Molecular Cell *24*, 853–865, December 28, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.molcel.2006.11.003

Phenotypic Consequences of Promoter-Mediated Transcriptional Noise

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Summary

A more complete understanding of the causes and effects of cell-cell variability in gene expression is needed to elucidate whether the resulting phenotypes are disadvantageous or confer some adaptive advantage. Here we show that increased variability in gene expression, affected by the sequence of the TATA box, can be beneficial after an acute change in environmental conditions. We rationally introduce mutations within the TATA region of an engineered *Saccharomyces cerevisiae GAL1* promoter and measure promoter responses that can be characterized as being either highly variable and rapid or steady and slow. We computationally illustrate how a stable transcription scaffold can result in "bursts" of gene expression, enabling rapid individual cell responses in the transient and increased cell-cell variability at steady state. We experimentally verify computational predictions that the rapid response and increased cell-cell variability enabled by TATA-containing promoters confer a clear benefit in the face of an acute environmental stress.

Introduction

The extent to which various cellular processes contribute to cell-cell heterogeneity in an isogenic population is a topic of considerable interest. Experimental studies involving expression of fluorescent reporters in single cells have linked the origin of this heterogeneity to a variety of sources including the biochemical processes of transcription and translation (Ozbudak et al., 2002; Elowitz et al., 2002; Blake et al., 2003; Raser and O'Shea, 2004; Golding et al., 2005), the remodeling of chromatin (Blake et al., 2003; Raser and O'Shea, 2004; Sato et al., 2004), the partitioning of proteins at cell division (Rosenfeld et al., 2005; Golding et al., 2005), and other, more general, factors affecting such processes across many genes within a single cell (Elowitz et al., 2002; Raser and O'Shea, 2004; Colman-Lerner et al., 2005; Volfson et al., 2006). In addition to studying these sources of stochasticity in gene expression, it is interesting to consider how the resulting variability can affect processes critical for cell proliferation within an environment. Noise in gene expression can be viewed as a detriment to cell function necessitating minimization (Becskei and Serrano, 2000; Fraser et al., 2004; Acar et al., 2005), as fluctuations in protein levels may disrupt intracellular signaling and cellular regulation. Gene expression variability may also be viewed as a beneficial trait to be exploited for cellular diversity (McAdams and Arkin, 1997; Becskei et al., 2001; Rao et al., 2002; Isaacs et al., 2003; Kramer and Fussenegger, 2005; Weinberger et al., 2005; Guido et al., 2006). It is still unclear, however, to what extent and under what circumstances cellular mechanisms are used to exploit gene expression variability to directly influence cell phenotype. It is therefore interesting to consider cases in which increased or decreased noise can be advantageous, and whether the underlying mechanisms provide an evolutionary advantage that would subject these processes to natural selection.

The rates of transition between general states of promoter occupancy and accessibility are critical in determining the magnitude of gene expression, and they have been implicated as a cause of gene expression variability (Kaern et al. [2005] provide an extensive review). Indeed, recent experimental work (Blake et al., 2003; Raser and O'Shea, 2004; Becskei et al., 2005) has suggested that variation in the rates of transition between different states of promoter activity may play an important role in determining the level of stochasticity in gene expression in *S. cerevisiae*. A variety of factors are important in mediating rates of transition between active and inactive promoter complexes, many of which are promoter specific (Cheng et al., 2002). The TATA box-binding protein (TBP) is one of the more critical of these factors, as it is highly conserved among eukaryotes and may be required for transcription of all yeast genes (Cormack and Struhl, 1992; Holstege et al., 1998; Kim and Iyer, 2004). Binding of TBP to a promoter region is an initial and often rate-limiting (Chatterjee and Struhl, 1995; Klages and Strubin, 1995; Kim and Iyer, 2004) step in transcription apparatus assembly and subsequent transcription initiation. A stable transcription scaffold, anchored by TBP, can lead to transcriptional reinitiation, a process associated with promoters that efficiently express genes at high levels (Zawel et al., 1995; Struhl, 1996). Transcriptional reinitiation occurs when a scaffold of transcription factors and cofactors containing TBP remains bound to the promoter region after RNA Pol II clearance, eliminating the need to reassemble a complete transcription apparatus de novo (Zawel et al.,

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1995; Yudkovsky et al., 2000). Successive binding of RNA Pol II leads to the production of a larger amount of mRNA in a shorter period of time (Yean and Gralla, 1999; Yudkovsky et al., 2000) and can result in pulsatile production of mRNA transcripts (Hume, 2000). There is a large body of evidence that supports this probabilistic view of transcription (Ko et al., 1990; Fiering et al., 1990; Ross et al., 1994; Walters et al., 1995).

Variation in the sequence of the highly conserved TATA box can have a direct effect on transcriptional activity (Chen and Struhl, 1988; Struhl, 1996; Hoopes et al., 1998). The TATA box sequence has been shown to be an important factor in determining the stability of a TBP-containing transcription scaffold (Stewart and Stargell, 2001) and can directly affect the extent of transcriptional reinitiation (Yean and Gralla, 1997; Yean and Gralla, 1999). Due to its importance as a conserved promoter element, the TATA box is an ideal target for modification to determine how transcription scaffold stability and promoter state transitions affect the level of cell-cell variability in gene expression (Blake et al., 2003; Raser and O'Shea, 2004). In fact, recent experimental work by Raser and O'Shea (Raser and O'Shea, 2004) demonstrated that mutations to the TATA box can affect gene expression noise. Raser and O'Shea theorized that these effects were a result of variation in the rates of promoter state transitions and only speculated on potential phenotypic consequences that may impact cell function (Raser and O'Shea, 2004). Here, we explore how variation in the binding stability of a transcription-mediating factor, TBP, modulated by rational, targeted mutation of the TATA box, can affect the level of variability in gene expression in *S. cerevisiae*. A combined experimental-computational approach is used to mechanistically describe how variation in binding stability of TBP to this defined promoter element can change both the speed and variability of promoter response within a cell population. Further, we provide experimental evidence that increased cell-cell variability can dramatically affect the ability of a cell population to rapidly respond to an acute stress, and we also present data demonstrating a phenotypic benefit to gene expression variability upon exposure to cellular stress.

These results illustrate a potentially critical role for transcription scaffold stability in mediating both speed and variability in response. Interestingly, recent studies in *S. cerevisiae* have demonstrated that genes expressed from promoters containing highly conserved TATA box sequences are associated with stress response, in contrast to the larger number of "TATA-less" promoters that function primarily in cellular "housekeeping" (Basehoar et al., 2004; Huisinga and Pugh, 2004; Zanton and Pugh, 2004; Newman et al., 2006; Tirosh et al., 2006). Our computational and experimental results support a mechanism by which TATA-containing promoters can enable a rapid individual cell response in the transient and increased cell-cell variability at steady state through the stochastic process of transcriptional bursting. We demonstrate that both the rapid response and increased cell-cell variability confer a clear benefit in the face of an environmental stress. Together, these findings indicate that noise in gene expression may be a promoter-specific, evolvable trait.

Results and Discussion

TATA Box Mutation Affects Both Transcriptional Activity and Gene Expression Variability

A previously engineered system (Blake et al., 2003) that integrates the *GAL1-10* bidirectional promoter from *S. cerevisiae* and the bacterial Tn*10 tet* repressor-operator system (Figure 1A) was used to study the effects of transcription scaffold stability on stochasticity in gene expression. Wild-type regulation of the *GAL1* and *GAL10* promoters has been characterized extensively (Johnston, 1987). Both promoters are coordinately activated through the action of the Gal4 activator protein that is involved in the recruitment of the SAGA and Mediator complexes (Bryant and Ptashne, 2003), which may remodel chromatin and lead to the binding of TBP and RNA Pol II. The *GAL1* promoter is well-suited for this study because it has a highly conserved consensus TATA box sequence (Basehoar et al., 2004) that has been shown to directly affect TBP occupancy (Selleck and Majors, 1988). Although expression from the *GAL1* promoter can be modulated by varying the amount of galactose in the growth media, integrating TetR-mediated control of transcription bypasses the complexity of native galactose regulation and any pleiotropic effects that may result from shifting carbon sources.

