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REPORT

Fus3-triggered Tec1 degradation modulates mating transcriptional output during the pheromone response

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The yeast transcription factor Ste12 controls both mating and filamentation pathways. Upon pheromone induction, the mitogen-activated protein kinases, Fus3 and Kss1, activate Ste12 by relieving the repression of two functionally redundant Ste12 inhibitors, Dig1 and Dig2. Mating genes are controlled by the Ste12/Dig1/Dig2 complex through Ste12-binding sites, whereas filamentation genes are regulated by the Tec1/Ste12/Dig1 complex through Tec1-binding sites. The two Ste12 complexes are mutually exclusive. During pheromone response, Tec1 is degraded upon phosphorylation by Fus3, preventing cross-activation of the filamentation pathway. Here, we show that a stable Tec1 also impairs the induction of mating genes. A mathematical model is developed to capture the dynamic formation of the two Ste12 complexes and their interactions with pathway-specific promoters. By model simulations and experimentation, we show that excess Tec1 can impair the mating transcriptional output because of its ability to sequester Ste12, and because of a novel function of Dig2 for the transcription of mating genes. We suggest that Fus3-triggered Tec1 degradation is an important part of the transcriptional induction of mating genes during the pheromone response.
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Introduction

Transcription factors in regulatory networks often have multiple functions during cellular responses. Identifying their roles in a complex network is fundamental to understanding the dynamics and performance of the network as a system. In the budding yeast *Saccharomyces cerevisiae*, the transcription factor Ste12 is regulated by the pheromone-responsive mitogen-activated protein kinase (MAPK) pathway, and it controls the expression of mating genes through Ste12-binding sites (PREs, pheromone-responsive elements) upstream of mating genes (Bardwell, 2005). During pheromone induction, the pheromone-responsive MAPKs, Fus3 and Kss1, regulate the transcriptional activity of the transcription factor Ste12 by relieving the repression of two functionally redundant Ste12 inhibitors, Dig1 and Dig2 (Cook *et al*, 1996; Tedford *et al*, 1997; Frey *et al*, 2000).

Ste12 also controls filamentous growth in yeast. It functions together with the transcription factor Tec1, which is specifically required for the filamentation pathway (Baur *et al*, 1997; Madhani and Fink, 1997). Ste12 controls two distinct developmental programs by forming two mutually exclusive complexes, Ste12/Dig1/Dig2 and Tec1/Ste12/Dig1 (Chou *et al*, 2006). In the Tec1/Ste12/Dig1 complex, Tec1 transcriptional activity is determined by Ste12, which is under Dig1 inhibition and the regulation by Fus3 and Kss1. Genome-wide analyses of Ste12- and Tec1-binding locations found that Ste12 and Tec1 are present at the promoters of filamentation genes despite the lack of a conserved Ste12-binding motif in most of these promoters (Zeitlinger *et al*, 2003; Borneman *et al*, 2007a, b). Therefore, most filamentation genes are regulated by the Tec1/Ste12/Dig1 complex through Tec1 binding to Tec1 control sites (TCSs).

The distribution of Ste12 between the two Ste12 complexes is likely determined by Tec1 level. Excess Tec1 can compete off Dig2 from Ste12 *in vitro* and *in vivo*, whereas excess Dig2 cannot compete off Tec1 from Ste12 (Chou *et al*, 2006). Importantly, Tec1 level is dynamically regulated by the MAPK pathway during the pheromone response. *TEC1* transcription is under the positive regulation of Ste12 and Tec1 by active Fus3 and Kss1, whereas active Fus3 can phosphorylate Tec1 and trigger the rapid degradation of Tec1 (Bao *et al*, 2004; Bruckner *et al*, 2004; Chou *et al*, 2004). This Fus3-activated Tec1 degradation is a key mechanism for preventing the expression of filamentation genes during the pheromone response.

The pheromone-responsive MAPK pathway and the transcription of mating genes have been modeled in several studies (Kofahl and Klipp, 2004; Komarova *et al*, 2005; Schaber *et al*, 2006; Shao *et al*, 2006; Wang *et al*, 2006; Bardwell *et al*, 2007; McClean *et al*, 2007; Paliwal *et al*, 2007). These studies emphasized MAPK activities. Transcriptional regulation by differential Ste12 complex formation has not been modeled before. Here, by modeling the dynamic formation of two Ste12 complexes and their binding to PREs of mating genes and TCSs of filamentation genes, and by correlating simulation results with the experimental data on the level of Tec1 and levels of expression from PREs and TCSs, we have identified a novel role of Tec1 in modulating the pheromone-responsive transcription level. We show that *TEC1* deletion results in an increased pheromone-responsive output, whereas a Tec1 stable mutant leads to a decreased pheromone-responsive output. Through simulations of the model and genetic analysis, we suggest that Tec1 modulates the level of pheromone-responsive output by sequestering Ste12 from the Ste12/Dig1/Dig2 complex. We also find that Dig2 is important for maximal Ste12 transcription activity.

