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Controlling gene expression timing through gene regulatory architecture

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Abstract

Gene networks typically involve the regulatory control of multiple genes with related function. This connectivity enables correlated control of the levels and timing of gene expression. Here we study how the timing of gene expression in networks can be encoded in the regulatory DNA of a gene. Using stochastic simulations, we examine the role of binding affinity, TF regulatory function and network size in controlling the mean first-passage time to reach a fixed fraction of steady-state expression for both an auto-regulated TF gene and a target gene. We also examine how the variability in first-passage time depends on these factors. We find that both network size and binding affinity can dramatically speed up or slow down the response time of network genes, in some cases predicting more than a 100-fold change compared to constitutive expression. Furthermore, these factors can also significantly impact the fidelity of this response. Importantly, these effects do not occur at "extremes" of network size or binding affinity, but rather in an intermediate window of either quantity.

Keywords: transcriptional regulation, resource competition, network motifs, auto-regulation, gene expression

Introduction

Transcription factors (TFs) play an essential role in controlling how a cell will respond to a stimulus through the regulation of gene expression (1; 2; 3). The gene regulatory code, inscribed in the DNA of each gene, specifies how the production of each gene product will be controlled in space, time and magnitude. A tremendous effort has focused on which regulatory proteins (transcription factors or TFs) regulate each gene, where they bind to accomplish their regulation and the mechanism by which the TF functions (4; 5; 6; 7; 8; 9). While this work has advanced our ability to read the regulatory code, an important consideration is how the timing of gene expression may also be encoded in the DNA. Although temporal patterns in expression can be arranged through a variety of mechanisms such as signalling (10; 11) or compartmentalization (12; 13; 14), here we will focus on timing encoded through transcriptional regulation. TFs are often part of a "single-input module" network motif where one auto-regulatory TF gene controls many genes

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(15; 16); typically these target genes are consecutive enzymes along a single physiological pathway and their temporal order in expression is crucial to ensure efficient response (17; 18; 19; 20). However, despite the prevalence of this regulatory motif in natural genetic circuits and the importance for efficient transcriptional programs, we can not predict or design temporal gene expression patterns from this motif based on the regulatory sequence of the regulated genes.

The natural timescales to reach steady-state of gene regulatory systems is set by the decay rate of the protein product (21). In bacteria such as *E. coli*, where most proteins are stable, the timescale is set by the division rate of the cell. Regardless of organism, natural regulatory systems seem to operate at a wide range of timescales sometimes requiring precise tuning (22; 17). The timing, and variability in timing, of cellular events such as metabolism and DNA damage response in *E. coli* (17; 18), meiosis in *S cerevisiae* (23), sporulation in *B. subtilis* (24) and apoptosis in human cell lines (25) have been identified as phenomena whose temporal dynamics are driven by transcriptional regulation. The network architecture will play a role in setting these timescales and the accompanied noise (26). It is known that auto-regulation can slow down (in the case of activation) or speed up (repression) the response time of an autoregulated gene (27; 21; 28). Furthermore, target genes in the network are impacted by these dynamics, each target gene in a network may have different regulatory DNA which may include differing number of TF binding sites, affinity for the TF or interactions with other TFs at those sites. Some studies have pointed to differential TF binding affinity at each target gene within a regulatory network as a driving force in setting timescales of expression (17; 18; 29; 30; 31; 32; 33; 34). However, other factors such as the size of the network (number and affinity of additional binding sites) likely play a significant role; TFs can target dozens to hundreds of specific sites in the genome and the presence of competitive sites can have a significant impact on the steady-state level of expression and noise of the genes in the network (35; 36; 37; 38; 39; 40; 41). Exploring the role regulatory architecture and competing binding sites play in tuning not only the average response time but also the precision of that response is an open question.

Here, we use stochastic simulations to characterize how a multitude of factors control the timing of gene expression (42). The primary focus of this work is auto-regulated TF genes and the target genes they regulate. The majority of TFs in *E. coli* are auto-regulated, and as such it is crucial to study this motif and its role in regulating the dynamics of gene expression of both itself and the other genes under its control within the network (4; 5). We start by examining auto-regulated genes alone and explore how details of the regulation such as binding affinity, TF regulatory role (activation or repression) and network size (number of TF binding sites) influence the mean first-passage time (MFPT) to reach 50% of the steady state expression level. Importantly, the MFPT is a single-cell measurement of response time; as opposed to measuring how long the bulk takes to reach a certain expression level on average. MFPT has been widely used to study biological processes(27; 43; 44; 45; 46). Furthermore, we also examine the robustness of the response; *i.e.* the variability in first-passage time at the single cell level; auto-regulation is known to impact the stability and noise of expression (47; 48), here we explore how these elements influence the variability of response of a gene within the network.

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Results

Model

The general model of gene expression for a self regulatory gene (TF-gene) is outlined in Fig. 1A. In this model, the TF-gene produces TFs that can bind to the promoter of the TF-gene with probability set by the binding-rate (k_{on}). The TF binding affinity is controlled through the unbinding rate (k_{off}), which is related to the sequence specificity of the binding site; higher affinity means lower unbinding rate and vice versa (49). The regulatory function of the TF is represented as a change to transcription rate when the TF is bound; the TF-gene transcribes at rate r when bound by a TF and r_0, when unoccupied. The ratio of the transcribing rates ($\alpha = r/r_0$) for the TF-gene determines whether the gene is self-activating ($\alpha > 1$), self-repressing ($\alpha < 1$) or constitutive ($\alpha = 1$). In later sections, we add the possibility of decoy TF binding sites to the model. Outlined in Fig. 1B, decoy TF binding sites bind a TF (thus making it unavailable to bind to the TF gene) but do not result in regulation (or do not regulate a gene whose product we consider). Decoy sites have the same TF binding rate as the TF gene but can have a distinct unbinding rate, $k_{\text{off,d}}$. For the sake of simplicity we consider N identical decoy sites, though in a real network these sites could have a spectrum of affinities. Finally, as outlined in Fig. 1C, we will explore the dynamics of a target gene. As is the case for decoys, the TF binding site for this gene binds TFs with the same rate as every other binding site and has a distinct unbinding rate, $k_{\text{off,t}}$. We also consider that the regulatory role of the TF may be different on this target gene and thus we define α_t as the ratio of TF-bound (r_t) over TF-unbound transcription rate ($r_{0,t}$) of this gene.

To explore the dynamics of this system, we employ a stochastic simulation algorithm to compute mean first passage time (MFPT) for gene expression to reach half of the steady state expression level (43; 50). The choice of threshold, 50% of the steady state expression, is arbitrary, but consistent with other work in the field (28; 51; 52), and the consequence of this choice is also discussed at points below. As demonstrated schematically in Fig. 1D, at time $t = 0$ the network is switched on and the genes start to express until the threshold is achieved. The time to reach the threshold is termed as "first passage time" (FPT) and is computed for each of $> 10^5$ individual trajectories (cells). The MFPT is obtained by taking the mean of the FPT distribution. We also calculate the noise in the timing of response represented by the CV which is the ratio of the standard deviation to the mean of the FPT distribution.

Role of TF-binding affinities in controlling expression timing of auto-regulated genes

We start by investigating the impact of TF-binding affinity in auto-regulatory motifs without any competing binding sites (decoy, Fig. 1B) or target genes (Fig. 1C). For both positive and negative auto-regulation motifs, the TF-binding affinity (k_{off}) impacts the mean first passage time (MFPT) to reach half of the Steady-state expression level (SSE) in a non-monotonic way. In Fig. 2A, we show the dependence of the MFPT on the binding affinity of a positive auto-regulatory gene. In the two extreme limits of affinity, the MFPT is a minimum approximately equal to that for a constitutive gene (set by the transcription and translation rates, roughly equal to one cell-cycle here; see supplementary Fig. S1A). When

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the binding affinity is very weak, the promoter is always free and expresses with a lower basal transcription rate r_0 and thus the MFPT is essentially the same as a constitutive gene with transcription rate r_0. On the other hand, when the binding affinity is strong, the promoter is always occupied by a TF and as such the MFPT is similar to a constitutive gene with transcription rate r. However, the MFPT is slightly higher than that of a constitutive gene due to the fact that the first binding event (to initiate the auto-activation) takes additional time and thus the MFPT is the sum of the MFPT of a constitutive gene and the average time to express the first TF to initiate the auto-activation. Interestingly, between these two extremes, the MFPT has an intermediate maximum at a specific value of TF binding affinity (k_{off}). The height of this intermediate maximum depends on the strength of the activation ($\alpha = r/r_0$), with lower strength corresponding to smaller maxima. This maxima indicates that in a specific range of binding affinity, the auto-activating TF gene can respond substantially slower than a constitutive gene, taking several generations to respond and reach the half maxima of steady-state expression. For a given α, altering translation rate shifts the position of maxima without affecting the peak MFPT (Fig. 2A, dashed curves). This shift vanishes if we replot the data as a function of steady state TF-occupancy rather than k_{off} (Fig. 2B); when plotted as a function of TF-occupancy the data for different translation rates collapses such that the maxima occurs at a specific value of TF occupancy for a given α regardless of the translational dynamics. However, the occupancy level where the maxima occurs still has a dependence on the strength of regulation, α.