Details of TBP-TATA box interaction (Wobbe and Struhl, 1990; Hoopes et al., 1998; Bareket-Samish et al., 2000) were used to rationally design several variants of the wild-type *GAL1* TATA box (TA-WT) that would affect TBP binding over a broad range (Figure 1B). The effect of each TATA box mutation on transcriptional activity was determined by flow cytometric measurement of the level of yEGFP output at full transcriptional induction (Figure 1B). Several TATA box variants showed expression levels that changed little relative to wild-type (TA-low1, TA-low2), while others resulted in intermediate (TA-int1, TA-int2) and more severe (TA-sev1, TA-sev2, TA-sev3) changes in expression level relative to wild-type. These data support extensive work highlighting the importance of the TATA box in determining expression activity of some promoters (Chen and Struhl, 1988; Struhl, 1996; Hoopes et al., 1998). Dose-response curves for each strain, obtained by growth in varying concentrations of ATc, showed similar induction thresholds and Hill coefficients (Figure 2A), indicating that TATA box mutations affect expression output without greatly affecting TetR repressibility. This allows consistent expression control across all strains at the same ATc induction level, with differences in expression relating only to changes in TATA box sequence.

Cell-cell variability was determined from these data by calculating the coefficient of variation (standard deviation/mean) of fluorescence histograms obtained by flow cytometric measurement of single cells within a small forward and side light-scattering gate (Ozbudak et al., 2002; Blake et al., 2003; Isaacs et al., 2003). Figure 2B shows how the level of cell-cell heterogeneity, or noise, in yEGFP fluorescence changes as a function of expression activity for the wild-type TATA (TA-WT) strain relative to strains containing mutations in the TATA box sequence. The highest levels of cell-cell

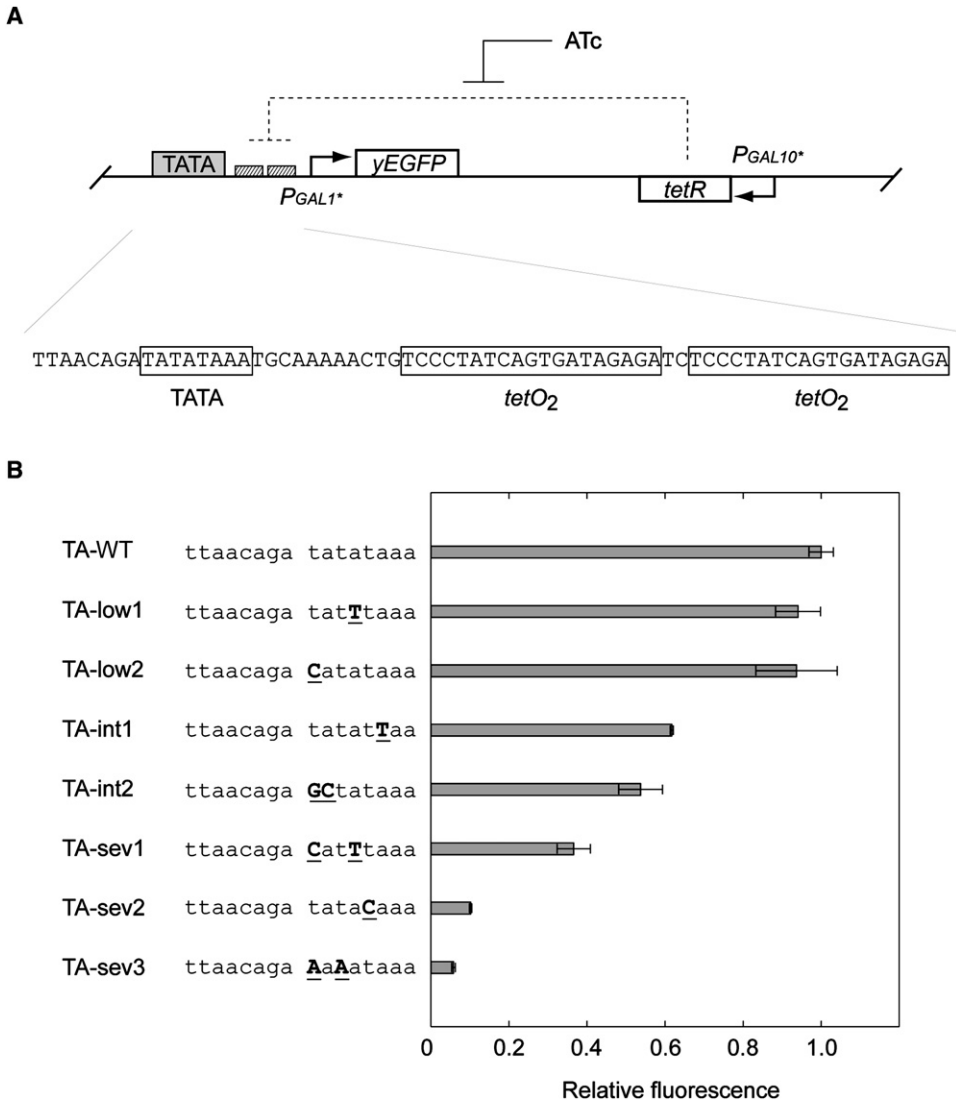

Figure 1. Engineered Expression Control and Variants of the *GAL1* TATA Box

(A) Engineered TetR control of the *GAL1* promoter (Blake et al., 2003). The Tet repressor gene (*tetR*), expressed from P_{GAL10*}, binds to tandem $tetO_2$ operators (hatched boxes) inserted downstream of the *GAL1* TATA box, repressing expression of the yeast-enhanced green fluorescent protein gene (*yEGFP*) from P_{GAL1*}. Addition of anhydrotetracycline (ATc) induces *yEGFP* expression.

(B) Expression levels of variant TATA strains are shown relative to TA-WT. Relative mean expression levels were determined at full transcriptional induction (250 ng ml^{-1} ATc, 2% galactose) by averaging yEGFP fluorescence obtained by three independent flow cytometric measurements of 30,000 cells gated based on size. Error bars are standard deviations.

variability for all strains are observed at low to intermediate transcriptional activity. However, the peak level of cell-cell variability decreases for strains with more severe TATA box mutation. Experiments were also conducted in which native promoter activation was altered by varying galactose concentration. As presented in Figure 2C, these data show the same general behavior as observed for nonnative ATc activation. Namely, promoters with mutated TATA sequences show lower levels of variability. These results are in support of a mechanism whereby an altered rate of transition between active and inactive promoter states, mediated by variation in the stability of a TBP-containing transcription scaffold, can have a dramatic effect on phenotypic variability.

