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STOCHASTIC DYNAMICS OF GENE TRANSCRIPTION

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Yan Xie, Student

Dr. Richard J. Kryscio, Major Professor

Arne Bathke, Ph.D., Director of Graduate Studies

STOCHASTIC DYNAMICS OF GENE TRANSCRIPTION

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in the College of Arts and Sciences
at the University of Kentucky

By
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Lexington, Kentucky

Director: Dr. Richard J. Kryscio, Professor of Statistics

Lexington, Kentucky

2011

ABSTRACT OF DISSERTATION

STOCHASTIC DYNAMICS OF GENE TRANSCRIPTION

Gene transcription in individual living cells is inevitably a stochastic and dynamic process. Little is known about how cells and organisms learn to balance the fidelity of transcriptional control and the stochasticity of transcription dynamics. In an effort to elucidate the contribution of environmental signals to this intricate balance, a Three State Model was recently proposed, and the transcription system was assumed to transit among three different functional states randomly.

In this work, we employ this model to demonstrate how the stochastic dynamics of gene transcription can be characterized by the three transition parameters. We compute the probability distribution of a zero transcript event and its conjugate, the distribution of the time durations in gene on or gene off periods, the transition frequency between system states, and the transcriptional bursting frequency. We also exemplify the mathematical results by the experimental data on prokaryotic and eukaryotic transcription.

The analysis reveals that no promoters will be definitely turned on to transcribe within a finite time period, no matter how strong the induction signals are applied, and how abundant the activators are available. Although stronger extrinsic signals could enhance promoter activation rate, the promoter creates an intrinsic ceiling that no signals could cross over in a finite time. Consequently, among a large population of isogenic cells, only a portion of the cells, but not the whole population, could be induced by environmental signals to express a particular gene within a finite time period. We prove that the gene on duration follows an exponential distribution, and the gene off intervals show a local maximum that is best described by assuming two sequential exponential process. The transition frequencies are determined by a system of stochastic differential equations, or equivalently, an iterative scheme of integral operators. We prove that for each positive integer n, there associates a unique time, called the peak instant, at which the n^{th} transcript synthesis cycle since time zero proceeds most likely. These moments constitute a time series preserving the nature order of n.

KEYWORDS: Stochastic Dynamics, Three State Model, Zero Transcript Event,
Transition Frequency, Frequency Burst

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December 12, 2011
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STOCHASTIC DYNAMICS OF GENE TRANSCRIPTION

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December 12, 2011

Dedicated to my husband, Zhiyong Xi, and my son, Andrew Xi

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TABLE OF CONTENTS

LIST OF FIGURES

Chapter 1

Introduction

Despite the intricacy of gene regulatory networks, transcription in individual living cells is inevitably a probabilistic and dynamic process. The timing and strength of transcriptional activation are determined by a succession of dynamic events, each with a given probability. The randomness of these events would produce fluctuations in the number of transcripts and proteins, constituting the phenotypic heterogeneity in a cell population (Kaufmann and van Oudenaarden, 2007; Maheshri and O'Shea, 2007; Raj and van Oudenaarden, 2008; Schrodinger, 1944). Although numerous protein factors responsible for transcriptional control have been identified, and many of their interactions have been discerned (Bushnell et al., 2004; Lemon and Tjian, 2000), surprisingly little is known about how cell and organisms learn to balance the fidelity of transcriptional control and the stochasticity of transcription process (Maheshri N and O'Shea, 2007; Raser and O'Shea, 2004).

Recent studies on stochastic gene expression have created revealing insights on the random nature of transcription (Austin et al., 2006; Elowitz et al., 2002; McAdams and Arkin, 1997; Ozbudak et al., 2002; Paulsson, 2004, 2005; Raser and O'Shea, 2004; Raj et al., 2010; Rosenfeld et al., 2005; Swain et al., 2002; Thattai and van Oudenaarden, 2001). These studies have offered compelling evidence in support of the model that, often, single genes are transcribed randomly and discontinuously, but not deterministically and continuously. The recent in vivo RNA detection technique, pioneered by the Singer laboratory (Bertrand et al., 1998), has allowed direct real-time observations of mRNA transcripts in individual living amoeba *Dictyostelium discoideum* cells (Chubb et al., 2006). Their findings have confirmed the Poisson scenario of transcriptional bursts, previously suggested by theoretical studies of Berg (1978), Rigney (1979), and Rigney and Schieve (1977). A transcriptional burst is characterized by relatively long periods of zero transcription interrupted by production of many transcripts in a quick succession. The bursts occurred randomly, but their durations fitted robustly with standard exponential decay curves (Chubb et al., 2006). Golding and his collaborators (Golding et al., 2005) have optimized the technique of Singer to allow precise counting of transcripts produced by a synthetic variant of the lac promoter in living Escherichia coli cells, and offered further statistical quantifications of the transcriptional bursts. Xie and colleagues (Cai et al., 2006; Taniguchi et al., 2010; Yu et al., 2006) have developed a novel assay allowing the detection of single protein molecules in E. coli cells, and confirmed the geometrical distribution of protein bursts suggested by Berg (1978) and Rigney (1979).

What causes stochasticity of gene transcription? This has been an intriguing open question in molecular genetics. In an earlier study, Ko (1991) proposed that it was caused by random switching between "gene on" and "gene off" states. This elegant proposition has been further explored theoretically (Kaern, 2005; Kepler and Elston, 2001; Paulsson, 2004, 2005; Peccoud and Ycart, 1995) and reinforced by experimental examples (Golding et al., 2006; Raj et al., 2006). For induced transcription, however, it remains unclear from this proposition how environmental signals contribute to the randomness of the transition between the on and off states.

In an effort to elucidate how the stochasticity of gene transcription profiles correlates with cellular conditions, a Three State Model was proposed (Tang, 2008, 2010) and further extended (Sun, Tang and Yu 2010a, 2010b). In the model, the transcription system consists of a gene, along with one type of sequence specific transcription factors (abbreviated as TF) that have cognate binding sites in the core gene promoter, an induction agent that can stimulate the specific binding of TF to the promoter DNA, and the intermediate agents sustaining their function linkage. It was assumed that the transcription system randomly rotates among three different functional states in the temporal order: ground state $Q \to$ excited state $Y \to$ engaged state E. The ground state is characterized by the lack of effective binding activity between TF and the core gene promoter, so there is no transcription initiation complex binding to the promoter, and no RNA polymerase II (Pol II) elongating the coding region either. If the TF and DNA binding is not followed by the assembling of basal transcription machinery at the transcription start site, then the system will remain in the ground state. If the binding can facilitate subsequent transcription activity effectively, then there could be multiple stable intermediate complexes formed in the pathway from the TF-DNA binding to the successful recruitment of Pol II at the transcription start site. We define the exit of the ground state, and so the entry of the excited state, to be the threshold moment when a particular complex satisfying the following property is formed: The interaction between the TF and the core promoter affects the formation and stability of this complex, but plays no essential role in the subsequent transcription activities before the system leaves the excited state. The exit of the excited state (or the entry of engaged state) is defined to be the instant at which the first phosphorylated Pol II is released from the transcription initiation complex to begin transcribing the gene. When the last engaged Pol II leaves the gene in this transcription cycle, the system returns back to the ground state.

The Three State Model was also supported by the genome-wide study performed by Zeitlinger et al. (2007). In their study, they analyzed global Pol II occupancy in a homogeneous population of mesodermal precursor cells from Toll10b Drosophila embryos. They carried out comprehensive Pol II chromatin immunoprecipitation microarray (ChIP-chip) assays and showed that the 13,448 protein coding genes could be classified into three distinct dynamic states: In 37% of all genes there were no Pol II binding altogether and genes remained silent; in about 7.5% of all genes Pol II was tightly restricted to the transcription start site; and in the rest of genes Pol II distributed throughout the entire transcription unit. Apparently, according to the Three State Model, the first class of genes resided uniformly in the ground state. The second class and third class genes remained in the excited state and the engaged state respectively. In the second class of genes, Pol II was engaged in transcription initiation but paused near the transcription start site. Pol II stalling in those genes suggests that the transition from transcription initiation to elongation could be a rate limiting step in the gene expression pathway (Core and Lis, 2008; Wade and Struhl, 2007).

When compared with previous studies on stochastic gene transcription, in which the system behaviors were divided into the "gene on" and "gene off" states (Kepler and Elston, 2001; Ko, 1991; Paulsson, 2004; Peccoud and Ycart, 1995; Raj et al., 2006), the engaged state E in the Three State Model is essentially identical to the "on" state, and the states Q and Y constitute the "off" state. The gene can only be transcribed when the system remains in the engaged state. Because transition between the system states is random, and the engaged state is interrupted by Q and Y, the Three State Model clearly indicates that the gene in individual cells is transcribed randomly and discontinuously.

This has been strongly supported by the experimental data and theoretical studies (Elowitz et al., 2002; McAdams and Arkin, 1997; Raser and O'Shea, 2004; Rosenfeld et al., 2005; Swain et al., 2002; Thattai and van Oudenaarden, 2001).

In the following chapters, we first revisit in detail the definition of Three State Model and demonstrate how the stochastic dynamics of gene transcription could be characterized by the transition parameters. Then we compute the probability distribution of a zero transcript event, the durations in gene on or gene off periods, the transition frequency, and the bursting frequency. We also exemplify the mathematical results by the experimental data on prokaryotic and eukaryotic transcription.