The non-monotonic behavior of the MFPT for the auto-activating gene can be understood by examining the relationship of two different quantities with k_{off}: the TF-production rate from a single TF (R) and the Steady state expression level (SSE). In this case, SSE is proportional to how much TF must be made by the gene to reach the FPT threshold and R is an approximation for the speed in which the system approaches that level (SSE has units of number of proteins and R is a rate of production); in practice R depends on time in this system and changes as the number of TFs increases, but we use this simplified rate of expression to gain intuition about the system. As such, the ratio of these quantities ((SSE/R) should tell us approximately the timescale of response of the gene. For the auto-activating gene, Fig. 2C shows the steady state expression level (blue curve) and the quantity $1/R$ (red curve) as a function of TF binding affinity, k_{off}. The steady state expression level decreases as the binding affinity is increased, from a maximal value of $\text{SSE} = rb/\gamma_m\gamma$ for a very strong affinity when the promoter is always occupied by a TF to $\text{SSE} = r_0 b/\gamma_m\gamma$ for very weak activation. The production rate R behaves in a similar fashion and we plot $1/R$ as a red curve on the same axis. The response time, which is then the product of the red and blue curves (SSE/R) will be non-monotonic with an intermediate maximum that approximately coincides with the maximum of the MFPT; although the maximum does not exactly coincide due to the simplifications in this calculation.

As shown in Fig. 2D, we see a similar phenomenon for an auto-repressing gene. In all cases, the MFPT is a minimum value, less than a cell-cycle (28), when the TF off-rate is very low (high affinity) and approaches the MFPT of a constitutive gene when the site is very weak. For auto-repression we once again see non-monotonic behavior with a minimum in MFPT less than one cell-cycle at an intermediate value of k_{off} (Fig. 2D blue and red curves). As the auto-regulatory strength, α is made smaller (stronger repression) the value of k_{off} at the minimum also gets smaller. When repression is "complete" ($\alpha = 0$), *i.e.*

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the transcription rate is 0 when bound by repressor, the intermediate minimum disappears (Fig. 2D, yellow curve). In Fig. 2E, we once again see that the MFPT is collapsed by plotting MFPT as a function of TF occupancy. We also see, like in auto-activation, the quantity SSE/R captures the non-monotonic behavior of the MFPT of an auto-repressing gene as well (Fig. 2F).

For an auto-activating gene, we can also examine how the magnitude of the peak in MFPT and the corresponding off-rate at the peak depends on the strength of auto-activation. We find that the peak in MFPT has a power-law dependence on auto-regulatory strength α and the power-law exponent is independent of the transcription and translation rates (see Fig. 3A). In this case, the exponent to the power-law is approximately 0.5, meaning for example that a 4-fold increase in the strength of activation will net a 2-fold increase in the peak MFPT. However, the arbitrary choice for MFPT threshold affects the power-law exponent in a monotonic way with lower thresholds having a slightly higher exponent and vice versa (Fig. 3B,C). Furthermore, the off-rate (k_{off}) corresponding to the MFPT peak collapses to a single power-law curve when scaled with basal expression rate, $r_0 b$ (see Fig. 3D). In this case, the power-law exponent is approximately 1, and as in our example above a 4-fold increase in activation strength would have a peak in the MFPT when the normalized off-rate is increased approximately 4-fold. Once again, the power-law exponent depends on the chosen value of the MFPT threshold and the exponent is higher for lower threshold and decreases monotonically as the threshold is increased (Fig. 3E,F). Furthermore, the MFPT for a constitutive gene can be estimated from the power-law fit by setting $\alpha = 1$. We plot the estimated MFPT and the MFPT computed from simulation for varying parameter ranges in Fig. S1B and find comparable results. For auto-repressing genes, the minima in MFPT as well as the off-rates corresponding to the minima deviate from this power-law behavior (see Supplementary Fig. S3 A,B).

The role of TF competition in controlling expression timing

Next, we investigate the effect of TF sharing with competing binding sites (decoy). Competing binding sites for the TF reduce the availability of free TF (TF not bound to any gene or competing binding sites). As a consequence, both the steady state expression (SSE) and MFPT is affected. As shown in Fig. 4A, the presence of competing binding sites in an auto-activating TF gene has effects similar to varying the TF-binding affinity in that the MFPT once again shows non-monotonic behavior. However, in this case the peak value of the MFPT can be significantly higher. The magnitude of the maxima depends on TF binding affinity with weaker sites displaying higher peaks (Fig. 4A red and blue curves). The red curve in Fig. 4A has an affinity chosen to be consistent with the strong LacI binding site *lacIO1* (38) and we see a predicted maximum in MFPT of 29 cell cycles. The maximum in MFPT becomes higher for weaker binding sites and can reach hundreds of cell-cycles for sites with affinities consistent with *lacO2* ($k_{\text{off}} \sim 0.01 s^{-1}$) or *lacO3* ($k_{\text{off}} \sim 0.6 s^{-1}$). This can be seen in Fig. 4C, where we plot a heatmap of MFPT in number of competing binding sites and affinity space. These timescales are exceptionally long compared to, for instance, the total duration of log phase growth of *E. coli* in lab conditions which is typically less than 10 generations. This implies that for specific "network sizes", the TF gene could not reach a significant fraction of its SSE level in a realistic time frame. The peaked MPFT behavior start to disappear as the affinity is further reduced (Fig. 4C).

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To understand the non-monotonic behavior of the MFPT as a function of decoy site number, we plot the steady state expression (SSE) level, the number of free TFs and the number of TF-bound decoy sites in Fig. 4B inset. Once again we will argue based on the balance between the SSE level (a fraction of which must be achieved to achieve the FPT) and the rate at which it gets there, which is a function of the number of free TFs (*i.e.* TFs not already bound to binding sites). When the decoy number is small (less than the constitutive expression level of roughly 390 proteins for this example), the presence of decoy sites do not alter the SSE significantly (solid blue line). However, in this regime, the free TF number reduces linearly with decoy number as the TFs bind to the available decoy sites (dashed line in Fig. 4B inset). This reduction in free TF will slow the production rate of TF and the MFPT will increase with decoy number in this regime. This decrease in free TF stops when the number of decoys is greater than the constitutive expression level, at this stage, very few if any TFs are free and the production rate will be roughly insensitive to decoy number. The SSE level in this regime will now decrease rapidly with decoy number signifying an overall decrease in the MFPT. The intermediate maxima will then occur approximately when the number of decoys and SSE levels of TF are comparable. It can also be seen from the heatmap (Fig. 4C) and Fig. 4A that that the number of decoys corresponding to the peak MFPT reduces as the affinity is lowered; low affinity also corresponds to lower SSE level for TFs. Unlike when affinity is varied, we do not find power-law behavior for the peak MFPT as a function of regulatory strength when decoys are varied (see Supplementary Fig. S3C,D).

