Effect of transcription reinitiation in stochastic gene expression

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Abstract. Gene expression (GE) is an inherently random or stochastic or noisy process. The randomness in different steps of GE, e.g., transcription, translation, degradation, etc., leading to cell-to-cell variations in mRNA and protein levels. This variation appears in organisms ranging from microbes to metazoans. Stochastic GE has important consequences for cellular function. The random fluctuations in protein levels produce variability in cellular behavior. It is beneficial in some contexts and harmful to others. These situations include stress response, metabolism, development, cell cycle, circadian rhythms, and aging. Different model studies e.g., constitutive, two-state, etc., reveal that the fluctuations in mRNA and protein levels arise from different steps of GE among which the steps in transcription have the maximum effect. The pulsatile mRNA production through RNAP-II based reinitiation of transcription is an important part of gene transcription. Though, the effect of that process on mRNA and protein levels is very little known. The addition of any biochemical step in the constitutive or two-state process generally decreases the mean and increases the Fano factor. In this study, we have shown that the RNAP-II based reinitiation process in gene transcription can have different effects on both mean and Fano factor at mRNA levels in different model systems. It decreases the mean and Fano factor both at the mRNA levels in the constitutive network whereas in other networks it can simultaneously increase or decrease both quantities or it can have mixed-effect at mRNA levels. We propose that a constitutive network with reinitiation behaves like a product independent negative feedback circuit whereas other networks

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behave as either product independent positive or negative or mixed feedback circuit.

**Keywords:** gene expression and regulation, molecular networks, stochastic processes

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**1. Introduction**

Gene expression (GE) is a fundamental cellular process consisting of several consecutive random steps like transcription, translation, degradation, etc. The random nature of the biochemical steps of gene expression is responsible for the stochastic or noisy production of mRNA and protein molecules. This stochasticity in gene expression gives rise to heterogeneity in an identical cell population and phenotypic variation. Phenotypic variation is generally attributed to genetic and environmental variation. Though it has been observed that genetically identical cells in a constant environment show significant phenotypic variation.

The origin and consequences of noise in stochastic gene expression have been studied extensively, both theoretically and experimentally, during the last three decades [1–20]. Several studies on both prokaryotic [6, 12] and eukaryotic systems [7, 8, 13, 17] suggest that gene transcription occurs in a discontinuous manner and that gives rise to fluctuating production of mRNAs and proteins. The random fluctuations in the number of mRNA and protein molecules in each cell constitute the noise. The cells must either exploit it, learn to cope with it, or overcome it using its internal noise suppression mechanisms. It can improve fitness by generating cellular heterogeneity in clonal cell
populations, thus enabling a fast response to varying environments [8]. Because of its functional importance in cellular processes, it is necessary and important to identify and dissect the biochemical processes that generate and control the noise.

The transcription is an important step in stochastic gene expression. It has been observed that the transcription process contributes maximum noise in protein level than any other biochemical steps in gene expression [4, 7–9, 11–13, 16, 17]. During the transcription process, different transcription factors (TFs) bind to multiple sites on regulatory DNA in response to intracellular or extracellular signals. On binding the regulatory systems, the TFs turn the gene into an active state from which a burst of mRNAs is produced. Transcriptional bursting has been observed across species and is one of the primary causes of variability in gene expression in cells and tissues [7, 8, 10, 12, 13, 21–23]. Many experimental observations are modeled with that burst mechanism [9, 11, 14, 16, 18, 24]. The mRNA synthesis from the active gene actually takes place through interactions with RNAP-II [25–28]. Experiments show that the RNAP-II based transcription, specific to eukaryotes, produces pulsatile mRNA production through reinitiation and is crucial to reproduce the experimental observations on noise at protein levels [7, 8]. The reinitiation of transcription introduces the third state of a gene along with the two states of the two-state model network. Recent research shows that the c-Fos gene in response to serum stimulation indicates that a third state along with the inactive and active states is essential to explain the experimental data on variance [21]. Noise strength or Fano factor at the mRNA level is unity for constitutive gene expression. The two-state model shows the super-Poissonian Fano factor at the mRNA level because of the random nature of the gene activation and deactivation. Only negative feedback in the two-state model network can reduce the noise in the mRNA levels but not below the unity or sub-Poissonian level. Saho and Zeitlinger propose, based on their experimental observations, that paused RNAP II prevents new initiation of transcription which may reduce noise [29]. Recent work on the two-state system with RNAP-II based transcriptional reinitiation process shows that the reinitiation step in transcription has the ability to reduce the noise strength or Fano factor at mRNA level below unity [30]. Reduction of noise strength can have important biological significance as it has different functional roles in cellular activity, development, and evolution [11, 15, 31]. The average level of proteins and the fluctuations about the average level can play crucial roles in different diseases [32–35]. An appropriate amount of both, the mean and noise strength at mRNA and protein levels, are therefore important for fine-tuned robust cellular processes [36].

In this paper, we have considered different gene expression models e.g., constitutive [20], two-state [11], and Suter [17] models, and studied the effect of transcription reinitiation on the mean and Fano factor at mRNA levels. The stochastic events at the gene activation and transcription process generally decrease the average and increase the Fano factor at mRNA and protein levels. But we show that reinitiation of transcription has the ability to control the average and Fano factor both at mRNA and protein levels in different combinations viz either it decreases the both or increases the both or shows mixed-effect (mean increases and Fano factor decreases or vice versa) in different model systems. From our exact analytical calculations, we find that the reinitiation of transcription behaves like product-independent negative feedback in the constitutive gene
Figure 1. Reaction scheme with rate constants for constitutive gene expression. $J_m$ ($J_p$) is the transcription (translation) rate constant and $k_m$ ($k_p$) is the mRNA (protein) degradation rate constant.

regulatory network. Whereas for the two-state and Suter model, the RNAP-II based transcription reinitiation behaves as either positive or negative or mixed feedback circuit depending on the rate constants of the biochemical steps. That is, the reinitiation process in gene transcription can simultaneously control the mean and Fano factor both at mRNA level.

2. Different gene expression models and analysis

2.1. Constitutive gene expression

The essential genes in the cell always produce mRNAs and proteins. The expression from essential genes is modeled by the constitutive network shown in figure 1. In that model, the gene is always assumed to be at the active state from which the mRNA synthesis takes place with rate constant $J_m$. The proteins are then synthesized from the newly born mRNAs. Both the mRNAs and proteins are degraded with rate constants $k_m$ and $k_p$ respectively. It is very easy to find out the expressions of mean, variance, and Fano factor for the constitutive gene expression at the steady-state by the master equation approach [37].

Let $p(n_1,n_2,t)$ be the probability density of $n_1$ mRNAs and $n_2$ proteins at time $t$. The rate of change of probability is given by the master equation [37]

$$\frac{\partial p(n_1,n_2,t)}{\partial t} = l J_m [p(n_1-1,n_2,t) - p(n_1,n_2,t)]$$

$$+ k_m [(n_1+1)p(n_1+1,n_2,t) - n_1p(n_1,n_2,t)]$$

$$+ J_p [n_1p(n_1,n_2-1,t) - n_1p(n_1,n_2,t)]$$

$$+ k_p [(n_2+1)p(n_1,n_2+1,t) - n_2p(n_1,n_2,t)]$$

(1)

where $l$ is the copy number of the gene.

