A Dynamic Model of Proteome Changes Reveals New Roles for Transcript Alteration in Yeast

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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.)

1st Editorial Decision 06 January 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 find the topic of your study of potential interest. However, they raise substantial concerns on your work, which, I am afraid to say, preclude its publication in its present form.

In general, the reviewers appreciated the detailed and rigorous dataset created in this work, and found the topic of potentially broad interest. Nonetheless, they felt that key conclusions based on the modeling analysis remained somewhat weakly supported, and they provide some potential alternate interpretations. While some of these concerns may be addressable with additional discussion, the reviewers clearly indicated that certain issues would require direct experimental testing. The two most important issues appear to be: 1) suppression of protein synthesis after salt treatment needs to be experimentally demonstrated to rule out possible dilution effects (noted by the first reviewer), and 2) the conclusions regarding ribosome redistribution and availability deserve direct testing (indicated strongly by the last reviewer, but also a concern of the first reviewer). The second reviewer also asks for additional discussion regarding the relevance of these results for our understanding of the salt stress response in yeast.

In addition, when preparing your revised work please take into consideration the following format and content issues:

1. Please note, that in addition to our capacity to host datasets in our supplementary information section, we provide a new functionality that allows readers to directly download the 'source data' associated with selected figure panels (e.g. <http://tinyurl.com/365zpej>). This sort of figure-

associated data may be particularly appropriate for this work. Guidelines have been pasted at the end of this email.

2. In general, Molecular Systems Biology requires that authors provide machine-readable versions of mathematical models as supplementary material. We strongly encourage authors to supply model files in SBML when appropriate, and to submit the models to a public database such as BioModels or JWS Online.

PLEASE NOTE As part of the EMBO Publications transparent editorial process initiative (see http://www.nature.com/msb/journal/v6/n1/full/msb201072.html), Molecular Systems Biology now publishes online a Review Process File with each accepted manuscript. Please be aware that in the event of acceptance, your cover letter/point-by-point document will be included as part of this file, which will be available to the scientific community. More information about this initiative is available in our Instructions to Authors, or by contacting our editorial office (msb@embo.org).

If you feel you can satisfactorily deal with these points and those listed by the referees, you may wish to submit a revised version of your manuscript. Please attach a covering letter giving details of the way in which you have handled each of the points raised by the referees. A revised manuscript will be once again subject to review and you probably understand that we can give you no guarantee at this stage that the eventual outcome will be favorable.

Yours sincerely,

Editor
Molecular Systems Biology

REFEREE REPORTS

Reviewer #1 (Remarks to the Author):

The advent of comprehensive measurements of mRNA and protein abundance have resulted in conflicting reports about the correlation between these two measures of gene expression. In this manuscript, Lee et al. weigh in on this important question with a careful analysis of dynamic changes in mRNA and protein levels during a stress response in budding yeast. They observe a qualitative difference between the effect of increases versus decreases in mRNA abundance; this effect is novel and has broad relevance for understanding how transcriptional regulation controls cell physiology. From a technical perspective, their measurements of protein abundance in particular are well-replicated and well-validated, and the high-quality data allows them to draw strong conclusions. I do have concerns with the authors' interpretation and modeling, as dicussed below. However, the work will have significant impact both for its methodological advances and for the exciting observations that they permit, and I strongly recommend its publication provided that these concerns can be addressed.

The authors do not distinguish carefully between the effects of cell growth and cell division in their mathematical model or in their qualitative discussion of their results. The authors show (e.g., in Figure S5) that salt treatment produces a significant block to cell division, but in many cases budding yeast continues to grow during cell cycle arrest. This continued growth would dilute pre-existing protein, regardless of whether cell division was occurring, and its absence would eliminate this dilution. The authors need to demonstrate that salt treatment blocks new protein synthesis in order to support their claim that growth arrest explains the maintenance of protein levels in the face of decreased mRNA abundance.

