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Transient transcriptional responses to stress are generated by opposing effects of mRNA production and degradation

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The state of the transcriptome reflects a balance between mRNA production and degradation. Yet how these two regulatory arms interact in shaping the kinetics of the transcriptome in response to environmental changes is not known. We subjected yeast to two stresses, one that induces a fast and transient response, and another that triggers a slow enduring response. We then used microarrays following transcriptional arrest to measure genome-wide decay profiles under each condition. We found condition-specific changes in mRNA decay rates and coordination between mRNA production and degradation. In the transient response, most induced genes were surprisingly destabilized, whereas repressed genes were somewhat stabilized, exhibiting counteraction between production and degradation. This strategy can reconcile high steady-state level with short response time among induced genes. In contrast, the stress that induces the slow response displays the more expected behavior, whereby most induced genes are stabilized, and repressed genes are destabilized. Our results show genome-wide interplay between mRNA production and degradation, and that alternative modes of such interplay determine the kinetics of the transcriptome in response to stress.
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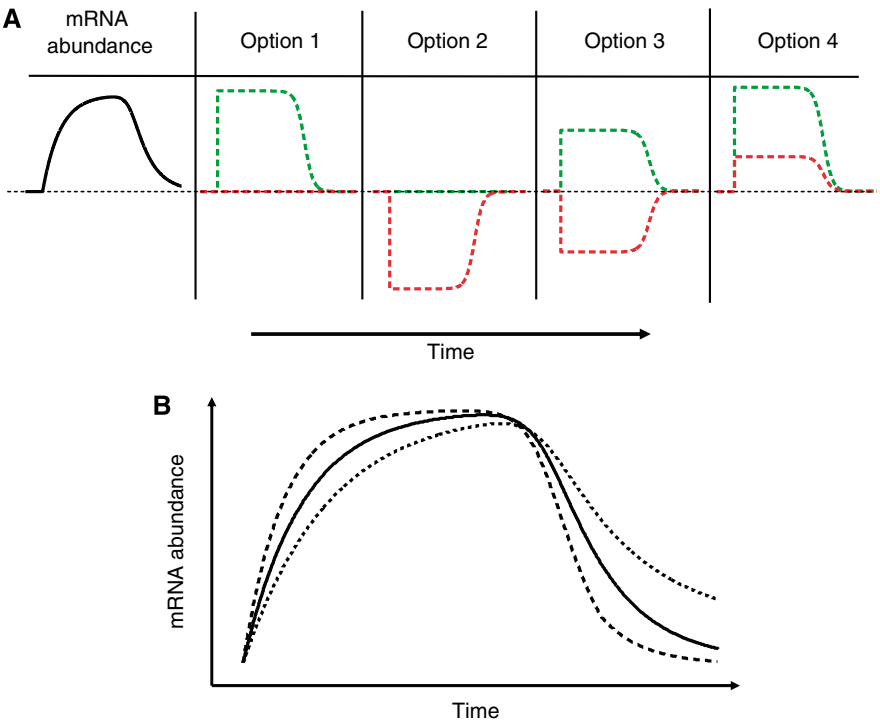
Introduction

In response to environmental stimuli, the mRNA abundance of a large fraction of the genome changes either by increasing or decreasing its levels (Gasch *et al*, 2000, 2001; Jelinsky *et al*, 2000; Gasch and Werner-Washburne, 2002). Clearly, to understand the state of the transcriptome under varying conditions, the role of both mRNA production and degradation must be examined. An increase in mRNA abundance in response to a stimulus may be achieved either by increasing the rate of transcription or by decreasing the rate of degradation. Likewise, a decrease in the transcript level can be achieved either by an increase in the rate of degradation or a decrease in the rate of production. More complex interplays between production and degradation are also possible. For instance, an increase in mRNA production rate might be accompanied by a decrease in degradation rate, leading to mRNA accumulation. Perhaps less intuitive is the possibility that an increase in transcript levels would be obtained by increasing both production and degradation rates, provided that the extent of production increase exceeds the elevation in the degradation rate (Box 1A). Whereas steady-state levels are simply determined by the ratio of production and degradation rates, the kinetic behavior is expected to be more complex, which is dependent on the actual rates, and hence different under the above regimens (Box 1B).

As a result, one may expect complex regimens of interplay between transcription induction and repression, and stabilization versus destabilization of mRNAs that will result in various effects on response kinetics (Perez-Ortin *et al*, 2007). Yet customary transcript abundance measurements, e.g. with microarrays, provide only the net values and do not provide information regarding the relative contribution of mRNA production and degradation.

Although still scarce when compared to transcription, the attention directed toward the control of mRNA degradation has

Box 1 Various potential relationships between mRNA production and degradation can produce similar mRNA abundance profiles but with distinct kinetics:



