Modeling and Analysis of the Macronutrient Signaling Network in Budding Yeast

Amogh Jalihal, Pavel Kraikivski, T. Murali, and John Tyson

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(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.)

RE: Manuscript #E20-02-0117
TITLE: Modeling and Analysis of the Macronutrient Signaling Network in Budding Yeast

Dear Dr. Tyson:

First, apologies for the delayed reviews. The reviewers, like all of us, got caught by the Covid-19 situation and could not return reviews on time.
It seems that the reviewers disagree here on the paper, with one reviewer having reservations. Please note the two misinterpretations identified by that reviewer. If you can fix this, and explain how you did so, perhaps we can reconsider the paper for publication.

Sincerely,

Leah Edelstein-Keshet
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Tyson,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments 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 haveopted out of publishing the review history.

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

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

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 manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

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

Reviewer #1 (Remarks to the Author):

In their manuscript on „Modeling and Analysis of Macronutrient Signaling of Budding Yeast" the authors describe a comprehensive effort to combine literature information on nutrient sensing cellular signaling networks and the activation of downstream transcription factors into a large model of nutrient signaling that is now able to represent complex responses to the presence of diverse macronutrients. They systematically use published experimental data to fit the model and also provide comprehensive tests and critical analysis of the goodness of fit (and also the weaknesses of fits). Overall this is a very helpful attempt to describe the response of yeast cells to nutrient changes and obtain an overview over the contribution of different parts of the cellular signaling network to the concerted answer.

The manuscript also contains a number of very useful approaches to deal with the remaining uncertainty about mechanisms and choice of parameters. Among them is the concept to select reaction mechanisms only from a small set of equations as provided in the standard component modeling framework. I also appreciate their approach to robustness analysis. It is also good to see the very carefully comparison of model prediction with a large set of individual reported experiments.

Some aspects should potentially be considered before publication:

Major
1) All parameter values are explored as relative values. It remains unclear how these parameters relate to real values with proper units (e.g. time units such as seconds or minutes, Mol, per gram dry weight). This would be very relevant, if one aims to combine these signaling models with metabolic models as the authors suggest. However, it would also be relevant to weight the relative importance of different regulation mechanisms. Given the vast amount of experimental data the authors collected, it should be possible to relate at least most of the parameter values to "real-world"-values with a unit.
2) Figure 2 nicely illustrates temporal behavior of same components after specific stimuli, here in comparison to experimental data. It would be very helpful to have a systematic overview of the time courses of the responses of the major variables to the 8 conditions as represented in Figure 4 (HC/HG, HC/HN,...) as additional supplement. As in supplement 4, just a flag whether the simulated behavior is in agreement with experiments or not or even not recorded would suffice. This would help to understand the physiological consequences of different nutrient changes and certainly also be a useful guide for further experiments.

Minor

P1, abstract - the authors speak about "... cellular responses to unpredictable changes ..." It should be discussed, why those nutrient changes are unpredictable given that yeast has gone through a long evolutionary time with always changing environment. And second, why it matters if the changes are predictable or not.

P23, Eq 2 - sigma remains unexplained
P33, Fig S5 - arrows unexplained

Reviewer #2 (Remarks to the Author):

Jalil et al. have set out to tackle a laudable goal -- to construct a global model for how various nutrient signals are integrated across signalling pathways in the budding yeast, Saccharomyces cerevisiae. This is no easy task, but if successful such a model should be generally useful for developing a deeper understanding of the often complex physiological responses that have been observed experimentally in yeast in studies that combine genetics and biochemistry.

The authors used a necessarily simplified version of the yeast signaling networks under question, and modeled them with a system of ODEs, and fit parameters for the model constrained by prior experimental data collected by a large number of previous studies.

While the approach used is reasonable, I note some concerning misinterpretations of some of the published experimental data. Below are two example taken from the text and Fig 2:

- p. 8: "The pde1Δpde2Δ strain shows an increase in cAMP levels compared to wt [49]." The reference here is Ma et al. 1999, Mol Cell Bio, 10(1): 91-104. The referenced figure is Fig 1A. However, Ma et al. show that the pde double mutant actually has **lower** cAMP levels than WT (the pde1 single mutant shows increased cAMP levels, the pde2 single mutant looks essentially like WT)

- p. 8: "Row C3 depicts trehalase (Nth1) levels after a glucose up-shift; trehalase levels remain low in a tpk3Δ mutant [8]." The reference they cite is Mbonyi et al. 1990, Mol Cell Bio p. 4518-4523. However, the Mbonyi et al. figure (Fig 5) shows trehalase levels when only one of the 3 PKA catalytic subunits (Tpk1, Tpk2, Tpk3) is present (i.e. the mutants in question are tpk1Δtpk2Δ, tpk1Δtpk3Δ, and tpk2Δtpk3Δ). The key point of the Mbonyi paper is that the Tpk3 catalytic subunit behaves quite differently than Tpk1 and Tpk2 w/respect to Trehalase levels. Jalihal et al misinterpret the data in the figure as illustrating a tpk3Δ mutant.

Also, the model of Jalihal et al. only considers a single parameter for PKA, so by definition it can't incorporate or simulate the findings of Mbonyi et al. which is concerned w/the behavior of the different subunits.

I haven't checked all the other example the authors give, but the misinterpetation of prior experiments suggests that the model may have been constrained inappropriately.

Minor comments
==========

* Fig 4 is a very hard to parse with the myriad abbreviations, arrows, and tiny bars. The authors should think about alternatives ways to convey this information about global cellular responses.

* The text several times refers to Pde3. There is no Pde3 in yeast, only the phosphodiesterases Pde1 and Pde2

Response to Reviewers

1 Reviewer #1 (Remarks to the Author):

In their manuscript on "Modeling and Analysis of Macronutrient Signaling of Budding Yeast" the authors describe a comprehensive effort to combine literature information on nutrient sensing cellular signaling networks and the activation of downstream transcription factors into a large model of nutrient signaling that is now able to represent complex responses to the presence of diverse macronutrients. They systematically use published experimental data to fit the model and also provide comprehensive tests and critical analysis of the goodness of fit (and also the weaknesses of fits). Overall this is a very helpful attempt to describe the response of yeast cells to nutrient changes and obtain an overview over the contribution of different parts of the cellular signaling network to the concerted answer.

The manuscript also contains a number of very useful approaches to deal with the remaining uncertainty about mechanisms and choice of parameters. Among them is the concept to select reaction mechanisms only from a small set of equations as provided in the standard component modeling framework. I also appreciate their approach to robustness analysis. It is also good to see the very carefully comparison of model prediction with a large set of individual reported experiments.

Some aspects should potentially be considered before publication.

1.1 Major comments

1. All parameter values are explored as relative values. It remains unclear how these parameters relate to real values with proper units (e.g. time units such as seconds or minutes, Mol, per gram dry weight). This would be very relevant, if one aims to combine these signaling models with metabolic models as the authors suggest. However, it would also be relevant to weight the relative importance of different regulation mechanisms. Given the vast amount of experimental data the authors collected, it should be possible to relate at least most of the parameter values to "real-world"-values with a unit.

   We thank the reviewer for this suggestion to consider proper units for the parameter values in our model. Since we use the Standard Component Modeling framework to construct the differential equations, the ω parameters, which capture the regulatory strengths of post-translational modifications, are dimensionless. Apart from these intrinsically dimensionless parameters, the model has time-scale parameters (k's and γ's) with units of reciprocal time min^{-1} and total abundances of all signaling components, which we set = 1 (i.e., all protein levels are expressed relative to their maximum level). Recently, we have found a resource on the Saccharomyces Genome Database (`https://yeastmine.yeastgenome.org/yeastmine/template.do?name=Gene_ProteinAbundance&scope=all`)that provides estimates of protein abundances in various media conditions across some prominent strains, curated from a variety of published sources. We downloaded the abundance data of the relevant catalytic subunits of the signaling

complexes present in our model and identified the maximum abundance across all strains grown in YEPD. These values are summarized in Table 1 below. We now provide a post-processing step in our model simulation code (`https://github.com/amoghpj/nutrient-signaling`) to scale the predicted protein activities to these curated estimates.

2. Figure 2 nicely illustrates temporal behavior of same components after specific stimuli, here in comparison to experimental data. It would be very helpful to have a systematic overview of the time courses of the responses of the major variables to the 8 conditions as represented in Figure 4 (HC/HG, HC/HN,...) as additional supplement. As in supplement 4, just a flag whether the simulated behavior is in agreement with experiments or not or even not recorded would suffice. This would help to understand the physiological consequences of different nutrient changes and certainly also be a useful guide for further experiments.

   Figures 12, 14, 13, 15 (in Section 4 , below) show the predicted dynamics across 100 randomly sampled parameter sets across the nutrient conditions and strains used to represent the global state space in Figure 4 of the main paper. To summarize these results, we created Figure 11 where we measured deviation of time courses from those predicted by the reference parameter set using the mean sum of squared errors (MSE). We selected a cutoff MSE based on visually inspecting the timecourse plots. The summary plot indicates the fraction of parameter sets producing MSE values less than the cutoff, shown in green. If greater than 90% of the parameter sets satisfy this cutoff we regard the simulation as being robust, and we indicate this by using the light green color. The bright colors indicate that less than 90% of the parameter sets satisfy the cutoff.

Variable	Estimate (molecules/cell)	Notes
Cyr1	4000	Max Cyr1
Gln1	23700	Max Gln1
Gcn2	4500	Max Gcn2
Sak	2000	Max Sak1
PKA	20000	Max Bcy1
Trehalase	12000	Only Nth1, neutral trehalase
Rtg13	2300	Min of Rtg1 and Rtg3
Ras	19000	Max Ras2 abundance
Gcn4	4600	Max Gcn4
Gln3	1600	Max Gln3 in YEPD
Dot6	8000	Sum of Dot6 and Tod6
PDE	40000	Sum of Pde1 and Pde2
Mig1	3000	Max Mig1
Tps1	26000	Max Tps1
TORC1	6000	Sum of Tor1 and Tor2
Gis1	5100	Max Gis1
Snf1	11300	Snf1 catalytic subunit
EGO	2700	Min of Gtr1 and Gtr2
Sch9	12000	Sch9 catalytic subunit
EGOGAP	300	Min of Lst4 and Lst7

Table 1: Estimates of protein abundances from YeastMine rounded to the nearest 100's. The column title "Notes" records the criteria used in estimating the abundance of the protein complex.

