The role of proteosome-mediated proteolysis in modulating potentially harmful transcription factor activity in Saccharomyces cerevisiae

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ABSTRACT

Motivation: The appropriate modulation of the stress response to variable environmental conditions is necessary to maintain sustained viability in Saccharomyces cerevisiae. Particularly, controlling the abundance of proteins that may have detrimental effects on cell growth is crucial for rapid recovery from stress-induced quiescence.

Results: Prompted by qualitative modeling of the nutrient starvation response in yeast, we investigated in vivo the effect of proteolysis after nutrient starvation showing that, for the Gis1 transcription factor at least, proteosome-mediated control is crucial for a rapid return to growth. Additional bioinformatics analyses show that potentially toxic transcriptional regulators have a significantly lower protein half-life, a higher fraction of unstructured regions and more potential PEST motifs than the non-detrimental ones. Furthermore, inhibiting proteosome activity tends to increase the expression of genes induced during the Environmental Stress Response more than those in the rest of the genome. Our combined results suggest that proteosome-mediated proteolysis of potentially toxic transcription factors tightly modulates the stress response in yeast.

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Supplementary information: Supplementary data are available at Bioinformatics online.

1 INTRODUCTION

A prompt and appropriate response to abrupt fluctuations in external conditions is crucial to survive stressful environmental changes, especially in unicellular organisms such as the yeast Saccharomyces cerevisiae. During the nutrient starvation, in order to ensure extended survival, S.cerevisiae cells exit the cell cycle at G1 and enter the quiescent state (called G0), but rapidly resume growth to enter the G1 phase. Overall, controlling the abundance of potentially toxic transcription factor activity is crucial for yeast. Proteosome-mediated proteolysis of potentially toxic transcription factors tightly modulates the stress response in yeast.

2 METHODS

2.1 Petri nets

We have built a qualitative logical model of nutrient starvation based on Petri nets. Petri nets are mathematically sound formalisms that can be used in systems biology to build and analyze coarse-grained models of complex processes (Bonzanni et al., 2009), taking advantage of the intuitive nature of their representation and the soundness of their foundation. The Petri net modeling framework used in this work has been described in an earlier article by Chaouiya et al. (2006). The states predicted by the model can be found in Supplementary Material. Statistical analyses of bioinformatics data were performed using R.

2.2 Gis1 overexpression at the transition phase

Wild-type (BY4742) cells were transformed with pCM190 (Gari et al., 1997) and pCM190-GIS1 (Zhang and Oliver, 2010). Transformants were grown on 30 April 2018

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This model includes the inhibition of Msn2/4 activity by TOR and TOR, PKA and Pho80/Pho85. (et al. represented Rim15 inhibition by TOR, PKA and Pho80/85 (Pedruzzi a necessary precondition for Msn2/4 repression. Similarly, we have represented Rim15 inhibition by TOR, PKA and Pho80/85 (Pedruzzi et al., 2003 and Wanke et al., 2005), which is represented as the recently discovered proteolytic control over Gis1 (Zhang and Oliver, 2010). Moreover, although full-length Gis1 is essential for PDS gene expression, the smaller Gis1 fragments, resulting from constitutive proteolysis by the proteasome, are also able to initiate transcription upon Rim15 activation (Zhang and Oliver, 2010). These data suggested that full-length Gis1 and its smaller variants activate the transcription of PDS genes cooperatively. Therefore, we concluded that our model needed to be refined by including the full-length protein and the smaller fragments separately, in order to fully capture the biological observations and increase the model’s accuracy. Different wiring choices were possible. One possibility, shown in Figure 2A, is to allow proteolytic activity to induce complete degradation of full-length Gis1. This is the behavior

Fig. 1. Model of nutrient starvation response in yeast. (A) Diagrammatic model depicting the proteolytic control over Gis1 and the regulation of Rim15 by TOR, PKA and Pho80/85. Ovals = nodes that represent ‘nodes’—proteins (e.g. PKA, Rim15, Gis1) and genes (PDS and STRE); colored squares = interactions. Arcs ending with an arrowhead (in blue) represent positive interactions (e.g. activations), while arcs ending with bars (in red) represent negative interactions (e.g. inhibitions). Note that if multiple arrows target the same square, all the sources are required at the same time. Dashed lines represent the interaction responsible for the discrepancy between the modeled and observed behaviors.