The authors also suggest that rapid decreases in transcript abundance may be important for redirecting protein biosynthesis towards stress-response proteins rather than for decreasing protein abundance per se. This is an interesting and reasonable hypothesis, but it seems unlikely that ribosomes are the limiting factor in this competition. Polysome profiles from studies such as Uesono et al. (2002) and Melamed et al. (2008) demonstrate that a large fraction of ribosomes are inactive during the early stress response period when the abundance of certain transcripts is declining and

cell growth is suppressed. This suggests that initiation factors, rather than ribosomes, limit translation following salt treatment and that this limitation may represent a regulated physiological response to constraints on protein biosynthetic capacity other than ribosomes. Furthermore, the authors never address whether the overall cellular mRNA concentration might change during salt stress, e.g. due to increases in mRNA turnover. Figure 4 should not report "percent cellular ribosomes" when conflicting data exists from more direct polysome profiling experiments. The authors should also avoid equating the "number of ribosomes", in the caption of Figure 4, with the "fraction of ribosomes", as shown in the figure.

Finally, the authors overlook the possibility that their measurements of mRNA abundance might include untranslated pools of mRNA whose abundance or composition changes during salt stress. For example, changes in the dynamics of nuclear export might affect the amount of nuclear poly-(A)+ RNA without significantly affecting the steady-state levels of cytoplasmic transcript. As mRNA abundance measurements should reflect the total amount of poly-(A)+ message while only the cytoplasmic fraction contributes to protein synthesis, the selective loss of a fraction of untranslated mRNAs could result in changes in mRNA levels that are not reflected in protein abundance.

Reviewer #2 (Remarks to the Author):

The manuscript by Lee and colleagues describes a coordinated quantitative proteomic and mRNA expression analysis of salt treated yeast. The authors carried out a time course analysis to determine the changes in mRNA and protein abundances over six hours and they took six time points during this time. Three biological replicates were analyzed at both the protein and mRNA levels. This is an insightful and well written manuscript on the correlation of mRNA and protein expression and has extensive mathematical modeling of the expression events to explain the results. The authors make a number of valuable observations regarding the relationship between changing mRNA and protein which are summarized in the discussion analyzing aspects like the estimated number of ribosomes released due to transcript repression is for redirection of translational capacity and transient changes in mRNA producing faster protein changes. In general, this is a well executed and valuable manuscript highly relevant for Molecular Systems Biology in need of minor revision.

One aspect of the manuscript that is somewhat concerning is the manuscript is written in general terms where the mRNA and protein expression events are considered in a broad sense. The specifics of the manuscript were this was an analysis of the salt response of yeast, and it is possible that the events described in the manuscript are specific or somewhat specific to this particular case. One idea that should be discussed more in the manuscript is have any of the prior publications on mRNA and protein expression, especially in yeast, drawn similar conclusions, especially the few where time course studies have been done. The question being how general are the mRNA and protein expression events described here? One simplistic but common theme seen recently in other time course work is there is certainly a delay in correlation of mRNA and protein expression changes. Here the authors found the highest correlation to be between the 60 min RNA time point and the 90 minute protein time point for induced RNA and the 60 min RNA time point and the 6 hr time point for repressed RNA. This could be a valuable topic to discuss in more detail in the context of recent literature. It suggests that proteins are not necessarily targeted for rapid degradation, for example.

The biological context of the manuscript is heavily slanted towards transcriptional and translational control. As a result, the biology of the salt response is somewhat ignored in the manuscript, but salt stress is a significant biological event. It would be valuable if the authors spent some time describing any new insights into the biology of the salt response that were elucidated in this study.

Reviewer #3 (Remarks to the Author):

Lee et al performed an experiment where they monitored in time course the response of yeast to osmotic stress and measured, in parallel and in triplicate, mRNA and protein levels. They observe a good correlation between up-regulation of mRNA and protein levels but no such corrections between down-regulated mRNA and protein levels. Down-regulation of mRNA levels does not seem to lead to similar down-regulation of protein levels. Employing a mathematical model the authors propose that protein levels do not drop because of a temporary growth arrest and altered protein stability. They further propose that the purpose for down-regulating mRNA levels is to (a)

actually prevent an increase of the protein levels in arrested cells and (b) to make available ribosomes for translating the up-regulated mRNAs. Finally the authors suggest that the transcriptional peak/over shoot observed for many stress-regulated genes allows a more rapid increase in the corresponding protein levels as compared to genes whose mRNA levels do not display such a peak.