1.2 Minor comments

- P1, abstract - the authors speak about "... cellular responses to unpredictable changes ..." It should be discussed, why those nutrient changes are unpredictable given that yeast has gone through a long evolutionary time with always changing environment. And second, why it matters if the changes are predictable or not.

  We have now modified the abstract to elaborate on the need for adapting to fluctuating nutrient environments

- P23, Eq 2 - sigma remains unexplained

  This term is now explained.

- P33, Fig S5 - arrows unexplained

  The explanation of the arrows is now mentioned in the caption.

2 Reviewer #2 (Remarks to the Author):

2.1 Major comments

Jalil et al. have set out to tackle a laudable goal – to construct a global model for how various nutrient signals are integrated across signalling pathways in the budding yeast, Saccharomyces cerevisiae. This is no easy task, but if successful such a model should be generally useful for developing a deeper understanding of the often complex physiological responses that have been observed experimentally in yeast in studies that combine genetics and biochemistry.

The authors used a necessarily simplified version of the yeast signaling networks under question, and modeled them with a system of ODEs, and fit parameters for the model constrained by prior experimental data collected by a large number of previous studies.

While the approach used is reasonable, I note some concerning misinterpretations of some of the published experimental data. Below are two example taken from the text and Fig 2:

1. p. 8: "The *pde1*Δ*pde2*Δ strain shows an increase in cAMP levels compared to wt [49]." The reference here is Ma et al. 1999, Mol Cell Bio, 10(1): 91-104. The referenced figure is Fig 1A. However, Ma et al. show that the pde double mutant actually has **lower** cAMP levels than WT (the pde1 single mutant shows increased cAMP levels, the pde2 single mutant looks essentially like WT)

   We thank the reviewer for pointing out this mismatch. The current version of the model is unable to capture the phenotype observed by Ma *et al.*. In the original publication, the authors explain the observation as follows:

   > Therefore, a likely explanation for the absence of the increases of cAMP in these strains is that the elevated PKA activity causes constitutively high feedback inhibition of cAMP synthesis.

   However our model simulations do not appear to support this claim. Moreover, Gonzales *et al.*, 2013 were able to recapitulate this phenotype by additionally assuming that PKA has a higher affinity for the phosphodiesterases than for its other binding partners Ira1 and Ira2. We are unable to represent such a mechanism in our model since the Type II equations which we use to model regulatory interactions is not suitable for representing direct enzyme binding and sequestration. In the face of this uncertainty we revise our claim as follows: We no longer

claim to capture the *pde1*Δ*pde2*Δ phenotype. Rather, the phenotype we do capture resembles that of a *pde1*Δ single deletion. We thank the reviewer for correcting our interpretation. Importantly, this change in interpretation does not affect the interpretation of other results as we do not attempt to model *pde1*Δ*pde2*Δ strain elsewhere in the paper.

2. p. 8: "Row C3 depicts trehalase (Nth1) levels after a glucose up-shift; trehalase levels remain low in a *tpk3*Δ mutant [8]." The reference they cite is Mbonyi et al. 1990, Mol Cell Bio p. 4518-4523. However, the Mbonyi et al. figure (Fig 5) shows trehalase levels when only one of the 3 PKA catalytic subunits (Tpk1, Tpk2, Tpk3) is present (i.e. the mutants in question are *tpk1*Δ *tpk2*Δ , *tpk1*Δ*tpk3*Δ , and *tpk2*Δ*tpk3*Δ). The key point of the Mbonyi paper is that the Tpk3 catalytic subunit behaves quite differently than Tpk1 and Tpk2 w/respect to Trehalase levels. Jalihal et al misinterpret the data in the figure as illustrating a *tpk3*Δ mutant. Also, the model of Jalihal et al. only considers a single parameter for PKA, so by definition it can't incorporate or simulate the findings of Mbonyi et al. which is concerned w/the behavior of the different subunits.

   We thank the reviewer for pointing out this oversight. Out of necessity, we do not attempt to model the different activities of the various Tpk subunits. That said, the data from Mbonyi *et al.* 1990 (Figure 5) indicates that trehalase specific activity is lower than the *wt* levels in all three strains namely the TPK1, TPK2, and the TPK3 strains. We have modified the description in the manuscript to reflect the claim that a strain with a single Tpk3 catalytic subunit demonstrates decreased trehalase activity.

3. I haven't checked all the other example the authors give, but the misinterpetation of prior experiments suggests that the model may have been constrained inappropriately.

   In view of the concerns of misinterpretation of the primary data, we have reviewed the data sources that we have used to constrain the model. The errors identified by the reviewer have been corrected. Moreover, in Section 3, we have compiled the original data sources (figures and tables) from the perturbation experiments used to interrogate our model.

2.2 Minor comments

1. Fig 4 is a very hard to parse with the myriad abbreviations, arrows, and tiny bars. The authors should think about alternatives ways to convey this information about global cellular responses.

   In order to provide a better overview, we have created Table 2 that records the fraction of parameter sets that predict that a transcription factor is 'on'. The color convention is the same as in the main figure. Light colors are robust predictions, with >90% of the parameter sets in consensus about the transcription factor state. Agreement with experimental data is indicated by a solid box, while model mismatch is indicated by a dashed box.

2. The text several times refers to Pde3. There is no Pde3 in yeast, only the phosphodiesterases Pde1 and Pde2

   We thank the reviewer for identifying this error. This has now been corrected.

3 Data sources and interpretation

This section provides the primary data sources for Figure 2I in the main paper. The data is ordered in the order of presentation in that figure. The simulation ID mentioned in each caption corresponds

to the ID of the entry in the data file (in YAML format) made available online at `https://github.com/amoghpj/nutrient-signaling/blob/master/data/yaml/perturbation-data.yaml`.

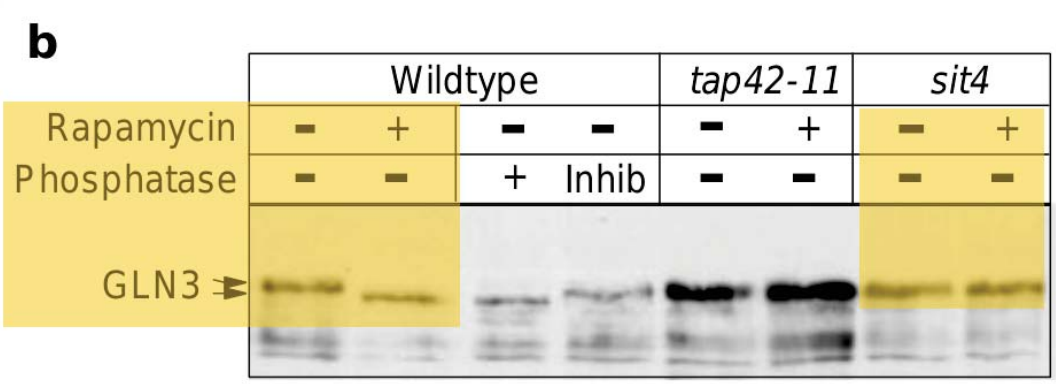

Figure 1: *sit4*Δ (simulation ID 12). Source: Beck, 1999 [1] Fig3B. The gel was quantified using ImageJ to estimate phosphorylated Gln3 in *wt* and *sit4*Δ before and after Rapamycin treatment.

TABLE 2. Expression of β-galactosidase from *GCN4-lacZ* fusions in wild-type and *gcn* strains under conditions of purine limitation

Strain/plasmid (genotype)	Analog	β-Galactosidase activity[a] (U) during growth in:			
		SD	SDade[b]	SC	SCade[c]
H1515/p180 (*GCN*)	None	7.3	8.0	8.6	9.0
	3AT	100	48	8.6	9.2
	azA	130	8.9	190	12
	azG	90	9.3	120	8.8
H1515/p227 (*GCN*)	None	630	790	760	640
	3AT	1,100	780	840	710
	azA	1,000	590	1,100	640
	azG	730	950	730	610
TD367/p180 (*gcn2*Δ)	None	11	11	12	12
	3AT	38	36	13	14
	azA	17	12	29	12
	azG	16	11	24	ND[d]
TD323/p180 (*sui2-S51A*)	None	11	9.9	11	12
	3AT	37	33	13	12
	azA	14	10	26	10
	azG	14	11	15	ND
TD392/p180 (*gcn3*Δ)	None	20	21	18	10
	3AT	54	55	21	12
	azA	25	20	41	10
	azG	28	24	30	ND

[a] Samples were assayed in duplicate with two to three transformants of each strain, except for the azG and SCade (see below) samples from the *gcn* mutants, for which assays were performed in duplicate with a single transformant. Standard errors were less than 15% except for seven samples (H1515/p227 in SDade with both no analog and azG, TD367 in SC with azG, and TD392 in SDade and SC with both no analog and azA) for which the standard error was between 16 and 25%.
[b] SDade, SD with minimal supplements (see Materials and Methods) and containing 0.6 mM adenine.
[c] SCade, SC (SD containing all 20 amino acids; see Materials and Methods) containing 0.6 mM adenine.
[d] ND, not determined.

Figure 2: *gcn2*Δ (simulation ID 5). Source: Rolfes, 1993 [8] Table 2. Gcn4-LacZ expression was measured in *wt* and *gcn2*Δ strains in SD medium with and without 3AT treatment to induce histidine starvation.

References

[1] Thomas Beck and Michael N. Hall. The Tor signalling pathway controls nuclear localization of nutrient-regulated transcription factors. *Nature*, 402(6762):689–692, 1999.