Computational Model of Gene Expression Identifies Transcriptional Bursting as a Critical Noise-Mediating Factor

To better conceptualize and understand the phenomenological effect of a stable transcription complex and transcriptional reinitiation on variability in gene expression, we developed a stochastic model of gene expression from P_{GAL1*}. The model explicitly takes into account transitions between various states of P_{GAL1*} promoter occupancy prior to transcription initiation, transcript elongation, and translation. In the model, five promoter states are defined by TetR, TBP, and RNA Pol II occupancy (Figure 3A and see Figure S3 in the Supplemental Data available with this article online), and these are further classified based on TBP occupancy as either OFF

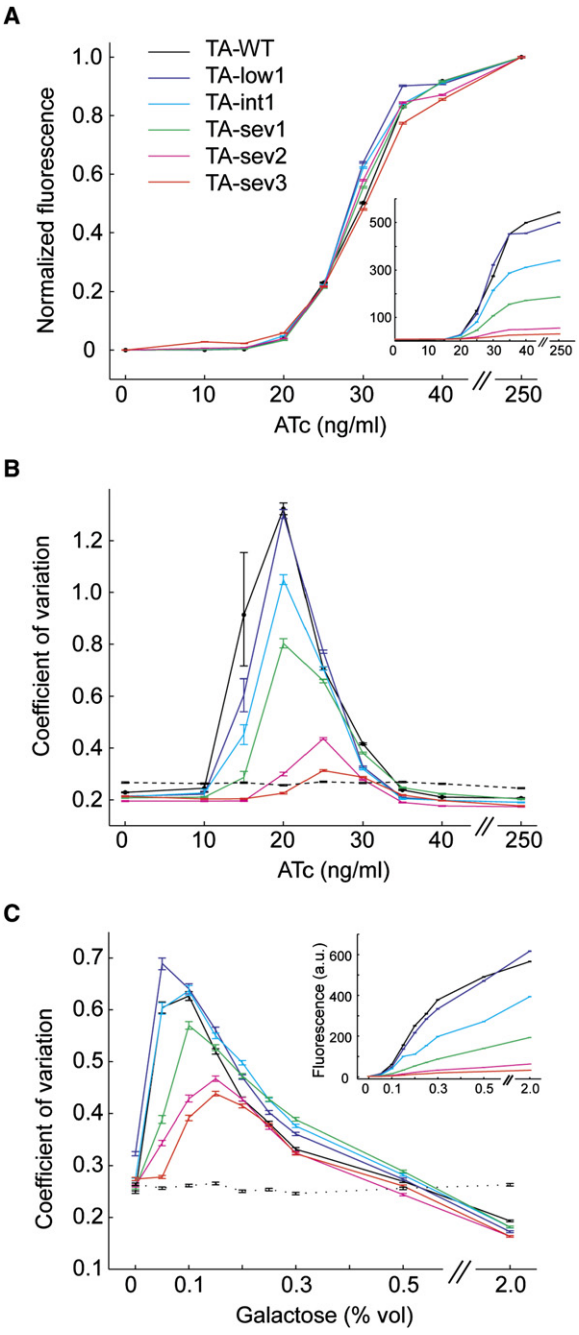

Figure 2. P_{GAL1*} Transcriptional Activity and Population Heterogeneity at Varying Levels of ATc and Galactose

(A) Normalized dose response of select P_{GAL1*} TATA variants to ATc. Data for each strain are individually normalized to conditions of no induction (0 ng/ml ATc, 2% galactose) and full induction (250 ng ml^{-1} ATc, 2% galactose). Data points represent normalized means obtained from 30,000 individual cell measurements, and error bars are 95% confidence intervals. Dose-response curve similarity among variants is reflected in nearly identical Hill coefficients (7.4 ± 0.5). (Inset) Prenormalized fluorescence data (a.u.) for each strain are plotted as a function of ATc concentration (ng ml^{-1}); error bars are 95% confidence intervals.

(B) Population heterogeneity of select P_{GAL1*} variants as a function of ATc. Data points represent noise values (standard deviation/mean) calculated from the prenormalized data in (A), and error bars are 95% confidence intervals. A strain lacking the *yEGFP* gene was also included in the analysis (dotted line) and represents the contribution from cellular autofluorescence to variability measurements.

(C) Population heterogeneity as a function of galactose concentration for strains in (A). Data points and error bars are calculated from 30,000 individual cells as in (B). (Inset) Mean fluorescence (a.u.) for each strain is plotted as a function of galactose concentration (percent galactose); error bars are 95% confidence intervals.

(TBP unbound) or ON (TBP bound). It is assumed that, in the presence of galactose, the Gal4 protein is in its active form, able to recruit transcription factors such as SAGA and Mediator to the promoter region. In addition, TetR protein bound at the promoter is assumed to prevent RNA Pol II binding, but not TBP binding. If the binding of TetR and TBP were exclusive, the model predicts that mutations to the TATA box would cause a horizontal shift of the ATc dose-response curve. This was not observed experimentally (see Figure 2A), so it was assumed that the binding of TetR and TBP was not exclusive (see also Supplemental Data, Section 4).

Mass action kinetics were used to determine the probability that P_{GAL1*} will reside in each of the various states of promoter occupancy and to reproduce the observed experimental effects of increasingly severe TATA box mutation on the ATc dose response (Figure 3B). TATA box mutations decrease TBP-DNA complex stability, and this was modeled by increasing the dissociation rate of TBP from the TATA box (Figure 3A, parameter γ_B), leaving all other parameters unchanged. Importantly, model simulations in which only the TBP-TATA association rate was changed in response to TATA box mutation showed a decrease in mean expression, but not the corresponding decrease in noise observed in Figure 2B (see Supplemental Data, Section 12). The result of these simulations, together with previous experimental work showing that mutations to the TATA box affect the rate of TBP dissociation at least an order of magnitude more than the rate of TBP association (Hoopes et al., 1998), was used to constrain our model.

To illustrate how random transitions between these states contribute to fluctuations in protein number, stochastic simulations (Gillespie, 1977) were run until the system reached steady state at various inducer concentrations (Figure 3B). At low ATc concentration, P_{GAL1*} resides with high probability in a repressed state, with TetR bound, resulting in low protein levels and low levels of noise in protein production. In contrast, at high inducer concentration, TetR is rarely bound, and transcription is frequently initiated, resulting in high protein levels and low levels of noise in protein output (Figure 3C). At intermediate levels of induction, however, the promoter is more likely to transition between an OFF (TBP-unbound) state and an ON (TBP-bound) state. A stable transcription scaffold increases the likelihood that, once in the ON state, the promoter will remain active, repeatedly recruiting RNA Pol II in the course of transcriptional reinitiation and production of new transcripts. As shown in Figure 3C, decreased TBP dissociation (low γ_B) results in higher levels of noise at the protein level, in agreement with experimental observations (Figure 2B). TATA box mutations that result in high γ_B, or a more unstable TBP-DNA complex, promote shorter bursts (eventually becoming single transcription initiation events) and lower levels of noise at the protein level (see Supplemental Data, Sections 8–10).

The effect of transcription scaffold stability on gene expression bursts and cell-cell variability is most evident in simulated single-cell time courses. Induction time courses for 100 cells were simulated following a stepwise increase in extracellular ATc concentration (Figure 3D). ATc was assumed to passively diffuse through the membrane, causing the intracellular inducer concentration to increase toward the extracellular value, as described by the equation $[ATc]_t = [ATc]_\infty [1 - \exp(-t/\theta)]$, where θ is the time constant characterizing ATc diffusion into the cell and $[ATc]_\infty$ is the fixed extracellular inducer concentration. Following an infusion-induced delay, protein is expressed at a rate determined by γ_B (dissociation rate of TBP from the TATA box), with increased values of γ_B resulting in slower rates of protein buildup (Figure 3D). Cells that are capable of forming stable transcription complexes at the promoter are characterized by abrupt changes in protein level (Figure 3F, top panel), resulting in a high degree of transient variability in response across the population (Figure 3E). Cells with decreased transcription scaffold stability, however, respond with steadily increasing protein levels (Figure 3F, bottom panel) and show substantially less variability in response (Figure 3E). The time spent in promoter state A (where all transcription factors, including RNA Pol II, are bound) is shown in Figure 3G for both cases presented in Figure 3F to further illustrate how promoter transitions affect variability in protein production. Greater transcription scaffold stability leads to greater time spent in the promoter ON state, producing greater bursts of protein (Figure 3G, top panel), in contrast to a promoter that is less able to form a stable scaffold (Figure 3G, bottom panel). These simulations indicate that both the speed of individual cell response and the variability of response across a population are linked to the ability of a responding promoter to facilitate stable binding of a transcription scaffold.

Experimental Dynamics of P_{GAL1*} Induction Response Reflect Computationally Predicted Gene Expression Bursts

The model of transcriptional induction presented in the previous section predicts that promoters on which the transcription complex is stable can facilitate "burst-like" expression of the downstream gene. This behavior is most evident in simulations of the temporal response of individual cells to an input stimulus (Figure 3F). Such behavior would cause individual cells within a genetically identical population to exhibit varying initial response times and magnitudes, resulting in a more rapid, heterogenous phenotypic response to a stimulus.