For a self-repressing gene, we find that with increasing decoy sites, the MFPT first decreases, attains a minimum value and then again goes up asymptotically reaching the MFPT for a constitutive gene with basal rate r_0 (Fig. 4D). In Fig. 4D, we see that at zero occupancy (achieved at high decoy numbers) the MFPT is approximately equal to the MFPT of a constitutive gene (black dashed line). Decreasing the decoy number increases the occupancy down to a minimum which is always less than one and is set by the TF-binding affinity and production rates. The MFPT at this point depends on TF-binding affinity which was shown in Fig. 2D and Fig. 4D. For complete auto-repression (Fig. 4D, dashed blue curve) the MFPT at zero decoy is close to 0.21 cell cycle consistent with (28). Interestingly, even for complete repression we see a slight dip in MFPT as decoys are introduced. In Fig. 4F, we show a heat map of MFPT in number of competing binding sites and affinity space. For $\alpha = 0.1$ the heat map shows a global minimum in MFPT corresponding to $k_{\text{off}} = 0.009s^{-1}$ and 80 decoys. Moreover, the qualitative behaviors are consistent for different parameter sets (see Fig. S2, where we have used cell division time of 38.5, 58 and 116 min).

The precision of expression timing depends on the nature of regulation

Next, we investigate the effect of TF-binding affinity and decoy size in controlling the noise of first passage time. We use coefficient of variation (CV) defined as the ratio of the standard deviation of first passage time and MFPT as a measure of noise. In Fig. 5C,F, we plot CV of first passage time versus MFPT for auto-activation or auto-repression if either the TF binding affinity or the number of decoys is used as a control parameter of the system. We know from Fig. 2 that for auto-activation the MFPT will first rise and then fall as the affinity is increased, Fig. 5C shows that despite degeneracy in MFPT (two different k_{off}

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values give the same MFPT), the associated noise in the MFPT is different for strong and weak binding sites with stronger binding leading to tighter distributions around the mean (Fig. 5A). For auto-repression (Fig. 5B,C) the story is similar but reversed; the MFPT is now less than 1 and the noise in MFPT is lower for weak binding compared to strong binding site having same MFPT. When number of decoys is used as a control parameter, the CV versus MFPT has trend qualitatively similar to affinity as the control parameter (Fig. 5F). In this case, zero competition (no decoy) leads to the minimum CV for auto-activation and maximum CV for auto-repression (Fig. 5D,E). Interestingly, for auto-repressing gene having very strong binding affinity, the FPT distribution is bimodal (Fig. 5B, bottom curve) and disappears for lower affinities. In all other scenarios, the distribution is unimodal.

The expression timing of a target gene depends on the nature of TF regulation

In this section, we explore the impact of a TF's mode of auto-regulation on the expression timing of a target gene. The TF gene can be expressed in three different ways: auto-activated ($\alpha > 1$), auto-repressed ($\alpha < 1$), and constitutively expressed ($\alpha = 1$). In Fig. 6 A and B, we plot the MFPT of an activated (Fig. 6A) or repressed (Fig. 6B) target gene as a function of TF off-rate at the target promoter. The colored lines in the plot represent different modes of auto-regulation of the TF (different values of α). In this plot, the number of total TFs is kept constant by adjusting the translation rate to counteract the effect of changing k_{off}.

As the TF-target affinity is decreased the MFPT increases irrespective of the TF auto-regulatory mode or the type of regulation of the target gene (activation, Fig. 6A or repression, Fig. 6B). For target repression, a lower TF-target affinity corresponds to higher target expression and thus a higher MFPT. On the other hand for target-activation a lower TF-target affinity reduces the target expression but at the same time the rate of production of target is also hampered due to lowering of the affinity which is more substantial. As a result the MFPT of the target gene increases with lowering affinity in case of activation. One interesting feature revealed from Fig. 6A,B is that the MFPT curves for different auto-regulatory strength of the TF gene (α) never intersect each other. The MFPT curve of a target gene regulated by an auto-repressed TF gene is always greater than the MFPT of a target gene under the control of an auto-activated TF gene and the constitutive TF gene defines the boundary of separation between the two cases (Fig. 6B). We get the opposite behavior when the target gene is activated by a TF gene. In this case, the MFPT curve of target gene regulated by an auto-repressed TF gene is always lower than the MFPT of target gene under the control of an auto-activated TF gene with the constitutive TF gene defining the separation between the two (Fig. 6A). This tells us that timing of expression of the TF-gene is the key factor for determining the timing of expression of target gene under control of the TF gene. To further explore this relationship, we plot the MFPT of a target gene as a function of MFPT of the TF-gene which regulates the target gene (Fig. 6C). We find that for target activation, the MFPT of the TF and target gene are positively correlated whereas for target repression it is anti-correlated. A useful way to view this data is to scale the MFPTs by the respective MFPT for constitutive TF expression ($\alpha = 1$). This is shown in Fig. 6C (also see Supp. Fig. S4A), where we see the MFPT-MFPT phase space is separated into four quadrants; 1) self-repressed gene repressing a target (RR), 2)

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self-repressed gene activating a target (RA), 3) self-activated gene repressing a target (AR), and 4) self-activated gene activating a target (AA). It further provides us with a tool to identify the mode of TF-gene by simply comparing the MFPT of a target gene regulated by an auto-regulated TF gene and MFPT of the same target gene regulated by a constitutive TF gene. For example, in case of target activation, MFPT of target gene under the control of auto-regulatory TF gene higher than that of under constitutive TF gene means that the TF gene is auto-activated and vice versa.

We further explore the expression timing relationship when the binding sequence for TF and target genes are exactly identical, which means the binding affinity of TFs being identical for both TF and target gene. In our previous work (38), we have shown that an auto-repressed gene and its target gene have different steady state expression levels even though their binding sequences are exactly identical. In Fig. 6D, we plot the MFPTs of the target gene which is regulated by an auto-regulated TF gene versus MFPT of the TF gene, the regulation being identical for both TF and target gene. Each curve is generated by tuning the TF affinity for TF and target gene while keeping other parameters constant. We find that the target gene is always slower than the TF gene to reach half of their respective steady state expression for negative regulation, i.e., target gene repressed by an auto-repressed TF gene (Fig. 6D, open squares. Also, see Supp Fig. S4B, where number of decoy sites are varied keeping the affinity fixed). The difference in their timing is maximum for high affinity binding sites and approaches to one-to-one line showing similar timing in expression as the affinity is lowered. For target gene activated by an auto-activated TF gene, we find that the target gene is faster than the TF gene, however, the difference is not significant (Fig. 6D, filled circles).

Discussion

The decay rate of proteins sets the natural timescale of the response of transcriptional networks (21); in bacteria where proteins are long-lived this timescale tends to be the division rate of the organism. Although specific network connections are known to speed or slow this response by several fold, extremely slow or fast responses have been observed in a growing number of systems (22; 25; 23; 24). Here, we systematically investigate the regulatory features that control gene expression timing of auto-regulated TFs, specifically in determining the mean first-passage time (MFPT) of reaching half of the steady-state level of expression. Interestingly, we find that auto-activation and auto-repression, around a specific range of TF binding affinities, are capable of slowing or speeding response times by orders of magnitude. Similarly, we find that extra TF binding sites, can alter the timing response in a more dramatic fashion. These extra binding sites may serve to regulate other promoters in the network or they may serve no specific function (*i.e.* decoys). Crucially, this means that for networks of particular size, a gene may not be realistically able to reach steady-state levels in physiologically reasonable timescales, in some extreme cases requiring hundreds of generations simply to reach half of the steady-state level. Despite arguments of the inherent interest of "steady-state levels" in such networks the concept is essentially irrelevant.

We also examine how the fidelity of single-cell timing depends on these physical parameters. Due to the intermediate extrema in MFPT, there are typically two values of

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TF binding affinity or decoy number that produce an identical "mean response", however these two regulatory parameter sets produce significantly different variance in timing among individual cells. As a result, for the majority of achievable MFPTs of an auto-regulatory gene there is both a "quiet" and "noisy" way to achieve a given response time. For auto-activation, higher off-rates and larger numbers of decoys produce noisier responses while the opposite is true for auto-repression. Just like for the case of average gene expression levels where it is argued that the ability to tune noise independent of mean can be important for fitness, it stands to reason the same holds for the speed of response of a gene's transcriptional response and thus the ability to reach similar average response times with very different levels of noise can be useful. Finally, we showed how the response of a target gene is controlled by the mode and details of regulation of the TF gene. Interestingly, for any mode of target regulation (activation or repression) the target gene MFPTs are well distinguished for different modes of TF gene regulation.

