A new cellular stress response that triggers centriolar satellite reorganization and ciliogenesis

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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.)

This manuscript was directly transferred with referee comments from a journal outside of the EMBO Publications family. Therefore, only reports obtained at The EMBO Journal are included here.

1st Editorial Decision 27 August 2013

Thank you again for transferring your manuscript on a new cellular stress response affecting centriolar satellites for our consideration. Both referees of the original submission kindly agreed to re-review the study for The EMBO Journal, and I am pleased to inform you that both of them consider the revised manuscript significantly strengthened. Consequently both reviewers are in principle supportive of publication, pending further addressing of a few remaining minor points.

One issue raised by both reviewers refers to the question whether cellular stress affects only selected satellite markers or satellite integrity in general, and both feel that this should be addressed with higher resolution images and some quantification. Referee 1 also brings up the point that the notion of two independent pathways should be further strengthened by an altered presentation of the respective data and by some additional data on MIB1 activation. From an editorial perspective, please carefully consider the nomenclature issue re. AZI1 vs Cep131, and please amend the manuscript text with brief Author Contribution and Conflict of Interest statements. Please also address the various minor points raised by the reviewers.

Following these few additional revisions, we shall be happy to accept this manuscript for publication in The EMBO Journal. You will find a link for resubmission of the final version below; at this stage please only upload files (text, main figures, combined supplementary information, a point-by-point response to the remaining comments as well as signed and completed license forms) that are directly relevant for the final version of the manuscript. Please do not hesitate to get back to me should you have any further questions in this regard.

Thank you again for the opportunity to consider this work for The EMBO Journal! I look forward to
receiving your final version.

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**REFeree REPORTS:**

Referee #1:

The authors highlight in this manuscript a new stress response that impacts on centriole satellite organization and ciliogenesis. They find that stress regulates satellites via two, partly independent, pathways: one in response to activation of p38 and the other in response to inactivation of the E3 ligase, MIB1. Moreover, they show that MIB1 is a novel satellite component that mono-ubiquitylates other satellite components, AZI1/Cep131 and PCM-1, and that MIB1 inactivation can trigger ciliogenesis in proliferating cells.

This is a revised manuscript that I originally reviewed for another journal. My impression of that first submission was that it was a potentially exciting story with high quality data. This view is maintained with this new version and the authors have responded well to most of my original concerns. However, there are a couple of important issues that need to be dealt with. If the authors can deal appropriately with these and the other minor points indicated below, then I would be happy to recommend publication.

**Major points**

1. The first major issue is whether cell stress causes specific loss of selected satellite markers or more general loss of satellite integrity. So in Fig. 1F, it's not clear to me why OFD1 localization remains unchanged if UV treatment causes loss of PCM-1. This result contradicts that of Lopes et al. (2011) who showed that localization of OFD1 to satellites was dependent on PCM-1. This needs to be explained; could it be that there is only partial PCM-1 displacement in response to stress as opposed to when it is depleted by siRNA? This could also mean that there is not selective protein loss but rather partial loss of satellite integrity. A direct comparison of high magnification images of PCM-1, OFD1 and AZI1 at satellites and centrosomes in cells treated with UV or depleted of PCM-1 may help to address this issue.

2. The second major issue concerns clarifying whether the p38 and MIB1 pathways are indeed, at least partly, independent. In my view, the result showing that the UV-induced inactivation of MIB1 is p38-independent (Fig. S4C) is an important result supporting the model of two independent pathways and so should be within the main figures and not the Supplementary Information. This conclusion should also be strengthened by determining MIB1 activity in response to stress with and without p38 depletion. The data shown in Fig. 5SA & B that MIB1 depletion doesn't prevent UV-induced loss of AZI1 and PCM-1 from satellites also supports the model of two independent pathways and would, in my opinion, be better in the main figures. Finally, the authors nicely highlight in their rebuttal letter and the Discussion that there are potentially two pathways by which stress disrupts satellites and induces ciliogenesis, p38 activation and MIB1 inactivation. Yet this does not come across well in the abstract. I recommend revising the Abstract to make sure this key message comes across.

**Minor points**

3. If the authors wish to keep the name AZI1, then they should at least indicate when it is first mentioned in the Results section that it is also referred to in the literature as Cep131.

4. In relation to Figs. 1 & 2, the authors mention in the text that the same results were obtained in other cell types, e.g. RPE1 cells. They should therefore mention in the text that the cells primarily used here were U2OS cells and not just mention it in the Figure Legend.