To experimentally test this model prediction, select strains with varying P_{GAL1*} TATA sequence were measured at several time points following a transcription-inducing stimulus. Two strains representing TATA box mutants with intermediate and more severe effects on expression efficiency (TA-int2 and TA-sev1) were studied together with two strains containing unaltered TATA boxes (TA-WT and codon mut). The codon mut strain harbors a promoter that is identical to the TA-WT P_{GAL1*} but contains a yEGFP gene modified by synonymous codon replacement, lowering the observed expression level to a value similar to the TA-int2 mutant. The codon mut and TA-int2 strains allow comparison of similar expression levels resulting from two distinct processes affecting expression level, namely transcription scaffold stability (TA-int2) and efficiency of translation (codon mut). Model simulation data presented in Figures 3D and 3E predict that changes in expression efficiency not related to the TATA box (e.g., codon mutations affecting rate of translation) should not greatly affect gene expression noise (compare "codon mut" and "TA-int" results in Figures 3D and 3E).

Figure 4A shows a monotonic increase in mean fluorescence as a function of time after ATc induction for all strains. Measured cell-cell variability (Figure 4B), however, increases sharply just as the populations begin to respond to the stimulus (i.e., show an increase in observed fluorescence levels), proceed to a peak level, then decrease toward the steady-state values presented in Figure 2B. Peak levels of noise are highest for strains carrying an unaltered TATA box (TA-WT and codon mut strains), while decreased heterogeneity is observed for the TA-int2 and TA-sev1 strains. Interestingly, although the TA-int2 and codon mut strains show similar response to ATc induction in terms of mean fluorescence (Figure 4A), the level of cell-cell heterogeneity in response differs remarkably (Figure 4B). The codon mut strain behaves much like the TA-WT strain in terms of response heterogeneity and shows a similar peak noise level as predicted in Figures 3D and 3E. Fluorescence histograms of individual cell measurements more clearly demonstrate this distinct behavior, with the TA-WT and codon mut strains both exhibiting a rapidly responding subset population (Figure 4C). The TA-int2 and TA-sev1 strains, by contrast, show a less-distinct subset of rapid responders, with lower levels of measured fluorescence (Figure 4C).

To more directly study transcriptional bursting in individual cell time courses, we expanded our analysis of cell behavior in response to TATA box mutation by employing a measurement technique that involves the combined use of a customized optical fiber array and fluorescence microscopy (Kuang et al., 2004). Each optical fiber in the array contains a microwell that fits a single yeast cell (well diameter $\sim 5\ \mu m$). Because each fiber array contains thousands of individual fibers, this system allows the simultaneous monitoring of fluorescence from many individual yeast cells over time. Control experiments were conducted to characterize measurement sensitivity and to determine to what extent fluctuations in fluorescence signal obtained from the optical fiber arrays are influenced by factors other than expression of yEGFP (see Supplemental Data, Section 2). Inherent fluctuations in measured fluorescence values resulting from thermal, light source, or voltage fluctuations were reduced by normalizing to the fluorescence signal obtained from interwell spaces.

Dilute cultures ($\sim 10^6$ cells ml^{-1}) of TA-WT and TA-sev1 cells were exposed to intermediate levels of ATc, then placed on separate optical fiber arrays for time course measurement. Measurements were taken every 10 min for several hours on approximately 100–500 individual cells maintained at 30°C in media containing 45 ng ml^{-1} ATc. Striking differences in behavior were observed between the TA-WT and TA-sev1 cells, with the TA-WT cells exhibiting sharp increases in fluorescence

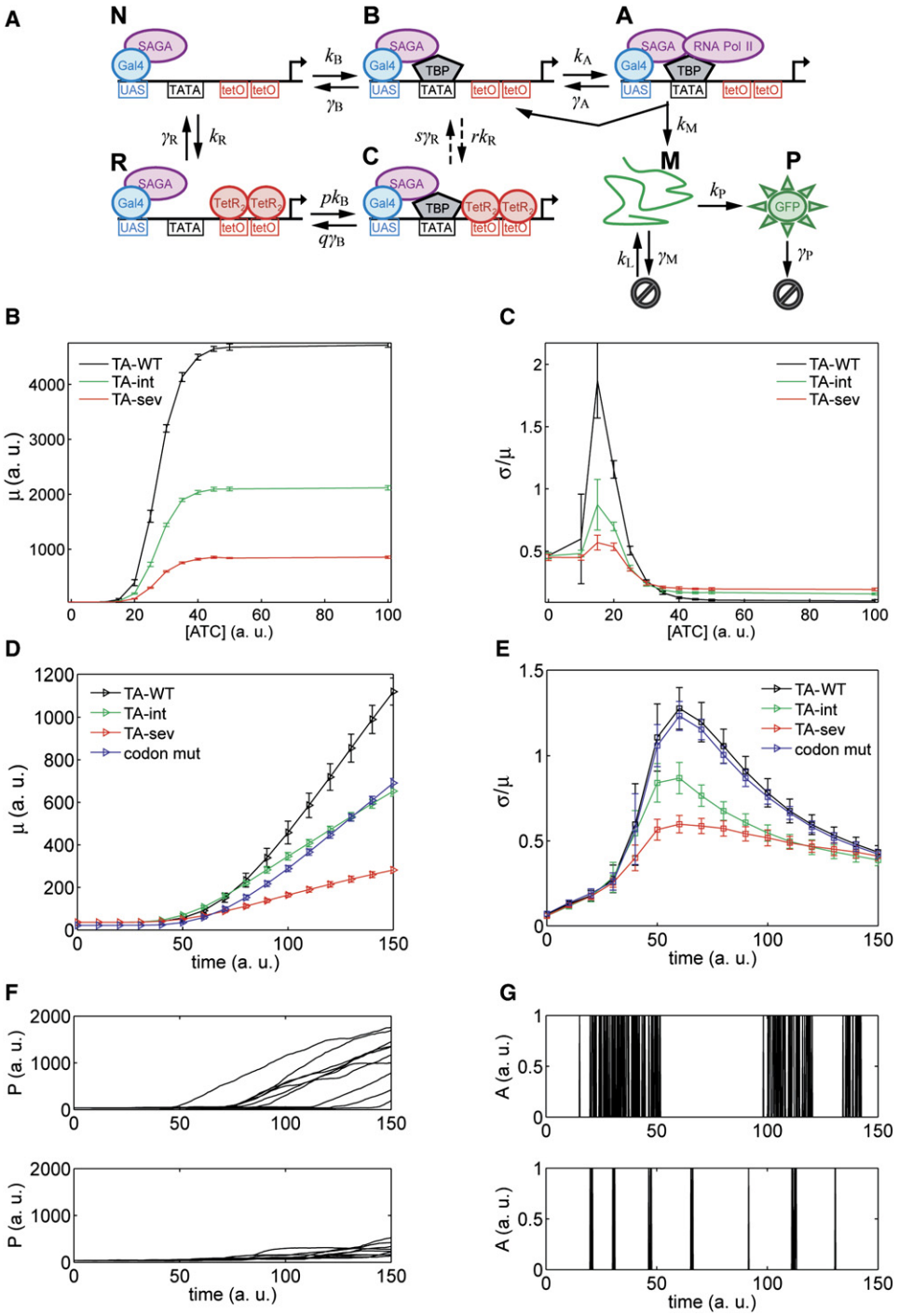

Figure 3. Modeling the Effects of Transcription Scaffold Stability on Gene Expression Variability

(A) The five promoter states (C, R, N, B, and A) correspond to binding of TetR, TBP, and RNA Pol II to promoter DNA in the following combinations: both TBP and TetR bound ("Combined" state C), only TetR bound ("Repressed" state R), only TBP bound ("Bound" state B), neither TBP nor TetR bound ("Neutral" state N), and TBP and RNA Pol II bound ("Active" state A). States C, R, and N (TBP not bound) represent inactive promoter states, while states B and A (TBP bound) represent active promoter states. The transition rate from B to N (and C to R) depends on TBP-DNA stability, while the transition rate from state N to R depends on the inducer concentration (see Supplemental Data, Section 4 and Figure S3). mRNA molecules (M) are created from state A with rate k_M and degraded with rate γ_M. mRNA molecules are translated into protein (P) at rate k_P, and protein molecules are degraded at rate γ_P. mRNA is also created at rate k_L as a result of promoter leakage (see Supplemental Data, Section 11).

