Intraflagellar transport during assembly of flagella of different length in *Trypanosoma brucei* isolated from tsetse flies

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MS TITLE: Intraflagellar transport during the assembly of flagella of different length in Trypanosoma brucei isolated from tsetse flies

AUTHORS: Eloise Bertiaux, Adeline Mallet, Brice Rotureau, and Philippe Bastin
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.

Indeed the reviewers are enthusiastic about publication. The issues raised are minor and can be clarified by small changes to the text; I do not expect you to require further experiments. Please do ask if you require any further information.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. 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

Trypanosoma brucei has flagella with various lengths, depending on stages in the left cycle, but molecular mechanism to determine the length of flagella is not known. Bertiaux and her colleagues investigated this question, taking interdisciplinary approach, such as TEM, FIB-SEM, immunofluorescent microscopy, fluorescent tagging and FRAP.
They examined flagella from Trypanosoma in cardia by TEM and FIB-SEM and revealed the geometry of IFT, which is novel information. Then they conducted optical microscopy studies to reveal correlation between the amount of IFT and flagellar length. For microscopy, they used fluorescent antibodies against IFT172, IFT22 and FLAM8. They also genetically tagged IFT81. It was known that the total amount of IFT and flagellar length have positive correlation. However,
this study demonstrated that this correlation is almost perfect proportion and independent of the stage of the lift cycle, suggesting that the density and frequency of IFT is even and thus cannot be the factor to regulate the length.
They also discovered IFT accumulated at the flagella tip by fluorescent microscopy of flagella with genetically tagged IFT81. Regarding molecular mechanism of length determination, the authors started from the “grow and lock” model, which they proposed in a previous paper, interpreted their results and investigated cells in short epimastigote with defected function of cell division. Interestingly this cell grew even shorter flagella. Possible mechanism was discussed.

Comments for the author

This manuscript provides a number of novel findings and extends interesting discussion. It is a significant cornerstone in flagella research and will inspire scientists in this field. This reviewer strongly recommends publication of this manuscript after the following minor points are addressed.

Minor points:
p.4, Line7: “main means” is not clear.
Fig.S1A: needs a scale bar to say “the cardia is a large organ (p.12)”.
Fig.2AB: needs to be presented differently as a proof of “multiple parasites”.
Maybe indication (enclosing) of each parasite will be helpful for readers to identify them.
p.13, Line11 “by FIB (Fig.S2C)”: Fig.S2C does not exist. Probably typo.
Bottom of p.13: to correlate Fig.3B and Fig.4 to discuss about length of IFTs, it would be helpful to present Fig.3B data perpendicularly – making 3D map by stacking FIB-SEM images and show a computational section including the entire length of IFT.
Fig.4B and Fig.5B: Apparently fluorescent microscopy data from TdT::IFT81 has more error (Fig.5B) than that from antibody-labelled one (Fig.4B). Why?
p. 17, Line 23 “This is in contrast to..”: The meaning of this sentence is unclear. “were” -> “where”?
bottom of p.21: what does it mean by “interpretation is difficult … because IFT is construction machinery”?
p. 23 third line from the bottom: locked and therefore can therefore elongate -> locked and therefore can elongate p. 47 (caption of video S1): Figure 2 -> Figure 3 in Video S1: begining -> beginning

Reviewer 2

Advance summary and potential significance to field

In this study, Bertiaux et al. examined the IFT and its dynamics in T. brucei at different developmental stages in the tse-tse flies. The results showed a positive correlation between flagellar IFT abundance and length, in all life stages. The unicellular T. brucei is capable of producing flagella of different length in different developmental stages, making it a good model to

understand the mechanisms of flagellar length regulation. Such experiments with in vivo developed parasites are technically challenging, which contributes to the highly descriptive nature of this work. The observations suggested that flagellar IFT amount/dynamics is unlikely to have a major regulatory role in flagellum length. However, many other possibilities such as IFT activity, train size, cargo loading, cargo abundance, disassembly pathways etc were not investigated.

