would suggest merging figures 6 -9 into a maximum of 2 figures*

We have merged the three penultimate figures into 2. It seemed inappropriate to merge the schematic figure with the data figures, but we can, of course, do that if required.

- *The cell margin and therefore what is segmented is not clear in the brightfield images of Figs 1b, 3b, d,. Highlighting the cell area would emphasise the difference between daughter cells.*

We note that the cell area was not measured – the brightfield was shown to illustrate how the cell edge became less well-defined as the cell began to spread onto the substrate, which was used to define the time interlude between anaphase and cell spreading (see the next point below). We have now added an additional image at Fig S2A for reader to appreciate the morphology change as cell division progresses, and described more precisely how the images were used to define the time interlude between anaphase and cell spreading.

- *The quantification in Fig 1b – how is the 'end of spreading' defined? There is a significant difference between the control daughter cells. I didn't find this graph/representation of the data clear.*

We apologise for this error, we should have said – 'start of spreading', and now explain this figure more clearly with an illustration (Fig S2A) and in lines 517-518, and 979-981.

- *Fig S2b – is this mislabelled? What is being shown in the +Ig grey scale image? Should it be HECD-1?*

We apologise, our previous labelling did not make clear that the stain was anti-mouse Ig, we have rectified this.

- *Fig 4b – This spindle looks very strange, like a monopolar spindle. Is this a representative example of spindle defects resulting from reduction of NuMA?*

The spindle is not monopolar but difficult to capture in the XY plane given the skewed spindle pole. To reassure the reader that the spindle is normal other than its orientation, we have included the associated xz plane in S4B(ii).

- *Fig 8a –does the Ecadherin signal disappear with scribble KD in interphase junctions, yet was retained in interphase junctions in Fig7a?*

No, Fig 8a images were taken at a plane suitable for analysing the nascent junction formation and so would not necessarily have picked up the interphase junction. To formally assess whether E-cadherin was retained at the myosin-enriched interphase junction, in Fig7A we used Myosin IIb to locate interphase junction for analysis, and showed negligible effect of Scribble KD on E-cad recruitment.

Reviewer 2.

In this manuscript, the authors suggest that a Scribble/E-cadherin complex regulates spindle orientation in single MCF10A cells and as a result daughter cell placement. The authors suggest that Scribble stabilises E-Cadherin at the cell cortex and at the retraction fibres during mitosis and is required for NuMA recruitment to the cortex. During telophase, Scribble relocates to the nascent cell-cell junction and promotes the formation of a new E-Cadherin based adhesion between the daughter cells. The main findings are quite similar to the study by Wang et al who also showed that loss of e-cadherin leads to SO defects in prostate epithelial cells both in vivo but also in an epithelial cell line. Although this manuscript does

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contain some interesting findings, these are not sufficiently developed and many of the author's conclusions are not supported by the provided data. As such, the manuscript is not suitable for publication at JCS in its current form.

Major points

1. *The manuscript title "A Scribble-E-cadherin complex controls symmetric daughter cell positioning by multiple mechanisms" does not reflect the findings and is not supported by the data. There is no evidence that Scribble and E-cadherin form a complex or that they interact presented in this manuscript! The authors need to change the title to reflect their findings or provide biochemical evidence for the existence of a complex.*

We have removed the word 'complex' from the title.

2. *The initial finding is that E-Cadherin is necessary for SO in single MCF10A cells. A role for cadherins in SO of single cells attached to the ECM would be quite interesting; however, this is not convincingly demonstrated by the authors. I am unable to rationally explain such a requirement, especially when one considers the author's data, which suggest that the cell does not only require cadherin expression but cadherin ligation, which is presumably what the inhibitory antibody (HECD-1) blocks. Given the above it would be critical for the authors to convincingly prove their point, yet in the first experiment using the inhibitory antibody HECD-1 no quantification is given for the SO defects elicited (Figure S2ii). Additionally, the XZ projection showing the antibody treated cell (Figure S2ii) suggests that the selected cell is detached. It would be important for authors to show more cells! Same with the one image provided in Figure 1c of an XZ projection after E-Cadherin siRNA. This image also suggests that the cell is detached (control is flat basally and cortical actin enrichment is absent basally as expected for an attached cell, while the KD cell is round basally with basal cortical actin enrichment, just like the antibody treated cell shown in Figure S2ii). As such, the main premise of the paper is undermined by the authors data, which suggest that their treatments somehow lead to poor cell attachment or detachment during mitosis which of course would lead to spindle misorientation with respect to the substrate due to loss of the spatial cues responsible for SO on planar substrates. Unfortunately, the same is true in Scribble KD exps (Figure 3a) where again the control cell is flat basally and cortical actin enrichment is absent in this region, while the KD cell is round basally with cortical actin enrichment. Based on the above, the authors need to convincingly show that the SO defects which form the basis of this manuscript do not stem from the detachment of cells from the substrate during mitosis.*

(1) We have performed quantification for spindle orientation under HECD treatment, as shown in FigS2D (previous S2C)
(2) We didn't mean to imply substrate dissociation upon knockdown, and we now have updated the representative images.