[2] M. Crauwels, M. C. V. Donaton, M. B. Pernambuco, J. Winderickx, J. H. de Winde, and J. M. Thevelein. The Sch9 protein kinase in the yeast *Saccharomyces cerevisiae* controls cAPK ac-

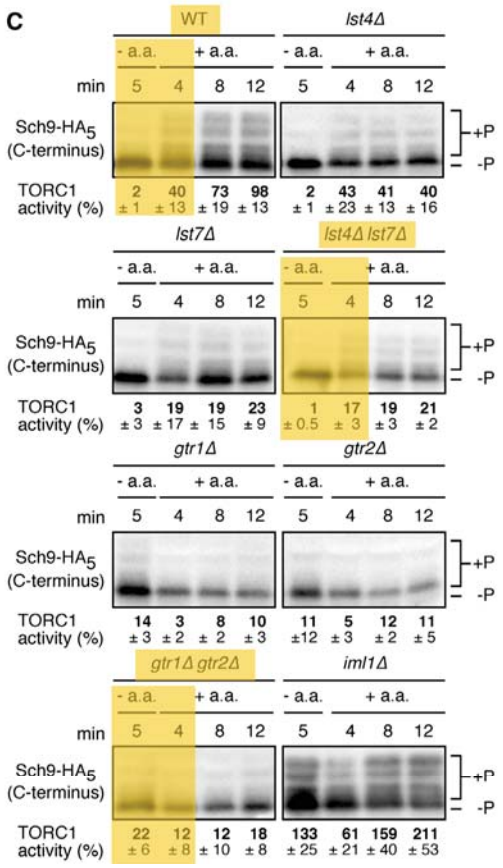

Figure 3: *gtr1*Δ *gtr2*Δ (4 m) (simulation ID 9) and *lst4*Δ *lst7*Δ (4 m) (simulation ID 10). Source: Peli-Gulli, 2015 [7] Fig 1C. Sch9 phosphorylation was measured using a pull down assay in *wt* and the *gtr1*Δ*gtr2*Δ and *lst4*Δ*lst7*Δ strains shortly after amino acid upshift. The reported TORC1 activity quantifications were used in our dataset.

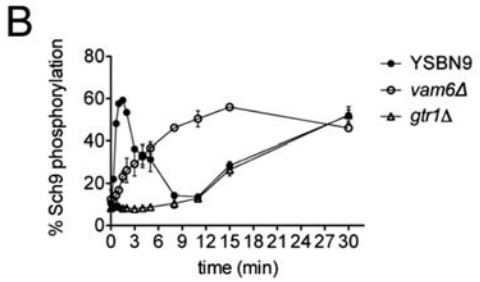

Figure 4: *gtr1*Δ (simulation ID 13). Source: Stracka, 2014 [9] Fig 6. Sch9 phohsphorylation measured over 30 minutes in response to glutamine readdition in *wt* and *gtr1*Δ strains.

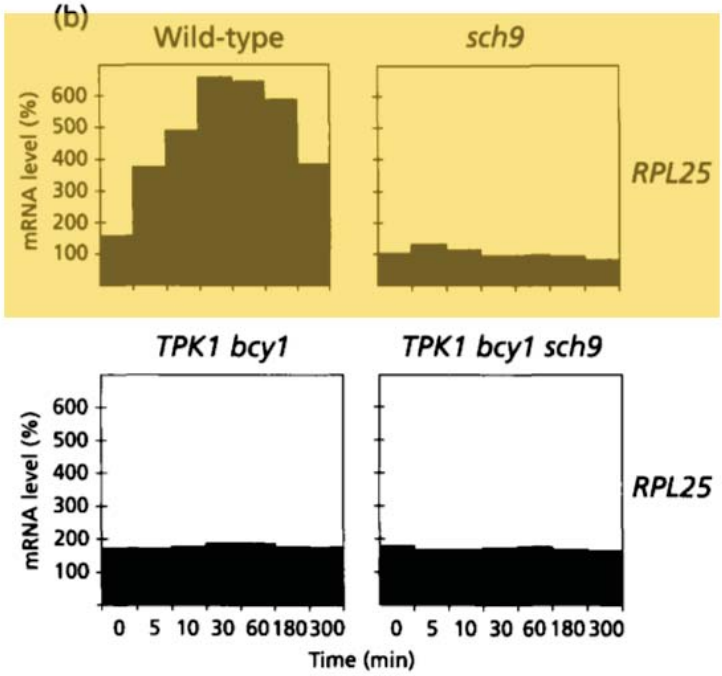

Figure 5: *sch9*Δ (simulation ID 4). Source: Crauwels, 1997 [2] Fig 9B. Relative RPL25 mRNA levels were measured in *wt* and *sch9*Δ strains over 300 minutes.

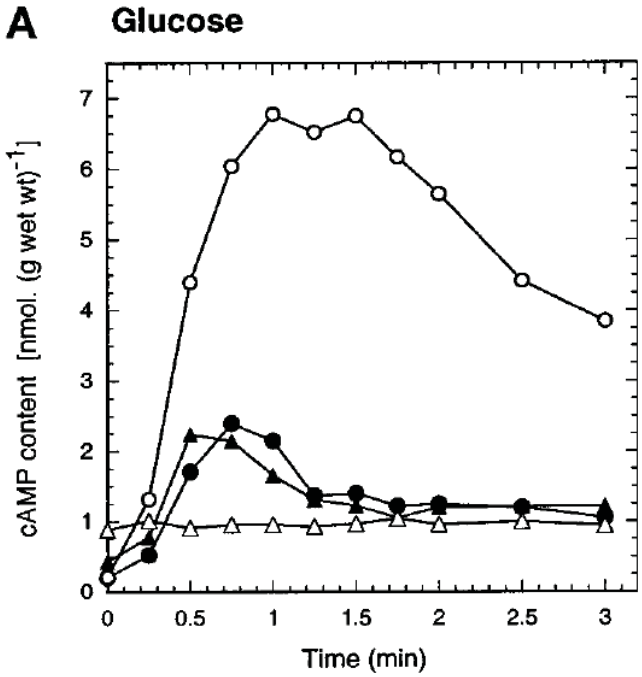

Figure 6: *pde*Δ (simulation ID 6). Source: Ma, 1999 [4] Fig 1A. cAMP was measured after a glucose upshift in *wt* and various pde strains.

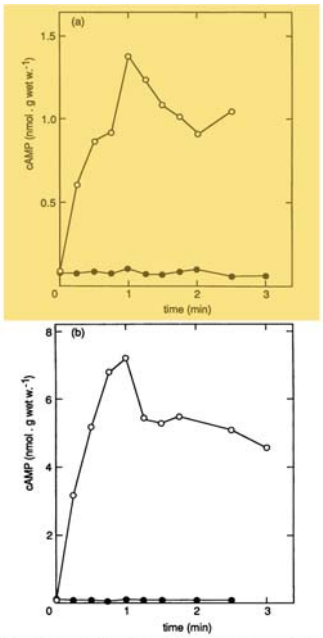

FIG. 2. Glucose-induced cAMP signal (a) and DNP-induced cAMP increase (b) at 32°C in derepressed cells of *ras1 ras2 bcy1* (●) and *bcy1* (○) strains.

Figure 7: *ras1*Δ*ras2*Δ*bcy1*Δ (simulation ID 8). Source: Mbonyi, 1990 [5] Fig 2A. cAMP was measured after a glucose upshift in *wt* and *ras1*Δ*ras2*Δ*bcy1*Δ strains in the first three minutes post shift.

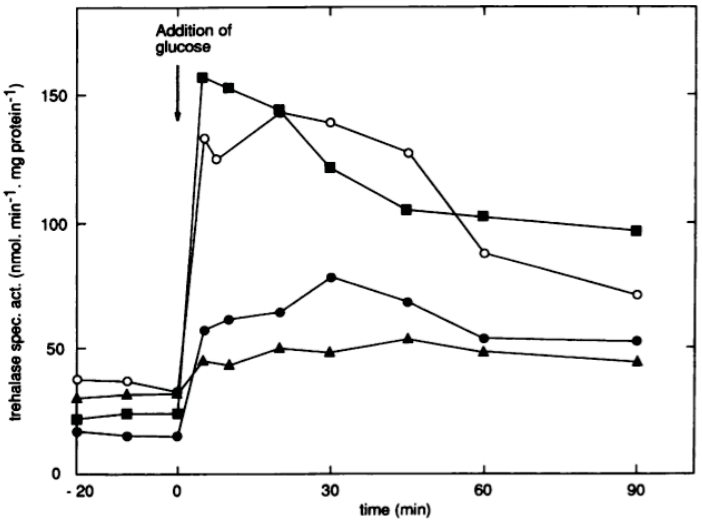

FIG. 5. Glucose-induced activation of trehalase in derepressed cells of strains with only one wild-type *TPK* gene. Shown are results for *TPK1* (●), *TPK2* (○), and *TPK3* (▲) in the wild-type form and for wild-type cells (*TPK1 TPK2 TPK3*) (■). The cells were suspended in YPG.

Figure 8: *tpk1*Δ *tpk2*Δ (simulation ID 7). Source: Mbonyi, 1990 [6] Fig 5. cAMP was measured after glucose upshift in *wt* and a *TPK3* strain.

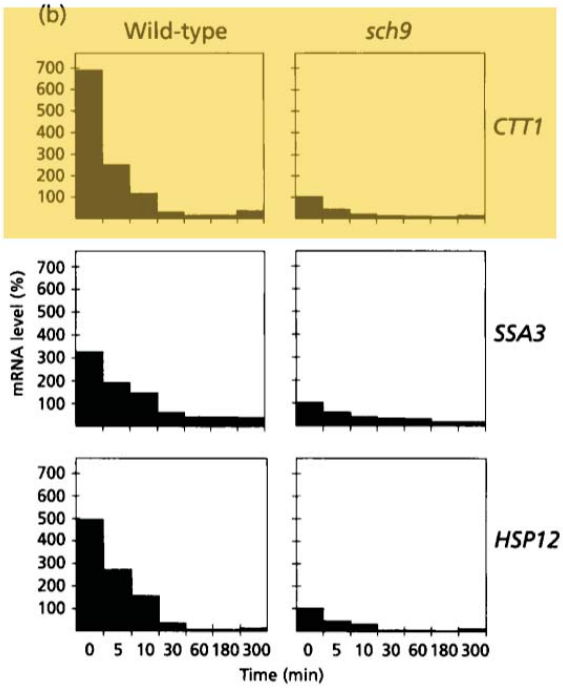

Figure 9: *sch9*Δ (simulation ID 3). Source: Crauwels, 1997 [2] Fig 6B. Gis1 activity was studied by measureing expression of its target CTT1 in response to glucose starvation in *wt* and *sch9*Δ strains.

