Whi5 is diluted and protein synthesis does not dramatically increase in pre-Start G1

Kurt Schmoller, Mike Lanz, Jacob Kim, Mardo Koivomagi, Yimiao Qu, Chao Tang, Igor Kukhtevich, Robert Schneider, Fabian Rudolf, David Moreno, Marti Aldea, Rafael Lucena, and Jan Skotheim

Corresponding author(s): Jan Skotheim, Stanford

Review Timeline:

| Event                  | Date       |
|------------------------|------------|
| Submission Date        | 2021-02-05 |
| Editorial Decision     | 2021-03-15 |
| Revision Received      | 2021-04-04 |
| Editorial Decision     | 2021-04-13 |
| Revision Received      | 2021-04-14 |
| Accepted               | 2021-04-16 |

Editor-in-Chief: Matthew Welch

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

RE: Manuscript #E21-01-0029
TITLE: "Whi5 is diluted and protein synthesis does not dramatically increase in pre-Start G1"

Dear Dr. Skotheim,

Your submission has now been reviewed by three experts whose comments are attached below. As you will see, they are somewhat divergent on their views about whether MBoC is the appropriate venue to publish your manuscript, but they make a number of interesting comments. I have discussed these points with the Editor in Chief, Matt Welch, and we agree 1) that there should be a venue to publish disagreements, 2) that the authors of the manuscripts you dispute should be given the opportunity to respond, and 3) that the manuscript should comment strictly on the scientific level.

I will be happy to see a revised manuscript that takes into account the reviewers' comments, notably toning down the text to remove any sarcasm or irony and simply sticking to the facts. The MTA should also be removed from supplements. The manuscript should stay purely on the scientific argument. I should be able to make a decision without further consultation with the reviewers.

Given the unusual format of your submission, your manuscript would be published as a letter to the editor, rather than a research paper. Upon acceptance, the authors of the two disputed papers will be invited to write a response, which will be published in the same issue as manuscript.

Sincerely,
Sophie Martin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Skotheim,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

Authors of Articles and Brief Communications whose manuscripts have returned for minor revision ("revise only") are encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract. Science Sketch Editors on the MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you
Reviewer #1 (Remarks to the Author):

Review of "Whi5 is diluted . . ." by Schmoller, . . . and Skotheim, for MBoC.

The authors discuss a controversy in the literature about whether the cell cycle regulator Whi5 is diluted by growth during G1 phase, and argue that two paper suggesting the contrary are mistaken.

In terms of purely scientific review, I think things are fairly clear. The science in this manuscript is good, and gives entirely plausible arguments as to how two other papers may have come to the wrong conclusion. It is important that disagreeing scientists have a mechanism for eventually agreeing on the facts. I am supportive.

In slightly more detail, Fig. 1 shows very plausibly that a common kind of error in taking means when means should not be taken gives a misleading conclusion in Litsios et al. This argument seems to me likely correct. My only small residual doubt is that I have not gone through the previous paper of Litsios in detail, and so am not completely certain that Litsios et al. actually made the arithmetic manipulations attributed to them here. It is often very difficult to understand exactly what arithmetic previous authors have done by reading their paper, and this affects both my ability to review, and the ability of Schmoller et al. to critique.

Likewise Fig. 2 is plausible and convincing and suggests that the mass spec data of Litsios et al. are inconclusive.

Fig. 3 was somewhat less convincing to me. Schmoller et al. argue that Litsios et al. " . . . did not account for fluorescence signal in the newly formed bud and instead only measured signal from the mother's cell body". One really needs the raw imaging data for this argument, and it is a pity that it was not available. Still, it is a plausible argument.

The language in this manuscript is quite strong and no doubt will be embarrassing for the authors of the Litsios et al. and Dorsey et al. papers. Still, as scientists, we do need to have a mechanism for agreeing on the facts, and clear language is part of that mechanism-so I support clear language. Also, in the absence of clear language and I guess some degree of embarrassment, bad science can spread unchecked. Still, there are a few places, maybe especially in regard to the Dorsey paper, where than language goes beyond the needed clarity. The sentence "We cannot leave unnoted the irony . . ." was entertaining to read, but maybe should not be published. But there are other sentences in this section that could be toned down without sacrificing clarity. If this manuscript were accepted, possibly the final language should be re-reviewed. Ideally the language should be kinder, but without sacrificing any clarity, to the extent that is possible.

If this Schmoller et al. manuscript were to be published, I think it would be beneficial if the authors of the Litsios et al. and Dorsey et al. papers were given a chance to respond simultaneously with publication. No doubt this is unusual and outside what MBoC normally does, but still it would be a very good thing in this case. We do not just want to come to agreement on what happens to Whi5, we want an example of how disagreeing scientists can come to agreement.

Reviewer #2 (Remarks to the Author):

See attached.