Comments for the author

1. The measurement of flagellar IFT.
T. brucei flagellum wraps along the cell body. It is thus difficult to perform accurate measurements of IFT fluorescence along the flagellum without the interference by cytoplasmic signal, in live or fixed cells. Perhaps the authors could explain in greater details how the measurements (as shown in Fig. 4B with near perfect correlation and Fig. 5B) was controlled, to rule out background or interference by cell body signals. Cells without IFT staining could be used as a control. Methanol fixation may remove some cytoplasmic and soluble IFT signals, and live cell measurements were not possible for the highly motile cells. Can TdTomato-IFT81 cells be fixed with PFA for the measurements of flagellar IFT in cells of all life stages?

2. On several occasions, it is stated that the total amount of IFT is correlated with flagellar length. Is it flagellar IFT or total cellular IFT that was measured? This need to be made clearer in the manuscript. Additionally, does the ROI include the concentrated spot of IFT at the flagellar base?

3. Page 24, shortening of existing flagellum was also observed in a trypanosomatid Leptomonas pyrrhocoris in a recent report (He et al., mBio 2019).

4. All live cell movies should have time stamp. Some additional labels of the base/tip of the flagella would help to orient the cells. For the FRAP movies please mark the ROI.

First revision

Author response to reviewers' comments

We are happy to read that reviewers enjoyed the manuscript and appreciated the difficulty to manipulate parasites extracted from tsetse flies. We thank the reviewers for their careful reading of the manuscript and their precise suggestions and corrections. We have addressed their comments in the point-by-by-point response letter. Modifications in the text are highlighted in yellow.

Reviewer 1 Advance Summary and Potential Significance to Field:
Trypanosoma brucei has flagella with various lengths, depending on stages in the left cycle, but molecular mechanism to determine the length of flagella is not known. Bertiaux and her colleagues investigated this question, taking interdisciplinary approach, such as TEM, FIB-SEM, immunofluorescent microscopy, fluorescent tagging and FRAP.
They examined flagella from Trypanosoma in cardia by TEM and FIB-SEM and revealed the geometry of IFT, which is novel information. Then they conducted optical microscopy studies to reveal correlation between the amount of IFT and flagellar length. For microscopy, they used fluorescent antibodies against IFT172, IFT22 and FLAM8. They also genetically tagged IFT81. It was known that the total amount of IFT and flagellar length have positive correlation. However, this study demonstrated that this correlation is almost perfect proportion and independent of the stage of the lift cycle, suggesting that the density and frequency of IFT is even and thus cannot be the factor to regulate the length. They also discovered IFT accumulated at the flagella tip by fluorescent microscopy of flagella with genetically tagged IFT81. Regarding molecular mechanism of length determination, the authors started from the "grow and lock" model, which they proposed in a previous paper, interpreted their results and investigated cells in short epimastigote with defected function of cell division. Interestingly this cell grew even shorter flagella. Possible mechanism was discussed.

Reviewer 1 Comments for the Author:
This manuscript provides a number of novel findings and extends interesting discussion. It is a significant cornerstone in flagella research and will inspire scientists in this field. This reviewer strongly recommends publication of this manuscript after the following minor points are addressed.

Minor points:
p.4, Line7: "main means" is not clear.

We have replaced "means" by "pathways"

Fig.S1A: needs a scale bar to say "the cardia is a large organ (p.12)".

We have added a scale bar as requested.

Fig.2AB: needs to be presented differently as a proof of "multiple parasites". Maybe indication (enclosing) of each parasite will be helpful for readers to identify them.

The figure has been modified as requested: we drew thick lines around the cell bodies to identify more easily individual cells.

p.13, Line11 "by FIB (Fig.S2C)": Fig.S2C does not exist. Probably typo.

Yes, it was Fig. S1. This has been corrected.

Bottom of p.13: to correlate Fig.3B and Fig.4 to discuss about length of IFTs, it would be helpful to present Fig.3B data perpendicularly – making 3D map by stacking FIB-SEM images and show a computational section including the entire length of IFT.

Thank you for this nice suggestion. We have generated the computational section that allows visualisation of the full train along the axoneme (new Fig. 3C).