The topic, namely how transcriptional and translational regulation interplay to mount appropriate cellular responses, is interesting and relevant. Unfortunately, the authors come to their main conclusions based on modeling and simulations while not making use of possibilities to test those experimentally. Most importantly, the ideas on ribosome redistribution and availability could have been supported by data on ribosome occupancy as they can be obtained from polysomes preparations. This could have been done from the same samples that were used to generate the mRNA and protein data.

The authors investigate the purpose of the transcriptional peak displayed by some genes after stress treatment, as mentioned above. Unfortunately, the authors measured mRNA levels first after 30min, instead of including a sample at 15min as well. Several genes respond very quickly. This means that in many cases the authors might have missed the initial peak.

Further points:
1. The paper is difficult to read. Especially the figures legends do not provide sufficient explanation to guide the reader. More information and explanation is needed in the main text and the supplement should only serve as a source for further information for the interested reader.
2. Why did the authors just use 0.7M NaCl? They may have a reason that should be explained.
3. The particular selection of genes in Fig. 2B might affect the interpretation. Are there genes behaving differently?
4. The terms "induced" and "repressed" in the context of array data should be avoided as those imply a transcriptional control mechanism. Up- and down-regulation should be used instead.

1st Revision - authors' response 02 May 2011

Thank you for handling our manuscript, "A Dynamic Model of Proteome Changes Reveals New Roles for Transcript Alteration in Yeast" by Lee and Topper et al, for Molecular Systems Biology. We were very happy that all three reviewers were enthusiastic about the manuscript and its publication in MSB, stating that the work is "[of] significant impact", "insightful and well written", and "interesting and relevant."

We submit a much-revised version in which we have addressed all of the reviewersí comments. In particular, we have added substantial experimental support of our model that transcript repression promotes redistribution of translational machinery during stress acclimation. We have analyzed a mutant lacking the transcriptional repressors Dot6p and Tod6p ñ this strain fails to repress ~250 transcripts in response to salt, allowing us to test the consequences of failed transcript reduction. In addition to microarray analysis of the double mutant (Dataset S3), we provide polysome profiles over time in wild-type and mutant cells responding to salt (Figure 4A and 4B) and follow polysome association of individual transcripts in the wild type and dot6 tod6 mutant (Figure 4C and 4D). Together, this work confirms the transient reduction in global protein synthesis after salt treatment and shows that failure to repress transcripts leads to continued association with ribosomes as cells acclimate. We have also reworked presentation and discussion of our model, clarified figure legends and text, added a supplemental figure panel to address cell growth arrest following salt treatment (Figure S6B), and provided an additional figure (Figure S3) and text discussing insights into the NaCl response, as requested by Reviewer #2.

Below we provide a detailed response to each of the reviewer comments (which are highlighted in blue). We hope the reviewers respond favorably to these changes, which we feel have strengthened the manuscript.

We have also gone to great lengths to make our modeling code available, as requested by the journal. Because of limitations in most existing SMBL interpreters, we implemented our modeling in the software COPASI, version 4.6 (available at http://www.copasi.org/tiki-read_article.php?articleId=33), so as to match the analysis reported in the manuscript. We provide the code and a short description of its usage for download with the manuscript as Dataset S4.

Reviewer #1

"The advent of comprehensive measurements of mRNA and protein abundance have resulted in conflicting reports about the correlation between these two measures of gene expression. In this manuscript, Lee et al. weigh in on this important question with a careful analysis of dynamic changes in mRNA and protein levels during a stress response in budding yeast. They observe a qualitative difference between the effect of increases versus decreases in mRNA abundance; this effect is novel and has broad relevance for understanding how transcriptional regulation controls cell physiology. From a technical perspective, their measurements of protein abundance in particular are well-replicated and well-validated, and the high-quality data allows them to draw strong conclusions. I do have concerns with the authors' interpretation and modeling, as discussed below. However, the work will have significant impact both for its methodological advances and for the exciting observations that they permit, and I strongly recommend its publication provided that these concerns can be addressed."

First, we thank the reviewer for their enthusiasm on the manuscript.

1. "The authors do not distinguish carefully between the effects of cell growth and cell division in their mathematical model or in their qualitative discussion of their results. The authors show (e.g., in Figure S5) that salt treatment produces a significant block to cell division, but in many cases budding yeast continues to grow during cell cycle arrest. This continued growth would dilute pre-existing protein, regardless of whether cell division was occurring, and its absence would eliminate this dilution."