Reviewer #3 (Remarks to the Author):

The question of how cell growth is coordinated with the cell cycle is an important, longstanding question that has been of considerable interest in recent years. A 2015 paper from the Skotheim lab demonstrated that as yeast cells grow in G1 it is dilution of the transcriptional repressor Whi5, and not accumulation of the G1 cyclin Cln3, that triggers entry into the cell cycle. Since that original publication, several groups have confirmed this finding in yeast and the Skotheim lab has shown that cell size is controlled by a similar mechanism in human cells. However, two recent papers challenge the main findings in the original yeast paper and come to the opposite conclusions regarding levels of Whi5 and Cln3 during G1. The current manuscript
reanalyzes the data from these conflicting studies and argues that their original conclusions were correct.

Overall, I find the authors' arguments clear and convincing. Their reanalysis brings to light serious issues with the two conflicting studies and makes a compelling argument that the conclusions in the original publication are correct. This paper will be of considerable interest to the field since it will help everyone to make sense of the seemingly conflicting results that have been published. I do not think the analysis or data presentation require any revision. However, I have a few suggestions for revisions to the text that could be made to improve the manuscript.

1. What is the source of the data that was replotted in Fig 1 and Fig 3? Citing specific source data files from Litsios would be useful.

2. On page 4, "Litsios et al. employed mass spectrometry to determine relative changes in Cln3 and Whi5 protein concentrations..." Citing the specific figures in Litsios that show these data would be helpful.

3. On page 6, "To shed light on this issue, we requested the raw microscopy files... We declined to sign the agreement and were therefore not given access to any of the unprocessed imaging data." Although the authors' frustration is understandable, it is sufficient to say that the raw microscopy files were not made available for reanalysis. It is unnecessarily confrontational to include the unsigned MTA, which doesn't support any scientific points or conclusions, as a supplemental file.

4. On page 7, "We cannot leave unnoted the irony that Dorsey et al. aim..." This sentence should be removed or rephrased to simply refute the scientific point without sarcasm or allusions to irony.
Detailed response to the Reviewers

Below is a detailed response to the points made by the reviewers. The reviewers’ comments are shown in **bold text**, while our responses are in normal font.

Reviewer #1 (Remarks to the Author):

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We agree with the reviewer that it is often hard to understand how exactly data have been averaged or normalized. We therefore contacted the authors of Litsios et al. to confirm our interpretation before submitting our manuscript. Prof Heinemann confirmed our representation of their analysis we show in Fig. 1.

Likewise Fig. 2 is plausible and convincing and suggests that the mass spec data of Litsios et al. are inconclusive.

Fig. 3 was somewhat less convincing to me. Schmoller et al. argue that Litsios et al. " . . . did not account for fluorescence signal in the newly formed bud and instead only measured signal from the mother's cell body". One really needs the raw imaging data for this argument, and it is a pity that it was not available. Still, it is a plausible argument.

We agree that having access to the raw data would have been useful. We tried repeatedly for several months to gain access to the raw data, which unfortunately (and shockingly) has been denied and would have only been accessible after signing a highly unusual and restrictive data transfer agreement (note that signing this agreement would have made writing an article like this using any of the raw data highly
The language in this manuscript is quite strong and no doubt will be embarrassing for the authors of the Litsios et al. and Dorsey et al. papers. Still, as scientists, we do need to have a mechanism for agreeing on the facts, and clear language is part of that mechanism—so I support clear language. Also, in the absence of clear language and I guess some degree of embarrassment, bad science can spread unchecked. Still, there are a few places, maybe especially in regard to the Dorsey paper, where than language goes beyond the needed clarity. The sentence "We cannot leave unnoted the irony . . ." was entertaining to read, but maybe should not be published. But there are other sentences in this section that could be toned down without sacrificing clarity. If this manuscript were accepted, possibly the final language should be re-reviewed. Ideally the language should be kinder, but without sacrificing any clarity, to the extent that is possible.

We revised our manuscript according to the suggestions of the reviewers.

If this Schmoller et al. manuscript were to be published, I think it would be beneficial if the authors of the Litsios et al. and Dorsey et al. papers were given a chance to respond simultaneously with publication. No doubt this is unusual and outside what MBoC normally does, but still it would be a very good thing in this case. We do not just want to come to agreement on what happens to Whi5, we want an example of how disagreeing scientists can come to agreement.
Reviewer #2 (Remarks to the Author):

