

Manuscript EMBOR-2012-36969

Adherens Junctions determine the apical position of the midbody during follicular epithelial cell division

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Review timeline:

Submission date:	14 December 2012
Editorial Decision:	17 January 2013
Revision received:	19 April 2013
Editorial Decision:	22 May 2013
Revision received:	24 May 2013
Accepted:	24 May 2013

Editor: Barbara Pauly

Transaction Report:

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

1st Editorial Decision

17 January 2013

Thank you very much for the submission of your research manuscript to our editorial office. I would like to apologize for the slight delay in getting back to you with a decision on your manuscript, which was due to the holiday period. We have now received the full set of reviews on your manuscript.

As the detailed reports are pasted below I will only repeat the main points here. You will see that reviewers 1 and 3 acknowledge the potential interest of the findings. While referee 1 only points out one issue with the data on Par-6 and Lgl, referee 3 has a number of concerns that would need to be addressed before the study can be published. This referee does not feel that the current data set provides conclusive evidence for the claims made. For example, s/he states that the evidence that asymmetric distribution of contractile ring components do not play a role in midbody positioning is not strong enough. In some cases, this referee feels that the data are over-interpreted and would need to be strengthened (see, for example his/her comments no. 3, 5, 7, 9). It also becomes clear from the comments of both referees 3 and 2 that it would be important to investigate the functional significance of AJ-mediated midbody positioning for the maintenance of epithelial integrity. This data would, in the opinion of referee 2, be required to raise the impact of the study, which s/he otherwise considers to be better suited for publication in a more specialized journal.

On balance, I would like to give you the opportunity to revise your manuscript, with the

understanding that the main concerns of the reviewers should be addressed. Acceptance of the manuscript will depend on a positive outcome of a second round of review and I should also remind you that it is EMBO reports policy to allow a single round of revision only and that therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. If you feel that this period is insufficient for a successful submission of your revised manuscript I can potentially extend this period slightly. Also, the length of the revised manuscript should not exceed roughly 28,000 characters (including spaces). Should you find the length constraints to be a problem, you may consider including some peripheral data in the form of Supplementary information. However, materials and methods essential for the repetition of the key experiments should be described in the main body of the text and may not be displayed as supplemental information only.

We have also started encouraging authors to submit the raw data for microscopical images and western blots to our editorial office. These data will be published online as part of the supplementary information. This is voluntary at the moment, but if you agree that this would be useful for readers I would like to invite you to supply these files when submitting the revised version of your study.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

We also welcome the submission of cover suggestions or motifs that might be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised form of your manuscript when it is ready. Should you in the meantime have any questions, please do not hesitate to contact me.

REFeree REPORTS:

Referee #1:

The manuscript by Morais-de-Sa & Sunkel explores how epithelial cells of the follicular epithelium undergo mitosis. Live imaging shows that following mitosis, the midbody re-localises to the apical side of the cell. The authors show that adherens junctions are responsible for positioning the midbody by attaching to the contractile ring. Overall, this is an interesting set of observations that is suitable for publication in EMBO Reports, as long as the following major criticism can be addressed.

Major point.

1. The title, abstract and majority of the manuscript are concerned with adherens junctions and mitosis, yet the authors also take a detour in Figure 3A-C to examine the apical-basal polarity determinants Par-6 and Lgl during mitosis. The authors report changes in these apical-basal polarity determinants but offer no mechanistic explanation for how these changes occur. Furthermore, the authors demonstrate no functional role for the observed changes in Par-6 or Lgl. Unless the authors can convincingly show both molecular mechanism and function for these changes in Par-6 and Lgl, then Fig3A-C should simply be removed from the manuscript.

Referee #2:

This paper investigates how epithelial cells position the midbody, previously known to be apically asymmetric. It uses *Drosophila* and shows that asymmetry is not due to asymmetric contractile ring but due to adherens junctions in the epithelium. It is well-executed experimentally and the S2 cell experiment is a nice demonstration of catenin-midbody relationship independent of other cell polarity. But the question of apical midbody positioning and the answer here that adherens junctions position them are not of as much general importance for the papers that EMBO Reports seeks to publish. The paper comes up short in terms of overall impact. There are no experiments that show functional significance of midbody positioning -such as that loss of apical midbody positioning breaks epithelial integrity. This is more suitable for a specialty cell biology journal. Also the paper provides little context for previous inquiries into the relationship between midbody, cleavage and cell polarity and junctions.

