Cooperation of the IFT-A complex with the IFT-B complex is required for ciliary retrograde protein trafficking and GPCR import

Takuya Kobayashi, Yamato Ishida, Tomoaki Hirano, Yohei Katoh, and Kazuhisa Nakayama

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(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 #E20-08-0556
TITLE: "Cooperation of the IFT-A complex with the IFT-B complex is required for ciliary retrograde protein trafficking and GPCR import"

Dear Dr. Nakayama:

Your manuscript has now been seen by two referees, whose comments are attached below. Both reviewers recommend the publication of the manuscript after minor revisions. I think that the manuscript should be accepted after minor revisions. Please send the revised manuscript via the web site.

Sincerely,
Kozo Kaibuchi
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Nakayama,

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.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

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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

Reviewer #1 (Remarks to the Author):

Using VIP assays and confirmations by co-IP, the authors map the IFT-A/IFT-B interaction to the IFT88/52 and IFT122/144 subcomplexes and go on to generate a truncation of IFT88 lacking the last 28aa that is defective in binding the IFT-A complex.

Introduction of this IFT88 mutant defective for IFT-A binding into Ift88 knockout cells results in:
-1/3rd shorter cilia
-2x decreased ciliation
-5x decrease in ciliary ARL13B levels
-very little entry of IFT-A into cilia
-No entry of GPR161 or SSTR3 into cilia
-accumulation of IFT-B in bulges at the tip -this is the most dramatic effect.

The last 3 phenotypes are also highly penetrant in IFT144-KO cells, further substantiating the conclusion that the IFT88ΔC28 is defective in IFT-A binding.

All in all, this is an outstanding study. The logic is sound throughout, the experimental quality is exceptional and the results are important. Must accept pending very minor modifications.

P.3 last line 'In addition to these complexes, the BBSome composed of eight BBS proteins participates in the export of ciliary membrane proteins, including GPCRs, by connecting GPCRs to IFT particles (Klink et al., 2017; Liu and Lechtreck, 2018; Nozaki et al., 2018; Ye et al., 2018; Nozaki et al., 2019).

The Klink citation does not fit, should be deleted.

P.4, second sentence of the second paragraph. 'Subsequently, these two complexes were shown to be coimmunoprecipitated from a Chlamydomonas flagellar fraction'. This statement is incomplete. Under most conditions, the IFT-A and IFT-B complexes do not associate but each migrates as a 16S complex on sucrose gradients. In the quoted paper, the IFT-A complex can be detected inIFT172 and IFT52 immunoprecipitates but these IP are performed in 25mM KCl and results should be viewed with caution.

Fig. 1E: the blots appear oddly saturated. A less aggressive gamma curve adjustment is recommended.

The conclusion that IFT46 participates in the binding of IFT-B to IFT-A is not well supported. It appears that the levels of IFT88 and IFT52 are elevated when these proteins are co-expressed with IFT46 (last lane of the right blot in Fig. 1G). The increased signal in the VIP assay when IFT88/52/46 are co-expressed compared to only 52 and 88 may thus be due to the stabilization of 88/52 by 46. It may be best to remove the dashed line between IFT144/122 and IFT46 in Fig. 1H.

P.8:' mChe-IFT88(Δα)-positive signals moving along the cilium did not look like particles (Video S3).' Examination of the video shows a very short shaft where any particle movement would be hard to discern. Video S4 is a bit clearer but still needs qualifier before using in support of the author's statement. To this reviewer, it appears that mChe-IFT88(Δα) cilia harbor a considerable amount of free IFT-B that masks any movement of IFT-B trains.

Reviewer #2 (Remarks to the Author):

Kobayashi, T., et al.

Nakayama's group have demonstrated that the binding between IFT-A and IFT-B complexes is critical for the interaction between IFT-144-IFT122 (from IFT-A) and IFT88-IFT52-IFT46 (from IFT-B). The authors also found that C-terminal amino acids on IFT-88 are important not only for the above complex formation but also for retrograde trafficking from the ciliary tip and the entry of GPCRs into cilia. This work is well designed and carefully carried out.

In general, one of important factors in protein expression experiments is the expression level of each protein in the cells, which may affect experimental results. The authors should address this issue especially in Fig. 4; in Fig.1, A-C if possible. In addition, some immunoblot bands appears to be saturated in Fig. 1E. The short exposure images should be also shown in supplemental figures if such data exist.

RE: Manuscript #E20-08-0556

TITLE: Cooperation of the IFT-A complex with the IFT-B complex is required for ciliary retrograde protein trafficking and GPCR import

Dear Dr. Kozo Kaibuchi, Monitoring Editor of MBoC,

Thank you very much for your valuable comments and those of the reviewers on our paper submitted to MBoC. I am sending herewith a revised version of #E20-08-0556. We have performed additional experiments and made alterations in the manuscript according to reviewers' comments.

I greatly appreciate your help concerning improvements of this paper. I hope that the revised manuscript is now acceptable for publication in MBoC.

Note that a new author, Yamato Ishida, has been added in the revised manuscript, because Yamato has performed the additional experiment.

Best regards,

Kazuhisa Nakayama, Ph.D.

Response to the comments of Reviewer #1:

P.3 last line 'In addition to these complexes, the BBSome composed of eight BBS proteins participates in the export of ciliary membrane proteins, including GPCRs, by connecting GPCRs to IFT particles (Klink et al., 2017; Liu and Lechtreck, 2018; Nozaki et al., 2018; Ye et al., 2018; Nozaki et al., 2019). The Klink citation does not fit, should be deleted.

