molecular systems biology

Mapping the interaction of Snf1 with TORC1 in Saccharomyces cerevisiae

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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 28 March 2011

Thank you again for submitting your work to Molecular Systems Biology. We have now heard back from the three referees whom we asked to evaluate your manuscript. As you will see from the reports below, the referees raise substantial concerns on your work, which, I am afraid to say, preclude its publication in its present stage.

While the reviewers find the general topic of the study potentially interesting and recognize the quality of the data, it appears that considerable additional experimentation and analysis are required to support the main conclusions of the present study. In particular, the points raised by reviewer #1 and to some extend by reviewer #3, seem to raise significant concerns with regard to the conclusiveness of the major claims made in this work.

In view of these concerns, which affect the main conclusions of the study, I am afraid I see no choice but to return the manuscript with the message that we cannot offer to publish it.

Nevertheless, in view of the interest expressed by the reviewers for the subject matter and your approach, we would not be opposed to consider a new submission which would extend the present work with the suitable additional analysis. Convincingly addressing the issue the potentially redundancy of tor1 and tor2 and providing additional support for some of the other claims seems essential in this regard.

This would have a new number and receipt date. We recognise that this may involve further experimentation and analysis, and we can give no guarantee about its eventual acceptability. However, if you do decide to follow this course then it would be helpful to enclose with your re-submission an account of how the work has been altered in response to the points raised in the

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present review.

I am sorry that the review of your work did not result in a more favourable outcome on this occasion, but I hope that you will not be discouraged from sending your work Molecular Systems Biology in the future.

Thank you for the opportunity to examine this work.

Yours sincerely,

Editor
Molecular Systems Biology

Reviewer #1 (Remarks to the Author):

The present paper uses a combination of transcriptomics and phosphoproteomics to examine the phenotypes of WT, SNF1, TOR1, and SNF1/TOR1 double deletion yeast under carbon and nitrogen limitation. The technology is very impressive, and augmented further by analysis also of amino acid and lipid levels. Unfortunately, due to questionable choice of experimental design and insufficient or inaccurate data interpretation, the paper does not achieve its potential. The figures are also not compelling.

Rather than addressing every interpretation in the text, I will focus on the main claims in the abstract:

1. That SNF1 regulates a much broader range of biological processes than TOR1. This conclusion, while perhaps correct, is not supported by the present experiments because they (1) do not disrput TOR2, which may be compensating for loss of TOR1, and (2) do not examine a well-fed condition (or nutrient upshift) where TOR activity might be more important than that of SNF1. In essence, the authors work in nutrient-limited conditions where SNF1 was known to be active, and TOR1 largely inactive, and then conclude from the weak phenotype of the TOR1 knockout that TOR1 doesn't matter.

2. That SNF1 regulates ammonia assimilation and amino acid biosynthesis through GDH3. The authors would need to examine the genetic interaction of GDH3 with SNF1 in controlling amino acid levels to substantiate their claim. Also, the bigger effect of SNF1 on glutamine than glutamate levels seems to conflict with GDH3 being the key regulated step.

3. That changes in amino acid levels can be sensed by TOR1, which further regulates AA biosynthetic genes through Gcn4. While this is already part of the canon, the present paper provides no data tying TOR1 activity to Gcn4. There is no good mechanism provided for the higher glutamine levels in the TOR1 knockout strains.

4. That fatty acid might be regulated by TOR1 through peroxisome and beta-oxidation. The provided data shows no statistically significant effect of TOR1 on fatty acids. The claims in the main text are overtly incorrect.

In summary, none of the overarching claims in this paper are supported by the data.

Reviewer #2 (Remarks to the Author):

GENERAL COMMENT

This is an interesting paper that addresses the difficult task of trying to establish the interactions between the products of genes Snf1 and Tor1 through systems biology analysis. Three levels of large scale datasets, transcrptome, phosphoproteome and metabolome, were analysed in two different nutrient conditions, C-limited (glucose) and N-limited (ammonia). These two genes code for serine/threonine protein kinases, both of which are important kinases involved in nutrient-

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induced signalling pathways. Tor1 protein senses nitrogen availability and regulates the cellular growth, but Snf1 protein (homologue of AMPK) also plays a role in nitrogen signaling. However, the hierarchy of the regulation between Snf1 and Tor1 in yeast remains unclear.