(**A**) A typical mRNA abundance profile plotted on the left (black) can be produced by utilizing changes in production and degradation in different ways. As an example, several options are plotted where the green and red lines represent the change in mRNA production and degradation, respectively, as a function of time. The first two options show that, at least hypothetically, either production or degradation alone can be modulated to produce an induction followed by relaxation of the mRNA abundance. The last two options show that a similar profile of mRNA abundance can be produced by changing both production and degradation either by counteracting each other or by working in the same direction. (**B**) Although induction can be achieved either by changes in production or degradation, the kinetics of induction and relaxation would depend on the levels of degradation. Following a simple kinetic model for changes in mRNA levels, which assumes zero-order production and first-order degradation: $dx/dt=\beta-\alpha x$, where x, β and α represent mRNA abundance, production rate and degradation rate, respectively, the change in mRNA abundance follows $\Delta x(t)=((\beta/\alpha)-x(0))(1-e^{-\alpha t})$, where $x(0)$ marks the level of x prior to induction. Thus, the response time, defined as the time at which half of the new steady-state level with respect to the former is achieved, is solely determined by the degradation rate, and is inversely proportional to it: $t_{1/2}=\ln(2)/\alpha$. Thus, increased decay rate is expected to result in a shorter response time. A reference mRNA abundance profile is plotted in a solid line, two profiles with increased and decreased decay rates are plotted in dashed and dotted lines, respectively. Production rates are changed accordingly to keep the same steady-state level. Increasing the decay rate results in faster kinetics both in the induction and relaxation phases, whereas a decrease in decay rate results in slower kinetics. The curves shown here were generated by solving the above differential equation. The dashed line thus represents an interesting case in which both production and degradation rates have increased, reconciling higher steady-state level with fast response time.

increased in recent years. Genome-wide measurements of mRNA half-lives have been conducted in several organisms, revealing interesting relationships between functional properties of genes and their decay rates (Bernstein *et al*, 2002; Wang *et al*, 2002; Yang *et al*, 2003; Grigull *et al*, 2004; Narsai *et al*, 2007). Genomic run-on methods were used to compare transcription rates to changes in mRNA abundance and concluded that changes in transcription rates are not sufficient to explain changes in mRNA abundances observed in response to stress (Fan *et al*, 2002; Garcia-Martinez *et al*, 2004). The regulation of mRNA stability by cis and trans-acting factors has also been investigated, both by extensive studies of specific examples (Xu *et al*, 1997; Wilusz *et al*, 2001; Parker and Song, 2004; Wilusz and Wilusz, 2004; Garneau *et al*, 2007) and also by genome-wide computational studies aiming to detect sequence features in 3′UTRs that are predicted to affect mRNA stability (Foat *et al*, 2005; Shalgi *et al*, 2005).

Despite this recent progress, the relationship between the control of mRNA production and degradation is poorly understood. Here, we investigate this interplay and study its effect on key kinetic parameters of the mRNA response to stress in the yeast *Saccharomyces cerevisiae*. We studied two intensively investigated conditions (Gasch *et al*, 2000, 2001; Jelinsky *et al*, 2000; Workman *et al*, 2006), an oxidative and a DNA-damaging stress selected such that they will differ in the kinetics of the mRNA response they induce. In oxidative stress, the majority of the responding genes show fast response followed by relaxation, whereas in the DNA damage experiment, the response is slow and long enduring. We found that changes in degradation counteract the changes in mRNA abundance for most of the genes under oxidative stress, whereas the opposite coupling, namely stabilization of induced genes, and destabilization of repressed genes, is observed for the majority of the transcripts during the

response to DNA damage. We show that the alternative types of interplays between mRNA production and degradation in each experiment shape the kinetic response of the transcriptome.

Results

The experimental design

We have chosen two environmental conditions, exposure to hydrogen peroxide (H_2O_2), which induces an oxidative stress, and exposure to methyl methanesulfonate (MMS), which induces DNA damage. We followed genome-wide response to these stresses using conventional microarray measurements of mRNA abundance (see Materials and methods). Under current parameters of each stress (see Materials and methods), the two conditions gave rise to distinct mRNA response kinetics. In oxidative stress, the majority of the responding genes showed, in agreement with previous measurements (Gasch *et al*, 2000), a fast transient response (see Supplementary information and Supplementary Figures S9–S11 for comparison with related experiments). In contrast, in the MMS experiment, the majority of the responding genes showed long enduring response with no relaxation within the measured time course, also in agreement with previous studies (Gasch *et al*, 2001) (Figure 1A). To decipher the ways in which modulation of mRNA decay rates is utilized by the cell to determine such different kinetic abundance profiles, we also performed in parallel mRNA decay experiments. In these experiments, the same stresses were applied, yet following the stress, we halted transcription in the cells and used microarrays to measure the decay kinetics of each gene under every condition. We realized that potential regulated change in mRNA stability in response to each stress may require active transcription of stability affecting genes. Thus, to build up their degradation control network, cells should be given a time interval between introduction of the stress and the transcriptional arrest. We therefore apply the stress and halt transcription after giving the cells a time interval to respond (see Materials and methods for details). We also carried out a reference decay experiment in which we measured the degradation kinetics of each gene after transcriptional arrest, yet without applying any of the above stresses (see schematic representation of the experiment design in Supplementary Figure S1).

