A function of profilin in force generation during malaria parasite motility independent of actin binding

Catherine A. Moreau, Katharina A. Quadt, Henni Piirainen, Hirdesh Kumar, Saligram P. Bhargav, Leanne Strauss, Niraj H. Tolja, Rebecca C. Wade, Joachim P. Spatz, Inari Kursula and Friedrich Frischknecht
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MS TITLE: Optical Tweezers Uncover a Function of Profilin in Force Generation During Malaria Parasite Motility Independent of Actin Binding

AUTHORS: Catherine A. Moreau, Katharina A. Quadt, Henni Piirainen, Hirdesh Kumar, Saligram P. Bhargav, Leanne Strauss, Niraj H. Tolja, Rebecca C., Joachim P. Spatz, Inari Kursula, and Friedrich Frischknecht
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.

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

Apicomplexa profilin has two unique features including an arm motif and an acidic loop. In addition to ADP exchange, profilin in Apicomplexa also has an actin sequestering function. A previous study by Moreau et al. found that the arm motif is important for actin binding, but the function of the acidic loop had not been explored. Here Moreau et al. study the role of the acidic loop with chimeric proteins that substituted the acidic loops between Toxoplasma, P. berghei and P. falciparum orthologs. The authors performed molecular dynamics simulations to calculate complex stability that led to the hypothesis that the acidic loop could impact actin-profilin complex stability. The authors produced the chimeric proteins recombinantly and found that they all sequestered actin similarly based on pyrene polymerization and co-pelleting assays. Next they replaced P. Bergheri profilin with the chimeras or falciparum profilin and assessed parasite transmission and motility on cover glass. The falciparum profilin strain had increased speed while the chimeras all had reduced speed. The authors then turned to measuring the force generation during motility with two optical trap assays. In this assay they found that the chimeric proteins produced the highest retrograde flow rate and the lowest force generation as determined by the ability of the mutants to push trapped beads out of the optical trap. The falciparum profilin performed similarly to the bergheri profilin for both retrograde transport and force generation. The authors conclude that lower force generation is inversely correlated with increased retrograde flow. How the acidic loop specifically impacts actin dynamics in Apicomplexa remains unclear, but the authors speculate it could be important for recruiting Apicomplexa specific motility factors.

The manuscript is clearly written and the experiments appear to be well executed. What slightly diminishes my enthusiasm is that the role of the acidic loop remains unclear. Still, the authors show it is important for force generation in gliding motility and that it is not directly involved in actin binding. This work is then the foundation for follow up studies.

Comments for the author

Major Comments:
1. The authors determine that the chimeras move more slowly in the coverglass motility assay while they find that retrograde flow is faster in the bead assay. Can the authors reconcile or comment on whether this is a conflicting result?

Minor comments:
2. JCS has a wide audience, it would be helpful for the readership if the authors include a diagram of a generic apicomplexan to show the IMC and hypothesized position of actin and myosin etc.

3. Upon the first use of “patent” could the authors define this. I believe it indicates that parasites are visible in the blood but this may not be the meaning.

4. Fig 1 should include Toxpoplasma profilin in the alignment

5. Figure S2 is called out before Figure S1

6. Table 2. Could the authors try to indicate units or what is counted.
For example, I believe for “growth rate” this is fold increase over 24 hours but that is not specified or easy to figure out.

7. The color scheme in Figure 4 is different than in Figures 2 and 3.
pBPfn-Pf loop should be yellow instead of green for consistency.

8. I think it would have been interesting to mutate berghei profilin acidic residues to polar residues to see how simply changing the charge would impact motility and force generation. Since this experiment would not test any specific hypothesis, I am suggesting the authors consider it it for future studies. For example, it might be a good control for identifying acidic loop binding proteins.