Overall, understanding the molecular features that dictate the timing in expression for genes is an important part of reading natural gene networks and designing efficient expression timing for synthetic networks.

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Materials and Methods

Model and Simulation methodology

Here, we describe a generalized model for an auto-regulatory TF-gene, one target gene and multiple competing binding sites. The TF-gene product , TF protein (X), binds to its own promoter (P_{TF}), to the promoter of the target gene (P_{Target}), and to the decoy sites (N) with a constant rate k_{on} per free TF per unit time. TF unbinds from the promoter with a sequence dependent off-rate (k_{off}) which determines the TF-binding affinity of the binding site. The off-rates of a bound TF from the promoters of the TF and target, and from the decoy sites are k_{off}, $k_{\mathrm{off,t}}$, and $k_{\mathrm{off,d}}$ per unit time, respectively. mRNAs are produced at the rate r_0 from a TF-free promoter (basal transcription) and rate r from a TF-bound promoter. The ratio of r_0 and r determines whether the gene is repressed ($r/r_0 < 1$) or activated ($r/r_0 > 1$). This ratio is termed auto-regulatory strength, α. Furthermore, $\alpha = 1$ describes a constitutive gene. mRNAs are translated into protein at the rate b. The mRNAs and the proteins are degraded at the rate γ_{m} and γ, respectively. The lifetime of protein in *E. coli* and many other organisms are much longer, typically 2-3 hrs, and hence the protein degradation, $\gamma = ln(2)/\tau$, is assumed to be due to dilution during cell division (τ). We further assume that all proteins (free protein, TF bound to promoter and TF bound to decoy sites) degrades with the same rate. The set of reactions describing the model above are listed below.

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$$
\begin{aligned}
m_{\mathrm{TF(Target)}} &\xrightarrow{r_0} m_{\mathrm{TF(Target)}} + 1 \quad \text{(Production of mRNA from TF free promoter)} \\
m_{\mathrm{TF(Target)}} &\xrightarrow{r} m_{\mathrm{TF(Target)}} + 1 \quad \text{(Production of mRNA from TF bound promoter)} \\
m_{\mathrm{TF}} &\xrightarrow{b} m_{\mathrm{TF}} + X \quad \text{(Translation of TF protein)} \\
m_{\mathrm{Target}} &\xrightarrow{b} m_{\mathrm{Target}} + Y \quad \text{(Translation of target protein)} \\
X + P_{\mathrm{TF(Target)}} &\xrightarrow{k_{\mathrm{on}}} XP_{\mathrm{TF(Target)}} \quad \text{(Binding of a TF to a promoter)} \\
XP_{\mathrm{TF(Target)}} &\xrightarrow{k_{\mathrm{off,TF(Target)}}} X + P_{\mathrm{TF(Target)}} \quad \text{(Unbinding of a TF from a promoter)} \\
m_{\mathrm{TF}} &\xrightarrow{\gamma_{\mathrm{m}}} m_{\mathrm{TF}} - 1 \quad \text{(Degradation of mRNA)} \\
m_{\mathrm{TF(Target)}} &\xrightarrow{\gamma_{\mathrm{m}}} m_{\mathrm{TF(Target)}} - 1 \quad \text{(Degradation of mRNA)} \\
X + N &\xrightarrow{k_{\mathrm{on}}} XN \quad \text{(Binding of a TF to decoy)} \\
XN &\xrightarrow{k_{\mathrm{off,Decoy}}} X + N \quad \text{(Unbinding of a TF from decoy)} \\
X, Y &\xrightarrow{\gamma} \phi \quad \text{(Degradation of TF protein)} \\
XP_{\mathrm{TF(Target)}} &\xrightarrow{\gamma} P_{\mathrm{TF(Target)}} \quad \text{(Degradation of TF from promoter-complex)} \\
XN &\xrightarrow{\gamma} N \quad \text{(Degradation of TF bound to Decoy)}
\end{aligned}
\tag{1}
$$

We implement the simulations for stochastic reaction systems using Gillespie's algorithm (42) in C programming, to compute mean first passage time (MFPT). The threshold for MPFT is set to 50% of steady state expression (SSE) unless specified. Simulations are run for sufficiently long time ($\sim 10^6$ s) to get rid of the transient dynamics and then steady state distributions (TF and target protein expressions) are obtained by sampling over time with a time interval (T_{S}), long enough for the slowest reaction to occur 20 times on average (T_{S} = 20 over rate for slowest reaction). Next, we run the simulations using the steady state expressions as input to obtain gene expression trajectories. At time $t = 0$, the network is switched on to express until the expression reaches a threshold. The time for each trajectory to reach that threshold is termed as first passage time and their mean is referred as mean first passage time (MFPT). MFPT and noise in first passage times are computed using at least 10^5 individual trajectories. For simulations, we use $k_{\mathrm{on}} = 0.0027/s$((53)), $\gamma_{\mathrm{m}} = 0.011/s$ ((54)) and $\gamma = 0.0001, 0.0002, 0.0003 s^{-1}$. These rates correspond to 90 sec average life for mRNA and a cell-cycle of 115, 56 and 38.5 min, respectively. k_{off} is varied in a range of $0.0002 - 100$ per TF per second to cover the binding affinities corresponding to a wide varieties of natural and synthetic promoters in *E.coli* such as *lacOID, lacO1, lacO2, lacO3*. Typical transcription rate in *E. coli* is of the order of 0.33 s^{-1} for constitutive gene (55), as such we use a basal rate in a range $r_0 = 0.001 - 0.05\ s^{-1}$ such that the ten -fold activation correspond to a transcription rate of $r_0 = 0.01 - 0.5\ s^{-1}$. Protein numbers in *E. coli* varies from few units to thousands (56) as such we vary translation rate (b) in a range of $0.025 - 0.1\ s^{-1}\mathrm{mRNA}^{-1}$.

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Figures

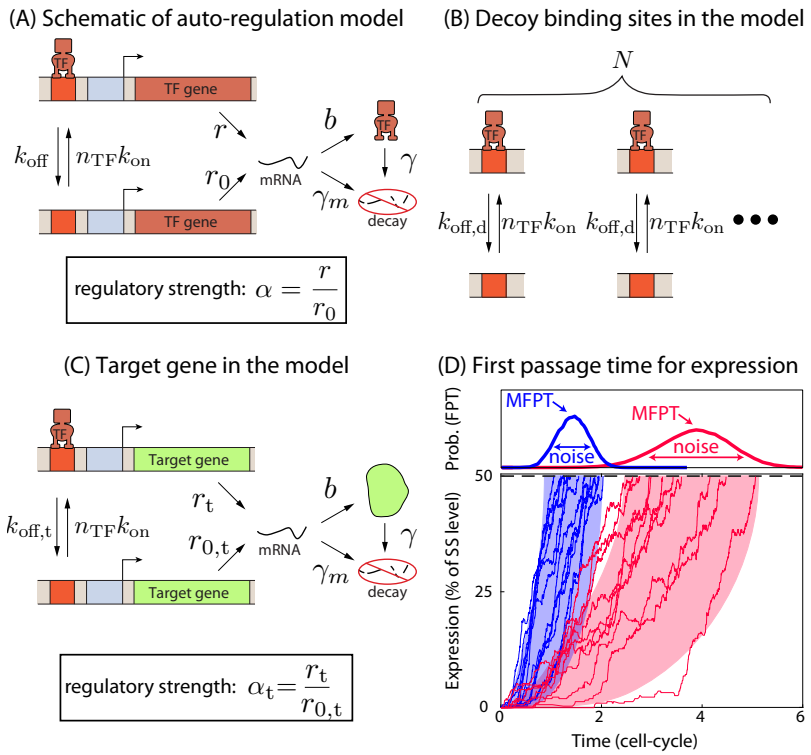

Figure 1: **Mean first passage time in a self regulated gene.** (A) Schematic of a self-regulating gene. (B) Schematic showing the reactions of TF binding/unbinding to competing decoy sites. (C) Reactions of a target gene regulated by the auto-regulatory TF gene. (D) First passage time is defined as the time for the gene expression to hit the threshold for the first time as shown here. For an auto-regulated gene, the mean first passage time (MFPT) and the distribution of FPT strongly depend on the strength of auto-regulation (α) and the TF-binding affinity.