The steady state solution of equation (1) for the constitutive gene expression process gives the mean ($\langle m^c \rangle$), variance ($\text{Var}^c_m$, $\text{Var}^c_p$) and Fano factor ($\text{FF}^c_m$, $\text{FF}^c_p$) of mRNAs and Fano factor ($\text{FF}^c_p$) of proteins and are given by (for $l = 1$)

$$\langle m^c \rangle = \frac{J_m}{k_m}; \quad \langle p^c \rangle = \langle m^c \rangle \frac{J_p}{k_p}$$

(2)
Figure 2. Reaction schemes with rate constants for constitutive gene expression with reinitiation (a) without reverse reaction and (b) with reverse reaction. The RNAP-II binds the gene \( (G) \) with rate constant \( k_1 \) and forms an initiation complex \( (G_c) \). \( k_2 \) is the dissociation rate constant of RNAP-II from the initiation complex. \( J_m \) \( (J_p) \) is the transcription (translation) rate constant and \( k_m \) \( (k_p) \) is the mRNA (protein) degradation rate constant.

\[
\text{Var}_m = \frac{J_m}{k_m}, \quad \text{FF}_m = \frac{\text{Var}_m}{\langle m \rangle} = 1
\]

\[
\text{Var}_p = \langle p' \rangle \frac{J_p + k_m + k_p}{k_m + k_p}, \quad \text{FF}_p = \frac{\text{Var}_p}{\langle p' \rangle} = \frac{J_p + k_m + k_p}{k_m + k_p}.
\]

The noise strength or Fano factor of mRNAs in constitutive GE is unity. That is a unique feature of the Poisson process and that can be taken as a reference to compare with other gene expression network models.

### 2.2. Constitutive gene expression with reinitiation

In constitutive gene expression, the binding and movement of RNAP-II are ignored. But in the actual process, the RNAP-II molecules bind the gene to form an initiation complex [25]. In the next step, the bound RNAP-II leaves the initiation complex and starts transcription along the gene. The gene then comes again into its normal state (figure 2(a)). In that process, it is assumed that bound RNAP-II must do transcription without any uncertainty. Though, that may not be possible always. There must be a finite probability that bound RNAP-II leaves the initiation complex without transcribing the gene. That is considered in figure 2(b).

To calculate the mean and variance/Fano factor, we consider the most general reaction scheme in figure 2(b). Let \( p(n_1, n_2, n_3, t) \) be the probability density of \( n_1 \) genes in the \( G_c \) state, \( n_2 \) mRNAs and \( n_3 \) proteins at time \( t \). The rate of change of probability density corresponding to the reaction scheme in figure 2(b) is given by the master equation [37]

\[
\frac{\partial p(n_1, n_2, n_3, t)}{\partial t} = k_1 [(l - (n_1 - 1)) p(n_1 - 1, n_2, n_3, t) - (l - n_1) p(n_1, n_2, n_3, t)] + k_2 [(n_1 + 1) p(n_1 + 1, n_2, n_3, t) - n_1 p(n_1, n_2, n_3, t)]
+ J_m [(n_1 + 1) p(n_1 + 1, n_2 - 1, n_3, t) - n_1 p(n_1, n_2, n_3, t)]
+ k_m [(n_2 + 1) p(n_1, n_2 + 1, n_3, t) - n_2 p(n_1, n_2, n_3, t)]
+ J_p [n_2 p(n_1, n_2, n_3 - 1, t) - n_2 p(n_1, n_2, n_3, t)]
+ k_p [(n_3 + 1) p(n_1, n_2, n_3 + 1, t) - n_3 p(n_1, n_2, n_3, t)].
\]
Equation (5) gives the mean, variance, and Fano factor at the steady state as (for \( l = 1 \))

\[
\langle m_{cwr} \rangle = \frac{k_1}{J_m + k_1 + k_2 k_m} J_m; \quad \langle p_{cwr} \rangle = \langle m_{cwr} \rangle \frac{J_p}{k_p} \tag{6}
\]

\[
\text{Var}_{m_{cwr}} = \frac{J_m k_1 \{(J_m + k_1 + k_2)(J_m + k_1 + k_2 + k_m) + k_1^2 k_2\}}{k_m (J_m + k_1 + k_2)^2 (J_m + k_1 + k_2 + k_m)}
\]

\[
= \langle m_{cwr} \rangle (1 - \frac{J_m k_1}{(J_m + k_1 + k_2)(J_m + k_1 + k_2 + k_m)}) \tag{7}
\]

\[
\text{FF}_{m_{cwr}} = 1 - \frac{J_m k_1}{(J_m + k_1 + k_2)(J_m + k_1 + k_2 + k_m)} \tag{8}
\]

\[
\text{FF}_{p_{cwr}} = \frac{\text{Var}_{p_{cwr}}}{\langle p_{cwr} \rangle} = 1 + \frac{J_p g_1}{(J_m + g_2)(J_m + g_2 + k_m)(J_m + g_2 + k_m + k_p)g_3} \tag{9}
\]

where \( g_1 = J_m^3 + g_2(g_2 + k_m)(g_2 + k_p) + J_m^2(2k_1 + 3k_2 + g_3) + J_m\{2k_1^2 + 3k_2^2 + k_m k_p + 2k_2 g_3 + k_1(5k_2 + g_3)\}, \ g_2 = k_1 + k_2, \ g_3 = k_m + k_p. \)

It is seen from equations (6)–(8) that the transcriptional reinitiation in constitutive GE process (figures 2(a) and (b)) decreases the mean and Fano factor at mRNA and protein levels in comparison to that the constitutive GE process without reinitiation (figure 1). Figures 3(a)–(c) show that the effect of reinitiation is strong enough at the lower values of \( k_1. \) We see from the figures that the mean mRNA level and FF\(_m\) approaches the value observed in the constitutive process for a given value of \( J_m \) and \( k_m \) for higher \( k_1. \) When \( k_1 \) increases from zero value, the Fano factor at mRNA levels decreases and attains a minimum value and then moves towards unity. The minimum of the Fano factor (FF\(_m\)) will occur at \( k_1 = \sqrt{(J_m + k_2)(J_m + k_2 + k_m)} \) (figure 3(b)). The equation (8) shows that the Fano factor has identical dependence on \( k_1 \) and \( J_m. \) As the rate constant \( k_2 \) increases, the degree of deviation of the Fano factor below unity decreases (figure 3(b)) because that decreases the mean mRNA levels (figure 3(a)). If one considers pre-initiation and initiation complexes in the transcriptional reinitiation process rather than only the initiation complex then the Fano factor further reduces below unity (appendix A).

To understand the effect of reinitiation of transcription on average and Fano factor at mRNA level, we consider the constitutive gene expression with reinitiation but without the reverse reaction \((k_2 = 0)\) (figure 2(a)). We see that the reinitiation in transcription reduces the mean mRNA level because the effective transcription rate is reduced by a factor \( \frac{k_1}{J_m + k_1}. \) That causes the reduction of variance at mRNA level. As a result, the noise strength or Fano factor (equation (8)) reduces below unity with the reinitiation of transcription.

