The interplay between the polar growth determinant DivIVA, the segregation protein ParA and their novel interaction partner PapM controls the Mycobacterium smegmatis cell cycle by modulation of DivIVA subcellular distribution

Izabela Matusiak, Agnieszka Strzałka, Patrycja Wadach, Martyna Gongerowska-Jac, Ewa Szwajczak, Aleksandra Szydłowska-Helbrych, Bernhard Kepplinger, Monika Pióro, and Dagmara Jakimowicz

Corresponding Author(s): Dagmara Jakimowicz, Uniwersytet Wroclawski Wydzial Biotechnologii

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Transaction Report:

August 3, 2023

Prof. Dagmara Jakimowicz
Uniwersytet Wroclawski Wydzial Biotechnologii
Department of Molecular Microbiology, Faculty of Biotechnology
Wroclaw
Poland

Re: Spectrum01752-23 (The interplay between the polar growth determinant DivIVA, the segregation protein ParA and their novel interaction partner PapM controls the Mycobacterium smegmatis cell cycle by modulation of DivIVA subcellular distribution)

Dear Dagmara,

Thank you for submitting your manuscript to Microbiology Spectrum. As we discussed recently, I encountered problems to identify suitable reviewers for your manuscript. One of the reviewers was not responsive anymore, and I have decided to proceed to the decision with only one reviewer. As you will see in the reviewer's comments, this reviewer appreciated your work and recommends publication once a few issues have been clarified. This reviewer is specifically concerned by the result of the DivIV phosphorylation, and suggests additional controls. She/he also requests more details on the biological replicates and statistical analyses. Based on the reviewer's opinion and recommendation, I encourage you to addreess the comments and invite you to submit a revised version of your work. When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript - we strongly recommend that you submit your paper within the next 60 days or reach out to me. Detailed instructions on submitting your revised paper are below.

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Sincerely,
Eric

Eric Cascales

Editor, Microbiology Spectrum

Reviewer comments:

Reviewer #1 (Comments for the Author):

Comments are uploaded as word file.

The authors focused on papM, which the authors newly found by using the bacterial two-hybrid (BTH) library of M. smegmatis genome. The interaction between PapM, ParA, and DivIVA were analyzed by BTH system, microscopic analysis in E.coli, and in vitro affinity chromatography. The function of PapM was further investigated in M. smegmatis, which originally expresses papM. The deletion of papM did not affect the cell growth but increased the immobile fraction of ParA and the establishment time of two daughter cell poles, while the overexpression extended the cell length when the authors used wild-type as the background. In addition, the authors showed some interesting results by deleting or overexpressing papM in parA deletion strains. This research is original and well written and provides a de novo binding partner of ParA. Overall, I agree with the idea that PapM interacts with ParA and DivIVA and co-relates to the cell segregation in M .smegmatis. However, I found some questionable arguments in the manuscripts. Please see the attached comments.
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Staff Comments:

Preparing Revision Guidelines
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• Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file.
• Manuscript: A .DOC version of the revised manuscript
• Figures: Editable, high-resolution, individual figure files are required at revision, TIFF or EPS files are preferred

For complete guidelines on revision requirements, please see the journal Submission and Review Process requirements at https://journals.asm.org/journal/Spectrum/submission-review-process. **Submissions of a paper that does not conform to Microbiology Spectrum guidelines will delay acceptance of your manuscript.** "

Please return the manuscript within 60 days; if you cannot complete the modification within this time period, please contact me. If you do not wish to modify the manuscript and prefer to submit it to another journal, please notify me of your decision immediately so that the manuscript may be formally withdrawn from consideration by Microbiology Spectrum.

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Corresponding authors may join or renew ASM membership to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at Service@asmusa.org.

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Response to Reviewers:

We would like to thank Reviewer for valuable comments. We have made suggested modifications, enhancing our manuscript impact and clarity. The detailed answers to comments are below.