It is difficult to try to elucidate the interactions, because a complex net of interactions connect genes to phenotypes, and consequently the phenotype observed when changing a particular nutritional stimulus, or when there is a genetic modi cation, re ects the action of a whole signalling network, which complicates the interpretation of the results. This is clear in this paper, where more than 600 genes have varied their expression in some of the conditions studied. In this context the work done in this manuscript, which is the continuation of previous work of the group, represents a step in the elucidation of the network that involve Snf1 and Tor1, and the conclusions (represented in Fig 6) amount to a sensible working hypothesis.

There are, however some points that need to be improved or clarified. Also as there are so much data I think that some errors have slipped in the text, which needs to be checked.

An interesting point in this Ms which is not discussed, is that although the genes Snf1 and Tor1 are almost silent in relation to growth, deleting them has an important effect at the level of metabolite expression. For example the deletion of Tor1, in N-limited conditions, induces a high increase in the aminoacid level compared to the reference (page 13, Fig 3), which is a nice illustration of the ideas discussed in the paper of Raamsdonk et al. (2001) Nature Biotechnol. 19, 45-50, and the commentary by Cornish-Bowden and C·rdenas (2001) Nature 409, 571-572, that concentrations of intermediates are more sensitive to mutations than metabolic fluxes are.

SPECIFIC COMMENTS

1) As one of the purposes of this work is to study the impact of carbon (glucose) or nitrogen (ammonia) limitation, it is important to indicate clearly the glucose and ammonia concentration of the culture medium that has been used in each case, which is not the case in the present Ms. Thus on page 19 it says "For the C-limited cultures, the medium composition was the same as used before (Zhang et al, 2010a). For N-limited cultivation, the medium was the same as the C-limited except that the concentrations for (NH4)2SO4 and glucose were 1.0g/l and 60.0 g/l, respectively.
But, in Zhang et al, 2010a it is not much clearer either. In the Chemostat cultivation it says "The medium composition was the same as the one used in batch cultivations except that the glucose concentration was 10 g/1." And in the Batch cultivations it says "Four litres of defined medium containing 30 g/l of glucose ..." Nowhere I was able to find the ammonia concentration.

2) The level of glucose in culture medium is very important in yeast, because on the one hand at high glucose level there is a repression of genes involved in the fermentation of other nutrients (glucose repression), but in addition the yeast will be doing only fermentation and not aerobic respiration. In limiting glucose concentration together with the release of the glucose repression, there is going to be oxidative phosphorylation.
Although the phenomenon of glucose repression is clearly stated in the Ms, together with the fact that Snf1 appears to be involved in releasing this repression, the fact that in one nutrient condition (glucose limited) the yeast will be doing oxidative phosphorylation while in the other (N-limited) will be doing fermentation is not discussed. This means a big change and consequently it is not surprising that the first principal component (PC1 in Figs, 1 and 2) distinguished the impact of nutrient condition (in the case of transcriptome accounts for 40% of total variance). This needs to be commented on.

3) As it was already known, neither Snf1 gene nor Tor1 deletion was lethal, and the effect on the growth was rather modest. However, no data for growth is given in N-limited conditions and there are no comments either. As the CO2 emission was used to determine the specific growth rate in the C-limited (glucose) conditions, I imagine that this method may not be adequate for conditions when glucose is not limiting, but spectroscopic measurements can be used. This point needs to be commented on.

4) As one of the mathematical tools used to integrate the data is "Principal Component Analysis" (not "principle component analysis", see point 10), it would be useful to say explicitly in Figure S2 that PCA was applied to decrease the dimension of the dataset (total number of the genes measured

(Fig S2A) and total number of phosphoproteins (FigS2B)) and therefore to decrease the redundancy of the data, as effects of some genes, are highly correlated with others.