Referee #3:

At the end of cytokinesis, the two daughter cells are transiently connected by a small intercellular bridge, called the midbody. It consists, among others, of microtubules and several other proteins, such as kinesins and kinases. One of the functions of the midbody is to localise the site of abscission. In epithelial cells, furrow ingression is asymmetric, in that it progresses from the basal side, resulting in an apically localised midbody. How the midbody itself is localised is not well understood.

Here, the authors studied the role of adherens junctions in the localisation of the midbody during cell division in the *Drosophila* follicular epithelium, a well-established *in vivo* system to study epithelial development and function. They confirm that, similar as in vertebrate epithelia, the furrow progresses from the basal side. They continue by analysing the localisation of proteins similar to those known to be asymmetrically localised during asymmetric cytokinesis in *C. elegans*. No asymmetric localisation was found for Spaghetti-squash (MRLC), Septin2 or RhoA, and asymmetry was not affected in mutants for Rho kinase (Rok). They further show that during mitosis, the apical proteins Par-6 and aPKC are no longer restricted to the apical side, and lateral Lgl is removed from the cortex. In contrast, components of the adherens junctions are maintained during mitosis, but are asymmetrically removed upon midbody formation. Midbody positioning is affected in arm and dlg mutant clones. Upon expression of a fusion protein between Echinoid-GFP and the intracellular domain of E-Cadherin in S2 cells, other proteins are recruited to the site of expression, such as Arm, alpha-Catenin and Bazooka, but not Par-6 or Dlg. In these cells, the midbody is more frequently recruited to the site where the fusion protein localises.

The data are well documented and interesting to researchers in this field. However, several of the conclusions drawn are not really justified by the data, and some of the results are over-interpreted. So there is an indication that, as stated in the title, adherens junction, or, more precisely, E-cadherin and Armadillo, play a role in positioning the midbody. I do not find compelling evidence that for the absence of asymmetry during midbody positioning.

1. The authors conclude that "... the mechanism underlying this process does not rely on intrinsic asymmetry of the CR structure, ..." (page 9). However, since they only used a few markers, this is an over-interpretation of the data. There can be some - still unknown - proteins that confer asymmetry. It has been published (Kechad et al., 2012) that Anillin is required in S2 cells for complete closure of the CR and the formation of the midbody ring.
2. A paper not mentioned by the authors (Lu, Roegiers, Jan and Jan, 2001) suggests a planar polarity cue provided by adherens junctions in neuroepithelial cells of the *Drosophila* embryo, which orients the mitotic spindle during symmetrical divisions. This cue depends on APC and EB1, raising the question whether these elements also play a role in positioning the midbody.
3. Throughout the text, the authors use the term "midbody" for a structure that is marked by different markers in different experiments, e. g. Jup-GFP, late staining of Sqh, Tubulin. Did they ever test whether the three components mark the same structure?

4. I do not understand the first sentence in the abstract. There is no evidence provided to show that the process of cytokinesis or the positioning of the midbody has any role in maintenance of epithelial integrity.

Additional points:

5. Page 7 and Fig. S2B: They write that AJs disassemble. What is shown is that Arm-GFP is not seen at the site opposite to the accumulation of Tubulin-RFP. Whether this is associated with a disassembly of the AJs, could only be determined by EM analysis. Can they really conclude from these pictures that the midbody "attaches" to AJs? What they see is that it is closely associated with Arm-positive staining.

6. Fig. 2C contains a typo, it should read p-MRLC (I guess).

7. The authors write (page 7): "... rok mutants do not affect AB polarity .. ", and in the legend to Fig. S1 they write: "... rok mutant clones display wild-type localisation of AJs near the apical domain." First, although the apical localisation of Arm suggests that the AJs are normal, they did not study it. So the only conclusion is that the mutant cells show wild-type localisation of Arm. Second, the authors themselves showed that cells can "partially depolarise", so without any additional marker the conclusion that the mutant clones "do not affect AB polarity" is premature and not justified by the data.