The expression for mean mRNA (equation (6)) with \( k_2 = 0 \) can be written as

\[
\langle m_{cwr} \rangle = \frac{k_1}{J_m + k_1 k_m} \frac{J_m}{1 + \beta \langle m^e \rangle}. \tag{10}
\]
Figure 3. Variation of (a) mean mRNA, (b) Fano factor at mRNA levels and (c) Fano factor at protein levels with $k_1$ for different values of $k_2$ with $J_m = 10$ and $k_m = 1$. The solid lines are drawn from analytical calculations and hollow circles are generated from the stochastic simulation based on the Gillespie algorithm [38].

This expression (equation (10)) is identical to the gain of a linear negative feedback amplifier with the feedback factor $\beta = \frac{km}{k_1}$ [39]. The expression for the Fano factor (equation (8)) also shows that the noise strength is reduced with the reinitiation of transcription.

We can also write from the equation (10)

$$\frac{d\langle m_{cwr}^{\text{rev}} \rangle}{\langle m_{cwr}^{\text{rev}} \rangle} = \frac{1}{1 + \beta \langle m_c \rangle} \left( \frac{d\langle m_c \rangle}{\langle m_c \rangle} \right).$$

Equation (11) shows that the percentage change in the mean mRNA levels with reinitiation is much less than that without reinitiation. That is reflected in the expression of the Fano factor of mRNA with transcriptional reinitiation (equation (8)). Therefore, as far as the mean and Fano factor is concerned, the equations (6) and (8) (with $k_2 = 0$) and the equations (10) and (11) clearly indicate that the reinitiation of transcription in gene expression behaves as a negative feedback loop in the regulatory network. It is important to note that the negative feedback in the gene regulatory network due to the reinitiation of transcription is product independent. It is entirely inherent to the gene transcription regulatory network because the mRNA and protein numbers do not have any role in its synthesis here. The deterministic rate equation for a gene regulatory network with an ordinary feedback loop, negative or positive, contains the product-dependent synthesis part [40, 41] but here the synthesis term is product independent and depends only on the reaction rate constants.

The mean mRNA with reverse reaction (equation (6)) can be expressed as

$$\langle m_{cwr}^{\text{rev}} \rangle = \frac{\langle m_c \rangle}{1 + \beta \langle m_c \rangle + \alpha \langle m_c \rangle}$$

where $\alpha = \frac{k_2}{k_1} J_m$.

From equations (6), (8) and (12), we see that the rate constant $k_2$ helps to reduce the mean mRNA level further but increases the Fano factor (figures 3(b) and (c)). So, the reverse transition with rate constant $k_2$ behaves like negative feedback for mean mRNA level but positive feedback for the Fano factor. Thus, the successful reinitiation of transcription behaves like a negative feedback loop whereas the unsuccessful reinitiation of transcription behaves like a mixed feedback loop. The effect of reinitiation in gene expression always reduces the Fano factor.
expression is observed at the mRNA level first. The nature of the variation in the Fano factor at the protein level is the same as that at mRNA level except for a change in scale (figure 3(c)). Therefore, the Fano factor at the protein level does not give any new information about the effect of reinitiation. So we keep our analysis up to the mRNA level in the rest of the paper.

2.3. Two-state gene expression without and with transcriptional reinitiation process

Regulation is ubiquitous in biological processes. The regulated gene expression without feedback in a cellular system is modeled by the two-state process. Many experimental results are explained with the help of two-state gene expression process [9–14, 19]. In that process, the gene can be in two possible states, active (G_a) and inactive (G_i) (figure 4(a)) and random transitions take place between the states. The mRNA synthesis occurs in bursts only from the active state of the gene. The mRNAs have a specific decay rate also.

Now, let us assume that there is l copy number of a particular gene that exists in the cell. Let p(n_1, n_2, t) be the probability that at time t and there are n_2 number of mRNAs with n_1 number of genes in the active state (G_a). The number of genes in the inactive states are (l – n_1). The time evaluation of the probability corresponding to the chemical reactions in figure 4(a) is given by the master equation [37]

\[
\frac{\partial p(n_1, n_2, t)}{\partial t} = k_a[(l - n_1 + 1)p(n_1 - 1, n_2, t) - (l - n_1)p(n_1, n_2, t)] \\
+ k_d(n_1 + 1)p(n_1 + 1, n_2, t) - n_1p(n_1, n_2, t)] \\
+ J_m[n_1n_2p(n_1, n_2 - 1, t) - n_1p(n_1, n_2, t)] \\
+ k_m[(n_2 + 1)p(n_1, n_2 + 1, t) - n_2p(n_1, n_2, t)].
\] (13)

Solving the equation (13), we can easily find out the mean, variance and Fano factor of mRNAs. They are given by

\[
\langle m_{\text{swtr}} \rangle = \frac{k_a J_m}{(k_a + k_d)k_m} \quad (14)
\]

\[
FF_m^{\text{swtr}} = 1 + \frac{J_m k_d}{(k_a + k_d)(k_m + k_a + k_d)}. \quad (15)
\]
Equations (14) and (15) show that the inclusion of inactive state and the random transitions between inactive and active states in the constitutive process reduces the mean and increases the Fano factor.

In figure 4(b), we consider the reinitiation of transcription along with the random transitions between the active and inactive states of the gene. In the transcription reinitiation step, an RNAP-II binds the gene in the active state and form an initiation complex $G_c$. Now, the bound RNAP-II has two choices, either it moves forward or backward. If it moves forward then again two events occur: mRNA synthesis and free up of the initiation complex. That is, the initiation complex again becomes an active state where free RNAP-II molecules can bind. The unsuccessful movement of RNAP-II from the initiation complex of the gene brings it back to the active state by dissociating the enzyme molecules. In the two-state gene expression model, the randomness due to the biochemical reactions in figure 4(b) is given by [30].

Let $p(n_1, n_2, n_3, t)$ be the probability that at time $t$ and there are $n_3$ number of mRNAs with $n_1$ number of genes in the active state ($G_a$) and $n_2$ number of genes in the initiation state ($G_c$). The number of gene in the inactive states are $(l - n_1 - n_2)$ with $l$ be the copy number of the gene. The time evaluation of the probability corresponding to the biochemical reactions in figure 4(b) is given by

$$\frac{\partial p(n_1, n_2, n_3, t)}{\partial t} = k_a [(l - n_1 - n_2 + 1)p(n_1 - 1, n_2, n_3, t) - (l - n_1 - n_2)p(n_1, n_2, n_3, t)]$$

$$+ k_d(n_1 + 1)p(n_1 + 1, n_2, n_3, t) - n_1p(n_1, n_2, n_3, t)]$$

$$+ k_1[(n_1 + 1)p(n_1 + 1, n_2 - 1, n_3, t) - n_1p(n_1, n_2, n_3, t)]$$

$$+ k_2((n_2 + 1)p(n_1 - 1, n_2 + 1, n_3, t) - n_2p(n_1, n_2, n_3, t)]$$

$$+ J_m[(n_2 + 1)p(n_1 - 1, n_2 + 1, n_3 - 1, t) - n_2p(n_1, n_2, n_3, t)]$$

$$+ k_m[(n_3 + 1)p(n_1, n_2, n_3 + 1, t) - n_3p(n_1, n_2, n_3, t)]]$$

(16)

The expressions of averages and Fano factors of mRNAs for the reaction scheme with the transcriptional reinitiation process in figure 4(b) are given by [30]

$$\langle m_{\text{swr}} \rangle = \frac{k_ao_1}{a_2} J_m$$

(17)

$$\text{FF}_{m}^{\text{swr}} = 1 + \frac{J_m k_3(a_2 - k_a a_1)}{a_2(a_1 k_m + a_2)}$$

(18)

where $a_1 = k_m + J_m + k_a + k_d + k_1 + k_2$ and $a_2 = k_ao_1 + k_dJ_m + k_dk_2 + k_1k_a + k_ao_2$.

