

Adaptable centriole biogenesis via the intrinsically disordered protein ALMS1

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Ozaki et al., start their response to the Reviewer's comments by providing a one page "General Remarks on Novelty" section. I found this section hard to follow. In the top figure, for example, I don't see why one needs to invoke the blue "ALMS1 extrinsic cues (memory?)" arrows in the middle to explain their data, and in the bottom figure I didn't understand what the magenta protein is and how this figure illustrates that ALMS1 is particularly suited to carry information/memory?

The subsequent answers to my specific points were also sometimes difficult to follow as the authors often made statements that I think are at least partially incorrect or seemed to have misunderstand the point I was trying to make. They seem to largely have taken the approach of arguing in their rebuttal letter why my points are not valid and then leaving the manuscript largely unchanged (at least in response to what I was asking for). I discuss their response to my first two points to illustrate these points and why I am not very satisfied with this approach; similar arguments would apply to most of the points they discuss in their rebuttal letter.

1a. I argued that the cartwheel normally makes multiple connections to the rest of the centriole, so it is hard to imagine how it can half-slide out of the daughter centriole to shed the lower half while retaining the upper half in the daughter. The authors answer by claiming that the cartwheel is very dynamic. This claim seems to be largely based on the observation that the cartwheel is ultimately degraded; but this is not the same as showing that the constituent proteins are constantly turning over in a way that would allow the cartwheel to easily slide out of the daughter. There is compelling evidence that a significant fraction of major structural cartwheel components like SAS-6 and SAS-4 do not turnover after they are incorporated into the cartwheel in worms and flies (Dammermann et al., JCB, 2008; Aydogan et al., JCB, 2018), and there is good evidence that this is also the case for SAS-6 in human cells (Keller et al., JCB 2014 – who report that in early S-phase ~40% of centriolar SAS-6 doesn't turnover, and this rises to ~75% in G2). This makes sense when one looks at the multiple interactions that would seem to lock the cartwheel to the outer centriole (e.g., Guichard et al., Curr. Biol., 2013).

They also claim in their response to this point that their observation of cartwheel splitting/shedding is corroborated by previous findings in *Drosophila*, citing Vidwans et al., JCS, 2003. There are certainly lots of unusual centriole configurations reported in this paper but to imply that this paper corroborates cartwheel splitting/shedding is disingenuous.

1b. I argued that the authors results could be explained if centrioles are prone to prematurely disengage during the extended, drug-induced, G2 period that they use to observe the phenomenon of splitting/shedding, and that this tendency might be exacerbated if ALMS1 function is perturbed (as ability to form a new centriole on the old mother seems to occur more easily in the ALMS1 mutant cells). In this scenario, ALMS1 would not have to be part of the engagement machinery for this to occur. Points 1-4 of their response do not counter this argument, and in point 5 the authors argue that their observations are inconsistent with premature disengagement throughout S and G2 (and this seems to be what they try to illustrate in the diagram they draw here). But I was clearly not suggesting this. I'm sure the centrioles don't prematurely disengage in S-phase (why would they?). I'm suggesting that they might disengage prematurely (i.e., not in mitosis) during the prolonged G2 arrest. I don't see anything in this response that counters this argument.

In summary, my main original criticism was that the authors were proposing a radically new idea based largely on studies of

cells in very non-physiological conditions, but they never make this clear to readers (and they phrase things in a way that often obscures this point). It would have been great if they had been able to get stronger data to support these conclusions (e.g., observing splitting/shedding in unperturbed cells) but I appreciated that this was likely to be difficult, so all I really asked was that they did a better job of highlighting the potential caveats and alternative explanations. Unfortunately, I have read the revised manuscript and I can't see that they have done either of these things. Thus, I remain of the opinion that this data shouldn't be published in this form as it will be too misleading to readers who are not experts in the field. This is a shame, as there are some interesting observations and stimulating ideas presented here.

Reviewer #2

(Remarks to the Author)

The authors have performed a remarkable rewrite of the manuscript and addressed my concerns. I think this paper will be an important addition to the literature.

Version 1:

Reviewer comments:

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I appreciate the authors efforts, but still have significant concerns that the mechanisms they propose here are unlikely to explain how canonical centriole duplication occurs in normal cells, and that they do not adequately convey to readers some of the caveats to the interpretation of their data. Nevertheless, I can see that the Editors and other Reviewers do not share these concerns, and I do not want to stand in the way of publishing what will no doubt be a provocative story that will hopefully stimulate debate and future work. I am therefore happy for this work to be published.

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Reviewer #1 (Remarks to the Author):

Ozaki et al., start their response to the Reviewer's comments by providing a one page "General Remarks on Novelty" section. I found this section hard to follow. In the top figure, for example, I don't see why one needs to invoke the blue "ALMS1 extrinsic cues (memory?)" arrows in the middle to explain their data, and in the bottom figure I didn't understand what the magenta protein is and how this figure illustrates that ALMS1 is particularly suited to carry information/memory?