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We appreciate this very accurate comment, indeed the use of phosphoablative mutant of DivIVA is the best approach to confirm the significance of DivIVA phosphorylation. We have now constructed appropriate expression construct: pETDuet pknB$_{KD}$ divIVAT74A and co-expressed these genes in presence of EGFP-ParA in E. coli BL21, finding that phosphoablative DivIV74A fully colocalised with EGFP-ParA in presence of His-PknB$_{KD}$. We have now included this result in the manuscript, as part of Fig. 2.

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to-noise ratio. Unfortunately at this stage we cannot change the colour of data points, without performing the whole data analysis.

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Indeed, approximately normal distribution of samples is listed as a requirement for the T-student test. However, given a sufficient sample size, this does not have to be the case. According to the Central Limit Theorem (CLT), repeated sampling from a non-normal population will produce a normal distribution of sampling means. This Normal distribution of an average underlies the validity of the T-student test. Non-parametric test such as Wilcoxon test are the most useful, when the sample size is small and can be misleading when used for large datasets. (Lumley et al, 2002, Fagerland, 2012)

We repeated the statistical analysis for data presented on figures 2 and 5, where sample's distributions were skewed and did not follow normal distribution using Wilcoxon rank-sum test (Mann–Whitney U test) instead of T-student test, with the same method for multiple comparisons correction (Holm). This analyses produced similar p-values with the exception of Figure 2H right panel and Fig. 5B. Therefore we exchanged those figures. We show below the comparison of p values obtained with both statistical approaches.

Figure	Panel	Comparison	T-student test p-value	Wilcoxon test p-value
2	H, left	ParA+DivIVA vs ParA + DivIVA + PapM	0.7330	0.5279
2	H, left	ParA+DivIVA vs ParA+DivIVA + PknB	3.6e-07	3.3e-08
2	H, left	ParA+DivIVA vs ParA+DivIVA + PapM + PknB	< 2e-16	< 2e-16
2	H, left	ParA+DivIVA + PknB vs ParA+DivIVA + PapM + PknB	< 2e-16	< 2e-16
2	H, right	DivIVA + PapM vs ParA + DivIVA + PapM	0.0082	0.2900
2	H, right	DivIVA + PapM vs ParA + DivIVA + PapM + PknB	6.0e-14	1.6e-08
2	H, right	DivIVA + PapM vs DivIVA + PapM + PknB	1.9e-09	4.4e-09
2	H, right	ParA + DivIVA + PapM vs ParA + DivIVA + PapM + PknB	0.0082	0.2300
2	H, right	ParA + DivIVA + PapM + PknB vs DivIVA + PapM + PknB	0.0230	0.2900
5	A	WT vs ΔpapM	0.2000	0.0900
5	A	WT vs ΔparA	0.0120	0.0190
5	B	WT vs ΔpapM	0.1300	0.0620
5	B	WT vs ΔparA	0.0270	0.1500

5	C	WT vs ΔpapM	0.3554	0.9747
5	C	WT vs ΔparA	0.0012	0.0006

-Minor points

1. Figure 1B. The anti-His western blotting is not a publication quality. Could the authors try once more? If this data is the representative data from biological replicates, authors should note this in the figure legend.

The results presented in figure 1 are representative of 4 independent experimental approaches which each time deliver the same clear result (while Western presented in figure1 was the best technical replicate) - we have now included this information in the figure legend.

2. Figure S1C does not support the idea "...interaction interface may be located within the linker between DivIVA coiled-coil domains..." (Line 56-57) and "phosphorylation site Thr74 potentially may be involved in PapM binding."(Line 392-393). The figure shows the blue colony in which the author used T18C-DivIVA III-IV, which does not include the linker.

We have now included the detailed information on the fragments used in BTH assay, which shows that T18C-DivIVA III-IV (68-143 aa) includes the phosphorylated Thr74. To be more precise, we have also rephrased the text in the result as follows: "interaction interface may encompass the linker between DivIVA coiled-coil domains and fragment of second coiled-coil…"

3. Figure 3C. This data should be shown as table format instead of figure.

OK, Fig 3C is now Table 1

4. Figure 3F. The annotations for images are missing.

Corrected, this figure is now Fig. 3A inset.