5) In table 1 instead of n.d. it would be better to put that the amount is lower than the detection threshold, whatever number that may be. It would be convenient to specify better what is "the specific growth rate" which is determined through the CO2 emission.

6) on page 8, lines 15-16th it says : ′ or Snf1 playing a compensatory rÙle ª. This is not very clear because in that case the double mutant should show an effect and it is not the case.

7) The Supplementary figure S2 is not mentioned in the text, which is a pity. It can be added on page 8, line 7 and on page 10, line 9

8) on page 11, lines 6-7 it says ... "(Bdf1, Ctr9, Eaf1, Leo1, Rph1, Sin3, and Spt6) were also found to be differentially phosphorylated only in the strains snf1 and snf1 tor1 ." "ONLY" does not apply to Ctr9 and Spt6 which also varied with tor1 deletion.
Similarly on page 12, lines 6-7 it says "the regulatory subunit of PKA pathway, was found to be less phosphorylated in the mutant strains snf1 and snf1 tor1 " This is not correct as in the double mutant, and in the strain with Tor1 deleted there is no effect.

9) A confusing aspect of this paper for the nonspecialist reader [though common in many papers] is the use of the same name for the gene and the corresponding one of the protein. The presentation could be much simpler if, for example, "Tor1" is used for the gene and "tor1protein" for the product of gene expression.

10) Although Snf1 kinase is homologous with AMPK , as it is not activated by AMP one needs to be cautious in the extrapolation from AMPK functional role to the one of Snf1.

11) How is the significance of the change defined in Figures S3, S4 ?
In the case of Figures S1 and S5 the meaning of the numbers are quite clear, as they represent the level of expression (log base 2)

12) The language needs some improvement. For example :
i) On Page 8, lines 5-6 it says "principle component analysis", the correct name of the mathematical procedure is "Principal Component Analysis ª. The same error in other parts of the text as the title of Fis 1 and 2, and Fig S2

ii) On page 11, line 21 it says : "certain proteins may be too low abundant"

iii) On page 14, line 11 says "impaired balance between protein translation and turnover as a ..." Probably what it is trying to say is that there is impaired balance between protein synthesis and degradation.

There are other examples of this type in the text.

Additional Comment : In several of the figures (as 5, S4, S5)it is difficult to see the data, even on the screen, but I imagine this will be solved at the Editorial office.

Reviewer #3 (Remarks to the Author):

This manuscript describes a systems biology approach to understanding the interaction between two nutrient signalling pathways in yeast. The Snf1 kinase signalling pathway in yeast is activated under conditions of nutrient and energy stress while the TORC1 pathway is activated under conditions of nutrient and energy abundance. Work from other groups in yeast and mammals have suggested that these pathways interact and more specifically that Snf1 downregulates TORC1. In this study, the authors use microarray data of mRNA abundance, phosphoprotein abundance and the abundance of other select metabolites to characterize these two signalling pathways and their interactions.

The execution of this study and methods used are excellent. The glaring fault in this study is the use

of the tor1 delete strain. Yeast encode two Tor kinases, Tor1 and Tor2. Tor1 and Tor2 can both assemble into the TORC1 complex. Tor2 is distinct from Tor1 in that it can also assemble into the TORC2 complex. Tor2 is essential for viability, presumably due to requirement for the TORC2 complex. In this study, the authors use a strain lacking the Tor1 kinase. A tor1 delete strain has some reduction in TORC1 activity. It might be a 5% reduction in activity or a 95% reduction. The fact that addition of rapamycin which completely blocks TORC1 causes much more severe growth defects than does tor1 deletion leads me to think the reduction in TORC1 activity caused by tor1 deletion is much closer to 5% than to 95%. Thus this study is really looking at strains with a partial loss in TORC1. I am sure the authors are well aware of this. However, it needs to be much more explicitly stated and discussed.