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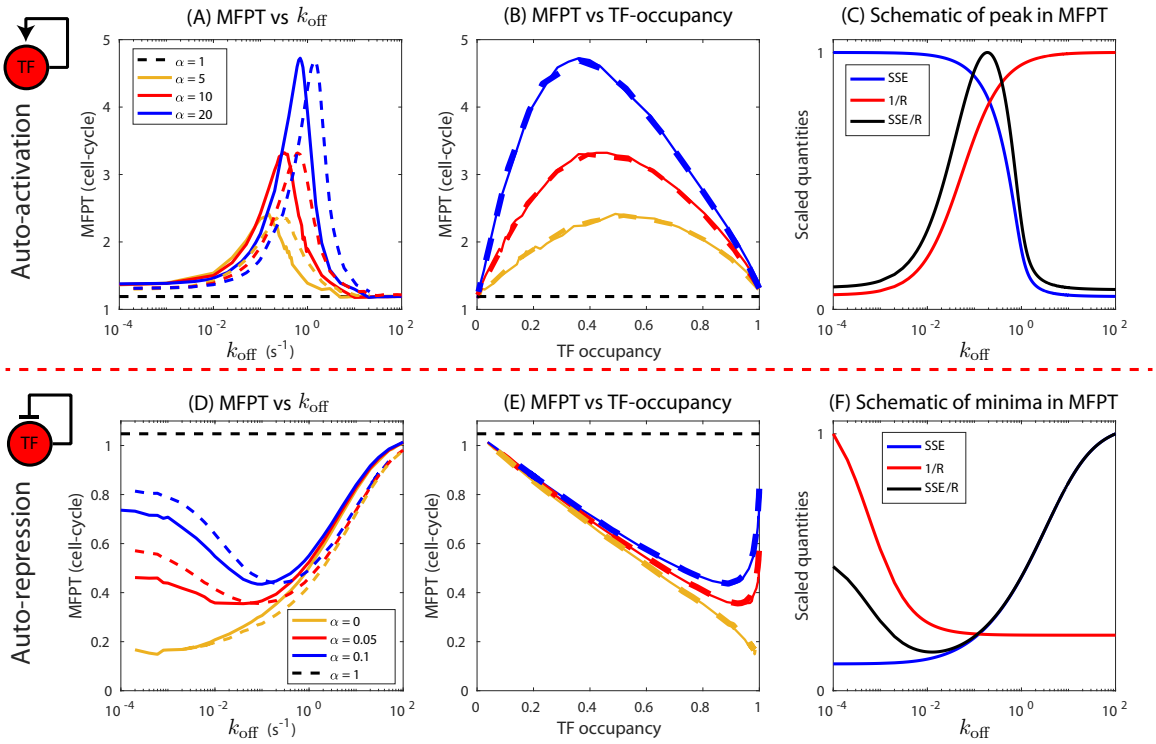

Figure 2: **Effect of TF-binding affinity on MFPT for auto-regulated genes without any competing binding sites.** (A, D) MFPT as a function of off-rate (binding affinity) of auto-activated gene and auto-repressed gene. Each curve (blue, red, yellow) is generated by keeping the transcription rate (r_0) and translation rate (b) fixed and varying only the binding affinity through k_{off}. Dashed curves correspond to doubling the translation rate ($2b$). Black dashed lines correspond to MFPT of a constitutive gene of transcription rate r_0. (B, E) MFPT as a function of TF-promoter occupancy. Solid and dashed curves are for translation rates b and $2b$, respectively. (C,F) Scaled steady state TF expression (SSE) and inverse of production rate ($1/R$) as a function of off-rate. The ratio of SSE and R (black curve) qualitatively predicts the behavior of the MFPT as a function of off-rate. For auto-activation we use $r_0 = 0.0025s^{-1}, b = 0.025s^{-1}\text{mRNA}^{-1}$; for auto-repression $r_0 = 0.05, b = 0.1s^{-1}\text{mRNA}^{-1}$.

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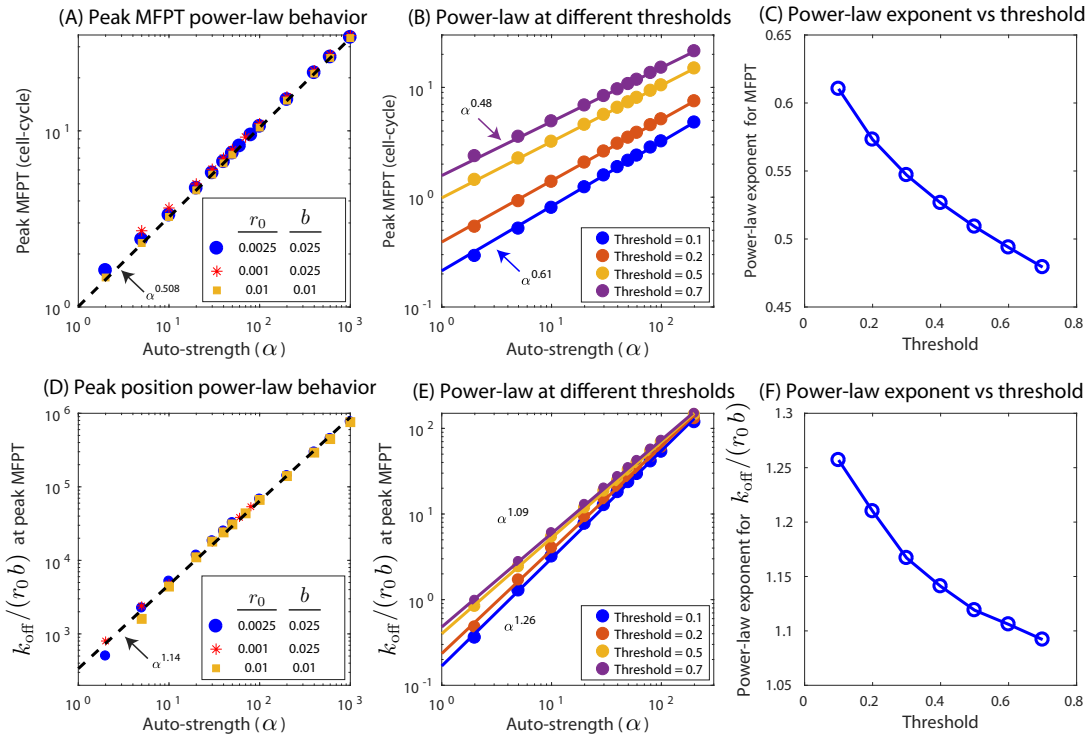

Figure 3: **Power-law behavior in auto-activated gene.** (A) Peak value of MFPT as a function of auto-regulation strength, α, shows a power-law behavior with approximate exponent of 0.5 irrespective of basal transcription or translation rates. (B) Peak MFPT for different expression thresholds have different exponents. (C)Power-law exponent of Peak MFPT versus auto-regulation strengths as a function of expression threshold. (D) The value of $k_{\rm off}/(r_0 b)$ at the peak MFPT also follows a power law behavior with exponent slightly larger than 1. (E) The exponent again depends on threshold. (F) The value of the exponent as a function of threshold.