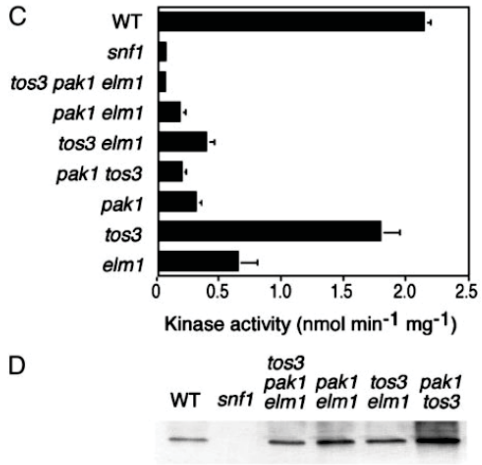

Fig. 2. Assays of Snf1 kinase activity. (*A* and *B*) WT and triple mutant cells expressing LexA-Snf1p or its T210A and K84R mutant derivatives [pRJ55, pRJ217, and pRJ215 (16)] were grown in selective SC plus 2% glucose. Proteins were immunoprecipitated from extracts (200 μg) with anti-LexA. (*A*) Immunoprecipitates were incubated in kinase buffer containing [γ-^{32}P]ATP and analyzed by SDS/PAGE and autoradiography. (*B*) Immunoprecipitates were analyzed by immunoblotting with anti-LexA. (*C*) Snf1 kinase activity was assayed by determining phosphorylation of the SAMS peptide (8, 30). A *snf1-K84R* mutant extract also showed no activity. (*D*) The assayed fractions were immunoblotted with anti-Snf1.

Figure 10: *sak1*Δ*tos3*Δ*elm1*Δ (simulation ID 11). Source: Hong, 2003 [3] Fig 2C. Snf1 kinase activity was quantified in *wt* and *sak1*Δ*tos3*Δ*elm1*Δ strains undergoing acute glucose starvation (Snf1 activating condition).

tivity and is required for nitrogen activation of the fermentable-growth-medium-induced (FGM) pathway. *Microbiology*, 143(8):2627–2637, 1997.

[3] S.-P. Hong, F. C. Leiper, A. Woods, D. Carling, and M. Carlson. Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases. *Proceedings of the National Academy of Sciences*, 100(15):8839–8843, 2003.

[4] P. Ma, S. Wera, P. Van Dijck, and J. M. Thevelein. The pde1-encoded low-affinity phosphodiesterase in the yeast *Saccharomyces cerevisiae* has a specific function in controlling agonist-induced cAMP signaling. *Molecular Biology of the Cell*, 10(1):91–104, 1999.

[5] K Mbonyi, M Beullens, K Detremerie, L Geerts, and J M Thevelein. Requirement of one functional Ras gene and inability of an oncogenic Ras variant to mediate the glucose-induced cyclic AMP signal in the yeast *Saccharomyces cerevisiae*. *Molecular and Cellular Biology*, 8(8):3051–3057, 1988.

[6] K Mbonyi, L van Aelst, J C Argüelles, A W Jans, and J M Thevelein. Glucose-induced hyperaccumulation of cyclic amp and defective glucose repression in yeast strains with reduced activity of cyclic amp-dependent protein kinase. *Molecular and Cellular Biology*, 10(9):4518–4523, 1990.

[7] Marie-Pierre Péli-Gulli, Alessandro Sardu, Nicolas Panchaud, Serena Raucci, and Claudio De Virgilio. Amino acids stimulate TORC1 through Lst4-lst7, a GTPase-activating protein complex for the Rag family GTPase Gtr2. *Cell Reports*, 13(1):1–7, 2015.

[8] R J Rolfes and A G Hinnebusch. Translation of the yeast transcriptional activator gcn4 is stimulated by purine limitation: Implications for activation of the protein kinase gcn2. *Molecular and Cellular Biology*, 13(8):5099–5111, 1993.

[9] D. Stracka, S. Jozefczuk, F. Rudroff, U. Sauer, and M. N. Hall. Nitrogen source activates Tor (target of rapamycin) complex 1 via glutamine and independently of Gtr/Rag proteins. *Journal of Biological Chemistry*, 289(36):25010–25020, 2014.

4 New results

Figure 11: Robust time courses of Snf1, Sch9, PKA, and cAMP across the indicated strains in the 8 qualitatively distinct nutrient states. The figure summarizes the deviation of simulated trajectories across 500 randomly sampled parameter sets with respect to that from the reference parameter set. We use mean sum of squared errors (MSE) to measure the deviation. Green and red bars indicate the fraction of parameter sets with MSE less than and greater than a chosen cutoff respectively. Light colors indicate robust timecourse, with greater than 90% of the parameter sets producing MSE less than a cutoff. Conversely, bright colors indicate fragile predictions with more than 10% of parameter sets making fragile predictions. (Note that no light red bars are visible in this figure. This suggests that nearly 100% of the parameter sets produce robust simulations under these conditions.)

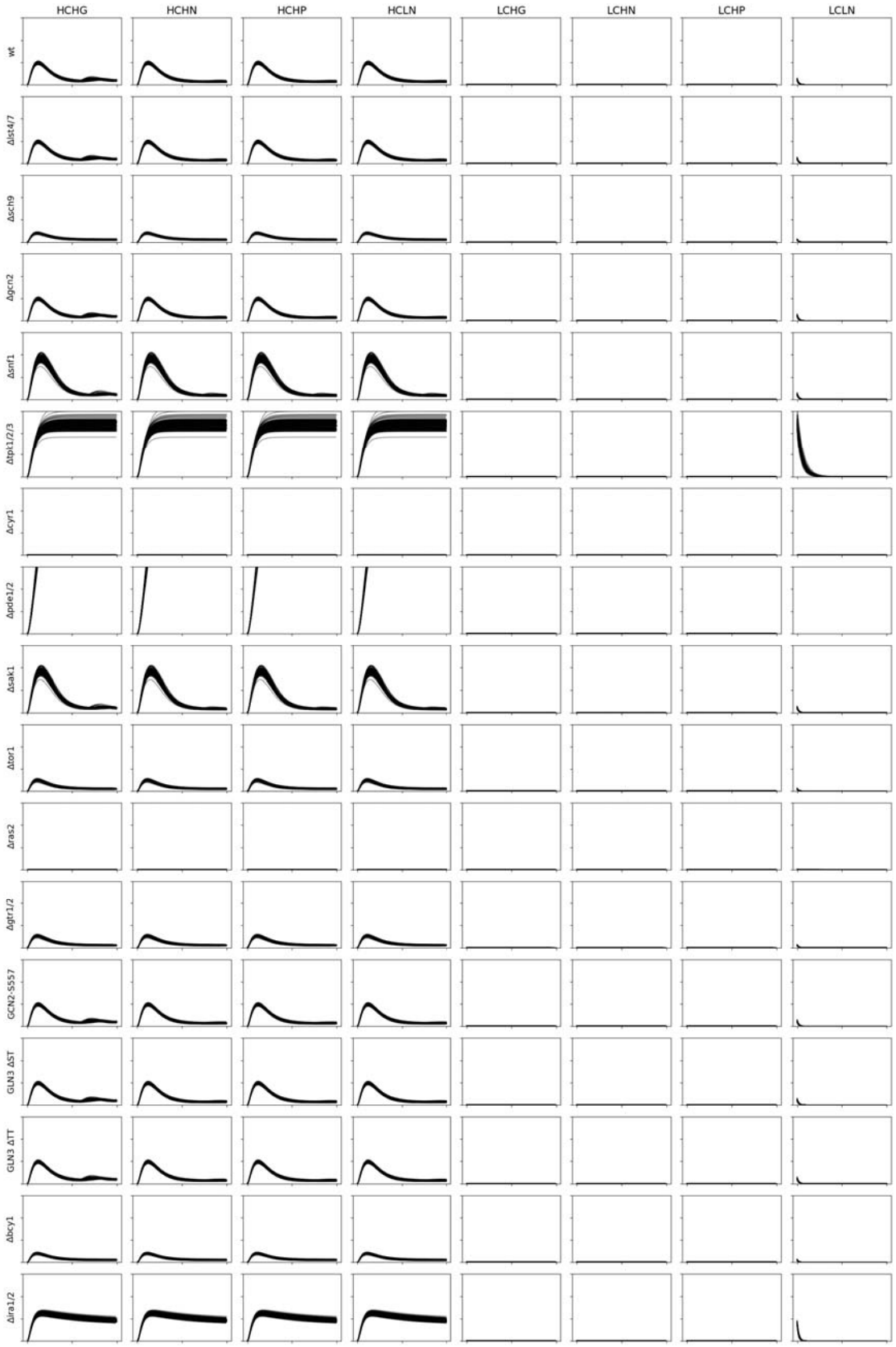

Figure 12: cAMP dynamics across 100 randomly sampled parameter sets.