Fig.4B and Fig.5B: Apparently fluorescent microscopy data from TdT::IFT81 has more error (Fig.5B) than that from antibody-labelled one (Fig.4B). Why?

This is correct. This is explained by the fact that live imaging implies a time parameter that is not present in fixed sample, hence adding one supplementary variable and inherently increasing variability

p. 17, Line 23 "This is in contrast to..": The meaning of this sentence is unclear. "were" -> "where"?

It is indeed "where". This has been corrected.

bottom of p.21: what does it mean by "interpretation is difficult … because IFT is construction machinery"?

The sentence has been deleted.

p. 23 third line from the bottom: locked and therefore can therefore elongate -> locked and therefore can elongate

This has been corrected.

p. 47 (caption of video S1): Figure 2 -> Figure 3

Corrected

in Video S1: begining -> beginning

This has been corrected.

Reviewer 2 Advance Summary and Potential Significance to Field:
In this study, Bertiaux et al. examined the IFT and its dynamics in T. brucei at different developmental stages in the tse-tse flies. The results showed a positive correlation between flagellar IFT abundance and length, in all life stages. The unicellular T. brucei is capable of producing flagella of different length in different developmental stages, making it a good model to understand the mechanisms of flagellar length regulation. Such experiments with in vivo developed parasites are technically challenging, which contributes to the highly descriptive nature of this work. The observations suggested that flagellar IFT amount/dynamics is unlikely to have a major regulatory role in flagellum length. However, many other possibilities such as IFT activity, train size, cargo loading, cargo abundance, disassembly pathways etc were not investigated.

This would be highly interesting and will be the subject of future studies. Cargo loading remains a challenge for trypanosomes since tagged tubulin fails to access the flagellum despite multiple attempts by several groups (Bastin et al., 1996; Sheriff et al, 2010).

Reviewer 2 Comments for the Author:
1. The measurement of flagellar IFT.
T. brucei flagellum wraps along the cell body. It is thus difficult to perform accurate measurements of IFT fluorescence along the flagellum without the interference by cytoplasmic signal, in live or fixed cells. Perhaps the authors could explain in greater details how the measurements (as shown in Fig. 4B with near perfect correlation and Fig. 5B) was controlled, to rule out background or interference by cell body signals. Cells without IFT staining could be used as a control.

Thanks for pointing this out. The procedure used to measure signal intensity is now described in the Materials and Methods section (see page 10).

In methanol fixation, the inherent dehydration means that the cell body loses a lot of volume and material, hence reducing background. We also noticed that the flagellum tends to lies on the side of the cell body, especially in cells at the long epimastigote stage on the surface of which the flagellum is positioned lengthways in a straight manner (see for example Fig. 4B and Fig. S2A).

Methanol fixation may remove some cytoplasmic and soluble IFT signals, and live cell measurements were not possible for the highly motile cells. Can TdTomato-IFT81 cells be fixed with PFA for the measurements of flagellar IFT in cells of all life stages?

Unfortunately, when these stages are fixed with paraformaldehyde, they do not adhere to glass slides, hence the number of cells visible by IFA is extremely low.

2. On several occasions, it is stated that the total amount of IFT is correlated with flagellar length. Is it flagellar IFT or total cellular IFT that was measured? This need to be made clearer in the manuscript. Additionally, does the ROI include the concentrated spot of IFT at the flagellar base?

We indeed measured flagellar IFT as defined at the bottom of page 14. We have made this clearer at other places in the manuscript (abstract, p.6, p.15, p.21)

3. Page 24, shortening of existing flagellum was also observed in a trypanosomatid Leptomonas pyrrhocoris in a recent report (He et al., mBio 2019).

This is interesting situation in a monogenetic species, we have added the reference (p24).

4. All live cell movies should have time stamp. Some additional labels of the base/tip of the

flagella would help to orient the cells. For the FRAP movies, please mark the ROI.

We have updated all the videos with additional labels. They all play at real time.

Second decision letter

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AUTHORS: Eloise Bertiaux, Adeline Mallet, Brice Rotureau, and Philippe Bastin
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.