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An atypical MAPK regulates translocation of GATA transcription factor in response to chemoattractant stimulation

Jeffrey A. Hadwiger, Huaqing Cai, Ramee G. Aranda and Saher Fatima
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Original submission

First decision letter

MS ID#: JOCES/2022/260148

MS TITLE: Atypical MAPK regulates translocation of GATA transcription factor in response to chemoattractant stimulation

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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 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.

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

The atypical MAP kinases, such as ErkB in Dictyostelium are an interesting set of proteins, whose activation route differs from conventional MAP kinases and whose functions are not clear. ErkB in Dictyostelium is required for chemotaxis to both folate and cyclic-AMP, and appears to be the most

important protein kinase in chemotaxis as judged by the number of chemotaxis proteins it phosphorylates. Yet it has been neglected over the years, with the Hadwiger laboratory being a notable exception.

This paper by Hadwiger et al shows that ErkB regulates the nuclear accumulation of the GATA transcription factor GtaC in response to cyclic-AMP and folic acid, which normally drive it from the nucleus. This response happens on the same time scale as ErkB activation and depends on consensus ErkB phosphorylation sites in GtaC.

These experiments depend on a GtaC-GFP reporter to show nuclear accumulation and are clear cut and convincing. They fall just short of showing that ErkB directly phosphorylates GtaC, rather than through an intermediate kinase. This would require more biochemistry – identify the sites phosphorylated on GtaC by mass-spectroscopy and better characterise the phosphorylations in vitro, using purified proteins.

Overall, this is a useful paper, but essentially it contains only a single result, whose significance for chemotaxis or ErkB signalling is not made clear. It is better suited to a short communication than a full paper.

Comments for the author

Fig 1. What developmental state were the cells in when stimulated with cAMP? The legend and methods suggest they were freshly starved. Classically such cells do not respond to cAMP. Please explain.
Fig 6. The development of all strains is absolutely terrible compared to wild-type KAx3. If this is the full degree of complementation obtained by expressing ErkB, then it is very poor. Does the lab parental strain (presumably KAx3, not JH10) behave like this? If so, it needs changing.
Development of delicate strains is often worse on growth plates than on non-nutrient agar, so it is worth checking the latter.

Reviewer 2

Advance summary and potential significance to field

In Dictyostelium secreted cAMP pulses acts both to coordinate aggregation and to induce the expression of genes required for aggregation and post-aggregative development. Earlier studies revealed that gene expression was dependent on the transcription factor GtaC, which transitioned in and out of the nucleus in reverse phase with the cAMP pulses. In this work the authors identified the atypical MAPKinase ErkB as the signaling intermediate for cAMP pulse induced nuclear exit of GtaC.

After establishing that in erkB null mutants gtaC remains nuclear localized after cAMP stimulation, the authors show that gtaC is phosphorylated by erkB after cAMP stimulation. ErkB was also found to mediate gtaC translocation by folate, the chemoattractant that is used by vegetative cells to find bacteria.
This effect requires the folate receptor far1 and its G-protein G4

They next show that erkB induced gtaC translocation requires phosphorylation at 4 erkB consensus phosphorylation sites in gtaC for optimal effect. The presence of these sites was also found to be required for the essential role of gtaC in Dictyostelium aggregation.

Overall this is a very solid piece of experimental work that provides the missing link in dynamic transcriptional regulation by oscillatory stimuli.
Since excitable systems that generate such stimuli are common throughout biology, this work and the study that precedes it are likely to become paradigms for transmission of complex sensory input.

Comments for the author

I have a few comments for improvement of the manuscript.
Criticisms lines 201-204. gtaC- cells were found to migrate further in response to folate stimulation, suggesting the gtaC suppresses chemotaxis. The authors should clarify why they consider folate chemotaxis to be enhanced by erkB, which activates gtaC translocation?
Line 91 insert "the" before presence
Line 222. the Nichols et al citation needs a date
Symbol sizes in the figures should be a bit larger. They are in most figures difficult to distinguish from each other at 100% page size.
The list of oligonucleotides in supplemental looks messy – convert to a regular table?