5. Figure S5A. Why was the papM overexpression in ParA deletion mutant weaker than that of wild-type? I am wondering if the datasets came from technical triplicate in single biological replication.

The dataset are from 4 biological replicates (we added this information into the figure legend), but we cannot explain why papM transcript levels are lower in absence of ParA. However, taking into account the high variation of *papM* transcript levels in wild type the difference between wild type and Δ*parA* mutant my be not significant.

6. Line 225, "parM" should be "papM".

corrected

7. Line 299-300, "This altered mobility could possibly result from modified ParA association with DNA which was earlier shown to predominantly affect ParA mobility". Does this

mean that PapM affects the DNA binding affinity of ParA? I think this is not supported by data.

We believe that suggested by PALM experiment change in the ParA mobility may be due to its altered DNA binding. Our earlier work showed that alterations of ParA-DNA binding have a large impact on ParA mobility. We have added the appropriate reference here.

8. Line 326. NADA mainly visualizes peptidoglycan remodeled by L,D-transpeptidase instead of nascent peptide glycan [PMID: 30198841].

Corrected (deleted "nascent").

9. Line 388-389 "Elevated levels of PapM could interfere with ParA dynamics impairing segrosomes separation." is not a clear sentence. Does this mean that PapMs capture non-DNA-binding ParA to interfere with ParA dynamics?

That is one of the possibilities that should be considered. We have rephrased the text as follows: "Elevated levels of PapM could interfere with ParA-DNA and/or ParA-DivIVA interaction affecting ParA dynamics and impairing segrosomes separation."

October 4, 2023

Prof. Dagmara Jakimowicz
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Dear Prof. Dagmara Jakimowicz:

Thank you for submitting your revised manuscript, which has been sent to the original reviewer. As you will see, this reviewer acknowledges that you properly addressed all the questions raised in the first round of review. One typo has however been noticed. I therefore invite you to make this last change and to submit your revised manuscript. I will then proceed to final acceptance.

When submitting the revised version of your paper, please provide (1) point-by-point responses to the issues raised by the reviewers as file type "Response to Reviewers," not in your cover letter, and (2) a PDF file that indicates the changes from the original submission (by highlighting or underlining the changes) as file type "Marked Up Manuscript - For Review Only". Please use this link to submit your revised manuscript. Detailed instructions on submitting your revised paper are below.

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Thank you for the privilege of reviewing your work. Below you will find instructions from the Microbiology Spectrum editorial office and comments generated during the review.

The ASM Journals program strives for constant improvement in our submission and publication process. Please tell us how we can improve your experience by taking this quick Author Survey.

Sincerely,

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Editor, Microbiology Spectrum

Reviewer comments:

Reviewer #1 (Comments for the Author):

The authors have done a great job in revising the manuscript and addressing the issues raised by the reviewer. Finding PapM as a new modulator of DivIVA-parA in *M.smegmatis* is a novel, interesting and intriguing.

The authors have addressed all my questions.

One minor comment- Right panels of figure 4 say "papA" and "parM". I believe those shoold be "parA" and "papM"

Preparing Revision Guidelines
To submit your modified manuscript, log onto the eJP submission site at https://spectrum.msubmit.net/cgi-bin/main.plex. Go to Author Tasks and click the appropriate manuscript title to begin the revision process. The information that you entered when you first submitted the paper will be displayed. Please update the information as necessary. Here are a few examples of required updates that authors must address:

• Point-by-point responses to the issues raised by the reviewers in a file named "Response to Reviewers," NOT IN YOUR COVER LETTER.
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For complete guidelines on revision requirements, please see the journal Submission and Review Process requirements at https://journals.asm.org/journal/Spectrum/submission-review-process. **Submissions of a paper that does not conform to Microbiology Spectrum guidelines will delay acceptance of your manuscript.** "

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Corresponding authors may join or renew ASM membership to obtain discounts on publication fees. Need to upgrade your membership level? Please contact Customer Service at Service@asmusa.org.

