FLUCTUATIONS OF mRNA DISTRIBUTIONS IN MULTIPLE PATHWAY ACTIVATED TRANSCRIPTION

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Abstract. Randomness in gene transcription can result in fluctuations (noise) of messenger RNA (mRNA) levels, leading to phenotypic plasticity in the isogenic populations of cells. Recent experimental studies indicate that multiple pathway activation mechanism plays an important role in the regulation of transcription noise and cell-to-cell variability. Previous theoretical studies on transcription noise have been emphasized on exact solutions and analysis for models with a single pathway or two cross-talking pathways. For stochastic models with more than two pathways, however, exact analytical results for fluctuations of mRNA levels have not been obtained yet. We develop a gene transcription model to examine the impact of multiple pathways on transcription noise for which the exact fluctuations of mRNA distributions are obtained. It is nontrivial to determine the analytical results for transcription fluctuations due to the high dimension of system parameter space. At the heart of our method lies the usage of the model’s intrinsic symmetry to simplify the complicated calculations. We show the symmetric relation among system parameters, which allows us to derive the analytical expressions of the dynamical and steady-state fluctuations and to characterize the nature of transcription noise. Our results not only can be reduced to previous ones in limiting cases but also indicate some differences between the three or more pathway model and the single or two pathway one. Our analytical approaches provide new insights into the role of multiple pathways in noise regulation and optimization. A further study for stochastic gene transcription involving multiple pathways may shed light on the relation between transcription fluctuation and genetic network architecture.

1. Introduction. An isogenic population of cells may exhibit a substantial phenotypic diversity, which can help increase the fitness of the organism in natural
selection and is largely attributed to the inherent fluctuation (noise) of gene transcription [9, 13, 25, 28, 29]. This intrinsic noise has been implicated in many important physiological processes, such as random lysis/lysogeny decision of bacteriophage λ [2], incomplete penetration of the nematode C. elegans [27], precision of morphogenesis in early embryos of D. melanogaster [14], and reactivation of HIV latency in isoclonal cells [8]. In large part, the intrinsic noise is due to the transcriptional bursting of messenger RNA (mRNA) molecules. It involves activation and inactivation of multiple signaling transduction pathways, remodeling of chromatin structures, and recruitment of transcription factors (TFs) to cognate binding sites in the gene promoter domains. Experimental studies over the past two decades allow us to track the nonlinear dynamics of transcription noise and to identify the underlying molecular mechanisms of transcriptional bursting, and to provide insight into the rate-limiting steps of transcription [13, 22, 28, 39]. However, it is not clear how transcriptional reprogramming regulates the behaviors of transcription noise, and it is still not clear how kinetic rates can be tuned to optimize noise in transcriptional reprogramming process.

The activation of transcription for a target gene is a tightly controlled process. Individual cells in living organisms are exposed to a set of signal molecules in their environment. They possess a collection of receptor proteins in response to these signal molecules. Receptors relay signals via signaling pathways, and the initiation of transcription is ultimately activated through the binding of downstream TFs at the cognate DNA binding sites in the gene promoter or enhancer domains (Fig. 1(a)). The transcription of many inducible genes in biological processes such as development and immunity is involved with entangling pathways [1, 7, 12, 15, 21, 32]. For instance, the transcription of the yeast glucose-regulated SUC2 gene is activated by AMPK/Snf1 and cAPK pathways [12], and the transcription of the mouse macrophage gene stimulated by Lipopolysaccharide is activated by p38 MAPK and JNK pathways [15].

Many important questions arise from these observations that remain to be answered: How does the rewire of multiple pathways affect transcription fluctuations? How can the kinetic rates of transcription be tuned to optimize transcription fluctuations in the presence of entangling pathways? To address these questions, we examine in this paper a model of multiple pathway activated transcription. It is not easy to obtain the analytical formulas for fluctuations of mRNA levels and to analyze their analytical properties since it involves a high dimensional space of system parameters. Luckily, we find an inherent relation among the system parameters. With the help of this relation, we are able to obtain the analytical expressions of the dynamical and steady-state fluctuations of mRNA distributions and to precisely characterize the nature of transcription noise. Previous studies on fluctuations in gene transcription have been emphasized on exact solutions and theoretical analysis for models with a single pathway [26, 29, 33] or two cross-talking pathways [35, 42]. The results in the current study not only extend previous ones in limiting cases but also reveal some crucial differences between the three or more pathway model and the single or two pathway one. Our theoretical analysis may provide new insights for better understanding of the relation between transcription fluctuation and the genetic network topology.

Let $M(t)$ denote the copy number of mRNA molecules of a target gene in a single cell at time $t$. The fluctuations of mRNA distributions of the target gene have frequently been described by the noise, the variance normalized by the
square of the mean, and the noise strength, the variance normalized by the mean [8, 18, 33, 34, 37, 38]. In Section 2, we establish a multiple pathway model and obtain the corresponding master equations and differential equations. In Section 3, we give our main results and the corresponding proofs, along with discussions on the implications in stochastic gene transcription. In Proposition 1, we find a symmetric relation among the system parameters, allowing us to derive the analytical symmetric forms of the dynamical and steady-state fluctuations of mRNA distributions and to precisely characterize their analytical properties. In Theorems 3.1 and 3.2, the exact dynamical mean value and second moment of $M(t)$ are respectively derived. In Theorems 3.3 and 3.4, we show the non-monotonic behaviors of the stationary noise strength $\Phi^*$ by varying the kinetic rates of transcription, from which the kinetic rates can be tuned to optimize the transcription fluctuations. We also show that $\Phi^*$ is close to one when the mean mRNA level $m^*$ is rare due to unstable transcription or ineffective mRNA synthesis, or in the opposite extreme case where $m^*$ is rich due to strong activation or stable transcription. Define the transcriptional efficiency $P_E^*$ as the probability that the gene is active. In Theorem 3.5, we characterize the dynamical dependence of both $\Phi^*$ on $P_E^*$ and $m^*$. In Theorem 3.6, we show that, given the same $m^*$, multiple pathways tend to induce noisier transcription at steady-state than a single pathway does. We conclude some of our findings in Section 4.

2. The model and equations.

2.1. The model. Gene transcription is a discontinuous and stochastic process that transits between gene active (ON) and inactive (OFF) states [16, 17, 28, 33]. The two-state model has been used to describe transcription kinetics in cells, from bacteria [28, 33], yeast [20, 44] to mammalian cells [8, 28]. In the model, as shown in the following diagram,

\[
\text{gene OFF} \xrightleftharpoons[\gamma]{\lambda} \text{gene ON} \xrightarrow{\nu} \text{mRNA} \xrightarrow{\delta} \emptyset, \tag{1}
\]

the gene is assumed to switch randomly between ON and OFF states, with rates $\lambda > 0$ and $\gamma > 0$. Transcripts are produced with rate $\nu > 0$ when the gene is active, and are degraded in rate $\delta > 0$ [28, 33]. The elegant postulation in the two-state model implies the durations in both ON and OFF states are independently and exponentially distributed. This model has been widely used to fit the experimental data and to yield insight into transcriptional bursting [8, 20, 28].

The transcription of inducible genes in physiological processes such as development and immunity is often activated by multiple signal transduction pathways [1, 7, 12, 15, 21, 32]. Eucaryotic gene transcription requires chromatin-remodeling activities and distinct chromatin remodelers cooperate for the dynamic activation of transcription. In the yeast glucose-regulated SUC2 gene, the SWI/SNF ATP-dependent chromatin-remodeling complex associates with the promoter in a biphasic manner. Moreover, two different histone acetyltransferases, Gcn5p and Esa1p, enhance the binding of SWI/SNF to the promoter during early transcription. Both AMPK/Snf1 and cAPK signaling pathways co-regulate the dynamic recruitment of SWI/SNF and Gcn5p to the SUC2 promoter and transcription of SUC2 [12]. Lipopolysaccharide, a cell wall component of gramnegative bacteria, activates macrophages via Toll like receptors. Upon this receptor ligation, the p38
MAPK pathway together with ERK1/2 and JNK pathways causes a highly orchestrated, transient induction of the inflammatory TNF, IER3, ZFP36, and IL1beta genes [15, 32]. Single-molecule RNA FISH experiments of eukaryotic genes suggest that the transition of the gene from ON to OFF state is a single rate-limiting biochemical step, and the transcriptional bursting is often regulated by modulating the transcription frequency instead of the average duration of the gene on period or the transcription rate [3, 11, 20, 24]. Bartman et al. [3] revealed that the β-globin enhancer predominantly augments transcriptional burst fraction of the β-globin gene, and observed that raising contact frequencies increases bursting frequency but not bursting size in the transcription of the β-globin gene. Fukaya et al. examined transcriptional bursting in living Drosophila embryos. They found different developmental enhancers positioned downstream of synthetic reporter genes produce transcriptional bursts with similar amplitude and duration but generate very different bursting frequencies [11]. Moreover, single cell imaging experiments of luciferase gene in mouse fibrin found that the probability density function of dwell time in the gene OFF state has a local maximum and the dwell time on gene OFF state could be appropriately described by a mixture of exponential distributions, suggesting that multiple gene OFF states may exist during transcriptional bursting [36].

Motivated by these observations, we continue our efforts in [34, 35, 42] to study the fluctuations of mRNA molecules in multiple signaling pathway activated transcription. Our model is a modified version of the classical two-state model where the
initiation of transcription is activated by multiple pathways. As shown in Fig. 1(b),
the initiation of transcription is viewed as a renewal process randomly switching
between gene OFF and gene ON states. We assume that there are \( n \) signaling
pathways, labelled as \( S_1, S_2, \ldots, S_n \), with constant probabilities to activate the
transcription of a target gene. We call these total \( n \) pathways \( S_1, S_2, \ldots, S_n \),
as a firing circuit, labelled as \( A_n \). The selection probabilities \( q_1, q_2, \ldots, q_n \) of these
signaling pathways satisfy

\[
q_i \in (0,1), \quad i = 1, 2, \ldots, n, \quad \text{and} \quad \sum_{i=1}^{n} q_i = 1. \tag{2}
\]

We assume further that:

(A1) When the gene is silent, the signaling pathway \( S_i \) has a constant probability
\( q_i \) to turn on the gene with a constant activation rate \( \lambda_i > 0 \).