Reviewer 2

Advance summary and potential significance to field

The manuscript entitled “Optical Tweezers Uncover a Function of Profilin in Force Generation During Malaria Parasite Motility Independent of Actin Binding” by Moreau et al, addresses an important issue of the Plasmodium sporozoite biology since motility is essential for this developmental stage to access the liver as final destination in the vertebrate host. Advances in understanding the sporozoite peculiar gliding type of motility have been achieved in the past by the Frischknecht team using a combination of molecular genetics, live imaging and biophysics. In this context, previous characterization of an Apicomplexa actin-binding profilin has highlighted that a phylum-restricted profilin domain (i.e. the β-hairpin arm) binds to parasite actin and fulfills the actin sequestering function of profilin. The present study now documents the functional contribution of the second domain featuring the Apicomplexan profilins, namely the acidic loop region that lies at the opposite side of the profilin actin binding domain. This loop had already been shown flexible in the case of P. falciparum profilin probably moving over the polyproline ligand (Schuler) and was known to substantially differ in amino acid sequences between rodent and human Plasmodium species and with the profilin of the related T. gondii Apicomplexa. Taking these differences into consideration and now applying complementary approaches spanning from first molecular simulations and then biochemistry, molecular genetics as well as optical particle trapping with optical tweezers, the authors have used three readouts. First they have analyzed whether and how a series of profilin chimeric constructs can modulate the profilin binding properties to recombinant parasite (P. berghei) actin; second, they have quantitatively assayed the effect of replacing endogenous profilin by the chimeric proteins on the life cycle progression using laboratory mice and P. berghei transgenic lines. Finally, they have quantitatively assayed in vitro the motile behavior the transgenic sporozoite by measuring a wide set of relevant parameters to accurately describe gliding motility including the challenging laser trap assay which monitor membrane flow and measure cortical forces with respect to motility. The assays are thoroughly designed and well executed and quantitatively analyzed for straightforward results. The conclusions are appropriate without any overstatements. To my opinion, apart from small comments for clarification purposes, this work represents a robust and detailed analysis with new findings to the contribution of profilin in vivo over the life cycle of Plasmodium and in vitro on the peculiar motility mode of the eukaryotic sporozoite stage. Considering the huge amount of work to study the transgenic lines in Anopheles mosquitoes and rodent hosts as well as the intriguing results on the large increase in the antero-posterior flow upon bead capping on the sporozoite surface induced by chimeric profilins, this work should certainly be of interest for the parasitology community but also for scientists investigating actin dynamics, membrane flow and cell migration in other cellular models. Therefore, I strongly recommend publication in Journal of Cell Science.

Comments for the author

Minor comments
-Abstract - Please would you correct "transmission of malaria" by malaria parasite or just Plasmodium.

-Intro line 46: Please specify "in vitro" (Profilin- monomer sequestering activity)

-Line 101: “Comparison between different Plasmodium species shows that there is little difference in the arm motif while T. gondii profilin has only a very short acidic loop (Figure 1B, C)”. In fact, said like that this is confusing since it is not the T. gondii Profilin that is shown with its short motif in red but the Pb mutant design that carries the Tg short loop. Please clarify.
-Line 107: “In addition to be shorter” : please remove to avoid repetition as this detail has been mentioned one sentence before.
-Line 153: “Interestingly, the effect of Plasmodium profilin was more pronounced on P. falciparum actin polymerization than on pig skeletal muscle actin”. Considering the differences in the y scale (Figure 2) and the gels presented in sup Figure 1, it might be good to emphasize on the high specificity of the parasite profiling for sequestering parasite actin.
-Line 165: clarify the sentence for the reader. Comparing the growth rates of the chimeric parasite lines to those of wild type P. berghei and the previously reported P. berghei line expressing P.

falciparum profilin showed that the parasites expressing the P. berghei profilin with the P. falciparum loop grew as fast as wild type P. berghei
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-Line 203: correct spelling actin-moysin motor for myosin. Is the retrograde flow dependent on myosin in the case of Plasmodium (which seems not to be for Toxoplasma, at least for myosin A)
It might be a good idea to restrict a bit the number of references since some are not really needed. For instances, the ref 9 cited line 70 and elsewhere might not be correct since to my knowledge the publication does not report formin activity (rabbit and parasite actin and mutants, and coronin).

First revision

Author response to reviewers' comments

Reviewer 1 Advance Summary and Potential Significance to Field:

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The falciparum profilin strain had increased speed while the chimeras all had reduced speed. The authors then turned to measuring the force generation during motility with two optical trap assays. In this assay they found that the chimeric proteins produced the highest retrograde flow rate and the lowest force generation as determined by the ability of the mutants to push trapped beads out of the optical trap. The falciparum profilin performed similarly to the berghei profilin for both retrograde transport and force generation. The authors conclude that lower force generation is inversely correlated with increased retrograde flow. How the acidic loop specifically impacts actin dynamics in Apicomplexa remains unclear, but the authors speculate it could be important for recruiting Apicomplexa specific motility factors.