Chapter 2

The Three State Model

In this chapter, we revisit in detail the definition of the Three State Model and demonstrate how the stochastic dynamics of gene transcription could be characterized by the transition parameters.

2.1 Introduction to the Three State Model

In an effort to elucidate how the stochasticity of gene transcription profiles correlates with cellular conditions, a Three State Model was proposed (Tang, 2008, 2010) and further extended (Sun, Tang and Yu 2010a, 2010b).

In the Three State Model, the transcription system consists of a gene, one type of transcription factor (TF) that can activate or repress the gene promoter, an induction agent that can induce specific binding of TF to the promoter, and other molecules sustaining their functional linkage.

The transcription system is assumed to exist in three different functional states: ground state Q, excited state Y, and engaged state E. The ground state is characterized by the lack of effective binding between the core gene promoter and TF, so there is no transcription initiation complex on the promoter, and no elongation by RNA polymerase II (Pol II). If the TF and DNA binding is not followed by the assembly of basal transcription machinery at the transcription start site, then the system will remain in the ground state. This occurs especially if the TF is a strong repressor of the gene promoter.

If the binding effectively facilitates subsequent transcription, then we define the exit of the ground state to be the threshold moment when a particular complex satisfying the following property is formed: The interaction between the TF and the core promoter affects the formation and stability of this complex, but plays no essential role in the subsequent transcription before the system leaves the excited state. There may not be a universal configuration of this particular complex, which could vary dramatically between cells and organisms.

The exit of the excited state is defined to be the instant at which the first Pol II is released from the transcription initiation complex to begin transcribing the gene. When the last engaged Pol II leaves the gene in this transcription cycle, the system returns back to the ground state.

The three functional states randomly rotates in the recurrent Markov chain $Q \xrightarrow{\kappa} Y \xrightarrow{\lambda} E \xrightarrow{\gamma} Q \xrightarrow{\kappa} \ldots$, where each arrow denotes an exponential distribution, and κ, λ, and γ are the corresponding transition rates. We treat the transition from Q to Y as an irreversible stochastic process, because we regard those TF/bindings that cannot induce the formation of the stable intermediate complex as sub-events of the ground state.

2.2 The parameters in the Three State Model

The parameter κ, the transition rate from Q to Y, is called the induction strength, that quantifies the effectiveness of the induction agents in transforming the system into the excited states. The value of κ depends not only on the amounts or concentrations of the agent, but also on how efficiently the binding of TF and promoter initiates the formation of the intermediate complex. Parameter λ, the transition rate from Y to E, is called the activation strength which quantifies the activation potential of the TF. Parameter γ, the transition rate from Y to E, is called the promoter fragility because a larger γ corresponds to a shorter life of the elongation state on average.

Parameter κ may or may not correlate with the binding affinity between the TF and the gene promoter in a parallel fashion: a strong binding may correspond to a small κ if the binding activity is ineffective in inducing system state transition. There is no apparent constraint on the nature of the induction agent: It can be a physical condition such as temperature or radiation, or a physiological condition such as starvation or DNA damage. More interestingly, it can be a biomolecule such as a growth factor that can induce binding of the TF to the gene promoter by turning on intracellular signal transduction pathways. In this case, any protein or RNA species within a pathway, including the target TF itself, can be taken as the induction agent in the model. This flexibility allows us to use the model to examine how the transcription profiles respond to perturbations of signal transduction networks. Because the concentration of inducing agents may change in time and space, κ can inherit the temporal variation and spatial heterogeneity. As a result, κ is not a constant, but a function of time and spatial variables, and further transfers the heterogeneities to the gene expression profiles.

The definition of the excited state makes it clear that the transition from Y to E is essentially independent of the cellular concentration of free specific TF and, thus, irrelevant to the induction agents. The same is true for the transition from E to Q. Therefore, λ and γ are mostly determined by the biochemical properties of the TF and the gene, both of which are intrinsic to the transcription system. In contrast to the temporal and spatial variation of κ, the parameters λ and γ can be approximately treated as constants. We define the pair (λ, γ) as the transcription mode allied to the TF and the gene in the transcription system. It is of great interest to test the hypothesis that this mode could be evolutionary conserved and would not vary notably among cells of closely related organisms.

In summary, the Three State Model deciphers transcription activation through the two parameters of distinct characteristics. The induction strength κ is mostly determined by environmental signals, and could inherit the temporal and spatial fluctuation of the signals. In contrast, the λ and γ are intrinsic to the TF and the gene, so they are approximately a constant and does not vary significantly within a homogeneous population of cells. By decomposing the activation dynamics into the impingement of environmental signals and the intrinsic response of activators and promoters, this model explicitly relates transcription stochasticity with the variability of micro-environmental conditions.

Chapter 3

Probability of a Zero Transcription Event in the Three State Model

In this chapter, we define and compute the distribution of the probability of zero transcription in the Three State Model, then define its conjugate, the probability of gene induction. We also discuss the properties of the two probabilities. Finally, we exemplify the mathematical results by the experimental data and simulation.

3.1 The distribution of the probability of a zero transcription event in the Three State Model

In order to test more directly how an induction agent affects the expression of a given gene, it has been helpful to work with the experimental assays where the gene remains silent before the application of the agent. Accordingly, we only gave the detailed mathematical analysis under the assumption that the transcription systems in cells are uniformly locked in ground states at time zero. The basic ideas of the analysis are similar when other types of initial conditions are more appropriate. For technical reasons, we only discuss the dynamics when the induction agents are kept at a constant level.

Theorem 3.1 Let $P_0(t)$ be the probability that the system has never arrived at the engaged state in $(0, t)$, then

$$P_0(t) = \begin{cases} \dfrac{\lambda}{\lambda - \kappa} e^{-\kappa t} + \dfrac{\kappa}{\kappa - \lambda} e^{-\lambda t}, & \kappa \neq \lambda \\ (1 + kt) e^{-kt} & k = \lambda \end{cases} \tag{3.1}$$

Proof of Theorem 3.1: Let $P_{1q}(t)$ be the probability that the transcription system has remained in the ground state since the application of the induction agents (time zero), and $P_{1y}(t)$ be the probability that the system is residing on the excited state Y at time t.

Then $P_0(t) = P_{1q}(t) + P_{1y}(t)$. We derive the probability distribution of $P_{1q}(t)$ and $P_{1y}(t)$ in the following paragraphs.

First, we find that $P_{1q}(t) = e^{-\kappa t}$. Here's the details for reasoning: Let X be the waiting time for transition from Q to Y, then $P(X \le t) = 1 - e^{-\kappa t}$ since X is distributed exponentially with parameter k. It follows that

$$P_{1q}(t) = \Pr ob(X > t) = 1 - \Pr ob(X \le t) = e^{-\kappa t}. \tag{3.2}$$

It's apparent that $P_{1q}(0) = 1$.

Second, we find that

$$P_{1y}(t) = \kappa e^{-\lambda t} \int_0^t e^{(\lambda-\kappa)s} ds = \begin{cases} \dfrac{\kappa}{\lambda - \kappa}(e^{-\kappa t} - e^{-\lambda t}) & \kappa \ne \lambda \\ \kappa t e^{-kt} & \kappa = \lambda \end{cases}. \tag{3.3}$$

To find $P_{1y}(t)$ analytically, we follow the transition rule of the Three State Model to derive the differential equation of $P_{1y}(t)$. It is presented in detail below, see Allen (2003) for mathematical background. This basic idea also helps us derive the system of master equations in Chapter 5 where the functions $P_{1q}(t)$ and $P_{1y}(t)$ are extended to $P_{nx}(t)$ with n ≥ 1 and $x = q, y,$ and e.

Here's the details to find $P_{1y}(t)$. Let Δt be an infinitesimal time increment. We first calculate $P_{1y}(t + \Delta t)$ in terms of $P_{1q}(t)$ and $P_{1y}(t)$. If the system has been remained in the ground state during the time period $(0, t)$, then it has a probability $\kappa \Delta t$ to shift to the excited state during the time interval (t, $t + \Delta t$) based on the definition of exponential distribution; this contributes $\kappa \Delta t P_{1q}(t)$ to $P_{1y}(t + \Delta t)$. If the system has arrived and stayed at the excited state in the time period $(0, t)$, then it has a probability $1 - \lambda \Delta t$ to remain at this state during the infinitesimal time interval; this contributes $(1-\lambda\Delta t)P_{1y}(t)$ to $P_{1y}(t+\Delta t)$. If the system has arrived and then left the excited state in the time period (0, t), then it contributes zero probability to $P_{1y}(t+\Delta t)$. In summary,

$$P_{1y}(t+\Delta t) = \kappa\Delta t P_{1q}(t) + (1-\lambda\Delta t)P_{1y}(t),$$

$$\text{or} \quad \frac{P_{1y}(t+\Delta t) - P_{1y}(t)}{\Delta t} = \kappa P_{1q}(t) - \lambda P_{1y}(t).$$