3 RESULTS

3.1 Model construction and analysis

In order to investigate the consistency and explanatory power of the available knowledge about the nutrient starvation response in yeast, we have constructed a dynamic computational model based on Petri nets (Reisig and Rozenberg, 1998). Petri nets can be depicted as graphs that contain two kinds of nodes: places, which represent resources and correspond to proteins and genes, and transitions, which represent interactions between places. Interactions can be either activations or inhibitions (Fig. 1B) and, during the course of the execution, each resource can change its state (in a Boolean fashion) from active to inactive (and vice versa) based on the surrounding interactions. Given a network topology, it is possible to execute the model and compare its behavior with the one observed empirically.

Due to the lack of fine-grained quantitative data, we captured the coarse-grained descriptive knowledge available in the form of a qualitative model firmly based on published experimental evidence. This model includes the inhibition of Msn2/4 activity by TOR and PKA (Beck and Hall, 1999; Görner et al., 1998), which is represented in Figure 1B by the red transitions connecting the TOR and PKA nodes to Msn2/4. Notice that the arc connecting TOR to the transition ends with an arrowhead, while the arc connecting the transition to Msn2/4 ends with a bar. This means that the availability of TOR is a necessary precondition for Msn2/4 repression. Similarly, we have represented Rim15 inhibition by TOR, PKA and Pho80/85 (Pedruzzi et al., 2003 and Wanke et al., 2005), the expression of STRE and the PDS genes upon Rim15 activation of Msn2/4 and Gis1, as well as the recently discovered proteolytic control over Gis1 (Zhang and Oliver, 2010).

After the construction of the network model, we analyzed its dynamics. By comparing our model with the experimental observations (Zhang and Oliver, 2010), we discovered a significant discrepancy in the behavior of Gis1 reproduced by the model. Our computational results (see Supplementary Material) suggested that only the full-length Gis1 was necessary for the activation of PDS genes. However, upon nutrient starvation or TORC1 inhibition, the abundance of full-length Gis1 decreases, which does not correspond to the increase of transcription activation of PDS genes (Zhang and Oliver, 2010). Moreover, although full-length Gis1 is essential for PDS gene expression, the smaller Gis1 fragments, resulting from constitutive proteolysis by the proteasome, are also able to initiate transcription upon Rim15 activation (Zhang and Oliver, 2010). These data suggested that full-length Gis1 and its smaller variants activate the transcription of PDS genes cooperatively. Therefore, we concluded that our model needed to be refined by including the full-length protein and the smaller fragments separately, in order to fully capture the biological observations and increase the model’s accuracy. Different wiring choices were possible. One possibility, shown in Figure 2A, is to allow proteolytic activity to induce complete degradation of full-length Gis1. This is the behavior

Fig. 2. Multiple possible wiring choices allow refinement of the model. Fragment of the model under refinement. The dashed interactions are more accurate alternatives than the dashed interaction in Figure 1B. Two alternative options are presented: (A) proteolytic activity induces complete degradation of the full-length Gis1 protein and simultaneous availability of cleaved Gis1 fragments. (B) Decoupling the production of cleaved Gis1 fragments and degradation of full-length protein allows partial depletion of the full-length Gis1.
observed during nutrient starvation; however, Gis1 is also subject to a constitutive, but partial degradation by the proteasome (Zhang and Oliver, 2010) during exponential growth. Therefore, an alternative modeling choice is to allow partial depletion of full-length Gis1. This can be accomplished by decoupling the availability of the cleaved Gis1 fragments from the complete degradation of the full-length protein (Fig. 2B). By refining our model as shown in Figure 2B, it qualitatively reproduced (see Supplementary Material) the behavior observed in Zhang and Oliver (2010).

