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In vivo imaging shows continued association of several IFT-A, IFT-B and dynein complexes while IFT trains U-turn at the tip

Jenna L. Wingfield, Betlehem Mekonnen, Ilaria Mengoni, Peiwei Liu, Mareike Jordan, Dennis Diener, Gaia Pigino and Karl Lechtreck
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Original submission

First decision letter

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MS TITLE: In vivo imaging of IFT proteins in Chlamydomonas indicates continued association of multiple IFT-A, IFT-B and IFT dynein complexes during anterograde to retrograde conversion of trains at the tip

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ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers gave favourable reports but raised some critical points that will require amendments to your manuscript. I hope that you will be able to carry these out because I would like to be able to accept your paper, depending on further comments from reviewers.

Specifically, my reading of the comments indicates that no further experimental work is required but that there are key areas where the text and figures might need some attention. If you happen to have any suitable tomograms as suggested by the reviewer then, as I am sure you know already, these would enhance the manuscript. That said, I do not expect further work to be necessary if you do not have those data sets already.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

Wingfield et al. performed a detailed and quantitative analysis of the dynamics of the IFT train in Chlamydomonas flagella using sophisticated live imaging and FRAP techniques.
It has been widely believed that the IFT train is disassembled at the tip of the flagellum reconstructed, and then retrogradely transported. In fact, in many review articles, IFT-A IFT-B, kinesin, and dynein are illustrated as disassembled and then reassembled into a complex at the tip of flagella or cilia. However, the authors' conclusion that the IFT train is converted to retrograde transport without disassembly is an important finding that may rewrite the conventional model of the IFT train turnaround. Furthermore, by using cryo-electron microscopy, the authors found that the structure of the fluorescent protein-fused IFT train is slightly distorted. This structural distortion is thought to weaken the interaction between the IFT complex and dynein. These results call to the attention of researchers who use fluorescent proteins for imaging the IFT train.

Overall, the experiments were carefully performed, and the quality of the data is high. The interpretation of the data also seems reasonable. Therefore, I think that this paper is very much worth publishing, although some minor corrections are required.

Comments for the author

Major comments
1. Have you ever observed the tip of a Chlamydomonas flagellum with a cryo-EM? Can you see the IFT train that is turning around? If such an electron micrograph is available conclusive evidence will support the authors' model.

2. There are previous studies that phosphorylation regulates the turnaround of the IFT train, but there is no mention of phosphorylation in this manuscript. The authors may think that phosphorylation is not necessary, but it would be nice to add a discussion on the role of phosphorylation in the turnaround of the IFT train. e.g., Liang, Y., Pang, Y., Wu, Q., Hu, Z., Han, X., Xu, Y., Deng, H., and Pan, J. (2014) FLA8/KIF3B Phosphorylation Regulates Kinesin-II Interaction with IFT-B to Control IFT Entry and Turnaround. Dev. Cell. 30, 585–597 Chaya, T., and Furukawa, T. (2021) Post-translational modification enzymes as key regulators of ciliary protein trafficking. J. Biochem. 10.1093/jb/mvab024

3. In this study, the authors showed that expression of IFT subunits fused with fluorescent proteins changes the structure of the IFT train and weakens its binding to dynein. If there is a problem with the use of fluorescent proteins, what live imaging alternatives can be considered?

Minor comments
1. It is generally known that the velocity of kinesin and dynein is temperature-dependent.
It would be better to cite previous studies or reviews. e.g., Yadav, S., and Kunwar, A. (2021) Temperature-Dependent Activity of Motor Proteins: Energetics and Their Implications for Collective Behavior. Front Cell Dev Biol. 9 610899

2. It is known that red fluorescent proteins such as DsRed and mCherry become two fragments by boiling, so the degraded band of mSc-IFT54 in Fig. 4A is probably due to the fragmentation of mSc when the protein is boiled for SDS-PAGE.
Gross, L. A., Baird, G. S., Hoffman, R. C., Baldridge, K. K., and Tsien, R. Y. (2000) The structure of the chromophore within DsRed, a red fluorescent protein from coral. Proc.
Natl. Acad. Sci. U. S. A. 97, 11990–11995