Results and discussion

A model for the dynamic formation of Ste12 complexes and transcription outputs of mating and filamentation pathways

To understand how Tec1 protein level affects the transcription outputs for mating and filamentation pathways quantitatively, we developed a mathematical model based on the molecular interactions illustrated in Box 1. The system includes formation of Ste12 complexes Tec1/Ste12/Dig1 and Ste12/Dig1/Dig2, and their binding to mating promoter PREs and filamentation promoter TCSs (Sprague and Thorner, 1992; Chou *et al*, 2006). The model also includes Fus3-activated Tec1 degradations and a positive feedback regulation of *TEC1* transcription (Oehlen and Cross, 1998; Bao *et al*, 2004; Chou *et al*, 2004). The inputs of the system are active Fus3 and Kss1 whose temporal dynamics are explicitly given in the model (Sabbagh *et al*, 2001). For simplicity, the pheromone level, which is assumed to be proportional to active Fus3 and Kss1 levels (Sabbagh *et al*, 2001), is not explicitly modeled.

During the pheromone response, Fus3 phosphorylation of Tec1 triggers Tec1 degradation, which ensures pathway specificity (Bao *et al*, 2004; Bruckner *et al*, 2004; Chou *et al*,

Box 1 A schematic diagram for biochemical reactions in the model.
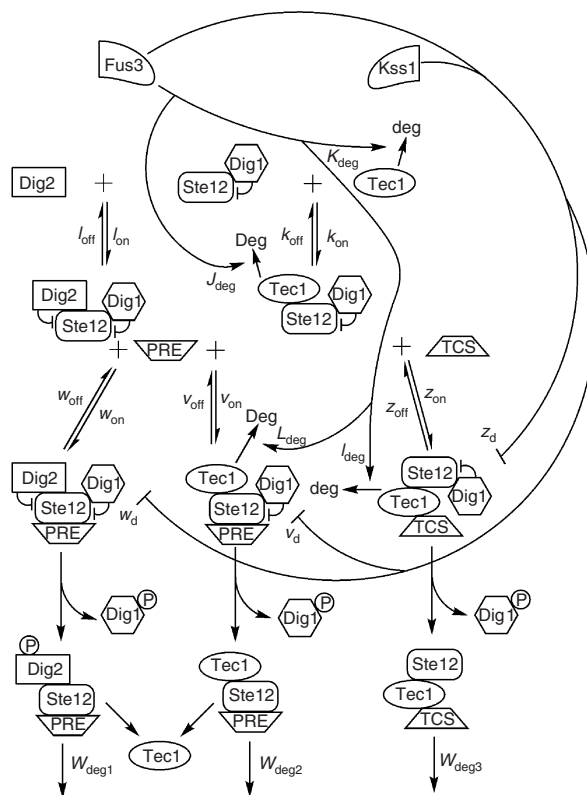
Box 1 The system consists of Tec1, Ste12, Dig1, Dig2, PRE, TCS and their reaction products. It is activated by pheromone through active Fus3 and Kss1 that in turn inhibit Dig1 and Dig2, and regulate Tec1 degradations. The three inhibition arrows originated from Fus3 and Kss1, along with the inhibition arrows from Dig1 and Dig2 to Ste12, represent activation of Ste12 activity through removing the inhibitions of Dig1 and Dig2 on Ste12. Dig1 in the system is not explicitly modeled, and only the complex Ste12/Dig1 is included in the system.

2004). Tec1[T273V], a stable Tec1 mutant that lacks a Fus3 phosphorylation site, shows increased *TCS-lacZ* expression in response to pheromone (Chou *et al*, 2004). Consistent with the previous publication, the level of *TCS-lacZ* expression increases during pheromone induction in the strain with *TEC1*[T273V] under its endogenous promoter (Figure 1A). To assist model simulations, temporal dynamics of both *PRE-lacZ* and *TCS*-lacZ during the pheromone response are also examined. In wild-type cells, the *PRE-lacZ* expression gradually increases, and reaches 100-fold of its initial level within a 2-h period, whereas the corresponding *TCS-lacZ* expression hardly changes (Figure 1A).

Simulations of the model gave a similar overall pattern of *PRE-lacZ* and *TCS-lacZ* dynamics and fold changes to that of the experimental data (Figure 1B). In addition, Tec1 dynamics in the simulation (Supplementary Figure S2) were consistent with the experimental observation (Chou *et al*, 2004) in which the level of Tec1 has a quick dip between 15 and 30 min after pheromone induction due to the Fus3-regulated degradation of Tec1, and then Tec1 gradually reaches a steady level due to
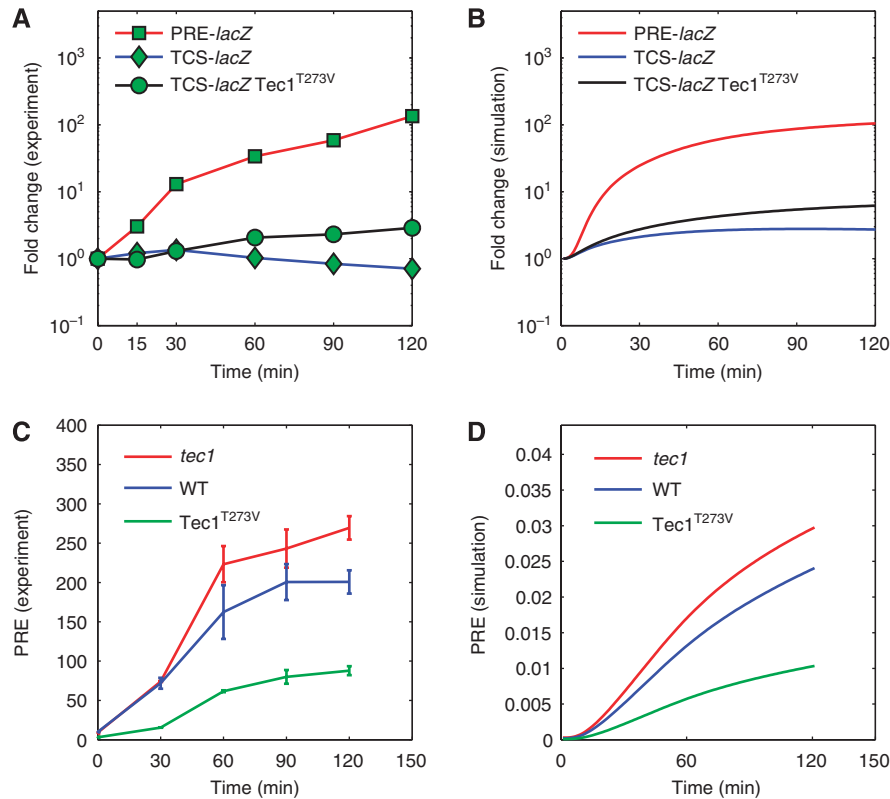
Figure 1 Tec1 degradation is important for proper PRE induction. PRE and TCS outputs as functions of time in both experiments and simulations are shown. (**A**) Experimental measurements of the relative fold activity for *PRE-lacZ* for wild-type cells (WT), *TCS-lacZ* for WT and *TCS-lacZ* for stable Tec1 strains, as functions of time after treatment with 5 μM α-factor. (**B**) Direct numerical simulations of (A) with parameters shown in Supplementary Tables 1 and 2. (**C**) Experimental measurements of *PRE-lacZ* output as a function of time for WT, *tec1* deletion and stable Tec1 strains. The system is treated with 200 nM α-factor. (**D**) One of the simulated cases from Figure 3B.

feedback activation of *TEC1* expression (Madhani and Fink, 1997). The simulation results also show that, in wild-type cells, the total amount of Ste12 and the total amount of Dig2 hardly change during the 2-h period after pheromone induction (Supplementary Figure S2), consistent with our previous experimental observations (Chou *et al*, 2004). To a certain degree, this consistency between the simulated results and the experimental data validates the model.

The rate constants used in the simulations of Figure 1B were chosen based on our previous studies of the two Ste12 complexes modeled in the system (Chou *et al*, 2006). The overall qualitative feature of the system, such as the relative positions of the three *lacZ* levels shown in Figure 1, and the dynamics of Tec1 and Ste12 levels were relatively robust with respect to changes of rate parameters in the model (Supplementary Figures S5 and S11). From the simulations, we found that the *TCS-lacZ* output during the pheromone response was very sensitive to the Tec1 degradation rate, a coefficient measuring the largest allowable Tec1 degradation regulated by Fus3. Consistent with the experimental data (Chou *et al*, 2004), for the case of zero degradation of Tec1 in the model, which correlates with the stable Tec1[T273V], the Tec1 level monotonically increases from the start, and the fold change of *TCS-lacZ* always increases in response to pheromone. On the other extreme, when Tec1 degradation is large, a case such as for wild-type *TEC1*, *TCS-lacZ* level decreases immediately after pheromone induction (Supplementary Figure S3). A large Tec1

degradation rate could also lead to *TCS-lacZ* reduction at late times of the pheromone induction (Supplementary Figure S3), which is similar to the measured *TCS-lacZ* profile shown in Figure 1A. Therefore, a 2- to 3-fold increase in the Tec1 degradation rate changed the *TCS-lacZ* output profile from a slight increase at late time points (Figure 1B) to reduction (Supplementary Figure S3). The dynamics of various Ste12 complexes in the simulations are presented in the Supplementary information as well (Supplementary Figure S2).

Fus3-activated Tec1 degradation during pheromone response is critical for proper transcriptional induction of mating genes

Excess Tec1 has been shown to reduce the amount of Ste12/ Dig1/Dig2 complex by replacing Dig2 from Ste12 both *in vitro* and *in vivo* (Chou *et al*, 2006). In addition, Tec1 is present on PRE elements, although at a lower level than Ste12 (Zeitlinger *et al*, 2003). Zeitlinger *et al* (2003) also observed a shift of Ste12 binding from the promoters of filamentation genes to that of mating genes upon pheromone treatment. The loss of Ste12 binding to the filamentation genes is likely due to the Fus3- triggered Tec1 degradation. These experimental data raised a question. Is Fus3-triggered Tec1 degradation important for the transcription induction output of the mating pathway? To address this, we measured *PRE-lacZ* in wild type, *tec1* deletion,

and stable $TEC1^{T273V}$ cells. The *PRE-lacZ* level in the *tec1* deletion cells after pheromone induction is slightly higher than that of wild-type cells; importantly, the *PRE-lacZ* level in the stable Tec1^{T273V} cells was dramatically lower than that in the wild-type cells (Figure 1C). The property of relative *PRE-lacZ* levels among the *tec1*, wild-type and $TEC1^{T273V}$ strains is preserved during the 2-h periods of pheromone induction, and remains true for several pheromone levels from 20 to 5 µM. Therefore, our data show that Tec1 can reduce pheromone-responsive PRE output. This suggests that degradation of Tec1, which is triggered by Fus3 phosphorylation, is a normal and important part of the transcription induction of mating genes during the pheromone response.

Ste12/Dig1/Dig2 is more effective in activating mating gene transcription than Tec1/Ste12/Dig1

To understand the underlying mechanisms for why excess Tec1 can hinder the transcriptional induction of mating genes, we performed model simulations to determine the constraints in reaction and interaction kinetics that can generate simulations similar to our experimental observations in Figure 1C. Specifically, we solved the system for many sets of reaction rates randomly chosen around the values based on *in vitro* experiments (Chou *et al*, 2006). We calculated the ratio of PRE output for the *tec1* deletion cells over PRE output for the wild-type cells, defined as R_1, and the ratio of PRE output for the wild-type cells over the stable $TEC1^{T273V}$ cells, defined as R_2, at various time points. To be consistent with the experimental observations in Figure 1C, both R_1 and R_2 obtained from the model should be larger than 1.

When the pheromone-induced activation rate of *PRE-lacZ* for Tec1/Ste12/Dig1 and Ste12/Dig1/Dig2 is set to be the same, we see that R_2 is always smaller than 1 (Figure 2A). For a wide range of reaction rates, the PRE output for stable $TEC1^{T273V}$ cells is always larger than the PRE output of the wild-type cells. In addition, R_2 seems to be an inverse function of R_1 (Figure 2A). This implies that the ratio of PRE output for $TEC1^{T273V}$ cells over PRE output for *tec1* deletion cells hardly changes from case to case in Figure 2A. However, when the
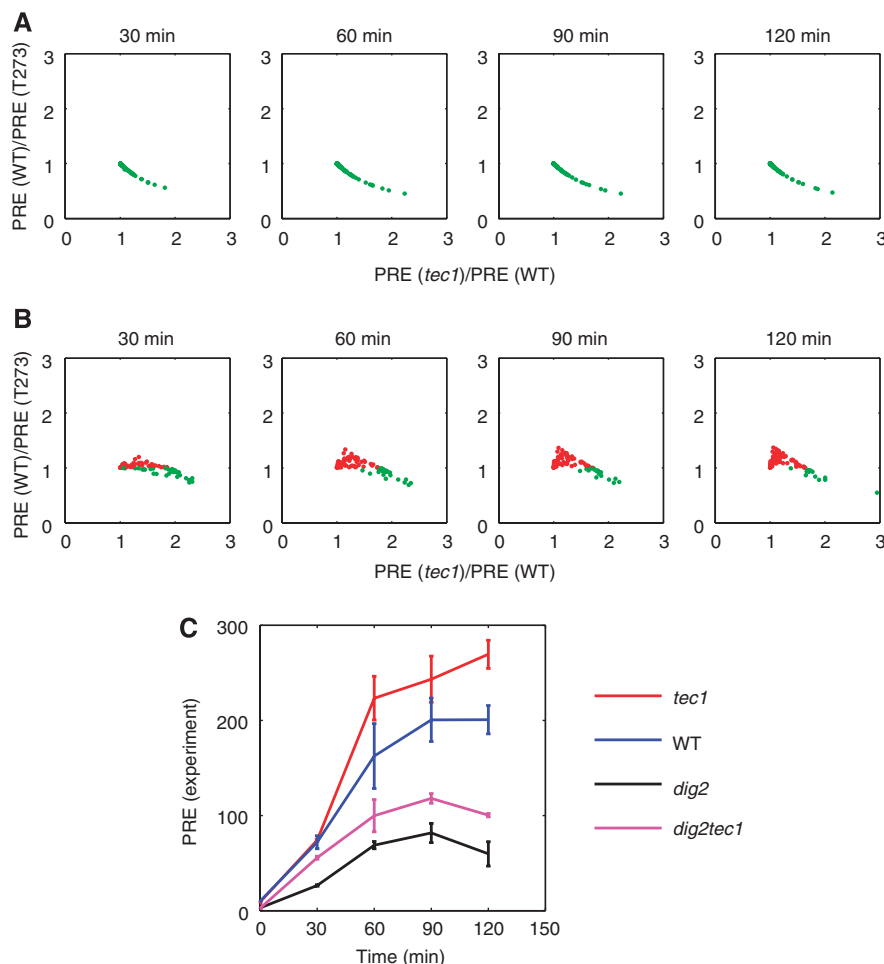

Figure 2 Strategy 1: Ste12/Dig1/Dig2 is more effective than Tec1/Ste12/Dig1 in the transcriptional induction of PREs. Ratio of PRE output for WT cells over PRE output for stable Tec1 strains (R_1) versus ratio of PRE output for the *tec1* deletion mutant over PRE output for WT cells (R_2) at four different times: 30, 60, 90 and 120 min. Each dot represents one case in which the reaction rates are randomly selected. Hundred cases are shown. Red dots represent the cases in which $R_1 > 1$ and $R_2 > 1$ at each time. (**A**) The overall activation rates of Fus3 on Tec1/Ste12/Dig1 and on Ste12/Dig1/Dig2 are assumed to be the same. (**B**) The overall activation rate of Fus3 on Tec1/Ste12/Dig1 is assumed to be half that of the rate on Ste12/Dig1/Dig2. (**C**) Experimental measurements of *PRE-lacZ* levels in WT, *dig2*, *tec1* and *dig2 tec1* mutants with 200 nM α-factor.

pheromone-induced activation rate for the complex Tec1/Ste12/Dig1 is set to be smaller than the activation rate for Ste12/Dig1/Dig2, R_1 and R_2 are both larger than 1 in our model simulations (Figure 2B). This strategy presumes that the Tec1/Ste12/Dig1 portion of the PRE output is produced less efficiently than the Ste12/Dig1/Dig2 portion of the PRE output. Under this assumption, both R_1 and R_2 are larger than 1 in most of the cases when other reaction rates randomly vary (red dots in Figure 2B). The overall pattern in Figure 2B is a relatively robust strategy (Supplementary Figures S4, S5 and S11). Therefore, our modeling suggests that the Ste12/Dig1/Dig2 complex may be more effective in the transcriptional activation of PREs than the Tec1/Ste12/Dig1 complex.

To validate the prediction from the model, we determined the pheromone-induced transcriptional activation from the Ste12/Dig1/Dig2 and Tec1/Ste12/Dig1 complexes by measuring *PRE-lacZ* in *tec1* mutants and *dig2* mutants, respectively. In the *tec1* mutant, *PRE-lacZ* expression should be induced exclusively by the Ste12/Dig1/Dig2 complex, whereas in the *dig2* mutant, *PRE-lacZ* expression is regulated only by the Tec1/Ste12/Dig1 complex. We found that the level of *PRE-lacZ* induced in the *dig2* mutant is about half of that found in wild-type cells (Figure 2C). There are two possibilities for this observation. Dig2 itself is required for full activation of the mating transcription. Alternatively, the effect of *dig2* deletion is caused solely by freeing Ste12 to form complex with Tec1, thus shifting Ste12 from PRE elements of mating genes to the TCS elements of filamentation gene promoters. However, we found that the *PRE-lacZ* induction in a *dig2 tec1* double mutant is very similar to that of a *dig2* mutant (Figure 2C). This supports the former, and is consistent with the prediction by our model that Ste12/Dig1/Dig2 is more effective in mating transcriptional induction than the Tec1/Ste12/Dig1 complex.

Tec1 can sequester Ste12 from forming the Ste12/Dig1/Dig2 complex to reduce the mating transcriptional output when the amount of Ste12 is limiting

We explored the model further by varying the amount of various molecules available in the system. We discovered that the amount of Ste12 in the system is important for the model to create the experimental data in Figure 1C. Simulations in Figure 2 assumed that the available free Ste12 in the system was similar to that of Tec1 and Dig2, as the number of Ste12, Tec1 and Dig2 molecules in a yeast cell are estimated to be 1920, 530 and 1310, respectively (Ghaemmaghami *et al*, 2003). By quantitative western blot analysis using Odyssey Infrared Imaging System (Lincoln, NE), we have determined that the relative amounts of these three proteins in Sigma strain background before exposing to pheromone are 1920 (Ste12), 615 (Tec1) and 691 (Dig2) (data not shown). When we reduced the amount of free Ste12 in the system to 30% or less of the level used in Figure 2, we found many cases in which $R_1 > 1$ and $R_2 > 1.4$ (Figure 3A, red dots) even when the PRE activation rates of Tec1/Ste12/Dig1 and Ste12/Dig1/Dig2 are the same. By correlating the five affinity rates for cases in which $R_1 > 1$ and $R_2 > 1.4$, a condition resembling the experimental observations in Figure 1C, we found another
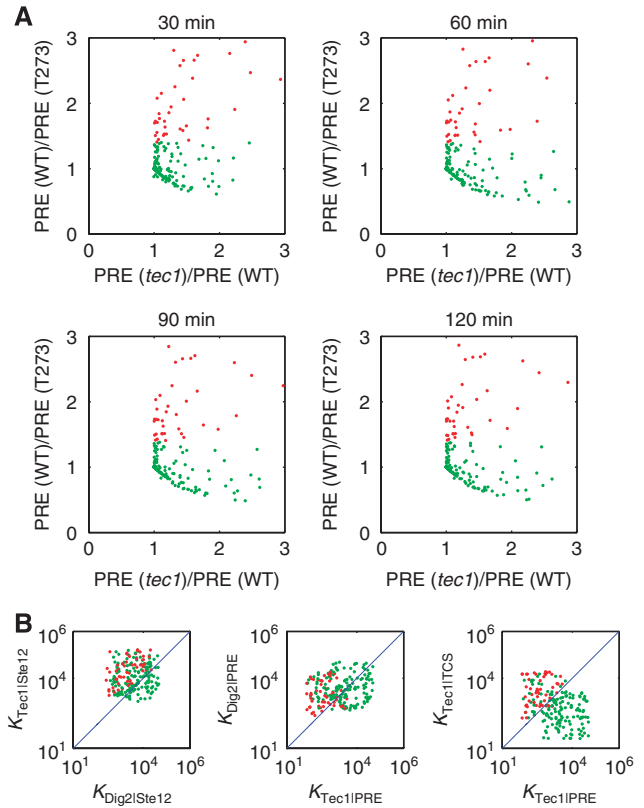

Figure 3 Emergence of strategy 2: Tec1 sequestration of Ste12 when free Ste12 in the system is limiting. (**A**) Same conditions as those in Figure 2A except the initial Ste12 amount in the system, which is 30% of the system in Figure 2A, and the total number of random cases is 200. Red dots represent the cases in which $R_1 > 1$ and $R_2 > 1.4$ at each time. (**B**) Correlations among the five binding affinity rates for the cases in (A); red dots represent the cases in which $R_1 > 1$ and $R_2 > 1.4$ at 120 min. Other points are represented in green. $K_{Tec1|Ste12}$ is the affinity rate between Ste12 and Tec1, and likewise for $K_{Dig2|Ste12}$, $K_{Dig2|PRE}$, $K_{Tec1|PRE}$ and $K_{Tec1|TCS}$.

strategy for the model to give simulated results that resemble the experimental data, as shown in Figure 3B (see Figure 1D for a typical case of Figure 3B). In this strategy, the binding affinity of Ste12 to Tec1 is likely to be higher than that to Dig2, and the binding affinity of Tec1/Ste12/Dig1 to TCSs is more likely to be higher than that to PREs (Figure 3B, left and right panels). The correlation between the binding affinities of Tec1/Ste12/Dig1 to PRE versus Ste12/Dig1/Dig2 to PREs is less clear (Figure 3B, middle panel). A study on the sensitivity of these correlations to the number of sample points, the initial amount of Ste12, the initial amount of PRE and TCS, and the choice of low bound of R_2 is presented in the Supplementary Figures S6–S10. We also found that the results from Figures 2 and 3 remained the same when a slightly different temporal dynamics of active Fus3 and Kss1 and their dependence on the pheromone level were used.

For most of the cases in Figure 3A that closely resemble the experimental observation, the affinity rates satisfy these two constraints. Interestingly, the two constraints are in very good agreement with *in vitro* and *in vivo* data. We have shown that excess Tec1 can replace Dig2 from binding to Ste12, whereas excess Dig2 cannot replace Tec1 from binding to Ste12, suggesting that Tec1 has a higher affinity for Ste12 than Dig2

(Chou *et al*, 2006). More Tec1 is found at the TCSs of filamentation promoters than the PREs of mating gene promoters (Chou *et al*, 2006). A genome-wide localization of Ste12 and Tec1 showed that they are present mostly at the promoters of filamentation genes, and Ste12 shifts to the promoters of mating genes upon pheromone induction (Zeitlinger *et al*, 2003). Therefore, both experimental measurements and modeling support that Tec1 can sequester Ste12 from the Ste12/Dig1/Dig2 complex to reduce the PRE output when Ste12 is limiting.

Discussion

Ste12 is a key regulator for both mating and filamentation pathways. It forms two mutually exclusive complexes Ste12/Dig1/Dig2 and Tec1/Ste12/Dig1 that control the transcription of mating and filamentation genes, respectively. Here, we developed a mathematical model to simulate dynamic formation of Ste12 complexes and their interactions with promoters. The positive feedback regulation of *TEC1* transcription and Fus3-activated Tec1 turnover are also featured in the model. The model can simulate experimental data on the induction of mating gene transcription, the dynamic change in Tec1 level and the transcription output of the filamentation pathway during the pheromone response. Our model simulation also supports the finding that Fus3-activated Tec1 degradation is critical in preventing the expression of filamentation genes during the pheromone response. More importantly, we show here that Fus3-activated Tec1 degradation is likely a part of the regulation for the transcriptional induction of mating genes during the pheromone response. Cells carrying the stable $TEC1^{T273V}$ mutant under its endogenous promoter showed a much reduced level of transcriptional induction for the mating pathway (Figure 1C). Our model simulations found two possible strategies to achieve the interference of mating transcriptional output by Tec1.

One mechanism is sequestration of Ste12 by Tec1. The two correlations discovered from random sampling in model simulations matched exactly with the experimental findings: Ste12 has a stronger binding affinity for Tec1 than Dig2 (Chou *et al*, 2006) and the Tec1/Ste12/Dig1 complex has a higher affinity for TCSs than PREs. This mechanism is in line with the finding that Ste12 distribution shifts from filamentation genes to mating genes in response to pheromone (Zeitlinger *et al*, 2003). A prerequisite of the sequestration mechanism is that free Ste12 available in the system is not abundant. Although the total number of Ste12 molecules is similar to the sum of that of Tec1 and Dig2 in a yeast cell (Ghaemmaghami *et al*, 2003), the available free Ste12 in the system for interaction with Tec1 and Dig2 could be lower, because Ste12 binds many proteins, including Mcm1 and **a**1. Indeed, overexpressing *TEC1* dramatically reduces the amount of Dig2 that is associated with Ste12 (Chou *et al*, 2006), suggesting that Ste12 is limiting *in vivo*. Therefore, Tec1 sequestration of Ste12 is a likely mechanism used in yeast, at least in cells with stable $TEC1^{T273V}$, as *TEC1* transcription is highly induced in response to pheromone.

Model simulations also predict that the Ste12/Dig1/Dig2 complex induces transcription from PREs more efficiently than

the Tec1/Ste12/Dig2 complex. In support of this, a *dig2* mutant is impaired in the induction of *PRE-lacZ*. This is not due to Tec1 sequestration of Ste12, as a *dig2 tec1* double mutant is similar to the *dig2* mutant. It is likely that Dig2 has dual functions. Dig2 is known to have an inhibitory function on Ste12, and this activity is redundant with Dig1 (Cook *et al*, 1996; Tedford *et al*, 1997; Chou *et al*, 2006). The inhibitory function of Dig2 is only revealed when Dig1 is also absent, as *dig2* deletion has no effect on the basal expression of *PRE-lacZ*, whereas *PRE-lacZ* is highly expressed in *dig1dig2* double mutants in the absence of pheromone (Chou *et al*, 2006). Our new finding that *dig2* (and *dig2 tec1*) mutants are impaired in the full induction of *PRE-lacZ* in response to pheromone suggests that Dig2 also has a novel positive role on Ste12. In support of this, we find, by immunoprecipitation of Ste12, that Dig2 is still bound to Ste12 while Dig1 is released from Ste12 during mating (data not shown). Therefore, failure to destroy Tec1 can lead to reduced Dig2/Ste12 complex formation and reduced mating transcriptional output.