Despite the limitations of this study caused by the redundancy of Tor1 and Tor2, this paper does advance our understanding. My only recommendation is that the authors amend the text to be very clear and distinct between tor1 and TORC1 and that they address the issue of residual TORC1 activity in a tor1 delete. It would be even better if they had some way to estimate what fraction of TORC1 activity is present in a tor1 delete.

New Submission 20 July 2011

Response to reviewers:

Reviewer #1 (Remarks to the Author):

The present paper uses a combination of transcriptomics and phosphoproteomics to examine the phenotypes of WT, SNF1, TOR1, and SNF1/TOR1 double deletion yeast under carbon and nitrogen limitation. The technology is very impressive, and augmented further by analysis also of amino acid and lipid levels. Unfortunately, due to questionable choice of experimental design and insufficient or inaccurate data interpretation, the paper does not achieve its potential. The figures are also not compelling.

> **We are quite confident on our experimental design, and new experimental data confirm that our tor1 deletion strain indeed have attenuated TORC1 activity. We have revised the paper accordingly.**
>
> **We do not quite understand the un-defined comment that the figures are not compelling, but we have revised figure 6 to make our point more clear.**

Rather than addressing every interpretation in the text, I will focus on the main claims in the abstract:

1. That SNF1 regulates a much broader range of biological processes than TOR1. This conclusion, while perhaps correct, is not supported by the present experiments because they (1) do not disrupt TOR2, which may be compensating for loss of TOR1, and (2) do not examine a well-fed condition (or nutrient upshift) where TOR activity might be more important than that of SNF1. In essence, the authors work in nutrient-limited conditions where SNF1 was known to be active, and TOR1 largely inactive, and then conclude from the weak phenotype of the TOR1 knockout that TOR1 doesn't matter.

> **We thank the reviewer for these comments, which are partially overlapping with the concern raised by Reviewer 3.**
>
> > **(1) We did (could) not delete *TOR2* gene because Tor2 is essential for TORC2 and disruption of this complex is lethal. In the revised MS we discussed the possibility that Tor2 may compensate for Tor1 function. However, our results (including new results added to the paper) showed that this is very unlikely for several reasons: 1) the *TOR2* gene was expressed at a level similar to the *TOR1* gene and the expression was unchanged in both the *tor1*Δ and the *snf1*Δ*tor1*Δ strain compared to the reference strain. This held true for both nutrient-limited conditions. 2) We did a follow-up experiment and clearly showed that Tor1 is responsible for a majority of the TORC1 activity and that Tor2 cannot fully compensate for the loss of Tor1 (similar studies were performed previously with different strain backgrounds and the same results were observed), and therefore *tor1*Δ could represent a knock-down, but not necessarily the complete disruption of TORC1. We have revised the MS and commented on the redundancy between Tor1 and Tor2.**
> >
> > **(2) The reviewer seemingly misunderstood the experimental design in this study. We examined two nutrient-limited conditions, with glucose (C-lim) and ammonia (N-lim) as the limiting nutrient. It is already known that Snf1 is mainly active at C-lim condition while inactive at N-lim. TORC1 was believed to be active at C-lim while the ammonia level is high. However, we showed that it is not the case and the TORC1 activity might be repressed under both nutrient-limited conditions, although we only used a mutant strain that can only represent the knock-down of TORC1. We have revised the conclusion in the MS to emphasize the specific conditions to avoid such misunderstandings.**

2. That SNF1 regulates ammonia assimilation and amino acid biosynthesis through GDH3. The authors would need to examine the genetic interaction of GDH3 with SNF1 in controlling amino acid levels to substantiate their claim. Also, the bigger effect of SNF1 on glutamine than glutamate levels seems to conflict with GDH3 being the key regulated step.