3. In Fig.6A, it is labeled as ift74-2 mSc-IFT54, but ift74-2 mSc-IFT74 is correct.

4. Some of the citations are not formatted correctly. Please correct them: Line 212-213 419-420, and 489.

Reviewer 2

Advance summary and potential significance to field

In this manuscript, Wingfield et al. use advanced live cell imaging approaches (TIRF, FRAP etc.) to investigate the dynamics of flagellar tip turn around of individual IFT system components in Chlamydomonas. Previous studies performed in Chlamydomonas as well as other organisms (e.g. C. elegans and trypanosomes) have led to the commonly accepted conception that IFT-A and IFT-B sub-complexes as well as kinesin-2 and dynein 2 motors dissociate from each other at the ciliary tip after their tip arrival in anterograde IFT trains, but this conception is not fully compatible with recent structural work from e.g. the Pigino lab (Jordan et al. 2018).

Here, the authors provide evidence indicating that IFT-A, IFT-B and dynein 2 from a specific anterograde train usually depart from the cilia tip in the same retrograde trains, indicating that upon conversion from (longer) anterograde IFT trains to (shorter) retrograde trains at the cilia tip, these components largely remain associated with each other, suggesting a direct conversion of anterograde to retrograde trains at the ciliary tip without major disassembly except for fragmentation of longer trains into shorter ones.

Overall the manuscript is well written and the quality of the imaging is data impressive (disclaimer: this reviewer is not an expert in the advanced imaging approaches used). The results should be of interest to the JCS readership, in particular people interested in cilia and IFT, as well as microtubule-based transport in general. I only have a few rather minor comments/suggestions to the authors that should be addressed before acceptance for publication.

Comments for the author

Main comments:
1) The authors show similar cilia tip dwell times for all IFT proteins, with no major differences between IFT-A and IFT-B components. As described on page 4, this is different from what was reported for C. elegans by Mijalkovic et al. 2018, where the authors show that IFT-B component OSM-6 appears to pause longer at the tip than IFT-A component CHE-11. Can the authors comment on this apparent difference between Chlamydomonas and C. elegans tip dwelling times for IFT – A/IFT-B proteins?

2) Table 1 and Lines 117-118: the authors state that all FP-expressing strains were expressed to rescue the corresponding mutants, but Table 1 only does not include rescue data for the new strains generated in this study. Please refer to correct section of the manuscript (Methods?) for relevant rescue data.

3) Figure 2D: are the observed differences statistically significant?

Minor comments:
Figure 2D: the two columns in magenta are almost of similar tone and the color code for mNG-IFT81 looks darker than both.
Figure 3G: text on x-axis is cropped/missing.
Line 52: Walther et al. 1994 and Kozminski et al. 1995 should also be cited here.
Line 58: Porter et al. 1999 and Pazour et al. 1999 should also be cited here.
Line 899 and 901: the same refernec (Pedersen et al. 2006) is listed twice.
Lines 106 and 310: the word “with” seems to be missing in the sentence.
Lines 212-213, 419-420, 489: references are not formatted correctly.

First revision

Author response to reviewers' comments

Reviewer 1 Advance summary and potential significance to field
Wingfield et al. performed a detailed and quantitative analysis of the dynamics of the IFT train in Chlamydomonas flagella using sophisticated live imaging and FRAP techniques. It has been widely believed that the IFT train is disassembled at the tip of the flagellum, reconstructed, and then retrogradely transported. In fact, in many review articles, IFT-A, IFT-B, kinesin, and dynein are illustrated as disassembled and then reassembled into a complex at the tip of flagella or cilia. However, the authors' conclusion that the IFT train is converted to retrograde transport without disassembly is an important finding that may rewrite the conventional model of the IFT train turnaround. Furthermore, by using cryo-electron microscopy, the authors found that the structure of the fluorescent protein-fused IFT train is slightly distorted. This structural distortion is thought to weaken the interaction between the IFT complex and dynein. These results call to the attention of researchers who use fluorescent proteins for imaging the IFT train.
Overall, the experiments were carefully performed, and the quality of the data is high. The interpretation of the data also seems reasonable. Therefore, I think that this paper is very much worth publishing, although some minor corrections are required.
We very much appreciate the positive comments of the reviewer.