First revision

Author response to reviewers' comments

Reviewer 1 Advance Summary and Potential Significance to Field:

The atypical MAP kinases, such as ErkB in Dictyostelium are an interesting set of proteins, whose activation route differs from conventional MAP kinases and whose functions are not clear. ErkB in Dictyostelium is required for chemotaxis to both folate and cyclic-AMP, and appears to be the most important protein kinase in chemotaxis as judged by the number of chemotaxis proteins it phosphorylates. Yet it has been neglected over the years, with the Hadwiger laboratory being a notable exception.

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These experiments depend on a GtaC-GFP reporter to show nuclear accumulation and are clear cut and convincing. They fall just short of showing that ErkB directly phosphorylates GtaC, rather than through an intermediate kinase. This would require more biochemistry – identify the sites phosphorylated on GtaC by mass-spectroscopy and better characterise the phosphorylations in vitro, using purified proteins.

Response: We acknowledge that the possibility of an intermediary protein kinase cannot be ruled out but the evidence presented in Fig. 2 (phosphorylation using immunoprecipitates of Erk2 and GFP-GtaC) and the previously reported analysis of Erk2 substrate specificity (Nichols et al., 2019) strongly support that Erk2 is phosphorylating GtaC directly. Note that the phosphorylation of GFP-GtaC in vitro is associated with immunoprecipitated Flag-Erk2 from cAMP stimulated cells and therefore recombinant Erk2 purified from bacteria or other sources might not function like Erk2 immunoprecipitated from stimulated Dictyostelium cells. The Nichols et al. report has shown purified Erk2 has a preference for the S/T-P-K/R motifs in vitro, such as those found and analyzed in GtaC. In addition, in vitro phosphorylation assays with purified protein do not always reflect in vivo interactions because protein kinases can phosphorylate nonphysiological targets in vitro. For example some MAPKs, including atypical MAPKs, can autophosphorylate in vitro but this type of phosphorylation seldom occurs in vivo.

Overall, this is a useful paper, but essentially it contains only a single result, whose significance for chemotaxis or ErkB signalling is not made clear. It is better suited to a short communication than a full paper.

Response: We respectfully disagree with the reviewer and believe this study should be a full paper. This study not only shows a requirement of Erk2 function for GtaC translocation but also reveals important insights into signaling initiated by chemoattractants. The study demonstrates 1) specificity among MAPKs in transcription factor regulation, 2) the regulation of GtaC in folate

responses, and 3) the importance of the Erk2 substrate sites on GtaC function in development. While more features or characteristics of atypical MAPK signaling are likely to be discovered in future work, the current study identifies a new role for atypical MAPKs in transcription factor regulation and challenges previous paradigms of GtaC function being exclusive to cAMP receptor mediated development.

Reviewer 1 Comments for the Author:
Fig 1. What developmental state were the cells in when stimulated with cAMP? The legend and methods suggest they were freshly starved. Classically such cells do not respond to cAMP. Please explain.

Response: The cAMP stimulation of strains expressing the GFP-GtaC reporter was conducted on freshly starved cells. Dictyostelium cells can respond to cAMP at many stages of development including at the onset of starvation as we have shown. Many studies have focused on cAMP responses in highly elongated cells after several hours of starvation and cAMP stimulation because features of cell polarity can be more easily observed and chemotactic movement is more directed.

Fig 6. The development of all strains is absolutely terrible compared to wild-type KAx3. If this is the full degree of complementation obtained by expressing ErkB, then it is very poor. Does the lab parental strain (presumably KAx3, not JH10) behave like this? If so, it needs changing. Development of delicate strains is often worse on growth plates than on non-nutrient agar, so it is worth checking the latter.

