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Regulation of dynamic polarity switching in bacteria by a Ras-like G-protein and its cognate GAP

Simone Leonardy, Mandy Miertzschke, Iryna Bulyha, Eva Sperling, Alfred Wittinghofer and Lotte Sogaard-Andersen

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Transaction Report:

(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.)

1st Editorial Decision 30 April 2010

Thank you very much for submitting your research paper for consideration to The EMBO Journal editorial office. Three expert referee's initially agreed to assess merits and suitability of your paper for our journal. I did receive two rather consistent and overwhelmingly positive reports. I am thus in the position to make a decision to prevent unnecessary delay and enable efficient proceedings for your study. As you will see, ref#1 has only a very minor suggestion for data presentation. Ref#2 asks to more carefully describe the functionality of the fusion construct used, also in comparison to function and localization described in a related study. Together with some minor points, this should be adequately addressed during revisions of the paper.

I do have to formerly remind you that it is EMBO_J policy to allow a single round of revisions only and that the ultimate decision on acceptance or rejection entirely depends on the content and strength of the final version of your manuscript.

I am very much looking forward receiving your revised manuscript.

Yours sincerely,

Editor
The EMBO Journal

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process initiative, please visit our website:
http://www.nature.com/emboj/about/process.html

REFEREE REPORTS

Referee #1 (Remarks to the Author):

This is an outstanding manuscript. The data are thorough, well controlled, and clearly support the authors' conclusions. It provides a significant advance in the field.

My only suggestion, which is very minor, is that in the model figure (7B) it would be helpful to the broader audience to indicate the direction of movement of the bacteria.

Referee #2 (Remarks to the Author):

In this manuscript, the authors present a nice set of in vitro and in vivo experiments in which they further characterize the previously described M. xanthus Ras-like-GTPase, MglA. Briefly, they show that protein localization and regulation of motility are linked and directly depend on the equilibrium between the GTP and GDP bound states of the MglA protein. The authors also identify in MglB the cognate GAP of MglA and in the Frz system a possible direct or indirect GEF regulator. The concert of activities of these regulators is responsible for the establishment of the correct polarity of MglA and in turn of the A- and S- motility machineries.
The paper is well written and fit a general readership. Also, I believe that the experimental data fully support the hypotheses presented in the discussion and that this work potentially adds to our knowledge about protein localization and regulation of motility.

Major comments:
1. Page 10, para 2. The authors claim to have obtained a functional MglA fusion. However, Fig. S2B shows that the YFP-MglA fusion protein is massively cut into MglA and YFP. The amount of MglA that results from the cleavage of YFP-MglA is comparable to the amount of wt MglA (interestingly the only fusion that it is not cleaved is YFP-MglAT26/27N in the mglA9, but not in the mglA+ background).
First of all, the MglA protein resulting from the cleavage of the YFP-MglA fusion and not the fusion itself could be responsible of the regained motility and reversal frequency phenotype of mglA9. Secondly, the presence of free YFP must be what leads to the high level of background observed in the fluorescence pictures shown in Fig. 2, 3 and 4. I do not believe that there is evidence that the described fusion is functional. I still believe that the experimental data support the hypotheses discussed in the manuscript, but the authors cannot describe YFP-MglA as a fully functional fusion.

2. Page 10, para 3. The authors say that the localization at the leading cell pole and distributed cluster of the MglA-YFP fusion published by Mauriello et al. might be due the partial functionality of this fusion. In fact, the YFP-MglA constructed by the authors is fully functional and localizes only at the leading cell pole. Firstly, I believe that the fully functionality of the YFP-MglA fusion described in this manuscript is arguable (see comment 1). Secondly, isn't it possible that the distributed clusters are not visible because of the high level of background seen in all pictures and probably due to the cleavage of the protein fusion (Fig. S2)?