We have addressed this point in two ways: by quantifying cell volume changes during the NaCl response and by clarifying the modeling text to address the potential issue.

We have carefully measured cell volume during the response to 0.7M NaCl using a flow cytometer and show that the cell volume does not change significantly as cells acclimate to NaCl. During the 60 min period after NaCl treatment, the median forward light scattering (proportionate to cell size) remains within 1.6% of pre-stress levels (see new supplemental Figure 6B). In contrast, controls show we can detect 15-30% changes in median forward scatter immediately after severe hypo- or hyperosmotic shock (due to cell swelling or shrinkage) or during synchronized cell-cycle progression (data not shown). The absence of significant changes after NaCl treatment is consistent with the near-constant optical density during this period, which reflects both cell size and cell number. Therefore, we conclude that cell volume does not change measurably due to cell growth during the arrest period, justifying our modeling assumption of constant cell volume.

2. "The authors need to demonstrate that salt treatment blocks new protein synthesis in order to support their claim that growth arrest explains the maintenance of protein levels in the face of decreased mRNA abundance."

We now provide several lines of evidence that translation is transiently reduced immediately after NaCl treatment. First, we provide a time course of polysome profiles during the NaCl response that shows the monosome:polysome ratio dramatically increases immediately after stress but recovers to near pre-stress levels ~45 min after acclimation (see new Figure 4). An increase in the monosome:polysome ratio is widely accepted to reflect reduced translation initiation; therefore, these results confirm the transient decrease in overall translation initiation immediately after NaCl treatment. We also followed polysome association of two repressed transcripts (ARX1, Figure 4, and NOP2, data not shown) and showed that both transcripts are associated with polysomes before

stress but are found largely in the monosome fraction 30 min after NaCl treatment, showing that these transcripts are not highly translated during this time.

These results are consistent with prior studies that have shown decreased 35S-methionine incorporation after NaCl treatment (see Uesono & Toh-E 1992, Varela et al. 1992, Blomberg 1995) ñ we did not to repeat those well-established experiments here, in part because it requires careful normalization to levels of methionine uptake (which is dramatically reduced after NaCl treatment, see Uesono and Toh-E 1992, Varela et al. 1992, Norbeck and Blomberg 1998).

Together, these results confirm the transient reduction in global protein translation during the period of cell-cycle arrest. Our main observation remains that significant reduction in transcript abundance does not lead to corresponding reductions in the encoded protein levels within the cell population.

3. "The authors also suggest that rapid decreases in transcript abundance may be important for redirecting protein biosynthesis towards stress-response proteins rather than for decreasing protein abundance per se. This is an interesting and reasonable hypothesis, but it seems unlikely that ribosomes are the limiting factor in this competition. Polysome profiles from studies such as Uesono et al. (2002) and Melamed et al. (2008) demonstrate that a large fraction of ribosomes are inactive during the early stress response period when the abundance of certain transcripts is declining and cell growth is suppressed. This suggests that initiation factors, rather than ribosomes, limit translation following salt treatment and that this limitation may represent a regulated physiological response to constraints on protein biosynthetic capacity other than ribosomes"

We agree with the reviewer that there are other possible reasons for limited translational capacity aside of ribosome availability. We have addressed this concern in several ways. First, we removed discussion of ribosome availability from the main text (and moved the original figure to Figure 7) and instead provide a brief presentation of this model, along with several alternatives, in the Discussion section. We also clarified the text to state that transcript repression redirects translational capacity as global translation is resuming, not immediately after NaCl treatment.

Second, we clarified the text to reflect the polysome time courses. These show that the dramatic increase in monosome-bound transcripts occurs at 5-15 min after NaCl treatment (see new Figure 4) whereas transcript repression does not peak until 30 min later, when global translation is resuming. This supports our conclusion that the main purpose of transcript reduction is not to reduce protein synthesis immediately after NaCl exposure, which is instead regulated at the level of translation initiation as Reviewer #1 points out.