The manuscript is clearly written and the experiments appear to be well executed. What slightly diminishes my enthusiasm is that the role of the acidic loop remains unclear. Still, the authors show it is important for force generation in gliding motility and that it is not directly involved in actin binding. This work is then the foundation for follow up studies.

A: We thank the reviewer for his/her generous remarks and the correct and concise summary of our work.

Reviewer 1 Comments for the Author: Major Comments:
1. The authors determine that the chimeras move more slowly in the coverglass motility assay while they find that retrograde flow is faster in the bead assay. Can the authors reconcile or comment on whether this is a conflicting result?

A: This appears at first sight indeed a conflicting result. We first described this phenomenon in our 2016 paper Quadt et al., in ACS Nano 2016 when investigating the surface protein TLP and also in the profilin paper Moreau et al in PLoS Pathogens 2017, when investigating arm mutants. We assume that retrograde flow is faster than forward motility as receptors flowing backwards are 'trapped' into adhesion sites that produce force leading to forward motility. WHY this faster flow is necessary, however, we don't know. In line with the second suggestion of this author we now provide a new introductory figure 1 and elaborate on this questions in the text associated with the figure and the figure legend. Hopefully the concept has become more clear.

Minor comments:
2. JCS has a wide audience, it would be helpful for the readership if the authors include a diagram of a generic apicomplexan to show the IMC and hypothesized position of actin and myosin etc.

A: Thanks for this suggestion. We now provide a new figure 1 and use this to introduce the concepts leading to this study.

3. Upon the first use of "patent" could the authors define this. I believe it indicates that parasites are visible in the blood but this may not be the meaning.

A: We deleted the first statement of patentcy but explained on the second occasion that patency is "the time it takes from injecting sporozoites to seeing first blood stage parasites" – line 284.

4. Fig 1 should include Toxpoplasma profilin in the alignment 5.Figure S2 is called out before Figure S1

A: Thanks for spotting this oversight, We added the T. gondii profilin sequence and Figure S1 is now Figure S5 and all other supplementary figures were adjusted to be called out in correct order.

6. Table 2. Could the authors try to indicate units or what is counted.
For example, I believe for "growth rate" this is fold increase over 24 hours but that is not specified or easy to figure out.

A: This is correct, we now state this and some other parameters more clearly in the table legend, see lines 718-724.

7. The color scheme in Figure 4 is different than in Figures 2 and 3. pBPfn-Pf loop should be yellow instead of green for consistency.

A: This was indeed a slight difference in hue, it is now corrected. All figures were also adjusted to Arial font as requested by the journal style.

8. I think it would have been interesting to mutate berghei profilin acidic residues to polar residues to see how simply changing the charge would impact motility and force generation. Since this experiment would not test any specific hypothesis, I am suggesting the authors consider it it for future studies. For example, it might be a good control for identifying acidic loop binding proteins.

A: We completely agree that there remains a lot to do and many options for further detailed work in trying to decipher how the proteins work together to make this cell move as fast as it does. We appreciate that the reviewer recognizes this good suggestion to be for future work.

Reviewer 2 Advance Summary and Potential Significance to Field:
The manuscript entitled "Optical Tweezers Uncover a Function of Profilin in Force Generation During Malaria Parasite Motility Independent of Actin Binding" by Moreau et al., addresses an

important issue of the Plasmodium sporozoite biology since motility is essential for this developmental stage to access the liver as final destination in the vertebrate host.
Advances in understanding the sporozoite peculiar gliding type of motility have been achieved in the past by the Frischknecht' team using a combination of molecular genetics, live imaging and biophysics. In this context, previous characterization of an Apicomplexa actin-binding profilin has highlighted that a phylum-restricted profilin domain (i.e. the ß-hairpin arm) binds to parasite actin and fulfills the actin sequestering function of profilin. The present study now documents the functional contribution of the second domain featuring the Apicomplexan profilins, namely the acidic loop region that lies at the opposite side of the profilin actin binding domain.
This loop had already been shown flexible in the case of P. falciparum profilin probably moving over the polyproline ligand (Schuler) and was known to substantially differ in amino acid sequences between rodent and human Plasmodium species and with the profilin of the related T. gondii Apicomplexa. Taking these differences into consideration and now applying complementary approaches spanning from first molecular simulations and then biochemistry, molecular genetics as well as optical particle trapping with optical tweezers, the authors have used three readouts. First they have analyzed whether and how a series of profilin chimeric constructs can modulate the profilin binding properties to recombinant parasite (P. berghei) actin; second, they have quantitatively assayed the effect of replacing endogenous profilin by the chimeric proteins on the life cycle progression using laboratory mice and P. berghei transgenic lines. Finally, they have quantitatively assayed in vitro the motile behavior the transgenic sporozoite by measuring a wide set of relevant parameters to accurately describe gliding motility including the challenging laser trap assay which monitor membrane flow and measure cortical forces with respect to motility.