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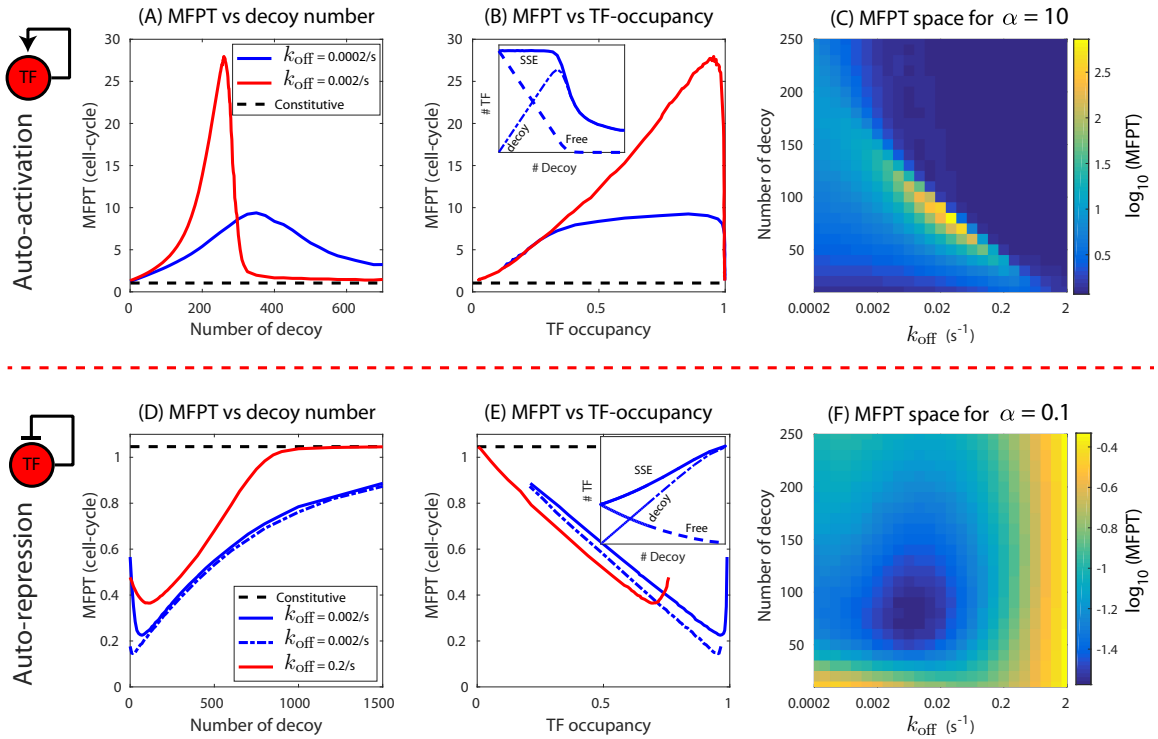

Figure 4: **MFPT of an auto-regulated gene in a network of competing binding sites.** The MFPT of an auto-activated gene as a function of (A) number of competitive binding sites and (B) steady-state occupancy of TF at the promoter when decoy number is varied. The red curve is for weaker TF binding while the blue curve is for stronger TF binding. Inset: MFPT as function of free TF in steady state. Black dashed lines correspond to MFPT of a constitutive gene of transcription rate r_0. (C) Heatmap showing phase space of MFPT in number of competing binding sites and affinity space for auto-activating gene ($\alpha = 10$). (D,E) The MFPT of an auto-repressed gene as a function of competing binding sites and steady-state occupancy. Dashed blue line correspond to complete repression ($\alpha = 0$). Inset: MFPT as function free TF in steady state. (F) Heatmap showing phase space of MFPT in number of competing binding sites and affinity space for auto-repressing gene ($\alpha = 0.1$). We use $\alpha = 10$, $b = 0.025s^{-1}\text{mRNA}^{-1}$, $r_0 = 0.0025s^{-1}$ (activation) and $\alpha = 0.1$, $b = 0.05s^{-1}\text{mRNA}^{-1}$, $r_0 = 0.05s^{-1}$ (repression). $\gamma = 0.0003/s$ is used for protein degradation corresponding to $\tau = 38.5$ min.

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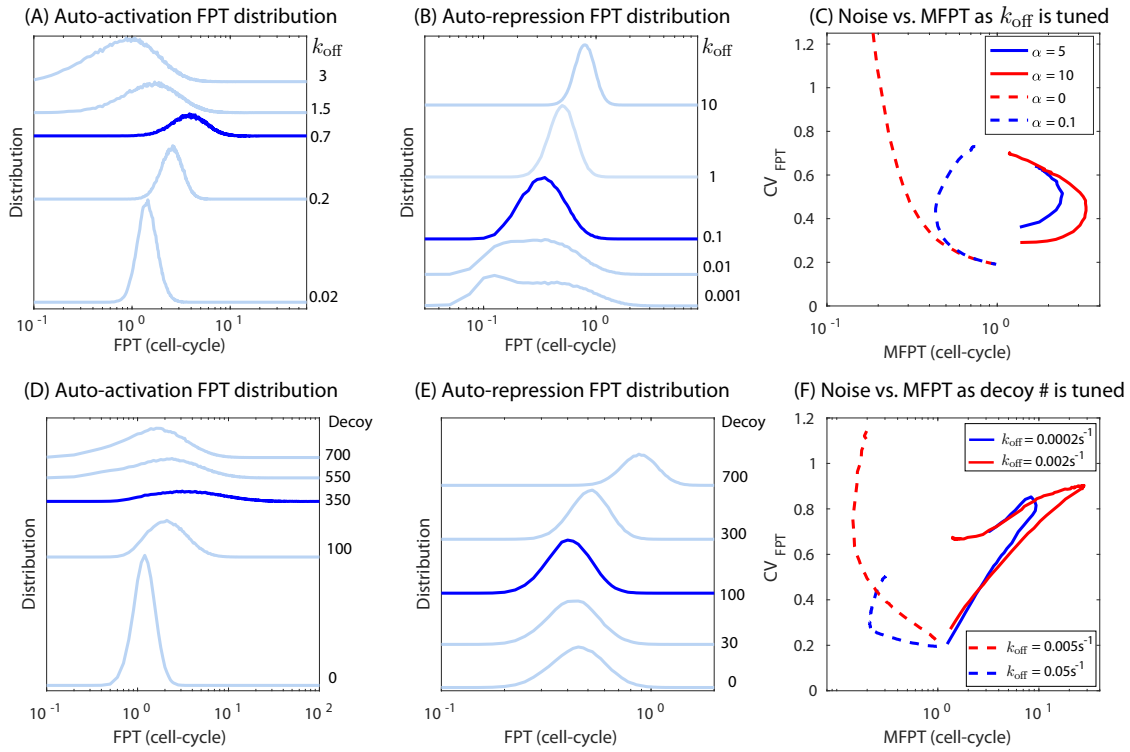

Figure 5: **Distribution of first passage time (FPT) of an auto-activated TF gene.** (A) The CV of first passage time as a function of MFPT when k_{off} is changed to vary MFPT for auto-activation (solid lines) and auto-repression (dashed lines) of differing regulatory strength. (B-C) FPT distribution for several values of k_{off} for (B) auto-activation and (C) auto-repression, the peak k_{off} is shown in dark blue. Parts (D-F) show the same information but for varying decoy instead.

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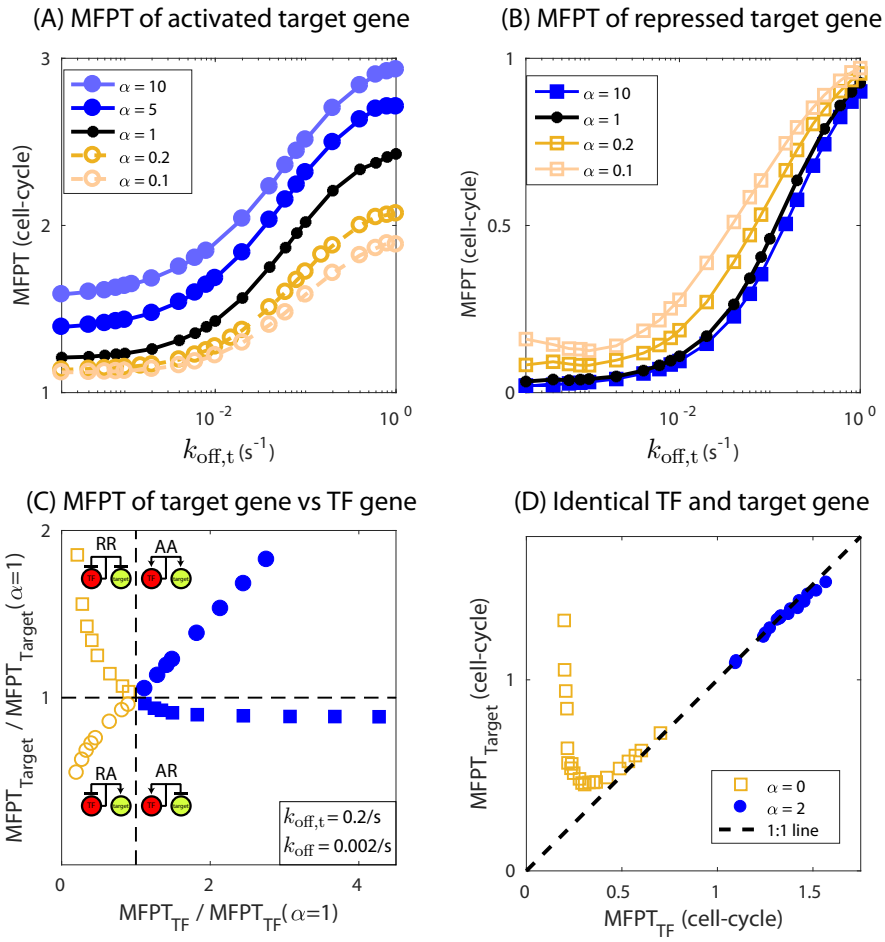