Finally, we provide a more direct test of our hypothesis by analyzing polysome association in the absence of transcript repression. We generated a mutant lacking the DOT6/TOD6 transcriptional repressors, which is unable to repress ~250 high-abundance transcripts in response to stress. This mutant behaves as wild type before stress, but has a defect acclimating to NaCl. In particular, we show that the ARX1 transcript, which fails to get reduced after NaCl treatment, remains associated with polysomes as global translation is resuming. This is consistent with our hypothesis (and a recent study by Scott et al., Science 2010) that failure to repress high-abundance transcripts leads to competition for ribosomes (and other translational machinery). Unfortunately, we were unable to quantify protein changes in the mutant using several available antibodies against Dot6p/Tod6p targets. We also tried several antibodies against induced proteins to look for delayed protein induction in the mutant but were unable to make strong statements due to technical limitations of the quantitative Westerns.

Nonetheless, our results demonstrate that failure to repress high-abundance transcripts consumes translational machinery during acclimation. We hope that these changes have addressed the reviewerís concerns.

4. "Furthermore, the authors never address whether the overall cellular mRNA concentration might change during salt stress, e.g. due to increases in mRNA turnover."

This reviewer raises an important point. It is possible that the total mass of mRNA in the cell decreases somewhat, especially since the most highly abundant mRNAs are transiently reduced after NaCl treatment. However, measuring absolute mRNA abundance is very difficult on microarrays;

instead, our measurements report on the relative abundance of each transcript with regard to the pool of mRNAs analyzed at each time point (a result of our - and almost all - microarray normalization protocols). Our microarray measurements agree well with quantitative RT-PCR analyses, which were normalized by an independent method (see supplemental Figure S7). The same holds for protein measurements based on quantitative Westerns normalized either to Act1p or to cell number (see supplemental Figure S1). Although our measurements reflect on changes of specific mRNAs relative to the population of transcripts, we do not believe that this could obscure any of our results or conclusions.

5. "Figure 4 should not report "percent cellular ribosomes" when conflicting data exists from more direct polysome profiling experiments. The authors should also avoid equating the "number of ribosomes", in the caption of Figure 4, with the "fraction of ribosomes", as shown in the figure."

We apologize for the confusing text. We have reworded the text and figure legend to reflect that we are plotting the fraction of the 171,000 ribosomes actively translating before stress. This figure has been moved to Figure 7 and reduced text has been moved to the Discussion section.

6 . "Finally, the authors overlook the possibility that their measurements of mRNA abundance might include untranslated pools of mRNA whose abundance or composition changes during salt stress. For example, changes in the dynamics of nuclear export might affect the amount of nuclear poly-(A)+ RNA without significantly affecting the steady-state levels of cytoplasmic transcript. As mRNA abundance measurements should reflect the total amount of poly-(A)+ message while only the cytoplasmic fraction contributes to protein synthesis, the selective loss of a fraction of untranslated mRNAs could result in changes in mRNA levels that are not reflected in protein abundance"
The reviewer raises an important point, namely that reduced abundance of some transcripts could reflect the disappearance of an untranslated pool, without affecting the pool of mRNAs that are being translated in the cytosol. However, several lines of evidence suggest this is unlikely to significantly affect our measurements. First, global analysis of polysome-bound transcripts from Arava et al. found that for the vast majority of genes, most of the poly-adenylated transcripts are actively engaged with ribosomes (i.e. cytosolic), and "abundant stored pools of nontranslationally engaged, polyadenylated mRNAs are uncommon or absent" for most transcripts. Their measurements were done with the same strain we use, grown under the same starting conditions; therefore, we assume that most of the measured polyadenylated transcripts in unstressed cells are engaged with ribosomes in the cytosol.

We also do not believe there to be large pools of unadenylated transcripts in these cells. We compared microarray measurements of the response to 0.7M NaCl when total RNA was labeled with an oligo-dT primer (enriching for polyadenylated transcripts) versus a mixture of random hexamers and oligo-dT primers (as done here). Regression analysis shows that the R2 and the slope of the fit (i.e. the fold-change in expression) are indistinguishable for experiments that probe total RNA vs. only polyadenylated RNA, for all the data and specifically for reduced transcripts shown in Figure 2. Therefore, we conclude that there must not be a large pool of unadenylated, nontranslated transcripts for most genes.