8. Fig. 3A, B: the authors write that Par-6 and aPKC are depolarised": I can't see any aPKC during mitosis. In addition, how do they know that they "... return apically when the midbody is positioned" without including a midbody marker? Perhaps they are synthesised de-novo?

9. Fig. 3D, E: they write: "... that the CR attaches apically to AJs formed with one of the neighbouring cells...". This phrasing implies a physical attachment, which they cannot conclude from the staining. The CR is just very close to E-cadherin. In addition, they cannot say that "... AJs opposite to the final place of midbody position disassemble", but only that E-cadherin disappears. I find this conclusion difficult to follow. In Fig. S2A, there are quite some non-dividing cells with gaps of Arm staining, so it could be that the adhesion belt is not in the same focal plane throughout.

10. Fig. 3F: the orientation is not clear. Does the boxed area present a surface view? Cells may have lost polarity already before they initiate mitosis, so many things could be affected, in particular, since there no other marker is used. Nevertheless, cells are not "unable to position the midbody" (page 8) (it adopts a position). Perhaps what they mean is that it does not adopt a position at a site the authors assume to be the apically site.

11. Fig. S4B: As far as I know, dlG mutant cells lose polarity, so how can the authors know where is apical and basal in these cells?

1st Revision - authors' response

19 April 2013

Response to reviewers' comments

The three referees acknowledge the interest of our study, but raised a number of pertinent points that we have addressed in this revised version. Importantly, reviewer 2 asked for data examining the relevance of apical midbody positioning, which we address on Figure 4 and Figure S8. Reviewer 3 raised important questions related to the relevance of an asymmetric distribution of contractile ring components to asymmetric cytokinesis, and the connection of the midbody and AJs, which we address with data presented in Figure 2A, Figure 3C, Figure S2 and Figure S5. In addition, we included three new papers that examine cytokinesis in epithelial cells and are were published during

revision (Founounou, Loyer et al. 2013; Guillot and Lecuit 2013; Herszterg, Leibfried et al. 2013) in the context of our discussion. A detailed answer to each reviewer's point is presented below.

Referee #1:

The manuscript by Morais-de-Sa & Sunkel explores how epithelial cells of the follicular epithelium undergo mitosis. Live imaging shows that following mitosis, the midbody re-localises to the apical side of the cell. The authors show that adherens junctions are responsible for positioning the midbody by attaching to the contractile ring. Overall, this is an interesting set of observations that is suitable for publication in EMBO Reports, as long as the following major criticism can be addressed.

Major point.

The title, abstract and majority of the manuscript are concerned with adherens junctions and mitosis, yet the authors also take a detour in Figure 3A-C to examine the apical-basal polarity determinants Par-6 and Lgl during mitosis. The authors report changes in these apical-basal polarity determinants but offer no mechanistic explanation for how these changes occur. Furthermore, the authors demonstrate no functional role for the observed changes in Par-6 or Lgl. Unless the authors can convincingly show both molecular mechanism and function for these changes in Par-6 and Lgl, then Fig3A-C should simply be removed from the manuscript.

At the moment, we do not fully understand the mechanism and functional role of Par-6 and Lgl re-organization during mitosis. Since exploring this question goes beyond the scope of this paper on midbody positioning, we followed the referee's suggestion and removed the part relevant to Lgl dynamics. Furthermore we moved the results referring to Par-6/aPKC depolarization to supplementary data (Fig. S4). Although we agree that this result is dispensable for the main conclusions of our study, we believe that it is sufficiently relevant to be included as supplemental data for following reasons: 1) It shows that the apical Par-6/aPKC distribution is not polarized at the onset of cytokinesis and so is not in place to directly determine asymmetric cytokinesis 2) It reveals that apical polarity needs to be re-established upon follicle cells division. Thus, it brings another angle to the relevance of apical midbody positioning, since for instance, apical determinants were shown to be delivered to the site of cytokinesis during MDCK cell polarization (Schluter, Pfarr et al. 2009).

Please note that Fig S4 was also included within this version as part of a response to comment 8 of reviewer 3.