The fundamental point of this manuscript is to buttress one claim, that the Whi5 concentration falls as budding yeast cells grow during G1, and to dispute another, that there are pulses of Cln3 expression during G1. Because the manuscript is almost entirely about the re-analysis of published data, the key question is whether this is a stand-alone paper or a commentary (often titled Matters Arising) about previous work. There are three possible positions on this question: 1) The issue is so important to biology in general that a rapid publication of a re-analysis of existing data is crucial and justifies a stand-alone paper. 2) The issue concerns a particular field and the journal that published the disputed paper should entertain a Matters Arising piece, which includes both a reinterpretation of published data, and an opportunity for the original authors to discuss and potentially dispute the reinterpretation. 3) The best option is for the parties with different interpretations to discuss their differences and either agree that one is correct or design and execute new experiments, performed in laboratories on both sides of the dispute, to try and resolve the discrepancy and jointly publish their findings if they disagree with previously published work. The third option is the best way of moving science forward but depends on a level of collegiality and open-mindedness about the outcome of experiments and the interpretation of data. The first is the least productive: if the original authors are not given a chance to respond to the reinterpretation of their work, they will then try to publish a challenge to the reinterpretation, which would produce a second round of reinterpretation, and so on, ad infinitum. The current authors’ response would be that they are taking issue with two separate papers and would like to publish a single response, demolishing the claims of both, rather than attacking them individually. I have some sympathy with this position, but I think when previous work is attacked, so forcefully, authors have a right to respond to the attack and readers should then be able to make their own judgement about the issues in dispute. My overall assessment is that there are genuine discrepancies in the way different authors have analyzed the same data and that the current manuscript points out a variety of ways in which the data analysis in two previous papers may have been flawed. But I also see ways in which the current authors are manipulating their presentation to persuade the reader that previous claims are worthless, which violates the standard that those who question previous claims should be both rigorous and fair to earlier work.

Major issues

I think the authors are being disingenuous in their attempt to dispute previous claims. Two examples follow. 1) Figure 1d gives the impression that that aligning cells at Start will fully mask the fall in Whi5 concentration and the text is ambiguous about what is to be expected: “it becomes immediately obvious that the resulting mean does not reflect the dynamics of dilution, but instead strongly depends on the distribution of pre-Start-G1 durations.” The simplest assumption is that this distribution is uniform and I therefore simulated an exponential decay in Whi5 concentration from birth and took 101 cells whose interval between birth and start varied smoothly between 0 and 1/l, where the normalized concentration of Whi5 falls as exp(-t/l). If I align them at start, the Whi5 concentration falls from 1 at time 0 to 0.63 at Start. This is smaller than the fall from 0 to 0.37 if the cells are aligned at birth, but the cartoon argument in Figure 1d that there will be no fall is false. Thus
the current authors are right that aligning traces at Start reduces the ability to detect Whi5 depletion but their argument that it abolishes it, made graphically in Fig 1d, is also incorrect. Resolving this type of discrepancy is why this work should be Matters Arising, so that the original authors can have the chance to point out errors in the current analysis.

We agree with the reviewer’s conclusion that aligning the traces at Start while normalizing at birth does not completely abolish the decrease in the mean. In fact, our example illustrated in Fig. 1d shows a decrease of about 25% (rather than 50%), so it is not entirely clear to us what the reviewer thinks we misrepresented. But given that the simulation performed by the reviewer demonstrated that aligning at Start resulted in an apparent dilution to 63% rather than 37%, we think that they agree with our point that averaging at Start results in a wrong reflection of the dynamics of the dilution. The exact consequences also depend on the distribution of G1 durations, which is why we directly compared the two approaches using the actual data (including the actual distribution of G1 duration) in Fig. 1e-g.

2) The current authors choose to reanalyze Cln3 concentrations rather than Cln3 amounts, justifying this choice by saying “protein concentrations are typically the more relevant quantities for kinase reactions, such as those driven by Cln3-Cdk1." But the Litsios paper discussed Cln3 amounts and it is entirely possible that it is the amount of protein rather than its concentration that matters. Choosing to analyze a different parameter than the one that previous authors reported seems like sleight of hand.

One important reason why we think it is useful to look at protein concentrations in the context of the claims made by Litsios et al. is that typically protein synthesis rate increases with cell size to maintain roughly constant concentrations. However, we see the point made by the reviewer that we should not add further confusion by putting the focus on the analysis of a different parameter. We rewrote the manuscript to emphasize our main point, which is that we do not see a dramatic (2-3 fold) global increase in protein synthesis prior to Start. We moved the previous Fig. 3a to the supporting information where it is now Fig. S8.

I am concerned that there is an ad hominem flavor to the manuscript with statements about “poor analysis methods” and “we cannot leave unnoted the irony”, reports on what previous authors did and did not provide and a discussion of a dispute about data transfer agreements. Since MBoC did not publish either of the papers that are being discussed, it is powerless to try and adjudicate disputes about data access, whereas the journals that originally published the papers that are being reanalyzed (Cell Systems and Nature Cell Biology) can request that authors follow their policies for sharing data.