We observed significantly lower levels of glutamine and glutamate (which serve as a major hub for the biosynthesis of many other amino acids) in the *snf1*Δ and *snf1*Δ*tor1*Δ strains at C-lim condition and discussed two possible mechanisms for this reduction. The transcriptome data supported the hypothesis that Snf1 regulates the expression of *GDH3* gene and therefore controls biosynthesis of amino acid. However, to elucidate the molecular mechanism by how Snf1 positively regulates *GDH3* requires extensive targeted studies (e.g. protein-protein interaction, protein-DNA interaction etc) and therefore is beyond the scope of the top-down approach applied in our study, which aimed to identify the coordination between Snf1 and TORC1 pathways at a global level. We have revised the conclusion to avoid confusion and commented on this point.

3. That changes in amino acid levels can be sensed by TOR1, which further regulates AA biosynthetic genes through Gcn4. While this is already part of the canon, the present paper provides no data tying TOR1 activity to Gcn4. There is no good mechanism provided for the higher glutamine levels in the TOR1 knockout strains.

It is known that TORC1 regulate amino acid biosynthesis via Gcn4 and we only used this mechanism to explain the observed changes in amino acid and the expression of the genes encoding the biosynthetic enzymes. We have revised the conclusion and added reference to the Gcn4 regulation to avoid potential confusions.

4. That fatty acid might be regulated by TOR1 through peroxisome and beta-oxidation. The provided data shows no statistically significant effect of TOR1 on fatty acids. The claims in the main text are overtly incorrect.

Our data clearly showed that some species of the FAs had significantly changed levels in *tor1*Δ and *snf1*Δ*tor1*Δ strains at either C-lim or N-lim, although the magnitudes were not as large as those observed in the *snf1*Δ strain, which was expected as Snf1 is well known for its key role in lipid biosynthesis. We therefore hypothesized that TORC1 might be involved in the regulation of FAs.

In summary, none of the overarching claims in this paper are supported by the data.

Reviewer #2 (Remarks to the Author):

GENERAL COMMENT

This is an interesting paper that addresses the difficult task of trying to establish the interactions between the products of genes Snf1 and Tor1 through systems biology analysis. Three levels of large scale datasets, transcrptome, phosphoproteome and metabolome, were analysed in two different nutrient conditions, C-limited (glucose) and N-limited (ammonia). These two genes code for serine/threonine protein kinases, both of which are important kinases involved in nutrient-induced signalling pathways. Tor1 protein senses nitrogen availability and regulates the cellular growth, but

Snf1 protein (homologue of AMPK) also plays a role in nitrogen signaling. However, the hierarchy of the regulation between Snf1 and Tor1 in yeast remains unclear.

It is difficult to try to elucidate the interactions, because a complex net of interactions connect genes to phenotypes, and consequently the phenotype observed when changing a particular nutritional stimulus, or when there is a genetic modiﬁcation, reﬂects the action of a whole signalling network, which complicates the interpretation of the results. This is clear in this paper, where more than 600 genes have varied their expression in some of the conditions studied. In this context the work done in this manuscript, which is the continuation of previous work of the group, represents a step in the elucidation of the network that involve Snf1 and Tor1, and the conclusions (represented in Fig 6) amount to a sensible working hypothesis.

We thank the reviewer for the nice comments.

There are, however some points that need to be improved or clarified. Also as there are so much data I think that some errors have slipped in the text, which needs to be checked.

An interesting point in this Ms which is not discussed, is that although the genes Snf1 and Tor1 are almost silent in relation to growth, deleting them has an important effect at the level of metabolite expression. For example the deletion of Tor1, in N-limited conditions, induces a high increase in the aminoacid level compared to the reference (page 13, Fig 3), which is a nice illustration of the ideas discussed in the paper of Raamsdonk et al. (2001) Nature Biotechnol. 19, 45-50, and the commentary by Cornish-Bowden and Cárdenas (2001) Nature 409, 571-572, that concentrations of intermediates are more sensitive to mutations than metabolic fluxes are.

This is a very good point and we have added a comment and reference to these papers in the text.