(B) Simulated dose-response curves for wild-type (TA-WT), intermediate (TA-int), and severe (TA-sev) TATA box variants. The mean protein copy number P was estimated from a single-cell time course (20,000 time units, 5,000 sample points) after the system reached steady state. Error bars correspond to the sampling error (standard deviation) calculated from ten simulations (see Supplemental Data, Section 5). A single parameter, γ_B, was varied to mimic the destabilizing effect of TATA box mutations (see Supplemental Data, Section 5).

(C) Steady-state noise levels as a function of inducer concentration for genotypes in (B). The coefficient of variation of protein copy number P was estimated from a long single-cell time course, as described for (B). Error bars correspond to the sampling error (standard deviation) calculated from ten simulations.

intensity over short periods of time, separated by variable periods of low activity (Figure 4D). In contrast, the TA-sev1 cells showed steady increases in fluorescence with few such bursts of activity (Figure 4E). These results demonstrate that both the frequency and magnitude of gene expression bursts can be modulated by TATA box sequence, reflecting the importance of transcription scaffold stability in determining gene expression variability as predicted by model simulations (Figures 3F and 3G).

Experiments both at the population (Figures 4B and 4C) and single-cell (Figures 4D and 4E) levels show that genes can exhibit distinct responses based on the sequence of their TATA boxes, and these responses can be categorized as either variable and rapid (wild-type TATA, TA-WT) or steady and slow (mutant TATA, TA-sev1). These results further validate the computational model describing a mechanism whereby stable transcription scaffolds enable bursts of gene expression (Figures 3F and 4D), leading to a subset of rapidly responding cells with elevated levels of protein (Figure 4C).

Bursts of Promoter Activity Enable a Rapid Response that Confers a Phenotypic Benefit upon Exposure to Cellular Stress

Our data show that promoters can have distinct responses, both in terms of speed and variability, based on TATA box sequence. Such differences in response can have dramatic effects on the ability of cells to adapt to a changing environment. Rapid response may be beneficial, for example, after an acute environmental change that requires a shift to an alternate metabolic pathway or necessitates the production of stress-mediating factors. Recent theoretical work (Thattai and van Oudenaarden, 2004; Wolf et al., 2005; Kussell and Leibler, 2005) has also shown that heterogeneous, or noisy, population-scale responses to stimuli may be beneficial under fluctuating environmental conditions.

To study the implications of different response types on cell behavior, we used the TA-WT and TA-sev1 versions of the engineered P_{GAL1*} promoter. Each of these promoters exhibits distinct expression behavior both in terms of variability and speed of individual cell response (Figure 4). We chose to monitor the ability of cells harboring these promoter variants to survive and propagate after exposure to an antibiotic that causes rapid cell death. We designed a system in which the action of the antibiotic is attenuated by addition of ATc, which causes induction of an antibiotic-resistance gene from either the TA-WT or TA-sev1 version of P_{GAL1*}. By controlling both the introduction of the antibiotic and the initiation of response, we can study how promoter response and the effects of transcriptional bursting correlate with cell growth and survival after an acute change in environmental conditions.

In strains containing the TA-WT and TA-sev1 versions of P_{GAL1*}, the *yEGFP* reporter gene was replaced with the *She ble* gene (ZeoR), conferring resistance to the antibiotic Zeocin (Invitrogen). Zeocin is a small peptide antibiotic of the bleomycin family that causes rapid cell death by binding and degrading cellular DNA (Berdy, 1980). Control experiments demonstrated that Zeocin concentrations greater than 1 mg ml^{-1} completely inhibited any detectable culture growth in the absence of ATc (no induction of ZeoR). Additionally, a substantial decrease in colony-forming units was detected 30 min after addition of 2 mg ml^{-1} Zeocin (see Supplemental Data, Section 3). The rapid antibiotic activity exhibited by the Zeocin toxin allows for a meaningful evaluation of the potential stress-response benefits of transcriptional bursting.

Growth of ZeoR-expressing TA-WT and TA-sev1 strains under select conditions of ATc induction and Zeocin exposure are shown in Figures 5A and 5B, respectively (see also Figures S14 and S15). TA-WT and TA-sev1 cultures that have been preinduced to steady-state levels of ZeoR production at 100 ng ml^{-1} ATc prior to exposure to 1.5 mg ml^{-1} Zeocin exhibit growth similar to that of cells grown in the absence of Zeocin (Figures 5A and 5B). Therefore, preconditioning cells to the Zeocin stress enables similar levels of cell growth despite differing levels of steady-state protein production from the TA-WT and TA-sev1 promoters (Figure 1B). However, when preconditioning is eliminated, and cells are exposed to ATc and Zeocin simultaneously, there is a significant difference in growth between the TA-WT and TA-sev1 strains. TA-WT cells are able to survive the challenge and propagate (Figure 5A), presumably due to a rapidly responding subset of cells, while the TA-sev1 cells show no detectable growth (Figure 5B). These data indicate that promoters enabling rapid individual cell response (e.g., through transcriptional bursting) can confer a fitness benefit in cases in which the expressed gene is necessary to combat cellular stress. These experimental results, combined with stochastic simulations showing the effect of TBP-TATA stability on gene expression bursts, highlight a potential feature of stress response genes. Namely, the TATA box promoter element may function to enable rapid response from a subset of a cellular population in the face of acute stress.

Population Heterogeneity Confers a Phenotypic Benefit upon Exposure to Cellular Stress

The results of the previous section demonstrate that the TATA box promoter element enables a rapid response that confers a distinct advantage for cells responding

(D) Simulated induction time course. An extracellular ATc concentration of 50 arbitrary units was applied. During the simulated time course, the intracellular inducer concentration $n(t)$ was updated every ten time steps in each of 100 model cells according to the diffusion equation. Variation of promoter states, mRNA, and protein copy number were modeled by stochastic simulation (see Supplemental Data, Section 6). The parameters for the TA-WT, TA-int, and TA-sev time courses are the same as in (B). The colors and legend indicate the same genotypes as in (B), with the addition of a codon mutant, codon mut, characterized by a reduced rate of translation.

(E) Simulated noise time course for genotypes in (D). The coefficients of variation in protein copy number P were calculated over a population of 100 cells, as described in (D). Error bars correspond to the sampling error (standard deviation) calculated from ten simulations.

(F) Simulated induction time course of protein copy number P in ten individual TA-WT (top) and TA-int (bottom) cells.

(G) Simulated steady-state time course of promoter state A in ten individual TA-WT (top) and TA-int (bottom) cells.