We appreciate the point and have added a statement to the results that, while it is possible that some of our measurements may reflect changes in the untranslated pool of mRNAs, prior experimental evidence suggests that such untranslated pools are likely to be very small for most transcripts (Arava et al. 2003), and thus most of the fold-changes in abundance correspond to the translated pool of mRNAs. Regardless, the main point ñ that significant transcript reduction observed on microarrays cannot be assumed to modulate corresponding protein levels ñ holds true.

Reviewer #2

"The manuscript by Lee and colleagues describes a coordinated quantitative proteomic and mRNA expression analysis of salt treated yeast. The authors carried out a time course analysis to determine the changes in mRNA and protein abundances over six hours and they took six time points during this time. Three biological replicates were analyzed at both the protein and mRNA

levels. This is an insightful and well written manuscript on the correlation of mRNA and protein expression and has extensive mathematical modeling of the expression events to explain the results. The authors make a number of valuable observations regarding the relationship between changing mRNA and protein which are summarized in the discussion analyzing aspects like the estimated number of ribosomes released due to transcript repression is for redirection of translational capacity and transient changes in mRNA producing faster protein changes. In general, this is a well executed and valuable manuscript highly relevant for Molecular Systems Biology in need of minor revision."

1. "One aspect of the manuscript that is somewhat concerning is the manuscript is written in general terms where the mRNA and protein expression events are considered in a broad sense. The specifics of the manuscript were this was an analysis of the salt response of yeast, and it is possible that the events described in the manuscript are specific or somewhat specific to this particular case. One idea that should be discussed more in the manuscript is have any of the prior publications on mRNA and protein expression, especially in yeast, drawn similar conclusions, especially the few where time course studies have been done. The question being how general are the mRNA and protein expression events described here?"

We thank the reviewer for these points. We have clarified throughout the manuscript that our results are specific to the NaCl response, and we have added a paragraph to the Discussion citing evidence that similar trends are evident (but not necessarily reported) in prior yeast proteomic datasets of other environmental responses. This suggests that the lack of significant protein reduction, despite reduced levels of the encoding transcripts, is likely common to several stress treatments beyond NaCl.

2. "One simplistic but common theme seen recently in other time course work is there is certainly a delay in correlation of mRNA and protein expression changes. Here the authors found the highest correlation to be between the 60 min RNA time point and the 90 minute protein time point for induced RNA and the 60 min RNA time point and the 6 hr time point for repressed RNA. This could be a valuable topic to discuss in more detail in the context of recent literature. It suggests that proteins are not necessarily targeted for rapid degradation, for example."

We have added a short presentation on this in the Discussion. Our results suggest that the delay between protein and mRNA is specific to each pair and determined in part by the mRNA dynamics. We highlight work of Fournier et al. (2010) that shows delayed changes in protein abundance after rapamycin treatment.

3. "The biological context of the manuscript is heavily slanted towards transcriptional and translational control. As a result, the biology of the salt response is somewhat ignored in the manuscript, but salt stress is a significant biological event. It would be valuable if the authors spent some time describing any new insights into the biology of the salt response that were elucidated in this study."

The reviewer raises a good point. We have added a new figure (Figure S3) and additional text to the main body and supplement that presents new insights into the NaCl response. Specifically, we present functional processes that are over-represented in proteins with the largest abundance changes, discuss the implications of different acclimation times of different functional processes, and compare the levels and dynamics of protein change to gene-fitness defects from prior studies of the yeast deletion library.

Reviewer #3

"Lee et al performed an experiment where they monitored in time course the response of yeast to osmotic stress and measured, in parallel and in triplicate, mRNA and protein levels. They observe a good correlation between up-regulation of mRNA and protein levels but no such corrections between down-regulated mRNA and protein levels. Down-regulation of mRNA levels does not seem

to lead to similar down-regulation of protein levels. Employing a mathematical model the authors propose that protein levels do not drop because of a temporary growth arrest and altered protein stability. They further propose that the purpose for down-regulating mRNA levels is to (a) actually prevent an increase of the protein levels in arrested cells and (b) to make available ribosomes for translating the up-regulated mRNAs. Finally the authors suggest that the transcriptional peak/over shoot observed for many stress-regulated genes allows a more rapid increase in the corresponding protein levels as compared to genes whose mRNA levels do not display such a peak."