3.2 Proteolytic control over Gis1 allows fast recovery from lag phase

The different causal wirings imply differences in the model behavior and may therefore suggest different roles for the proteolytic control. In order to understand the evolutionary advantages of the different proteolytic controls over Gis1 in the context of nutrient response, we were prompted to investigate its physiological role. GIS1 overexpression leads to accumulation of the full-length protein and is toxic to cell growth (Pedruzzi et al., 2000; Zhang and Oliver, 2010). Inhibition of the proteasome function results in hyperactivation of PDS genes in nutrient-starved conditions (Zhang and Oliver, 2010). Knowing that growth and budding are suspended in stationary phase, we performed an experiment to determine whether the proteolytic control over Gis1 is necessary for survival of cells entering the stationary phase, the recovery of cells from glucose starvation or both.

Wild-type yeast cells were transformed with plasmid pCM190 or the same plasmid bearing the GIS1 gene under the control of the repressible promoter, tetO. Cells were grown in the presence of doxycycline to early stationary phase, washed and resuspended in medium with no glucose or doxycycline for 36 h. There is no difference in viability between cells bearing the empty plasmid and those carrying the tetO-GIS1 plasmid (data not shown). Glucose and doxycycline were added to allow cells to resume growth. As shown in Figure 3, cells harboring the tetO-GIS1 plasmid display a 15% longer lag phase than those bearing the empty plasmid, suggesting that GIS1 overexpression during the transition to quiescence, delays the subsequent resumption of exponential growth on re-addition of nutrients. These data indicate that proteolytic degradation of Gis1 by the proteasome may provide cells with an important evolutionary advantage, since periods of nutrient availability and may therefore suggest different roles for the proteolytic control.

3.3 Predicting that toxic transcriptional regulators are subject to lighter proteolytic control

Prompted by the proteolytic regulation of Gis1 and its physiological implications, we went on to inquire if, in general, the stress response is restrained by the proteasome. We adopted two strategies: the first to discover whether toxic transcription factors are likely to be controlled post-translationally by the proteasome, and the second to find out whether proteasome inhibition allows transcription factors normally targeted by the proteasome to elicit a stress response.

3.3.1 Toxic transcriptional regulators have lower half-life

To monitor the validity of our hypothesis, we performed a sequence of bioinformatics analyses. First, we partitioned the known yeast transcriptional regulators into two disjoint sets. The first set contained 75 potentially toxic regulators and was created by filtering the set of 796 genes, whose overexpression was found to be detrimental for cell growth (Sopko et al., 2006) using the GO annotation ‘transcription regulator activity’ (GO:0030528). The second set contained 251 non-toxic regulators and was built by filtering the whole yeast genome with the same GO annotation after removing the toxic genes contained in the first set. Detailed data are available as Supplementary Material.

With our first analysis, we assessed whether the protein half-lives of toxic regulators are shorter than those of non-toxic regulators, using the protein half-life measurements of Belle et al. (2006). Since the measurements are not normally distributed (P < 10^{-13}; Shapiro–Wilk test), we computed the Wilcoxon rank sum test under the null hypothesis that the median difference between the two measurement sets is zero and the alternative hypothesis that the median half-life of the toxic transcription factors is less than that of the non-toxic ones. The null hypothesis has been discarded with the statistically significant value of P = 5.54 × 10^{-3} (Fig. 4A). Note that it was not possible to find measurements for all the proteins in the two sets. We also analyzed the mRNA half-life data (Wang et al., 2002) for the transcripts of the toxic and the non-toxic TFs and found no significant difference between the two (P = 0.256; Wilcoxon test), supporting the hypothesis that a significant portion of the control over the toxic TFs is exerted post-translationally (Fig. 4B).

3.3.2 Toxic transcriptional regulators have a higher fraction of unstructured regions

The availability of many intrinsically unstructured proteins (IUPs) is regulated via proteolytic degradation...
We analyzed the number of potential PEST motifs in the protein Wilcoxon test) than those of non-toxic ones (blue), while (Wilcoxon test) tend to be higher than those for the rest of the genome.

Investigated whether proteolytic control could contribute to modulating the stress response by checking transcriptional changes modulating the stress response by checking transcriptional changes mediated degradation plays a significant role in the regulation of the activity of potentially detrimental TFs.