Letting $\Delta t \to 0$, we have the following reasoning: The limit yields the equation

$$\frac{dP_{1y}(t)}{dt} = \kappa P_{1q}(t) - \lambda P_{1y}(t)$$

$$\Rightarrow \frac{dP_{1y}(t)}{dt} + \lambda P_{1y}(t) = \kappa P_{1q}(t)$$

$$\Rightarrow \frac{dP_{1y}(t)}{dt} e^{\lambda t} + P_{1y}(t)e^{\lambda t}\lambda = e^{\lambda t}\kappa P_{1q}(t) \text{ since multiplying both sides by } e^{\lambda t}$$

$$\Rightarrow \frac{d(P_{1y}(t)e^{\lambda t})}{dt} = e^{\lambda t}\kappa P_{1q}(t) \text{ since } d(P_{1y}(t)e^{\lambda t}) = \frac{dP_{1y}(t)}{dt} e^{\lambda t} + P_{1y}(t)e^{\lambda t}\lambda$$

$$\Rightarrow \int_0^t \frac{d(P_{1y}(s)e^{\lambda s})}{ds} ds = \int_0^t e^{\lambda s}\kappa P_{1q}(s) ds$$

$$\Rightarrow P_{1y}(s)e^{\lambda s}\Big|_{s=0}^{s=t} = \int_0^t e^{\lambda s}\kappa P_{1q}(s) ds$$

$$\Rightarrow P_{1y}(t)e^{\lambda t} - P_{1y}(0) = \int_0^t e^{\lambda s}\kappa P_{1q}(s) ds$$

$$\Rightarrow P_{1y}(t)e^{\lambda t} = \int_0^t e^{\lambda s}\kappa P_{1q}(s) ds \text{ since } P_{1y}(0) = 0$$

$$\Rightarrow P_{1y}(t) = e^{-\lambda t}\int_0^t e^{\lambda s}\kappa P_{1q}(s)ds$$

$$\Rightarrow P_{1y}(t) = e^{-\lambda t}\int_0^t e^{\lambda s}\kappa e^{-\kappa s}ds \text{ since } P_{1q}(s) = e^{-\kappa s} \text{ by (3.2)}$$

$$\Rightarrow P_{1y}(t) = \kappa e^{-\lambda t}\int_0^t e^{(\lambda-\kappa)s}ds$$

$$\Rightarrow P_{1y}(t) = \begin{cases} \kappa e^{-\lambda t}\left.\dfrac{e^{(\lambda-\kappa)s}}{\lambda-\kappa}\right|_{s=0}^{s=t} & \kappa \neq \lambda \\ \kappa e^{-\lambda t} s\Big|_{s=0}^{s=t} & \kappa = \lambda \end{cases}$$

$$\Rightarrow P_{1y}(t) = \begin{cases} \kappa e^{-\lambda t}\left(\dfrac{e^{(\lambda-\kappa)t}}{\lambda-\kappa} - \dfrac{1}{\lambda-\kappa}\right) & \kappa \neq \lambda \\ \kappa e^{-\lambda t}(t-0) & \kappa = \lambda \end{cases}$$

$$\Rightarrow P_{1y}(t) = \begin{cases} \dfrac{\kappa}{\lambda-\kappa}(e^{-\kappa t} - e^{-\lambda t}) & \kappa \neq \lambda \\ \kappa t e^{-kt} & \kappa = \lambda \end{cases}$$

So $P_0(t)$, the probability that the system has never arrived at the engaged state in $(0,t)$, is as given below

$$P_0(t) = \begin{cases} \dfrac{\lambda}{\lambda-\kappa}e^{-\kappa t} + \dfrac{\kappa}{\kappa-\lambda}e^{-\lambda t}, & \kappa \neq \lambda \\ (1+kt)e^{-kt} & \kappa = \lambda \end{cases}$$

After the system enters the engaged state, it is possible that there is a short time delay before the nascent mRNA emerge from the elongating Pol II. This delay is usually insignificant compared to the average residency time of the three functional states. Therefore, $P_0(t)$ equals approximately the probability that no gene transcripts have been produced since the application of the induction agents. When the expression profile of a homogeneous population of cells is measured, $P_0(t)$ can be used to estimate the portion of silent cells during the time interval $(0,t)$.

3.2 Conjugate of probability of a zero transcription event

Because the probability of a zero transcription event $P_0(t)$ provides a measure of the proportion of silent cells, its conjugate $P_{in}(t)=1-P_0(t)$, called the induction probability, can be used to estimate the proportion of the cells where the gene has been induced to elongation state at least once since time zero.

3.3 Properties of probability of a zero transcription event and its conjugate

The properties of probability of zero transcription event $P_0(t)$ and its conjugate $P_{in}(t)$ are given as follows:

1) $P_0(t)\equiv 1$ and $P_{in}(t)\equiv 0$ if either κ=0 or λ=0. It means that the system remains silent for all $t\geq 0$ if either κ=0 or λ=0. This justifies the necessity of the induction agents and the regulatory activators for transcription initiation.

2) $P_{in}(t)$, as a function of κ, is increasing. That is, $P_{in}(t)$ increases as the induction signal is strengthened, which is consistent with our intuitive perception that stronger induction signals activate more promoters. The proof for $P_{in}(t)$, as a function of κ is increasing, is given as follows: Write $P_{in}(t,\kappa)$ for $P_{in}(t)$ to indicate its dependence on the induction strength κ explicitly. Then differentiating $P_{in}(t,\kappa)$ with respect to κ gives, for all $\kappa\neq\lambda$,

$$\frac{dP_{in}(t)}{d\kappa}=\frac{d(1-\frac{\lambda}{\lambda-\kappa}e^{-\kappa t}-\frac{\kappa}{\kappa-\lambda}e^{-\lambda t})}{d\kappa}$$

$$= -\frac{\lambda}{(\lambda-\kappa)^2}e^{-\kappa t} + \frac{\lambda t}{\lambda-\kappa}e^{-\kappa t} - \frac{(\kappa-\lambda)-\kappa}{(\kappa-\lambda)^2}e^{-\lambda t}$$

$$= -\frac{\lambda}{(\lambda-\kappa)^2}e^{-\kappa t} + \frac{\lambda t}{\lambda-\kappa}e^{-\kappa t} - \frac{-\lambda}{(\kappa-\lambda)^2}e^{-\lambda t}$$

$$= -\frac{\lambda e^{-\kappa t}}{(\lambda-\kappa)^2}[1-(\lambda-\kappa)t-e^{-(\lambda-\kappa)t}] \qquad > 0 \qquad \text{because } 1+x<e^x \text{ as long as x} \neq 0.$$

3) $\lim_{t\to\infty} P_0(t) = 0$ and $\lim_{t\to\infty} P_{in}(t) = 1$ as long as κ and λ are positive. It predicts that any promoter will be turned on to transcribe eventually, if it is kept constantly stimulated, and the specific transcription factors are continually available.

4) $P_{in}(t) < \lim_{\kappa\to\infty} P_{in}(t) = 1 - e^{-\lambda t}$ for all $\kappa > 0$ and $\lambda > 0$ because $P_{in}(t)$ as a function of κ is increasing, which is due to $dP_{in}(t,\kappa)/d\kappa > 0$. Therefore, the induction probability $P_{in}(t)$ is always strictly < 1, even if the induction signals are exceedingly strong. The upper limit $1-e^{-\lambda t}$ is determined by the intrinsic parameter λ, and is independent of κ. This suggests that no promoters will be definitely turned on to transcribe within a finite time period, no matter how strong the induction signals are applied, and how abundant the activators are available. Although stronger extrinsic signals could enhance promoter activation rate, the promoter creates an intrinsic ceiling that no signals could cross over in a finite time. Consequently, among a large population of isogenic cells, only a portion of the cells, but not the whole population, could be induced by environmental signals to express a particular gene within a finite time period. These statements indicate that a weak and continual external signal within a long time period may provoke a more potent transcriptional response than a strong and transient signal does within a short time period.

3.4 Example and simulation result for probability of a zero transcription event

In this section we demonstrate how formula for $P_0(t)$ could be utilized to estimate the probability of zero transcriptions, by using the transcription system of Golding et al. (2005) in the clonal population of living *E. coli* cells.

First, we describe the transition system briefly. This system consists of two components: the tagging protein and the RNA target. The tagging protein, named MS2-GFP, is a fusion of the MS2 coat protein to a green fluorescent protein (GFP). And the RNA target contains the coding region for a red fluorescence protein (Campbell et al., 2002), followed by a tandem array of 96 MS2 binding sites. The MS2 coat protein in MS2-GFP can recognize the MS2 binding sites in the RNA target, and the GPF in the MS2-GFP allows the detection and measurement of this RNA target by fluorescence microscopy and image analysis. In the experiment, the expression of MS2-GFP at optimal levels was first induced by adding anhydrotetracycline in the system. The RNA target (RNA transcripts) were then induced by Isopropyl-β-D- thiogalactopyranoside (IPTG) under the control of a $P_{\text{lac/ara}}$ promoter (Lutz and Bujard, 1997). The amount of RNA transcripts were imaged and measured at different time points by fluorescence microscopy.

Golding et al. (2005) estimated $P_0(t)$ by the fraction of cells having no tagged RNA because $P_0(t)$ can be used to estimate the portion of silent cells during the time interval $(0,t)$ when the expression profile of a homogeneous population of cells is measured. They found the measured $P_0(t)$, as a function of time, decreased exponentially at a decay rate $0.032 \pm 0.005 \text{ min}^{-1}$ (see '+' and 'O' in Figure 3.1). However, the estimated decay rate was $0.014 \pm 0.02 \text{ min}^{-1}$ based on their theoretical prediction of the first-order transcription model $P_0(t) = e^{-\kappa_1 t}$ (see dash line in Figure 3.1). This estimated number is about four times larger than the measured decay rate $0.032 \pm 0.005 \text{ min}^{-1}$. They attributed this discrepancy to the stochastic nature of mRNA synthesis and elimination.

