Journal of
Cell Science

Microtubule polyglutamylation is important for regulating cytoskeletal architecture and motility in *Trypanosoma brucei*

Jana Jentzsch, Adal Sabri, Konstatin Speckner, Gertrud Lallinger-Kube, Matthias Weiss and Klaus Ersfeld
DOI: 10.1242/jcs.248047

Editor: Michael Way

Review timeline

Original submission:	27 April 2020
Editorial decision:	4 June 2020
First revision received:	30 July 2020
Accepted:	30 July 2020

Original submission

First decision letter

MS ID#: JOCES/2020/248047

MS TITLE: Microtubule polyglutamylation is important for the regulation of cytoskeletal architecture and motility in Trypanosoma brucei

AUTHORS: Jana Jentzsch, Adal Sabri, Konstatin Speckner, Gertrud Lallinger-Kube, Matthias Weiss, and Klaus Ersfeld
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

As you will see, all three reviewers were positive but raise a number of criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

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.

© 2020. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

Reviewer 1

Advance summary and potential significance to field

The authors have studied the role of glutamylation on microtubules in the parasite T. brucei and have shown the microtubule based-result phenotypes resulting from knockdown of two putative tubulin polyglutamylases, TTLL6A and TTLL12B. In the RNAi knockdown phenotypes, they show disorganization of sub-pellicular microtubules, with the resulting disintegration of the posterior pole, loss of plus-end binding microtubules, alteration in microtubule dynamics and changes in swimming patterns. The work is explained and discussed in detail and with precision, with coherent and logical hypotheses.

This work provides novel insights into trypanosome biology and makes a significant and novel contribution to our understanding of cell biology, it is of broad interest to the cell biology community. However, there are some points to be addressed before it reaches a publishable level.

Comments for the author

This work provides novel insights into trypanosome biology and makes a significant and novel contribution to our understanding of cell biology, it is of broad interest to the cell biology community. However, there are some points to be addressed before it reaches a publishable level.

Major

It is possible to purify flagella from trypanosomes and observe the associated microtubule structures. The MTQ is a quartet of microtubules that originate in the basal body of the flagellum. It would be interesting and important to look at the status, (absence /presence and glutamylation status), of the MTQ before and after RNAi. This would provide some insights as to whether these mts are associated with the “glove” phenotype or not. This could be done by IFA.

Figure 3A shows a W/T cell probed with YL1/2, and the subsequent increase in this signal after RNAi knockdown of TTLL6A-RNAi and TTLL12B-RNAi, which I agree looks real. However, the WT signal is not the normal signal observed with YL1/2 on trypanosomes. If I am not mistaken, the original work using YL1/2 on T.brucei W/t cytoskeletons showed bright labeling on the posterior of the cell as well as basal bodies. Perhaps the authors have diluted YL1/2 because the labeling is extremely high almost at saturation point in RNAi induced cells. However, I feel that the original W/T signal must be demonstrated in this work.

Figure 3C. The question of whether EB1 fails to be recruited to microtubule posterior ends after TTLL6A-RNAi or TTLL12B-RNAi knockdown is not addressed in this experiment. If the monoclonal to EB1 functions by western blot, it should be used on W/T non-induced and RNAi induced cells in western blots to define whether EB1 is absent, degraded, or in low amounts in the cell. If the monoclonal does not blot the encoding gene should be endogenously tagged.

Minor

None

Reviewer 2

Advance summary and potential significance to field

In this study, the authors examined the cellular phenotypes upon RNAi silencing of two putative TTLL in T. brucei, 6A and 12B. Depletion of 6A or 12B both led to decreased tubulin glutamylation in T. brucei subpellicular microtubules and flagellar axoneme. Changes in cell morphology, microtubule dynamics, EB1 localization and cell motility mode were also found.

A previous study by Casanova et al. (International J. for Parasitology, 2015) examined the RNAi effects of seven T. brucei TTLL proteins, 1, 4A, 4B, 4C, 6A, 6B and 9. Despite successful

© 2020. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

knockdown (by Northern blots), no growth phenotypes were observed with the exception of 4A and 6B. The current study re-visited 6A and extended to 12B.

