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Regulation of intrinsic polarity establishment by a differentiation-type MAPK pathway in *S. cerevisiae*

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First decision letter

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MS TITLE: Regulation of an Intrinsic Polarity Establishment Pathway by a Differentiation-Type MAPK Pathway

AUTHORS: Aditi Prabhakar, Jacky Chow, Alan Siegel, and Paul Cullen
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 substantial 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 manuscript by Prabhakar et al. reports the involvement of the yeast filamentous growth MAPK pathway in the regulation of bud emergence. By using genetic, biochemical and time-lapse

microscopy approaches, the authors demonstrate the importance of a properly regulated fMAPK pathway for correct polarity establishment under nutrient-limiting conditions that favor filamentous/invasive growth. The study is well carried out and contributes to a better understanding of the crosstalk between the fMAPK pathway and bud morphogenesis, although it would benefit from addressing some points before publication.

Comments for the author

Line 117. Two “likely” in the same sentence. It should be rephrased.

Line 130. pGFP-MBS2 does not seem to express a hyperactive version of Mbs2.
This should be clarified.

Line 190. “the localization defect was statistically significant (Fig. 1F)”.
Quantification is shown in Fig. 1E instead of Fig. 1F.

Line 352. The authors state that “The formation of multiple growth sites correlated with fMAPK pathway activity (Fig. 5, B and C)”. This is true for hiperactive MSB2Δ100-818 and STE11-4, which induce a significant increase in Kss1 phosphorylation, but not for GAL-SHO1. Overexpression of SHO1 leads to the formation of multiple growth sites similarly to STE11-4 (Fig. 5B) but the Kss1 phosphorylation level is low, similar to that of the wild-type (Fig. 5C).
In addition, total Kss1 levels in cells expressing MSB2Δ100-818 and STE11-4 are lower than expected by the positive feedback operating in the fMAPK pathway (Fig. 5C, middle blot). These discrepancies should be explained.

Line 401. Fig. S10 is missing in the supplement file.

Reviewer 2

Advance summary and potential significance to field

Summary:
The paper from Prabhakar et al. described a cross talk between the polarity pathway and the fMAPK pathway that impacts bud emergence and filamentous growth. The key findings in the paper are interesting and very well supported with the experiments and are in line with previously published work. I enjoyed reading this manuscript. Overall, this paper uses a range of techniques to identify and has elegantly elucidated how fMAPK pathway regulates transcription of polarity target genes and GTP-Cdc42p levels, to increase the rate of bud emergence during filamentous growth. It is a very strong and interesting paper, which is clearly written and largely convincing. The authors should hence address the following key questions to improve the manuscript prior to acceptance.

Comments for the author

1. Authors should discuss and provide some evidence for the role of Boi1 and Boi2 in the fMAPK pathway.
2. Authors should test the genetic interaction between the fMAPK pathway mutants with septin organisation kinases (Elm1 and Gin4).
3. It was shown that Msb1 promotes Cdc42 function in early bud development and and that it inhibits Rho1 activity specifically in small budded cells. Over production of Msb1 is known to rescue cdc24 and cdc42 mutants at restrictive temperature and to affects cell morphology and septic organisation (Liao et al., 2013).
Authors should show the role of Msb1 in fMAPK pathway in this manuscript.
4. Authors should monitor actin dynamics in live cell imaging using Lifeact-GFP as a marker instead of staining with rhodamine phalloiidin. This will provide more insight into the changes of actin cytoskeleton in filamentous cells.

First revision

Author response to reviewers' comments

Response to Reviewer #1
Advance summary and potential significance to field
The manuscript by Prabhakar et al. reports the involvement of the yeast filamentous growth MAPK pathway in the regulation of bud emergence. By using genetic, biochemical and time-lapse microscopy approaches, the authors demonstrate the importance of a properly regulated fMAPK pathway for correct polarity establishment under nutrient-limiting conditions that favor filamentous/invasive growth. The study is well carried out and contributes to a better understanding of the crosstalk between the fMAPK pathway and bud morphogenesis, although it would benefit from addressing some points before publication.

Thanks for taking the time to review the manuscript and for your supportive comments.