The variation of mean mRNA levels for both the scenarios, with and without reinitiation, are plotted with the rate constants $k_a$, $k_d$ and $J_m$ in figure 5. We see from the figures
Figure 5. Variation of mean mRNA levels with and without reinitiation as a function of (a) $k_a$, (b) $k_d$ and (c) $J_m$ corresponding to the figure (4). The solid (dashed) lines are drawn from exact analytical expressions in equation (14) (equation (17)). The hollow circles are generated using the stochastic simulation based on the Gillespie algorithm. The rate constants are $k_1 = 50$, $k_2 = 1$, $k_m = 1$, $k_d = 10$ and $J_m = 10$ in (a), $k_a = 10$ and $J_m = 10$, in (b) and $k_a = 10$ and $k_d = 10$ in (c).

Figure 6. Plot of Fano factor at mRNA level versus (a) $k_a$, (b) $k_d$ and (c) $J_m$ with and without reinitiation. The solid (dashed) lines are drawn from exact analytical expressions (equations (15) and (18)). The hollow circles are obtained from stochastic simulation based on the Gillespie algorithm. The rate constants are $k_1 = 50$, $k_2 = 1$, $k_m = 1$, $k_d = 10$ and $J_m = 10$ in (a), $k_a = 10$ and $J_m = 10$ in (b) and $k_a = 10$ and $k_d = 10$ in (c).

that the reinitiation of transcription helps to keep the mean mRNA levels at higher values for lower values of $k_a$ and $J_m$ and for almost all values of $k_d$. Fano factor remains lower for all values of $k_a$, $k_d$ and at lower values of $J_m$ (figures 6(a)–(c)). If one considers pre-initiation and initiation complexes in the transcriptional reinitiation process [25] rather than only the initiation complex then the Fano factor further reduces below unity (appendix B). Fano factor can also be higher due to the transcriptional reinitiation process with other sets of rate constants [7, 30]. The Fano factor can have three different phases, Poissonian (FF = 1), super-Poissonian (FF > 1) and sub-Poissonian (FF < 1), when plotted against $k_1$ with $k_a$, $k_d$ and $J_m$ as parameters as shown in figures 7(a) and (b) and in reference [30] respectively. The rate constants $k_1 = 6.34$ and $k_d = 5$ in figures 7(a) and (b) respectively can be considered as the critical value of that rate constants as that values sharply divide the super-Poissonian and sub-Poissonian Fano factor regimes [30].
Figure 7. Plot of Fano factor with the variation of $k_1$ with parameter (a) $k_a$ and (b) $k_d$. The other rate constants are $J_m = 10, k_m = 1, k_2 = 1, k_d = 10$ in (a) and $k_a = 5$ in (b). The solid lines are drawn from analytical expression (equation (18)) and hollow circles are obtained from the stochastic simulation based on the Gillespie algorithm.

The expression of mean mRNA level (equation (17)) can be written as

$$\langle m_{tsw} \rangle = \frac{\langle m_{tswtr} \rangle}{1 - \beta_1 \langle m_{tswtr} \rangle + \beta_2 \langle m_{tswtr} \rangle}$$  \hspace{1cm} (19)

where $\beta_1 = \frac{k_d k_m}{k_m J_m}$ and $\beta_2 = \frac{(k_a + k_d)(J_m + k_2)k_m}{k_a k_1 J_m}$.

The expression of Fano factor (equation (18)) can also be expressed as

$$\text{FF}_{m_{tsw}} = 1 - \gamma_1 \langle m_{tsw} \rangle + \gamma_2 \langle m_{tsw} \rangle$$  \hspace{1cm} (20)

where $\gamma_1 = \frac{k_m k_1 (k_a + k_d)}{(a_1 J_m + a_2) a_2}$ and $\gamma_2 = \frac{k_m k_1 (k_a + k_d)}{(a_1 J_m + a_2) k_a}$.

In general, for $\beta_1 = 0$ and $\beta_2 \neq 0$ ($\beta_2 = 0$ and $\beta_1 \neq 0$), the expression for mean in equation (19) looks like the expression of the gain with linear negative (positive) feedback network in electronic circuit with $\beta_2$ ($\beta_1$) as the feedback factor. For the non-zero value of $\beta_1$ and $\beta_2$, the expression for mean in equation (19) can be considered as the mean mRNA from a network with mixed feedback, i.e. both positive and negative feedback. Therefore, $\beta_1$ ($\beta_2$) is working here as the positive (negative) feedback factor. For $\beta_1 > \beta_2$ ($\beta_1 < \beta_2$) the positive (negative) feedback nature dominates and the mean mRNA level with reinitiation ($\langle m_{tsw} \rangle$) becomes higher (lower) than that without reinitiation process ($\langle m_{tswtr} \rangle$). Again, as far as the Fano factor is concerned, the positive (negative) feedback nature dominates for $\gamma_2 > \gamma_1$ ($\gamma_2 < \gamma_1$). The expression of mean mRNA (equation (19)) and Fano factor (equation (20)) shows that the two-state gene regulatory network with reinitiation of transcription (figure 4(b)) can behave as mixed feedback network.

The mean mRNA level and Fano factor can be higher or lower due to the reinitiation of transcription compared to the two-state gene expression process without reinitiation. From equations (14) and (17) or from equation (19), we have the condition of higher average mRNA level in presence of reinitiation of transcription as

$$\beta_1 > \beta_2 \quad \text{or} \quad (J_m + k_2) < \frac{k_d k_1}{k_a + k_d}.$$  \hspace{1cm} (21)
Figure 8. The plot of $(J_m + k_2)$ versus (a) $k_a$ and (b) $k_d$ shows the four different regions. The rate constants are taken as $k_1 = 50$ and $k_m = 1$ and $k_d = 10$ (in (a)), and $k_a = 10$ (in (b)). The condition given in equation (21) is satisfied in regions I and IV whereas the condition given in equation (22) is satisfied in regions I and II.

From equation (18) or (20), we have the condition of sub-Poissonian Fano factor as \[ \gamma_1 > \gamma_2 \text{ or } (J_m + k_2) < \frac{k_a}{k_d}(k_a + k_d + k_m). \] (22)

Two conditions in equations (21) and (22) divide the whole permissible space in $(k_a, J_m + k_2)$ and $(k_d, J_m + k_2)$ space into four different regions with different conditions of Fano factor and mean mRNA level. The four regions are identified as: region I: $F_{\text{Ftswr}} < 1$ and $r > 1$; region II: $F_{\text{Ftswr}} < 1$ and $r < 1$; region III: $F_{\text{Ftswr}} > 1$ and $r < 1$; region IV: $F_{\text{Ftswr}} > 1$ and $r > 1$; where $r = \langle m_{\text{tswr}} \rangle / \langle m_{\text{tswtr}} \rangle$ and shown in figure (8). The mean and Fano factor of mRNA can be in any one of the four regions depending on the rate constants $k_a, k_d, k_1, J_m + k_2$ and $k_m$. The rate constants $k_a$ and $k_d$ are generally function of TFs and therefore, can be modulated [7, 8, 34]. Thus the mean and Fano factor at the mRNA level can be changed according to the cellular requirement by changing the number of TFs in the cell.