Response: We believe the reviewer has mistakenly mentioned "expressing ErkB" but the development displayed in Fig. 6 is actually gtaC$^-$ cells expressing different allelic constructs of GFP-GtaC. The development of these strains on bacterial lawns in Fig. 6 is not as robust as KAx3 or JH10 and this is likely due to the heterologous expression of the GFP-GtaC alleles from the act15 promoter. As in the case with many genes expressed using this commonly used promoter the complementation of mutants is often not fully restored but sufficient enough to show allelic differences. As mentioned in the results section, excessive expression of GFP-GtaC can be toxic to cells and so it is not surprising that the gtaC- aggregation defective phenotype is not fully restored by vectors heterologously expressing GFP-GtaC.

Reviewer 2 Advance Summary and Potential Significance to Field:
In Dictyostelium secreted cAMP pulses acts both to coordinate aggregation and to induce the expression of genes required for aggregation and post-aggregative development. Earlier studies revealed that gene expression was dependent on the transcription factor GtaC, which transitioned in and out of the nucleus in reverse phase with the cAMP pulses. In this work the authors identified the atypical MAPKinase ErkB as the signaling intermediate for cAMP pulse induced nuclear exit of GtaC.

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Reviewer 2 Comments for the Author:
I have a few comments for improvement of the manuscript.

Criticisms
lines 201-204. gtaC- cells were found to migrate further in response to folate stimulation, suggesting the gtaC suppresses chemotaxis. The authors should clarify why they consider folate chemotaxis to be enhanced by erkB, which activates gtaC translocation?

Response: We appreciate the reviewer's suggestion and have revised with the following - "This result suggests that folate chemotaxis might be enhanced by Erk2 mediated translocation of GtaC, possibly through the loss of GtaC repression of the folate receptor gene (Santhanam et al., 2015)."

Line 91 insert "the" before presence

Response: The missing "the" has been inserted.

Line 222. the Nichols et al citation needs a date

Response: The citation has been corrected.

Symbol sizes in the figures should be a bit larger. They are in most figures difficult to distinguish from each other at 100% page size.

Response: The symbol sizes have been enlarged.

The list of oligonucleotides in supplemental looks messy – convert to a regular table?

Response: The list has been converted to a table.

Second decision letter

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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

The authors show that the atypical MAP kinase, Erk2, of Dictyostelium is required for chemo-attractants to drive the transcription factor GtaC out of the nucleus and that the putative Erk2 phosphorylation sites on GtaC are needed for this function. They also show, as expected, that the response to folate requires the folate receptor and G protein.

Comments for the author

Unfortunately, the authors have declined to make any substantial revisions to this paper. There appear to be no new experiments and just minor tweaks to the text.

Some of the other experimental effects are very marginal. For instance, squint as one may at Figure 2 (A) it is difficult to be convinced that there is a difference in the mobility shifts of GFP-GtaC between WT and erk2-cells, though differences are apparent between WT and erk1- and erk2o/e cells. Nichols et al (2019) found a commercial antibody that recognizes Erk2 phosphorylations, and I am surprised that it was not used to re-probe these blots. I had also hoped that an attempt would be made to discover the actual phosphorylation sites on GtaC by mass-spectroscopy. I remain unconvinced that the very poor complementation shown in Figure 6 is an adequate basis for functional studies.
It also does not help that the paper is difficult reading.
A concise statement of the results would make a nice short paper, but regretfully I do not see this revision as suitable for JCS.

Reviewer 2

Advance summary and potential significance to field

In Dictyostelium secreted cAMP pulses acts both to coordinate aggregation and to induce the expression of genes required for aggregation and post-aggregative development. Earlier studies revealed that gene expression was dependent on the transcription factor GtaC, which transitioned in and out of the nucleus in reverse phase with the cAMP pulses. In this work the authors identified the atypical MAPKinase ErkB as the signaling intermediate for cAMP pulse induced nuclear exit of GtaC.

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Both the scope and experimentation of the manuscript are quite extensive and I disagree with reviewer 1 that the work is more suitable for a short communication

All my previous comments on the manuscript have been addressed

Comments for the author

All my previous comments on the manuscript have been addressed