(A2) The gene switches from ON state to OFF state with a constant rate \( \gamma > 0 \).

(A3) When the system is in gene ON state, the copy number of mRNA molecules
is controlled by a simple birth and death process with a constant rate \( \nu > 0 \)
and a constant rate \( \delta > 0 \), respectively.

As in Felmer et al. [10], Sun et al. [34, 34], Tang [37, 38], and Yu et al. [42], we
call \( \lambda_i \) the induction strength of signaling pathway \( S_i \) for \( i = 1, 2, \ldots, n \), and \( \gamma \) the
fragility of the gene. By labeling the induction strengths appropriately, we may
assume further that

\[
\lambda_1 < \lambda_2 < \cdots < \lambda_{n-1} < \lambda_n. \tag{3}
\]

2.2. The master equations. For each \( t \geq 0 \), we let the random variable \( X(t) \) be
the state of a target gene. Denote \( X(t) = O_i \) if the gene is OFF but the pathway
\( S_i \) is turned on, and \( X(t) = E \) if the gene is ON. Let \( M(t) \) denote the copy number
of mRNA molecules in a single cell. To describe the master equations, define the
probabilities

\[
P_E(m,t) = \text{Prob}\{M(t) = m, X(t) = E\}, \quad m = 0, 1, 2, \ldots, \tag{4}
\]

and

\[
P_i(m,t) = \text{Prob}\{M(t) = m, X(t) = O_i\}, \quad i = 1, 2, \ldots, n, \quad m = 0, 1, 2, \ldots. \tag{5}
\]

| Initial State | Terminal State | Transition Probability |
|---------------|----------------|------------------------|
| (a) \( (E, m) \) | \( (E, m) \) | \( P_E(m,t) \cdot (1 - \nu h)(1 - \gamma h)(1 - m\delta h) \) |
| (b) \( (E, m + 1) \) | \( (E, m) \) | \( P_E(m + 1, t) \cdot (m + 1)\delta h \) |
| (c) \( (E, m - 1) \) | \( (E, m) \) | \( P_E(m - 1, t) \cdot \nu h \) |
| (d) \( (O_i, m) \) | \( (E, m) \) | \( P_i(m, t) \cdot \lambda_i h \) |

**Table 1.** The initial states at time \( t \) and transition probabilities toward
the terminal state \( (E, m) \) at time \( t + h \). If the gene is ON with \( m \) copies
of the mRNA molecules at \( t + h \), then all of the initial states at time
\( t \), listed in (a), (b), (c), and (d), can reach \( (E, m) \) with a transition
probability of zero or first order of \( h \).

To obtain the time evolution of \( P_E(m,t) \), we assume that the gene is ON with
\( m \) copies of the mRNA molecules at time \( t + h \) for an infinitesimal time increment
Then one of the state transition events in Table 1 occurs during time interval 
\((t, t + h)\). After adding all of the probabilities listed in Table 1, we obtain 
\[ P_E(m, t + h) = P_E(m, t) \cdot (1 - \nu h)(1 - \gamma h)(1 - m\delta h) \]
\[ + P_E(m - 1, t) \cdot \nu h + \sum_{i=1}^{n} P_i(m, t) \cdot \lambda_i h \]
\[ + P_E(m + 1, t) \cdot (m + 1)\delta h. \] (6)

Dividing (6) by \(h\) and letting \(h \to 0\) give
\[ \frac{dP_E(m, t)}{dt} = \sum_{i=1}^{n} \lambda_i P_i(m, t) - \gamma P_E(m, t) + \nu[P_E(m - 1, t) - P_E(m, t)] \]
\[ + \delta[(m + 1)P_E(m + 1, t) - mP_E(m, t)], \] (7)

where \(P_E(-1, t) = 0\) by convention. Similarly, the time evolution of \(P_i(m, t)\) is 
given as
\[ \frac{dP_i(m, t)}{dt} = -\lambda_i P_i(m, t) + q_i \gamma P_E(m, t) + \delta[(m + 1)P_i(m + 1, t) - mP_i(m, t)]. \] (8)

Assume that the gene is OFF within all cells at the initial time \(t = 0\), and the 
residual mRNA molecules are disregarded at \(t = 0\). Then the initial condition reads 
as follows
\[ P_i(0, 0) = q_i, \quad P_i(m, 0) = 0 \text{ for } m > 0, \quad \text{and } P_E(m, 0) = 0 \text{ for } m \geq 0. \] (9)

2.3. The differential equations. The fluctuations of the copy number of mRNA 
molecules \(M(t)\) for the target gene in a single cell have frequently been described 
by the noise \(\eta^2(t)\), the ratio of the variance \(\sigma^2(t)\) to the square of the mean \(m(t)\), 
and the noise strength \(\phi(t)\), the variance normalized by the mean:
\[ \eta^2(t) = \frac{\sigma^2(t)}{m^2(t)} \quad \text{and} \quad \phi(t) = \frac{\sigma^2(t)}{m(t)}, \text{ where } \sigma^2(t) = \mathbb{E}(M^2(t)) - m^2(t). \] (10)

Obviously, we need to obtain the mean \(m(t)\) and the second moment \(\mu_2(t) = \mathbb{E}(M^2(t))\) to determine the noise and the noise strength. To determine the time 
evolution of the mean \(m(t)\), we first introduce the dynamical utilization probability 
of pathway \(S_i\):
\[ P_i(t) = \sum_{m=0}^{\infty} P_i(m, t), \] (11)
and the dynamical transcriptional efficiency:
\[ P_E(t) = \sum_{m=0}^{\infty} P_E(m, t). \] (12)

They are related to \(m(t)\) and \(\mu_2(t)\) by
\[ m(t) = \mathbb{E}(M(t)) = \sum_{i=1}^{n} \sum_{k=0}^{\infty} kP_i(k, t) + \sum_{k=0}^{\infty} kP_E(k, t), \] (13)
and
\[ \mu_2(t) = \mathbb{E}(M^2(t)) = \sum_{i=1}^{n} \sum_{k=0}^{\infty} k^2P_i(k, t) + \sum_{k=0}^{\infty} k^2P_E(k, t). \] (14)
Differentiating (11) and substituting (7) give us that
\[
dP_i(t) \frac{dt}{dt} = -\lambda_i P_i(t) + q_i \gamma P_E(t) + \delta \sum_{k=0}^{\infty} [(k+1)P_i(k+1,t) - kP_i(k,t)].
\] (15)

It follows from (9) that the initial condition of \( P_i(t) \) satisfies \( P_i(0) = q_i \). Therefore, noticing \( \sum_{k=0}^{\infty} [(k+1)P_i(k+1,t) - kP_i(k,t)] = 0 \), we obtain the differential equations
\[
dP_i(t) = -\lambda_i P_i(t) + q_i \gamma P_E(t), \quad P_i(0) = q_i, \quad i = 1, 2, \ldots, n.
\] (16)

Similarly, we obtain
\[
dP_E(t) = \sum_{i=1}^{n} \lambda_i P_i(t) - \gamma_i P_E(t), \quad P_E(0) = 0.
\] (17)

By differentiating (13) with respect to \( t \), and substituting (7) and (8), we find
\[
\frac{dm(t)}{dt} = \nu \sum_{k=0}^{\infty} k[P_E(k-1,t) - P_E(k,t)]
+ \delta \sum_{i=1}^{n} \sum_{k=0}^{\infty} [k(k+1)P_i(k+1,t) - k^2P_i(k,t)]
+ \delta \sum_{k=0}^{\infty} [k(k+1)P_E(k+1,t) - k^2P_E(k,t)].
\] (18)

We also find
\[
\sum_{k=0}^{\infty} k[P_E(k-1,t) - P_E(k,t)] = \sum_{k=0}^{\infty} (k+1)P_E(k,t) - \sum_{k=0}^{\infty} kP_E(k,t)
= \sum_{k=0}^{\infty} P_E(k,t) = P_E(t).
\] (19)

Similarly, we obtain that the last two terms of (18) equals \(-\delta m(t)\). Putting the initial condition (9) into (13) gives \( m(0) = 0 \). These calculations tell us that
\[
\frac{dm(t)}{dt} = -\delta m(t) + \nu P_E(t), \quad m(0) = 0.
\] (20)

Now we are in a position to determine the time evolution of the second moment \( \mu_2(t) \). We introduce the following conditional mean values
\[
m_i(t) = \sum_{k=0}^{\infty} kP_i(k,t), \quad i = 1, 2, \ldots, n, \quad \text{and} \quad m_E(t) = \sum_{k=0}^{\infty} kP_E(k,t).
\] (21)

In terms of (7) and (8), we derive the time evolutions of \( m_E(t) \) and \( m_i(t) \) as
\[
\begin{cases}
\frac{dm_E(t)}{dt} = \sum_{i=1}^{n} \lambda_i m_i(t) - (\gamma + \delta) m_E(t) + \nu P_E(t), \\
\frac{dm_i(t)}{dt} = -\lambda_i m_i(t) + q_i \gamma m_E(t),
\end{cases}
\] (22)
with the initial conditions \( m_E(0) = 0 \) and \( m_i(0) = 0 \) for \( i = 1, 2, \cdots, n \). After multiplying (7) and (8) by \( k^2 \) and taking the sum, we obtain

\[
\frac{d\mu_2(t)}{dt} = \nu \sum_{k=0}^{\infty} k^2[P_E(k-1, t) - P_E(k, t)] \\
+ \delta \sum_{k=0}^{\infty} k^2[(k+1)P_E(k+1, t) - kP_E(k, t)] \\
+ \delta \sum_{i=1}^{n} \sum_{k=0}^{\infty} k^2[(k+1)P_i(k+1, t) - P_i(k, t)].
\]