The majority of the genes showed exponential decay in all three decay experiments, suggesting constant rate of degradation throughout time (Supplementary Figure S7). As carried out earlier for non-stressful conditions (Wang *et al*, 2002), we could calculate the half-life of each gene in each of the three conditions. Interestingly, the decay rates of some entire gene modules showed coherent change in a given stress when compared with the reference condition or with the other stress, indicating extensive condition-specific regulation of mRNA

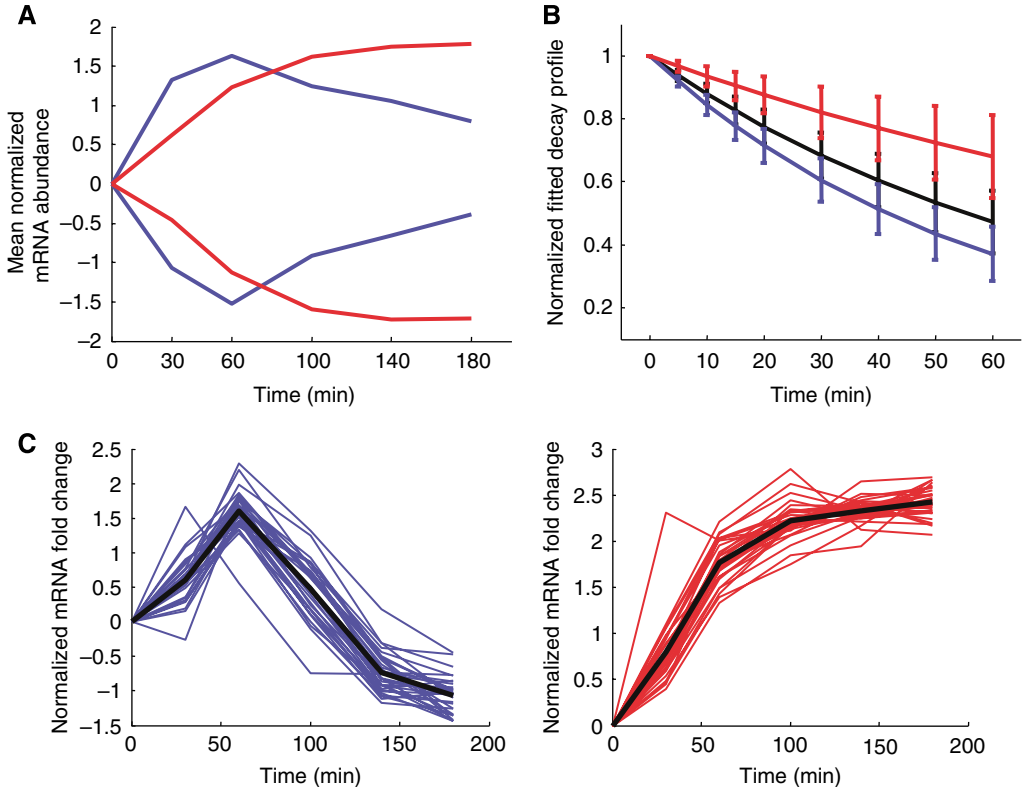


Figure 1 Distinct transcriptome responses at the two conditions. (**A**) Mean expression profile of all induced and repressed genes (fold change >2) in oxidative and MMS stress (blue and red curves, respectively). (**B**) The proteasomal genes as an example for a group of genes showing coherent change in mRNA stability in response to each stress. The mean of the fitted decay profiles is shown; black, blue and red represent the reference, oxidative stress and DNA damage conditions, respectively. (**C**) The mRNA abundance profiles of the proteasomal genes (after mean and variance normalization) are shown for the oxidative stress and DNA damage stress (left and right panels, respectively).

decay. Figure 1B shows an example of the average change in decay rate of the genes that encode the 26S proteasome. Although the genes show clear destabilization relative to the reference condition in response to the oxidative stress, they are significantly stabilized on treatment with MMS. Interestingly, although the stability of the proteasomal genes changes in opposite directions in the two conditions, their mRNA abundance profiles (measured in the experiment that did not involve transcriptional arrest) show a clear net induction in both the stabilizing and the destabilizing conditions. Yet the kinetics of the response is markedly different, featuring fast relaxation in the oxidative stress, and sustained response in MMS (Figure 1C). Although *a priori* the fast relaxation seen in oxidative stress could result from decrease in transcription alone, it may alternatively be also due to destabilization of the proteasomal genes in this condition. In the following, we take a systematic genome-wide view to study the interplay between the change in mRNA abundance and the change in mRNA stability, with the aim of deciphering the relative role of production and degradation in shaping transcript kinetics.

Alternative interplays between changes in mRNA stability and changes in mRNA abundance

On the basis of mRNA abundance measurements, we characterize each gene in every condition by the maximal fold change of the gene's mRNA abundance levels following the stress, and by the time at which such maximal fold change is attained (see Materials and methods). A third parameter that we use for this characterization is the log ratio of the half-life of the gene's mRNA in a given stress and it's half-life in the reference condition. This ratio serves as a measure of the stabilization/destabilization of the gene's mRNA in the stress (see Materials and methods). To investigate, in every condition, the interplay between changes in mRNA abundance and changes in mRNA degradation rates, we assessed the correlation between maximal fold change of each gene to the half-life ratio in a given condition (Figure 2). We found two opposing relationships in the two stressful conditions. Examining the response to MMS, we observe an opposite, and at first more intuitive trend. In this condition, induced genes show a tendency toward stabilization, whereas repressed genes show a tendency toward destabilization (Figure 2). In the oxidative stress, we found a surprising negative correlation—genes whose mRNAs are induced in response to the stress are typically destabilized, whereas repressed genes show a weaker, though still significant, tendency toward stabilization.