"The topic, namely how transcriptional and translational regulation interplay to mount appropriate cellular responses, is interesting and relevant. Unfortunately, the authors come to their main conclusions based on modeling and simulations while not making use of possibilities to test those experimentally. Most importantly, the ideas on ribosome redistribution and availability could have been supported by data on ribosome occupancy as they can be obtained from polysomes preparations. This could have been done from the same samples that were used to generate the mRNA and protein data."

In accordance with the requests of Reviewer #3, we have added substantial biological experiments to the manuscript, including: 1) time-course analysis of polysome profiles in wild-type cells; 2) analysis of gene expression and polysome profiles in a dot6 tod6 mutant that fails to repress ~250 transcripts in response to NaCl; and 3) characterization of polysome association of individual transcripts in wild-type and dot6 tod6 cells to show the effect of transcript repression on ribosome association. Our results show that failure to repress high-abundance transcripts does not affect the reduced translation initiation immediately after NaCl treatment (Figure 4B), but it does lead to continued polysome association of the remaining transcripts as the mutant acclimates to NaCl (Figure 4C).

As described above (see Reviewer #1 response), we also moved discussion of the model, along with several alternatives, to the Discussion section. We believe that the additional experiments requested by this reviewer strengthen and complement the proteomic analysis and modeling in the manuscript.

"The authors investigate the purpose of the transcriptional peak displayed by some genes after stress treatment, as mentioned above. Unfortunately, the authors measured mRNA levels first after 30min, instead of including a sample at 15min as well. Several genes respond very quickly. This means that in many cases the authors might have missed the initial peak."

We apologize that our reasoning for choosing these time points was omitted from the prior manuscript. The choice of time points was based on other time courses in our lab, which showed that the vast majority of increased transcripts peak at or after 30 min (compared to an earlier 15 minute time point) at this dose of NaCl. A statement has been added that most transcripts peak at or later than 30 min after this dose of salt treatment. To ensure that our results are not influenced by transcripts peaking before 30 min, we reanalyzed dynamic patterns (Figure 5 and text) after removing all genes whose fold-change at 15 min was greater than or equal to the fold-change at 30 minutes. This removed 13 of the 140 transcripts analyzed in Figure 5. All of the original results have been recapitulated (in fact many of the p-values are now significantly lower) and thus none of the conclusions are due to rapidly changing transcripts. Figure 5 and statistics mentioned in the text have been updated to reflect the new analysis.

1. "The paper is difficult to read. Especially the figures legends do not provide sufficient explanation to guide the reader. More information and explanation is needed in the main text and the supplement should only serve as a source for further information for the interested reader."

We apologize for the confusion and have expanded and clarified many of the figure legends and the text to improve the readability.

2. "Why did the authors just use 0.7M NaCl? They may have a reason that should be explained."

We have added a statement (Page 4) that this dose of salt provides a robust physiological response (including large gene expression changes) but results in high viability and eventual resumption of

cell growth. Lower doses do not produce large transcriptome changes, while higher doses do not support robust resumption of cell growth.

3. "The particular selection of genes in Fig. 2B might affect the interpretation. Are there genes behaving differently?"

We have clarified the figure legend to make this clearer. Figure 2A and 2B show average log2 changes of transcripts and proteins, for all transcripts with statistically significant changes in abundance (FDR < 0.05) whose corresponding proteins were measured in triplicate (to minimize the effects of technical noise on the correlation). No other selection criteria were applied. The main point of Figure 2B is to show that changes in transcript abundance are a good predictor of changes in protein abundance. The only RNA-protein pairs that behave significantly differently are those for which protein changed significantly but RNA did not - this class is discussed in detail in the text and Figure 6C.

4. "The terms "induced" and "repressed" in the context of array data should be avoided as those imply a transcriptional control mechanism. Up- and down-regulation should be used instead."

We agree with the reviewer and have removed all references to ëinducedí and ërepressedí transcripts and instead refer to transcripts with ëincreasedí or ëdecreasedí abundance throughout the manuscript.

2nd Editorial Decision 30 May 2011

Thank you again for submitting your work to Molecular Systems Biology. We have now heard back from the three referees who agreed to evaluate your revised study. As you will see, the referees were satisfied with the revisions made to this work, and are now supportive of publication. We have, however, a series of minor format and data issues that we would like to ask you to address in a final revision of this work.