Figure 6: **Expression timing of target gene is dictated by the nature of TF regulation.** (A, B) MFPT of a target gene as a function of binding affinity of TF to the target gene ($k_{\mathrm{off,t}}$). Filled symbols represent the TF being auto-activated ($\alpha > 1$), open symbols represent when the TF gene is auto-repressed ($\alpha < 1$), and black asterisks are when the TF gene is constitutive ($\alpha = 1$). Circles represent target gene activation whereas squares represent target gene being repressed by the TF gene. (C) MFPT of target gene as a function of MFPT of TF gene. α is varied from from 0 to 50 while keeping $k_{\mathrm{off}} = 0.002/s$ and $k_{\mathrm{off,t}} = 0.2/s$ constant. The MFPTs of TF and target genes are normalized by their respective MFPTs when α is one or equivalently when TF gene is constitutive. Translation rate of the TF gene is adjusted to achieve constant level of TF number (~ 50) for varying binding affinities and α in (A-C). (D) Plot showing asymmetry in the expression timing of TF and target gene when they have identical binding affinity, transcription and translation rates. Target gene repressed by an auto-repressed TF gene always has higher MFPT compared to the TF gene. Target gene activated by an auto-activated TF gene, on contrary, do not show any significant differences (target gene being moderately faster than the TF gene).

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Supporting Figures

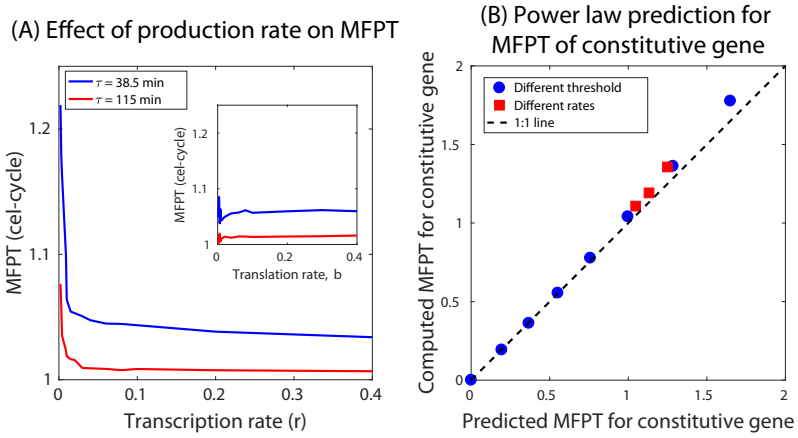

Figure S1: (A) Effect of transcription and translation rates on MFPT of a constitutive gene. At low transcription rates the MFPT of constitutive gene is usually higher than that at higher transcription rate. Inset: The translation rate on the other hand does not alter MFPT significantly. (B) Comparison of MFPT of a constitutive gene computed from simulation and predicted from the the power-law fit of peak MFPT versus α by setting $\alpha = 1$. For the power-law fit data point corresponding to $\alpha = 1$ is excluded, since $\alpha = 1$ is a constitutive gene having constant MFPT as a function of k_{off}.

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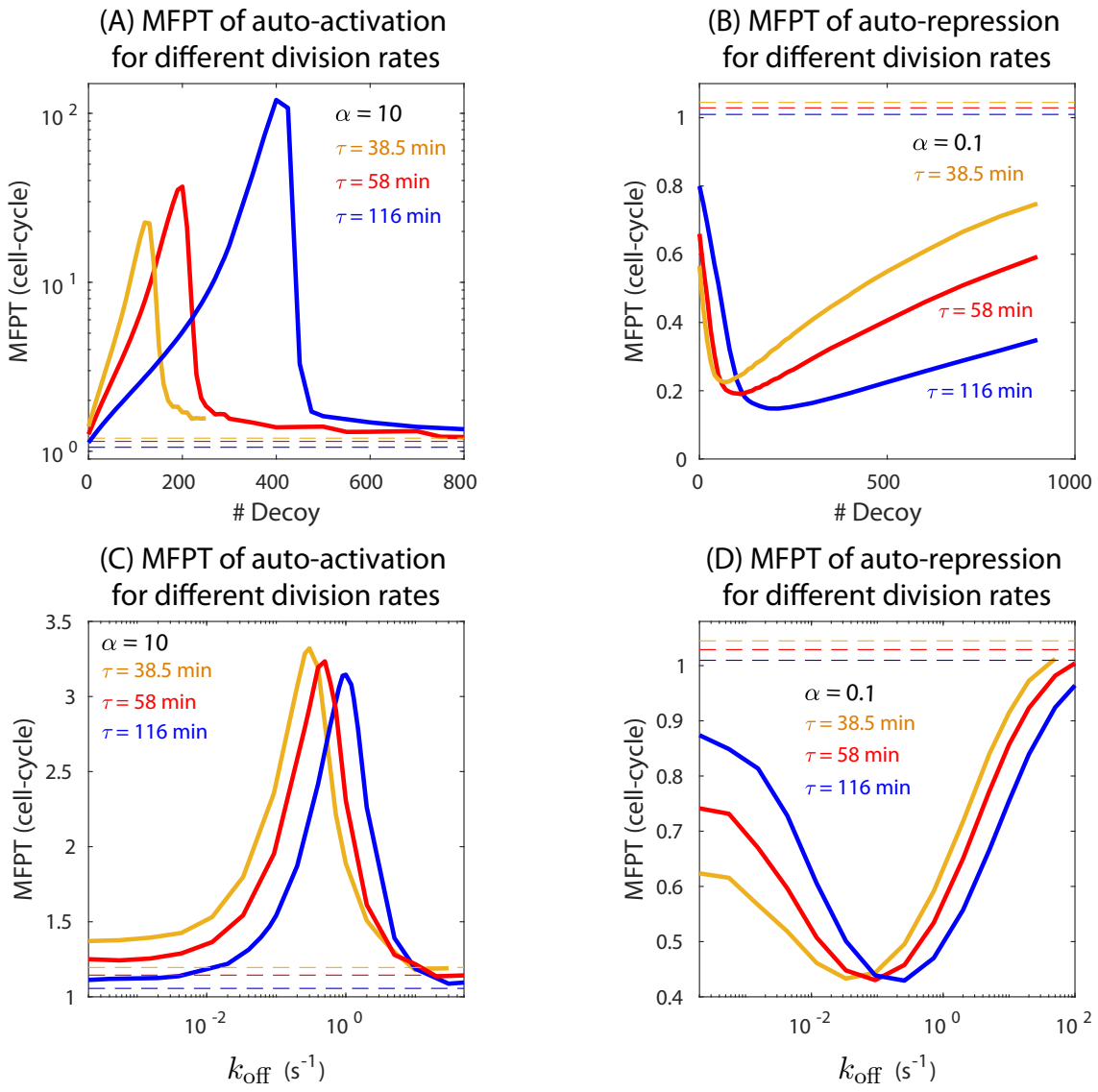

Figure S2: Effect of TF-binding affinity and competing binding sites on MFPT for auto-regulated genes for different cell division rates, $\tau = 38.5$ min (yellow), 58 min (red) and 116 min (blue). The TF gene is auto-activated with (A,C) or auto-repressed (B,D). Dashed lines correspond to MFPT of a constitutive gene of transcription rate r_0. Parameters used to generate the figure: $\alpha = 10$, $r_0 = 0.0025\ s^{-1}$, $b = 0.025\ s^{-1}\text{mRNA}^{-1}$(auto-activation). $\alpha = 0.1$,$r_0 = 0.05\ s^{-1}$, $b = 0.05\ s^{-1}\text{mRNA}^{-1}$(auto-repression). $k_{\text{off}} = 0.002 s^{-1}$ is used for (A,B).