We further mined the data with respect to the dynamics of different functional categories using gene ontology (GO) (www.geneontology.org). We have first identified GO categories that are enriched among the induced and repressed genes (see Supplementary information), and found that these are in good agreement with classical analyses (Gasch *et al*, 2000). We then asked, separately for GO categories that are enriched among repressed or induced genes, whether the genes belonging to them are significantly stabilized or destabilized (Table I and Supplementary information). We used two separate statistical tests, one that asks whether enriched categories have a general tendency toward stabilization or destabilization, and a second test to check whether specific groups behave anomalously when compared to the general tendency of induced or repressed genes (see Materials and methods). In oxidative stress, induced genes have a strong tendency toward destabilization, whereas the opposite is observed following DNA damage, and indeed most enriched categories, particularly genes involved in response to stimuli, and oxidoreductase activity, behave according to this trend. Repressed genes in both conditions show a less clear trend, and enriched categories are found both stabilized and destabilized. Ribosomal proteins and rRNA metabolism proteins represent an interesting deviation from the (rather weak) genome-wide trend—these genes are repressed in response to oxidative stress, yet they undergo destabilization. This probably explains why these genes show less transient repression compared to the general repression in this condition.

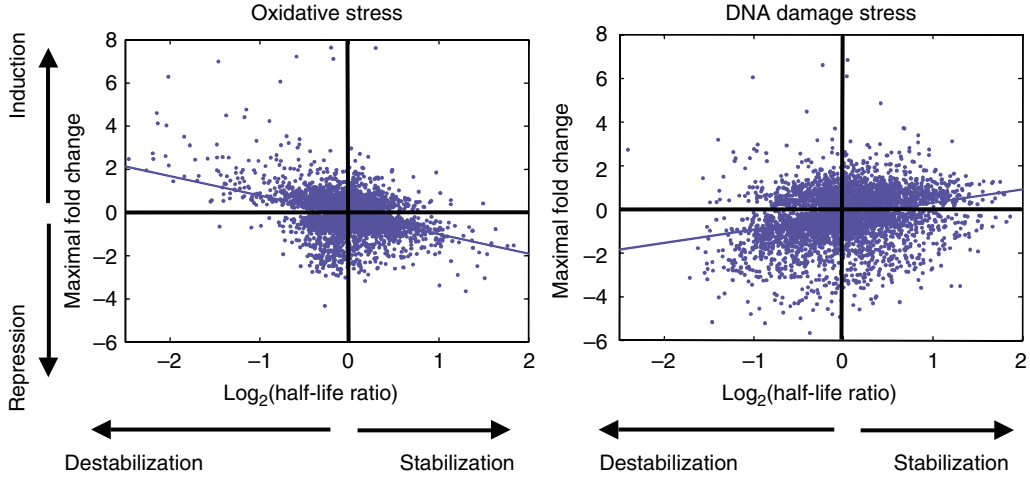


Figure 2 Distinct relationships between the change in mRNA abundance and the change in stability between the two conditions. For each stress, the change in mRNA stability relative to the reference state ($\log_2(t_{1/2\ \text{stress}}/t_{1/2\ \text{reference}})$) is plotted against the maximal fold change (defined as described in Materials and methods). Two different trends are observed, a negative trend ($R=-0.38$, p-value $< 3 \times 10^{-150}$) for the oxidative stress and a positive trend for the DNA damage stress ($R=0.27$, p-value $< 4.2 \times 10^{-70}$).

Table I Sample categories that are both enriched among the induced and repressed genes and show a significant tendency toward stabilization or destabilization

Induced genes						Repressed genes					
Destabilized (78%)			Stabilized (22%)			Destabilized (47%)			Stabilized (53%)		
Category name	*t*-test *P*-value	χ^2 *P*-value	Category name	*t*-test *P*-value	χ^2 *P*-value	Category name	*t*-test *P*-value	χ^2 *P*-value	Category name	*t*-test *P*-value	χ^2 *P*-value
Oxidative stress											
Mitochondrion	6.7E−10	0.056				Ribosome biogenesis	0.17	1.80E−04	Nuclease activity	0.011	4.90E−04
Response to stimulus	1.1E−5	0.58				Nucleolus	0.005	4.40E−06	Bud neck	1.40E−04	0.0079
Oxidoreductase activity	1.3E−4	0.75				Nuclear lumen	0.34	1.30E−04	Ribonuclease activity	0.0033	0.0024
Response to abiotic stimulus	3.2E−4	0.25				RNA metabolism	0.75	0.02	Biopolymer metabolism	0.0042	0.77
Cofactor metabolism	0.003	0.72				rRNA metabolism	0.71	0.05	Cytoplasmic exosome	0.0069	0.014
Destabilized (36%)			Stabilized (64%)			Destabilized (66%)			Stabilized (34%)		
Category name	*t*-test *P*-value	χ^2 *P*-value	Category name	*t*-test *P*-value	χ^2 *P*-value	Category name	*t*-test *P*-value	χ^2 *P*-value	Category name	*t*-test *P*-value	χ^2 *P*-value
DNA damage											
			Proteolysis	6.10E−05	0.0079	Translation	7.20E−56	6.20E−14	Nuclear part	0.045	3.30E−15
			Cellular macromolecule catabolism	1.30E−04	0.04	Ribosome	1.10E−52	7.30E−13	Nucleolus	0.0019	2.30E−15
			Carbohydrate metabolism	6.80E−04	0.02	Protein biosynthesis	1.00E−50	3.40E−13	RNA processing	0.0013	9.30E−14
			Protein catabolism	0.0012	0.038	Sterol metabolism	1.50E−07	0.0021	Biopolymer metabolism	0.8	2.70E−09
			Ubiquitin cycle	0.0056	0.038	Lipid biosynthesis	2.10E−07	0.0055	Methyltransferase activity	0.042	3.70E−04