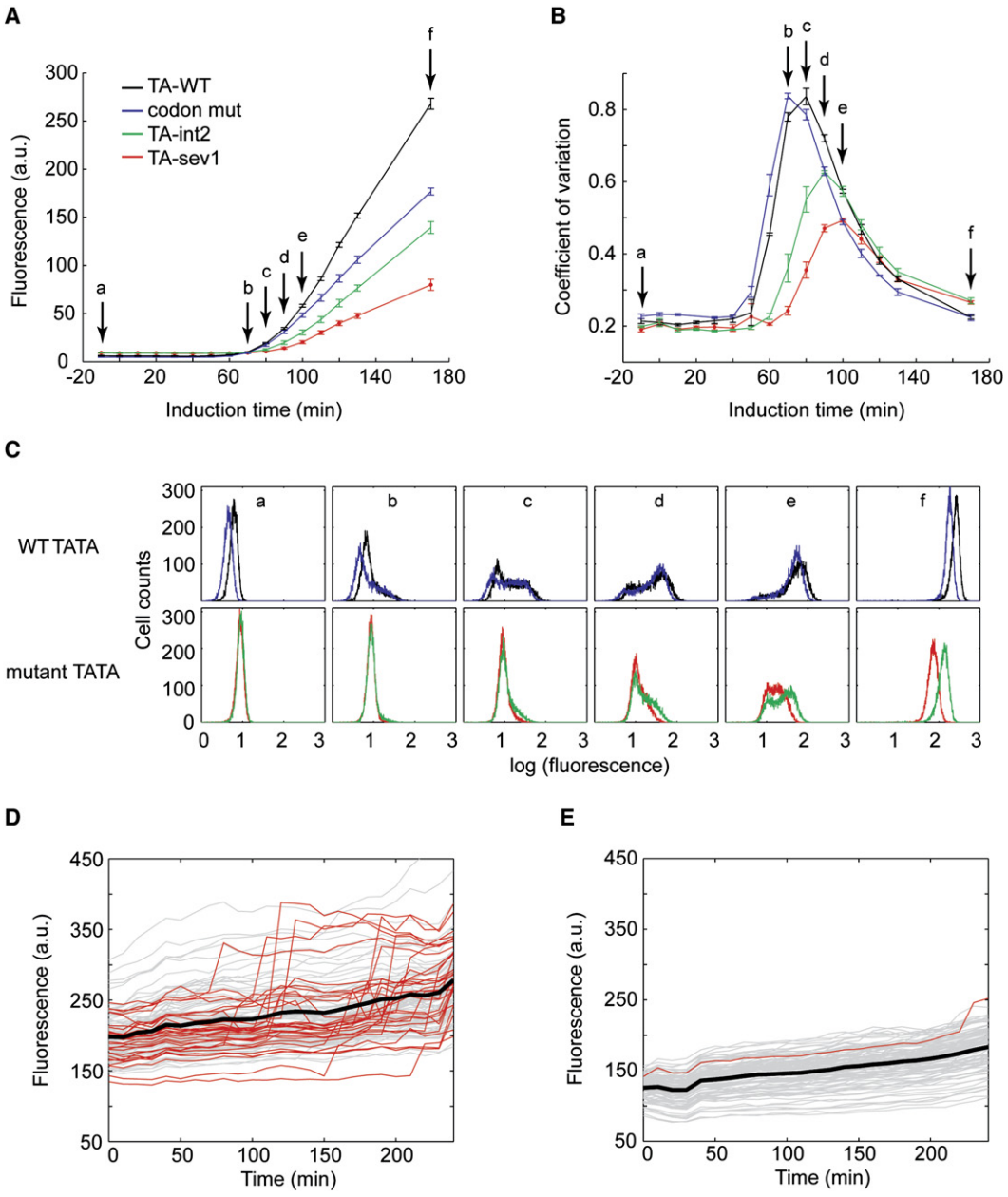

Figure 4. P_{GAL1^*} Induction and Heterogeneity of Response

(A) Change in fluorescence as a function of ATc induction time. Each data point represents the mean of three independent measurements of 15,000 gated cells, and error bars are standard deviations.

(B) Change in population heterogeneity as a function of ATc induction time for cultures in (A). TA-WT and codon mut strains contain identical promoter regions and show near-identical levels of population heterogeneity in response to the ATc stimulus. TA-int2 and TA-sev1 strains contain mutated TATA boxes and show lower levels of population heterogeneity in response to the ATc stimulus. At 170 min postinduction, all strains approach their steady-state levels of population heterogeneity shown in Figure 2B. Each data point represents the mean of three independent measurements of 15,000 gated cells, and error bars are standard deviations.

(C) Fluorescence histograms for select points in (A) and (B).

(D) Individual TA-WT cell fluorescence as a function of induction time measured by optical fiber arrays (Kuang et al., 2004). Each gray trace represents fluorescence measurements obtained every 10 min from an individual cell, and black traces are the average of all data points at a given measurement time. Red traces highlight cells that exhibit changes in fluorescence greater than ten times the average change in fluorescence from one time point to the next.

(E) Optical fiber measurements for the TA-sev1 strain. Data were obtained and are plotted as described in (D).

to stress. Although the observation that rapid response is a beneficial feature of a stress-response gene is unsurprising, it is interesting to consider whether the accompanying increase in variability observed from TATA-containing promoters may also confer a benefit. A combination of the unique features of the experimental system under investigation and a predictive computational model allowed investigation of whether heterogeneity in

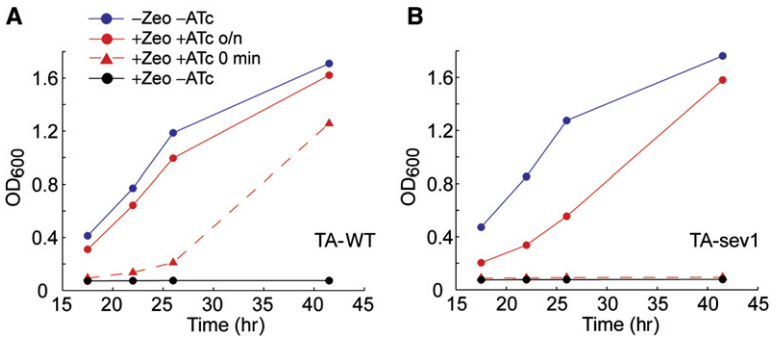

Figure 5. Effect of Promoter Response Time on Culture Growth after Exposure to Zeocin Antibiotic

(A) ZeoR-expressing TA-WT culture growth in varying conditions of Zeocin antibiotic and ATc induction of ZeoR. Cultures grown for 18 hr in the presence (o/n) or absence of ATc were diluted 1:100 into microplate wells containing media with (1) no Zeocin and no ATc, (2) 1.5 mg ml^{-1} Zeocin and no ATc, or (3) 1.5 mg ml Zeocin and 100 ng ml^{-1} ATc, and OD_{600} was measured over a period of approximately 40 hr. Cells preinduced with ATc (o/n) and exposed to condition 3 (solid red curve) showed an increase in OD_{600} in a manner similar to cells grown in the absence of ATc and Zeocin (condition 1, blue curve). Cells grown in the absence of ATc that were simultaneously exposed to ATc and Zeocin (condition 3, dashed red curve) were able to propagate, while those exposed only to Zeocin (condition 2, black curve) did not show any increase in OD_{600}.

(B) ZeoR-expressing TA-sev1 strain growth in conditions as described in (A). No increase in OD_{600} is detected after cells grown in the absence of ATc were simultaneously exposed to 100 ng ml^{-1} ATc and 1.5 mg ml^{-1} Zeocin (dashed red curve).

the expression of a stress response gene affects population survival upon exposure to cellular stress.

The stochastic model was used to identify conditions in which TA-WT and TA-sev exhibited similar levels of gene expression but differing levels of cell-cell variability, thereby allowing meaningful study of the effects of such heterogeneity on population viability. By computationally varying ATc concentrations while calculating both mean and coefficient of variation (CV) for TA-WT (γ_B = 0.05) and TA-sev (γ_B = 1.0), ATc concentrations of 20 and 25 ng ml^{-1} were identified as appropriate concentrations for TA-WT and TA-sev1 induction levels, respectively (Figure 6A). Experimental data (Figure 6B) confirm that at these steady-state induction levels, TA-WT and TA-sev1 strains exhibit similar mean levels of expression (TA-WT mean = 101 a.u. and TA-sev1 mean = 100 a.u.) but different levels of cell-cell variability (TA-WT CV = 1.2 and TA-sev1 CV = 0.6).