Response: We thank the reviewer for the comment. We propose a model in the discussion that uses general terms to discuss how ALMS1, as an IDP outside of the CS, cartwheel, and procentriole, causes centriole biogenesis in ways that are both adaptable and transmittable. Because of the limited space, perhaps it would be helpful to prepare a separate perspective article addressing the novel abstract framework suggested by this study.

The subsequent answers to my specific points were also sometimes difficult to follow as the authors often made statements that I think are at least partially incorrect or seemed to have misunderstand the point I was trying to make. They seem to largely have taken the approach of arguing in their rebuttal letter why my points are not valid and then leaving the manuscript largely unchanged (at least in response to what I was asking for). I discuss their response to my first two points to illustrate these points and why I am not very satisfied with this approach; similar arguments would apply to most of the points they discuss in their rebuttal letter.

Response: We apologize for any confusion we may have caused. New data supporting cartwheel splitting/shedding has been added (Fig. 2j & k, see below), which is highlighted in yellow in the revised manuscript.

1a. I argued that the cartwheel normally makes multiple connections to the rest of the centriole, so it is hard to imagine how it can half-slide out of the daughter centriole to shed the lower half while retaining the upper half in the daughter. The authors answer by claiming that the cartwheel is very dynamic. This claim seems to be largely based on the observation that the cartwheel is ultimately degraded; but this is not the same as showing that the constituent proteins are constantly turning over in a way that would allow the cartwheel to easily slide out of the daughter. There is compelling evidence that a significant fraction of major structural cartwheel components like SAS-6 and SAS-4 do not turnover after they are incorporated into the cartwheel in worms and flies (Dammermann et al., JCB, 2008; Aydogan et al., JCB, 2018), and there is good evidence that this is also the case for SAS-6 in human cells (Keller et al., JCB 2014 – who report that in early S-phase ~40% of centriolar SAS-6 doesn't turnover, and this rises to ~75% in G2). This makes sense when one looks at the multiple interactions that would seem to lock the cartwheel to the outer centriole (e.g., Guichard et al., Curr. Biol., 2013). They also claim in their response to this point that their observation of cartwheel splitting/shedding is corroborated by previous findings in Drosophila, citing Vidwans et al., JCS, 2003. There are certainly lots of unusual centriole configurations reported in this paper but to imply that this paper corroborates cartwheel splitting/shedding is disingenuous.

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We appreciate the reviewer's comment and apologize for any confusion we caused. All changes made in response to the reviewer's suggestions are highlighted in yellow in the manuscript. Below we first address **1b** with new data, followed by the response to **1a**.

Response to 1b:

ALMS1 mutant centrioles undergo premature disengagement during G2 arrest. The disengagement, however, was observed to occur in concert with cartwheel splitting/shedding. ExM and Ex-dSTORM show the intermediate phenotype, including "physical splitting" in the lumen or "partial shedding" from the lumen. We do not see a net gain or loss of the cartwheel signal before and after its splitting/shedding. The subsequent discovery of ALMS1's function in mediating the assembly-disassembly dynamics of CSs, which impacts cartwheel assembly-disassembly dynamics, results in a coherent notion that ALMS1 is not directly implicated in centriole engagement/disengagement.

Importantly, this finding is consistent with what is already known. There are a number of close connections between changes in cartwheel dynamics and centriole disengagement: (i) The initiation of cartwheel removal at late G2/M is immediately followed by centriole disengagement in M; (ii) the inhibition of cartwheel removal at late G2/M by PLK1 inhibition concurrently inhibits disengagement; (iii) persistent SAS-6 recruitment after procentriole formation is required for maintaining centriole engagement from S to G2, with the whole configuration being lost in mitosis through CDK1 phosphorylation (*Huang et al., 2022, JBC*); and (iv) cartwheel splitting/shedding occurs concurrently with disengagement during G2 arrest under ALMS1 mutations (*this study*).

In contrast, what the reviewer proposed in the comment is a scenario where centriole disengagement occurs without cartwheel loss or changes in cartwheel dynamics (followed by new cartwheel growth at the disengaged mother). To our knowledge, there have been no reports of centriole disengagement, whether premature or not, occurring without a corresponding alteration in cartwheel dynamics. We think that a potential causal relationship between cartwheel dynamics and centriole disengagement may exist at late G2/M, an idea that aligns with the existing literature and the results of the current study.

However, we concur that augmenting our finding of cartwheel splitting/shedding with additional data is beneficial and important. Below are the texts and figure numbers in the manuscript that go with the new data:

“Notably, the shed cartwheel was frequently observed attached to the adjacent disengaging mother centriole at irregular positions or angles (Fig. 2F), a pattern contrasting with the typical

growth of new cartwheels, which consistently occurs at the sides of mother centrioles in orthogonal arrangements (refer to Fig. 2j & k below for further details)."

"Furthermore, by delineating the nascent, short procentriole tethered to the host centriole regarding its position and orientation (Fig. 2j & k), we observed that a considerable proportion of these procentrioles are either developing at atypical sites, such as beneath the proximal end of the host centriole (Fig. 2k, left), or are oriented at angles beyond the typical range established by wild-type centrioles (Fig. 2k, right), which aligns with the stochastic nature of cartwheel shedding-tethering during centriole disengagement."