Our formula to estimate $P_0(t)$ performs better than that suggested by Golding and his colleagues (see solid line in Figure 3.1) . In the plot of logarithm of $P_0(t)$ versus time, our estimated $P_0(t)$ fits the actual data much better than that in Golding et al. (2005) does (solid line versus dash line in Figure 3.1). Our curve is not identically a straight line, but is nevertheless well approximated by a straight line with slope = -0.03125, which is very close to the measured decay rate 0.032 .

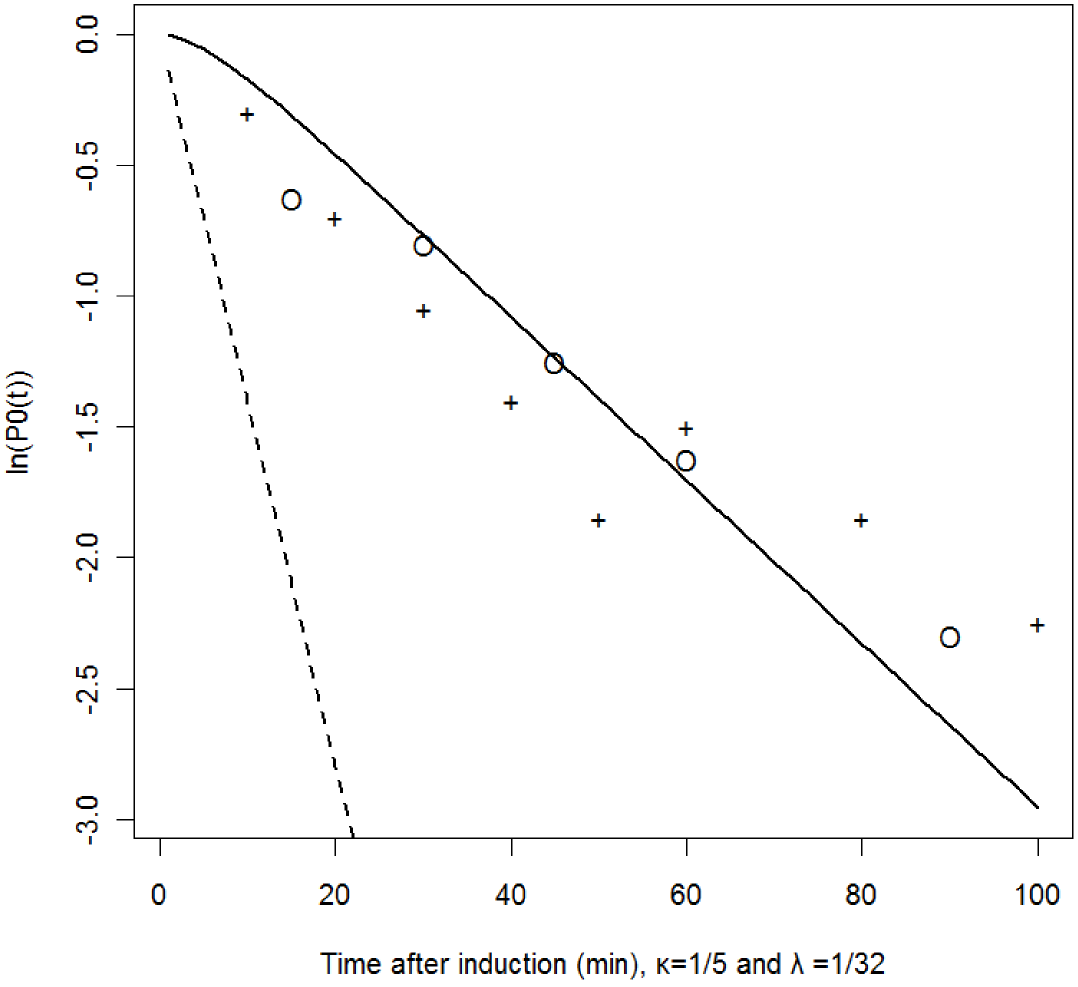

Figure 3. 1 Probability of zero transcriptions since induction (fraction of cells having no tagged RNA ($P_0(t)$) as a function of time after induction t). Data $(+, \mathrm{O}, \Delta)$ are from Golding et al. (2005). Dashed line is the theoretical prediction of the first-order transcription model $P_0(t) = e^{-\kappa_1 t}$ by Golding and his colleagues, with $\kappa_1 = 0.14$. The actual decline is about four times slower, with a rate of approximately 0.032 $\min^{-1}$. Solid

line is the theoretical prediction by our model which is

$$P_0(t) = \frac{\lambda}{\lambda-\kappa} e^{-\kappa t} + \frac{\kappa}{\kappa-\lambda} e^{-\lambda t}, \qquad \kappa \neq \lambda, \text{ with } \kappa{=}1/5 \ \text{min}^{-1} \text{ and } \lambda = 1/32 \ \text{min}^{-1}.$$

The details for estimating κ, λ and γ were given here. By (3.3), $P_0(t)$ is determined by the induction strength κ and the activation strength λ, and is independent of the rates of mRNA production and elimination. As Golding and his colleagues had estimated that the mean transcription inactivity periods $\Delta t_{OFF} \approx 37$ min and the mean activity periods $\Delta t_{ON} \approx 6$ min, we take $\kappa^{-1} + \lambda^{-1}$=37 min, and $1/\gamma = 6$ min. Although no further information is currently available to estimate κ and λ separately, we assume that κ is much bigger than λ, because IPTG was added at optimal amounts and the promoter was observed to be fully activated when the data were collected. This consideration suggests us to take $1/\kappa{=}5$ min and $1/\lambda = 32$ min (that is, $\kappa{=}0.2 \ \text{min}^{-1}$ and $\lambda = 0.03125 \ \text{min}^{-1}$) in our simulation, see Fig. 1.

Chapter 4

Durations in Gene Off and Gene On Periods in the Three State Model

In this chapter, we first compute the distribution of durations in gene off or gene on periods in the Three State Model. Then we discuss the properties of the distributions. Finally, we exemplify the mathematical results by the experimental data and simulation.

4.1 The distribution of durations in gene off and gene on periods in the Three State Model

Theorem 4.1.1 Let $P_{off}(t)$ and $f_{off}(t)$ be the cumulative and density distribution function of the duration in the gene off period respectively, then

1) $$P_{off}(t) = \begin{cases} 1 - \dfrac{\lambda e^{-\kappa t} - \kappa e^{-\lambda t}}{\lambda - \kappa} & for\, \kappa \neq \lambda \\ 1 - \kappa t e^{-\kappa t} - e^{-\kappa t} & for\, \kappa = \lambda \end{cases}$$

2) $$f_{off}(t) = \begin{cases} \dfrac{\kappa\lambda}{\lambda - \kappa}(e^{-kt} - e^{-\lambda t}) & for\, \kappa \neq \lambda \\ \kappa^2 t e^{-\kappa t} & for\, \kappa = \lambda \end{cases}$$

3) The average duration in the gene off period is $1/\kappa + 1/\lambda$.

Theorem 4.1.2 Let $P_{on}(t)$ and $f_{on}(t)$ be the cumulative and density distribution function of the duration in the gene on period respectively, then

1) $P_{on}(t) = 1 - e^{-\gamma t}$

2) $f_{on}(t) = \gamma e^{-\gamma t}$

3) The average duration in the gene on period is $1/\gamma$.

Proof for Theorem 4.1.1:

1) Show

$$P_{off}(t)=\begin{cases}1-\dfrac{\lambda e^{-\kappa t}-\kappa e^{-\lambda t}}{\lambda-\kappa} & for\ \kappa\neq\lambda\\ 1-\kappa t e^{-\kappa t}-e^{-\kappa t} & for\ \kappa=\lambda\end{cases}\quad .$$

Let X be the waiting time for the system transiting from state Q to state Y, then $f_X(t)=\kappa e^{-\kappa t}$ since X is exponentially distributed with parameter κ. Let S be the waiting time for the system transiting from state Y to state E, then $f_S(t)=\lambda e^{-\lambda t}$ because S is exponentially distributed with parameter λ. The joint density function of X and S is $\kappa e^{-\kappa s}\lambda e^{-\lambda s}$ because X and S are independent.