We revised the wording according to the suggestions of the reviewers and removed any reference to the data transfer agreement. From a scientific point of view, the ideal approach would have been to directly analyze the raw microscopy files associated to the experiments to identify the origin of the conflicting results. In fact, we offered to Dr. Heinemann to share data, strains, and analysis pipelines, even before
Litsios et al. was published as we became aware of this work at a scientific conference. We then requested the raw imaging files several times after publication. Since the fact that we did not get access to the raw data (which is in direct conflict to the data sharing policy of NCB) limits our ability to identify the origins of conflicting results, we think it is valid for us to clarify somewhere in the manuscript that we did not get access to the original data.

**Minor issue**

**The list of references should have a complete list of all authors.**

We adjusted the references to MBoC style.
Reviewer #3 (Remarks to the Author):

The question of how cell growth is coordinate with the cell cycle is an important, longstanding question that has been of considerable interest in recent years. A 2015 paper from the Skotheim lab demonstrated that as yeast cells grow in G1 it is dilution of the transcriptional repressor Whi5, and not accumulation of the G1 cyclin Cln3, that triggers entry into the cell cycle. Since that original publication, several groups have confirmed this finding in yeast and the Skotheim lab has shown that cell size is controlled by a similar mechanism in human cells. However, two recent papers challenge the main findings in the original yeast paper and come to the opposite conclusions regarding levels of Whi5 and Cln3 during G1. The current manuscript reanalyzes the data from these conflicting studies and argues that their original conclusions were correct.

Overall, I find the authors' arguments clear and convincing. Their reanalysis brings to light serious issues with the two conflicting studies and makes a compelling argument that the conclusions in the original publication are correct. This paper will be of considerable interest to the field since it will help everyone to make sense of the seemingly conflicting results that have been published. I do not think the analysis or data presentation require any revision. However, I have a few suggestions for revisions to the text that could be made to improve the manuscript.

1. What is the source of the data that was replotted in Fig 1 and Fig 3? Citing specific source data files from Litsios would be useful.

   The data plotted in Fig. 1 were received from the authors of Litsios et al. upon request. The data used in the previous Fig. 3a (now moved to the supplement) were obtained from the source file (on github) provided in Litsios et al. We now clarified this in the manuscript.

2. On page 4, "Litsios et al. employed mass spectrometry to determine relative changes in Cln3 and Whi5 protein concentrations..." Citing the specific figures in Litsios that show these data would be helpful.

   We added references to the figures in Litsios et al. as suggested.

3. On page 6, "To shed light on this issue, we requested the raw microscopy files... We declined to sign the agreement and were therefore not given access to any of the unprocessed imaging data." Although the authors' frustration is understandable, it is sufficient to say that the raw microscopy files were not made available for reanalysis. It is unnecessarily confrontational to include the unsigned MTA, which doesn't support any scientific points or conclusions, as a supplemental file.

   We removed the MTA and any references to it from the manuscript.
4. On page 7, "We cannot leave unnoted the irony that Dorsey et al. aim..." This sentence should be removed or rephrased to simply refute the scientific point without sarcasm or allusions to irony.

We changed this section and others that use less formal language as suggested by the reviewers.
Dear Dr. Skotheim,

Thank you for revising your submission. I will be happy to accept it as a letter to the editor for publication in MBoC, but I must first ask you to remove the abstract before I can formally do so. Feel free to remodel slightly the start of your introduction if you want to blend in your main message at the start of your text.

As previously explained, we will then invite the authors of the two disputed papers to write a response.

Best wishes,
Sophie Martin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Skotheim,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor’s decision letter above and the reviewer comments (if any) below.

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Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

Authors of Articles and Brief Communications whose manuscripts have returned for minor revision ("revise only") are encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract. Science Sketch Editors on the MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,
Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org
April 14, 2021

To: Dr. Sophie Martin
Monitoring Editor
Molecular Biology of the Cell

We are submitting a revised manuscript titled ‘Whi5 is diluted and protein synthesis does not dramatically increase in pre-Start G1’ for publication as a letter to the editor in Molecular Biology of the Cell. We have revised the text to remove the abstract and integrate that information with the first two paragraphs of the text as you requested. We have also generated a separate supporting information file and uploaded publication quality figures.

Thank you for your time and attention that you have given to the publication process of our work. We are very glad that MBoC will publish this piece so that we can communicate with our field some points that are quite important for us to make.

Sincerely,

Dr. Jan M. Skotheim
Professor
Department of Biology
Stanford University
3rd Editorial Decision

April 16, 2021

RE: Manuscript #E21-01-0029RR
TITLE: "Whi5 is diluted and protein synthesis does not dramatically increase in pre-Start G1"

Dear Dr Skotheim,

I am pleased to accept your letter for publication in MBoC.

Best wishes,
Sophie Martin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Skotheim:

Congratulations on the acceptance of your manuscript.

We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org