We have removed Klink et al. (2017) in the revised manuscript.

P.4, second sentence of the second paragraph. 'Subsequently, these two complexes were shown to be coimmunoprecipitated from a Chlamydomonas flagellar fraction'. This statement is incomplete. Under most conditions, the IFT-A and IFT-B complexes do not associate but each migrates as a 16S complex on sucrose gradients. In the quoted paper, the IFT-A complex can be detected in IFT72 and IFT52 immunoprecipitates but these IP are performed in 25mM KCl and results should be viewed with caution.

We have changed the sentence pointed by the reviewer as follows.
' A subsequent study confirmed that IFT-A proteins can be coimmunoprecipitated with IFT-B proteins from a *Chlamydomonas* flagellar fraction under certain conditions (Qin et al., 2004).'

Fig. 1E: the blots appear oddly saturated. A less aggressive gamma curve adjustment is recommended.

For an unknown reason, the quality of images shown in Fig. 1 of the unrevised manuscript was considerably degraded upon conversion of the original jpg file to pdf. We therefore think that the quality of the original images in the Fig. 1 jpg file is good. In particular, the saturation of the immunoblot bands in Fig. 1E is eliminated (also see the response to the reviewer #2 comment).

The conclusion that IFT46 participates in the binding of IFT-B to IFT-A is not well supported. It appears that the levels of IFT88 and IFT52 are elevated when these proteins are co-expressed with IFT46 (last lane of the right blot in Fig. 1G). The increased signal in the VIP assay when IFT88/52/46 are co-expressed compared to only 52 and 88 may thus be due to the stabilization of 88/52 by 46. It may be best to remove the dashed line between IFT144/122 and IFT46 in Fig. 1H.

According to the reviewer's comment, we have removed the dashed line between IFT144/122 and IFT46 in revised Fig. 1H, and made following alterations in the text.
Page 2, Abstract, line 6: '–IFT46' has been removed.
Page 4, Introduction, line 3 from the bottom: '–IFT46' has been removed.

Page 5: The bottom sentence has been changed to 'These results indicate that the IFT88–IFT52 from IFT-B, and IFT144–IFT122 from IFT-A make major contributions to the IFT-A–IFT-B interface (Fig. 1H), and that IFT46 may play an auxiliary role in the IFT-A–IFT-B interaction.'

P.8:' mChe-IFT88(Δα)-positive signals moving along the cilium did not look like particles (Video S3).' Examination of the video shows a very short shaft where any particle movement would be hard to discern. Video S4 is a bit clearer but still needs qualifier before using in support of the author's statement. To this reviewer, it appears that mChe-IFT88(Δα) cilia harbor a considerable amount of free IFT-B that masks any movement of IFT-B trains.

Using our own TIRF microscope, we could not have taken better quality videos. We have therefore toned down the corresponding statements as follows.
Page 8, line 18: 'Furthermore, mChe-IFT88(Δα)-positive signals moving along the cilium (Video S3) appeared to be different from distinct particle-like signals of mChe-IFT88(WT) expressed in control RPE1 and *IFT88*-KO cells (Videos S1 and S2), and rather resembled those of EGFP-IFT88(WT) expressed in *IFT144*-KO cells (Video S4), in which the IFT-A complex cannot be assembled nor loaded onto the anterograde trains due to the absence of the essential core subunit, IFT144 (Hirano et al., 2017).'

Response to the comments of Reviewer #2:

In general, one of important factors in protein expression experiments is the expression level of each protein in the cells, which may affect experimental results. The authors should address this issue especially in Fig. 4; in Fig.1, A-C if possible. In addition, some immunoblot bands appears to be saturated in Fig. 1E. The short exposure images should be also shown in supplemental figures if such data exist.

Response to the comment 'The authors should address this issue especially in Fig. 4':
As pointed by the reviewer, the expression levels of certain proteins in individual cells are variable. However, we confirmed the fluorescence signals of mChe-fused IFT88 constructs in individual cells (Fig. 4, B–E, H, I, J, and K), and subjected only the cells with confirmed protein expression to statistical analysis. We believe that the search for statistically significant differences (Fig. 4, F, G, L, and M) is based on the inclusion of such variations in expression levels. Therefore, we have not performed additional experiments in response to this comment.

Response to the comment 'in Fig.1, A-C if possible. In addition, some immunoblot bands appears to be saturated in Fig. 1E':

For an unknown reason, the quality of images shown in Fig. 1 of the unrevised manuscript was considerably degraded upon conversion of the original jpg file to pdf. We therefore think that the quality of the original images in the Fig. 1 jpg file is good. In particular, the saturation of the immunoblot bands in Fig. 1E is eliminated. Even so, we have performed again the experiment shown in Fig. 1C because the upper left image was saturated. Therefore, Fig. 1 has been revised.

RE: Manuscript #E20-08-0556R
TITLE: "Cooperation of the IFT-A complex with the IFT-B complex is required for ciliary retrograde protein trafficking and GPCR import"

Dear Dr. Nakayama:

Thank you for sending us the re-revised manuscript. I have carefully read the new version and found that you almost fully revised the manuscript according to the reviewer's suggestion. I am pleased to accept your manuscript for publication in Mol Biol Cell.

Sincerely,
Kozo Kaibuchi
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Nakayama:

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.

Within approximately four weeks you will receive a PDF page proof of your article.

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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
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