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An image analysis method to survey the dynamics of polar protein abundance in the regulation of tip growth

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Original submission

First decision letter

MS ID#: JOCES/2020/252064

MS TITLE: A dynamic survey of the links between polar proteins abundance and tip growth

AUTHORS: Sarah Taheraly, Dmitry Ershov, Serge Dmitrieff, and Nicolas Minc
ARTICLE TYPE: Tools and Resources

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.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

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

This manuscript tests a hypothesis that the local concentration of certain polarity factors may determine the rate of tip growth in fission yeast. Improved semi-automated methods for tip growth measurements are developed, allowing for measuring growth at each tip of the cell. Fluorescence intensities of polarity factors in a variety of cases, including natural differences between tips, show that growing tips often contain "saturating" amounts of these factors that do not correlate well with growth rate. These findings suggest that growth rate is not only set by the numbers of these polarity factors at the tip. Interesting examples of differential growth rates at tips in the same cell are explored.

In general, this study provides important quantitative data on fission yeast growth and polarity factors that will be foundational for this field. The work is of high technical quality, and I appreciate the quantitative nature of the data.
It is also very well written. I think it is appropriate for publication and have only a few minor comments.

Comments for the author

The title "dynamic survey" should be reconsidered. The survey is not what is dynamic here.

Can the authors see if the total concentrations (in the whole cell) of representative polarity factors is maintained through the cell cycle? A large cell cycle fluctuation such as at NETO would affect interpretations about competition between tips and ideas about dilution, for instance. This data could be obtained from the preexisting images, or the authors might be able to cite publish data in some cases.

Can the authors give some sense when in the cell cycle does the cell reach the plateau in growth rate (relative to concentration of polarity factors at the tip)?
For instance, is it the beginning of G2 phase, or near the end?

The authors should add in the discussion that the activity of proteins (such as by regulation by phosphorylation) rather than absolute numbers of proteins might be the limiting factor.

Figure 2D is confusing. Instead of grouping at each time point, I suggest to group each cell at 3 time points together, with the fiducial point fixed to better show OE and NE growth.

Figure 3, 6. The pink outlines distract from the white signal; naive readers may not see that the white is the relevant signal. Suggest to change the colors to highlight the GFP signals better.

Figure 7D could use more thorough analyses and improved presentation. The vast majority of the points, which are between 0-0.5, do suggest a positive correlation, but a few outliers with high Rab lead the eye. Can the data at the lower Rab values be expanded and/ or analyzed a different way? Can the points corresponding to germling and mature hyphal be labeled, and if appropriate analyzed separately?

The claim that tea1 cells grow 2-3x faster needs to be qualified that it is in comparison of NEs. "tea1Δ cells provided the most striking effect, because while total cell growth speed was nearly identical to that of WT cells, the NEs that grew elongated 2-3X faster than NEs in WT."

Can the authors comment on Cdc42 et al., oscillations between cell tips (Das et al., 2012). Might this somehow coordinate competition between the cell tips?
They may also consider discussing more the cell wall assembly fluctuations (Davi et al), and how these may affect growth rate, and how the Knapp et al paper suggests that increased concentration of the proteome correlates with more rapid growth. I see that these papers are cited under a broad mention, but a more specific discussion would be interesting for the reader.

Reviewer 2

Advance summary and potential significance to field

This is a very nice manuscript from Minc and colleagues describing a cell segmentation tool and its application to study the relation between polar growth and the factors that regulated it in tip-growing fungi. I think the manuscript is a good fit for the JCS tool and resource section. The image analysis tool presented will be useful to the fission yeast and filamentous yeast communities and will help bring more quantitative data analysis. I only have a few minor comments for improvement of the manuscript and its general usefulness, which should be addressed before publication.

Comments for the author

My main comment relates to the description of the method. This is done in the first paragraph of the results, with rather little detail. More detail is provided in the methods and also in the files on the Minc lab website, where one can download the MatLab package, which comes with a user manual. Since the paper is proposed as a tool description, the method would benefit from more information in the first section of the results. In particular, it is necessary to describe it in very practical terms, perhaps with a supplementary figure guiding the reader through the various steps of the segmentation and quantification process (see for instance a recent nice way of doing it in a paper presenting the concept of “super-plots”: Lord et al, JCB 2020). A large number of readers with little quantitative image analysis expertise will be intimidated and may not use the method if that’s not available. The files available on the Minc lab website could also be provided as supporting files with the paper.

I would also suggest modifying the title to emphasize the tool development. Perhaps, something like: A quantitative image analysis tool reveals links between polar protein abundance and tip growth.

Minor comments:

Is the abbreviation "mic" meant to be µm?

At the top of page 8, the “end of septation” may be better described as the time of cell separation.