SPECIFIC COMMENTS

1) As one of the purposes of this work is to study the impact of carbon (glucose) or nitrogen (ammonia) limitation, it is important to indicate clearly the glucose and ammonia concentration of the culture medium that has been used in each case, which is not the case in the present Ms. Thus on page 19 it says "For the C-limited cultures, the medium composition was the same as used before (Zhang et al, 2010a). For N-limited cultivation, the medium was the same as the C-limited except that the concentrations for $(NH_4)_2SO_4$ and glucose were 1.0g/l and 60.0 g/l, respectively.

But, in Zhang et al, 2010a it is not much clearer either. In the Chemostat cultivation it says "The medium composition was the same as the one used in batch cultivations except that the glucose concentration was 10 g/1." And in the Batch cultivations it says "Four litres of defined medium containing 30 g/l of glucose ..." Nowhere I was able to find the ammonia concentration.

We have revised the section of M&M and describe the media composition clearly.

2) The level of glucose in culture medium is very important in yeast, because on the one hand at high glucose level there is a repression of genes involved in the fermentation of other nutrients (glucose repression), but in addition the yeast will be doing only fermentation and not aerobic respiration. In limiting glucose concentration together with the release of the glucose repression, there is going to be oxidative phosphorylation.

Although the phenomenon of glucose repression is clearly stated in the Ms, together with the fact that Snf1 appears to be involved in releasing this repression, the fact that in one nutrient condition (glucose limited) the yeast will be doing oxidative phosphorylation while in the other (N-limited) will be doing fermentation is not discussed. This means a big change and consequently it is not surprising

that the first principal component (PC1 in Figs, 1 and 2) distinguished the impact of nutrient condition (in the case of transcriptome accounts for 40% of total variance). This needs to be commented on.

We thank the reviewer for the helpful suggestion. Indeed it is true that the medium had a great impact on the whole metabolism of the cell, which was reflected by the PC1. We have revised the MS and commented on that point.

3) As it was already known, neither Snf1 gene nor Tor1 deletion was lethal, and the effect on the growth was rather modest. However, no data for growth is given in N-limited conditions and there are no comments either. As the CO2 emission was used to determine the specific growth rate in the C-limited (glucose) conditions, I imagine that this method may not be adequate for conditions when glucose is not limiting, but spectroscopic measurements can be used. This point needs to be commented on.

The maximum specific growth rate for each strain was only determined based on the CO_2 emission from the batch culture (i.e. no limiting nutrient) before they were shifted to the chemostat culture. However, the specific growth rate in C- or N-limited cultures was fixed by the dilution rate (0.1 h^{-1}). We have revised the MS to make this more clear.

4) As one of the mathematical tools used to integrate the data is "Principal Component Analysis" (not "principle component analysis", see point 10), it would be useful to say explicitly in Figure S2 that PCA was applied to decrease the dimension of the dataset (total number of the genes measured (Fig S2A) and total number of phosphoproteins (FigS2B)) and therefore to decrease the redundancy of the data, as effects of some genes, are highly correlated with others.

We have corrected the name and added the explanation in the MS.

5) In table 1 instead of n.d. it would be better to put that the amount is lower than the detection threshold, whatever number that may be. It would be convenient to specify better what is "the specific growth rate" which is determined through the CO2 emission.

We have replaced the n.d. (not detected) with specific thresholds, which is less than 0.002 Cmol $Cmol^{-1}$. A comment is added to the table that the maximum specific growth rate was determined based on CO_2.

6) on page 8, lines 15-16th it says : « or Snf1 playing a compensatory rôle ». This is not very clear because in that case the double mutant should show an effect and it is not the case.

We removed the speculation that Snf1 is playing a compensatory role.

7) The Supplementary figure S2 is not mentioned in the text, which is a pity. It can be added on page 8, line 7 and on page 10, line 9

We have added the supplementary figure in the main text as the reviewer suggested.

8) on page 11, lines 6-7 it says ... "(Bdf1, Ctr9, Eaf1, Leo1, Rph1, Sin3, and Spt6) were also found to be differentially phosphorylated only in the strains snf1Δ and snf1Δtor1Δ." "ONLY" does not apply to Ctr9 and Spt6 which also varied with tor1 deletion.