In the two-state process (figure 4(a)) Fano factor is always greater than unity and there is a specific mean mRNA level depending on the rate constants $k_a, k_d, J_m$ and $k_m$. But, as reinitiation of transcription is added in the two-state gene expression process, we get four different options on Fano factor and mean mRNA in the $(k_a, J_m + k_2)$ (figure 8(a)) or $(k_d, J_m + k_2)$ (figure 8(b)) space. Four distinct regions in the parameter space are obtained by two intersecting curves corresponding to the two conditions given in equations (21) and (22). The intersecting point of the two curves gives the condition $r = 1$ and $F_{\text{m}} = 1$ simultaneously. With respect to the feedback features of a network, we see that the gene regulatory network (figure 4(b)) behaves like a network with inherent negative (positive) feedback in region II (region IV). In regions I and III, the two-state network with reinitiation of transcription behaves like a gene regulation with inherent mixed feedback. The mixed feedback nature is of two types: either it behaves as negative feedback for the mean ($r < 1$) and positive feedback for Fano factor ($F_{\text{m}} > 1$) or it behaves as positive feedback for mean ($r > 1$) and negative feedback for Fano factor ($F_{\text{m}} < 1$).
Now, we can express $k_1$ in terms of $k_a$, $k_d$ and $k_m$ with the equality conditions in equations (21) and (22), as

$$k_1 = \frac{k_a}{k_d^2}(k_a + k_d)(k_a + k_d + k_m). \tag{23}$$

The equation (22) with the equality sign gives the critical condition ($\text{FF}_{\text{swr}} = 1$) and that condition is satisfied at all the points on the surface in figure 9(a). The equation (21) with the equality sign gives the condition $r = 1$ and all the points on the surface in figure 9(b) satisfy that condition (the ratio $k_a/k_d$ is chosen as independent variable to avoid the assignment of constant value to any one of them). Both the conditions, $r = 1$ and $\text{FF}_{\text{swr}} = 1$, are satisfied at all the points on the surface in figure 9(c) provided the value of $(J_m + k_2)$ is chosen from the surface of figure 9(a) for the same $k_a$ and $k_d$ as in figure 9(c). For a given $k_a$, $k_d$ and $k_m$, the value of the rate constants $k_1$ from equation (23) and $(J_m + k_2)$ from equation (22) (with equality) can be obtained to have both the conditions $r = 1$ and $\text{FF}_{\text{swr}} = 1$. The value of rate constants $k_1$ and $(J_m + k_2)$ in the pink shaded region of the plot in figure 9(d) satisfy the conditions $r = 1$ and $\text{FF}_{\text{swr}} = 1$ simultaneously.
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Figure 10. Reaction scheme with rate constants for two-state gene expression (Suter model) (a) without and (b) with reinitiation. \( k_b \) (\( k_d \)) is the activation (deactivation) rate constant and \( k_a \) is the rate constant for transition from \( G \) to \( G_1 \). \( k_1 \) is the rate constant of initiation complex formation and \( k_2 \) is the rate constant of dissociation of RNAP-II from initiation complex. \( J_m \) is the transcription rate constant and \( k_m \) is the mRNA degradation rate constant.

2.4. Two-state gene expression (Suter model) without and with reinitiation

The regulated gene expression is an important and essential property of a complex cellular system. Though many experimental results are modeled with the two-state process, Suter et al [17] observe something different in the mammalian system. They observe gamma-distributed off time in gene regulation rather than the exponentially distributed off time in the two-state process. Suter et al model their experimental observation by a gene regulatory network shown in figure 10(a). In their model network, the gene can be in three possible states, one active (\( G_2 \)) and two inactive states (\( G \) and \( G_1 \)) and random transitions take place between the states according to the reaction scheme in figure 10(a). The mRNA synthesis takes place only from the active state of the gene with rate constant \( J_m \).

The master equation corresponding to the figure 10(a) is given by

\[
\frac{\partial p(n_1, n_2, n_3, t)}{\partial t} = k_a[(l - n_1 - n_2 + 1)p(n_1 - 1, n_2, n_3, t) - (l - n_1 - n_2)p(n_1, n_2, n_3, t)] + k_b[(n_2 + 1)p(n_1, n_2 + 1, n_3, t) - n_2p(n_1, n_2, n_3, t)] + J_m[n_2p(n_1, n_2, n_3 - 1, t) - n_2p(n_1, n_2, n_3, t)] + k_m[(n_3 + 1)p(n_1, n_2, n_3 + 1, t) - n_3p(n_1, n_2, n_3, t)].
\]

The expression for mean and Fano factor at mRNA level corresponding to figure 10(a) are given by (for \( l = 1 \))

\[
\langle m^{\text{surwtr}} \rangle = \frac{J_m k_a k_b}{(k_a k_b + k_a k_d + k_b k_d)k_m} = \frac{J_m k_a k_b}{C_1 k_m}
\]

\[
\text{FF}_{m}^{\text{surwtr}} = 1 + \frac{J_m k_d [(k_b + k_m)(k_a + k_b) + k_a^2]}{k_a (k_b + k_d) + k_b k_d \{(k_b + k_m)(k_d + k_m) + k_a (k_b + k_d + k_m)\}}
\]

\[
= 1 + \frac{J_m k_d [(k_b + k_m)(k_a + k_b) + k_a^2]}{C_1 C_2}
\]

where \( C_1 = k_a (k_b + k_d) + k_b k_d \) and \( C_2 = (k_b + k_m)(k_d + k_m) + k_a (k_b + k_d + k_m) \).
Now let us consider the gene transcriptional regulatory network with the reinitiation of transcription by RNAP-II (figure 10(b)). We have the master equation corresponding to the figure 10(b) as

\[
\frac{\partial p(n_1, n_2, n_3, n_4, t)}{\partial t} = k_a \{ [l - (n_1 - 1 + n_2 + n_3)p(n_1 - 1, n_2, n_3, n_4, t) \\
- \{l - (n_1 + n_2 + n_3)\}p(n_1, n_2, n_3, n_4, t) \\
+ k_b[(n_1 + 1)p(n_1 + 1, n_2 - 1, n_3, n_4, t) - n_1p(n_1, n_2, n_3, n_4, t)] \\
+ k_d[(n_2 + 1)p(n_1, n_2 + 1, n_3, n_4, t) - n_2p(n_1, n_2, n_3, n_4, t)] \\
+ k_1[(n_3 + 1)p(n_1, n_2 + 1, n_3 - 1, n_4, t) - n_3p(n_1, n_2, n_3, n_4, t)] \\
+ k_2 [(n_4 + 1)p(n_1, n_2 - 1, n_3 + 1, n_4 - 1, t) \\
- n_4p(n_1, n_2, n_3, n_4, t)] + J_m [(n_3 + 1) \\
\times p(n_1, n_2 - 1, n_3 + 1, n_4 - 1, t) - n_3p(n_1, n_2, n_3, n_4, t)] \\
+ k_m[(n_4 + 1)p(n_1, n_2, n_3, n_4 + 1, t) - n_4p(n_1, n_2, n_3, n_4, t)]. \quad (27)
\]