By using the equality

\[
\sum_{k=0}^{\infty} k^2[(k+1)P_E(k+1, t) - kP_E(k, t)] = \sum_{k=1}^{\infty} [k(k-1)^2P_E(k, t) - k^3P_E(k, t)] \\
= \sum_{k=0}^{\infty} (-2k^2 + k)P_E(k, t),
\]

and the similar ones for \( P_i(k, t) \), we rewrite \( \mu_2'(t) \) as

\[
\frac{d\mu_2(t)}{dt} = \sum_{k=0}^{\infty} \left\{ (-2k^2 + k)\delta \left( \sum_{i=1}^{n} P_i(k, t) + P_E(k, t) \right) \right\} \\
+ \sum_{k=0}^{\infty} \nu k^2[P_E(k-1, t) - P_E(k, t)] \\
= -2\delta \mu_2(t) + \delta m(t) + \nu \sum_{k=0}^{\infty} k^2[P_E(k-1, t) - P_E(k, t)].
\]

We notice that the last sum equals \( \nu P_E(t) + 2\nu m_E(t) \) by definitions (13) and (21), and \( \mu_2(0) = 0 \) by the initial condition (9). Finally we get

\[
\frac{d\mu_2(t)}{dt} = -2\delta \mu_2(t) + \delta m(t) + \nu P_E(t) + 2\nu m_E(t), \quad \mu_2(0) = 0. \quad (23)
\]

3. Results and proofs.

3.1. Analytical expressions of \( m(t) \) and \( \mu_2(t) \). It is clear from (10) that the noise \( \eta^2(t) \) and the noise strength \( \phi(t) \) are completely determined by the mean \( m(t) \) and the second moment \( \mu_2(t) \). To determine the analytical expressions of \( m(t) \) and \( \mu_2(t) \), we firstly need to solve the transcriptional efficiency \( P_E(t) \) defined in (12). It tells us the probability that the gene is active at a time \( t \). We see from (16) and (17) that \( P_E(t) \) can be solved from the initial value problem of the homogeneous linear system

\[
\begin{cases}
\frac{dP_k(t)}{dt} = -\lambda_k P_k(t) + q_k \gamma P_E(t), \\
\frac{dP_E(t)}{dt} = \sum_{i=1}^{n} \lambda_i P_i(t) - \gamma P_E(t),
\end{cases}
\quad (24)
\]
with the initial conditions $P_E(0) = 0$ and $P_k(0) = q_k$ for $k = 1, 2, \cdots, n$, and the
matrix of constant coefficients

$$B = \begin{pmatrix}
-\lambda_1 & 0 & \cdots & 0 & q_1 \gamma \\
0 & -\lambda_2 & \cdots & 0 & q_2 \gamma \\
\vdots & \vdots & \ddots & \vdots & \vdots \\
0 & 0 & \cdots & -\lambda_n & q_n \gamma \\
\lambda_1 & \lambda_2 & \cdots & \lambda_n & -\gamma
\end{pmatrix}.$$  

(25)

It has been obtained in Sun et al. [35] that the set of eigenvalues of the matrix $B$
contains $\alpha_0 = 0$ and $n$ distinct negative numbers, denoted by $-\alpha_1$, $-\alpha_2$, $\cdots$, and
$-\alpha_n$, with

$$\lambda_1 < \alpha_1 < \lambda_2 < \alpha_2 < \cdots < \lambda_{n-1} < \alpha_{n-1} < \lambda_n < \alpha_n.$$  

(26)

**Proposition 1.** The negative eigenvalues $-\alpha_1$, $-\alpha_2$, $\cdots$, and $-\alpha_n$ of the matrix
$B$ satisfy the identity

$$\prod_{i=1}^{n} (x + \alpha_i) = \prod_{i=1}^{n} (x + \lambda_i) \left[ 1 + \sum_{i=1}^{n} \frac{q_i \gamma}{x + \lambda_i} \right].$$  

(27)

It is clear that identity (27) is invariant if $(q_i, \lambda_i)$ interchanges $(q_j, \lambda_j)$ for $1 \leq i, j \leq n$. We mention that identity (27) will play important roles in our later
analysis.

**Proof.** Let $I$ denote the $(n+1) \times (n+1)$ identity matrix. The eigenvalues of $B$ are
the roots of the characteristic equation

$$f_B(x) = x \prod_{i=1}^{n} (x + \alpha_i) = \det(xI - B) = 0.$$

For $1 \leq i \leq n$, by multiplying the $i$-th column of $xI - B$ with $q_i \gamma/(x + \lambda_i)$ and
adding the product to the last column to make the first $n$ entries vanish, we get

$$x \prod_{i=1}^{n} (x + \alpha_i) = (x + \lambda_1)(x + \lambda_2) \cdots (x + \lambda_n) \left[ (x + \gamma) - \sum_{i=1}^{n} \frac{q_i \lambda_i \gamma}{x + \lambda_i} \right]$$

$$= x \prod_{i=1}^{n} (x + \lambda_i) \left[ 1 + \sum_{i=1}^{n} \frac{q_i \gamma}{x + \lambda_i} \right],$$  

(28)

which implies that (27) holds and this finishes the proof of Proposition 1.  

By solving the initial value problem (24), we have found in Sun et al. [35] that

$$P_E(t) = \frac{\lambda_1 \lambda_2 \cdots \lambda_n}{\alpha_1 \alpha_2 \cdots \alpha_n} - \sum_{k=1}^{n} \beta_k e^{-\alpha_k t}, \quad \beta_k = \frac{\sum_{i=1}^{n} q_i \lambda_i \prod_{j \neq i} (\lambda_j - \alpha_k)}{\alpha_k \prod_{j \neq k} (\lambda_j - \alpha_k)}.$$  

(29)

Let $P_E^* = \lim_{t \to \infty} P_E(t)$ denote the steady-state transcriptional efficiency. Then it follows from (27) and (29) that

$$P_E^* = \frac{\lambda_1 \lambda_2 \cdots \lambda_n}{\alpha_1 \alpha_2 \cdots \alpha_n} \left[ 1 + \gamma \sum_{i=1}^{n} \frac{q_i \lambda_i - 1}{\lambda_i} \right]^{-1} = \frac{\gamma^{-1}}{\gamma^{-1} + \sum_{i=1}^{n} q_i \lambda_i^{-1}}.$$  

(30)

By solving the initial value problem (20) and using the exact form of $P_E(t)$ given in (29), the analytical expression of $m(t)$ can be obtained as follows:
Theorem 3.1. Assume that $\delta \neq \alpha_i$ for $i = 1, 2, \cdots, n$. The mean $m(t) = E[M(t)]$ of the copy number of mRNA molecules $M(t)$ takes the form

$$m(t) = \frac{\nu}{\delta} P^*_E - \sum_{k=1}^{n} \frac{\nu \beta_k}{\beta - \alpha_k} e^{-\alpha_k t} - \frac{\nu \sum_{i=1}^{n} q_i \lambda_i \prod_{j \neq i} (\lambda_j - \delta)}{\delta \prod_{j=1}^{n} (\alpha_j - \delta)} e^{-\delta t}. \quad (31)$$

When $\delta$ equals some $\alpha_i$, identity (31) is not well-defined literally. However, we can still use (31) to obtain $m(t)$ by taking limit $\delta \to \alpha_i$.

Proof. We will derive (31) by solving (20) via the Laplace transform. Recall that $f$ for a function $F$ where the numerator $F$ has the partial fraction decomposition

$$L(F(s)) = \int_0^\infty e^{-st} f(t) dt.$$

By using the Laplace transform to equation (20), and noting that $P_E(0) = 0$, we change (20) into an algebraic equation

$$(s + \delta)L(m(t)) = \nu L(P_E(t)). \quad (32)$$

We see from equations (29) and (30) that the Laplace transform of $P_E(t)$ is given as

$$L(P_E(t)) = \frac{P^*_E}{s} + \sum_{j=1}^{n} \frac{\beta_j}{s + \alpha_j} = \frac{P^*_E \prod_{j=1}^{n} (s + \alpha_j) + s \sum_{j=1}^{n} \beta_j \prod_{j \neq i} (s + \alpha_i)}{s \prod_{j=1}^{n} (s + \alpha_j)}. \quad (33)$$

It follows that

$$L(m(t)) = \frac{F_m(s)}{s(s + \delta) \prod_{j=1}^{n} (s + \alpha_j)},$$

where the numerator $F_m(s) = \nu P^*_E \prod_{j=1}^{n} (s + \alpha_j) + \nu s \sum_{j=1}^{n} \beta_j \prod_{j \neq i} (s + \alpha_i)$. Assume that $L(m(t))$ has the partial fraction decomposition

$$L(m(t)) = \frac{a_0}{s} + \frac{b_0}{s + \delta} + \sum_{j=1}^{n} \frac{c_j}{s + \alpha_j}. \quad (34)$$

The undetermined coefficients can be derived from the equation

$$F_m(s) = a_0 (s + \delta) \prod_{j=1}^{n} (s + \alpha_j) + b_0 s \prod_{j=1}^{n} (s + \alpha_j) + s(s + \delta) \sum_{j=1}^{n} c_j \prod_{i \neq j} (s + \alpha_i).$$

Substituting $s = 0$ into this equation gives us that $F_m(0) = a_0 \delta \alpha_1 \alpha_2 \cdots \alpha_n$, from which follows $a_0 = \nu / \delta \cdot P^*_E$. The remaining coefficients can be determined by the similar arguments. We omit the tedious process here. By using the inverse Laplace transform to (34) we derive (31). The proof of Theorem 3.1 is completed. \qed