Similarly on page 12, lines 6-7 it says "the regulatory subunit of PKA pathway, was found to be less phosphorylated in the mutant strains snf1Δ and snf1Δtor1Δ" This is not correct as in the double mutant, and in the strain with Tor1 deleted there is no effect.

We carefully revised the MS and removed the incorrect statements.

9) A confusing aspect of this paper for the nonspecialist reader [though common in many papers] is the use of the same name for the gene and the corresponding one of the protein. The presentation could be much simpler if, for example, "Tor1" is used for the gene and "tor1protein" for the product of gene expression.

We thank the reviewer for a good suggestion and indeed that will avoid the confusion for non-specialist readers. However, we followed the standard nomenclature for the journal, i.e. all genes are capitalized italic letters indicate wild type, while the lower case means deletion, and the proteins are non-italic, with only the first letter capitalized. We will modify this if requested by the editor.

10) Although Snf1 kinase is homologous with AMPK, as it is not activated by AMP one needs to be cautious in the extrapolation from AMPK functional role to the one of Snf1.

We thank the reviewer for an excellent point and we totally agree with this. We have carefully revised the MS to avoid any possible ambiguousness.

11) How is the significance of the change defined in Figures S3, S4?

In the case of Figures S1 and S5 the meaning of the numbers are quite clear, as they represent the level of expression (log base 2)

The significance in these figure was presented as a score calculated by BAMarray version 3.0 (Ishwaran et al, 2006). Green (negative values): significantly lower; yellow (positive values): significantly higher; black (zero): insignificant.

12) The language needs some improvement. For example:

i) On Page 8, lines 5-6 it says "principle component analysis", the correct name of the mathematical procedure is "Principal Component Analysis ». The same error in other parts of the text as the title of Fis 1 and 2, and Fig S2

ii) On page 11, line 21 it says : "certain proteins may be too low abundant"

iii) On page 14, line 11 says "impaired balance between protein translation and turnover as a ..."

Probably what it is trying to say is that there is impaired balance between protein synthesis and degradation.

There are other examples of this type in the text.

We have carefully revised the MS and hopefully it is much easier to read now.

Additional Comment: In several of the figures (as 5, S4, S5) it is difficult to see the data, even on the screen, but I imagine this will be solved at the Editorial office.

We have tried to increase the contrast of the numbers and make it readable. But, we found the contract numbers become rather distractive as we originally meant to use the color to indicate the changes. However, we will keep this open and make necessary changes if requested by the editor.

Reviewer #3 (Remarks to the Author):

Review for Molecular Systems Biology

Author: Nielsen

Date: Feb 25, 2011

Title: Mapping the interaction of Snf1 with TORC1...

This manuscript describes a systems biology approach to understanding the interaction between two nutrient signalling pathways in yeast. The Snf1 kinase signalling pathway in yeast is activated under conditions of nutrient and energy stress while the TORC1 pathway is activated under conditions of nutrient and energy abundance. Work from other groups in yeast and mammals have suggested that these pathways interact and more specifically that Snf1 downregulates TORC1. In this study, the authors use microarray data of mRNA abundance, phosphoprotein abundance and the abundance of other select metabolites to characterize these two signalling pathways and their interactions.

The execution of this study and methods used are excellent. The glaring fault in this study is the use of the tor1 delete strain. Yeast encode two Tor kinases, Tor1 and Tor2. Tor1 and Tor2 can both assemble into the TORC1 complex. Tor2 is distinct from Tor1 in that it can also assemble into the TORC2 complex. Tor2 is essential for viability, presumably due to requirement for the TORC2 complex. In this study, the authors use a strain lacking the Tor1 kinase. A tor1 delete strain has some reduction in TORC1 activity. It might be a 5% reduction in activity or a 95% reduction. The fact that addition of rapamycin which completely blocks TORC1 causes much more severe growth defects than does tor1 deletion leads me to think the reduction in TORC1 activity caused by tor1 deletion is much closer to 5% than to 95%. Thus this study is really looking at strains with a partial loss in TORC1. I am sure the authors are well aware of this. However, it needs to be much more explicitly stated and discussed.