Reviewer 1 Comments for the author
Major comments
1. Have you ever observed the tip of a Chlamydomonas flagellum with a cryo-EM? Can you see the IFT train that is turning around? If such an electron micrograph is available, conclusive evidence will support the authors' model.

R: A micrograph of an IFT train at the flagellar tip is shown in Nievergelt et al. (Fig. 3C, D. bioRxivs). The train in that tomographic slice possesses the typical high-defined structure of anterograde trains in its proximal region but loses this a structure in its distal region, where it has lost contact to the doublet microtubule. Whether that off-track distal region of the train assumes the configuration typical for retrograde trains remains unclear.

We now included these observations in the discussion:
“Such trains have been described by standard TEM (Dentler, 2005; Pedersen et al., 2006) and, in tomographic analyses, such trains appear to lose the highly defined structure of anterograde trains in their distal regions, which have lost contact to the underlying doublet microtubules (Jordan et al., 2018; Nievergelt et al., 2021).”

2. There are previous studies that phosphorylation regulates the turnaround of the IFT train, but there is no mention of phosphorylation in this manuscript. The authors may think that phosphorylation is not necessary, but it would be nice to add a discussion on the role of phosphorylation in the turnaround of the IFT train.
e.g., Liang, Y., Pang, Y., Wu, Q., Hu, Z., Han, X., Xu, Y., Deng, H., and Pan, J. (2014) FLA8/KIF3B Phosphorylation Regulates Kinesin-II Interaction with IFT-B to Control IFT Entry and Turnaround. Dev. Cell. 30, 585–597 Chaya, T., and Furukawa, T. (2021) Post-translational modification enzymes as key regulators of ciliary protein trafficking. J. Biochem. 10.1093/jb/mvab024

R: We agree that these studies should have been discussed and we included them into the revised manuscript. However, we kept the discussion of kinesin-2 phosphorylation brief as we do not analyze kinesin-2 and its phosphorylation in this study.

The flowing phrases were modified or added:
Introduction: The release of kinesin-2 at the tip likely involves its phosphorylation by a calcium-dependent kinase (Liang et al., 2014).

Discussion: Previous studies established that kinesin-2 or at least its KAP subunit is released from IFT at the tip, a process which possibly involves phosphorylation of its KIF3B/FLA8 subunit (Engel et al., 2009; Liang et al., 2014).

Discussion: This suggests that the specific structural and biochemical environment of the flagellar tip region (Chaya and Furukawa, 2021; Louka et al., 2018) are not required for train conversion.

3. In this study, the authors showed that expression of IFT subunits fused with fluorescent proteins changes the structure of the IFT train and weakens its binding to dynein. If there is a problem with the use of fluorescent proteins, what live imaging alternatives can be considered?

R: The following was added to the discussion:
Placement of fluorescent proteins tags informed by protein and train structure, longer linkers between the fluorescent tag and the target protein, and smaller tags such as the cysteine-tag in combination with a fluorescent payload or the split GFP technique are alternatives approaches, which could reduce tag-induced anomalies of IFT (Backer et al., 2007; Kamiyama et al., 2016).

Minor comments
1. It is generally known that the velocity of kinesin and dynein is temperature-dependent. It would be better to cite previous studies or reviews.
e.g., Yadav, S., and Kunwar, A. (2021) Temperature-Dependent Activity of Motor Proteins: Energetics and Their Implications for Collective Behavior. Front Cell Dev Biol. 9, 610899

R: We agree that our observation on temperature-dependency of IFT is not at all surprising. Nevertheless, we briefly reported these data using a supplementary figure to explain the variations we observed in train velocity and the speed of turnaround at the tip between the individual single tagged strains. We modified the corresponding part of the results by adding (p8):

“These observations were expected since the temperature dependency of motor protein activity and cellular transports are well documented (Yadav and Kunwar, 2021).”