In what follows, we will give the analytical expression of $\mu_2(t)$. To present the exact form of $\mu_2(t)$ more neatly, for $k = 1, 2, \cdots, n$, we first introduce some notations as follows:

$$d_1 = -\frac{\nu}{\delta} \left[ 1 + \frac{2\nu}{\delta} P^*_E \right] \left[ P^*_E - \sum_{j=1}^{n} \frac{\delta \beta_j}{(\alpha_j - \delta)} \right], \quad (35)$$

$$d_2 = \frac{\nu^2}{\delta \beta} \prod_{j=1}^{n} (\lambda_j - \delta) \left[ P^*_E - \sum_{j=1}^{n} \frac{2\delta \beta_j}{(\alpha_j - 2\delta)} \right], \quad (36)$$
the Laplace transform to equation (39), we transform (39) into an algebraic equation 

\[ a_k = \frac{\nu \beta_k}{\delta - \alpha_k} \left[ 1 + \frac{2\nu}{2\delta - \alpha_k} \prod_{i=1}^{n} \frac{\delta + \lambda_i - \alpha_k}{\delta + \alpha_i - \alpha_k} \right], \quad (37) \]

and 

\[ b_k = \frac{2\nu^2}{\alpha_k(\delta + \alpha_k)(\delta - \alpha_k)} \prod_{i\neq k}(\alpha_i - \alpha_k) \left[ P_E^* - \sum_{j=1}^{n} \frac{(\delta + \alpha_k)\beta_j}{\alpha_j - \delta - \alpha_k} \right], \quad (38) \]

where \( \beta_j \) for \( j = 1, 2, \cdots, n \) are given in equation (29).

By solving the initial value problem (22) and (23) and using the exact forms of \( P_E(t) \) as well as of \( m(t) \), the analytical expression of \( \mu_2(t) \) can be gained as follows:

**Theorem 3.2.** Assume that the conditions of Theorem 3.1 are satisfied. The second moment \( \mu_2(t) = E[M^2(t)] \) of the copy number of mRNA molecules \( M(t) \) takes the form

\[ \mu_2(t) = \frac{\nu}{\delta} P_E^* \left[ 1 + \frac{\nu}{\delta} \prod_{i=1}^{n} \frac{\delta + \lambda_i}{\delta + \alpha_i} \right] + d_1 e^{-\delta t} + d_2 e^{-2\delta t} \]

\[ + \sum_{k=1}^{n} a_k e^{-\alpha_k t} + \sum_{k=1}^{n} b_k e^{-(\delta + \alpha_k) t}, \quad (39) \]

where \( d_1, d_2, a_k, \) and \( b_k \) are given respectively in (35), (36), (37), and (38).

**Proof.** We will obtain (39) from (23) by applying the Laplace transform. By using the Laplace transform to equation (39), we transform (39) into an algebraic equation 

\[ (s + 2\delta)L(\mu_2(t)) = \delta L(m(t)) + \nu L(P_E(t)) + 2\nu L(m_E(t)). \quad (40) \]

To determine \( L(\mu_2(t)) \), we need to obtain \( L(m_E(t)) \). Using the Laplace transform to (22) gives us a system of algebraic equations

\[ \begin{cases} 
(s + \delta + \lambda_i)L(m_i(t)) - q_i \gamma L(m_E(t)) = 0, \\
- \sum_{i=1}^{n} \lambda_i L(m_i(t)) + (s + \delta + \gamma)L(m_E(t)) = \nu L(P_E(t)).
\end{cases} \quad (41) \]

The determinant of the coefficient matrix for this linear system equals

\[ \prod_{i=1}^{n} (s + \delta + \lambda_i) \left[ (s + \delta + \gamma) - \sum_{i=1}^{n} \frac{q_i \gamma}{s + \delta + \lambda_i} \right] \]

\[ = (s + \delta) \prod_{i=1}^{n} (s + \delta + \lambda_i) \left[ 1 + \sum_{i=1}^{n} \frac{q_i \gamma}{s + \delta + \lambda_i} \right]. \]

It follows from (27) that the determinant of the coefficient matrix for the linear system (41) equals

\[ (s + \delta) \prod_{i=1}^{n} (s + \delta + \alpha_i). \]

Hence we find

\[ L(m_E(t)) = \frac{\nu}{s + \delta} \prod_{i=1}^{n} \frac{s + \delta + \lambda_i}{s + \delta + \alpha_i} \cdot L(P_E(t)). \quad (42) \]

In terms of (32), (40), and (42), we obtain

\[ L(\mu_2(t)) = \frac{\nu \left[ (s + 2\delta) \prod_{i=1}^{n} (s + \delta + \alpha_i) + 2\nu \prod_{i=1}^{n} (s + \delta + \lambda_i) \right]}{(s + \delta)(s + 2\delta) \prod_{i=1}^{n} (s + \delta + \alpha_i)} L(P_E(t)). \]
In view of (33), the Laplace transform of \( \mu_2(t) \) can be given as

\[
L(\mu_2(t)) = \frac{F_\mu(s)}{s(s+\delta)(s+2\delta)} \prod_{i=1}^{n}(s+\alpha_i) \cdot \prod_{i=1}^{n}(s+\delta+\alpha_i),
\]

where the numerator \( F_\mu(s) \) equals

\[
\nu \left[ (s+2\delta) \prod_{i=1}^{n}(s+\delta+\alpha_i) + 2\nu \prod_{i=1}^{n}(s+\delta+\lambda_i) \right]
\times \left[ P_\nu^* \prod_{i=1}^{n}(s+\alpha_i) + s \sum_{j=1}^{n} \beta_j \prod_{i \neq j}(s+\alpha_i) \right].
\]

Assume that \( L(\mu_2(t)) \) has the partial fraction decomposition

\[
L(\mu_2(t)) = \frac{d_0}{s} + \frac{d_1}{s+\delta} + \frac{d_2}{s+2\delta} + \sum_{j=1}^{n} \frac{a_j}{s+\alpha_j} + \sum_{j=1}^{n} \frac{b_j}{s+\delta+\alpha_j}.
\] (43)

The undetermined coefficients can be determined by the equality

\[
F_\mu(s) = \prod_{i=1}^{n}(s+\alpha_i)(s+\delta+\alpha_i) \cdot \left[ \sum_{j=0}^{2} d_j \prod_{i \neq j}(s+i\delta) \right]
+ \prod_{i=0}^{2}(s+i\delta) \cdot \prod_{i=1}^{n}(s+\alpha_i) \cdot \left[ \sum_{j=1}^{n} a_j \prod_{i \neq j}(s+\alpha_i) \right]
+ \prod_{i=0}^{2}(s+i\delta) \cdot \prod_{i=1}^{n}(s+\alpha_i) \cdot \left[ \sum_{j=1}^{n} b_j \prod_{i \neq j}(s+\delta+\alpha_i) \right].
\]

Substituting \( s = 0 \) into this equation, it follows that

\[
F_\mu(0) = 2d_0\delta^2 \prod_{i=1}^{n}\alpha_i(\delta+\alpha_i),
\]

from which we have

\[
d_0 = \frac{\nu [2\delta \prod_{i=1}^{n}(\delta+\alpha_i) + 2\nu \prod_{i=1}^{n}(\delta+\lambda_i)] \cdot [P_\nu^* \prod_{i=1}^{n}\alpha_i]}{2\delta^2 \prod_{i=1}^{n}\alpha_i(\delta+\alpha_i)}
= \frac{\nu P_\nu^* \left[ \delta \prod_{i=1}^{n}(\delta+\alpha_i) + \nu \prod_{i=1}^{n}(\delta+\lambda_i) \right]}{\delta^2 \prod_{i=1}^{n}(\delta+\alpha_i)}
= \frac{\nu P_\nu^*}{\delta} \left[ 1 + \frac{\nu \prod_{i=1}^{n}(\delta+\lambda_i)}{\delta \prod_{i=1}^{n}(\delta+\alpha_i)} \right].
\]

The remaining coefficients can be derived by the similar arguments. We omit the tedious calculations. Taking advantage of the inverse Laplace transform to (43) gives us (39). The proof of Theorem 3.2 is completed.

\[\square\]

3.2. Noise regulation and optimization. By using the exact expressions in Theorems 3.1 and 3.2, the analytical properties of transcription fluctuations at steady states can be precisely obtained. These obtained results provide new insights into noise regulation and optimization of stochastic gene transcription. Let \( m^*, \eta^2*, \) and \( \Phi^* \) denote the steady-state (stationary) values of the mean \( m(t) \), the noise
It is clear from (31) that \( m(t) \) approaches exponentially to its constant term:

\[
m^* = \lim_{t \to +\infty} m(t) = \frac{\nu \lambda_1 \lambda_2 \cdots \lambda_n}{\delta \alpha_1 \alpha_2 \cdots \alpha_n} = \frac{\nu}{\delta} P_E^* = \frac{\nu}{\delta} \gamma^{-1} \cdot \frac{\gamma^{-1}}{\sum_{i=1}^{n} q_i \lambda_i}. \tag{44}
\]

Similarly, the stationary second moment \( \mu^*_2 \) can be given as

\[
\mu^*_2 = \lim_{t \to +\infty} \mu_2(t) = \frac{\nu}{\delta} P_E^* \left[ 1 + \frac{\nu}{\delta} \prod_{i=1}^{n} \frac{\delta + \lambda_i}{\delta + \alpha_i} - 1 \right]. \tag{45}
\]