Referee #2:

This paper investigates how epithelial cells position the midbody, previously known to be apically asymmetric. It uses Drosophila and shows that asymmetry is not due to asymmetric contractile ring but due to adherens junctions in the epithelium. It is well-executed experimentally and the S2 cell experiment is a nice demonstration of catenin-midbody relationship independent of other cell polarity. But the question of apical midbody positioning and the answer here that adherens junctions position them are not of as much general importance for the papers that EMBO Reports seeks to publish. The paper comes up short in terms of overall impact. There are no experiments that show functional significance of midbody positioning -such as that loss of apical midbody positioning breaks epithelial integrity. This is more suitable for a specialty cell biology journal. Also the paper provides little context for previous inquiries into the relationship between midbody, cleavage and cell polarity and junctions.

In this revised version, we explored the importance of midbody positioning and tested the outcome of mispositioning the midbody along the AB axis of the tissue (Fig S8 and Fig 4). We find that midbody localization in the apical domain is followed by actin accumulation at the apical interface between daughter cells, which starts around the midbody and coincides with the transient accumulation of the ARP2/3 complex (Fig.S8A-S8C, and Movie S9). Importantly, we show that when we misposition the midbody, such as in *arm* mutants, actin polymerization occurs around the midbody wherever it is positioned along the AB axis, showing a more direct association between midbody position and the place of F-actin polymerization (Fig. S8D). However, due to the lack of total AJ integrity, *arm* mutants cannot be used to access the effect of midbody mispositioning on tissue organization. We induced the ectopic localization of AJ-intracellular components to the basal domain of the follicle epithelium via a fusion with a basal specific transmembranar protein (Fig. 4A). Basal localization of AJ-components allows the maintenance of endogenous apical AJs (Fig. 4A). However since it occasionally disrupted midbody positioning, this tool provided us the opportunity to analyze by live imaging the effect of midbody mispositioning within a context where the surrounding epithelia maintained its adhesion and AB polarity. Our data reveals that positioning the midbody and its related actin polymerization more basally results in the formation of invaginations of the epithelium, which are characterized by a basal shift of the apical interface between daughter cells relatively to AB axis of the surrounding tissue (Fig 4B, Fig4C, and Movie S10). We therefore proposed that the position of the midbody determines where newly formed actin filament arrays will be able to form AJs clusters, stabilizing the position of the new apical interface. We are aware that we do not have a response to for how exactly this wave of actin polymerization stabilizes the formation of a new apical junction. However, it is consistent with the widely accepted view that a dense F-actin network stabilizes AJs at the apical side of epithelial cells during interphase and also reports that show that the formation of an actin scaffold where AJs complexes can cluster is required for the expansion of cell-cell contacts in cells undergoing mesenchymal to epithelial transition, or as mammalian epithelial cells establish attachment sites (Vasioukhin, Bauer et al. 2000; Verma, Shewan et al. 2004; Yamada and Nelson 2007),

As discussed in the final part of the current manuscript, other papers addressing cytokinesis in different *Drosophila* epithelial tissues were published during revision. Although *Herszterg et al., 2013* thoroughly examine the role of midbody related actin polymerization on the extension of the apical interface between daughter epithelial cells. Our experiments support this feature of the midbody within a different epithelial context and add that midbody mispositioning results in the formation an apical interface basally shifted in relation to the plane of the surrounding epithelium. This shows that the position of the midbody can impact on tissue architecture by positioning the daughter cell junction along the AB axis of the tissue.

Referee #3:

At the end of cytokinesis, the two daughter cells are transiently connected by a small intercellular bridge, called the midbody. It consists, among others, of microtubules and several other proteins, such as kinesins and kinases. One of the functions of the midbody is to localise the site of abscission.

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The data are well documented and interesting to researchers in this field. However, several of the conclusions drawn are not really justified by the data, and some of the results are over-interpreted. So there is an indication that, as stated in the title, adherens junction, or, more precisely, E-cadherin and Armadillo, play a role in positioning the midbody. I do not find compelling evidence that for the absence of asymmetry during midbody positioning.

1. The authors conclude that "... the mechanism underlying this process does not rely on intrinsic asymmetry of the CR structure, ..." (page 9). However, since they only used a few markers, this is an over-interpretation of the data. There can be some - still unknown - proteins that confer asymmetry. It has been published (Kechad et al., 2012) that Anillin is required in S2 cells for complete closure of the CR and the formation of the midbody ring.