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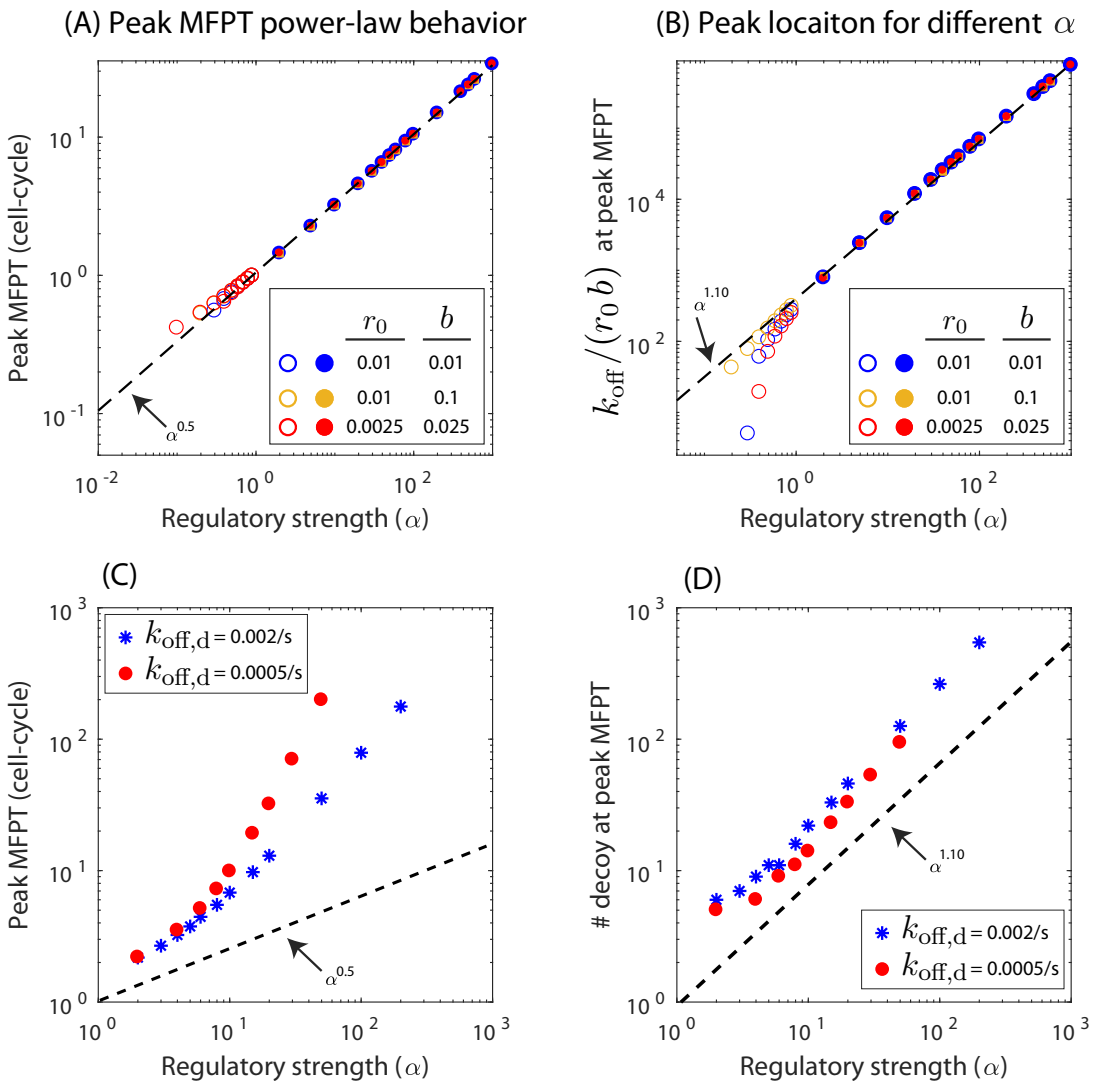

Figure S3: (A,B) Power-law behavior in self-repressing gene when affinity is varied. For an auto-repressing gene both the peak MFPT (A) and $k_{\rm off}$ at the peak (B) deviate from the power-law fit as a function of auto-regulatory strength α. Black dashed lines corresponds to power-law fit for auto-activating TF gene (i.e., $\alpha > 1$). Open circles are auto-activation ($\alpha > 1$) and filled circles are auto-repression ($\alpha < 1$). (C,D) Peak MFPT and number of decoys at the peak versus regulatory strength (α) when decoy is tuned. We do not observe any power-law behavior. Parameters: $k_{\rm off} = 0.002s^{-1}$, $r_0 = 0.001s^{-1}$, $b = 0.01s^{-1}$mRNA^{-1}.

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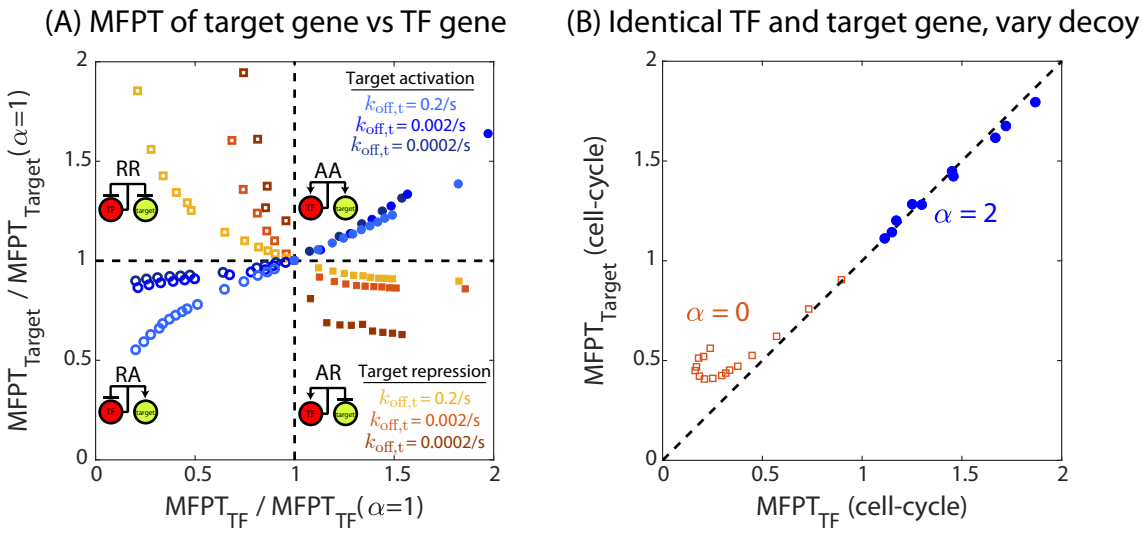

Figure S4: Expression timing of target gene depends on the nature of TF regulation. (A) MFPT of target gene as a function of MFPT of TF gene. α is varied from from 0 to 50 while keeping $k_{\text{off}} = 0.002s^{-1}$ and $k_{\text{off,t}} = 0.0002s^{-1}$, $0.002s^{-1}$, $0.2s^{-1}$ constant. The MFPTs of TF and target genes are normalized by their respective MFPTs when α is one or equivalently when TF gene is constitutive. Translation rate of the TF gene is adjusted to achieve constant level of TF number (~ 50). The TF gene is auto-activated (filled symbols) or auto-repressed (open symbols) and the target gene is activated (circles) or repressed (squares). (D) Plot showing asymmetry in the expression timing of TF and target gene when they have identical TF-binding affinity, transcription and translation rates. Decoy binding sites are varied along the curve from 0 to 300 for $\alpha = 2$ (blue) and $\alpha = 0$ (brown).