5. In Fig. 1D, there appears to be a reduction in the amount of AZI1 in both cytoskeletal and cytoplasmic fractions as if this protein is being generally degraded rather than just delocalized. This is not seen though in Fig. 2E. I suggest replacing the AZI1 blot in Fig. 1D with a more representative one assuming that Fig. 2E is the more consistent result.

6. The y-axis on Fig. 4A could be relabeled to indicate that the SILAC ratio relates to differences in ubiquitylated proteins recovered.
Referee #2:

The authors have addressed all my original comments in a satisfactory manner. I do accept the technical challenge regarding the identification of the ubiquitylation site on AZI1. Based on Fig. 1F I am not entirely convinced about the authors' claim that centriolar satellites remain intact after UV and siRNA treatments of PCM1 or AZI1. In the majority of cells OFD1 is strongly centrosomal (whether in mock or UV-treated cells) and its satellite localisation is not particularly striking (perhaps the cytoplasmic background is too high). Close-ups might help to demonstrate the point better. Including some quantification for the volume/spread of OFD1 staining could further strengthen the data. Figure legend is missing for Fig. 1F.

1st Revision - authors' response 13 September 2013

Referee #1

This is a revised manuscript that I originally reviewed for another journal. My impression of that first submission was that it was a potentially exciting story with high quality data. This view is maintained with this new version and the authors have responded well to most of my original concerns. However, there are a couple of important issues that need to be dealt with. If the authors can deal appropriately with these and the other minor points indicated below, then I would be happy to recommend publication.

Major points

1. The first major issue is whether cell stress causes specific loss of selected satellite markers or more general loss of satellite integrity. So in Fig. 1F, it's not clear to me why OFD1 localization remains unchanged if UV treatment causes loss of PCM-1. This result contradicts that of Lopes et al. (2011) who showed that localization of OFD1 to satellites was dependent on PCM-1. This needs to be explained; could it be that there is only partial PCM-1 displacement in response to stress as opposed to when it is depleted by siRNA? This could also mean that there is not selective protein loss but rather partial loss of satellite integrity. A direct comparison of high magnification images of PCM-1, OFD1 and AZI1 at satellites and centrosomes in cells treated with UV or depleted of PCM-1 may help to address this issue.

These are important issues to consider. In the study by Lopes et al., OFD1 localization to satellites was assessed several days after siRNA-mediated depletion of PCM1. Under these conditions OFD1 localization to satellites is indeed PCM1-dependent; we have made similar observations. In fact, data from the literature as well as our own findings suggest that removal of virtually any centriolar satellite factor will cause a gradual, general dissolution of this structure over the course of a couple of days, the normal time-frame for experiments involving siRNA-mediated knockdowns. What is important to point out here is that the stress-induced reorganization of centriolar satellites represents a very different scenario from such siRNA-based experiments, in that rather than inducing effects resulting from long-term depletion of an individual protein, the stress-induced impact on centriolar satellite architecture that we observe is a much more rapid response. In the great majority of experiments we analyzed centriolar satellite status 1 h after cell stress, but in fact these effects can also be seen much earlier. It is highly plausible that given the removal of PCM1, OFD1 will eventually also dissociate from satellites at late stages after UV, but it is clear that at 1 h after UV, PCM1 is all but fully expelled from satellites while OFD1 is retained. As such, we believe our observations are not in disagreement with those made by Lopes et al., but that they simply reflect two very different, and not directly comparable, scenarios. To more clearly illustrate that only selected factors are acutely displaced from satellites, and as suggested by the Reviewer, we have included in the new Suppl. Fig. S2 representative high magnification images showing PCM1 and OFD1 localization to centriolar satellites before and after UV treatment, and we have included quantification of these data in the new Fig. 1G. We believe these additional data further illustrate and strengthen the notion that only selected factors are acutely expelled from satellites in response to cell stress, as opposed to overall dissolution of this structure.

2. The second major issue concerns clarifying whether the p38 and MIB1 pathways are indeed, at
least partly, independent. In my view, the result showing that the UV-induced inactivation of MIB1 is p38-independent (Fig. S4C) is an important result supporting the model of two independent pathways and so should be within the main figures and not the Supplementary Information. This conclusion should also be strengthened by determining MIB1 activity in response to stress with and without p38 depletion. The data shown in Fig. 5SA & B that MIB1 depletion doesn’t prevent UV-induced loss of AZI1 and PCM-1 from satellites also supports the model of two independent pathways and would, in my opinion, be better in the main figures. Finally, the authors nicely highlight in their rebuttal letter and the Discussion that there are potentially two pathways by which stress disrupts satellites and induces ciliogenesis, p38 activation and MIB1 inactivation. Yet this does not come across well in the abstract. I recommend revising the Abstract to make sure this key message comes across.