Following this reviewer suggestion, we have now included the analysis of the distribution of Anillin and the septin Peanut during constriction (Fig. 2A, Fig S3A and Movie S1), showing that these proteins do not present a basal enrichment that would support their role on basal to apical furrowing. In addition, we now show that although *pnut* mutant follicle cells often fail cytokinesis (Fig. S3B and Fig. S3C), they position the midbody on the apical domain whenever they manage to go further through cytokinesis until midbody formation (Fig. S3C). In conjunction with our previous data on Septin 2, Rho1 and p-Myosin symmetry, we show that 1) Anillin and septins, which drive asymmetric furrowing *C.elegans*, or 2) an asymmetry on the activated state of Myosin do not underline asymmetric constriction in the follicular epithelium. Thus, although we cannot rule out an asymmetry of proteins whose role in asymmetric furrowing is still unexplored, this new version provides more compelling evidence in support that polarized constriction does not result from an intrinsic asymmetry of CR components.

2. A paper not mentioned by the authors (Lu, Roegiers, Jan and Jan, 2001) suggests a planar polarity cue provided by adherens junctions in neuroepithelial cells of the *Drosophila* embryo, which orients the mitotic spindle during symmetrical divisions. This cue depends on APC and EB1, raising the question whether these elements also play a role in positioning the midbody.

Lu et al., 2001 suggests that AJs/APC/EB1 orient mitotic spindles to control the symmetric division in the *Drosophila* ventral neuroectoderm epithelium. This is supported by the reported Cadherin function in regulating spindle orientation and APC localization in mammalian cells (den Elzen, Buttery et al. 2009). However, this mechanism does not seem to be conserved in all epithelial cells

types (McCartney, Price et al. 2006) (Woolner and Papalopulu 2012). Moreover, during the analysis of midbody positioning in *arm* mutants we noticed that spindle orientation was always nearly parallel to the epithelial monolayer, suggesting that AJs are not required to orient the spindle in the follicular epithelium. Nevertheless, we appreciate this suggestion as it raises the possibility that orienting midbody associated microtubules would be relevant for AJs-dependent midbody positioning. Following the reviewers question, we analyzed midbody positioning in clones of double mutants for the two *Drosophila* APC proteins *apc* and *apc2* and in *eb1* mutants, using alleles that have previously validated the role of these proteins in different contexts, including the analysis of APC requirement for orientation of asymmetric cell division (Elliott, Cullen et al. 2005; McCartney, Price et al. 2006). None of these mutant conditions produced any defect in midbody positioning, suggesting that Eb1 and APC protein function is not essential for apical midbody position (Fig R1).

Since the dynamics and organization of the microtubules associated with the mitotic spindle are very different from midbody microtubules, and given that we could not detect an effect of *eb1* and *apc* *apc2* mutants on midbody positioning, we believe that this data does not add much to the main argument of the paper and decided not to include it in the manuscript.