The duration in the gene off period is the duration that the system is in state Q or state Y. It is also the waiting time for the system transiting from state Q to state Y, plus the waiting time for the system transiting from state Y to state E. So it's the sum of durations in two sequential exponential process. Therefore, it follows that

$$P_{off}(t)=P(X+S<t)=P(X<t-S)$$

$$=\int_0^t\Big(\int_0^{t-x}f(x,s)ds\Big)dx$$

$$=\int_0^t\Big(\int_0^{t-x}(\kappa e^{-\kappa x}\lambda e^{-\lambda s})ds\Big)dx \qquad \text{since} \qquad f(x,s)=\kappa e^{-\kappa s}\lambda e^{-\lambda s}$$

$$=\int_0^t \kappa e^{-\kappa x}\Big(\int_0^{t-x}(\lambda e^{-\lambda s})ds\Big)dx$$

$$=\int_0^t \kappa e^{-\kappa x}\Big(-e^{-\lambda s}\Big|_{s=0}^{s=t-x}\Big)dx$$

$$=\int_0^t \kappa e^{-\kappa x}(1-e^{-\lambda(t-x)})dx$$

$$= \int_0^t \kappa e^{-\kappa x} dx - \int_0^t \kappa e^{-\lambda t} e^{-(\kappa-\lambda)x} dx$$

$$= -e^{-\kappa x}\Big|_{x=0}^{x=t} - \kappa e^{-\lambda t} \int_0^t e^{-(\kappa-\lambda)x} dx$$

$$= \begin{cases} 1 - e^{-\kappa t} - \kappa e^{-\lambda t} \dfrac{e^{-(\kappa-\lambda)x}}{-(\kappa-\lambda)}\Bigg|_{x=0}^{x=t} & for\, \kappa \neq \lambda \\ 1 - e^{-\kappa t} - \kappa e^{-\kappa t} x\Big|_{x=0}^{x=t} & for\, \kappa = \lambda \end{cases}$$

$$= \begin{cases} 1 - e^{-\kappa t} + \dfrac{\kappa e^{-\lambda t} e^{-(\kappa-\lambda)t}}{\kappa - \lambda} - \dfrac{\kappa e^{-\lambda t}}{\kappa - \lambda} & for\, \kappa \neq \lambda \\ 1 - e^{-\kappa t} - \kappa t e^{-\kappa t} & for\, \kappa = \lambda \end{cases}$$

$$= \begin{cases} 1 - e^{-\kappa t} + \dfrac{\kappa e^{-\kappa t}}{\kappa - \lambda} - \dfrac{\kappa e^{-\lambda t}}{\kappa - \lambda} & for\, \kappa \neq \lambda \\ 1 - \kappa t e^{-\kappa t} - e^{-\kappa t} & for\, \kappa = \lambda \end{cases}$$

$$= \begin{cases} 1 + \dfrac{\lambda e^{-\kappa t}}{\kappa - \lambda} - \dfrac{\kappa e^{-\lambda t}}{\kappa - \lambda} & for\, \kappa \neq \lambda \\ 1 - \kappa t e^{-\kappa t} - e^{-\kappa t} & for\, \kappa = \lambda \end{cases}$$

$$= \begin{cases} 1 - \dfrac{\lambda e^{-\kappa t}}{\lambda - \kappa} + \dfrac{\kappa e^{-\lambda t}}{\lambda - \kappa} & for\, \kappa \neq \lambda \\ 1 - \kappa t e^{-\kappa t} - e^{-\kappa t} & for\, \kappa = \lambda \end{cases}$$

2) Show

$$f_{off}(t) = \begin{cases} \dfrac{\kappa\lambda}{\lambda - \kappa}(e^{-kt} - e^{-\lambda t}) & for\, \kappa \neq \lambda \\ \kappa^2 t e^{-\kappa t} & for\, \kappa = \lambda \end{cases} .$$

For $\kappa \neq \lambda$,

$$f_{off}(t) = \frac{dP_{off}(t)}{dt} = \frac{d(1 - \frac{\lambda e^{-\kappa t}}{\lambda - \kappa} + \frac{\kappa e^{-\lambda t}}{\lambda - \kappa})}{dt} = \frac{-\lambda e^{-\kappa t}(-\kappa) + \kappa e^{-\lambda t}(-\lambda)}{\lambda - \kappa} = \frac{\kappa\lambda}{\lambda - \kappa}(e^{-kt} - e^{-\lambda t})$$

For $\kappa = \lambda$,

$$f_{off}(t) = \frac{d(1-\kappa te^{-\kappa t} - e^{-\kappa t})}{dt} = -\kappa(e^{-\kappa t} + te^{-\kappa t}(-\kappa)) - e^{-\kappa t}(-\kappa) = \kappa^2 te^{-\kappa t}$$

3) Show the average duration in the gene off period is $1/\kappa + 1/\lambda$.

For $\kappa \neq \lambda$, the average duration in the gene off period $= \int_0^\infty tf_{off}(t)dt$

$$= \int_0^\infty \frac{\kappa\lambda t}{\lambda - k}(e^{-\kappa t} - e^{-\lambda t})dt$$

$$= \frac{\lambda}{\lambda - k}\int_0^\infty t\kappa e^{-\kappa t}dt - \frac{\kappa}{\lambda - k}\int_0^\infty t\lambda e^{-\lambda t})dt$$

$$= \frac{\lambda}{\lambda - k}\frac{1}{k} - \frac{k}{\lambda - k}\frac{1}{\lambda} \qquad \sin ce \quad \int_0^\infty t\kappa e^{-\kappa t}dt \quad is \quad mean \quad of \quad \exp(\lambda)$$

$$= \frac{\lambda^2 - k^2}{(\lambda - k)k\lambda}$$

$$= \frac{1}{k} + \frac{1}{\lambda}$$

For $\kappa \neq \lambda$, the average duration in the gene off period $= \int_0^\infty tf_{off}(t)dt$

$$= \int_0^\infty \kappa^2 t^2 e^{-\kappa t}dt$$

$$= \int_0^\infty -\kappa t^2 d(e^{-\kappa t})$$

$$= -(\kappa t^2 e^{-\kappa t}\Big|_{t=0}^{\infty} - \int_0^{\infty} e^{-\kappa t} d(\kappa t^2)) \text{ since } \int_a^b u(v)' dx = uv\Big|_a^b - \int_a^b (u)' v dx$$

$$= \int_0^{\infty} e^{-\kappa t} d(\kappa t^2)) \qquad \sin ce \quad \kappa t^2 e^{-\kappa t}\Big|_{t=0}^{\infty} = 0$$

$$= \int_0^{\infty} 2tke^{-\kappa t} dt) \qquad \sin ce \quad \int_0^{\infty} t\kappa e^{-\kappa t} dt \quad is \quad mean \quad of \quad \exp(\lambda)$$

$$= 2/k$$

$$= 1/\kappa + 1/\lambda \qquad \sin ce \quad \lambda = \kappa$$

Proof for Theorem 4.1.2:

The Proof for Theorem 4.1.2 is straightforward. The duration in the gene on period is the duration that the system is in state E, and it is also the waiting time for the system transiting from state E to state Q, which is exponentially distributed with parameter γ. So we have

1) $P_{on}(t) = 1 - e^{-\gamma t}$

2) $f_{on}(t) = \gamma e^{-\gamma t}$

3) The average duration in the gene on period is $1/\gamma$.

4.2 Properties of the density distribution function of the duration in the gene off period

The properties of $f_{off}(t)$, the density distribution function of the duration in the gene off period are given as follows:

1) If κ and λ differ dramatically, then the density function $f_{off}(t)$ can be well approximated by a standard exponentially decaying function. This occurs in particular when the gene is fully activated and the transition step from excited state to engaged state

is relatively slow, in which case κ is large and λ is small, leading to $f_{off}(t) \approx \lambda e^{-\lambda t}$. Conversely, if the induction signals are weak but the transition from excited state to engaged state is fast, then κ is small, but λ is large, yielding $f_{off}(t) \approx \kappa e^{-\kappa t}$. In both cases, the distribution of the durations in the gene off period fits an exponential decay curve, and the logarithm of the distribution fits a straight line whose slope is either $-\lambda$ or $-\kappa$. (see solid and dash lines in Figure 4.1 and Figure 4.2)

2) However, if κ and λ are close, then the logarithm of the distribution deviates from the straight line. Because

$$\log(f_{off}(t)) = -\lambda t + \log\frac{\kappa\lambda}{\kappa-\lambda}(1-e^{-(\lambda-\kappa)t}) = -\kappa t + \log\frac{\kappa\lambda}{\lambda-\kappa}(1-e^{-(\kappa-\lambda)t}),$$

the deviation is determined by the two residual log terms behind $-\lambda t$ or $-\kappa t$. (see dotted lines in Figure 4.2)

3) In the limiting case when $\kappa = \lambda$, we have

$$\log(\lambda P_{1y}(t)) = -\kappa t + 2\log\kappa + \log t,$$

which differs from the linear part by $\log t$ (see dot-dash lines in Figure 4.2). This difference seems not to be significant, but rather it suggests a noticeable deviation of experimental data from straight lines.

