# In vivo imaging reveals independent intraflagellar transport of the nexin-dynein regulatory complex subunits DRC2 and DRC4

Sahana Saravanan, Douglas Trischler, Raqual Bower, Mary Porter, and Karl Lechtreck

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## Review Timeline:

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| Submission Date: | 2022-11-20 |
| Editorial Decision: | 2022-12-09 |
| Revision Received: | 2022-12-18 |
| Accepted: | 2022-12-19 |

*Editor-in-Chief: Matthew Welch*

## 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.)

RE: Manuscript #E22-11-0524
TITLE: "In vivo imaging reveals independent intraflagellar transport of the nexin-dynein regulatory complex subunits DRC2 and DRC4"

Dear Karl,
Your paper has now been reviewed by two experts in the field, who both found it of great interest and recommend it be published essentially as is. In the interests of due diligence I read it too and I find myself in complete agreement - this is another tour de force paper from your lab! I do think it would make sense to fix the typos that were pointed out, at your discretion, which should be very easy, and if you can send back a revised version, I will accept it with no need for further review. Thank you for sending this interesting work to MBoC!

Sincerely,
Wallace Marshall
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Lechtreck,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

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Reviewer #1 (Remarks to the Author):

Cilia are built at their distal tip and many components require the intraflagellar transport (IFT) system for ciliary entry and movement to the site of assembly. Some axonemal structures such as the outer dynein arms, inner arm I1/f and radial spokes, require the IFT system for axonemal assembly, and are attached to IFT particles via specific adaptor molecules. An important question is whether this paradigm applies to all axonemal substructure. The nexin-dynein regulatory complex (N-DRC) is an 11 subunit complex that links doublet microtubules, contacts both inner and outer dynein arms and the radial spokes and is involved in regulating ciliary beating. This complex consists of a base plate attached to the A-tubule of one doublet formed from a DRC1/2 heterodimer and a DRC4 homodimer with other components attached more distally. In this paper, the authors use mutants lacking various DRC components combined with expression of GFP/mCherry-tagged versions to follow the transport and assembly of the key DRC2 and DRC4 components by TIRF microscopy. They find that these proteins are, in most cases, transported independently of each other on distinct IFT particle trains. These data suggest that the N-DRC is mostly assembled in a step-wise fashion in the growing cilia from individual components rather than being pre-assembled in cytoplasm. However, they do occasionally observe co-transport at a rate several times greater than would be expected by chance suggesting that some (small?) fraction of DRC2/4 coassemble in cytoplasm prior to transport. They also observe that assembly of DRC4 into the distal region of the axoneme depends on DRC2 but that assembly in the proximal region does not. This is a fascinating observation although the mechanism remains undetermined. I wondered if the authors might add comment on how they envisage this might occur and/or if this implies differences in the N-DRC along the axonemal length. In total, this work reveals a distinct mechanism for the formation of this axonemal substructure and further amplifies the complexity and variability of IFT-driven processes. The manuscript is well written and clear. The data are compelling and well presented. I do not have any major suggestions for improvement.

There are a few typos:
p.4 l.7 - "mutants" should be singular
p.4 l.11 - should read "... the more peripheral..."
p.8 l.2 - delete "was"
p.10 l.1 - "indicated" what?
p.10 l.6 - delete "add to table" - this strain is in the table.

Reviewer #2 (Remarks to the Author):

Saravanan et al. expand the previous analyses of the mechanism of transport of microtubule-associated complexes in eukaryotic flagella by TIRF analysis of fluorescently tagged N-DRC proteins. Unlike three other flagellar complexes (radial spokes and two axonemal dyneins) that pre-form in the cytoplasm and are transported as a unit through interaction with IFT adaptors, these DRC proteins appear to travel primarily on separate IFT complexes, not as complexes. This has relevance to the still quite poorly understood mechanisms of cargo recognition by the IFT system.

Tagged transgenes for DRC2 and DRC4 were expressed in mutant backgrounds, and the data support an ability of both tagged products to function similarly to the wild type gene products and rescue DRC assembly and flagellar motility (and, in the case of double mutants expressing both proteins, flagellar assembly). Expression levels in whole cells were not tested, but levels expressed in flagella were adequate to support the observed motility changes, and overexpression has not been commonly observed in this organism and is thus unlikely to be a factor in the slightly sub-par rescues that were observed.

Quantitative analysis of a large number of transport events provided excellent support for the overall conclusion that DRC2 and DRC4 infrequently transport together on the same IFT trains in pseudo-wild type cells, and that each can still be transported in the mutation-induced absence of the other.

The manuscript contained several typographic errors that should be easily fixed:
The (Choksi et al., 2014) reference (p. 4) is extraneous and should be deleted.
The words "attempt to" appear duplicated (p. 7).
The description of variable expression levels within the population is described (p. 8) as "the signal strength was fluctuated between cells". This terminology is confusing and inaccurate, as fluctuation generally refers to a change over time.
On p. 8, line 3, "DRC4-mS" should be "DRC4-mC". On p. 9, line 17, a hyphen is missing in "RSP4-sfGFP".
Subheading on the first line of p. 10 is incomplete.
(Bower et l., 2018) reference on p. 11, line 17 is duplicated.
On p. 16, two parentheses are left open in the description of antibodies.

RE: Manuscript #E22-11-0524R
TITLE: "In vivo imaging reveals independent intraflagellar transport of the nexin-dynein regulatory complex subunits DRC2 and DRC4"
Dear Karl,
With these new changes, I am happy to officially accept your manuscript for publication in MBoC. Thank you for sending us this interesting work!

best regards,

Wallace Marshall
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Lechtreck:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

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