3. *The authors claim that E-Cadherin is enriched at the RFs and active integrins are not! Their images however do not reveal any enrichment of E-Cadherin on the RFs compared to the cortex. Had they generated a simple intensity profile this would be clear. In Figure 1a it's evident on the XZ view that most of the E-Cadherin signal is on the cortex, not on the RFs. Every transmembrane protein would be expected to display a similar distribution and be found on the RFs, since they are membrane protrusions; it in no way implies a functional role at the RFs. They also claim that no active integrins are detected (Figure 1a), but they are using 12G10, an antibody against extended open headpiece integrins (fully activated typically ligand bound integrins) which one would not expect to be found on RFs other than perhaps at the basalmost region where the RFs are in contact with the substrate. Had they used a total integrin beta 1 antibody the distribution would be very similar to that of E-Cadherin. If the authors believe that E-Cadherin is selectively enriched at the RFs, they could use a membrane tethered FP and quantify the ratio of the FP to that of E-Cadherin on the cortex vs RFs.*

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We were not meaning to imply that E-cadherin was higher in the RFs than in the cortex, and to prevent this misconception, wee have changed the wording to 'expressed' in the RFs. We agree with (and have stated in the manuscript) that this antibody is likely to bind active integrin (ligand-bound), but do not feel that the transition from predominantly E-cadherin to predominantly integrin β1 (Fig 1A) implies a non-technical explanation. Further, the dramatic reduction in E-cadherin upon depletion of Scribble (Fig 2C) provides compelling evidence that this is not just passive recruitment of membrane proteins. We performed further characterisation of the retraction fibres in an earlier version of this manuscript (https://www.biorxiv.org/content/10.1101/2021.04.15.440081v1.full.pdf, Fig 2) which we removed to simplify the paper, but would be happy to include if you feel it necessary).

4*. The premise that cadherins may be required, or play a role in SO in single cells needs to be further examined in other cell types and using other approaches. Do other epithelial cells require cadherin expression to orient their spindles? Single MCF10A cells presumably act as mesenchymal cells in the absence of tight junctions and adherens junctions forming with neighbouring cells. Do non polar cells, such as HeLa cells, display spindle orientation defects if N-Cadherin is downregulated or blocked? Does expression of E-Cadherin DN constructs such as cadherin constructs lacking the ectodomain which would behave similar to the wild type protein bound with the inhibitory antibody elicit SO defects? How about constructs lacking the cytoplasmic tail? Additionally, the authors should also test other function blocking cadherin antibodies, since some of these have been shown to also affect cell-ECM interactions. These experiments would go a long way towards providing convincing evidence that cadherin function is necessary for SO responses to ECM substrate cues.*

We agree further exploration of this phenomenon would be interesting in future studies. However, such experiments are beyond the scope of this study.

5*. The link between scribble and e cadherin is tentative at best. For some reason the authors initially carry out a couple of experiments using E-Cadherin inhibition and downregulation and then move on to say Scribble down regulation elicits the same defects and focus on Scribble for the rest of the study. If Scribble elicits SO defects via E-Cadherin downregulation, this can be tested quite easily by overexpression of E-Cadherin in their scribble shRNA cells. Does exogenous E-Cadherin rescue the SO defects?*

Many papers have shown a functional connection between Scribble and E-Cadherin, but the relationship is clearly complex and context-specific (as described in the manuscript), and we do not agree that a rescue experiment would be either simple or conclusive. From our experience, over-expression of E-cadherin in MCF10A cells caused defective localisation of E-cadherin *per se*, which will confound interpretation. Given that Scribble acts post-transcriptionally on E-cadherin levels during cell division, it would be difficult to achieve equivalent levels for a fair rescue. In addition, we have shown that E-cadherin is necessary for spindle orientation, but have no evidence that it is sufficient to mediate the phenotype caused by Scribble deletion. A lack of rescue by exogenous E-cadherin would therefore not necessarily provide information on the relationship between E-cadherin and Scribble in this context.