The mean mRNA and Fano factor corresponding to figure 10(b) are given by (for \(l = 1\))

\[
\langle m^{\text{suwr}} \rangle = \frac{J_m k_1 k_a k_b}{[k_a k_b (J_m + k_1 + k_2) + (J_m + k_2)(k_a k_d + k_b k_d)]k_m}
\quad (28)
\]

\[
\text{FF}^{\text{suwr}}_m = 1 - \frac{J_m k_1 k_a k_b}{[k_1 k_a k_b + (J_m + k_2)C_1]k_m} + \frac{J_m C_3}{[C_3 + (J_m + k_2 + k_m)C_2]k_m}
\quad (29)
\]

where \(C_3 = k_1(k_a + k_m)(k_b + k_m)\).

Figures 11(a)–(d) show the variation of mean mRNA number with the rate constants \(k_a, k_b, k_d\) and \(J_m\) respectively. All figures show that the reinitiation of transcription favours the higher mean mRNA levels with reinitiation of transcription.

The variation of Fano factors are plotted with the rate constants \(k_a, k_b, k_d\) and \(J_m\) respectively in figures 12(a)–(d). The figures show that the Fano factor is always lower with the reinitiation of gene transcription. The variation of the Fano factor with the rate constant \(k_1\) is shown in figure 13. The figures also show that the three different regimes of Fano factor viz, Poissonian, sub-Poissonian and super-Poissonian, are likely to occur with the reinitiation of transcription. That is a unique feature of the Fano factor for a gene regulatory network with reinitiation of transcription. In the Suter model, \(k_b\) is an extra parameter by which the mean and Fano factor can be controlled. It can be shown that for higher \(k_b\) (\(k_b > 30\)) the Suter model merges to the two-state model.

The expression of mean mRNA (equation (28)) can also be expressed as

\[
\langle m^{\text{suwr}} \rangle = \frac{\langle m^{\text{suwr}} \rangle}{1 - \delta_1 \langle m^{\text{suwr}} \rangle + \delta_2 \langle m^{\text{suwr}} \rangle}
\quad (30)
\]

where \(\delta_1 = \frac{k_d k_m (k_a + k_b)}{k_a k_b J_m}\) and \(\delta_2 = \frac{C_1 (J_m + k_2) k_m}{k_a k_d J_m k_b}\). Here \(\delta_1(\delta_2)\) is working as the positive (negative) feedback factor. For \(\delta_1 > \delta_2\) (\(\delta_1 < \delta_2\)) the positive (negative) feedback nature
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Figure 11. Variation of mean mRNA with (a) $k_a$, (b) $k_b$, (c) $k_d$, and (d) $J_m$. The dashed (solid) lines are from exact analytical calculations corresponding to equation (28) (equation (25)). The hollow circles are generated from the stochastic simulation based on the Gillespie algorithm. The rate constants are $k_1 = 50$, $k_2 = 1$, $k_m = 1$. In (a) $k_d = 10$, $k_b = 20$ and $J_m = 10$. In (b) $k_a = 10$, $k_d = 10$ and $J_m = 10$. In (c) $k_a = 10$, $k_b = 20$ and $J_m = 10$. In (d) $k_a = 10$, $k_d = 10$ and $k_b = 20$.

dominates and the mean mRNA level with reinitiation ($\langle m_{\text{suwr}} \rangle$) becomes higher (lower) than that without reinitiation process ($\langle m_{\text{suwtr}} \rangle$).

The expression of Fano factor (equation (18)) can also be expressed as

$$FF_{m_{\text{suwr}}} = 1 - B_1\langle m_{\text{suwtr}} \rangle + B_2\langle m_{\text{suwtr}} \rangle$$

(31)

where $B_1 = \frac{(J_m + k_2)}{k_1} + \frac{k_m}{C_1}$ and $B_2 = \frac{C_3}{(C_3 + (J_m + k_2 + k_m)C_3)} k_2 k_0$.

Again, as far as the Fano factor is concerned, the positive (negative) feedback nature dominates for $B_2 > B_1$ ($B_2 < B_1$). The expressions of mean mRNA (equation (30)) and Fano factor (equation (31)) show that the gene regulatory network following Suter model with reinitiation of transcription (figure 10(b)) can also behave as mixed feedback network.

We see that the Fano factor is reduced by the transcriptional reinitiation process as observed in two-state also. From the equations (30) and (31) we find that the average mRNA level can be greater with the reinitiation in gene transcription provided

$$J_m < \frac{k_1 k_d (k_1 + k_b)}{k_d k_b + k_a k_d + k_b k_d}$$

(32)

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Figure 12. Variation of Fano factor at mRNA level \( \text{FF}_m \) with different conditions. Dashed (solid) lines are drawn from analytical calculation for figure 10(b) (figure 10(a)). The hollow circles are generated from the stochastic simulation based on the Gillespie algorithm. The rate constants are \( k_1 = 50, k_2 = 1, k_m = 1 \). In (a) \( k_d = 10, k_b = 20 \) and \( J_m = 10 \). In (b) \( k_a = 10, k_d = 10 \) and \( J_m = 10 \). In (c) \( k_a = 10, k_b = 20 \) and \( J_m = 10 \). In (d) \( k_a = 10, k_d = 10 \) and \( k_b = 20 \).

From equation (18), we have the condition of sub-Poissonian Fano factor as \[ (J_m + k_2) \frac{k_a k_b \{(k_b + k_m)(k_d + k_m) + k_a(k_b + k_d + k_m)}{k_a^2 + k_b^2 + k_a k_b + k_a k_m + k_b k_m}. \] (33)

Here also, two conditions in equations (32) and (33) divide the whole permissible space in \((k_a, J_m + k_2)\) and \((k_d, J_m + k_2)\) space into four different regions with different conditions of Fano factor and mean mRNA levels. The four regions are identified as: region I: \( \text{FF}^{\text{suwr}}_m < 1 \) and \( s > 1 \); region II: \( \text{FF}^{\text{suwr}}_m < 1 \) and \( s < 1 \); region III: \( \text{FF}^{\text{suwr}}_m > 1 \) and \( s < 1 \); region IV: \( \text{FF}^{\text{suwr}}_m > 1 \) and \( s > 1 \); where \( s = \langle m^{\text{suwr}} \rangle / \langle m^{\text{suwr}} \rangle \) and shown in figure 14. The mean and Fano factor of mRNA can be in any one of the four regions depending on the rate constants \( k_a, k_b, k_d, k_1, J_m + k_2 \) and \( k_m \).