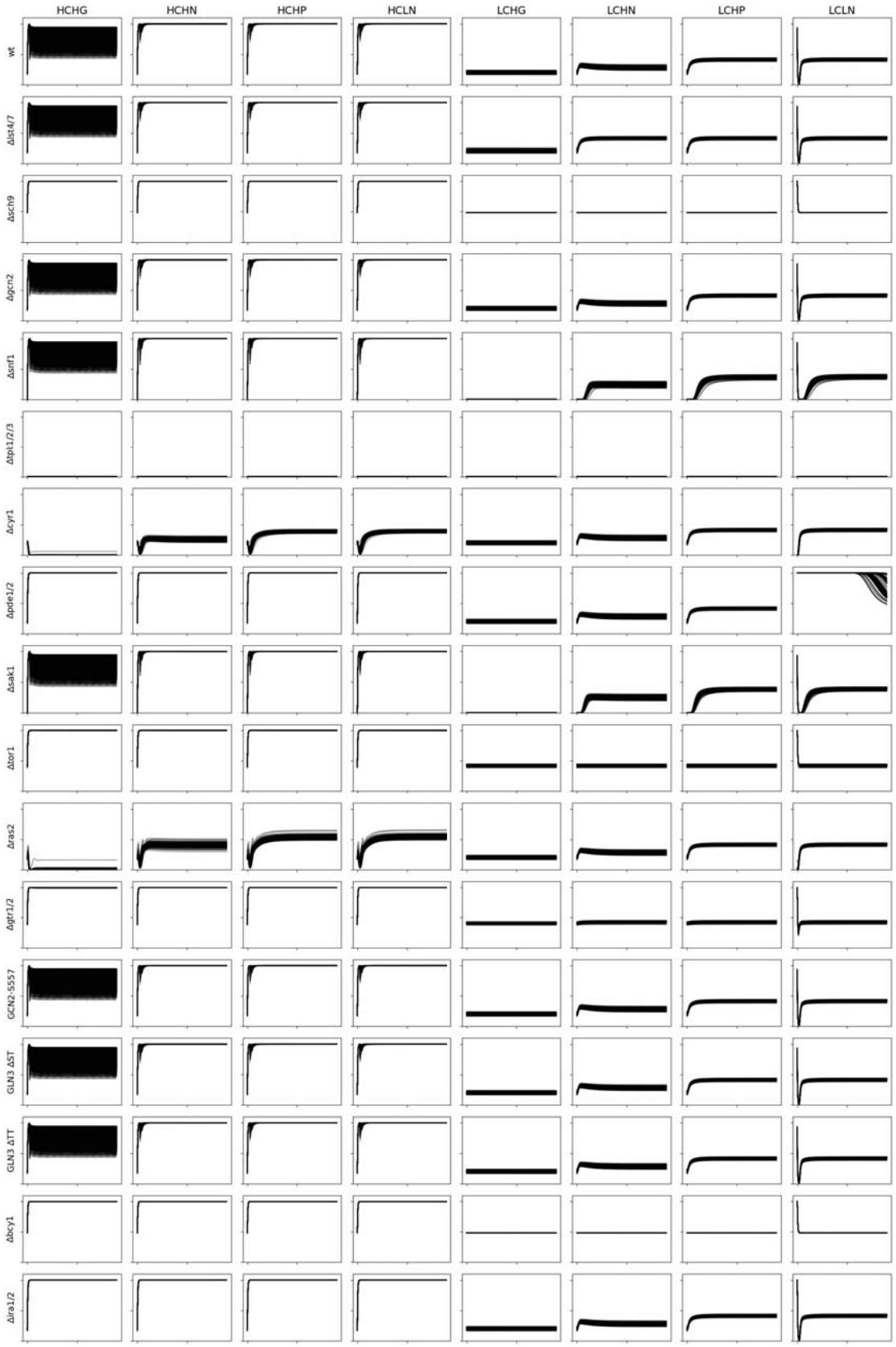

Figure 13: PKA dynamics across 100 randomly sampled parameter sets.

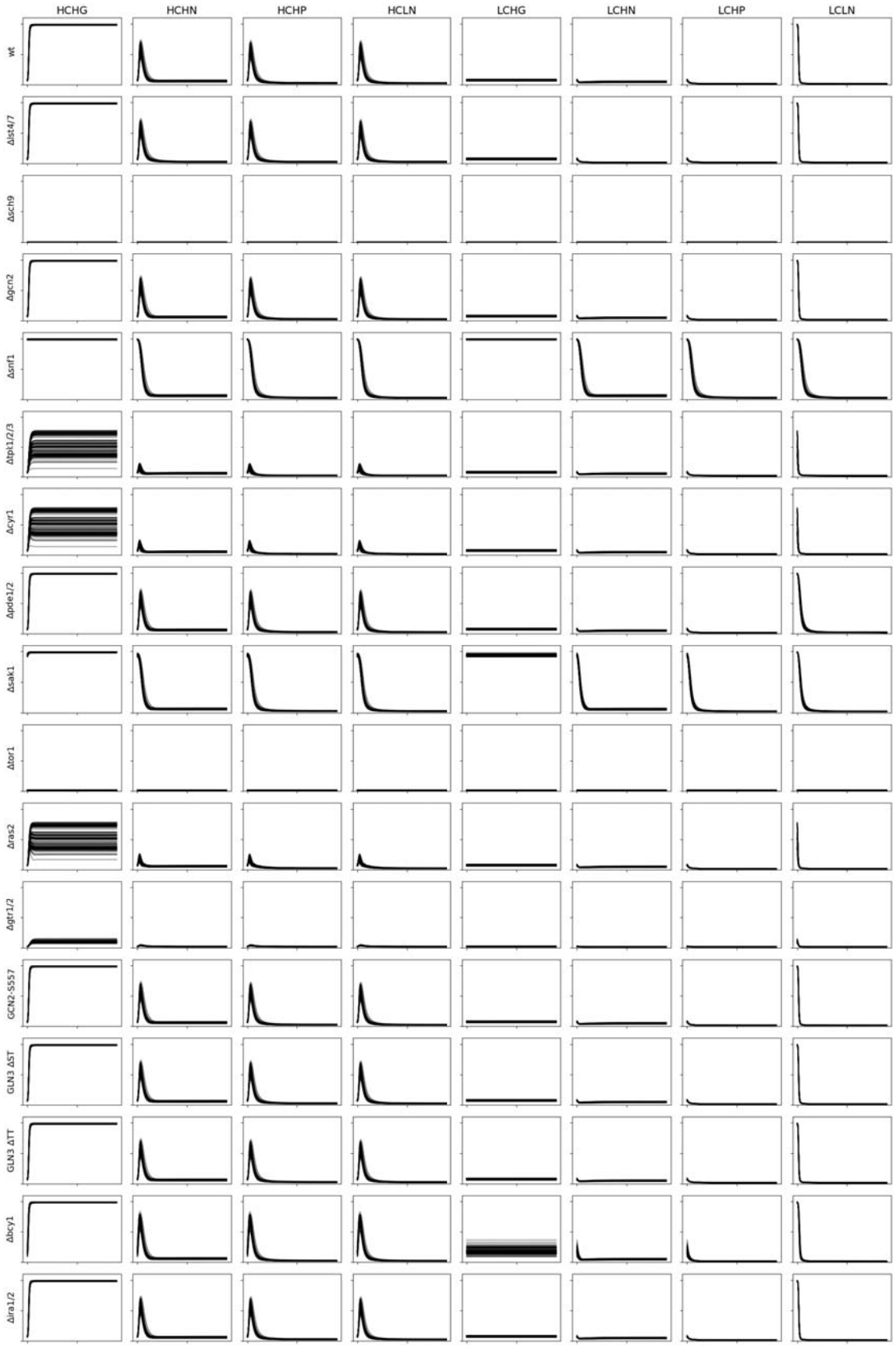

Figure 14: Sch9 dynamics across 100 randomly sampled parameter sets.

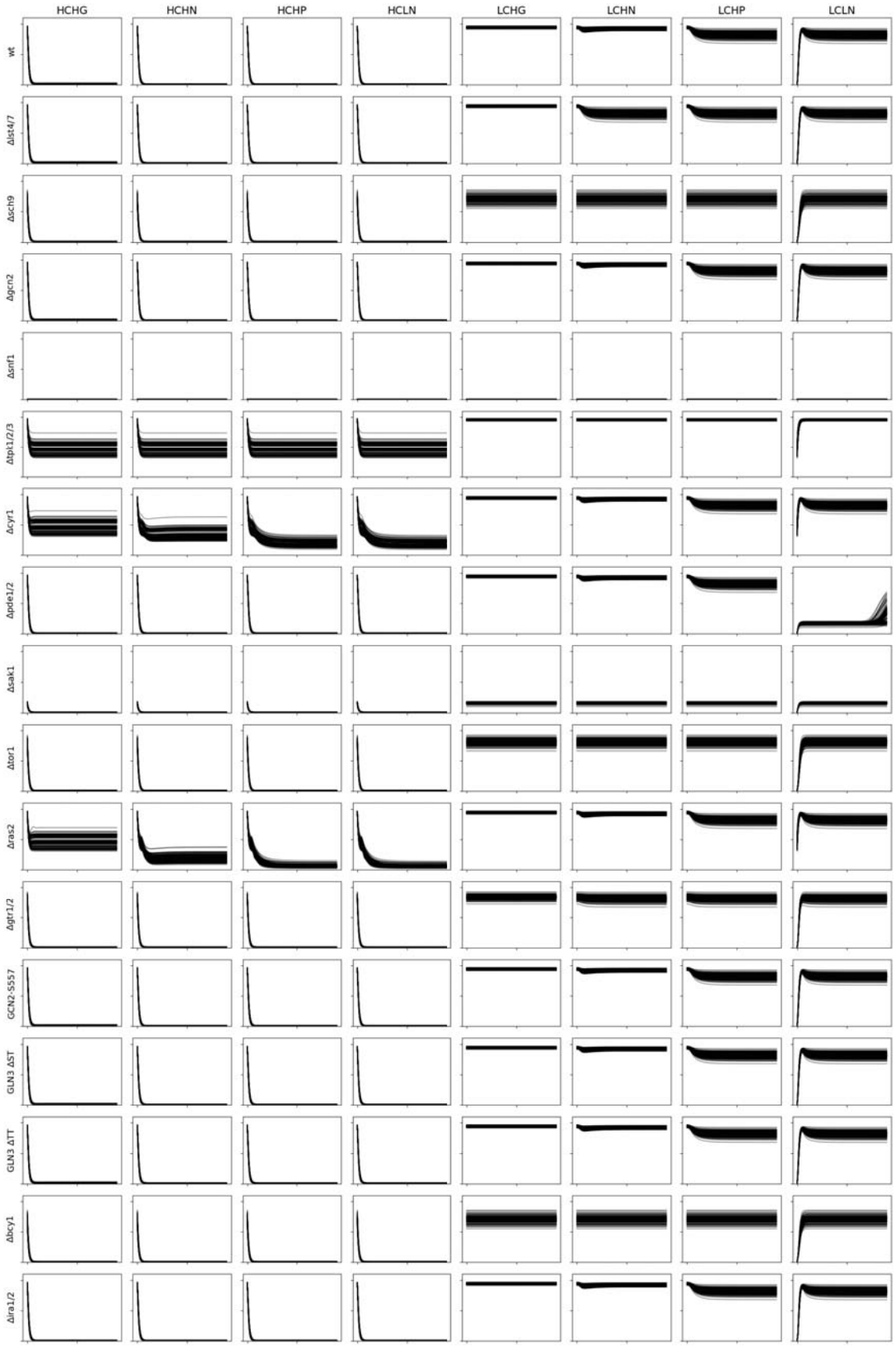

Figure 15: Snf1 dynamics across 100 randomly sampled parameter sets.