6. *In an effort to explain how loss of E-Cadherin or Scribble leads to spindle orientation defects, the authors suggest that somehow loss of Scribble prevents association of NuMA with the cell cortex. Without numa, astral microtubules would indeed fail to anchor on the cortex, leading to spindle misorientation. However, the authors a) fail to show what happens to LGN localization in E-Cadherin KD cells or Scribble KD cells?*

As the reviewer agreed, NuMA plays a critical role in spindle orientation. In addition, an impact of E-cadherin and Scribble on LGN localisation has been demonstrated in (Wang 2018A) (Gloerich 2017). Given our demonstration that NuMA is mislocalised in Scribble-depleted anaphase cells therefore strongly suggests that Scribble mediates spindle orientation via the LGN/NuMA complex, thus we feel testing mislocalisation of LGN per se would not add substantive new knowledge. We

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also note that LGN antibodies were applicable only with methanol fixation but Scribble antibodies were not compatible with that, so there was a technical limitation to test LGN localisation upon Scribble depletion; and many previous studies of LGN localisation have involved GFP-tagged constructs, so not a simple experiment.
b) *although they show that Dlg fails to associate with the cortex in Scribble KD, they fail to show if Dlg is affected in E-Cadherin KD cells and c) the authors need to also examine if Gai, which typically recruits LGN on the mitotic cortex, still associates with the cortex in E-Cadherin and Scribble KD cells.*

There is clearly much to be done to determine all the players in this novel process. However, we believe further examination of each component is not in the scope of this initial discovery paper.

***7**. The authors make a very interesting observation that in the absence of Scribble, E-Cadherin is selectively lost during mitosis but they largely ignore it. The fact that Scribble controls the levels of E-Cadherin is known, since Lohia et al.2012 showed that loss of Scribble enhanced endocytosis of E-Cadherin by weakening the E-Cadherin-p120-catenin interaction, leading to the accumulation of E-Cadherin in the Golgi apparatus (https://doi.org/10.1371/journal.pone.0051130). Given that a drastic decrease of E-Cadherin is detected specifically during mitosis in MCF10A cells, it is possible that Scribble may play a role in the stabilisation of E-Cadherin specifically during mitosis. This would be an important finding worth examining further. It is also possible that Scribble and E-Cadherin form a complex as the authors suggest and this may happen specifically during mitosis. The authors could check if this is the case, as well as the impact of Scribble on E-Cadherin stability, by carrying out experiments in synchronised cells using IP and western blotting*

We appreciate that the reviewer agreed that this finding is interesting. As mentioned above, many studies have attempted to elucidate how Scribble and E-Cadherin regulate each other. Our finding of differences in regulation at interphase versus mitosis, might provide new opportunities, but we do not believe it is in the scope of this manuscript to solve this puzzle. In our experience attempts to enrich metaphase MCF10A cells led to detachment, including interphase cells, which confounded interpretation. However, we have added further discussion (lines 204-6, 391-395), including the possible relevance of the Lohia paper.

Reviewer 3 Advance Summary and Potential Significance to Field...

This paper from the Russell lab identifies a role for E-cadherin and Scribble in spindle orientation of MCF10A cells grown on plastic. This study is well-controlled and has beautiful imaging of the cells.

Reviewer 3 Comments for the Author...

This paper has already undergone a substantial revision in JCB. I therefore only have minor comments.

Western blots and their quantifications (or alternative method) need to be included to show the extent of E-cadherin knock-down (siRNA-mediated silencing of E-cadherin in Fig. 1), Scribble (siRNA-mediated silencing in Fig. 4,5). I realise that Fig. S3c shows that Scribble is not visible in the knock-down cells (shRNA and siRNA) as observed by immunofluorescence, but only one image is shown.

For siEcad, we provided intensity quantification at Fig1C, and now included low zoom images of confluent cells in FigS2C to demonstrate the reduction in E-cadherin expression by Ecad knockdown. For siScrib, in addition to the images shown in S3C, the flow cytometry data in Fig5B quantifies loss of Scribble. We hope that's sufficient to accommodate the reviewer's concerns.

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Park, J., and J.E. Schwarzbauer. 2014. Mammary epithelial cell interactions with fibronectin stimulate epithelial-mesenchymal transition. *Oncogene*. 33:1649-1657.

Second decision letter

MS ID#: JOCES/2022/260547

MS TITLE: Scribble and E-cadherin cooperate to control symmetric daughter cell positioning by multiple mechanisms

AUTHORS: Anchi S Chann, Ye Chen, Tanja Kinwel, Patrick Humbert, and Sarah M Russell
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.

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