Therefore, it follows from equations (10), (44), and (45) that

\[
\eta^2 = \lim_{t \to +\infty} \eta^2(t) = \frac{\mu^*_2 - m^{*2}}{m^*} = \frac{1}{P_E^*} \left[ \frac{\delta}{\nu} + \prod_{i=1}^{n} \frac{\delta + \lambda_i}{\delta + \alpha_i} - 1 \right] - 1 \tag{46}
\]

and

\[
\Phi^* = \lim_{t \to +\infty} \phi(t) = \frac{\mu^*_2 - m^{*2}}{m^*} = \frac{1}{m^*} + \frac{\gamma \delta}{1 + \gamma \sum_{i=1}^{n} q_i (\delta + \lambda_i)^{-1}} \tag{47}
\]

We see from (27), (30), and (44) that the stationary noise \( \eta^2 = \) and the stationary noise strength \( \Phi^* \) can be rewritten respectively as

\[
\eta^2 = \left( 1 + \gamma \sum_{i=1}^{n} q_i \lambda_i^{-1} \right) \left[ \frac{\delta}{\nu} + \frac{1}{1 + \gamma \sum_{i=1}^{n} q_i (\delta + \lambda_i)^{-1}} \right] - 1 = \frac{\delta}{\nu} - \gamma \sum_{i=1}^{n} q_i \lambda_i^{-1} \left( \sum_{i=1}^{n} q_i \lambda_i^{-1} \right) + \gamma \delta \sum_{i=1}^{n} q_i \left( (\delta + \lambda_i)^{-1} \right) \tag{48}
\]

and

\[
\Phi^* = 1 + \frac{\nu}{\delta} \left[ \frac{1}{1 + \gamma \sum_{i=1}^{n} q_i (\delta + \lambda_i)^{-1}} - \frac{1}{1 + \gamma \sum_{i=1}^{n} q_i \lambda_i^{-1}} \right] = 1 + \frac{\nu \gamma}{1 + \gamma \sum_{i=1}^{n} q_i \lambda_i^{-1}} \cdot \frac{\sum_{i=1}^{n} q_i \lambda_i^{-1} (\delta + \lambda_i)^{-1}}{1 + \gamma \sum_{i=1}^{n} q_i \lambda_i^{-1}}. \tag{49}
\]

3.2.1. Dependence of transcription fluctuations on system parameters. Clearly, we can see from (44) that the mean \( m^* \) increases with \( \nu > 0 \), and decreases with either \( \delta > 0 \) or \( \gamma > 0 \). To explore the characteristics of transcription fluctuations, we first analyze the dynamical dependence of \( \eta^2 \) and \( \Phi^* \) on system parameters of transcription. Our next results show that the noise strength \( \Phi^* \) can develop non-monotonic behaviors by modulating the gene fragility or the activation strengths.

**Theorem 3.3.** Assume that the conditions of Theorem 3.1 are satisfied.

(i) The noise \( \eta^2 \) increases with either \( \delta > 0 \) or \( \gamma > 0 \), and decreases with \( \nu > 0 \).

(ii) The noise strength \( \Phi^* \) increases with \( \nu > 0 \), and decreases with \( \delta > 0 \).

(iii) When \( \gamma > 0 \) varies, \( \Phi^* \) initially increases until reaching the peak uniquely at some \( \gamma^* \), and then decreases thereafter.
(iv) The stationary noise strength $\Phi^*$ is greater than one, with the residual either independent of $\delta$ or being controlled by the product of $\nu/\delta$ and $(1 - P_E^*)$:

$$1 < \Phi^* < 1 + \min \left\{ \frac{\nu \gamma \sum_{i=1}^n q_i \lambda_i^{-2}}{1 + \gamma \sum_{i=1}^n q_i \lambda_i^{-1}} \nu (1 - P_E^*) \right\}.$$  \hfill (50)

(v) The stationary noise $\eta^{2*}$ is greater than the inverse of $m^*$, with the residual being controlled by $(1 - P_E^*)/P_E^*$:

$$\frac{1}{m^*} < \eta^{2*} < \frac{1}{m^*} + \frac{1 - P_E^*}{P_E^*}.$$  \hfill (51)

In Theorem 3.3, we derive the optimal gene fragility $\gamma^*$ at which the noise strength enhancement is maximal by regulating $\gamma$. In this case, as the fragility $\gamma$ varies, the noise strength initially increases, reaches a peak at $\gamma = \gamma^*$, and subsequently decreases. We also determine the boundedness of $\eta^{2*}$ and $\Phi^*$. Our estimate (51) indicates that a very rare transcription ($m^* \ll 1$) can cause a large value of $\eta^{2*}$. No matter how many pathways co-regulate the initiation of transcription, as long as the gene is strongly activated ($P_E^* \approx 1$), $\eta^{2*}$ must be close to the inverse of $m^*$.

We see from (50) that $\Phi^* \approx 1$ when the gene is stably transcribed ($\gamma \ll 1$), or the gene is strongly activated ($P_E^* \approx 1$). In this case, the mRNA production can be rich. We also see from (50) that $\Phi^* \approx 1$ when the gene is ineffectively synthesized ($\nu/\delta \ll 1$), or the gene is too fragile to be transcribed ($\gamma \gg 1$). In this case, the mRNA production can be rare. These two opposite extreme cases may provide insights on explaining why $\Phi^* \approx 1$ has been often observed in experiments [6, 31].

**Proof.** Since both $\delta/\nu$ and $1/[1 + \gamma \sum_{i=1}^n q_i (\delta + \lambda_i)^{-1}]$ increase with $\delta > 0$, it follows from the first equation of (48) that $\eta^{2*}$ increases with $\delta > 0$. Similarly, we see from the second equation of (48) that $\eta^{2*}$ increases with $\gamma > 0$, and decreases with $\nu > 0$. This completes the proof of claim (i) in Theorem 3.3.

It is clear from (49) that $\Phi^*$ increases with $\nu > 0$. Next, we will prove $\Phi^*$ decreases with $\delta > 0$. To do so, we need to prove

$$\frac{\partial}{\partial \delta} \left[ \sum_{i=1}^n \frac{q_i \lambda_i^{-1}}{1 + \gamma \sum_{i=1}^n q_i (\delta + \lambda_i)^{-1}} \right] = \frac{G}{(1 + \gamma \sum_{i=1}^n q_i (\delta + \lambda_i)^{-1})^2} < 0,$$  \hfill (52)

where the numerator $G$ equals

$$G = - \sum_{1 \leq i < j \leq n} \frac{\gamma q_i q_j (\lambda_i - \lambda_j)^2}{\lambda_i \lambda_j (\delta + \lambda_i)^2 (\delta + \lambda_j)^2} - \sum_{i=1}^n \frac{q_i}{\lambda_i (\delta + \lambda_i)^2}.$$  

After the tedious calculations, we find that

$$G = - \sum_{1 \leq i < j \leq n} \frac{\gamma q_i q_j (\lambda_i - \lambda_j)^2}{\lambda_i \lambda_j (\delta + \lambda_i)^2 (\delta + \lambda_j)^2} - \sum_{i=1}^n \frac{q_i}{\lambda_i (\delta + \lambda_i)^2} < 0.$$  

Thus the inequality in (52) holds and the proof of claim (ii) in Theorem 3.3 is finished.

We can see from (49) that

$$\frac{\partial \Phi^*}{\partial \gamma} = \nu \sum_{i=1}^n \frac{q_i}{\lambda_i (\delta + \lambda_i)} \cdot \frac{1 - \gamma^2 \sum_{i=1}^n q_i \lambda_i^{-1} \cdot \sum_{i=1}^n q_i (\delta + \lambda_i)^{-1}}{(1 + \gamma \sum_{i=1}^n q_i \lambda_i^{-1})(1 + \gamma \sum_{i=1}^n q_i (\delta + \lambda_i)^{-1})^2}.$$
It follows that $\partial \Phi^*/\partial \gamma = 0$ if and only if $\gamma = \gamma^*$ where

$$\gamma^* = \frac{1}{\sqrt{\sum_{i=1}^{n} q_i \lambda_i^{-1} \cdot \sum_{i=1}^{n} q_i (\delta + \lambda_i)^{-1}}}.$$ (53)

and $\partial \Phi^*/\partial \gamma > 0$ for $\gamma \in (0, \gamma^*)$, and $\partial \Phi^*/\partial \gamma < 0$ for $\gamma > \gamma^*$. Therefore, $\Phi^*$ peaks uniquely at $\gamma^*$, increases for $\gamma \in (0, \gamma^*)$, and decreases for $\gamma > \gamma^*$. This finishes the proof of claim (iii) in Theorem 3.3.

It is clear from (48) and (49) that

$$\frac{1}{m^*} < \eta^2*$$ and $1 < \Phi^*$. (54)

We can see from (27) that

$$\prod_{i=1}^{n} \frac{\delta + \lambda_i}{\delta + \alpha_i} = \frac{1}{1 + \sum_{i=1}^{n} q_i \gamma (\delta + \lambda_i)^{-1}}$$ (55)

strictly increases with $\delta$. Thus it follows from (47) and (55) that

$$\Phi^* < 1 + \frac{\nu}{\delta} \left[ \lim_{\delta \to \infty} \frac{1}{1 + \sum_{i=1}^{n} q_i \gamma (\delta + \lambda_i)^{-1}} - P_E^* \right]$$

$$= 1 + \frac{\nu}{\delta} \left[ 1 - P_E^* \right].$$ (56)

Since $\Phi^*$ decreases with $\delta$, in view of (49), we have

$$\Phi^* < 1 + \frac{\nu \gamma}{1 + \gamma \sum_{i=1}^{n} q_i \lambda_i^{-1}} \cdot \lim_{\delta \to 0} \frac{1}{1 + \gamma \sum_{i=1}^{n} q_i (\delta + \lambda_i)^{-1}}$$

$$= 1 + \frac{\nu \gamma}{1 + \gamma \sum_{i=1}^{n} q_i \lambda_i^{-2}}.$$ (57)

In terms of (54), (56), and (57), the estimate in (50) holds. This finishes the proof of claim (iv) in Theorem 3.3. We can also see from (10), (44), and (57) that

$$\eta^2* = \frac{\Phi^*}{m^*} < \frac{1}{m^*} + \frac{1 - P_E^*}{P_E^*},$$

and hence (51) follows. The proof of Theorem 3.3 is completed.