In Fig 2C, it is not clear what comparison the statistics refer to. The text states that new end growth was still significantly smaller than old end growth, but the stars in the figure seem to indicate comparison between WT and cdc25-22. The issue is similar in other panels, for instance S2C, where the text compares across tips, but the figure gives significance across genotypes. This should be clarified.

The reading of panels 2K-L is a bit difficult. The message that growth in tea1Δ irrespective of whether this is at the old or new end is faster than in either WT end is clear, but the labelling is confusing. For instance, the meaning of the green flat curve is not immediately clear. I eventually understood that it represents the old end growth of tea1Δ cells that grow at the new end. The overlay of all curves is masking many colors. Perhaps splitting that in several plots would help.

There are a few typos, for instance than instead of that 9 lines before end of p. 9

The data strongly suggest there is a limiting factor, but none of the components they investigated is limiting. The authors suggest at the end of the discussion that the limiting effect arises from more complex feedback with cell wall regulation. Alternatively, there could be one factor that is directly limiting, but just has not been identified yet. This could be suggested as alternative hypothesis.

First revision

Author response to reviewers' comments

Reviewer 1 Advance Summary and Potential Significance to Field:
This manuscript tests a hypothesis that the local concentration of certain polarity factors may determine the rate of tip growth in fission yeast. Improved semi-automated methods for tip growth measurements are developed, allowing for measuring growth at each tip of the cell. Fluorescence intensities of polarity factors in a variety of cases, including natural differences between tips, show that growing tips often contain "saturating" amounts of these factors that do not correlate well with growth rate. These findings suggest that growth rate is not only set by the numbers of these polarity factors at the tip. Interesting examples of differential growth rates at tips in the same cell are explored.

In general, this study provides important quantitative data on fission yeast growth and polarity factors that will be foundational for this field. The work is of high technical quality, and I appreciate the quantitative nature of the data. It is also very well written. I think it is appropriate for publication and have only a few minor comments.

We thank the referee for her/his positive comments on our work

Reviewer 1 Comments for the Author: The title "dynamic survey" should be reconsidered. The survey is not what is dynamic here.

We have now changed the title, to address this comment, and one from Reviewer 2 (see below).

Can the authors see if the total concentrations (in the whole cell) of representative polarity factors is maintained through the cell cycle? A large cell cycle fluctuation such as at NETO would affect interpretations about competition between tips and ideas about dilution, for instance. This data could be obtained from the preexisting images, or the authors might be able to cite publish data in some cases.

Our methodology, as presented here has been designed to compute the polarized accumulation of specific factors at cell tips. As such, we directly account for photobleaching effects by normalizing the signal at cell tips to that in the cytoplasm. Its application to compute the total concentration of representative factors, is largely feasible, but requires an independent assessment of photobleaching (e.g. by filming fixed cells expressing different polar factors). Re-calibrating all our experiments would take a large amount of time, and is tangential to the method and outputs we report here. Following referee's suggestion, we have now updated the text to clearly explain these aspects of our work, and refer to Knapp et al. 2019, where the total concentration of factors including bgs4, pom1 and sec6, and others is quantified during the cell cycle, and suggests no net global variations in total protein concentration over time.

Can the authors give some sense when in the cell cycle does the cell reach the plateau in growth rate (relative to concentration of polarity factors at the tip)? For instance, is it the beginning of G2 phase, or near the end?

We thank the referee for this suggestion, we have now clarified that cells appear to reach a plateau in the total growth speed toward the end of G2 indeed.

The authors should add in the discussion that the activity of proteins (such as by regulation by phosphorylation) rather than absolute numbers of proteins might be the limiting factor.

We agree, and have now added this possibility in the discussion.

Figure 2D is confusing. Instead of grouping at each time point, I suggest to group each cell at 3 time points together, with the fiducial point fixed to better show OE and NE growth.

We have now modified this figure panel accordingly, and hope it is better.

Figure 3, 6. The pink outlines distract from the white signal; naive readers may not see that the white is the relevant signal. Suggest to change the colors to highlight the GFP signals better.

We have now replaced the lectin signals with dotted lines marking the cell outlines based on the lectin signals. We hope this is improved.

Figure 7D could use more thorough analyses and improved presentation. The vast majority of the points, which are between 0-0.5, do suggest a positive correlation, but a few outliers with high Rab lead the eye. Can the data at the lower Rab values be expanded and/ or analyzed a different way? Can the points corresponding to germling and mature hyphal be labeled, and if appropriate, analyzed separately?

We thank the referee for suggesting this. We have now represented this analysis by separating points from germlings and mature hyphae. This highlights a clear saturation in growth rates for hyphae for a large range of Rab concentration, and a more dose-dependence behavior followed by a saturation plateau for germlings.