1. Line 117. Two “likely” in the same sentence. It should be rephrased.

The original sentence-
“Given that Rho GTPases are functionally connected to MAPK pathways in higher eukaryotes, it is likely that polarity establishment is more likely to be subject to input from MAPK and other pathways than is currently appreciated.”

has now been modified to-
“Given that Rho GTPases are functionally connected to MAPK pathways in higher eukaryotes, polarity establishment might be generally regulated by MAPK and other pathways.”

2. Line 130. pGFP-MBS2 does not seem to express a hyperactive version of Mbs2. This should be clarified.

The original sentence did not clarify that GFP-Msb2p expresses a hyperactive version of the protein.
“Hyperactive versions of Msb2p (pGFP-MSB2) weakly suppressed the growth defect of the cdc24-4 mutant (Fig. 1B).”

We have included this fact in the revised manuscript and modified the sentence as follows-
“Insertion of GFP into the extracellular domain of Msb2p results in a version of the protein that hyperactivates the fMAPK pathway(Adhikari et al., 2015; Vadaie et al., 2008). pGFP-MSB2 weakly suppressed the growth defect of the cdc24-4 mutant (Fig. 1B).”

3. Line 190. “the localization defect was statistically significant (Fig. 1F)”. Quantification is shown in Fig. 1E instead of Fig. 1F.

The error has been corrected. The quantification of proper septin localization is reported in panel G.

4. Line 352. The authors state that “The formation of multiple growth sites correlated with fMAPK pathway activity (Fig. 5, B and C)”. This is true for hiperactive MSB2Δ100-818 and STE11-4, which induce a significant increase in Kss1 phosphorylation, but not for GAL-SHO1. Overexpression of SHO1 leads to the formation of multiple growth sites similarly to STE11-4 (Fig. 5B) but the Kss1 phosphorylation level is low, similar to that of the wild-type (Fig. 5C).

We agree that overexpression of Sho1p shows high levels of multiple growth sites but have wild type levels of Kss1p phosphorylation. This might be because GAL-SHO1 has a unique phenotype where cells make hyper elongated buds, which might result in the formation of multiple growth sites. Thus, we have rephrased this sentence to make it more accurate.

“Generally speaking, the formation of multiple growth sites correlated with fMAPK pathway activity (Fig. 5, B and C). One exception was GAL-SHO1, which showed high levels of multiple growth site formation (Fig. 5B), yet modestly induced fMAPK pathway activity (Fig. 5C). This may be because

overexpression of SHO1 induces a unique cell morphology where cells have hyper elongated buds (Fig. 5D).

5. In addition, total Kss1 levels in cells expressing MSB2Δ100-818 and STE11-4 are lower than expected by the positive feedback operating in the fMAPK pathway (Fig. 5C, middle blot). These discrepancies should be explained.

This is a good point, and although we do not know the reason for this phenotype, it could be due to negative feedback. In the revised manuscript, we included the sentences: “In some fMAPK hyperactive mutants (MSB2Δ100-818 and STE11-4) the levels of total Kss1p were lower than would be expected by positive feedback. Although the reason for this is not known, it could be due to the presence of negative feedback that acts to attenuate the activated pathway.”

6. Line 401. Fig. S10 is missing in the supplement file.

All supplement files have been updated. We did not remove data but reduced the number of files, as specified by the journal’s guidelines. We have made sure that all figures and supplemental figures have been referred to properly in the revised manuscript.

Response to Reviewer #2
Summary:
The paper from Prabhakar et al. described a cross talk between the polarity pathway and the fMAPK pathway that impacts bud emergence and filamentous growth. The key findings in the paper are interesting and very well supported with the experiments and are in line with previously published work. I enjoyed reading this manuscript. Overall, this paper uses a range of techniques to identify and has elegantly elucidated how fMAPK pathway regulates transcription of polarity target genes and GTP-Cdc42p levels, to increase the rate of bud emergence during filamentous growth. It is a very strong and interesting paper, which is clearly written and largely convincing. The authors should hence address the following key questions to improve the manuscript prior to acceptance.

Thanks for taking the time to review the manuscript and for your supportive comments.

Authors should discuss and provide some evidence for the role of Boi1 and Boi2 in the fMAPK pathway.