Minor comments:
3. Page 4, para 2. The authors introduce the concept of polarity and how polarity is established and maintained. However, following are examples of more general protein localization such as the inhibition of the FtsZ cell division machinery, the binding of proteins to different region of the chromosome and the recognition of negative and positive curvature during the B. subitlis spore formation. The authors should begin the paragraph introducing the concept of protein localization in bacteria.

4. Page 4, para 2. "Protein localization may be positively regulated by trans-acting factors". It would be helpful for the readers if the authors provide an example.

5. Page 6, para 2 (Mauriello et al., 2009). Isn't it 2010? Check over the manuscript.

6. Page 6, para 2. Eliminate the following sentence: "These observations taken together with the finding that MglA has GTPase activity (Mauriello et al, 2009) and that Ras-like proteins function in cell polarity in eukaryotes made us hypothesize that MglA is involved in regulating the polarity of motility proteins." And substitute with " It has also been shown that MglA has a GTPase activity (Mauriello et al., 2010)."

7. Page 11, para 1. Instead of "several minutes" the author should give a number. From Fig. 2B' looks like it could be one minute, but from figure 2B looks more like two.

8. Page 11, para 1. How long does a cell stop for during a reversal? From Fig. 2B it looks like the cell stops between minutes 3 and 4 and re-starts moving between minutes 5 and 6. So, can a stop be as long as 3 minutes?

9. Page 11, para 2. Does the cluster described for the YFP-MglAG21V fusion oscillate from pole to pole at the same velocity than the cell?

10. Fig. 5A (table). Make it clear in the legend that the Kds shown in the table were determined in the presence of MglB.

11. Legend for Fig. 5B. "stabilizes" instead of "stabilises".

12. Page 17, last para. At which pole does PilT localize in mglAT26/27N non moving cells?

13. Fig. 2, 3, 4. I think that it should be mentioned somewhere in the text or in the figure legend that the high background in the fluorescence pictures might be due to the cleavage of the YFP-MglA fusion (see comment 1).

14. Fig. 6H and 6I. I believe that these plots are inverted as they show that PilT is at leading pole.

15. The use of "+" in YFP-MglA+ or MglA+ is confusing as it is omitted sometimes in the text or in the figure legend. Could the author clarify the use of "+" or just get rid of it?

16. Legend for Fig. S5A. The authors mention for the first time "focal adhesion complexes" that I believe are the ones described by Mignot et al. (2007). Can the authors mention them in the introduction (for example in page 6, para1).

1st Revision - Authors' Response 05 May 2010

Referee 1:

This is an outstanding manuscript. The data are thorough, well controlled, and clearly support the authors' conclusions. It provides a significant advance in the field.

My only suggestion, which is very minor, is that in the model figure (7B) it would be helpful to the broader audience to indicate the direction of movement of the bacteria.

Reply: We have now included arrows to indicate the direction of movement.

Referee 2:

In this manuscript, the authors present a nice set of in vitro and in vivo experiments in which they further characterize the previously described M. xanthus Ras-like-GTPase, MglA. Briefly, they show that protein localization and regulation of motility are linked and directly depend on the equilibrium between the GTP and GDP bound states of the MglA protein. The authors also identify in MglB the cognate GAP of MglA and in the Frz system a possible direct or indirect GEF regulator.

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First of all, the MglA protein resulting from the cleavage of the YFP-MglA fusion and not the fusion itself could be responsible of the regained motility and reversal frequency phenotype of mglA9. Secondly, the presence of free YFP must be what leads to the high level of background observed in the fluorescence pictures shown in Fig. 2, 3 and 4. I do not believe that there is evidence that the described fusion is functional. I still believe that the experimental data support the hypotheses discussed in the manuscript, but the authors cannot describe YFP-MglA as a fully functional fusion.

Reply: On p.11, para 2 we have modified the description of the MglA-YFP fusion and removed the word "fully". Also we have included that a more detailed description of the degradation products arising from the MglA-YFP fusion.