All categories in this table have passed an initial hypergeometric test that indicated that they are enriched in the group of induced or repressed genes irrespective of their tendency toward stabilization or destabilization. Then two tests were performed on the half-life change of the group of genes that were both annotated by a specific category and induced or repressed: a one-sided *t*-test was performed to check whether the mean half-life change was significantly different from zero, indicating stabilization or destabilization. To look for groups of genes that behave anomalously, a χ^2 test was performed on the number of stabilized and destabilized genes in the intersection between the category genes and the group of induced or repressed genes when compared with all induced or repressed genes. The full list of enriched categories is given in Supplementary data set 2. Cases where both tests display a low *P*-value (as 'translation' in DNA damage repression and destabilization) indicate that the trend is stronger than the trend of the reference group but in the same direction.

Fast transcriptional responses are accompanied by an opposing force of degradation

We then investigated whether the correlation between the mRNA abundance fold change to the change in stability is different, within each condition, between genes that display different kinetics. For that, we grouped the genes in each condition according to the time of attainment of the maximal fold change. We then plotted again, for each group separately, the change in mRNA stability against the maximal fold change (also plotted are the corresponding mRNA abundance profiles in each set; Figure 3A). In both conditions, fast induced genes show strong destabilization, whereas fast repressed genes show stabilization. For instance, among the induced genes that peak in the first 40 min following the stress, a 2- to 4-fold reduction in half-life when compared with the reference