1. Thank you for supplying the proteomics data as supplementary material. We strongly encourage you to also deposit this data, in a machine-readable format (e.g. mzML), in one of the major public databases, for example Pride (http://www.ebi.ac.uk/pride/), while following the MIAPE recommendations (http://www.psidev.info/index.php?q=node/91). After deposition, the dataset accession number should be included in the Methods section of the main manuscript. Please contact us if this will pose a problem.

2. In addition to our capacity to host datasets in our supplementary information section, we provide a new functionality that allows readers to directly download the 'source data' associated with selected figure panels (e.g. <http://tinyurl.com/365zpej>). This sort of figure-associated data may be particularly appropriate for Fig. 7. A document with figure source data guidelines has been attached.

3. Our synopsis format can only include Figures (one or two), taken directly from the main manuscript. The figure legend included in the current synopsis appears to describe a figure that is a different from the existing manuscript figures.

4. We currently have two files submitted as "Dataset 4" -- one is the COPASI model files and the other is the excel-based modeling. Thank you for providing this detailed supplementary material. I think it would be best to rename the excel-based modeling file as "Dataset 5" and briefly describe the distinction between the two in the Supplementary Information pdf.

Thank you for submitting this paper to Molecular Systems Biology.

Yours sincerely,

Editor
Molecular Systems Biology

REFEREE REPORTS

Reviewer #1 (Remarks to the Author):

Lee et al. have revised their manuscript substantially and added new evidence that transcript reduction during osmotic stress serves principally to free up protein biosynthetic capacity, rather than to directly decrease protein levels. The authors directly test the role of transcript repression using dot6D/tod6D yeast, which fail to repress a large class of abundant transcripts following osmotic shock. This strain shows defects in polysome recovery, persistent polysome association of target messages, and defects in recovery from osmotic stress. These defects are consistent with the authors' model and provide independent support for the physiological importance of transcript repression.

Beyond these new experiments, the authors have clarified important aspects of the osmotic response in a manner that addresses my concerns. They have shown that osmotic stress blocks cell growth and protein synthesis, thereby explaining how levels of stable proteins are maintained following transcript reduction. They have also shown that the acute repression of cell growth operates on a different timescale than transcript reduction. The existence of a large pool of unoccpuied ribosomes in the early stress response is not an objection to the ribosome competition model, because this competition acts later.

The authors have verified key assumptions in their model of osmotic stress and have used dot6D/tod6D mutants to provide direct evidence for their model. The ribosome competition phenomenon investigated by the authors may have rather broad implications for understanding genome-wide gene expression. The revisions have strengthened the paper substantially and fully addressed my concerns, and I recommend its publication.

Reviewer #2 (Remarks to the Author):

The authors have fully addressed my concerns over the first version of the manuscript. The revised manuscript is acceptable for publication. It will make an important contribution to the field.

Reviewer #3 (Remarks to the Author):

The authors have done an excellent job in responding to the referee comments and made significant efforts to provide further data. I was happy to read that also the authors believed that performing the analysis that I previously suggested (employing polysomal RNA) has strengthened the manuscript.

From my perspective the paper is now ready for acceptance.

2nd Revision - authors' response 14 June 2011

Thank you for handling our manuscript, "A Dynamic Model of Proteome Changes Reveals New Roles for Transcript Alteration in Yeast" by Lee and Topper et al, for Molecular Systems Biology. We are thrilled that the reviewers were so positive about the revised manuscript.

We have addressed the remaining requests of the journal in the following ways:

1. We provide both .raw and mzML files of proteomic data on our website (http://www.chem.wisc.edu/~coon/Downloads/Lee_MSB_2011/) as well as in the Proteome Commons Tranche public repository. A statement with our url and the Tranche accession number

have been provide in the Methods in section "LC MS/MS."

2. We also provide the source data for Figure 7, as requested.

3. We have updated the Extended Synopsis to contain the full legend for Figure 2 of the manuscript, and have slightly updated the text to fit in the required word limit.

4. We have separated the modeling codes into the Excel code used in our analysis (now labeled "Dataset 4") and the COPASI code and instructions (labeled as Dataset 5"). A statement to this effect has been added to the Supplemental Information.

Please let us know if there is any other information you need. We look forward to seeing the manuscript in print.