3. Throughout the text, the authors use the term "midbody" for a structure that is marked by

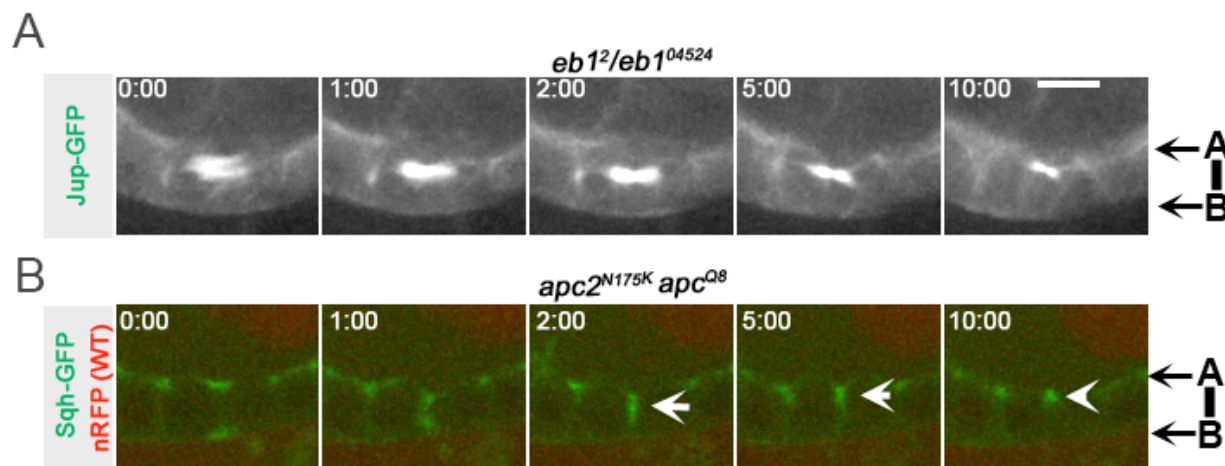


Figure R1: (A) *eb1* (100 %, n = 36) and (B) *apc apc2* (100%, n = 11) mutants maintain apical midbody positioning marked by the midbody microtubules with Jup-GFP (A) or Sqh to label cross-section of the CR until MR formation (B) *apc2*^{N175K} *apc*^{Q8} clones are marked by absence of RFP (B).

different markers in different experiments, e. g. Jup-GFP, late staining of Sqh, Tubulin. Did they ever test whether the three components mark the same structure?

Jup-GFP is reported to be a microtubule binding protein that can be used to follow microtubule dynamics (Karpova, Bobinnec et al. 2006). We added this reference and the following sentence to the supplementary methods: “Stocks with endogenous expression of the microtubule binding protein Jup-GFP were used to label microtubules”. So, Jup-GFP and Tubulin label the microtubules associated with the midbody, whereas Sqh-GFP labels initially the contractile ring and late the

midbody ring that surrounds the midbody. We used the term midbody for simplification, and also because the central structure labeled by these different markers is the midbody. To clarify this within the paper we added an end-on view of the contractile ring, which shows the midbody associated microtubules compacting within the contractile ring during the CR to midbody ring transition (Fig. S1A).

Following this comment, we also decided to include in the paper FigS1B, which shows midbody position labeled by a different marker of the midbody and Anillin to mark the midbody ring. We therefore added the following sentence:

“This can be visualized using midbody markers, such as Pavarotti, or markers of the midbody ring such as Spaghetti squash (Sqh, *Drosophila* Myosin II regulatory light chain (MRLC)) and Anillin...”

4. I do not understand the first sentence in the abstract. There is no evidence provided to show that the process of cytokinesis or the positioning of the midbody has any role in maintenance of epithelial integrity.

We agree with this comment and we removed this sentence as we rephrased the abstract to account for data addressing the functional relevance of apical midbody positioning as requested by reviewer 2.

5. Page 7 and Fig. S2B: They write that AJs disassemble. What is shown is that Arm-GFP is not seen at the site opposite to the accumulation of Tubulin-RFP. Whether this is associated with a disassembly of the AJs, could only be determined by EM analysis. Can they really conclude from these pictures that the midbody "attaches" to AJs? What they see is that it is closely associated with Arm-positive staining.

We agree with the reviewer that the way we wrote this sentence can be misleading since we do not have sufficient evidence to show AJ disassembly at the site opposite to the midbody localization. We rephrased the sentence in the text “The levels of AJs components E-CAD, α -catenin and Armadillo (Arm, *Drosophila* β -catenin) are reduced at the side where the CR is uncoupled...”. In addition we added figures showing α -catenin-GFP imaging during follicle cell cytokinesis to support that the main components of AJs display a similar behaviour along the apical side (Fig. S5B, and Movie S4).

To elucidate the close association of the midbody to AJ, we present now in Figure 3C/Fig S5F a TEM picture showing that the midbody (visualized as highly electron-dense material that is associated with microtubules) is positioned in close proximity to AJs formed with a neighboring cell. As referred in the discussion it is still unclear how AJ associate with the CR to mediate midbody recruitment, and the most likely explanation is that it occurs either via a connection to actin filaments or other components of the CR, lately MR, and so not directly to the midbody. We therefore agree that we cannot accurately imply direct attachment to the midbody and rephrased the text accordingly.

6. Fig. 2C contains a typo, it should read p-MRLC (I guess).

We have made this change.

7. The authors write (page 7): "... rok mutants do not affect AB polarity .. ", and in the legend to Fig. S1 they write: "... rok mutant clones display wild-type localisation of AJs near the apical domain." First, although the apical localisation of Arm suggests that the AJs are normal, they did not study it. So the only conclusion is that the mutant cells show wild-type localisation of Arm. Second, the authors themselves showed that cells can "partially depolarise", so without any additional marker the conclusion that the mutant clones "do not affect AB polarity" is premature and not justified by the data.