We agree with these helpful suggestions. As suggested by the Reviewer, we moved the data showing that UV-induced MIB1 inactivation is independent of p38 activity (former Suppl. Fig. S4C) to the main figures (now incorporated into Fig. 4B). In addition, we used p38 siRNA to show that depletion of the kinase by this approach also did not compromise UV-induced downregulation of MIB1 E3 ligase activity (new Suppl. Fig. S5C), corroborating the notion that MIB1 inactivation in response to UV is independent of p38 activity. Finally, we rephrased the Abstract slightly to further emphasize that two parallel and independent pathways underlie cell stress-induced centriolar satellite reorganization and ciliogenesis.

Minor points

3. If the authors wish to keep the name AZI1, then they should at least indicate when it is first mentioned in the Results section that it is also referred to in the literature as Cep131.

Following discussions with the Editor, we have decided to keep the name AZI1 as there seems to be no clear preference for either AZI1 or CEP131 in the literature, and because AZI1 is the official name for the protein in the Uniprot database. To avoid confusion, we now refer to the protein as AZI1/CEP131 on its first mention in the Abstract, Introduction, and Results sections.

4. In relation to Figs. 1 & 2, the authors mention in the text that the same results were obtained in other cell types, e.g. RPE1 cells. They should therefore mention in the text that the cells primarily used here were U2OS cells and not just mention it in the Figure Legend.

We agree, and the text has been amended accordingly.

5. In Fig. 1D, there appears to be a reduction in the amount of AZI1 in both cytoskeletal and cytoplasmic fractions as if this protein is being generally degraded rather than just delocalized. This is not seen though in Fig. 2E. I suggest replacing the AZI1 blot in Fig. 1D with a more representative one assuming that Fig. 2E is the more consistent result.

We agree with this notion. We have performed these fractionation experiments multiple times, and indeed the general trend is that the abundance of AZI1 in the cytoplasmic fraction does not significantly change in response to UV, unlike what was shown in Fig. 1D. We have therefore replaced this experiment with a replicate, and more representative, experiment in which the levels of cytoplasmic AZI1 do not differ significantly between mock- and UV-treated samples.

6. The y-axis on Fig. 4A could be relabeled to indicate that the SILAC ratio relates to differences in ubiquitylated proteins recovered.

This is a good suggestion, which we have incorporated into Fig. 4A in the revised manuscript.

Referee #2:

The authors have addressed all my original comments in a satisfactory manner. I do accept the technical challenge regarding the identification of the ubiquitylation site on AZI1.

Based on Fig. 1F I am not entirely convinced about the authors’ claim that centriolar satellites remain intact after UV and siRNA treatments of PCM1 or AZI1. In the majority of cells OFD1 is
strongly centrosomal (whether in mock or UV-treated cells) and its satellite localisation is not particularly striking (perhaps the cytoplasmic background is too high). Close-ups might help to demonstrate the point better. Including some quantification for the volume/spread of OFD1 staining could further strengthen the data. Figure legend is missing for Fig. 1F.

This is a valid point. In the new Suppl. Fig. S2, we have included representative images showing close-ups of OFD1 (and PCM1) localization to centriolar satellites. The Reviewer is indeed right that PCM1 has a much more dominant presence in satellites compared to OFD1, whose localization pattern at centriolar satellites is also confined to a somewhat smaller area surrounding the centrosomes, which admittedly made it difficult to fully appreciate OFD1 localization to satellites solely based on the data in Fig. 1F. We do not know the reason for the lack of full colocalization between PCM1 and OFD1 in centriolar satellites. Still, we believe the images shown in Suppl. Fig. S2 (and Fig. 1F) together with quantification of PCM1 and OFD1 localization to centriolar satellites before and after UV treatment (new data shown in Fig. 1G) clearly demonstrate that OFD1 but not PCM1 is retained at satellites at 1 h after exposure to UV, suggesting that centriolar satellites *per se* are not acutely disassembled after cell stress.

The missing figure legend for Fig. 1F has been added in the revised manuscript.