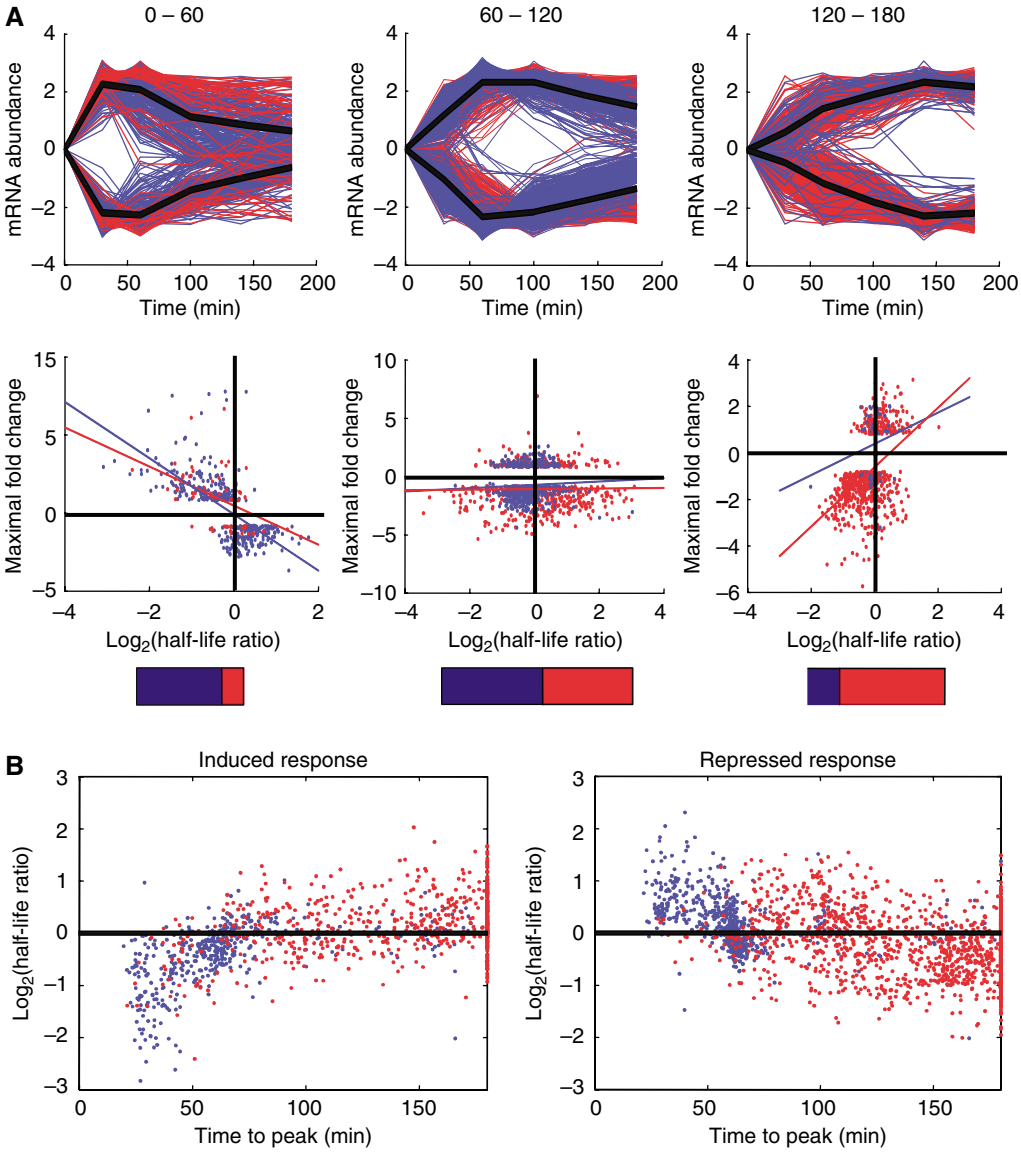


Figure 3 (**A**) Relationship between changes in mRNA abundance to changes in mRNA stability in different kinetic regimens. For both conditions, mRNA profiles are grouped according to the time point at which the maximal fold change is attained (0–60, 60–120 and 120–180 minutes). For each such group, we plot both normalized (mean and variance) mRNA abundance profiles (upper panels) and, as was done in Figure 2, the relationship between the maximal mRNA abundance fold changes to the changes in mRNA stability relative to the reference condition (lower panels). We joined in each plot profiles from the two conditions, blue and red correspond to oxidative and MMS stress, respectively. The first group of genes, which mostly consists of profiles in the oxidative stress condition, displays transient kinetics and an early peak, in the first 40 min following the stress. These genes display an opposite relationship between the changes in mRNA stability and the net change in mRNA abundance. As we progress to groups of genes that attained a later peak, the negative correlation is replaced with a positive correlation. The last group, which displays long enduring kinetics, shows a positive correlation between the changes in mRNA abundance to the change in mRNA stability. The bars below each group represent the relative amount of genes from each stress represented in each group. (**B**) Changes in mRNA stability determine response duration. The time at which the maximal fold change in mRNA abundance is attained against the half-life change is plotted separately for induced and repressed genes. The different colors represent the two conditions, blue and red for oxidative and DNA damage stress, respectively. Opposite trends are observed between induced and repressed genes. In addition, both conditions display similar trends with difference in the amount of genes that display transient versus endured response.

condition is common. In contrast, in both conditions, genes that display a slow long enduring response show a positive correlation between changes in the mRNA abundance level and changes in stability. Among these genes, induction is accompanied by stabilization, whereas repression is often seen among the destabilized genes. It is therefore apparent that in both conditions similar basic kinetic trends are obeyed: for genes in which a change in degradation rates counteracts the direction of change in mRNA abundance, a fast transient response is seen. In contrast, in both conditions, a long enduring response is seen if upregulated genes are also stabilized, and downregulated genes are destabilized. The two conditions thus only differ in the relative proportion of genes that display the transient versus the long enduring response as apparent from the horizontal bars at the bottom of Figure 3A.

To investigate quantitatively how changes in mRNA stability in response to stress affect the duration of mRNA abundance response, we plotted the half-life ratio against the time of maximal fold change attainment for induced and repressed genes separately (Figure 3B). We see a clear correspondence between the two quantities. For induced genes, in both conditions, short responses are obtained in proportionality to the extent of destabilization in the stress. In contrast, repressed genes in both conditions show the opposite trend—shorter times to maximal fold change are observed among the stabilized genes. We propose that destabilization of transient induced genes and stabilization of transient repressed genes accelerate the relaxation phase back to the (lower and higher, respectively) base level. In this respect, the picture with induced and repressed transient genes is consistent: in both cases, counteraction between production and degradation is suggested to facilitate the fast relaxation of the initial response.

These results suggest that mRNA decay is a key feature regulating the response duration of mRNA abundance.

As stated above, an increase in decay rate, as seen for the transient induced genes, is expected to shorten the response time (Box 1). Thus, it is possible that for these genes, the increase in decay rate serves not only to achieve fast relaxation but also to accelerate the response to the new stressful conditions. Reassuringly, for these fast transient induced genes the destabilization starts rather early, even before these genes attain their maximal level (Supplementary Figure S2), revealing a potential to affect the response time.

Discussion

We report an intriguing relationship between changes in mRNA abundance and changes in mRNA degradation in response to stress. We show that this relationship varies between conditions, and among genes with different kinetics within the same condition. In particular, among the transient genes, which attain maximal fold change, and begin relaxation within the first 60 min, it appears that the change in the mRNA abundance level is counteracted by the change in degradation rate.

Clearly, the destabilization of transiently induced genes has to be compensated by a large increase in transcription rates, otherwise a net increased level would not be obtained. Likewise, fast repressed transient genes must experience a decrease in production rate, as at the degradation level they are actually stabilized. Thus, although we deduce transcription rates indirectly, we can still conclude in this case, that transcription rate has increased among the induced destabilized genes and decreased among the repressed stabilized genes. A more direct indication that among destabilized genes transcription rates are predominantly increased, and that among stabilized genes transcription rates are most reduced, may be obtained from the measurements done recently by Molina-Navarro *et al* (2008). Reassuringly, re-analysis of their data strongly shows exactly that trend (Supplementary Figure S13). Among genes that respond either by induction or repression of above twofold change, genes which, by our measurements, are destabilized show a mean increase in transcription rate of above 60% in most time points, whereas genes that we found to be stabilized show a general decrease in transcription rate of about 40%. An interesting conclusion regarding the fast transient responding genes is that changes in transcription rates alone (increase or decrease) determine the *direction* of the response (induction or repression). Stability changes appear not to determine the direction of the net response as they actually occur in opposite directions. Yet our results ascribe a major role to changes in degradation—these, along with likely changes at the transcriptional level, appear to impact the speed and relaxation properties of the response. These conclusions are in line with the study of Perez-Ortin *et al* (2007) who showed for the *STL1* gene in yeast that the measured transcription rate profile was not sufficient to explain a transient mRNA abundance response after osmotic stress; an increase in degradation rate had to be assumed to achieve a rapid decrease back to the basal level. The combined increase in both production and degradation rates, observed at the transient induced genes, may thus represent an interesting strategy, as it allows the acceleration of the response, without compromise of the maximal expression level (see Box 1).