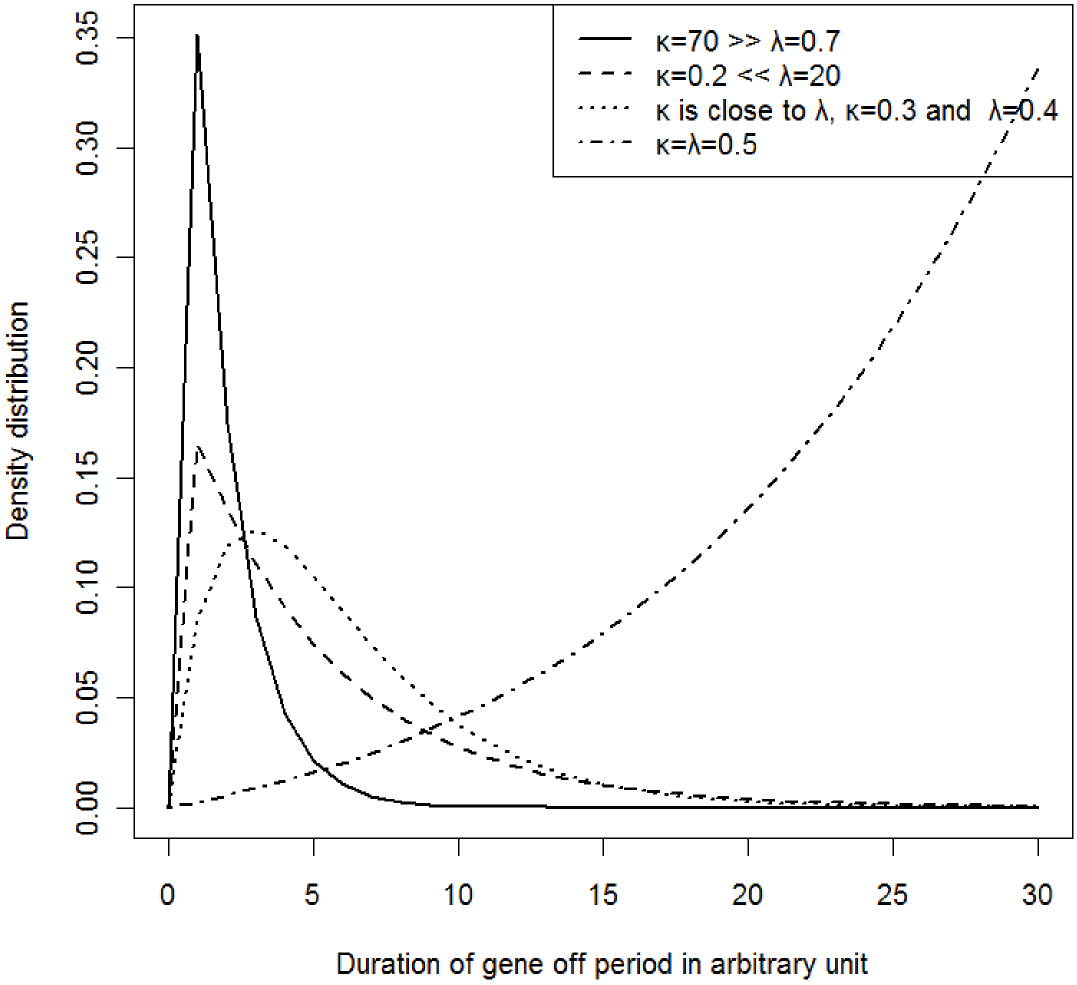

Figure 4.1 Plot of duration of gene off period versus density distribution $f_{off}(t)$. If the values of κ and λ differ dramatically, then the density function $f_{off}(t)$ can be well approximated by a standard exponentially decaying function (solid and dash lines).

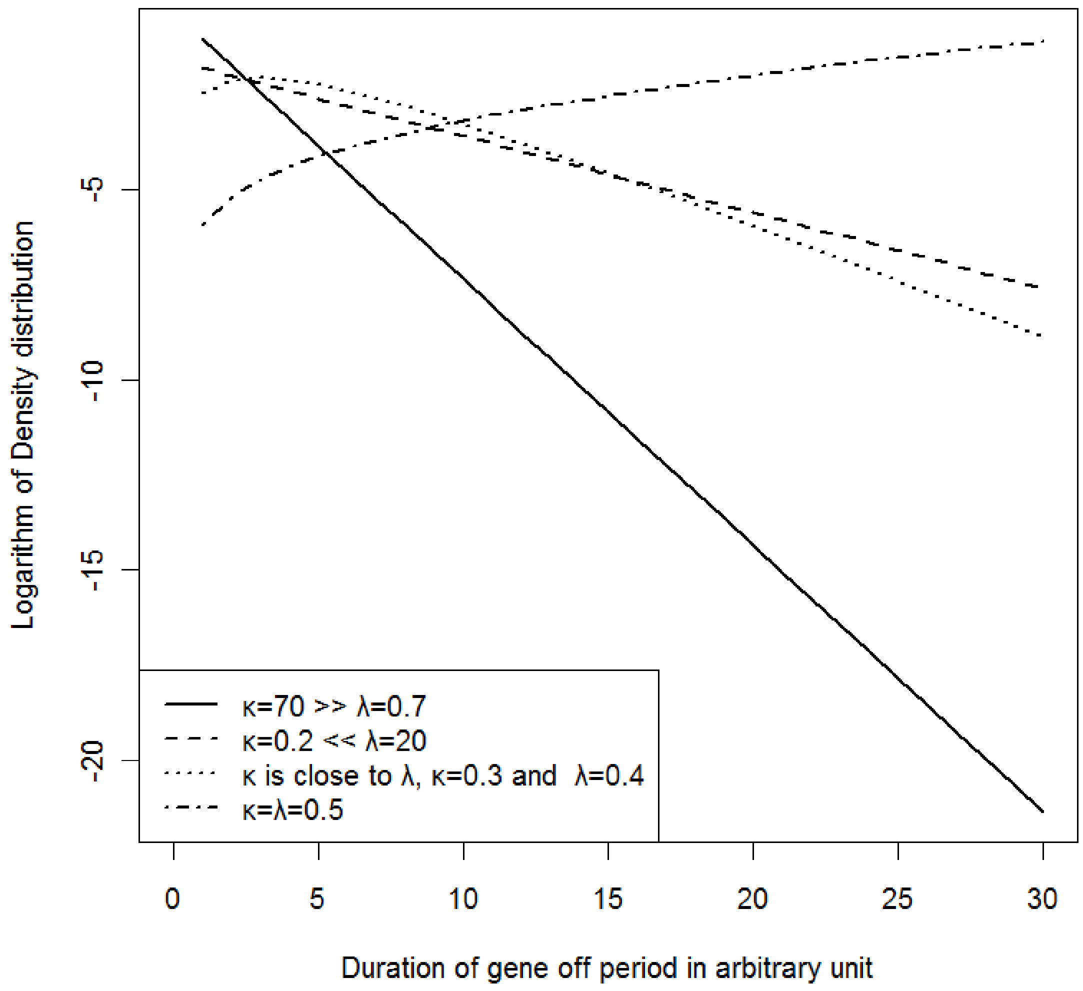

Figure 4.2 Plot of duration of gene off period versus logarithm of density distribution $\log(f_{off}(t))$. If the values of κ and λ differ dramatically, $\log(f_{off}(t))$ fits a straight line whose slope is either $-\lambda$ or $-\kappa$ (see solid and dash lines). If the values of κ and λ are close, $\log(f_{off}(t))$ deviates from the straight line by the two residual log terms, $\log\frac{\kappa\lambda}{\kappa-\lambda}(1-e^{-(\lambda-\kappa)t})$ or $\log\frac{\kappa\lambda}{\lambda-\kappa}(1-e^{-(\kappa-\lambda)t})$ (see dotted lines). In the limiting case when $\kappa=\lambda$, $\log(f_{off}(t))$ differs from the linear part by $\log t$ (see dot-dash line).

4.3 Example and simulation result for the density distribution function of the duration in the gene off and gene on period

The gene transcription was directly monitored at several studies. Raj et al. (2006) observed an ON/OFF expression pattern for the master regulatory gene of intestinal differentiation. Chubb et al. (2006) observed the Poisson scenario of transcriptional bursts. A transcriptional burst is characterized by relatively long periods of zero transcription interrupted by production of many transcripts in a quick succession. Chubb and his collaborators found the bursts occurred randomly, but their durations fitted robustly with standard exponential decay curves. Golding et al. (2005) have observed that the transcription is characterized periods of inactivity, followed by period of activity. They also confirmed that the period of activity could be described by exponentials.

Recently, Suter et al. (2011) found in their study that the gene on interval followed an exponential distribution, and the gene off interval showed a local maximum that was best described by assuming two sequential exponential process (see Figure 4.3). The findings of Suter and his collaborators confirmed our theorem for the duration of gene off and gene on period.