2. It is known that red fluorescent proteins such as DsRed and mCherry become two fragments by boiling, so the degraded band of mSc-IFT54 in Fig. 4A is probably due to the fragmentation of mSc when the protein is boiled for SDS-PAGE.
Gross, L. A., Baird, G. S., Hoffman, R. C., Baldridge, K. K., and Tsien, R. Y. (2000) The structure of the chromophore within DsRed, a red fluorescent protein from coral. Proc. Natl. Acad. Sci. U. S. A. 97, 11990–11995

R: We appreciate the expertise advice of the reviewer. I learned something new. The possibility that the observed fragmentation of tagged proteins was caused by boiling has been added to the Materials and methods-section and to the figure legend on p. 8.

Figure legend:
Anti-IFT54 stained endogenous IFT54 in wild type; in the experimental strain, this antibody stained mSc-IFT54, the unprocessed BLE-mSc-IFT54, a likely mSc-IFT54 degradation product (?), which could have formed during boiling of the samples (Gross et al., 2000), and ift54, the non-functional truncated product of the ift54-2 mutant.

M&M:
As previously described, cleavage of the 2A sequence is not always complete, resulting in two or more bands of the tagged protein in western blots (Lechtreck et al., 2018). Further, boiling of red fluorescence protein SDS-samples can lead to fragmentation and the formation of additional bands (Gross et al., 2000).

3. In Fig.6A, it is labeled as ift74-2 mSc-IFT54, but ift74-2 mSc-IFT74 is correct.
R: Good catch. The mistake has been corrected.

4. Some of the citations are not formatted correctly. Please correct them: Line 212-213,

419-420, and 489.
R: The references are now correctly formatted.

Reviewer 2 Advance summary and potential significance to field
In this manuscript, Wingfield et al. use advanced live cell imaging approaches (TIRF, FRAP etc.) to investigate the dynamics of flagellar tip turn around of individual IFT system components in Chlamydomonas. Previous studies performed in Chlamydomonas as well as other organisms (e.g. C. elegans and trypanosomes) have led to the commonly accepted conception that IFT-A and IFT-B sub-complexes as well as kinesin-2 and dynein 2 motors dissociate from each other at the ciliary tip after their tip arrival in anterograde IFT trains, but this conception is not fully compatible with recent structural work from e.g. the Pigino lab (Jordan et al. 2018).
Here, the authors provide evidence indicating that IFT-A, IFT-B and dynein 2 from a specific anterograde train usually depart from the cilia tip in the same retrograde trains, indicating that upon conversion from (longer) anterograde IFT trains to (shorter) retrograde trains at the cilia tip, these components largely remain associated with each other, suggesting a direct conversion of anterograde to retrograde trains at the ciliary tip without major disassembly except for fragmentation of longer trains into shorter ones.
Overall the manuscript is well written and the quality of the imaging is data impressive (disclaimer: this reviewer is not an expert in the advanced imaging approaches used). The results should be of interest to the JCS readership, in particular people interested in cilia and IFT, as well as microtubule-based transport in general. I only have a few rather minor comments/suggestions to the authors that should be addressed before acceptance for publication.
We thank the referee for this positive assessment of our work.

Reviewer 2 Comments for the author

Main comments:

1) The authors show similar cilia tip dwell times for all IFT proteins, with no major differences between IFT-A and IFT-B components. As described on page 4, this is different from what was reported for C. elegans by Mijalkovic et al. 2018, where the authors show that IFT-B component OSM-6 appears to pause longer at the tip than IFT-A component CHE-11. Can the authors comment on this apparent difference between Chlamydomonas and C. elegans tip dwelling times for IFT – A/IFT-B proteins?

R: We agree that the observation in C. elegans, which support a different model, deserve more attention. In the revised discussion, we describe and discuss the differences in tip turn around and other aspects of IFT between Chlamydomonas and C. elegans.