**Theorem 3.4.** Assume that the conditions of Theorem 3.1 are satisfied.

(I) The noise strength $\Phi^*$ decreases with $\lambda_1 \in (0, \lambda_2)$.

(II) If $n = 2$, then when $\lambda_2$ varies, $\Phi^*$ reaches the minimum uniquely at some $\lambda_2^*$, decreases for $\lambda_2 \in (\lambda_1, \lambda_2^*)$, and increases for $\lambda_2 > \lambda_2^*$.

(III) Let $n > 2$. For $\lambda_j \in (\lambda_{j-1}, \lambda_{j+1})$, $j = 2, 3, \cdots, n - 1$, we have:

(i) If the inequality

$$\sum_{i=1, i \neq j}^{n} q_i (\lambda_{j-1} - \lambda_i) \geq \frac{1}{\gamma} \lambda_i (\delta + \lambda_i)$$ (58)

holds, then $\Phi^*$ increases with $\lambda_j \in (\lambda_{j-1}, \lambda_{j+1})$.

(ii) If the inequality

$$\sum_{i=1, i \neq j}^{n} q_i (\lambda_{j+1} - \lambda_i) \leq \frac{1}{\gamma} \lambda_i (\delta + \lambda_i)$$ (59)
holds, then $\Phi^*$ decreases with $\lambda_j \in (\lambda_{j-1}, \lambda_{j+1})$.

(iii) If the inequality

$$\frac{n}{\lambda_i(\delta + \lambda_i)} \leq \frac{1}{\gamma} \leq \frac{n}{\lambda_i(\delta + \lambda_i)}$$

holds, then when $\lambda_j$ varies, $\Phi^*$ reaches the minimum uniquely at some $\lambda_j^*$, decreases for $\lambda_j \in (\lambda_{j-1}, \lambda_j^*)$, and increases for $\lambda_j \in (\lambda_j^*, \lambda_{j+1})$.

(iv) If $n > 2$, then when $\lambda_n$ varies, $\Phi^*$ reaches the minimum uniquely at some $\lambda_n^*$, decreases for $\lambda_n \in (\lambda_{n-1}, \lambda_n^*)$, and increases for $\lambda_n > \lambda_n^*$, by providing

$$\sum_{i=1, i \neq j}^{n-2} \frac{q_i(\lambda_{n-1} - \lambda_i)}{\lambda_i(\delta + \lambda_i)} < \frac{1}{\gamma}$$

If $n > 2$ and inequality (61) reverses, then $\Phi^*$ increases with $\lambda_n \in (\lambda_{n-1}, \infty)$.

Theorem 3.4 indicates that the gene fragility $\gamma$ plays important roles in the determination of the dynamical dependence of $\Phi^*$ on the activation strengths. If the left side of (58) is positive and the gene is too fragile to be transcribed ($\gamma \gg 1$), then inequality (58) holds, and hence $\Phi^*$ increases with $\lambda_j \in (\lambda_{j-1}, \lambda_j^*)$ for $j = 2, 3, \ldots, n-1$. If the gene is stably transcribed ($\gamma \ll 1$), then inequality (59) holds, and hence $\Phi^*$ decreases with $\lambda_j \in (\lambda_{j-1}, \lambda_j^*)$. If the gene fragility is normal, as shown in inequality (60), then the optimal induction strength $\lambda_j^*$ at which the noise strength reduction is maximal can be found. In this case, as the activation strength $\lambda_j$ varies, the noise strength first decreases until reaching a minimum value at $\lambda_j = \lambda_j^*$ and then increases.

It is seen from Theorem 3.4 that, if the transcription is activated by two cross-talking pathways, or $n = 2$, then $\Phi^*$ develops non-monotonic behavior as the strongest activation strength varies. However, if the transcription is activated by more than two pathways, or $n > 2$, then $\Phi^*$ increases with the strongest activation strength when $\gamma \gg 1$. The obtained result indicates that there are differences between the multiple pathways ($n > 2$) and the two cross-talking ones ($n = 2$).

We further show our results in Fig. 2, where there exist four entangling pathways to activate the gene transcription, or $n = 4$, and we fix $\lambda_1 = 0.15, \lambda_2 = 0.26, \lambda_3 = 10.18, \nu = 2.1$, and $\delta = 1$. We assume that the selection probabilities of these signaling pathways $q_1 = 0.3, q_2 = 0.25, q_3 = 0.15, q_4 = 0.3$. We note in this case that

$$\sum_{i=1, i \neq 3}^{4} \frac{q_i(\lambda_2 - \lambda_i)}{\lambda_i(\delta + \lambda_i)} \approx 0.165 \quad \text{and} \quad \sum_{i=1, i \neq 3}^{4} \frac{q_i(\lambda_4 - \lambda_i)}{\lambda_i(\delta + \lambda_i)} \approx 25.014.$$
Figure 2. Different dynamical behaviors of the noise strength $\Phi^*$ on $\lambda_3$. The three curves are generated by the analytical form (47) with $n = 4$, $q_1 = 0.3$, $q_2 = 0.25$, $q_3 = 0.15$, $q_4 = 0.3$, $\lambda_1 = 0.15$, $\lambda_2 = 0.26$, $\lambda_4 = 10.18$, $\nu = 2.1$, $\delta = 1$, and $\gamma$ respectively equals 10.65, 0.03, and 0.56 in (a), (b), and (c).

Proof. It follows from (47) that

$$\Phi^* = 1 + \frac{\nu}{\delta} \left[ \frac{1}{1 + \gamma \sum_{i=1}^{n} q_i (\delta + \lambda_i)^{-1}} - \frac{1}{1 + \gamma \sum_{i=1}^{n} q_i \lambda_i^{-1}} \right].$$

After differentiating $\Phi^*$ with respect to $\lambda_j$ for $j = 1, 2, \cdots, n$, and then doing some simplifications, we find

$$\frac{\partial \Phi^*}{\partial \lambda_j} = \frac{\nu \gamma q_j}{\delta} \left[ \frac{(A + B) \lambda_j + 2 \gamma q_j + A \delta}{[(B - A) \lambda_j - A \delta][A(\delta + \lambda_j) + \gamma q_j]^2[B \lambda_j + \gamma q_j]^2} \right],$$

where

$$A = 1 + \gamma \sum_{i \neq j} q_i (\delta + \lambda_i)^{-1} \quad \text{and} \quad B = 1 + \gamma \sum_{i \neq j} q_i \lambda_i^{-1}.$$

It follows that $\partial \Phi^*/\partial \lambda_j = 0$ if and only if $\lambda_j = \lambda_j^*$ where

$$\lambda_j^* = \frac{\gamma^{-1} + \sum_{i \neq j} q_i (\delta + \lambda_i)^{-1}}{\sum_{i \neq j} q_i \lambda_i^{-1}(\delta + \lambda_i)^{-1}}, \quad (62)$$
and \( \partial \Phi^*/\partial \lambda_j < 0 \) for \( \lambda_j \in (0, \lambda_j^*) \), and \( \partial \Phi^*/\partial \lambda_j > 0 \) for \( \lambda_j > \lambda_j^* \). Since \( \lambda_j \in (\lambda_j-1, \lambda_j+1) \), to determine the dependence of \( \Phi^* \) on \( \lambda_j \), we need to examine the location of \( \lambda_j^* \) relative to the interval \( (\lambda_j-1, \lambda_j+1) \), where \( \lambda_0 = 0 \) and \( \lambda_{n+1} = +\infty \) by convention.

When \( j = 1 \), it is clear that

\[
\lambda_1^* = \frac{\gamma^{-1} + \sum_{i=2}^{n} q_i (\delta + \lambda_i)^{-1}}{\sum_{i=2}^{n} \gamma_i (\delta + \lambda_i)^{-1}}.
\]

We can see from (3) that

\[
\lambda_2 \sum_{i=2}^{n} q_i \lambda_i^{-1} (\delta + \lambda_i)^{-1} < \sum_{i=2}^{n} q_i (\delta + \lambda_i)^{-1} < \frac{1}{\gamma} + \sum_{i=2}^{n} q_i (\delta + \lambda_i)^{-1},
\]

which implies \( \lambda_2 < \lambda_1^* \), and so is \( \partial \Phi^*/\partial \lambda_1 < 0 \) for \( \lambda_1 \in (0, \lambda_2) \). Thus \( \Phi^* \) decreases with \( \lambda_1 \in (0, \lambda_2) \). The proof for the remaining cases can be done by the similar arguments. We omit the elementary and tedious process here. The proof of Theorem 3.4 is completed.

3.2.2. Dependence of transcription fluctuations on \( P_E^* \) and \( m^* \). It is seen from identity (44) that the stationary mean \( m^* \) is linearly dependent on the transcriptional efficiency \( P_E^* \). However, it is seen from identities (46) and (47) that the transcription noise can be nonlinearly dependent on \( P_E^* \) or \( m^* \). Naturally, it is of interest to study the relation between transcription noise and \( P_E^* \) or \( m^* \) for our multiple pathway model. Here comes the result.

**Theorem 3.5.** (i) Let \( \gamma > 0 \) change \( P_E^* \) or \( m^* \), i.e., \( P_E^* \) is \( P_E^*(\gamma) \) or \( m^* = m^*(\gamma) \), and the other transcription parameters be fixed. When \( P_E^* > 0 \) \( (m^* > 0) \) varies, \( \Phi^* \) peaks at some \( P_0 \) \( (m_0^* = \nu/\delta \cdot P_0) \), increases for \( P_E^* \in (0, P_0) \) \( (m^* \in (0, m_0^*)) \), and decreases for \( P_E^* > P_0 \) \( (m^* > m_0^*) \). Specially, when \( P_E^* \geq 0.5 \) \( (m^* \geq 0.5\nu/\delta) \), \( \Phi^* \) decreases with \( P_E^* \) \( (m^*) \).