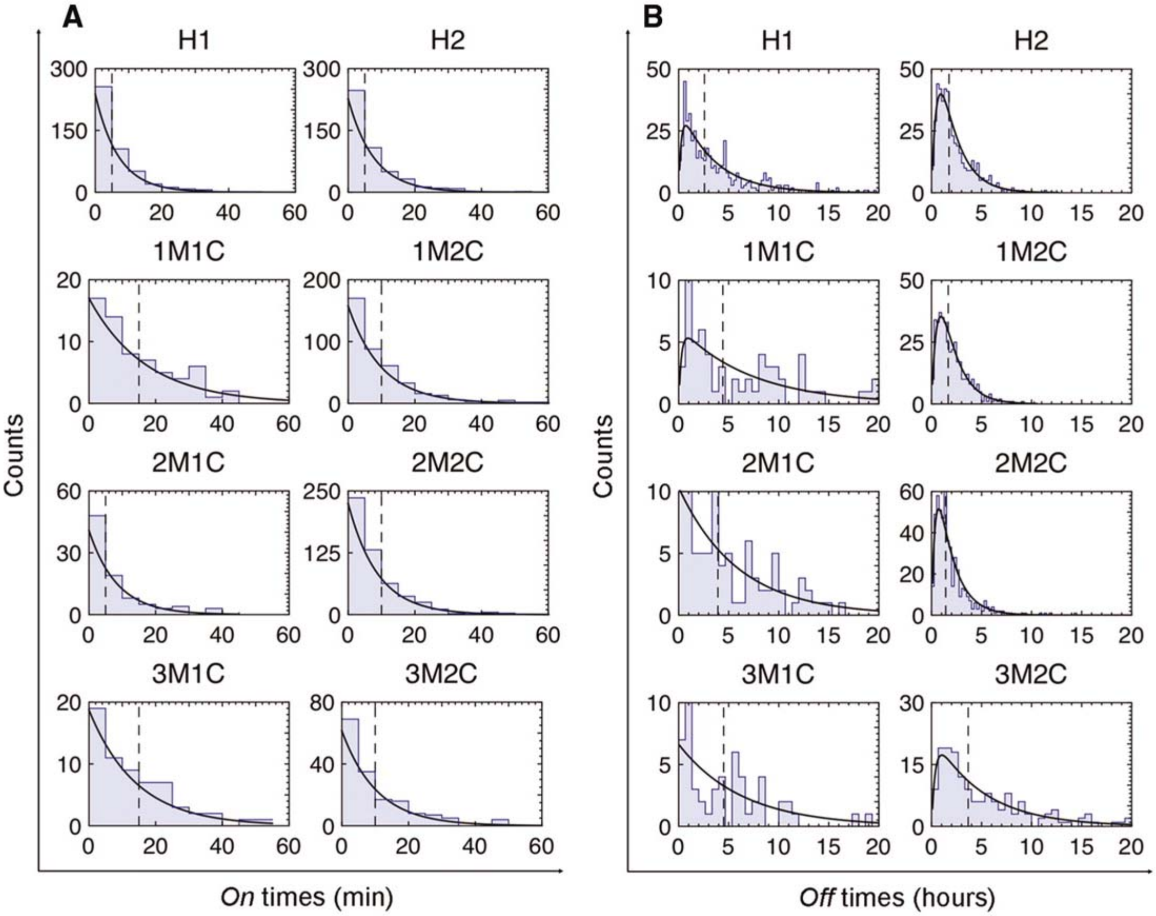

Figure 4.3 Distribution of the duration of gene on and off. (A) Distribution of “on” intervals; black lines show exponential fits. (B) Distribution of “off” intervals. Black lines show best fits to “two-step” model (Suter et al. (2011)).

Chapter 5

The Transition Frequencies in System States in the Three State Model

To understand the stochastic dynamics of transcription activities more thoroughly, we also explore how frequently the states in the three state system are transformed. For instance, for a given time t and an integer n, we estimate how likely the gene has been transcribed exactly n times during the time period $(0, t)$. In this chapter, we extend the definitions of $P_{1q}(t)$ and $P_{1y}(t)$ to a larger class of transition frequency probability $P_{nx}(t)$, $n \geq 1$, and $x = q, y,$ and e. Also we introduce the system of master equations governing these functions, $P_{nx}(t)$, derived by Tang (Tang 2008, 2010) utilizing the transition rule of the Three State Model. We also derive the formulae to compute $P_{3q}(t)$ and $P_{3y}(t)$ using the master equation. Finally we derive and discuss the properties of these transition frequency probability.

5.1 The transition frequency probabilities and the master equations

If the system is in the ground (excited, or engaged) state at any given time $t \geq 0$, then we define $X(t) = q$ (y or e). Let $N(t)$ be a discrete random variable that counts the number of transition events among these states. We say that

$$(N(t), X(t)) = (n, x),\ n \in \{1, 2, 3, ...\} \text{ and } x \in \{q, y, e\},$$

if the transcription system is residing on the state X at time t, and has visited X exactly n times (including the current visiting) since time zero. The transition of the pair of random variable $(N(t), X(t))$ follows the unidirectional infinite Markov chain

$$(1,q) \xrightarrow{\kappa} (1,y) \xrightarrow{\lambda} (1,e) \xrightarrow{\gamma} ...(n,q) \xrightarrow{\kappa} (n,y) \xrightarrow{\lambda} (n,e) \xrightarrow{\gamma} ... \qquad (5.1)$$

Apparently, each state in this chain can only be reached by earlier states, but not by later ones. For any pair of different states in the chain, there is a unique pathway connecting them. The connecting pathway is not reversible, so that the two states do not "communicate". This chain is reducible, and all states are transient because each can only be visited by the transcription system once. See Allen (2003, Chapters 2 and 5) for classification of Markov chains.

Define $P_{nx}(t) = \Pr ob\{(N(t), X(t)) = (n, x)\}$, then $P_{nx}(t)$ gives the probability that the system is residing on the state X the n^{th} time at time t, so is called transition frequency probability. For instance, $P_{1q}(t)$ is the probability that the system has remained in the ground state since time zero, and $P_{1y}(t)$ is the probability that the system is residing on excited state the first time. Because the system must reside on one and only one state of the chain (5.1) at any given time $t > 0$, we have the conservation relations

$$\sum_{n=1}^{\infty}(P_{nq}(t) + P_{ny}(t) + P_{ne}(t)) = 1 \text{ for all } t > 0. \tag{5.2}$$

We assume that the system is in the ground state at time $t = 0,$ and so maintains the initial condition

$$P_{1q}(0) = 1,\ P_{1y}(0) = P_{1e}(0) = 0, \text{ and } P_{nq}(0) = P_{ny}(0) = P_{ne}(0) = 0 \text{ for } n > 1. \tag{5.3}$$

Clearly $P_{1q}(t) = e^{-\kappa t}$ because the transition $(1, q) \xrightarrow{\kappa} (1, y)$ is exponentially distributed. The rest of the transition frequency probability $P_{nx}(t)$ satisfy the following master equations which was derived by Tang (Tang 2008, 2010) following the same logic to find $P_{1y}(t)$ in Section 3.1:

$$\frac{dP_{ny}(t)}{dt} = \kappa P_{nq}(t) - \lambda P_{ny}(t) \tag{5.4}$$

$$\frac{dP_{ne}(t)}{dt} = \lambda P_{ny}(t) - \gamma P_{ne}(t) \tag{5.5}$$

$$\frac{dP_{(n+1)q}(t)}{dt} = -\kappa P_{(n+1)q}(t) + \gamma P_{ne}(t) \tag{5.6}$$

Using the initial condition (5.3), this system of master equations can be transformed into the iterative integration scheme (Hirsch, 2003)

$$P_{ny}(t) = \kappa \int_0^t e^{\lambda(s-t)} P_{nq}(s)ds \tag{5.7}$$

$$P_{ne}(t) = \lambda \int_0^t e^{\gamma(s-t)} P_{ny}(s)ds \tag{5.8}$$

$$P_{(n+1)q}(t) = \gamma \int_0^t e^{\kappa(s-t)} P_{ne}(s)ds\,. \tag{5.9}$$

Here's how (5.7) can be obtained from (5.4) and the condition $P_{ny}(0) = 0$.

$$\frac{dP_{ny}(t)}{dt} = \kappa P_{nq}(t) - \lambda P_{ny}(t)$$

$$\Rightarrow P_{ny}^{'}(s) + \lambda P_{ny}(s) = \kappa P_{nq}(s)$$

$\Rightarrow P_{ny}^{'}(s)e^{\lambda s} + P_{ny}(s)e^{\lambda s}\lambda = \kappa P_{nq}(s)e^{\lambda s}$ since multiplying both sides by $e^{(\lambda s)}$

$\Rightarrow [P_{ny}(s)e^{\lambda s}]^{'} = \kappa P_{nq}(s)e^{\lambda s}$ since $(uv)' = u^{'}v + uv^{'}$

$\Rightarrow \int_0^t [P_{ny}(s)e^{\lambda s}]^{'} ds = \int_0^t \kappa P_{nq}(s)e^{\lambda s} ds$ since integrating this equation over $(0, t)$

$$\Rightarrow P_{ny}(s)e^{\lambda s}\Big|_{s=0}^{s=t} = \int_0^t \kappa P_{nq}(s)e^{\lambda s} ds$$

$$\Rightarrow P_{ny}(t)e^{\lambda t} - P_{ny}(0)e^{s\times 0} = \int_0^t \kappa P_{nq}(s)e^{\lambda s} ds$$

$$\Rightarrow P_{ny}(t)e^{\lambda t} = \int_0^t \kappa P_{nq}(s)e^{\lambda s}ds \quad \text{since } P_{ny}(0) = 0 \text{ by (5.3)}$$

$$\Rightarrow P_{ny}(t) = \kappa\int_0^t e^{\lambda(s-t)}P_{nq}(s)ds$$

Also (5.8) and (5.9) can be obtained from (5.5) and (5.6) similarly.

The integral forms (5.7)-(5.9) provide a straightforward iterative scheme for finding all transition frequency probabilities $P_{nx}(t)$, starting from known formula $P_{1q}(t) = e^{-\kappa t}$. For example, inserting $P_{1q}(t) = e^{-\kappa t}$ into (5.7) and evaluating the integral, we can find $P_{1y}(t)$. By inserting $P_{1y}(t)$ into (5.8) we can find $P_{1e}(t)$; continuing the process iteratively we could obtain $P_{2q}(t)$, $P_{2y}(t)$, and so on.