“In C. elegans, IFT dynein, the anterograde motor OSM-3 motor, and IFT-A turnaround almost instantaneously after arriving at the tip whereas the IFT-B protein OSM-6 pauses considerably longer tentatively indicating that the IFT-B complex dissociates from the other IFT complexes and then re-associated with material derived from later trains (Mijalkovic et al., 2017). These observations add to the already known differences in IFT between C. elegans and Chlamydomonas: In the former, anterograde IFT uses two distinct anterograde motors, the BBSome participates in IFT train assembly, stabilization and tip turnaround, and the kinesin-2 motors move by retrograde IFT; retrograde IFT of kinesin-2 was also observed in mammalian cilia (Ou et al., 2005; Wei et al., 2012; Williams et al., 2014).”

The C. elegans studies are based on single color imaging of a single marker for the IFT-B complex. In Chlamydomonas, several IFT-B proteins have been analyzed by several laboratories with essentially identical outcomes (i.e., dwell period of ~2 s). Similar to Chlamydomonas, GFP tagging and high laser intensities have the intrinsic risk to affect IFT. In the 2018 paper from the Peterman lab (Oswald et al. Cell Reports), they write: “We cannot, however, exclude that the prolonged, relatively high intensity illumination required for single-molecule detection somewhat alters IFT-train dynamics.”

2) Table 1 and Lines 117-118: the authors state that all FP-expressing strains were expressed to rescue

the corresponding mutants, but Table 1 only does not include rescue data for the new strains generated in this study. Please refer to correct section of the manuscript (Methods?) for relevant rescue data.

R: Indeed, the table does not provide documentation about the rescue phenotypes and it was cited here to provide the reader with an easy overview of the strains used.
We reformulated the phrase in question and added more information to the material and methods to make it clear that expression of the FP-tagged IFT proteins restores the ability to assemble flagella to the flagella-less parental mutant strains. We also refer to Fig. S5 showing the IFT data for most of the rescue strains. The presence of flagella in the IFT-FP recue strains is well documented in the figures.

Lines 117-118: "; expression of the proteins restored IFT and flagellar assembly in the corresponding mutants (Fig. S5E, Table S1, and Material and Methods)."

AND

Materials and methods: The bald ift81-1 and ift74-2 mutants have been previously described (Brown et al., 2015; Kubo et al., 2016). ift81-1 was rescued as assessed by the restoration of flagellar assembly and IFT using a construct consisting of the aphVIII selectable marker gene and the genomic region of IFT81 fused at its N-terminus to mNeonGreen codon-adapted for C. reinhardtii.

3) Figure 2D: are the observed differences statistically significant?
R: A statistical analysis of the data using t-test was performed and the data were included into the revised figure and are described in the revised figure legend.

Minor comments:
Figure 2D: the two columns in magenta are almost of similar tone and the color code for mNG-IFT81 looks darker than both.
R: The color scheme was altered to increase clarity.

Figure 3G: text on x-axis is cropped/missing.
R: The parts were only cropped in the combined word/pdf file used for submission. We apologize for this oversight.

Line 52: Walther et al. 1994 and Kozminski et al. 1995 should also be cited here.
R: The references were added as suggested.

Line 58: Porter et al. 1999 and Pazour et al. 1999 should also be cited here.
R: Done.

Line 899 and 901: the same refernec (Pedersen et al. 2006) is listed twice.
R: Unfortunately, not all references were properly formatted (as more than one Endnote library was used during the preparation of the manuscript). The problem was corrected.

Lines 106 and 310: the word "with" seems to be missing in the sentence.
R: We read the paragraphs at the indicated positions and could not find the mistake?

Lines 212-213, 419-420, 489: references are not formatted correctly.
R: See above.

The requested revisions pushed the word count over the 8,000 word journal limit and we condensed the text somewhat in various places to ensure that the manuscript has less than 8,000 words. Several smaller edits, corrections of typos etc. were made throughout the manuscript.

Second decision letter

MS ID#: JOCES/2021/259010

MS TITLE: In vivo imaging shows continued association of several IFT A, B and dynein complexes while IFT trains U-turn at the tip

AUTHORS: Jenna L Wingfield, Betlehem T Mekonnen, Ilaria Mengoni, Peiwei Liu, Mareike Jordan, Dennis R Diener, Gaia Pigino, and Karl F. Lechtreck
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks. Thank you for your careful revisions to the manuscript. I did not consider it necessary to return this to the reviewers and so no new reports are available.