Model simulations predict that, although these two populations have similar mean expression levels, their ability to survive when the expressed protein is necessary for response to a stress agent should differ. Cell death was modeled simply by assuming that individual cells perish unless a stress-response protein is expressed above a threshold level that is proportional to the concentration of the stress agent. Lower concentrations of stress agent correspond to lower thresholds required for survival and, therefore, a greater percent viability across the population. For the sake of simplicity, our model included only two phenotypic outcomes: survival and death. The effect of stress was to divide the cell population into two subpopulations corresponding to these phenotypes based on their protein expression levels. By using simulated distributions as presented in Figure 6A, the model was used to predict cell survival for TA-WT and TA-sev populations as a function of stress level (Figure 6C). Interestingly, greater levels of cell-cell heterogeneity are predicted to be disadvantageous at low levels of stress. The converse is true, however, at high stress levels in which increased cell-cell variability results in a clear fitness benefit.

To test this prediction, TA-WT and TA-sev1 strains were induced to steady-state levels of ZeoR expression with 20 and 25 ng ml^{-1} ATc, respectively, where yEGFP expression data indicate that mean protein output is similar but expression variability differs. These strains were then exposed to varying levels of Zeocin, and growth measurements were taken at varying intervals. After 28 hr growth, the experimental observations correlate remarkably well with our model predictions, showing increased viability for the population with greater cell-cell variability at high levels of Zeocin antibiotic (Figure 6D). In addition, experimental results show that increased cell-cell variability can be less advantageous at low levels of stress, as predicted by the model. This result likely reflects the fact that a population with a high level of cell-cell variability (e.g., TA-WT) will have a greater number of cells below the protein production threshold necessary for survival than a population with a low level of cell-cell variability (e.g., TA-sev1). However, as stress levels increase and the threshold shifts, the TA-WT cell population will have a greater number of cells above the threshold than the TA-sev1 population, resulting in a survival advantage (Figures 6C and 6D).

These results demonstrate that a particular genetic component, the TATA box, can dramatically influence the level of noise in gene expression and that there is a distinct phenotypic benefit to the resulting increased cell-cell variability of TATA-containing promoters. These data support the claim that noise in gene expression can be beneficial when the expressed gene is required for stress response. Two populations responding with similar mean expression levels exhibit drastically different stress responses based on differing levels of cell-cell variability. These computational and experimental results describe a mechanism whereby TATA-containing promoters can enable rapid individual cell responses in the transient and increased cell-cell variability at steady state through the stochastic process of transcriptional bursting. Both the rapid response and increased cell-cell variability confer a clear benefit in the face of an environmental stress.

Recent global analyses of the significance of TATA box sequence in *S. cerevisiae* genes revealed that TATA-containing genes are associated with inducible responses to stress-related factors, in contrast to TATA-less genes, which are primarily associated with constitutive housekeeping function (Basehoar et al., 2004; Huisinga and Pugh, 2004; Zanton and Pugh, 2004; Newman et al., 2006; Tirosh et al., 2006). These

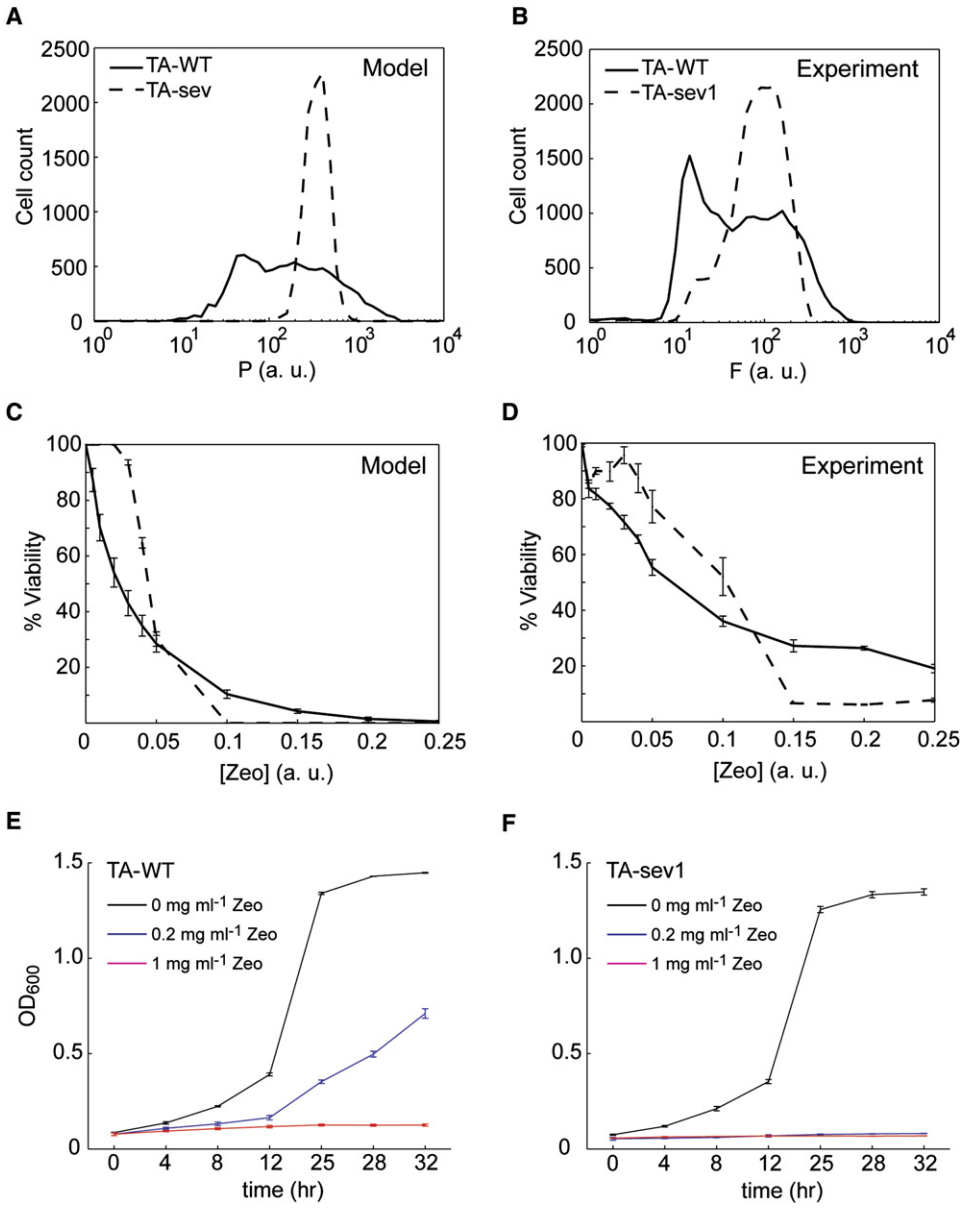

Figure 6. Phenotypic Benefit of Cell-Cell Heterogeneity upon Exposure to Zeocin Antibiotic

(A) Simulated distributions of steady-state protein expression from TA-WT induced with 20 ng ml^{-1} ATc (mean = 315.2, CV = 404.6/315.2 = 1.3) and TA-sev induced with 25 ng ml^{-1} ATc (mean = 367.8, CV = 109.0/367.8 = 0.3).

(B) Fluorescence histograms obtained by flow cytometric measurement of 30,000 individual cells from TA-WT and TA-sev1 populations expressing steady-state levels of yEGFP at 20 and 25 ng ml^{-1} ATc induction, respectively. TA-WT (mean = 101.9, CV = 125.4/101.9 = 1.2) and TA-sev1 (mean = 100.4, CV = 61.8/100.4 = 0.6) populations have approximately the same mean expression levels but different coefficients of variation.

(C) Simulated kill curves. Viability was calculated as the number of cells that survive in the presence of a given Zeocin concentration, normalized by the total number of cells (N_T = 10,000). Cell survival was modeled assuming that cells perish unless a stress-response protein is expressed above a threshold that is proportional to the concentration of the stress agent. The proportionality constant, 10^4, was chosen based on the simulated protein expression histograms such that all cells perish for a Zeocin concentration of 0.25 mg ml^{-1}. Data points represent means from five independent simulations, and error bars are standard deviations.