4.1 Alternative presentation of state space results

Strain	HCHG						HCHN						HCHP						HCLN					
	Gis1	Mig1	Dot6	Gcn4	Rtg13	Gln3	Gis1	Mig1	Dot6	Gcn4	Rtg13	Gln3	Gis1	Mig1	Dot6	Gcn4	Rtg13	Gln3	Gis1	Mig1	Dot6	Gcn4	Rtg13	Gln3
wt	0	98	4	0	3	1	0	100	43	88	100	99	0	100	53	100	100	100	0	100	53	100	100	100
Δlst4/7	0	98	4	0	3	1	0	100	53	100	100	100	0	100	53	100	100	100	0	100	53	100	100	100
Δsch9	0	100	58	100	2	1	0	100	58	100	100	99	0	100	58	100	100	100	0	100	58	100	100	100
Δgcn2	0	98	4	0	3	1	0	100	43	0	100	99	0	100	53	0	100	100	0	100	53	0	100	100
Δsnf1	0	98	4	0	2	0	0	100	43	87	100	97	0	100	53	100	100	100	0	100	53	100	100	100
Δtpk1/2/3	98	3	93	4	47	100	100	3	100	91	100	100	100	3	100	100	100	100	100	3	100	100	100	100
Δcyr1	98	3	93	3	47	100	92	11	99	91	100	100	31	33	98	100	100	100	29	35	98	100	100	100
Δpde1/2	0	100	3	0	2	1	0	100	42	88	100	99	0	100	52	100	100	100	0	100	53	100	100	100
Δsak1	0	98	4	0	2	0	0	100	43	88	100	99	0	100	53	100	100	100	0	100	53	100	100	100
Δtor1	0	100	55	100	100	100	0	100	55	100	100	100	0	100	55	100	100	100	0	100	55	100	100	100
Δras2	98	3	93	2	47	100	31	35	96	90	100	100	5	80	94	100	100	100	4	81	94	100	100	100
Δgtr1/2	0	100	36	55	97	99	0	100	55	100	100	100	0	100	55	100	100	100	0	100	55	100	100	100
GCN2-S557	0	98	4	100	3	1	0	100	43	100	100	99	0	100	53	100	100	100	0	100	53	100	100	100
GLN3 ΔST	0	98	4	0	3	0	0	100	43	88	100	97	0	100	53	100	100	100	0	100	53	100	100	100
GLN3 ΔTT	0	98	4	0	3	0	0	100	43	88	100	0	0	100	53	100	100	0	0	100	53	100	100	0
Δbcy1	0	100	3	0	2	1	0	100	43	88	100	99	0	100	53	100	100	100	0	100	53	100	100	100
Δira1/2	0	100	3	0	2	1	0	100	42	88	100	99	0	100	52	100	100	100	0	100	53	100	100	100

Strain	LCHG						LCHN						LCHP						LCLN					
	Gis1	Mig1	Dot6	Gcn4	Rtg13	Gln3	Gis1	Mig1	Dot6	Gcn4	Rtg13	Gln3	Gis1	Mig1	Dot6	Gcn4	Rtg13	Gln3	Gis1	Mig1	Dot6	Gcn4	Rtg13	Gln3
wt	100	2	100	79	97	100	88	3	99	94	100	100	19	7	98	100	100	100	19	7	98	100	100	100
Δlst4/7	99	2	100	82	97	100	19	7	98	100	100	100	19	7	98	100	100	100	19	7	98	100	100	100
Δsch9	5	15	97	100	83	100	5	15	97	100	100	100	5	15	97	100	100	100	5	15	97	100	100	100
Δgcn2	100	2	100	0	97	100	88	3	99	0	100	100	19	7	98	0	100	100	19	7	98	0	100	100
Δsnf1	95	6	61	0	2	0	93	22	99	87	100	97	42	49	99	100	100	100	38	51	98	100	100	100
Δtpk1/2/3	100	1	100	82	98	100	100	1	100	94	100	100	100	1	100	100	100	100	100	1	100	100	100	100
Δcyr1	100	2	100	79	97	100	88	3	99	94	100	100	19	7	98	100	100	100	19	7	98	100	100	100
Δpde1/2	100	2	100	79	97	100	88	3	99	94	100	100	19	7	98	100	100	100	19	7	98	100	100	100
Δsak1	96	4	69	0	7	100	92	14	99	89	100	100	32	34	98	100	100	100	30	35	98	100	100	100
Δtor1	17	8	98	100	100	100	17	8	98	100	100	100	17	8	98	100	100	100	17	8	98	100	100	100
Δras2	100	2	100	79	97	100	88	3	99	94	100	100	19	7	98	100	100	100	19	7	98	100	100	100
Δgtr1/2	26	6	98	100	100	100	18	8	98	100	100	100	18	8	98	100	100	100	18	8	98	100	100	100
GCN2-S557	100	2	100	100	97	100	88	3	99	100	100	100	19	7	98	100	100	100	19	7	98	100	100	100
GLN3 ΔST	100	2	100	79	97	95	88	3	99	94	100	98	19	7	98	100	100	100	19	7	98	100	100	100
GLN3 ΔTT	100	2	100	79	97	100	88	3	99	94	100	100	19	7	98	100	100	100	19	7	98	100	100	100
Δbcy1	2	15	85	9	83	100	4	15	96	92	100	100	4	15	97	100	100	100	4	15	97	100	100	100
Δira1/2	100	2	100	79	97	100	88	3	99	94	100	100	19	7	98	100	100	100	19	7	98	100	100	100

Table 2: Summary of predicted global cellular state. Each cell contains the precentage of parameter sets that predicted the given transcription factor to be 'on' in the given strain under a specific nutrient condition. Light colors represent robust predictions where greater than 90% of the parameter sets are in consensus regarding the state of a transcription factor. Bright colors represent fragile predictions. Boxes around cells indicate the presence of experimental data. Solid boxes indicate agreement with experiment while dashed boxes indicate a mismatch. The color of the box indicates the state of the transcription factor as interpreted from experimental data, provided at `https://github.com/amoghpj/nutrient-signaling/blob/master/data/csv/tf-state-experimental-evidence.csv`

RE: Manuscript #E20-02-0117R
TITLE: Modeling and Analysis of the Macronutrient Signaling Network in Budding Yeast

Dear Authors,

First, we are sorry for the delay in this response, due in part to non-responsive referee I, and our desire to avoid simple rejection based on reviewer 2.

A consultation with another editor resulted in the opinion that your paper has many positive features, including 1) it summarizes a lot of past experimental work in a coherent manner, with careful reading; 2) it creates a complex model that was useful in stimulating experiments. This required insightful ways to simplify biology without creating "apparent biology" emerging unintentionally from some of the simplifying assumptions.

It seems that reviewer 2 is still not convinced, and we ask that you consider their comments and make another attempt to address them. Once this is done, we will ask a different reviewer to weigh in on the outcome.

Sorry that we cannot simply accept the paper as is, and that in these chaotic days, it has proven so tricky to get reviewers.

Sincerely,

Leah Edelstein-Keshet
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Tyson,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments 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 haveopted out of publishing the review history.

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

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

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 manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker

Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #2 (Remarks to the Author):

* The authors response seems to indicate that mis-specification of several terms in the initial model (see previous review) did not substantively change the findings. This seems somewhat surprising, but the authors offer no explanation for this in their response. At a minimum, I think this needs to be addressed.

* In their response, the authors write "...the data from Mbonyi et al. 1990 (Figure 5) indicates that trehalase specific activity is lower than the wt levels in all three strains...". That is not the case, the strain that still possesses TPK2 (i.e. tpk1delta tpk3delta) is essentially wild type in trehalase activity.

* Since the authors are collapsing/lumping together the PKA catalytic subunits into a single species in their model they need to explain how they have resolved conflicting data on the phenotypes associated with the different Tpks. This is also true of the phosphodiesterases, Pde1 and Pde2. At the minimum they should note in the manuscript that mutant phenotypes differ depending on which Tpk or which Pde is considered. Better yet, they might consider exploring how the model behaves when fit to alternate parameters based on the different phenotypes of the TPKs or PDEs.

* I am unable to understand how the authors derived the results shown in Fig. 4c, which shows the predicted cellular states under different nutrient conditions for a variety of mutant background. Many of the molecular species (e.g. ira1/2 and lst4/7) considered in this figure are not terms in the mathematical model (S1.1) so it's not clear where such predictions come from (insufficient details in methods and/or supplemental materials)

* There is still reference to the non-existent PDE3. p. 7 "In our model, 'PDE' is a single variable representing phosphodiesterases Pde1/2/3."

Response to Reviewers - 2

We have revised our manuscript a second time to respond to the valid concerns of Referee #2. Specifically, the changes affect the Section "Testing the model against observed phenotypes of mutant strains". In the earlier version, we attempted to account qualitatively for the observed phenotypes of 18 mutant strains of budding yeast including three experiments involving the *mig1*Δ*snf1*Δ*pde2*Δ. Since we no longer claim to model the Pde1 subunit (as explained below, we now study only 15 mutant strains. This change affects the values reported in Figure 3 and Table 2 of the main paper, and Table S1 in the supplemental material, but does not change the overall interpretation of the results.

1. The authors response seems to indicate that mis-specification of several terms in the initial model (see previous review) did not substantively change the findings. This seems somewhat surprising, but the authors offer no explanation for this in their response. At a minimum, I think this needs to be addressed.