As suggested by the reviewer, we investigated the role of Boi1p and Boi2p in regulating the fMAPK pathway. Based on two assays, we did not find evidence to support a role for Boi1p and Boi2p in regulating the fMAPK pathway. We have included those tests in the revised manuscript (Fig. S4).

Authors should test the genetic interaction between the fMAPK pathway mutants with septin organization kinases (Elm1 and Gin4).

We investigated the genetic interaction between a hyperactive fMAPK allele, STE11-4, and septin organization kinases, Elm1p and Gin4p, by monitoring growth and cell morphology at various temperatures. The elm1Δ mutant showed abnormally elongated morphologies at all temperatures while a point mutation in GIN4 (gin4G377T) resulted in the formation of elongated cells at the restrictive temperature. Neither growth nor cell morphologies were impacted by the fMAPK pathway. This data has also been included in the revised manuscript (Fig. S2).

It was shown that Msb1 promotes Cdc42 function in early bud development and and that it inhibits Rho1 activity specifically in small budded cells. Over production of Msb1 is known to rescue cdc24 and cdc42 mutants at restrictive temperature and to affects cell morphology and septic organisation (Liao et al., 2013). Authors should show the role of Msb1 in fMAPK pathway in this manuscript.

Thanks to the reviewer for these interesting comments. We have cited this paper and explored the role of Msb1p in the regulation of the fMAPK pathway. Msb1p did not regulate the fMAPK pathway based on immunoblot analysis of Kss1p phosphorylation and FUS1-HIS3 growth reporter, which in the absence of an intact mating pathway (ste4Δ) gives a fMAPK-dependent response (McCaffrey et al., 1987). We have included this data in the revised manuscript (Fig. S4).

Authors should monitor actin dynamics in live cell imaging using Lifeact-GFP as a marker instead of staining with rhodamine phalloiidin. This will provide more insight into the changes of actin cytoskeleton in filamentous cells.

We monitored actin dynamics in live cells using Abp140-YFP, which is a marker for the actin cables and the molecular reporter for Lifeact-GFP (Asakura et al., 1998; Riedl et al., 2008; Yang and Pon, 2002). The fluorescence reporter localized to multiple surfaces in hyperactive fMAPK mutants, and we have included this data in the revised manuscript in (Fig. 6B). This figure replaced Fig. 6B, which was moved to the supplement (S6A).
Here is the revised text: Actin dynamics in live cells using Abp140-YFP, a marker for the actin cables (Asakura et al., 1998; Riedl et al., 2008; Yang and Pon, 2002), showed multiple growth sites in the hyperactive fMAPK mutants compared to wild-type cells (Fig. 6B). Actin staining in filamentous cells using rhodamine phalloidin also showed polarized actin cytoskeleton at more than one site (Fig. S6A-C).

In addition to the suggestions recommended by the reviewers, following changes were also made to the manuscript.
1. The running title was shortened to meet length requirements.
2. Changes to the title were made for clarity.
3. Current addresses of authors were provided to comply with the journal guidelines.
4. The number of keywords were reduced to comply with the journal guidelines.
5. A summary statement was included.
6. Changes to the abstract were made for clarity and to meet length requirements.
7. Changes were made to the layout of the figures to ensure effective use of space based on the journal guidelines.
8. In Fig. 5 and 6, the label “S+AA No Glu” was changed to “S+AA” throughout.
9. Changes to the figure legends were made for clarity and to simplify interpretation by the reader, as well as to account for suggestions made by the JCS Checklist.
10. The number of supplemental files were reduced but no data was removed to meet length requirements.
11. Line 340 “its localization throughout the cell cycle has not been explored” was changed to “whether it localizes to presumptive bud sites is not known” for clarity.
12. The abbreviations, acknowledgements and Table 1 (strain table) were updated.

REFERENCES INCLUDED IN THE REVISED MS

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Second decision letter

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AUTHORS: Aditi Prabhakar, Jacky Chow, Alan Siegel, and Paul Cullen
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

Authors have satisfactorely addressed the points I raised in my report.

Comments for the author

Authors have satisfactorely addressed the points I raised in my report.

Reviewer 2

Advance summary and potential significance to field

The authors have done a nice job of addressing every reviewer's comment. In particular, they have greatly strengthened the paper with additional experiments. This is a very nice story. I except that it will be appreciated by the cell biology community.

Comments for the author

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