(D) Experimental kill curves. Viability was determined by normalizing OD_{600} measurement for cultures grown in the presence of Zeocin to an identical dilution grown the absence of Zeocin. Data points are the means from three independent cultures at 28 hr growth in the presence of indicated amounts of Zeocin, and error bars are standard deviations.

(E and F) Experimental growth time course at selected Zeocin concentrations for TA-WT and TA-sev1 strains, respectively. Data points are means of three independent cultures, and error bars are standard deviations.

differences may reflect distinct modes of transcriptional regulation that predominate for TATA-containing and TATA-less genes (Kuras et al., 2000; Huisinga and Pugh, 2004). Such studies have identified a significant difference in both regulation and response between genes involved in environmental and stress response and those genes that are not associated with stress response. Our findings provide mechanistic insight into these biological observations. We computationally and experimentally show that a promoter containing a

consensus TATA sequence is more likely to enable gene expression bursts, through increased transcription scaffold stability, directly influencing the level of noise in gene expression. Our results demonstrate that this type of response can be beneficial when encountering cellular stress and highlight a key phenotypic consequence of gene expression noise.

Experimental Procedures

Strains and Growth

The *S. cerevisiae* YPH500 strain (α, *ura3-52*, *lys2-801*, *ade2-101*, *trp1*Δ63, *his3*Δ200, *leu2*Δ 1, obtained from Stratagene) was used as the host for all plasmid integrations. Yeast strains created in this study were constructed by targeted integration of plasmids containing the *TRP1* selectable marker to the *GAL1-10* promoter region of chromosome II. Plasmids were linearized within the *GAL1-10* promoter region by AgeI digestion, and 0.5–2 μg was used for transformation of yeast cells following a modified lithium acetate procedure (Stearns et al., 1990). Targeted integrations were confirmed by PCR screening as described (Chen et al., 1995).

Cultures were grown in synthetic dropout (SD) media (6.7 g L^{-1} yeast nitrogen base without amino acids, 1.92 g L^{-1} dropout supplement without tryptophan, and 76 mg L^{-1} adenine, all from Sigma) with 2% galactose. In experiments in which galactose concentration varied, cells were grown in 2% raffinose. Anhydrotetracycline (ACROS Organics) was added to the growth medium at concentrations ranging from 10 to 250 ng ml^{-1}, and Zeocin (0.1 g ml^{-1}, Invitrogen) was added at concentrations ranging from 0.5 to 2 mg ml^{-1} as indicated in the text.

The *E. coli* XL10-Gold strain (Stratagene) was used as a host for all plasmid construction. Competent bacterial cells were made and transformed using a modified one-step preparation (Ausubel et al., 1987).

Plasmid Construction and Promoter Mutation

pRS4D1 (Blake et al., 2003) was used as a template for all *GAL1* TATA box mutations. Engineered TetR-mediated repression of the *GAL1* promoter has been described previously (Blake et al., 2003). For ZeoR experiments, the *Sh ble* gene was PCR amplified from pcDNA4/TO (Invitrogen) and subcloned into the BamHI and XhoI sites of pRS4D1 (and variant plasmids carrying P_{GAL1^*} TATA mutations), replacing the *yEGFP* gene.

All TATA box mutations were introduced by PCR. Custom primers from Integrated DNA Technologies (Coralville, Iowa) were used to introduce point mutations within the *GAL1* TATA box as described in Supplemental Data, Section 1. Either Pfu*Turbo* polymerase (Stratagene) or the Expand Long Template Kit (Roche) was used with a PTC-100 Programmable Thermal Controller (MJ Research) for all PCR amplification. Restriction endonucleases and T4 DNA ligase were from New England Biolabs (Beverly, Massachusetts). All mutations were sequence-verified with 2× coverage (Agencourt, Beverly, Massachusetts).

yEGFP Expression Experiments

Individual colonies were used to inoculate SD galactose media, and cultures were grown at 30°C and 300 rpm for 15–20 hr. For steady-state experiments, cultures were grown for 15–18 hr in the presence of 10–250 ng ml^{-1} ATc. Cultures were then diluted to OD_{600} 0.1 in media containing the appropriate ATc concentration and grown for an additional 4–6 hr prior to flow cytometric measurement. Induction time course experiments were conducted similarly, except that cells were initially grown in the absence of ATc. After dilution to OD_{600} 0.1, cultures were grown for 3–4 hr prior to induction with 45 ng ml^{-1} ATc. Periodic measurements were taken as indicated in the text. All OD_{600} measurements were obtained with a Tecan SpectraFluor Plus instrument in absorbance mode.

ZeoR Expression Experiments

Individual colonies of ZeoR-expressing strains were used to inoculate SD galactose media, and cultures were grown as described above. Cells were either preinduced with ATc or grown in the absence of ATc as indicated in the text. After dilution to OD_{600} 0.2, specified cultures were preinduced 30–120 min prior to Zeocin exposure with 55–100 ng ml^{-1} ATc. All cultures were then diluted 1:100 in microplates (Costar) containing prewarmed media (30°C) with varying combinations of ATc and Zeocin as indicated in the text. Microplates were placed in a static 30°C incubator. For growth assay, OD_{600} readings were taken as indicated.

Flow Cytometry and Data Analysis

All flow cytometry data were obtained using a Becton-Dickinson FACSCalibur instrument with a 15 mW 488 nm argon-ion laser and a 515–545 nm emission filter (FL1-H). In preparation for flow cytometric analysis, cells were pelleted and resuspended in 0.22 μm filtered PBS (GIBCO, pH 7.1). Twenty thousand to eighty thousand cells (events) were collected within a small forward and side light scatter gate. Flow cytometry data were analyzed using Matlab (The MathWorks, Inc.).

Optical Fiber Array Measurement and Analysis

Optical fiber array design and fabrication are described elsewhere (Kuang et al., 2004).

Cultures were initiated from single colonies as described above. After 15–20 hr growth, cultures were diluted to OD_{600} 0.15 in SD galactose media and grown for an additional 3–4 hr prior to induction with 45 ng ml^{-1} ATc. After 45 min, cultures were diluted 1:4 to $\sim 4 \times 10^6$ cells ml^{-1} in media containing 45 ng ml^{-1} ATc prewarmed to 30°C. A 10 μl aliquot was loaded on the array, and the apparatus was centrifuged twice at 4000 rpm for 2.5 min each, rotating the fiber between each centrifugation. The array was then placed in a 30°C chamber mounted on an Olympus inverted microscope (model IX81, Olympus America Inc., Melville, New York), and fluorescent images were acquired every 10 min from the proximal end of the fiber using an Hamamatsu CCD camera (model Orca-ER, Hamamatsu, Japan) as previously described (Kuang et al., 2004). Raw fluorescence values were normalized by subtracting the average fluorescence obtained from interwell spaces at each time point.

Model and Simulations

Mass action kinetics and stochastic simulations were used to model gene expression from the engineered P_{GAL1^*}. Parameters used in the calculations were obtained from the literature, where available, and otherwise were estimated based on previous work (Blake et al., 2003); parameter values are provided in the Supplemental Data. The model was implemented using the chemical kinetics simulation software Dizzy (Ramsey et al., 2005). The mean and noise steady-state values were calculated over time from a single cell time course. A complete description of the model and stochastic simulations are presented in the Supplemental Data. Data and software are available at http://www.bu.edu/abl/data.html.

Supplemental Data

Supplemental Data include 13 sections composed of text, 15 figures, and two tables and can be found with this article online at http://www.molecule.org/cgi/content/full/24/6/853/DC1/.

Acknowledgments

This work was supported by the National Institutes of Health and The National Science Foundation.

Received: February 11, 2006
Revised: August 7, 2006
Accepted: November 1, 2006
Published: December 28, 2006

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