Figure 14 shows the four different regions in the \((k_a, J_m + k_2)\) (figure 14(a)) or \((k_d, J_m + k_2)\) (figure 14(b)) space in Suter model. With the gradual decrease in the rate constant \( k_b \), the area of the region II decreases gradually and the two curves intersect at higher (lower) value of \( k_a \) (\( k_d \)) in figure 14(a) (figure 14(b)). A cell can choose any one of the four regions according to its functional requirement by modulating the rate constants \( k_a, k_d \) or \( k_b \).
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Figure 13. Variation of Fano factor with the rate constant $k_1$ with (a) $k_a$ (b) $k_b$ (c) $k_d$ and (d) $J_m$ as parameter. The solid lines are drawn from analytical expression (equation (29)) and hollow circles are obtained from the stochastic simulation based on the Gillespie algorithm. The rate constants are taken as $k_d = 10$, $k_b = 20$, $k_1 = 50$, $k_2 = 1$, $J_m = 10$ and $k_m = 1$ in (a). The rate constants are $k_a = 10$, $k_d = 10$, $k_1 = 50$, $k_2 = 1$, $J_m = 10$ and $k_m = 1$ in (b). In (c), the rate constants are $k_a = 10$, $k_b = 20$, $k_1 = 50$, $k_2 = 1$, $J_m = 10$ and $k_m = 1$. In (d), the rate constants are $k_a = 10$, $k_d = 10$, $k_b = 20$, $k_1 = 50$, $k_2 = 1$, and $k_m = 1$.

Figure 14. The plot of $(J_m + k_2)$ versus (a) $k_a$ and (b) $k_d$ shows the four different regions. The rate constants are taken as $k_b = 20$, $k_1 = 50$, $k_m = 1$, $k_d = 10$ (in (a)), and $k_a = 10$ (in (b)). The condition given in equation (32) is satisfied in regions I and IV whereas the condition given in equation (33) is satisfied in regions I and II.

From equations (32) and (33) (with equality sign in both the equations), we can have the rate constant $k_1$ in terms of $k_a$, $k_b$, $k_d$ and $k_m$ as

$$k_1 = \frac{k_a k_b (k_a k_b + k_a k_d + k_b k_d) \{(k_b + k_m)(k_d + k_m) + k_a (k_b + k_d + k_m)\}}{k_d^2 (k_a + k_b)} \left(\frac{k_d^2 + k_b^2 + k_a k_b + k_a k_m + k_b k_m}{k_a + k_b}\right).$$

(34)
3. Conclusion

In this article, we study the effect of transcriptional reinitiation by RNAP-II in gene expression. Transcriptional reinitiation is an important step in gene expression though it is ignored in most of the model networks assuming it has an insignificant role in mRNA and protein levels. But, Blake et al identify that reinitiation of transcription can be crucial in eukaryotic systems [7, 8]. To find out the effect of transcriptional reinitiation on phenotypic variability, we consider different gene regulatory networks, with and without the reinitiation step. We find the analytical expression of mean and Fano factor at mRNA level at steady state for constitutive, two-state, and Suter model.
We compare our analytical results with the results obtained from stochastic simulation using the Gillespie algorithm. In this work, the rate constants are chosen from different works [11, 30]. When the constitutive gene network is analyzed in presence of reinitiation, we observe that the mean mRNA level and Fano factor both are reduced. That is happened due to the reduction of the effective transcription rate of mRNA synthesis. The behaviour is similar to a negative feedback amplifier which reduces the gain and noise. Though, there is a fundamental difference between a negative feedback amplifier in electronic circuits and the observed negative feedback like behavior in the constitutive gene network with transcriptional reinitiation. In the electronic negative feedback circuit, a fraction of the output voltage is fed back to the input but in the present reinitiation-based gene regulatory circuit the gene product (mRNAs or proteins) is not involved at all in the synthesis process. Thus the reinitiation based negative feedback in constitutive gene transcription is entirely inherent in nature. Again, with the non-zero rate constant of reverse reaction, i.e. with the unsuccessful transcription from the initiation complex, the average mRNA level further decreases but the Fano factor increases than before but still remains in the sub-Poissonian region. Thus, even with the reverse reaction, the reinitiation of transcription in the constitutive model has the capability to decrease the Fano factor below unity.

Then we study the two-state gene expression model with transcriptional reinitiation. In two-state model without the reinitiation the Fano factor at mRNA level is higher than unity due to random transitions between the active and inactive states of the gene. Now, with the reinitiation of transcription in the two-state model, we observe four different phenotypic outcomes ($r < 1$ and $\text{FF}_{\text{m}}^{\text{tswr}} < 1$; $r > 1$ and $\text{FF}_{\text{m}}^{\text{tswr}} > 1$; $r < 1$ and $\text{FF}_{\text{m}}^{\text{tswr}} > 1$; $r > 1$ and $\text{FF}_{\text{m}}^{\text{tswr}} < 1$; where $r = \langle m^{\text{tswr}} \rangle / \langle m^{\text{tswr}} \rangle$) depending on the rate constants of the biochemical reactions. Similar behaviour is observed for the Suter model also. Though the mean mRNA level is higher and the Fano factor is lower over a wide region of parameter variation in the Suter model. We find that the gene regulatory network like the two-state and Suter model with RNAP-II based transcriptional reinitiation process can behave as either the inherent negative ($r < 1$ and $\text{FF}_{\text{m}}^{\text{tswr}} < 1$) or positive ($r > 1$ and $\text{FF}_{\text{m}}^{\text{tswr}} > 1$) or mixed feedback process ($r < 1$ and $\text{FF}_{\text{m}}^{\text{tswr}} > 1$; $r > 1$ and $\text{FF}_{\text{m}}^{\text{tswr}} < 1$).

It has been observed that the noise or fluctuations in mRNA/protein level can be detrimental or beneficial for cellular activities [8, 11, 35, 44, 46]. Noise in gene expression also plays an important role in cellular behaviour and disease control [35, 44, 45, 47]. Some study shows that the appropriate functioning of the cellular system requires a specific average level of mRNA and protein [32–34]. Recent work shows that reinitiation of transcription has the capability to reduce the Fano factor below unity, i.e. to the sub-Poissonian regime in the two-state process [30]. We now observe that transcriptional reinitiation has an important role not only in controlling the Fano factor but also the average at the mRNA level over a wide region of parameter space. In the two-state gene expression process, the cellular system can regulate its mean and Fano factor by controlling the rate constants responsible for random transitions between the gene states. In that process, the Fano factor can be reduced up to unity for large $k_a$ and small $k_d$. But, in presence of reinitiation of gene transcription, the cellular system can decrease the Fano factor below unity at a lower value of $k_a$. At the same time, the average mRNA
level compared to the two-state process can be increased. This is very much important to control diseases like haploinsufficiency [32–35]. The noise in gene expression can also be the survival strategy for cells in adverse environmental conditions [44, 45, 47]. The reinitiation of gene expression can also be helpful in such situations by selecting the higher Fano factor and appropriate mean mRNA and protein levels. It has been observed that the protein from essential genes has less variability compared to the non-essential genes [47]. The cellular system adopted many strategies to control the noise in mRNA and protein levels among which higher transcription rate and lower translation rate is an important one [47, 48]. The reinitiation of transcription can also be a crucial step to keep low noise levels at mRNA and protein levels. However, this noise minimization is found to be energetically expensive. It has been shown that a specific average and fluctuations level of mRNA/protein is subject to the energy consumption of cells [49]. To maintain a higher average level and lower fluctuations requires higher energy consumption [49, 50]. Thus the low noise and higher average levels are expected to be advantageous only when the benefit of reducing noise in a particular gene’s expression outweighs this cost. The reinitiation of transcription step in the gene regulatory network can help the cellular system to choose the rate constants in such a way so that the energy consumption and mean and noise in mRNA/protein levels can be optimized.

