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CPAP promotes timely cilium disassembly to maintain neural progenitor pool

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Transaction Report:

Please note that the manuscript was previously reviewed at another journal and submitted here including the original referee comments, which were taken into consideration in the editorial decision-making process. These reports are not subject to EMBO's transparent review process policy and can therefore not be published. The report below was provided by an arbitrating referee who had access to both the manuscript and the previous referee comments.

(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 27 January 2016

Thank you for the submission of your manuscript entitled "CPAP promotes timely cilium disassembly to maintain neural progenitor pool" and please accept my apologies for the delay in getting back to you, but we have only now received the comments from the referee, which I copy below.

As you can see from his/her comments, the referee is rather supportive of your work, but point out to a number of concerns that will require your attention before your manuscript can be published in The EMBO Journal. I will not repeat here these issues, which essentially refer to further controls and clarifications. Please notice that they may require additional experimental evidence in at least some cases.

REFEREE REPORT

Referee #1:

The authors look at the role of a Seckel associated mutation in CPAP, also called Sas4, in cilium biogenesis and cell cycle progression. A proportion of fibroblasts derived from a Seckel patient are longer than controls and have abnormal centriole appendages. The proportion of fibroblasts in S-phase are decreased, and reentry into the cell cycle upon serum addition is slightly slowed. Ciliary disassembly may be slower and some form of ciliary remnant may be present in a portion of Seckel fibroblasts in mitosis. What this ciliary remnant corresponds to (does it have an axoneme?) is not clear.

CPAP interacts with a number of other centrosomal proteins, such as HDAC6, OFD1, NDE-1, CDK5RAP2, gamma tubulin and alpha tubulin. Given the diverse centrosomal proteins that seem to associate with CPAP, the analysis should be extended to some non-interacting centrosomal proteins, confirming the specificity of the assay.

The CC5 domain affected in this case of Seckel syndrome is sufficient to recapitulate many of these interactions. Several of these interacting proteins show reduced localization to the basal body in the Seckel fibroblasts, although this should be quantitated similar to what was done in Figure 2.

In vitro Seckel derived iPSCs show increased rapidity of differentiation into neurons. The Aurora kinase inhibitor Tozasertib increases ciliary length and also promotes differentiation, recapitulating some aspects of the Seckel cell model. Expression of a different mutant form of CPAP (E1235V) also caused a slight increase in neuronal differentiation. Cerebral organoids made from Seckel iPSC cells show reduced subventricular zones.

Two additional points remain to be addressed. Why aren't the cilia of Seckel fibroblasts longer than controls as measured in Fig. 2D? This seems to be inconsistent with the data in Fig. 1.

It is not possible to appreciate the OFD1 knockdown in Fig 5A or the CPAP knockdown in Fig 5E.

1st Revision - authors' response 03 February 2016

Our response to the reviewer is given in bold.

The authors look at the role of a Seckel associated mutation in CPAP, also called Sas4, in cilium biogenesis and cell cycle progression. A proportion of fibroblasts derived from a Seckel patient are longer than controls and have abnormal centriole appendages. The proportion of fibroblasts in S-phase are decreased, and reentry into the cell cycle upon serum addition is slightly slowed. Ciliary disassembly may be slower and some form of ciliary remnant may be present in a portion of Seckel fibroblasts in mitosis. What this ciliary remnant corresponds to (does it have an axoneme?) is not clear.

We are thankful to the reviewer for evaluating our work and providing us with positive feedback to improve our manuscript further.

The ciliary remnant that is positive for Arl13b is a ciliary membrane as was also observed by Paridaen et al., Cell 2013. Electron microscopy analyses by Paridaen et al., Cell 2013 reveals that the ciliary membrane is present near to a centriole. In addition, in mammalian cells the basal body is converted into a centriole during cell cycle re-entry (Dutcher et al, 2000). From these, it appears that the remnant we seldom observe (only in a very tiny number of cells, Fig. 2f) is a ciliary membrane. We have now explained it better in the manuscript (Page: 5 as well as the Figure legend 2F)

CPAP interacts with a number of other centrosomal proteins, such as HDAC6, OFD1, NDE-1, CDK5RAP2, gamma tubulin and alpha tubulin. Given the diverse centrosomal proteins that seem to associate with CPAP, the analysis should be extended to some non-interacting centrosomal proteins, confirming the specificity of the assay.

Very constructive point and we appreciate it. We indeed provided a Western blot showing Cap350, a centrosomal protein that does not co-purify with endogenous CPAP (Fig. 3a, c and

d) indicating the specificity of CPAP interaction with other CDC components. Perhaps, we have not explained it well in the figure legend.

To further strengthen this point that CPAP interacting proteins are indeed specific, we have now included another marker ODF2 (a mother centriolar appendage protein) that does not seem to co-purify with CPAP. This new information is now included and described in the legend for Fig. 3.

The CC5 domain affected in this case of Seckel syndrome is sufficient to recapitulate many of these interactions. Several of these interacting proteins show reduced localization to the basal body in the Seckel fibroblasts, although this should be quantitated similar to what was done in Figure 2.

The reviewer is referring to Fig. S6 and discusses about the reduced recruitment of CDC components (Aurora A, OFD1 and Nde1) in Seckel fibroblasts. To quantify the amount of protein being recruited in Seckel cells as compared to wild type cells, we have provided intensity quantification just beneath immunofluorescence panels in Fig. S6.

In vitro Seckel derived iPSCs show increased rapidity of differentiation into neurons. The Aurora kinase inhibitor Tozasertib increases ciliary length and also promotes differentiation, recapitulating some aspects of the Seckel cell model. Expression of a different mutant form of CPAP (E1235V) also caused a slight increase in neuronal differentiation. Cerebral organoids made from Seckel iPSC cells show reduced subventricular zones.

We appreciate this reviewer to find evaluate our disease modeling experiments

Two additional points remain to be addressed. Why aren't the cilia of Seckel fibroblasts longer than controls as measured in Fig. 2D? This seems to be inconsistent with the data in Fig. 1.

In Fig. 1, we analyzed cells growing asynchronously in the presence of serum. Whereas, in Fig. 2d, we analyzed the rate of cilium disassembly of Seckel and WT cells after they were synchronized at G_0 by serum starvation for a prolonged period of 96 hrs. This is also depicted (Fig. S2a). This treatment causes both Seckel and WT cells to have equivalent cilia length. This allows us to estimate the rate of cilia disassembly upon serum stimulation.

Possibly, this point was not evident from the previous version. This is now fixed in the revised version (In the main text as well as at the figure legend). Page: 5, at the beginning of the last paragraph.

It is not possible to appreciate the OFD1 knockdown in Fig 5A or the CPAP knockdown in Fig 5E.

We have now provided better Western blots for Fig. 5a and 5e.

2nd Editorial Decision 05 February 2016

I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Jay Gopalakrishnan
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2015-93679

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Figure legeneds 1, 2, 4, 5, 6, S2, S3, S4, S5, S6, S10 and S12 contain the details of sample size chosen and statistic test applied to derive the significance.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes. Figure legeneds 1, 2, 4, 5, 6, S2, S3, S4, S5, S6, S10 and S12 contain the details of sample size chosen and statistic test applied to derive the significance.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes
Is there an estimate of variation within each group of data?	Yes
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

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http://figshare.com

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6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Method section contains the details of the reagents including citation and catalogs that are appropriate
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	All the cell lines used in this study were routinely tested and for mycoplasma and are mycoplasma free

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D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Referred

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
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F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
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22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA