

Tec1 Reduces Pheromone Responsive Transcription Output by Differential Ste12 Complex Formation

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Introduction

Transcription factors in regulatory networks often have multiple functions during cellular responses. Identifying their roles is fundamental to understanding the dynamics and performance of a network as a system. In the budding yeast *Saccharomyces cerevisiae*, the transcription factor Ste12 is downstream of the pheromone responsive mitogen-activated protein kinase (MAPK) pathway and controls both mating and filamentous growth [1]. The transcription factor Tec1 is specifically required for the filamentation pathway [2]. During mating, the MAPKs, Fus3 and Kss1, regulate the transcriptional activity of Ste12 by relieving the repression of Dig1 and Dig2. Mating genes are regulated by Ste12 through Ste12 binding sites (PREs, pheromone responsive elements).

Ste12 forms two mutually exclusive complexes, Ste12/Dig1/Dig2 and Tec1/Ste12/Dig1, through the mechanism of competitive binding of Ste12 with Tec1 or Dig2 [3]. Filamentation genes are regulated by the Tec1/Ste12/Dig1 complex through Tec1 binding to Tec1 control sites (TCSs) [3]. Tec1 transcriptional activity is determined by the associated Ste12/Dig1. The level of Tec1 is dynamically regulated during the pheromone response. *TEC1* transcription is under the positive regulation of Ste12 by Fus3 and Kss1, while active Fus3 can phosphorylate Tec1 and trigger the rapid degradation of Tec1. This Fus3-activated Tec1 degradation is a key mechanism for preventing the expression of filamentation genes during the pheromone response [4, 5].

Results - Tec1 reduces the mating transcriptional output

Here, we developed a model that emphasizes the dynamic formation of Ste12 complexes Tec1/Ste12/Dig1 and Ste12/Dig1/Dig2, and their binding to mating promoter PREs and filamentation promoter TCSs. It also highlights the dynamic regulation of the level of Tec1. Simulations of the model gave similar temporal dynamics of Tec1 level and the fold changes of PRE and TCS expression to that of the experimental data (Fig. 1A,B).

We found a novel role for Tec1 in pheromone responsive output. *TEC1* deletion resulted in increased expression from PREs, whereas a Tec1 stable mutant led to a decreased level of expression from PREs (Fig.1C). Similar results could be obtained by model simulations (Fig.1D). Therefore, through our genetic analyses and model simulations, we suggest that Tec1 reduces the mating transcriptional output.

Strategies - Tec1 reduces mating transcript output by sequestering Ste12 from the Ste12/Dig1/Dig2 complex

To identify the underlying mechanisms responsible for the role of Tec1 on PRE output, we analyzed the model to determine constraints on biochemical kinetics under which the model can realize the experimental observation in Figure 1C. We studied simplified models and solved the full model for many sets of reaction rates randomly chosen around the values based on in-vitro experiments.

When Ste12 is abundant in the system, we found one possible strategy to achieve Figure 1C. In this strategy, the Tec1/Ste12/Dig1 portion of the PRE output is produced less *efficiently* than the Ste12/Dig1/Dig2 portion of the PRE output. This inefficiency may be achieved by requiring *unequal* inhibition/activation of Dig1 on Tec1/Ste12/Dig1 and Ste12/Dig1/Dig2. Although this is a very robust strategy by modeling, current experimental data displays no direct evidence for this.

When Ste12 in the system is not abundant, we found another possible strategy (Figure 2). In this mechanism, the affinity rate for Ste12 to bind Tec1 needs to be higher than that for Dig2; the affinity for PREs to bind Ste12/Dig1/Dig2 need to be higher than for Tec1/Ste12/Dig1. Both parameters are in good agreement with the *in vitro* and *in vivo* measurements [3]. In fact, an excess amount of Tec1 has been shown to reduce the amount of Dig2/Ste12/Dig1 complex by replacing Dig2 in the complex. Also, the amount of Ste12 molecules in yeast is similar to the sum of Tec1 and Dig2 molecules [6]. Therefore, based on our modeling and experimental measurements, we suggest that Tec1 reduces the mating transcriptional output by sequestering Ste12 from the Ste12/Dig1/Dig2 complex through competitive binding to Ste12.

References

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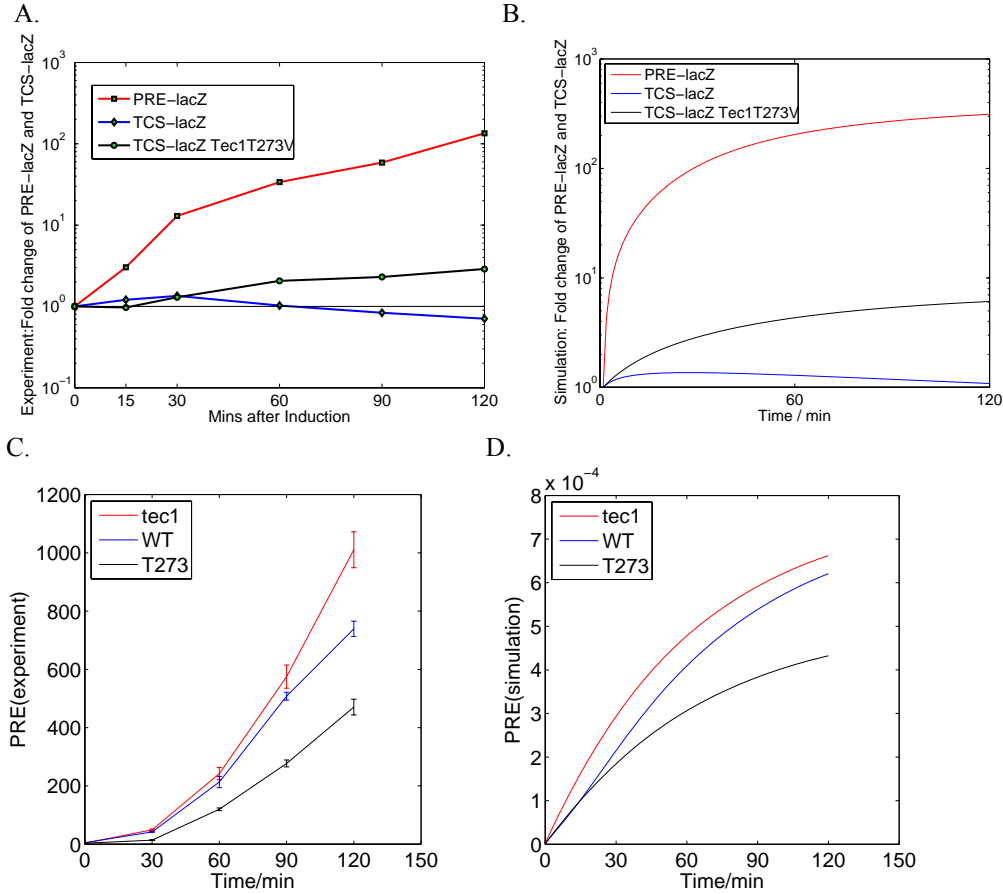


Figure 1. **Comparisons between experiments and simulations.** (A) Experimental measurement of the relative fold activity for PRE-*lacZ* for wild type cells (WT), TCS-*lacZ* for WT, and TCS-*lacZ* for stable TEC1^{T273V} as function of time after treated with 5 μ M α factor. (C) Experimental measurement of PRE-*lacZ* for wild-type, *tec1* and stable TEC1^{T273V} in response to 5 μ M α factor. (B) and (D): Direct numerical simulations of the model under the conditions of (A) and (C), respectively.

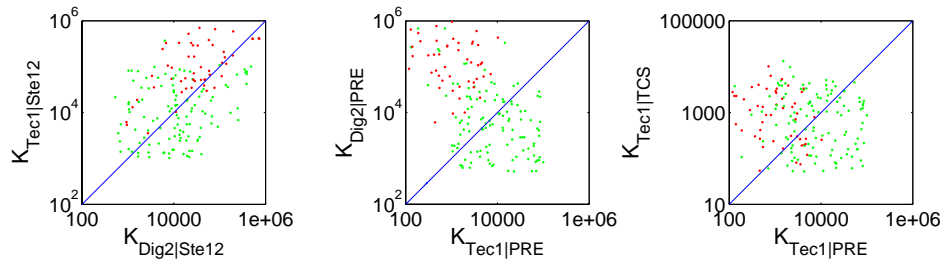


Figure 2. **Emergence of a strategy for Tec1 to reduce the PRE output.** Correlations among the five binding affinity rates; $K_{Tec1|Ste12}$ is the affinity rate between Ste12 and Tec1, and similarly for $K_{Dig2|Ste12}$, $K_{Dig2|PRE}$, $K_{Tec1|PRE}$, and $K_{Tec1|TCS}$. Each dot represents one simulation using one set of randomly chosen parameters. Red dots represent the cases in which the experiments in Figure 1C are realized; green dots are points not consistent with the experiments in Figure 1C.