Response to 1a:

(1). The mechanism underlying the interaction between the cartwheel and the centriole lumen has not been carefully characterized; it remains largely unclear about (i) how static, dynamic, or labile the interaction is, (ii) whether the interaction is under additional regulations, and (iii) whether the interaction is sufficient to maintain the cartwheel in the lumen without other factors like the CS. **We thus do not feel comfortable discussing or invoking this little-known interaction in the current study. Additional thoughts are provided below in point (5).**

(2). The existing literature, including that provided by the reviewer, suggests that at least in human cells the cartwheel is a relatively dynamic structure (it continues to lose and recruit SAS-6 throughout interphase), as compared to the centriole backbone.

(3). The similar centriole triplet seen in CDK1-compromised *Drosophila* tissues supports the idea that the phenotype is specific to partial CDK1 inhibition. **We have revised the sentence in the manuscript to make it clear.**

(4) For cartwheel shedding, we have made it clear that it is a phenotype seen specifically under ALMS1 mutations or partial CDK1 inhibition. The shedding behavior per se, however, is consistent with the above notion that the cartwheel is a relatively dynamic structure.

(5). Additional thoughts to share with the reviewer and editor (not included in the manuscript):

- Our study shows that CS assembly directly drives cartwheel assembly, not vice versa, and that CS disassembly timely precedes or correlates with cartwheel disassembly. This raises an idea that, perhaps upon CS disassembly at G2/M, the cartwheel grown by the CS in S phase becomes less stable and cannot be maintained properly in the lumen, leading to cartwheel loss in mitosis.
- Unlike human cells, in some organisms like worms, flies, and unicellular species, a complete removal of the embedded cartwheel from the centriole lumen does not occur in mitosis. Instead, at least in some cases (flies), the new cartwheel synthesis starts in anaphase of mitosis.
- Interestingly, however, a 50% reduction of the embedded cartwheel after its assembly in S phase was reported in early worm embryos that lack a G2 phase.

Similarly, dynamic fluctuation of the cartwheel length (the height of the stack) within the centriole lumen also occurs in some unicellular species.

- It is therefore complicated to make a direct comparison to human cells.
- All these suggest that, at least in human cells, and perhaps in other species as well, the connection between the cartwheel and centriole is not static/rigid but under regulation such that cartwheel dynamics, including cartwheel expansion, splitting, reduction, and removal, can occur naturally in different species.

In summary, my main original criticism was that the authors were proposing a radically new idea based largely on studies of cells in very non-physiological conditions, but they never make this clear to readers (and they phrase things in a way that often obscures this point). It would have been great if they had been able to get stronger data to support these conclusions (e.g., observing splitting/shedding in unperturbed cells) but I appreciated that this was likely to be difficult, so all I really asked was that they did a better job of highlighting the potential caveats and alternative explanations. Unfortunately, I have read the revised manuscript and I can't see that they have done either of these things. Thus, I remain of the opinion that this data shouldn't be published in this form as it will be too misleading to readers who are not experts in the field. This is a shame, as there are some interesting observations and stimulating ideas presented here.

Response: We have carefully checked and ensured that the experimental conditions for all our results are correctly stated. **For cartwheel splitting/shedding or its related discussion and notion, we make sure that it always comes with the stated condition where the phenotype was observed or discussed (i.e., under ALMS1 mutations or partial CDK1 inhibition).**

Please note that cartwheel shedding observed here under disease and non-physiological conditions is not merely a negative, loss-of-function phenotype; rather, it causes a positive, gain-of-function outcome where centrioles and cartwheels robustly enforce each other's assembly. We highlighted this striking mechanism in the discussion because of its causal power for centriole formation without invoking a single initiating cause, as compared to a near-complete lack of understanding of how the initial order emerges from disorder during the normal/WT centriole assembly. It is possible that a similar cartwheel-shedding mechanism is preserved in some lineages of life for centriole biogenesis according to their specific biological or evolutionary needs—but such a mechanism is clearly not compatible with the lifestyle of vertebrates and thus suppressed in WT cells (as shown in our results). **We have revised the relevant part of the discussion to reflect this point.**

Reviewer #2 (Remarks to the Author):

The authors have performed a remarkable rewrite of the manuscript and addressed my concerns. I think this paper will be an important addition to the literature.

We thank the reviewer for the positive remarks.

Reviewer #1 (Remarks to the Author):

I appreciate the authors efforts, but still have significant concerns that the mechanisms they propose here are unlikely to explain how canonical centriole duplication occurs in normal cells, and that they do not adequately convey to readers some of the caveats to the interpretation of their data. Nevertheless, I can see that the Editors and other Reviewers do not share these concerns, and I do not want to stand in the way of publishing what will no doubt be a provocative story that will hopefully stimulate debate and future work. I am therefore happy for this work to be published.

Response: We thank the reviewer for the comment and positive remarks. As the comments lack specific details, we are unable to address them constructively. Using centriole biogenesis as the system, our results shed light on the principle underlying the emergence of order (macroscopic objects) from disorder (microscopic parts)—the defining hallmark of our physical world. More studies are certainly needed to fully understand the impact of our work on emergent phenomena, including but not limited to centriole duplication.