Comments for the author

1. The in vivo polyglutamylase activities of 6A and 12B.
The enzymatic activities were established using cells depleted of 6A or 12B by RNAi. RNAi cells showed reduced GT335 and polyE signal by immunoblots and immunostaining. In my opinion, these observations did not provide direct evidence for the enzymatic activities of 6A and 12B. It is possible that depletion of 6A or 12B affect microtubule association with other proteins and only indirectly affect microtubule polyglutamylation. In vitro polyglutamylase activities assays using purified TTLL or overexpressed TTLL (as in Casanova et al., 2015) may be the best to establish the enzymatic functions.

2. Inference of EB-1 function.
EB-1 labelling at the posterior end was lost in TTLL6A and 12B-RNAi cells. And based on this observation, the authors hypothesized that EB-1 recruitment failure was a contributing factor to the cytoskeletal disorganization. This logic appears flawed. Depletion of TTLL6A and 12B, polyglutamylase or not, may affect microtubule association with many proteins and thus affect microtubule organization in EB-1-independant manner(s). EB-1 seems to be the only one tested in this paper, but have other microtubule-associated proteins been tested?

3. Motility assays.
T. brucei motility is a complex behavior that can be affected by many factors including cell body size, cell cycle, flagellar length, flagellum structures, how flagellum is attached to the cell body etc. The observed motility phenotype in TTLL6A and 12B-RNAi cells was therefore not too surprising, considering their gross effects on the entire cytoskeleton. It would however, be more interesting if one could tease apart the actual cause of this change. Could it be changes in flagellum structure and/or flagellum beating motion? Or changes in cell body rigidity?

Other points—
1. It is not clear why only 6A and 12B were selected for this study?

2. Fig. 2C. Please consider a thinner line or other way to highlight the posterior region of the RNAi cells. The thick dotted line currently used made it difficult to see the (lack of) GT335 labelling in this region.

Reviewer 3

Advance summary and potential significance to field

This is a focussed paper that provides a clean set of experiments defining the phenotype of mutational analysis of two putative TTLs. The tubular code ....what do all these modifications do?...has been around a good while. It os becoming clearer that they are important. This study that is able to define differences in the many microtubule arrays in one cell by using the trypanosome is valuable. The phenotypes are surprising. It will be of general interest and I recommend publication.

Comments for the author

This is an interesting paper that details the characterisation of two proteins that are likely members of a large tubulin polyglutamylase family. They study them in the trypanosome which again turns out to provide a very tractable system in that it has good cell biology and reverse genetics, but also, the highly defined and spatially separated microtubule arrays allow the definition of functions of spindle and cytoplasmic arrays.

The results are rather surprising and fascinating and contribute significantly to the idea of different members of this family having particular functions on particular microtubule arrays. More than that they show very clearly the particular effects of these enzymes on microtubule dynamics and function – motility – in the microtubule arrays,

© 2020. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

The results are rather surprising, the phenotypes are novel and will speak to the general cell biology community interested in the unfolding story of the “tubulin post-translational code”.

I have only a few minor points:

1. It would be useful to put, either I the text, or in the supplementary a description of the nomenclature of the NINE TTLs in the trypanosome genome. It will be a bit confusing for the lay reader t try to sort out nine TTLs (line 109) yet the two studied here are TTL6A and TTL12B. some guidance here would be useful.
2. Is diffusive motility the best word to describe this form of motility…it suggested motility by diffusion and this is not the case …the cells appear to be inherently motile but not productively so in terms of directed locomotion.
3. The English is good in the manuscript but there are a few areas where issues arise through wrong use that produces a misleading conclusion. These are small errors but useful if the authors might just relook at the manuscript. Examples (there are others) include:
• Line 377….was not analysed in this study …should be ..that study
• Line 435 ,,,ciliar..change to ciliary…
• Line 443. Precise mechanisms how …change to precise mechanism of how…
etc

First revision

Author response to reviewers' comments

General comments
Abstract and Title has been shortened to comply with word limits. Grammar and spelling has been corrected.

Response to referees

Reviewer 1

1. We have attempted to look at the MTQ on isolated flagella, but did not obtain any meaningful data, possibly due to the limited resolution of conventional light microscopy. However, since this is an ongoing project, we will try to gain access to a superresolution microscope to revisit this question.

2. Regarding the YL1/2 staining, it is correct that a higher concentration of this antibody results in a more typical posterior staining of the cytoskeleton. We have included a supplementary figure to illustrate this. However, in both cases the intensity of YL1/2 staining is strongly increased in TTLL-depleted cells. The effect is much better visible at lower YL1/2 concentrations.