On the other hand, for genes that display a high endurance response, both up- and downregulated, it is possible that the direction of the response is both due to transcriptional and degradation effects, as for these genes both factors do not counteract each other. A potential explanation for the behavior seen among the long endurance responsive genes could be that changes in mRNA stability are used to maintain and enhance the changes in transcription, i.e. induced genes are further stabilized, whereas repressed genes are also degraded faster.

Increase in both production and degradation can account for a transient response if the increase in degradation is slower than the increase in production. Under these assumptions, for a short time period, production rate may be higher than degradation rate. In this period, mRNA level may overshoot when compared with the final steady state, and the relaxation is attained when degradation rate exceeds the rate of production (Supplementary Figure S3). Assuming that an external signal control both production and degradation rates, this model produces a relaxation of the response in mRNA even if the external signal (e.g. hydrogen peroxide in the present case) is still ON. Support to this idea is given by the fact that the levels of hydrogen peroxide are decreased only slightly throughout the experiment (not shown) in agreement with previous studies (Gasch *et al*, 2000), whereas the mRNA

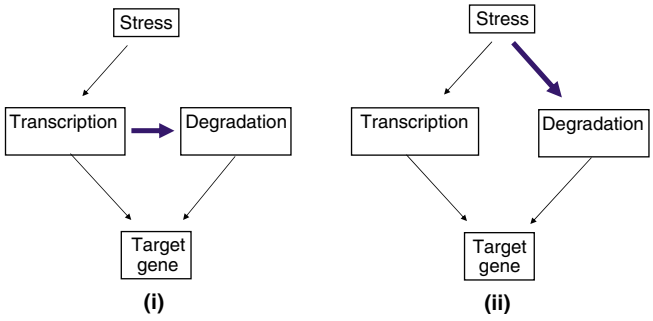


Figure 4 Two alternative models that might account for the observed coordination between transcription and degradation. Our results suggest coordination between changes in transcription to changes in mRNA degradation; this might be achieved by at least two alternative models: The first (i) suggests direct coupling between transcription and degradation meaning that mRNA degradation is directly affected by the rates of production. According to a possible alternative model (ii), the sensor of the stress activates a transcriptional response and, independently of that, it also induces a change in stability of the transcripts.

response begins to relax after an hour. Although concomitant increase in both production and degradation rates is an energetically costly solution, it might have an important advantage as it accelerates both the response to the stress and the relaxation back toward fast growing mode.

Finally, the idea of counteraction between production and degradation suggests coordination of transcriptional and mRNA degradation in the cell. Recently, experimental evidence suggested one potential mechanism that could account for this interplay. Lotan *et al* (2005, 2007) have shown that two subunits of RNA polymerase II, Rpb4p and Rpb7p, are involved in mRNA decay by enhancing both deadenylation and decapping. This suggests that counteraction could be achieved through direct coupling between transcription and degradation, namely that degradation rates are directly affected by changes in the rates of transcription. An alternative model is that the sensor of the stress activates a transcriptional response and, independently of that, it also induces a change in stability of the transcripts (Figure 4). This latter model has a topology reminiscent of a feed-forward loop, a recurring motif in many regulatory networks (Milo *et al*, 2002; Mangan *et al*, 2006), which was curiously found to accelerate response time to nutrient changes (Mangan *et al*, 2006). In this respect, it is noteworthy that recent observations made in the mammalian network spanned by microRNAs and transcription factors have proposed that similar coupling between transcription and post-transcription regulators may be implemented through similar in-coherent feed-forward loops (Shalgi *et al*, 2007; Sinha *et al*, 2008). Future work will be needed to determine the relative contribution of these models, or yet alternative ones, to the interplay seen here between the transcriptional and post-transcriptional regulation.

Materials and methods

Strains and growth conditions

Two types of experiments were conducted: experiments measuring mRNA abundance and mRNA decay experiments. Changes in mRNA abundance were measured in two separate experiments in response to both conditions described. mRNA decay experiments were performed in three different conditions: two for each of the stressful conditions and a reference condition, which was actually performed in two independent biological replicates as described below.

All experiments were carried out using the *S. cerevisiae* strain Y262 carrying a temperature-sensitive mutation in RNA polymerase II (Nonet *et al*, 1987) (*Matα ura3–52 his4–539 rpb1-1*). For all experiments, cells were grown in YPD medium (2% yeast extract, 1% peptone and 1% dextrose) at 26°C to the concentration of 2×10^7 cells/ml. Cells were then treated either by 0.1% MMS (Sigma-Aldrich) or 0.3 mM H_2O_2 (Frutarom Ltd). As a control experiment, no reagent was added. For mRNA abundance measurements, following each treatment, aliquots (15 ml) were removed at the following time points: 0, 30, 60, 100, 140 and 180 min and frozen in liquid nitrogen. RNA was extracted using MasterPure™ (Epicenter Biotechnologies). The quality of the RNA was assessed using the Bioanalyzer 2100 platform (Agilent); the samples were then processed and hybridized to Affymetrix yeast 2.0 microarrays using the Affymetrix GeneChip system according to manufacturer's instructions.