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2	H, left	ParA+DivIVA vs ParA+DivIVA + PapM + PknB	< 2e-16	< 2e-16
2	H, left	ParA+DivIVA + PknB vs ParA+DivIVA + PapM + PknB	< 2e-16	< 2e-16
2	H, right	DivIVA + PapM vs ParA + DivIVA + PapM	0.0082	0.2900
2	H, right	DivIVA + PapM vs ParA + DivIVA + PapM + PknB	6.0e-14	1.6e-08
2	H, right	DivIVA + PapM vs DivIVA + PapM + PknB	1.9e-09	4.4e-09
2	H, right	ParA + DivIVA + PapM vs ParA + DivIVA + PapM + PknB	0.0082	0.2300
2	H, right	ParA + DivIVA + PapM + PknB vs DivIVA + PapM + PknB	0.0230	0.2900
5	A	WT vs ΔpapM	0.2000	0.0900
5	A	WT vs ΔparA	0.0120	0.0190
5	B	WT vs ΔpapM	0.1300	0.0620
5	B	WT vs ΔparA	0.0270	0.1500

| 5 | C | WT vs ΔpapM | 0.3554 | 0.9747 |
| 5 | C | WT vs ΔparA | 0.0012 | 0.0006 |

-Minor points

1. Figure 1B. The anti-His western blotting is not a publication quality. Could the authors try once more? If this data is the representative data from biological replicates, authors should note this in the figure legend.

The results presented in figure 1 are representative of 4 independent experimental approaches which each time deliver the same clear result (while Western presented in figure1 was the best technical replicate) - we have now included this information in the figure legend.

2. Figure S1C does not support the idea "...interaction interface may be located within the linker between DivIVA coiled-coil domains..." (Line 56-57) and "phosphorylation site Thr74 potentially may be involved in PapM binding."(Line 392-393). The figure shows the blue colony in which the author used T18C-DivIVA III-IV, which does not include the linker.

We have now included the detailed information on the fragments used in BTH assay, which shows that T18C-DivIVA III-IV (68-143 aa) includes the phosphorylated Thr74. To be more precise, we have also rephrased the text in the result as follows: "interaction interface may encompass the linker between DivIVA coiled-coil domains and fragment of second coiled-coil…"

3. Figure 3C. This data should be shown as table format instead of figure.

OK, Fig 3C is now Table 1

4. Figure 3F. The annotations for images are missing.

Corrected, this figure is now Fig. 3A inset.

5. Figure S5A. Why was the papM overexpression in ParA deletion mutant weaker than that of wild-type? I am wondering if the datasets came from technical triplicate in single biological replication.

The dataset are from 4 biological replicates (we added this information into the figure legend), but we cannot explain why papM transcript levels are lower in absence of ParA. However, taking into account the high variation of *papM* transcript levels in wild type the difference between wild type and Δ*parA* mutant my be not significant.

6. Line 225, "parM" should be "papM".

corrected

7. Line 299-300, "This altered mobility could possibly result from modified ParA association with DNA which was earlier shown to predominantly affect ParA mobility". Does this

mean that PapM affects the DNA binding affinity of ParA? I think this is not supported by data.

We believe that suggested by PALM experiment change in the ParA mobility may be due to its altered DNA binding. Our earlier work showed that alterations of ParA-DNA binding have a large impact on ParA mobility. We have added the appropriate reference here.

8. Line 326. NADA mainly visualizes peptidoglycan remodeled by L,D-transpeptidase instead of nascent peptide glycan [PMID: 30198841].

Corrected (deleted "nascent").

9. Line 388-389 "Elevated levels of PapM could interfere with ParA dynamics impairing segrosomes separation." is not a clear sentence. Does this mean that PapMs capture non-DNA-binding ParA to interfere with ParA dynamics?

That is one of the possibilities that should be considered. We have rephrased the text as follows:

"Elevated levels of PapM could interfere with ParA-DNA and/or ParA-DivIVA interaction affecting ParA dynamics and impairing segrosomes separation."

October 6, 2023

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Re: Spectrum01752-23R2 (The interplay between the polar growth determinant DivIVA, the segregation protein ParA and their novel interaction partner PapM controls the Mycobacterium smegmatis cell cycle by modulation of DivIVA subcellular distribution)

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