The assays are thoroughly designed and well executed and quantitatively analyzed for straightforward results. The conclusions are appropriate without any overstatements. To my opinion, apart from small comments for clarification purposes, this work represents a robust and detailed analysis with new findings to the contribution of profilin in vivo over the life cycle of Plasmodium and in vitro on the peculiar motility mode of the eukaryotic sporozoite stage. Considering the huge amount of work to study the transgenic lines in Anopheles mosquitoes and rodent hosts as well as the intriguing results on the large increase in the antero-posterior flow upon bead capping on the sporozoite surface induced by chimeric profilins, this work should certainly be of interest for the parasitology community but also for scientists investigating actin dynamics, membrane flow and cell migration in other cellular models. Therefore, I strongly recommend publication in Journal of Cell Science.

A: We thank this reviewer for her/his encouraging words and appreciation of our work.

Reviewer 2 Comments for the Author: Minor comments
-Abstract - Please would you correct "transmission of malaria" by malaria parasite or just Plasmodium.

A: Thanks for spotting this, we now state "malaria-causing parasites", line 37.

-Intro line 46: Please specify "in vitro" (Profilin- monomer sequestering activity)
A: we included the mention "in vitro" and changed the sentence accordingly, so that it stays correct. Now line 59.

-Line 101: "Comparison between different Plasmodium species shows that there is little difference in the arm motif while T. gondii profilin has only a very short acidic loop (Figure 1B, C)". In fact, said like that this is confusing since it is not the T. gondii Profilin that is shown with its short motif in red but the Pb mutant design that carries the Tg short loop. Please clarify.

A: This was indeed a mis-stated sentence. It now reads: "Comparison between different Apicomplexan species shows that there is little difference in the arm motif[5] while *T. gondii* profilin has only a very short acidic loop compared with the profilins in *Plasmodium spp* (Figure 2B, C)." – lines 124-127.

-Line 107: "In addition to be shorter" : please remove to avoid repetition as this detail has been mentioned one sentence before.

A: done.

-Line 153: “Interestingly, the effect of Plasmodium profilin was more pronounced on P. falciparum actin polymerization than on pig skeletal muscle actin”. Considering the differences in the y scale (Figure 2) and the gels presented in sup Figure 1, it might be good to emphasize on the high specificity of the parasite profilin for sequestering parasite actin.

A: This is a tricky issue that clearly deserves more attention. We already see that there are quite some differences in vivo between expressing P. falciparum and P. berghei profilin suggesting the evolution between the two parasites. So one could argue that the difference to human profilin-actin interaction is not that strikingly different. On the other hand, it appears that Apicomplexan profilin sequesters actin rather than (or in addition to) promoting actin polymerization. In a sense we would like to keep the focus here on the profilin loops, but also felt obliged to show the data with mammalian actin to rise curiosity. We ask the reviewer to kindly allow us to keep the text a concise as it currently is. We nevertheless added a small phrase to the sentence now ending in “… suggesting an important co-evolutionary constraint” to highlight the specificity as suggested by the reviewer – now line 175.

-Line 165: clarify the sentence for the reader. Comparing the growth rates of the chimeric parasite lines to those of wild type P. berghei and the previously reported P. berghei line expressing P. falciparum profilin showed that the parasites expressing the P. berghei profilin with the P. falciparum loop grew as fast as wild type P. berghei

A: This was too long a sentence. Thanks for spotting. We now split it in two (lines 186-192) and also added info to table 2, as also requested by reviewer 1 (line 718-724). Hopefully this makes for easier understanding.