For measuring mRNA decay, a similar protocol was applied albeit with the following modifications: 25 or 40 min following addition of H_2O_2 or MMS (respectively), temperature was abruptly raised to the restrictive temperature of 37°C by the addition of an equal volume of a medium pre-warmed to 49°C. This step inactivated the RNA polymerase II and therefore stopped transcription. Following the temperature shift, aliquots were removed at the following time points: 0, 5, 10, 15, 20, 30, 40, 50 and 60 min and were processed and hybridized as described earlier. Time point zero in each of the four decay experiments was hybridized to arrays twice independently, representing two technical replicas. The purpose of this replica is two: first, it allows the validation of the normalization method—the ratio between the mRNA levels and spiked in RNA was found to be constant in replicates of the same time point (see Supplementary information for more details). In addition, the technical replica allows the assessment of the reproducibility of array hybridizations (Supplementary Figure S8). We also performed one complete biological replica of an entire decay experiment, at the reference condition. Although estimated half-life values can vary between replicas of the same condition, these analyses strongly suggested that the variation due to replicates is much smaller than the variation in estimated half-life across conditions (see Supplementary information).

Data preprocessing

Most preprocessing algorithms use a normalization step to bring all samples to have the same global distribution of intensity values. This is done under the assumption that the mean intensities of all samples should be similar, and deviations, between samples, represent technical artifacts that result from a difference in the processes that the samples undergo till hybridization and scanning. A unique aspect of mRNA decay measurements is that the above assumption, that there is no change in the total levels of mRNAs, is by definition not valid: due to transcription inhibition, we do expect a global decrease in the total amount of mRNA. Thus, for normalization between time points, we used an internal standard that was mixed with each RNA sample. This standard contained a pool of four—*in vitro* transcribed *Bacillus subtilis* RNAs ('spiked in'), each in a different concentration (poly(A) control kit supplied by Affymetrix). Each transcript was represented on the

microarrays by several probe sets. For a more detailed explanation on the preprocessing procedure, see Supplementary information and Supplementary Figures S4–S8.

Determination of mRNA half-lives

We represent the stability of an mRNA in each condition using a half-life that is derived from the measured decay profile in each condition. Each transcript decay profile is zero transformed by dividing each gene's decay profile by the mean measured expression value of the two replicates at time point zero, then assuming a constant decay rate throughout the course of the experiment the decay profile is fitted to a first-order exponential decay model, $y(t)=y(0)\cdot e^{-kt}$, from which the fitted decay constant k is used to calculate a gene-specific half-life in each condition, $t_{1/2}=\ln(2)/k$. Only genes for which a relatively good fit is achieved (R-square >0.7) are taken for further analysis ($\sim$70% of the genes).

Determination of the maximal fold change and the time at which it is attained

Responsive genes were defined as having an absolute fold change of above twofold for at least one time point out of the mRNA abundance measurements. These measurements were then used to approximate the full response by fitting the profiles of these genes to a cubic spline with breaks at each measured time point. The maximal point was taken as the point where both the spline derivatives were equal to zero and the fitted spline value reached the maximal absolute value (maximal for induced genes and minimal for repressed genes). For genes with a fitted spline that was constantly increasing or decreasing throughout the whole time course, the last point of the time course was considered the maximal point. It is important to note that the results presented in this paper will not change qualitatively if the data would be treated as a discrete time course containing only the measured time points.

Data mining

We have used two data sets, GO (http://www.geneontology.org/) and KEGG pathways (http://www.genome.ad.jp/kegg/pathway.html), to mine the data with respect to the dynamics of different functional categories. For each category, we took the intersection between the annotated genes to the induced/repressed genes in each condition and performed three separate statistical tests. First, we used the hypergeometric distribution to check which categories are enriched in the induced and repressed sets of genes using only the data on changes in mRNA abundance. Two additional tests were performed on the logarithm of the half-life ratio for each intersection. A one-sided t-test was performed to check whether it deviates significantly from zero, indicating a tendency for stabilization or destabilization. Additionally, to test whether some groups of genes behave anomalously when compared with the general trend of induced or repressed genes, we used a χ^2 test on the number of destabilized/stabilized genes in the intersection when compared with the expected number based on the percentage of destabilized genes in all induced or repressed genes. A representative sample of the results is given in Table I and the full data set is provided as part of the Supplementary information.

Single-gene measurements of decay and mRNA abundance with RT–PCR

We performed real-time PCR experiments on selected genes to verify the array-derived decay profiles and changes in mRNA abundance (see Supplementary Figure 14A–D). For mRNA quantification, a 2 μg aliquot of total RNA was reverse transcribed using random primers. RT–PCR was performed using 480 SYBR Green I Master (Roche) Reagent on LightCycler 480 Real-Time PCR system (Roche).

Expression data

The entire expression data set, including decay profiles in the reference condition, and under oxidative and MMS stresses, along with mRNA abundance profiles in each of the stress conditions, is deposited in the GEO databases (GSE12222). The data set and additional supplementary information is also available on line at http://longitude.weizmann.ac.il/pub/papers/Shalem2008_mRNAdecay/suppl/, and at MSB website.

Supplementary information

Supplementary information is available at the *Molecular Systems Biology* website (www.nature.com/msb).

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Conflict of interest

The authors declare that they have no conflict of interest.

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