**Appendix A. Constitutive gene expression with pre-initiation and initiation complexes**

The biochemical reactions for constitutive gene expression with pre-initiation and initiation complexes are shown in figure 16. The RNAP-II molecule binds the active gene and forms a pre-initiation complex $G_t$. Then further modifications in that produce the initiation complex from which mRNA synthesis takes place [25].

Let, there are $l$ copy number of a particular gene exist in the cell. The master equation describing the rate of change of probability $P(n_1,n_2,n_3,t)$ with $n_3$ number of mRNAs and $n_1$ number of genes in the pre-initiation state ($G_t$) and $n_2$ number of genes in the initiation complex ($G_c$) is given by

$$
\frac{\partial p(n_1,n_2,n_3,t)}{\partial t} = k_1[(l - n_1 - n_2 + 1)p(n_1 - 1,n_2, n_3,t) - (l - n_1 - n_2)p(n_1, n_2, n_3,t)] \\
+ k_2[(n_1 + 1)p(n_1 + 1,n_2 - 1, n_3,t) - n_1p(n_1, n_2, n_3,t)] \\
+ J_m[(n_2 + 1)p(n_1, n_2 + 1, n_3 - 1, t) - n_2p(n_1, n_2, n_3, t)] \\
+ k_m[(n_3 + 1)p(n_1, n_2, n_3 + 1, t) - n_3p(n_1, n_2, n_3, t)].
$$

If the reinitiation process happens in two states (as shown in figure (16)) then the expression of mean mRNA and Fano factor at mRNA level are given by

$$
\langle m_{\text{r}}^{\text{cwts}} \rangle = \frac{k_1 k_2}{J_m k_1 + J_m k_2 + k_1 k_2 k_m};
$$

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Figure 16. Reaction scheme with the rate constants for constitutive gene expression with pre-initiation and initiation complexes. $G$ is the open active state and $k_1$ is the rate constant for the open active to the pre-initiation complex formation. $k_2$ is the rate constant for the pre-initiation to the initiation complex formation. $J_m$ is the transcription rate constant and $k_m$ is the mRNA degradation rate constant.

Figure 17. Variation of mean mRNA and Fano factor with $J_m$ for the rate constants $k_1 = k_2 = 4$ and $k_m = 1$.

\[
FF_m^\text{wrt} = 1 - \frac{J_m k_1 k_2 (J_m + k_1 + k_2 + k_m)}{(J_m k_1 + J_m k_2 + k_1 k_2) \{J_m (k_1 + k_2 + k_m) + (k_1 + k_m)(k_2 + k_m)\}}.
\]

(37)

Figure (17) shows that consideration of the pre-initiation complex in the transcription initiation process results the decrease in mean and Fano factor further.

Appendix B. Two-state gene expression with pre-initiation and initiation complexes

The biochemical reactions for the two-state gene activation process with pre-initiation and initiation steps are shown in figure 18. The gene state $G_t$ is the pre-initiation complex and $G_c$ is the initiation complex. From the initiation complex, the RNAP-II starts transcription for mRNA synthesis and the gene turns into an open active state.
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Let \( p(n_1, n_2, n_3, n_4, t) \) be the probability that at time \( t \), there are \( n_4 \) number of mRNAs with \( n_1 \) number of genes in the active state \((G_a)\), \( n_2 \) number of genes in the pre-initiation state \((G_i)\) and \( n_3 \) number of genes in the transcription initiation complex \((G_c)\). The number of gene in the inactive state \((G_i)\) are \((l - n_1 - n_2 - n_3)\) with \( l \) being the copy number of a particular gene. The time evaluation of the probability is given by the master equation

\[
\frac{\partial p(n_1, n_2, n_3, n_4, t)}{\partial t} = k_a \left[ \{ l - (n_1 - 1 + n_2 + n_3) \} p(n_1 - 1, n_2, n_3, n_4, t) \right. \\
- \left. \{ l - (n_1 + n_2 + n_3) \} p(n_1, n_2, n_3, n_4, t) \right] \\
+ k_d [(n_1 + 1) p(n_1 + 1, n_2, n_3, n_4, t) - n_1 p(n_1, n_2, n_3, n_4, t)] \\
+ k_1 [(n_1 + 1) p(n_1 + 1, n_2 - 1, n_3, n_4, t) - n_1 p(n_1, n_2, n_3, n_4, t)] \\
+ k_2 [(n_2 + 1) p(n_1 - 1, n_2 + 1, n_3, n_4, t) - n_2 p(n_1, n_2, n_3, n_4, t)] \\
+ k_3 [(n_2 + 1) p(n_1, n_2 - 1, n_3 - 1, n_4, t) - n_2 p(n_1, n_2, n_3, n_4, t)] \\
+ k_4 [(n_3 + 1) p(n_1, n_2 - 1, n_3 + 1, n_4, t) - n_3 p(n_1, n_2, n_3, n_4, t)] \\
+ J_m [(n_4 + 1) p(n_1, n_2, n_3, n_4 + 1, t) \\
- n_4 p(n_1, n_2, n_3, n_4, t)] \\
\]

The expressions for mean mRNA and Fano factor for mRNA \((\text{FF}_m)\) are given by

\[
\langle m^{\text{lawrrth}} \rangle = \frac{k_a}{k_m} \frac{b_0}{(b_1 + b_3)} 
\]

\[
\text{FF}_{m}^{\text{lawrrth}} = 1 - \langle m^{\text{lawrrth}} \rangle 
+ \frac{(k_a + k_m) b_0}{\left[ k_m \{ b_1 + J_m (k_d (b_5 - k_1) + (k_a + k_m) b_3) \} + k_a (k_2 (k_4 + k_m) + b_6 (k_1 + k_m)) \right]} 
\]

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Figure 19. Plot of mean mRNA and Fano factor corresponding to the figure (18) with $J_m$ for the rate constants $k_a = k_d = 10$, $k_2 = k_4 = 1$, $k_m = 1$ and three different sets of $k_1$ and $k_3$. The solid line corresponds to the two-state process without reinitiation (figure 4(a)).

where $b_0 = J_m k_1 k_3$, $b_1 = J_m (k_4 k_1 + k_4 k_2 + k_3 k_3 + k_4 k_3 + k_1 k_3 + k_4 k_1 + k_4 k_3 + k_2 k_4 + k_2 k_4 + k_2 k_4)$, $b_2 = k_m (k_4 k_1 + k_4 k_3 + k_1 k_3 + k_4 k_1 + k_4 k_3)$, $b_3 = k_m (k_1 k_1 + k_1 k_4 + k_2 k_4) + k_2 k_3 k_4$, $b_4 = k_m^2 (k_4 + k_1 + k_2 + k_3 + k_4 + k_m)$, $b_5 = (k_1 + k_2 + k_3 + k_m)$, $b_6 = (k_4 + k_4 + k_m)$, $b_7 = k_m k_1 k_3 + b_2 + b_4$.

Figure 19 shows that the mean and the Fano factor can be controlled in better ways by controlling the rate constants $k_1$ and $k_3$.

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