The claim that tea1 cells grow 2-3x faster needs to be qualified that it is in comparison of NEs. “tea1Δ cells provided the most striking effect, because while total cell growth speed was nearly identical to that of WT cells, the NEs that grew elongated 2-3X faster than NEs in WT.”

We agree that our statement was misleading. We have now clarified that old ends can grow up to 1.7X times faster and that new end grow 3X faster.

Can the authors comment on Cdc42 et al., oscillations between cell tips (Das et al., 2012). Might this somehow coordinate competition between the cell tips? They may also consider discussing more the cell wall assembly fluctuations (Davi et al), and how these may affect growth rate, and how the Knapp et al paper suggests that increased concentration of the proteome correlates with more rapid growth. I see that these papers are cited under a broad mention, but a more specific discussion would be interesting for the reader.

We have now expanded our discussion to mention these different aspects better.

Reviewer 2 Advance Summary and Potential Significance to Field:
This is a very nice manuscript from Minc and colleagues describing a cell segmentation tool and its application to study the relation between polar growth and the factors that regulated it in tip-growing fungi. I think the manuscript is a good fit for the JCS tool and resource section. The image analysis tool presented will be useful to the fission yeast and filamentous yeast communities and will help bring more quantitative data analysis. I only have a few minor comments for improvement of the manuscript and its general usefulness, which should be addressed before publication.

We thank the referee for her/his positive assessment of our work.

Reviewer 2 Comments for the Author:
My main comment relates to the description of the method. This is done in the first paragraph of the results, with rather little detail. More detail is provided in the methods and also in the files on the Minc lab website, where one can download the MatLab package, which comes with a user manual. Since the paper is proposed as a tool description, the method would benefit from more information in the first section of the results. In particular, it is necessary to describe it in very practical terms, perhaps with a supplementary figure guiding the reader through the various steps of the segmentation and quantification process (see for instance a recent nice way of doing it in a paper presenting the concept of “super-plots”: Lord et al, JCB 2020). A large number of readers with little quantitative image analysis expertise will be intimidated and may not use the method if that’s not available. The files available on the Minc lab website could also be provided as supporting files with the paper.

We agree, and have now included a new supplementary figure for the method, as well as have expanded the first part of the text to better detail the methodology. We have also placed a direct download link in supplementary material for the software package as it is too large for uploading on the submission server (~2 GB).

I would also suggest modifying the title to emphasize the tool development. Perhaps, something like: A quantitative image analysis tool reveals links between polar protein abundance and tip growth.

We agree, and have now modified the title (see also reply to ref #1 comment).

Minor comments:
Is the abbreviation "mic" meant to be µm?

Yes, we apologize for this typo that we have now corrected

At the top of page 8, the “end of septation” may be better described as the time of cell separation.

In fission yeast, cells may become visually separated sometimes much later than at the end of septation. Thus, although we agree that the end of septation indeed corresponds to the physical separation of daughter cells’ cytoplasms, we prefer to keep end of septation to avoid confusion.

In Fig 2C, it is not clear what comparison the statistics refer to. The text states that new end growth was still significantly smaller than old end growth, but the stars in the figure seem to indicate comparison between WT and cdc25-22. The issue is similar in other panels, for instance S2C, where the text compares across tips, but the figure gives significance across genotypes. This should be clarified.

We apologize for this confusion, we have now changed this figure panel. For panel S2C, we have now specified in the text that the significance refers to a comparison between genotypes. We hope it is clearer.

The reading of panels 2K-L is a bit difficult. The message that growth in tea1Δ irrespective of whether this is at the old or new end is faster than in either WT end is clear, but the labelling is confusing. For instance, the meaning of the green flat curve is not immediately clear. I eventually understood that it represents the old end growth of tea1Δ cells that grow at the new end. The overlay of all curves is masking many colors. Perhaps splitting that in several plots would help.

We have followed suggestions, and now generated four independent graphs for *tea1Δ* cells (2 for the new end growing and 2 for the old end growing). We hope it is clearer now.

There are a few typos, for instance than instead of that 9 lines before end of p. 9

We did not understand why we need to replace the word “than” with “that” in this sentence. However, we have now made a systematic effort to remove all typos from the MS.

The data strongly suggest there is a limiting factor, but none of the components they investigated is limiting. The authors suggest at the end of the discussion that the limiting effect arises from more complex feedback with cell wall regulation. Alternatively, there could be one factor that is directly limiting, but just has not been identified yet. This could be suggested as alternative hypothesis.

We agree, and had already mentioned this alternative explanation. We have now improved our discussion, to make it even clearer.

Second decision letter

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AUTHORS: Sarah Taheraly, Dmitry Ershov, Serge Dmitrieff, and Nicolas Minc
ARTICLE TYPE: Tools and Resources

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.