Despite the limitations of this study caused by the redundancy of Tor1 and Tor2, this paper does advance our understanding. My only recommendation is that the authors amend the text to be very clear and distinct between tor1 and TORC1 and that they address the issue of residual TORC1 activity in a tor1 delete. It would be even better if they had some way to estimate what fraction of TORC1 activity is present in a tor1 delete.

> **We thank the reviewer for the comment. We have performed a follow-up study to assess the remaining TORC1 activity in the *tor1*Δ strain and further discussed the redundancy of Tor1 and Tor2 in the MS. Please see the response to reviewer 1.**

2nd Editorial Decision 20 August 2011

Thank you again for submitting your work to Molecular Systems Biology. We have now heard back from the two referees who accepted to evaluate the revised study. As you will see, referee #2 and #3 are now supportive and I am pleased to inform you that we will be able to accept your study for publication pending the following minor amendments:

- please include the microarray accession number at the end of the microarray section in Materials & Methods.
- the phopshoproteomics dataset should be deposited in a public database (eg PRIDE, Tranche). Please include the respective accession/link/url at the end of to the phosphoproteomics section in Materials & Methods.
- the amino acid and FA measurements should be included in supplementary information in a format that would allow others to reproduce the essential aspects of the analysis and reuse/compare/integrate your data in other studies.

Thank you for submitting this paper to Molecular Systems Biology.

Yours sincerely,

Editor
Molecular Systems Biology

http://www.nature.com/msb

Referee reports

Reviewer #2 (Remarks to the Author):

The authors have responded to the concerns I raised with the earlier version of this paper by including discussion in the text and data in Fig. S1

Reviewer #3 (Remarks to the Author):

I have looked at the revised version of this manuscript and I think that the points raised have been addressed.

I would like to repeat what I said the first time. I think that this is an interesting paper that addresses the difficult task of trying to establish the interactions between the products of genes Snf1 and Tor1 by systems biology analysis. It is difficult because a complex net of interactions connect genes to phenotypes. This is clear in this paper, where more than 600 genes have varied their expression in some of the conditions studied. In this context the work done in this manuscript, represents a step in the elucidation of the network that involve Snf1 and Tor1, and the conclusions amount to a sensible working hypothesis. It is not realistic to expect work like this to arrive at an absolute truth.

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Response to the editor's points:

- Please include the microarray accession number at the end of the microarray section in Materials &
Methods.
The accession numbers for Affymetrix microarray data is added in M&M (page 19 line 1).

- The phopshoproteomics dataset should be deposited in a public database (eg PRIDE, Tranche). Please include the respective accession/link/url at the end of to the phosphoproteomics section in Materials & Methods.

The link for phosphoproteome data is added in M&M (page 20 line 13-15)

- The amino acid and FA measurements should be included in supplementary information in a format that would allow others to reproduce the essential aspects of the analysis and reuse/compare/integrate your data in other studies.

The data for AA and FA (both as biological triplicates) are included in the supplementary information as Table S3 and S4.

Reviewer #2 (Remarks to the Author):

The authors have responded to the concerns I raised with the earlier version of this paper by including discussion in the text and data in Fig. S1

Reviewer #3 (Remarks to the Author):

I have looked at the revised version of this manuscript and I think that the points raised have been addressed.

I would like to repeat what I said the first time. I think that this is an interesting paper that addresses the difficult task of trying to establish the interactions between the products of genes Snf1 and Tor1 by systems biology analysis. It is difficult because a complex net of interactions connect genes to phenotypes. This is clear in this paper, where more than 600 genes have varied their expression in some of the conditions studied. In this context the work done in this manuscript, represents a step in the elucidation of the network that involve Snf1 and Tor1, and the conclusions amount to a sensible working hypothesis. It is not realistic to expect work like this to arrive at an absolute truth.

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