   The points raised by the Reviewer in their review of our original submission have helped us correct our erroneous representation of some experimental data. We would like to clarify that our new representation of the curated experimental data does indeed affect the search for parameter sets with acceptable cost-of-fit to the data, which in turn will affect the confidence in our predictions -for example, in the global nutrient-shift analysis (main text Figure 4). After having corrected our interpretation of experiments (discussed below), we have repeated the parameter search, the comparison to qualitative experimental data (Figure 3) and the predictions for global cellular responses to nutrient shifts (Figure 4). We find that the results from these revised simulations are largely unchanged from our initial submission, except for some additional "fragile" predictions. Specifically, we observe that with the latest set of parameter values, our predictions for the Dot6 state under carbon starvation conditions (LC) are fragile compared to the original version of the model, although a majority of the parameter sets still predict that Dot6 will be "ON" in LC conditions. Below we compare the results from the new parameter search (Figure 1 below) with the results in our initial submission.

1. In their response, the authors write "... the data from Mbonyi et al. 1990 (Figure 5) indicates that trehalase specific activity is lower than the wt levels in all three strains...". That is not the case, the strain that still possesses TPK2 (i.e. tpk1delta tpk3delta) is essentially wild type in trehalase activity.

   To calibrate the model in Figure 2(I) in the main text, we have used data from Figure 5 Mbonyi *et al.*, 1990 (reproduced below for ease of comparison). Figure 2(I) compares the steady states of cAMP level between our model prediction and the Mbonyi data. While we agree with the reviewer that the timecourse of the *tpk1*Δ*tpk3*Δ strain initially appears similar to *wt* strain, the final timepoint at 90 minutes is still lower than that in *wt*. For our steady

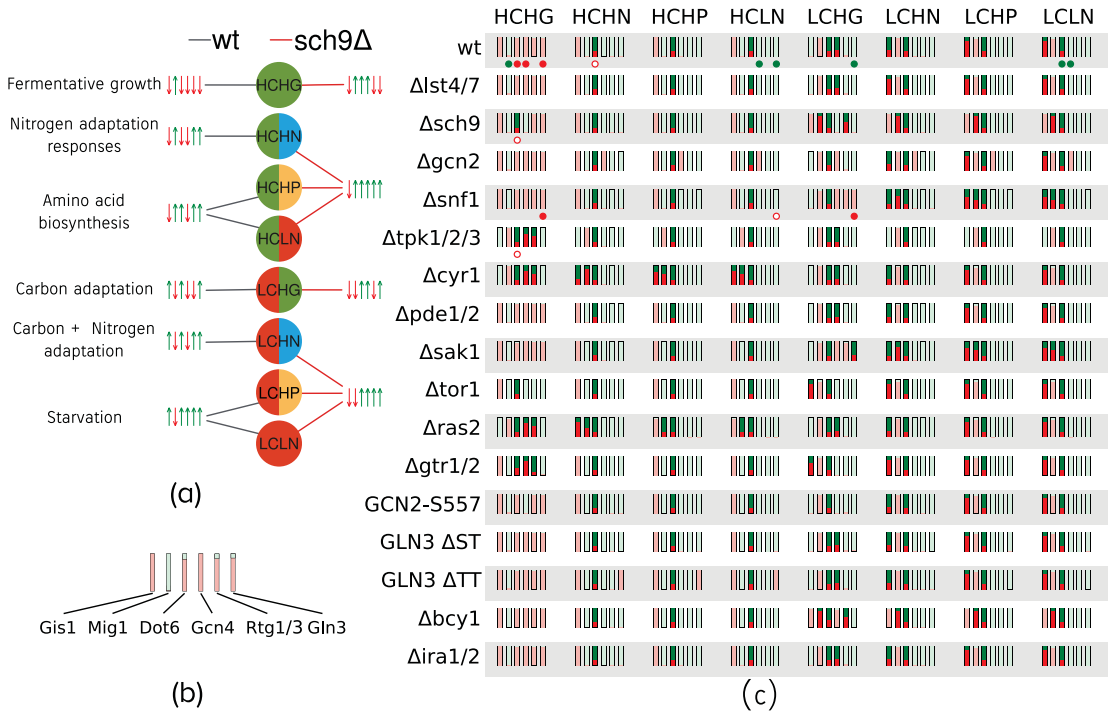

Figure 1: Global state space predictions from repeated parameter search.

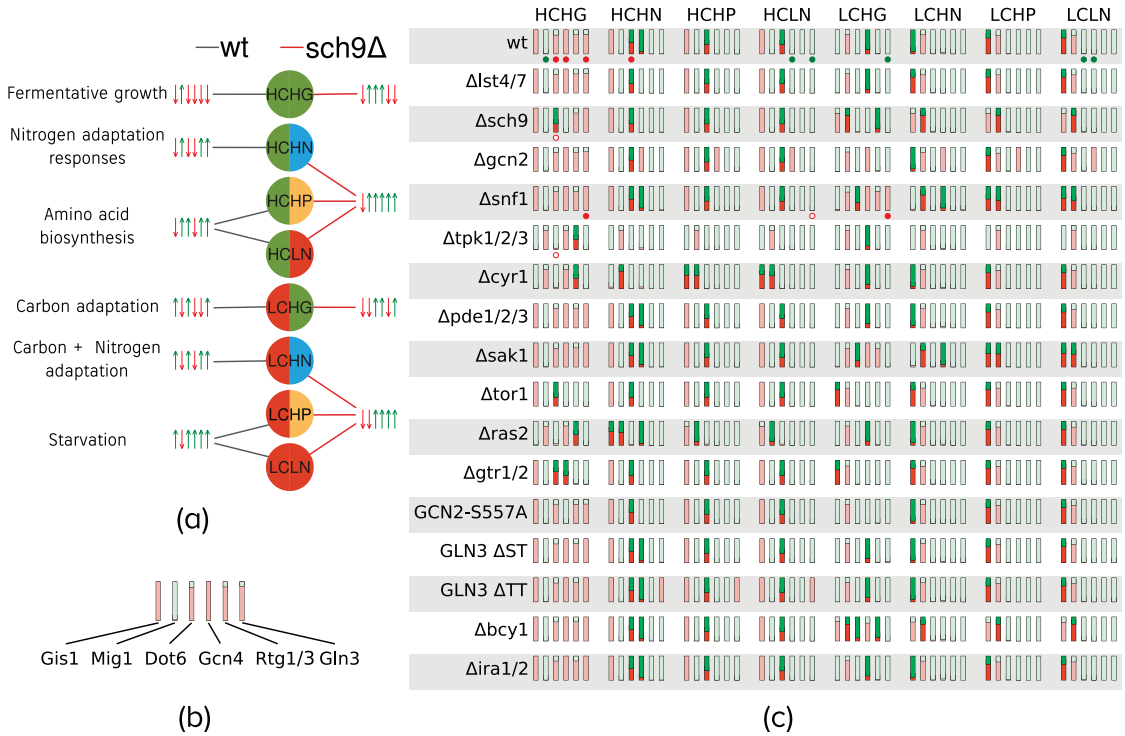

Figure 2: Global state space predictions from initial manuscript submission.

state analysis, which considers only the initial and final time points, our interpretation of the Mbonyi data remains valid. Nonetheless, we acknowledge in the revised text that the initial response of the TPK2 strain to glucose addition is quite different from the response of the TPK1 and TPK3 strains, suggesting that our 'naive' assumption of interchangeability of the three isoforms is incorrect.

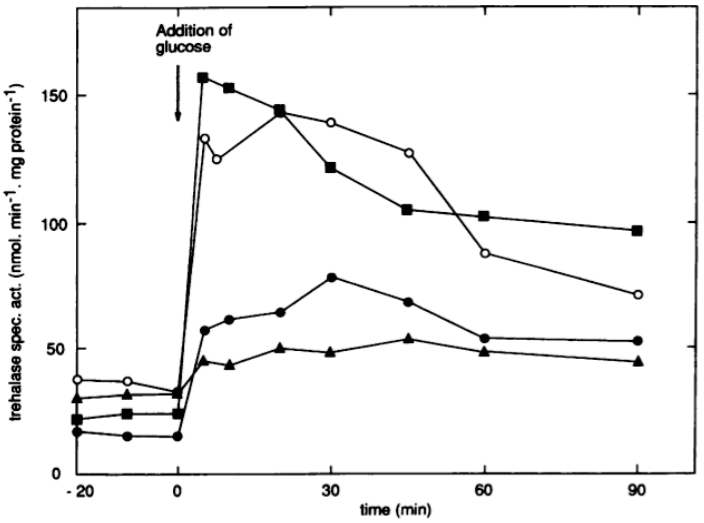

FIG. 5. Glucose-induced activation of trehalase in derepressed cells of strains with only one wild-type *TPK* gene. Shown are results for *TPK1* (●), *TPK2* (○), and *TPK3* (▲) in the wild-type form and for wild-type cells (*TPK1 TPK2 TPK3*) (■). The cells were suspended in YPG.

2. Since the authors are collapsing/lumping together the PKA catalytic subunits into a single species in their model they need to explain how they have resolved conflicting data on the phenotypes associated with the different Tpks. This is also true of the phosphodiesterases, Pde1 and Pde2. At the minimum they should note in the manuscript that mutant phenotypes differ depending on which Tpk or which Pde is considered. Better yet, they might consider exploring how the model behaves when fit to alternate parameters based on the different phenotypes of the TPKs or PDEs.

   We thank the reviewer for raising this point. The original manuscript lacked a clear explanation of how we sought to explain mutant data when we don't explicitly represent the catalytic subunits of PKA and PDE in our mathematical model. We have added the following discussion to the "Section S1.1 Kinetic expressions" of the revised manuscript.