We must draw the attention of the referee for the paper we cited next to this sentence "Since *rok* mutants do not affect AB polarity (Fig S1C and [18])". This previous paper refers that *rok* mutants retain polarity as data not show. Thus, following reviewer's concern we show that in addition to the AJ-marker Arm, the markers of apical polarity (aPKC) and basolateral polarity (Dlg) maintain their normal localization in *rok* mutant background (Fig. S3D). We believe that this further supports that AB polarization is retained in *rok* mutant tissue.

8. Fig. 3A, B: the authors write that Par-6 and aPKC are depolarised": I can't see any aPKC during mitosis. In addition, how do they know that they "... return apically when the midbody is positioned" without including a midbody marker? Perhaps they are synthesised de-novo?

Following referee's 1 suggestion, we moved this data to Fig S4.

In order to clarify depolarization of Par-6 and aPKC during mitosis, we changed this figure to include sequential frames of Par-6 with higher exposition and as a single channel, where it is clear that Par-6 localizes around the cortex and within the cytoplasm of mitotic follicle cells (Fig S4A). Similarly, staining with anti-aPKC in fixed samples shows that during mitosis aPKC is no longer apically enriched (Fig S4C).

Nevertheless, we agree that this data does not elucidate whether there is some protein degradation followed by new synthesis upon cell division. Accordingly, we rephrased the sentence to "apical enrichment of Par-6 and aPKC is only similar to interphase levels when the midbody is positioned apically". The midbody is now marked by its associated microtubules (Fig S4B and S4C). These figures show that at the beginning of cytokinesis (before the compactation of the microtubules associated with the midbody), the apical domain is not fully re-polarized, and so that full apical polarization is established upon midbody positioning.

9. Fig. 3D, E: they write: "... that the CR attaches apically to AJs formed with one of the neighbouring cells...". This phrasing implies a physical attachment, which they cannot conclude from the staining. The CR is just very close to E-cadherin

As we describe in our response to comment 5, we agree that we cannot show direct physical attachment and just very close association of the CR to apical AJs formed with one neighboring cell. We added an additional longitudinal time-lapse projection presenting an end-on view of the ring, which allows the visualization of the AJ formed with both neighbor cells within same plane of the

contractile ring, showing how AJ remain coupled to the contractile ring until midbody formation (Fig S5A).

In addition, they cannot say that "... AJs opposite to the final place of midbody position disassemble", but only that E-cadherin disappears. I find this conclusion difficult to follow.

We rephrased this sentence accordingly to our response to previous comments:

“The levels of AJs components...are reduced at the side where the CR is uncoupled suggesting that (yellow arrows in Fig 3B, S5B and S5C) anisotropic distribution of AJs anchoring points correlates with the asymmetry of midbody localization...”. Although most follicle cells display off-centred position, we now added to FigS5D examples of some cells that show centred position of the midbody and relate this to a symmetric organization of AJs components. We consider that this data further supports the relevance of AJ to determine midbody positioning, by showing that the organization of the AJ interface in the region of furrowing determines the asymmetry of midbody localization within the apical side.

In Fig. S2A, there are quite some non-dividing cells with gaps of Arm staining, so it could be that the adhesion belt is not in the same focal plane throughout.

We aim that all the focal planes that include the AJ belt of the dividing cell, or other region of interest are used for the time-lapse projections at the surface of the egg chamber. However we can easily fail to include AJ of other non-dividing cell, which due to the oval shape of the egg chambers present AJs in a different plane. Since we recognize that this could be confusing, we replaced this figure with Fig S5B.

10. Fig. 3F: the orientation is not clear. Does the boxed area present a surface view? Cells may have lost polarity already before they initiate mitosis, so many things could be affected, in particular, since there no other marker is used. Nevertheless, cells are not "unable to position the midbody" (page 8) (it adopts a position). Perhaps what they mean is that it does not adopt a position at a site the authors assume to be the apically site.

(Fig 3F of previous version, which is now Fig 3D) presents a longitudinal view showing the interface between the follicle cell monolayer and the germline. We enlarged both frame on the left that represents all egg chamber, and the frames from the time-lapse projections, which made this new version clearer (Fig. 3D).