Given the high number of potential PEST motifs found in rapidly degraded proteins, we predicted that proteasome-mediated degradation plays a significant role in the regulation of the activity of potentially detrimental TFs.

3.3.3 Toxic TFs contain more potential PEST motifs Sequence regions rich in proline (P), glutamic acid (E), serine (S) and threonine (T) are found in many rapidly degraded proteins and have been suggested to serve as signals for proteolysis (Rogers et al., 2008). Therefore, for both the toxic and non-toxic regulators, we computed (using Disopred2; Ward et al., 2004) the fraction of the amino acids predicted to form unstructured regions is significantly higher in toxic than in non-toxic proteins (P = 2.48 × 10^-4; Wilcoxon test). (D) After 120 min of proteasome inhibition by MG132, transcription rates of UES genes (P = 8.26 × 10^-16; Wilcoxon test) and ESR induced genes (P < 2.2 × 10^-16; Wilcoxon test) tend to be higher than those for the rest of the genome.

We found that the fold changes of the UES genes tend to be higher than for the rest of the genome (P = 3.7 × 10^-17; Wilcoxon test), supporting the hypothesis that proteasome-mediated degradation plays a significant role in the regulation of the activity of potentially detrimental TFs.

3.3.4 The proteasome modulates the expression of a significant fraction of genes induced by environmental stress Finally, we investigated whether proteolytic control could contribute to modulating the stress response by checking transcriptional changes after proteasome inhibition. A previous study has shown that 23% of all yeast genes (1386 mRNAs) increase their rate of transcription by a factor of 1.5 or more (6% increase more than 2 times) after 120 min treatment with the proteasome inhibitor MG132 (Dembla-Rajpal et al., 2004). We extracted the data for the Universally Expressed at Starvation (UES) genes (Wu et al., 2004); these genes are controlled by Gis1 and Msn2—two TFs known to be under proteolytic control. We found that the fold changes of the UES genes tend to be higher than for the rest of the genome (P = 8.26 × 10^-16; Wilcoxon test). Further, extending the analysis of the effect of inhibiting proteasome activity on the induction of gene transcription in the Environmental Stress Response (ESR; Gasch et al., 2000), we predicted the number of transcription factors than that for non-toxic regulators (Gsponer et al., 2008). Therefore, for both the toxic and non-toxic regulators, we computed (using Disopred2; Ward et al., 2004) the fraction of the amino acids predicted to form unstructured regions is significantly higher in toxic than in non-toxic proteins (P = 2.48 × 10^-4; Wilcoxon test). (D) After 120 min of proteasome inhibition by MG132, transcription rates of UES genes (P = 8.26 × 10^-16; Wilcoxon test) and ESR induced genes (P < 2.2 × 10^-16; Wilcoxon test) tend to be higher than those for the rest of the genome.

To summarize, our work suggests that proteasome-mediated proteolysis of TFs tightly modulates the stress response in yeast. This hypothesis is the result of the integration of computational and in vivo analysis. Our computational model highlighted the particular behavior of the proteolytic control, suggesting further in vivo investigations. Our in vivo experiments showed that, for the Gis1 transcription factor at least, proteasome-mediated control is crucial for a rapid return to growth after nutrient starvation, which may give yeast cells an important selective advantage over their competitors. Finally, our bioinformatics analyses generalized our in vivo observations to the class of potentially toxic transcription factors that control the stress response in yeast.

ACKNOWLEDGEMENTS
We would like to thank A. Feenstra, W. Fokkink and J. Heringa for helpful discussions. Part of this work was done, while NB was an intern at Microsoft Research Cambridge.

FunDing: Work in the Cambridge Systems Biology Centre was supported by BBiSC (Grant BB/C505140/2 awarded to S.G.O.); Work in the Centre for Integrative Bioinformatics VU was supported in part by ENFIN, a Network of Excellence funded by the European Commission within its FP6 Program, under the thematic area ‘Life Sciences, genomics and biotechnology for health’, contract number LSHG-CT-2005-518254.

Conflict of Interest: none declared.

REFERENCES
Proteosome-mediated proteolysis modulates stress response in yeast