   > The experimental data used to calibrate the model comprises genetic perturbations to individual catalytic subunits of the complex regulators. For the most part, it is beyond the scope of the current model to represent the activities of individual subunits, and we treat a regulatory complex as a single entity in the model. However, this assumption fails in cases where experimental evidence indicates that the catalytic subunits have distinct activities. We discuss two such instances below.
   >
   > (a) PKA has three catalytic subunits Tpk1, Tpk2, and Tpk3. Experimental data from strains with single TPK subunits (cf. Mbonyi *et al.* 1990) indicate that each of these subunits have distinct catalytic activities, where TPK1 and TPK3 exhibit similar activities, while TPK2 is similar to *wt*. The model currently is

able to capture the phenotypes of strains with Tpk1 and Tpk3 subunits by setting the total amount of PKA to 0.3 of the *wt* activity. However this assumption is not sufficient to model the strain with a single Tpk2 subunit. #, and we currently do not attempt to model the Tpk2 subunit of PKA.

(b) PDE has two catalytic subunits, Pde1 and Pde2. The *pde1*Δ, *pde2*Δ, and the *pde1*Δ*pde2*Δ strains all show very different cAMP phenotypes with respect to glucose upshifts (cf. Ma 1999, Figure 1A). Our model succeeds in capturing the phenotype of only the *pde1*Δ strain, i.e. the PDE2 subunit. #We do not attempt to model the *pde2*Δ strain.

In both the above cases, a single model representation is unable to explain the behavior of various catalytic subunits. A goal for a future version of the model is to incorporate data specific to various subunits by uniquely representing the various genetic mutant strains.

3. I am unable to understand how the authors derived the results shown in Fig. 4c, which shows the predicted cellular states under different nutrient conditions for a variety of mutant background. Many of the molecular species (e.g. ira1/2 and lst4/7) considered in this figure are not terms in the mathematical model (S1.1) so it's not clear where such predictions come from (insufficient details in methods and/or supplemental materials)

   We thank the reviewer for raising this concern. We have now added Table S4 which lists how each of the 16 mutants is represented. Briefly, 11 of 16 mutants are gene deletions, where the gene correspond(s) to variable(s) in the model. These mutations are represented by setting the total activity of the corresponding variable to 0. Specifically, LST4/7 corresponds to the EGOGAP variable in the model, as noted in the 'Nitrogen Sensing' paragraph in the Section 'A proposal for the nutrient signaling network in budding yeast'. Two of the remaining five mutants (*bcy1*Δ and *ira1/2*Δ) are also gene deletions, but they do not correspond to variables in the model; rather, they mediate regulatory interactions that are present in the model, and these interactions are represented by 'interaction parameters' in the Type II equations. Three of the remaining five mutations are gene truncations (GLN3 ΔST and GLN3 ΔTT) or mutations (GCN2-S557) that effectively remove a regulatory interaction from the model. These mutant strains are also represented by modifying interaction parameters that appear in Type II equations. This explanation is added to the first paragraph of Section S4.

4. There is still reference to the non-existent PDE3. p. 7 "In our model, 'PDE' is a single variable representing phosphodiesterases Pde1/2/3."

   This oversight has now been fixed.

RE: Manuscript #E20-02-0117RR
TITLE: Modeling and Analysis of the Macronutrient Signaling Network in Budding Yeast

Dear Dr. Tyson:

Apologies for the long delay, where we tried and failed to get the 2nd review, and also for the long trajectory of this paper. As you can see, the single reviewer is overall positive, though on the fence about suitability of this paper. We can proceed if you are willing to add a bit of discussion that this reviewer requests on "role that cross-talk plays in the nutrient response system. " In particular (She/he) suggests "a section specifically addressing crosstalk between the pathways". If you can do so, and highlight that new section in red font so I can easily find it, then I'd accept the paper forthwith.

Sincerely,

Leah Edelstein-Keshet
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Tyson,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

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

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

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 manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

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

Reviewer #3 (Remarks to the Author):

This is an unusual paper in that it is focused on (1) converting the literature about nutrient signaling in yeast (particularly, nitrogen, glucose and carbon signaling) into a comprehensive mathematical model and (2) testing if the resulting model can recapitulate previous observations in wild-type and a variety of mutant strains. It does not provide any new biological insights or test any specific hypotheses (although the authors discuss areas, such as Rtg1/3 activity, where the model is not well constrained by available data). So ultimately the value of Jalihal et. al. is as an aggregation of the literature and a "state of the field" or benchmark model. From this perspective I think Jalihal et al is sound, the modelling seems to have been done carefully, and the tests of the robustness are very comprehensive. In line with this Jalihal et al has been carefully reviewed previously and the authors have made the appropriate revisions.

So, the question then, is does a paper of this style/type (with no new insights into biology) belong in MBoC, or a more specialized journal? I lean towards saying it does belong in MBOC because the literature in this important area has gotten so complex that a model of the type presented by Jalihal can help move the field forward by delineating the critical elements in the signaling network. However, as written, the paper does not do this: Specifically, what researchers in the field need is an assessment of the role that cross-talk plays in the nutrient response system.

The molecular mechanisms underlying signaling through the PKA, Snf1 and TOR signaling pathways are well established, but the literature explaining how and when crosstalk between these pathways comes into play is much weaker and often contradictory. Jalihal et al has already addressed the role of crosstalk in their study of robustness (or could easily do so), by asking how well their network performs in the absence of each type of pathway-pathway interaction. Therefore, I suggest that the authors add a section specifically addressing crosstalk between the pathways (ie what happens when the various mechanisms of PKA and TOR crosstalk are removed, then Snf1 and TOR etc) so that the paper is useful for a broader audience. In doing so the authors should be careful to address whether the specific mechanisms of cross-talk in their model are supported by the data (for example via experiments in mutants), or if it just important to have crosstalk between one type of nutrient signaling pathway and another. For example, does Snf1 regulate TORC1, or is there just support for glucose/energy dependent regulation of TOR.

Response to Reviewers - 3

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We thank the reviewer for these suggestions. We have added a new section focusing on Snf1-TORC1 interactions. Specifically, we address the experimental evidence that Snf1 does not mediate the formation of Tor1 foci or the inhibition of TORC1 - phosphorylation of Sch9. To explain these observations would require supplementing the model with a novel interaction from carbon-sensing to TORC1 inhibition. Because we don't have any guidance from molecular biologists as to what this interaction might be, we hesitate to add a new, speculative interaction to a model that is already very complex.

The new section is presented below.

Crosstalk interactions between the carbon and nitrogen signaling pathways

In Figure 1 we include two well-documented interactions by which the carbon and nitrogen signaling pathways cross-talk: Snf1 inhibition of the TORC1 complex, and Sch9 inhibition of PKA. In the previous section we investigated how deletions of Snf1 and Sch9 affect global metabolic responses in terms of regulatory signals impinging on transcription factor readouts. In this section, by looking more closely at the role of Snf1 in communicating carbon-status to the nitrogen signaling pathway through TORC1, we conclude that our model oversimplifies the interaction and will need to be revised when further experimental studies resolve the discrepancy between the formation of TORC1 protein aggregates and the inhibition of TORC1 kinase activity on Sch9.

The effects of glucose starvation and refeeding on the activity of the TORC1 complex have been studied by Hughes-Hallett *et al*.(Hallett *et al.*, 2015) and by Prouteau

et al.(Prouteau *et al.*, 2017). Both groups observe that, in response to glucose starvation, (1) components of TORC1 complexes rapidly form dense protein aggregates and (2) Sch9 protein is rapidly dephosphorylated (indicating a loss of TORC1 activity). Hughes-Hallett et al. reported that YFP-Kog1 dissociates from TORC1 complexes (monitored by GFP-Tor1), forming 'Kog1-foci'. The formation of Kog1 foci was slowed considerably in *snf1Δ* mutants and, even more so, in cells expressing Kog1 proteins with serine-to-alanine mutations at Snf1-phosphorylation sites, clearly indicating Snf1 involvement in the formation of Kog1-foci. Nonetheless, the dephosphorylation and re-phosphorylation of Sch9 in response to glucose withdrawal and re-addition was no different in these mutant cells compared to wild-type (their Figure 5), suggesting a complicated relationship between Kog1 aggregation and TORC1 activity. Prouteau et al. confirmed the formation of TORC1 foci microscopically, using GFP-Kog1 and GFP-Tor1 labelled cells. In electron micrographs they observed large, toroidal aggregates of TORC1 forming under glucose-starvation conditions. Nonetheless, they observed (in their Extended Data Figure 3) no appreciable differences between wild-type and *snf1Δ* mutants in terms of either GFP-TOR1 foci formation or Sch9 dephosphorylation in response to glucose starvation (and reversed by glucose refeeding). These results suggest that the formation of 'Kog1-foci' but not 'Tor1-foci' is strongly dependent on Snf1 signaling, but TORC1 activity (in terms of Sch9 phosphorylation) is not dependent on Snf1.

Our model, at present, cannot account for these complex relationships because it does not distinguish between TORC1 aggregation and TORC1 kinase activity. In our network diagram (Figure 1), the signal from Snf1 to TORC1 seems to represent the formation of Kog1 foci but not the inhibition of TORC1 kinase activity. There seems to be some other signal(s) from carbon-sensing to TORC1 signaling that is not included in our model; perhaps, a direct link from glucose-receptors in the cell membrane to the guanine exchange factor that converts EGOC-GDP (an activator of TORC1 kinase) into EGOC-GTP. Until we have more convincing experimental evidence of the molecular identity of this 'other signal,' it seems premature to model these interactions based on speculative assumptions.

Hallett, JEH, Luo, X, and Capaldi, AP (2015). Snf1/AMPK promotes the formation of Kog1/Raptor-bodies to increase the activation threshold of TORC1 in budding yeast. eLife 4, nil.

Prouteau, M, Desfosses, A, Sieben, C, Bourgoint, C, Mozaffari, NL, Demurtas, D, Mitra, AK, Guichard, P, Manley, S, and Loewith, R (2017). TORC1 organized in inhibited domains (TOROIDs) regulate TORC1 activity. Nature 550, 265–269.

RE: Manuscript #E20-02-0117RRR
TITLE: "Modeling and Analysis of the Macronutrient Signaling Network in Budding Yeast"

Dear Dr. Tyson:

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell.

Congratulations for the final acceptance, and apologies for the very long process of review and revision.

Sincerely,
Leah Edelstein-Keshet
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Tyson:

Congratulations on the acceptance of your manuscript.

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