(ii) Let \( \lambda_j > 0 \) change \( P_E^* \) or \( m^* \) for \( j = 1, 2, \cdots, n \), i.e., \( P_E^* = P_E^*(\lambda_j) \) or \( m^* = m^*(\lambda_j) \), and the other transcription parameters be fixed. When \( P_E^* > 0 \) \( (m^* > 0) \) varies, \( \Phi^* \) peaks at some \( P_j \) \( (m_j^* = \nu/\delta \cdot P_j) \), increases for \( P_E^* \in (0, P_j) \) \( (m^* \in (0, m_j^*)) \), and decreases for \( P_E^* > P_j \) \( (m^* > m_j^*) \).

The complex relation between the transcription noise strength \( \Phi^* \) and the transcriptional efficiency \( P_E^* \) or the mean transcription level \( m^* \) has been explored in some studies [4, 18, 31, 33]. It has been respectively observed by Blake et al. [4] and Sanchez et al. [31] in yeast that \( \Phi^* \) initially grows up until reaching its peak and decays down thereafter when \( P_E^* \) increases. It has also been respectively observed the similar up-down dependance of \( \Phi^* \) on \( m^* \) in *Escherichia coli* by Jones et al. [18] and So et al. [33]. It is clear that both \( P_E^* \) and \( m^* \) increase as either \( \gamma \) decreases or \( \lambda_j \) increases. In addition, Theorem 3.5 tells us that \( \Phi^* \) first increases and then decreases with either \( P_E^* \) or \( m^* \). Taken together, it suggests that the gene fragility and the induction strength may play parallel roles in the up-down dependence of \( \Phi^* \) on \( P_E^* \) or \( m^* \). Since \( \gamma \leq \lambda_1 \) implies that \( P_E^* > 0.5 \) and \( m^* \geq 0.5\nu/\delta \), we see from Theorem 3.5 (i) that, if \( \gamma \leq \lambda_1 \), then \( \Phi^* \) decreases with either \( P_E^* \geq 0.5 \) or \( m^* \geq 0.5\nu/\delta \). This result suggests that increasing the number of either transcribing cells or transcripts by down-regulating the gene fragility will bring down transcription noise if all the induction strengths of pathways keep surpassing the gene fragility.
FLUCTUATIONS BY MULTIPLE PATHWAYS

Figure 3. Nonlinear dependance of the noise strength $\Phi^*$ on $P^*_E$.
The up and down dependance curve of $\Phi^*$ for $0.005 < P^*_E < 0.997$ is
generated by varying $\gamma$ from 0.0001 to 5.5, where $\lambda_1 = 0.015$, $\lambda_2 = 1.53$,
$\nu = 7.5$, $\delta = 0.1$, and $q_1 = q_2 = 0.5$.

We further show the nonlinear dependance of the noise strength $\Phi^*$ on $P^*_E$ in
Fig. 3, where there exist two cross-talking pathways to activate the gene transcrip-
tion, or $n = 2$, and we fix $\lambda_1 = 0.015$, $\lambda_2 = 1.53$, $\nu = 7.5$, $\delta = 0.1$, and $q_1 = q_2 = 0.5$.
To obtain a wider range of $P^*_E$ within the limit $[0, 1]$, we let $\gamma$ vary from 0.0001 to 5.5.
Both numerical simulation in Fig. 3 and Theorem 3.5 show that $\Phi^*$ first increases
and then decreases with $P^*_E$.

Proof. In view of (44), we just need to verify the dependence of $\Phi^*$ on the trans-
scriptional efficiency $P^*_E$. Equation (30) gives us

$$\gamma = \frac{P^*_E - 1}{\sum_{i=1}^n q_i \lambda_i^{-1}},$$

from which it follows that

$$\frac{\partial \gamma}{\partial P^*_E} = -\frac{1}{P^*_E \sum_{i=1}^n q_i \lambda_i^{-1}}.$$

After substituting (55) into (47) and then differentiating (47) with respect to $P^*_E$,
we obtain

$$\frac{\partial \Phi^*}{\partial P^*_E} = \frac{\nu}{\delta} \left[ \frac{\partial\gamma}{\partial P^*_E} \left[ \frac{1 \gamma \sum_{i=1}^n q_i (\delta + \lambda_i)^{-1}}{1 + \gamma \sum_{i=1}^n q_i (\delta + \lambda_i)^{-1}} - 1 \right] \right].$$

We note that

$$\frac{\partial \gamma}{\partial P^*_E} \cdot \frac{\partial}{\partial \gamma} \left[ \frac{1 \gamma \sum_{i=1}^n q_i (\delta + \lambda_i)^{-1}}{1 + \gamma \sum_{i=1}^n q_i (\delta + \lambda_i)^{-1}} \right].$$
the induction strength \( \lambda \) the transcription of the same gene can be also activated by a single pathway with activated transcription to the case with a single pathway activated transcription. If \( \nu/\delta \) is encouraging to compare models that produce the same mean transcription level.

3.3. **Multiple pathways induce noisier transcription.** It is seen from our estimate (51) that the large change of \( \eta^2* \) can be contributed to the large change of \( m^* \). Then, to determine the impact of multiple pathways on transcription noise, it is encouraging to compare models that produce the same mean transcription level. To address this issue, for a target gene with fixed fragility \( \gamma \) and effective transcription synthesis rate \( \nu/\delta \), we will compare its noise for the case with multiple pathway activated transcription to the case with a single pathway activated transcription. If the transcription of the same gene can be also activated by a single pathway with the induction strength \( \lambda \), then it follows from (44) that the gene will give rise to the same \( m^* \) if and only if

\[
\frac{1}{\lambda} = \frac{q_1}{\lambda_1} + \frac{q_2}{\lambda_2} + \cdots + \frac{q_n}{\lambda_n}. \tag{63}
\]

We let \( \eta^2* \) and \( \Phi^*_1 \) respectively denote the stationary noise and the stationary noise strength for the gene with the single pathway activated transcription. The following result indicates that multiple pathways tend to induce noisier transcription.

**Theorem 3.6.** For a target gene with fixed fragility \( \gamma \) and effective transcription synthesis rate \( \nu/\delta \), if identity (63) holds, then

\[
\eta^2* > \eta^2_1 \quad \text{and} \quad \Phi^* > \Phi^*_1. \tag{64}
\]

**Proof.** Clearly, it is seen from (63) that the activations by the single pathway as well as the multiple pathways respectively give rise to the same \( m^* \). We will just
prove the second inequality $\Phi^* > \Phi_1^*$ in (69), since $\eta^{2*} > \eta_2^{2*}$ follows from $\Phi^* > \Phi_1^*$ immediately by the definition of $\eta^{2*}$. Equation (47) gives us that

$$\Phi^* = 1 + \frac{\nu}{\delta} \left[ \frac{1}{1 + \gamma \sum_{i=1}^{n} q_i (\delta + \lambda_i)^{-1}} - \frac{1}{1 + \gamma \lambda^{-1}} \right],$$

from which it follows by taking limit $\lambda_i \to \lambda$ that

$$\Phi_1^* = 1 + \frac{\nu}{\delta} \left[ \frac{1}{1 + \gamma (\delta + \lambda)^{-1}} - \frac{1}{1 + \gamma \lambda^{-1}} \right].$$

In terms of the above two equations, we find

$$\Phi^* > \Phi_1^* \iff \frac{1}{\delta + \lambda} > \sum_{i=1}^{n} \frac{q_i}{\delta + \lambda_i}.$$  

We note that the last inequality holds if and only if

$$\frac{1}{\delta \lambda^{-1} + 1} < \sum_{i=1}^{n} \frac{q_i}{\delta \lambda_i^{-1} + 1}. \quad (65)$$

Since the function $f(x) = 1/(\delta x + 1)$ is convex for $x > 0$, it follows from relations (2), (3), and (63) by using the property of convex function that inequality (65) holds, and so is $\Phi^* > \Phi_1^*$. This completes the proof of Theorem 3.6. \hfill \square

For the target gene with fixed fragility $\gamma$ and effective transcription synthesis rate $\nu/\delta$, we assume that its transcription can also be activated by another firing circuit $A_N$ of $N$ pathways, with the number $N$ of the pathways satisfying

$$n > N > 1,$$  

and the induction strength $\Lambda_j$ and the corresponding pathway probability $Q_j$ of the $j$-th pathway for $j = 1, 2, \cdots, N$. It is desirable to compare transcription noise for the transcription being activated respectively by the two distinct firing circuits $A_n$ and $A_N$. Motivated by Theorem 3.6, we are interested in the following question: In which case the activation by $A_n$ will create noisier transcription? It is very involved in the choice of the transcription parameters. We will examine the following case.

We assume that the total $n$ pathways of the circuit $A_n$ can be classified into non-overlapping $N$ groups, labelled as $G_1, G_2, \cdots, G_N$, and so that the probability $\sum_{i \in G_j} q_i$ of the activation by the group $G_j$ equals the probability $Q_j$ of the $j$-th pathway of the circuit $A_N$ for $j = 1, 2, \cdots, N$, i.e.,

$$\{1, 2, \cdots, n\} = \bigcup_{j=1}^{N} G_j, \quad G_j \bigcap G_k = \emptyset \text{ if } j \neq k,$$

and

$$Q_j = \sum_{i \in G_j} q_i \text{ for } j = 1, 2, \cdots, N. \quad (67)$$

To satisfy the constraint that the same stationary transcription level $m^*$ will be produced, we assume further that

$$\frac{Q_j}{\Lambda_j} = \sum_{i \in G_j} \frac{q_i}{\lambda_i}, \quad j = 1, 2, \cdots, N. \quad (68)$$

**Proposition 2.** For a target gene with fixed fragility $\gamma$ and effective transcription synthesis rate $\nu/\delta$, if conditions (66), (67), and (68) hold, then

$$\eta^{2*} > \eta_2^{2*} \text{ and } \Phi^* > \Phi_1^*.$$  

(69)
where $\eta_N^2$ and $\Phi^*_N$ denote respectively the stationary noise and the stationary noise strength in the $N$ pathway system.