2. Page 10, para 3. The authors say that the localization at the leading cell pole and distributed cluster of the MglA-YFP fusion published by Mauriello et al. might be due the partial functionality of this fusion. In fact, the YFP-MglA constructed by the authors is fully functional and localizes only at the leading cell pole. Firstly, I believe that the fully functionality of the YFP-MglA fusion described in this manuscript is arguable (see comment 1). Secondly, isn't it possible that the distributed clusters are not visible because of the high level of background seen in all pictures and probably due to the cleavage of the protein fusion (Fig. S2)?

Reply: On p.12, para 1 we have now included that the high background fluorescence observed with out MglA-YFP fusion could mask the adhesion complexes.

Minor comments:

3. Page 4, para 2. The authors introduce the concept of polarity and how polarity is established and maintained. However, following are examples of more general protein localization such as the inhibition of the FtsZ cell division machinery, the binding of proteins to different region of the chromosome and the recognition of negative and positive curvature during the B. subitlis spore formation. The authors should begin the paragraph introducing the concept of protein localization in bacteria.

Reply: On p. 4, para 1 we have now included a sentence to introduce the concept of polarity in bacteria.

4. Page 4, para 2. "Protein localization may be positively regulated by trans-acting factors". It would be helpful for the readers if the authors provide an example.

Reply: Changed as suggested by including the DivIVA recruitment of MinJ.

5. Page 6, para 2 (Mauriello et al., 2009). Isn't it 2010? Check over the manuscript.

Reply: Corrected as suggested.

6. Page 6, para 2. Eliminate the following sentence: "These observations taken together with the finding that MglA has GTPase activity (Mauriello et al, 2009) and that Ras-like proteins function in cell polarity in eukaryotes made us hypothesize that MglA is involved in regulating the polarity of motility proteins." And substitute with " It has also been shown that MglA has a GTPase activity (Mauriello et al., 2010)."

Reply: Removed as suggested.

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Reply: Changed as suggested.

8. Page 11, para 1. How long does a cell stop for during a reversal? From Fig. 2B it looks like the cell stops between minutes 3 and 4 and re-starts moving between minutes 5 and 6. So, can a stop be as long as 3 minutes?

Reply: On p. 12, para 1 we have now included a sentence that a stop lasts for seconds to minutes.

9. Page 11, para 2. Does the cluster described for the YFP-MglAG21V fusion oscillate from pole to pole at the same velocity than the cell?

Reply: On p. 13, para 1 we have now included the mean velocity of a cluster (1.3±0.2 µm/min). This velocity is lower than the velocity of a cell. However, we do not believe that we have an understanding of the system that allows us to discuss these two velocities against each other.

10. Fig. 5A (table). Make it clear in the legend that the Kds shown in the table were determined in the presence of MglB.

Reply: Changed as suggested.

11. Legend for Fig. 5B. "stabilizes" instead of "stabilises".

Reply: Changed as suggested.

12. Page 17, last para. At which pole does PilT localize in mglAT26/27N non moving cells?

Reply: On p. 19, para this information has now been included.

13. Fig. 2, 3, 4. I think that it should be mentioned somewhere in the text or in the figure legend that the high background in the fluorescence pictures might be due to the cleavage of the YFP-MglA fusion (see comment 1).

Reply: Has been included as suggested (See also our replies to comment 1 and 2).

14. Fig. 6H and 6I. I believe that these plots are inverted as they show that PilT is at leading pole.

Reply: Corrected as suggested.

15. The use of "+" in YFP-MglA+ or MglA+ is confusing as it is omitted sometimes in the text or in the figure legend. Could the author clarify the use of "+" or just get rid of it?

Reply: We have corrected the manuscript and figures to make sure that we consistently use "+" when we discuss the properties of the wild type protein.

16. Legend for Fig. S5A. The authors mention for the first time "focal adhesion complexes" that I believe are the ones described by Mignot et al. (2007). Can the authors mention them in the introduction (for example in page 6, para1).

Reply: We have now introduced the focal adhesion complexes on p. 6, para 2.