It is well known that disruption of AJs disturbs organization and polarity of the epithelial tissue (Cox, Kirkpatrick et al. 1996; Muller and Wieschaus 1996; Tanentzapf, Smith et al. 2000). Although this is observed in the follicle epithelium, loss of AJ allows the gross maintenance of an epithelial monolayer in this context (Tanentzapf, Smith et al. 2000, Fig S6A). The follicle epithelium always contains a putative apical side contacting the germline. During the measurement of midbody positioning we always consider the apical domain as the surface that contacts the germline, normalizing midbody position to the length of the AB axis of the surround epithelial monolayer, as

stated in the supplementary methods. This AB referential is shown as example in Fig3D and we clarified this point by stating in the manuscript: “*arm* mutant cells disrupt midbody positioning on the putative apical domain, defined as the surface that contacts the germline”. We cannot exclude that other things are affected within a tissue with disrupted AJs, and so we did not state with this data if AJs were required directly for midbody positioning. Instead, the more direct role of AJs in positioning the midbody is better supported by the recruitment of the midbody to the edges that can form AJs in follicle cells with intracellular asymmetric distribution of AJ (Fig 3F) or by its recruitment to polarized AJ components in otherwise non-polarized S2 cells (Fig 3G-3I).

11. Fig. S4B: As far as I know, *dlg* mutant cells lose polarity, so how can the authors know where is apical and basal in these cells?

dlg mutants indeed lose polarity, which allows the formation of multiple layers of epithelial tissue and the formation of AJs misplaced along the AB axis (Fig. S7A As replied to comment 10, we defined apical as the surface that contacts the germline (schemes on Figure S7B.). By disrupting the organization of the monolayer contacting the germline we could randomly misposition AJ along this putative axis and test if it affected midbody positioning. Accordingly, it was stated in the figure legend “It should be noted that only dividing cells that had direct contact with the germline were used for quantification”. As we understand how this point arose and to make it clearer we added the following to the legend “...were used for quantification, so that apical could be defined as the side contacting the germline and basal the opposite side”

2nd Editorial Decision

22 May 2013

Thank you for your patience while we were waiting to get feedback from the referees on your revised study. We have now received the enclosed reports from the referees that were asked to assess it. I am happy to tell you that both reviewers now support publication of your study in EMBO reports. Referee 3 still has two minor suggestions that I would like you to incorporate before we proceed with the official acceptance of your manuscript. You can either send us the modified file as an email attachment or upload it through our website, whatever is easiest for you.

I might have mentioned before that we have started encouraging authors to submit the raw data of biochemical (western blots etc) and/or microscopical images to our editorial office. These data will be published online as part of the supplementary information. This is voluntary at the moment, but if you agree that this would be useful for readers I would like to invite you to supply these files by sending them as email attachments or by uploading them as additional files.

I look forward to seeing a new revised version of your manuscript as soon as possible.

REFEREE REPORTS:

Referee #1:

I am satisfied with the revisions to the manuscript and I now support publication in EMBO Reports.

Referee #3:

The authors have done a great job in addressing the points I raised and improved the manuscript.

There are two more points left:

1. Fig. S7B needs some more explanation, in particular the cartoon. They write that blue marks the AJs, but they also mark the MT in blue? They should explain the dotted blue line (I guess this is the "normal" AJ) and red staining and should explain the abbreviations (GL, FC). Although they explain what they mean by apical, I would write apical and basal in this context with quotation marks, i.e. as "apical" and "basal".
2. This point was not raised in my first comments, so I leave it to the authors to consider: Since they only used the follicle epithelium, and since it is known that epithelia may behave differently, they may include in their title "follicular" epithelial cell division.

2nd Revision - authors' response

24 May 2013

Please find enclosed two files containing the modification you requested in the acceptance letter of our manuscript.

One point regarding comment 2 of referee#3 is that the modification of the title as requested increases the number of characters of the title and might just be over the limit.

The second point is how do we proceed now. Do we submit a new version by adding it or do we wait for you to provide a link to submit the original files.

Please let me know so that we can complete the process as soon as possible.

3rd Editorial Decision

24 May 2013

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: emboreports@embo.org]. If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.