The proof of Propositions 2 is similar to that of Theorem 3.6 and hence we omit it to avoid repetition. We can see from Theorem 3.6 that multiple pathways tend to increase transcription noise with the constraint that the mean transcription level is fixed. It is of interest to examine the behaviors of noise as the induction strengths of multiple pathways vary. Assume for mathematical simplicity that the transcription of the target gene is regulated by two cross-talking pathways, or $n = 2$. We further assume that the induction strengths $\lambda_1$ and $\lambda_2$ are changed subject to the constraint that the mean $m^*$ is fixed. Our next result shows that the monotonic behaviors of both $\Phi^*$ and $\eta^2$ as the strength $\lambda_2$ or $\lambda_1$ varies, with the condition that the mean $m^*$ is fixed.

**Proposition 3.** Let $n = 2$. For a target gene with fixed fragility $\gamma$, effective transcription synthesis rate $\nu/\delta$, and pathway probability $q_1$, if the mean $m^*$ is fixed, then both $\Phi^*$ and $\eta^2$ increase with the stronger induction strength $\lambda_2 > \lambda_1$.

![Figure 4](image)

**Figure 4.** Distinct dynamical behaviors of the noise strength $\Phi^*$ on $\lambda_2$ with or without the constraint on the mean $m^*$. The two curves are generated by the analytical form (47) with $n = 2$, $\gamma = 0.3$, $\nu = 15.4$, $\delta = 1$, and $q_1 = q_2 = 0.5$. (a) The mean transcriptional level is fixed as $m^* = 4.5$. (b) No constraint on the mean $m^*$ and $\lambda_1 = 0.0856$.

It is interesting to compare the conclusions for our Proposition 3 and Theorem 3.4. In Theorem 3.4, if $n = 2$, then when the stronger strength $\lambda_2 \in (\lambda_1, +\infty)$ varies, $\Phi^*$ shows the non-monotonic behavior: $\Phi^*$ first decreases until reaching the uniquely valley and then goes up. However, Proposition 3 indicates that $\Phi^*$ of two cross-talking pathways develops only increasing behavior with the constraint that the mean $m^*$ is fixed, while $\Phi^*$ of the activation by a single pathway remains unchanged. Thus the obtained results suggest that the choice of reference model with a proper constraint plays a significant role in the regulations of transcription noise.

We further show our results in Fig. 4, where there has two cross-talking pathways, or $n = 2$, and we fix $\gamma = 0.3$, $\nu = 15.4$, $\delta = 1$, and $q_1 = q_2 = 0.5$. We can see from Fig. 4(a) that $\Phi^*$ increases with the stronger induction strength $\lambda_2$ for the case where the mean transcriptional level is fixed as $m^* = 4.5$. On the other hand,
for the case without the constraint on the mean $m^*$, as shown in Fig. 4(b), $\Phi^*$ first decreases until reaching the uniquely valley and then goes up when the stronger strength $\lambda_2 > \lambda_1 = 0.0856$ varies.

Proof. Since the mean $m^*$ is fixed and $\eta^{2*} = \Phi^{*}/m^*$, if $\Phi^*$ increases with $\lambda_2$, then so does $\eta^{2*}$. We just need to prove the claim for $\Phi^*$. It follows from (44) that

$$\lambda_1 = \frac{q_1 \lambda_2}{a \lambda_2 - q_2} > 0, \quad a = \frac{\nu}{\delta m^*\gamma} - \frac{1}{\gamma} > 0. \quad (70)$$

As $\lambda_2 > \lambda_1$, it is clear from (70) that

$$1 - a \lambda_2 < 0. \quad (71)$$

We note that

$$\frac{\partial \Phi^*}{\partial \lambda_2} > 0 \iff \frac{\partial H}{\partial \lambda_2} < 0, \quad H = \frac{q_1}{\delta + \lambda_1} + \frac{q_2}{\delta + \lambda_2}. \quad (72)$$

After substituting the identity of $\lambda_1$ in (70) into the expression of $H$, and differentiating $H$ with respect to $\lambda_2$, and doing some simple calculations, it follows from (71) that

$$\frac{\partial H}{\partial \lambda_2} = \frac{q_2 \delta (1 - a \lambda_2)[q_1 (\delta + 2 \lambda_2) + \delta (a \lambda_2 - q_2)]}{(\delta + \lambda_2)^2 [\delta (a \lambda_2 - q_2) + q_1 \lambda_2]^2} < 0. \quad (73)$$

This verifies relation (72) and hence $\Phi^*$ increases with $\lambda_2 > \lambda_1$. The proof of Propositions 3 is finished.

4. Conclusion and discussion. Gene transcription is a discontinuous and stochastic process [28, 33]. The inherent noise of gene transcription can result in fluctuations of mRNA levels and lead to substantial phenotypic plasticity in the isogenic populations of cells. Recent experimental studies indicate that the transcription of common genes in physiological processes such as development and immunity is often activated by multiple signal transduction pathways [12, 15, 32], and the multiple pathways play important roles in the regulation of noise in stochastic gene transcription. Previous theoretical studies on fluctuations in gene transcription have been emphasized on exact solutions and the analysis for models with a single pathway [26, 29, 33, 40] or two cross-talking pathways [30, 34, 35, 42]. However, for stochastic models of gene expression with more than two pathways, exact analytical results for the fluctuations of mRNA levels have not been obtained so far. Motivated by these observations, we study the fluctuations of mRNA molecules in multiple signaling pathway activated transcription.

In Theorems 3.1 and 3.2, the exact dynamical mean value and second moment of the copy number of mRNA molecules are respectively derived, which allows us to determine the dynamical as well as the steady-state noise and noise strength. Our obtained exact solutions can be reduced to previous ones in limiting cases and can provide a basis for further analysis of the key features of transcription fluctuations.

In Theorem 3.3, we derive the optimal fragility $\gamma^*$ at which the noise strength enhancement is maximal by regulating the gene fragility $\gamma$. In this case, as the fragility $\gamma$ varies, the noise strength $\Phi^*$ initially increases, reaches a peak at $\gamma = \gamma^*$, and subsequently decreases. We also can see from Theorem 3.3 that, the noise strength $\Phi^*$ will be close to one when the gene is stably transcribed or strongly activated which leads to the rich mRNA production, or when the gene is ineffectively synthesized or unstably transcribed which leads to the rare mRNA production.
These two opposite extreme cases may partly explain why $\Phi^* \approx 1$ has been often observed in experiments [6, 31].

Theorem 3.4 indicates that the fragility $\gamma$ plays an important role in the determination of the dynamical dependence of $\Phi^*$ on the induction strengths $\lambda^*_j$ for $j = 1, 2, \cdots, n$. If the gene fragility is normal and is located in a certain parameter region, then the optimal strength $\lambda^*_j$ at which the noise strength reduction is maximal can be found. Theorem 4 also suggests that, if the transcription is activated by two cross-talking pathways, or $n = 2$, then $\Phi^*$ develops non-monotonic behavior as the strongest induction strength varies. However, if the transcription is activated by more than two pathways, or $n > 2$, then $\Phi^*$ shows only increasing behavior as the strongest induction strength varies. These results indicate that there are differences between multiple pathways ($n > 2$) and two cross-talking ones ($n = 2$).

Theorems 3.3 and 3.4 show us the non-monotonous response of transcription noise to model parameters in multiple pathway activation model. Similar non-monotonous fashion also has been predicted in stochastic gene expression models with auto-regulation or bursting at the protein level [5, 19]. We hope to examine further the nonlinear fluctuations of stochastic gene expression by varying the underlying parameters in more general cases when auto-regulation or bursting at the protein level is involved in multiple pathway model.

The complex relation between noise strength $\Phi^*$ and transcriptional efficiency $P^*_E$ or mean mRNA level $m^*$ has been examined in some studies [4, 18, 31, 33]. It has been respectively observed the up and down dependance of $\Phi^*$ on $P^*_E$ [4, 31] and $m^*$ [18, 33]. Our Theorem 3.5 tells us that, $\Phi^*$ first increases and then decreases with either $P^*_E$ or $m^*$, as the gene fragility $\gamma$ varies. It may partially explain the mechanism of the up-down dependance of $\Phi^*$ on $P^*_E$ or $m^*$. The obtained result suggests that, if all the induction strengths of pathways keep surpassing the gene fragility, then the increasing in either the number of transcribing cells or the mean of transcripts by down-regulating the gene fragility will lead to the reduction of transcription noise. Similar to our results shown in Theorem 3.5, the up and down mean-noise relationship in a multi-on gene model at the transcription level was also described [41]. Taken together, the up and down dependance between transcription noise on transcriptional efficiency or mean mRNA level seems a universal feature of gene transcription.

For a gene with fixed fragility $\gamma$ and effective transcription synthesis rate $\nu/\delta$, we compare its noise when the transcription is respectively activated by distinct firing circuits, including the single pathway system. Our Theorem 3.6 indicates that multiple pathways tend to increase transcription noise with the constraint that the mean transcription level is fixed. We look forward to examining transcription fluctuations in models with more details such as external stimulation by random signals [23] and mRNA degradation by two pathways [43], when multiple pathways are involved in transcription activation. A further study for stochastic gene transcription involving multiple signaling pathways may lead to the better understanding of the relation between transcription fluctuation and genetic network architecture.

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