3. As the anti EB1 mAb is of IgM isotype Western blotting is not that straightforward, However, the reviewers comment prompted us to optimise the procedure and we have now included a Western blot. In fact, this was a very valuable suggestion, because the blot shows that EB1 is still present in the cells, but no longer part of the cytoskeleton fraction.

Reviewer 2

1. The reviewer is right in suggesting that biochemical enzyme assay would be valuable. In fact, such assays have been done by Casanova et al. (2015) for TTLL6A, but not for TTLL12B. However, there are serious problems with this approach. Firstly, the analysis by Casanova showed that apparently only one or two of the tested seven TTLLs show activity. This is unlikely because the sequences strongly suggest that all tested TTLLs are genuine polyglutamylases. However, our

© 2020. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

data provide a clue as to why those tests were by-and-large negative. Mammalian TTLLs appear not to be specific for tubulin as substrate, but have been shown to polyglutamylate a range of other proteins, such as the acidic nuclear proteins NAP1 and ANP32B. In contrast, polyglutamylation in T. brucei appears to be highly tubulin-specific (see Western blot in Figure S2). Therefore, the conventional biochemical test established for mammalian TTLLs using NAP1 and ANP32 peptides are unlikely to work with T. brucei TTLLs and this might explain the surprising results of Casanova et al. Therefore, we would need to establish a novel, robust test to characterise the catalytic properties of the TTLLs of T. brucei. Whilst this is highly desirable, it is, in our opinion, beyond the scope of this initial study.

2. Regarding the comments about EB1, we refer to our reply (3) to reviewer 1. We did test the distribution of the cytoskeleton-associated protein CAP5.5, but found no obvious alteration (that is the reason to use CAP5.5 in some experiments as control for the cytoskeletal fraction). However, the loss of EB1 localisation was an important observation and we will, of course, examine the impact of MT-PTMs on other cytoskeleton-associated proteins in the course of this project. This, however, requires the generation of in situ tagged cell lines in TTLL-RNAi-capable cells or the generation of antibodies against a selection of known cytoskeletal proteins. Both was not feasible within the time frame of this resubmission. (Unfortunately, excellent antibodies generated against a number of cytoskeleton associated proteins in T. brucei in the past, mainly by Tom Seebecks lab, are no longer available.)

3. Regarding motility assays it is correct that the disintegration of the cellular cytoskeleton would probably lead to motility defects, independent of any flagellar defects. To minimise this possibility, we have chosen only cells for motility analysis that appeared of normal morphology (see line 247). However, we are aware and have discussed this, that it is still difficult to safely assign a motility defect to a flagellar phenotype. To further strengthen a functional correlation between the observed decreased axonemal polyglutamylation and aberrant motility, we have included an additional data set showing flagellar beat frequencies in wild-type and TTLL-depleted cells. These data show, more clearly for TTLL12B than for TTLL6A, a slight shift towards higher frequencies. Such a minor phenotype is not entirely unexpected, because data from the Oda group (e.g. Kubo and Oda, 2017) show a very subtle change in the interaction of dynein-regulatory proteins of the axoneme of Chlamydomonas after a mutational analysis.

4. We performed RNAi analysis on all TTLLs of T. brucei. Only 6A and 12B had this specific impact on the cytoskeletal integrity. We observed additional phenotypes with other TTLLs, but they are distinct and therefore not subject of this particular study.

5. We have removed the dotted lines in Fig. 2. The DIC images are sufficient to visualise the outlines of the cells.

Reviewer 3

1. We have included a table of all T. brucei TTLLs to help with nomenclature.

2. Apparently, the term ”super-diffusive” and “diffuse motility” is the standard way for biophysicists to describe motility, independent of a particular organism. It is correct that the TTLL-depleted cells do not move by thermal diffusion.

3. Changes to grammar etc. have been made.

© 2020. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).

Second decision letter

MS ID#: JOCES/2020/248047

MS TITLE: Microtubule polyglutamylation is important for regulating cytoskeletal architecture and motility in Trypanosoma brucei

AUTHORS: Jana Jentzsch, Adal Sabri, Konstatin Speckner, Gertrud Lallinger-Kube, Matthias Weiss, and Klaus Ersfeld
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.

© 2020. Published by The Company of Biologists under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/).