What is the significance of Fus3-triggered Tec1 degradation in pheromone-responsive mating? It may play a role in sensing the duration, dosage or gradient of pheromone. For example, by making the pheromone response sluggish early on, Tec1 may insert a measure of quality control to ensure that yeast cells ignore pheromone stimuli that come from either internal noise or perhaps some environmental condition. The significance of this regulation can only be realized when cells are observed at the single-cell level in their physiological conditions. Some of the observed differences between the two MAPKs Fus3 and Kss1 in pheromone sensing at the single-cell level could be mediated through the Fus3-specific activation of Tec1 degradation (Colman-Lerner *et al*, 2005; Paliwal *et al*, 2007).

Materials and methods

Modeling

The model aims to study properties arising from interactions and regulations among several transcriptional factors in the yeast mating pathway. It consists of Tec1, Ste12, Dig1, Dig2, PRE, TCS, and their reaction products and the final signals. Dig1 in the system is not explicitly modeled, and only the Ste12/Dig1 complex is included in the system. The ordinary differential equations consist of mass actions for the concentrations of Tec1, Ste12/Dig1, Dig2, Ste12/Dig1/Dig2, Ste12/Dig1/Tec1, and the PRE-binding site, the TCS-binding site, PRE/Ste12/Dig1/Dig2, PRE/Ste12/Dig1/Tec1 and TCS/Ste12/Dig1/Tec1.

The system is activated by pheromone through active Fus3 and Kss1 that are two input functions resembling the experimentally observed temporal dynamics for Fus3's and Kss1's responses to pheromone (Sabbagh *et al*, 2001). To be consistent with the same experimental observation, the level of active Fus3 and Kss1 is assumed to be positively correlated with the level of pheromone. On the basis of experimental results (Bao *et al*, 2004), a positive feedback of active Fus3 on Tec1 degradation and a positive feedback of PRE signal on Tec1 expression are built in the model. The inhibition role of active Fus3 and Kss1 on Dig1 (Bardwell, 2005) is modeled through activation of the inactive complexes involving Dig1 by active Fus3 and Kss1. For the case of stable Tec1, such as Tec1^{T273V}, the degradation of Tec1 is set to be zero; for the case of the *TEC1* deletion, the initial concentration of Tec1 is set to be zero. A complete model is listed in the Supplementary information.

Before the induction of pheromone, we first allow the system to develop for 3 h due to the presence of basal levels of Fus3 and Kss1. The rate constants in the model are chosen based on the biochemical

analysis of various complexes involving Tec1, Ste12, PRE and TCS as in Chou *et al* (2006). A list of the parameters and a sensitivity analysis of rate constants are given in the Supplementary information. The ordinary differential equations, which resulted from the model, are numerically calculated using the Runge-Kutta 45 solver implemented in Matlab.

Yeast strain construction and β-galactosidase assay

tec1::*KanR* (HLY3665), *dig2*::*TRP1* (HLY3316) and *tec1*::*KanR dig2*::*TRP1* (HLY3662) strains were constructed in the wild-type strain 10560-4A (*MATα ura3-52 his3*::*hisG leu2*::*hisG trp1*::*hisG*) as described in Chou *et al* (2006). The yeast strains were transformed with a *PRE-lacZ* reporter (Bardwell *et al*, 2001) or a *TCS-lacZ* reporter (Chou *et al*, 2004) for β-galactosidase assays. The *TEC1p-TEC1/CEN* (pHL741) and *TEC1p-TEC1*^{T273V} (pHL742) are described in Chou *et al* (2004). Cells from fresh overnight cultures in a selective medium were diluted into YEPD medium to 0.2 OD_{600} and were grown for 4 h at 30°C. α-Factor was added to a final concentration of 5, 200 or 20 nM. The cells were collected at 0, 30, 60, 90, and 120 min. β-Galactosidase assays were performed as described (Rose and Botstein, 1983) with the addition of protease inhibitors in the cell-breaking buffer (0.1 M Tris, pH 8.0; 20% glycerol, 0.5 mM PMSF, 2 mM benzamidine, 1 μM leupeptin, 2 μM pepstatin, 4 μM chymostatin, 2.6 μM aprotinin). β-Galactosidase units were calculated by $OD_{420} \times (1.7/0.0045) \times 1000/\text{time (min)} \times \text{volume (μl)} \times \text{concentration (μg/μl)}$.

Supplementary information

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

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