-Line 461: Could you specify the number of midguts of infected mosquito that were checked to enumerate oocysts

A: We counted at least 50 infected midguts to arrive at our data. This is stated in the materials and methods section, but we now also state it in the legend to table 2 – line 720.

-Line 185-186: “Those expressing P. falciparum profilin containing the loop of T. gondii were gliding much more robustly than those just expressing the P. falciparum profilin (Figure 3E). When I looked at the figure panel, I see an increase in the class of persistently moving spz, but is this what is defined as “robust” by opposition in “partially”. Could this be clarified (may be persistent versus intermittent?) as I did not find explanation in the M&M.

A: This is correct, it should read persistently and mention the increased percentage instead of just stating “robustly”, we changed the sentence accordingly. It reads: “…but curiously, those expressing *P. falciparum* profilin containing the loop of *T. gondii* showed a higher percentage of persistently moveing sporozoites than those just expressing the *P. falciparum* profilin“ – lines 208-211.

-Line 203: correct spelling actin-moysin motor for myosin. Is the retrograde flow dependent on myosin in the case of Plasmodium (which seems not to be for Toxoplasma, at least for myosin A)

A: We corrected the typo. We assume it is myosin dependent and just generated our first series of myosin mutants, but will still need a few more months to analyze them.

It might be a good idea to restrict a bit the number of references since some are not really needed. For instances, the ref 9 cited lane 70 and elsewhere might not be correct since to my knowledge the publication does not report formin activity (rabbit and parasite actin and mutants, and coronin).

A: At 60 references we hope to not have overdone referencing. We refer here to a supplementary figure in reference 9 that shows the localization of formin. To give this more prominence we will

include a similar image in a review article that we are preparing, as also other colleagues are not aware of this experiment.

Second decision letter

MS ID#: JOCES/2019/233775

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AUTHORS: Catherine A Moreau, Katharina A Quadt, Henni Piirainen, Hirdesh Kumar, Saligram P Bhargav, Leanne Strauss, Niraj H Tolja, Rebecca C Wade, Joachim P Spatz, Inari Kursula, and Friedrich Frischknecht
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.

Reviewer 1

Advance summary and potential significance to field

Please see initial review.

Comments for the author

As indicated in my original review, the study was well performed and I had only minor comments mostly about clarification. The authors have addressed all of my comments and I have no remaining concerns. I recommend the manuscript be accepted for publication.

Reviewer 2

Advance summary and potential significance to field

The manuscript entitled “Optical Tweezers Uncover a Function of Profilin in Force Generation During Malaria Parasite Motility Independent of Actin Binding” by Moreau et al, addresses an important issue of the Plasmodium sporozoite biology since motility is essential for this developmental stage to access the liver as final destination in the vertebrate host. Previous characterization of an Apicomplexa actin-binding profilin has highlighted that a phylum-restricted profilin domain (i.e. the β-hairpin arm) binds to parasite actin and fulfills the actin sequestering function of profilin. The present study now documents the functional contribution of the second domain featuring the Apicomplexan profilins, namely the acidic loop region that lies at the opposite side of the profilin actin binding domain. This loop had already been shown flexible in the case of P. falciparum profilin and was known to substantially differ in amino acid sequences between rodent and human Plasmodium species and with the profilin of the related T. gondii Apicomplexa. Taking these differences into consideration and applying first molecular simulations and then biochemistry, molecular genetics as well as optical tweezers, the authors have used three readouts. First they have analyzed whether and how a series of profilin chimeric constructs can modulate the profilin binding properties to recombinant parasite (P. berghei) actin; second, they have quantitatively assayed the effect of replacing endogenous profilin by the chimeric proteins on the life cycle progression using laboratory mice and P. berghei transgenic lines. Finally, they brought careful multi-parameter analysis of the motile behavior of the transgenic sporozoite and in particular monitor membrane flow and measure cortical forces with respect to motility using optical tweezers.

The detailed characterization of the transgenic mosquitoe motile behavior together with the intriguing influence of chimeric profilins on the antero-posterior bead flow on sporozoite surface make this work of significant interest for the parasitology community but also for scientists investigating actin dynamics, membrane flow and cell migration in other cellular models.

Comments for the author

The minor comments raised in my original review have all been properly addressed in this revised form. To my opinion, this nice and robust piece of work is ready for publication in JCS and should definitively be appreciated by the field of parasitology and beyond by the scientists interested in cel motility. Congratulations.