Tang (Tang 2010) also suggested another way to compute $P_{nx}(t)$. Here's the summary of the computation method. Let

$$\kappa_{ij} = \frac{1}{(\kappa-\lambda)^i(\kappa-\gamma)^j}, \lambda_{ij} = \frac{1}{(\lambda-\gamma)^i(\lambda-\kappa)^j}, \gamma_{ij} = \frac{1}{(\gamma-\kappa)^i(\gamma-\lambda)^j}, \text{ and}$$

$$E_{ij}(t) = \kappa_{ij}e^{-\kappa t} + \lambda_{ij}e^{-\lambda t} + \gamma_{ij}e^{-\gamma t}.$$

Define master operator as

$$L(f(t)) = \kappa\lambda\gamma\int_0^t E_{11}(t-s)f(s)ds. \tag{5.10}$$

The prominent property of the master operator L,

$$P_{nx}(t) = L(P_{(n-1)x}(t)),$$

can be used to compute $P_{nx}(t)$. The expressions for $P_{1q}(t)$, $P_{1y}(t)$, $P_{1e}(t)$, $P_{2q}(t)$, $P_{2y}(t)$, $P_{2e}(t)$, $P_{3e}(t)$ were given by Tang (2010) and Flemer et al. (2009) as follows:

$$P_{1q}(t) = e^{-\kappa t} \tag{5.11}$$

$$P_{1y}(t) = \begin{cases} \dfrac{\kappa}{\lambda - \kappa}(e^{-\kappa t} - e^{-\lambda t}) & \kappa \neq \lambda \\ \kappa t e^{-kt} & \kappa = \lambda \end{cases} \tag{5.12}$$

$$P_{1e}(t) = \kappa\lambda E_{11}(t) \tag{5.13}$$

$$P_{2q}(t) = \kappa\lambda\gamma(\kappa_{11} t e^{-\kappa t} + (\kappa_{12} + \kappa_{21})e^{-\kappa t} - \lambda_{12} e^{-\lambda t} - \gamma_{21} e^{-\gamma t}) \tag{5.14}$$

$$P_{2y}(t) = -\kappa^2\lambda\gamma(\kappa_{21} t e^{-\kappa t} + \lambda_{12} t e^{-\lambda t} + E_{22}(t) + 2\kappa_{31} e^{-\kappa t} + 2\lambda_{13} e^{-\lambda t} - 2\gamma_{22} e^{-\gamma t}) \tag{5.15}$$

$$P_{2e}(t) = \kappa^2\lambda^2\gamma(tE_{22}(t) + 2E_{23}(t) + 2E_{32}(t)) \tag{5.16}$$

$$\begin{aligned} P_{3e}(t) = \kappa^3\lambda^3\gamma^2 (& t^2 E_{33}(t)/2 + 3t(E_{34}(t) + E_{43}(t)) + 3E_{35}(t) + 5E_{44}(t) + 3E_{53}(t) \\ &+ (\lambda_{22}\kappa_{31} + \gamma_{22}\kappa_{13})e^{-\kappa t} + (\kappa_{22}\lambda_{13} + \gamma_{22}\lambda_{31})e^{-\lambda t} + (\kappa_{22}\gamma_{31} + \lambda_{22}\gamma_{13})e^{-\gamma t} \\ &+ 2\kappa_{11}(\lambda_{24} + \gamma_{33})e^{-\kappa t} + 2\lambda_{11}(\gamma_{24} + \kappa_{33})e^{-\lambda t} + 2\gamma_{11}(\kappa_{24} + \lambda_{33})e^{-\gamma t} \\ &+ 2\kappa_{11}(\lambda_{33} + \gamma_{42})e^{-\kappa t} + 2\lambda_{11}(\gamma_{33} + \kappa_{42})e^{-\lambda t} + 2\gamma_{11}(\kappa_{33} + \lambda_{42})e^{-\gamma t}) \end{aligned} \tag{5.17}$$

Given below is the expressions for $P_{3q}(t)$, $P_{3y}(t)$ found by us using master operator L:

$$P_{3q}(t) = \kappa\lambda\gamma(\kappa_{11} L(te^{-\kappa t}) + (\kappa_{12} + \kappa_{21})L(e^{-\kappa t}) - \lambda_{12} L(e^{-\lambda t}) - \gamma_{21} L(e^{-\gamma t})) \tag{5.18}$$

$$\begin{aligned} P_{3y}(t) = -\kappa^2\lambda\gamma(&\kappa_{21} L(te^{-\kappa t}) + (\kappa_{22} + 2\kappa_{31})L(e^{-\kappa t}) + \lambda_{12} L(te^{-\lambda t}) + \\ &(\lambda_{22} + 2\lambda_{13})L(e^{-\lambda t}) - 2\gamma_{22} L(e^{-\gamma t})) \end{aligned} \tag{5.19}$$

Here, $L(e^{-\kappa t})$, $L(e^{-\lambda t})$, and $L(e^{-\gamma t})$ were shown by Tang (2010) as follows:

$$L(e^{-\kappa t}) = \kappa\lambda\gamma(\kappa_{11} t e^{-\kappa t} + (\kappa_{12} + \kappa_{21})e^{-\kappa t} - \lambda_{12} e^{-\lambda t} - \gamma_{21} e^{-\gamma t})$$

$$L(e^{-\lambda t}) = \kappa\lambda\gamma(\lambda_{11} t e^{-\lambda t} + (\lambda_{12} + \lambda_{21})e^{-\lambda t} - \gamma_{12} e^{-\gamma t} - \kappa_{21} e^{-\kappa t})$$

$$L(e^{-\gamma t}) = \kappa\lambda\gamma(\gamma_{11} t e^{-\gamma t} + (\gamma_{12} + \gamma_{21})e^{-\gamma t} - \kappa_{12} e^{-\kappa t} - \gamma_{21} e^{-\gamma t})$$

And the expression of $L(te^{-\kappa t})$ and $L(te^{-\lambda t})$ derived by us are as follows:

$$L(te^{-\kappa t}) = \kappa\lambda\gamma(\frac{\kappa_{11}}{2}t^2e^{-\kappa t} + (\frac{\lambda_{11}}{\lambda-\kappa} + \frac{\gamma_{11}}{\gamma-\kappa})te^{-\kappa t} - (\frac{\lambda_{11}}{(\lambda-\kappa)^2} + \frac{\gamma_{11}}{(\gamma-\kappa)^2})e^{-\kappa t}$$
$$+ \frac{\lambda_{11}}{(\lambda-\kappa)^2}e^{-\lambda t} + \frac{\gamma_{11}}{(\gamma-\kappa)^2}e^{-\gamma t})$$

$$L(te^{-\lambda t}) = \kappa\lambda\gamma(\frac{\lambda_{11}}{2}t^2e^{-\lambda t} + (\frac{\kappa_{11}}{\kappa-\lambda} + \frac{\gamma_{11}}{\gamma-\lambda})te^{-\lambda t} - (\frac{\kappa_{11}}{(\kappa-\lambda)^2} + \frac{\gamma_{11}}{(\gamma-\lambda)^2})e^{-\lambda t}$$
$$+ \frac{\kappa_{11}}{(\kappa-\lambda)^2}e^{-\kappa t} + \frac{\gamma_{11}}{(\gamma-\lambda)^2}e^{-\gamma t})$$

Here are the details to get $L(te^{-\kappa t})$ and $L(te^{-\lambda t})$:

$$L(te^{-\kappa t}) = \kappa\lambda\gamma(\int_0^t (\kappa_{11}e^{-\kappa(t-s)} + \lambda_{11}e^{-\lambda(t-s)} + \gamma_{11}e^{-\gamma(t-s)})se^{-\kappa s}ds)$$

since $E_{ij}(t) = \kappa_{ij}e^{-\kappa t} + \lambda_{ij}e^{-\lambda t} + \gamma_{ij}e^{-\gamma t}$ and $L(f(t)) = \kappa\lambda\gamma\int_0^t E_{11}(t-s)f(s)ds$

$$= \kappa\lambda\gamma(\int_0^t (\kappa_{11}se^{-\kappa t} + \lambda_{11}se^{(\lambda-\kappa)s-\lambda t} + \gamma_{11}se^{(\gamma-\kappa)s-\gamma t})ds)$$

$$= \kappa\lambda\gamma(\frac{\kappa_{11}}{2}s^2e^{-\kappa t}\Big|_{s=0}^{s=t} + \lambda_{11}e^{-\lambda t}\int_0^t se^{(\lambda-\kappa)s}ds + \gamma_{11}e^{-\gamma t}\int_0^t se^{(\gamma-\kappa)s}ds)$$

$$= \kappa\lambda\gamma(\frac{\kappa_{11}}{2}t^2e^{-\kappa t} + \lambda_{11}e^{-\lambda t}\frac{1}{\lambda-\kappa}\int_0^t sde^{(\lambda-\kappa)s} + \gamma_{11}e^{-\gamma t}\frac{1}{\gamma-\kappa}\int_0^t sde^{(\gamma-\kappa)s})$$

$$= \kappa\lambda\gamma(\frac{\kappa_{11}}{2}t^2e^{-\kappa t} + \lambda_{11}e^{-\lambda t}\frac{1}{\lambda-\kappa}(se^{(\lambda-\kappa)s}\Big|_{s=0}^{s=t} - \int_0^t e^{(\lambda-\kappa)s}ds)$$

$$+ \gamma_{11}e^{-\gamma t}\frac{1}{\gamma-\kappa}(se^{(\gamma-\kappa)s}\Big|_{s=0}^{s=t} - \int_0^t e^{(\gamma-\kappa)s}ds))$$ since $\int_a^b u(v)'dx = uv\Big|_a^b - \int_a^b (u)'vdx$

$$= \kappa\lambda\gamma(\frac{\kappa_{11}}{2}t^2e^{-\kappa t} + \lambda_{11}e^{-\lambda t}\frac{1}{\lambda-\kappa}(te^{(\lambda-\kappa)t} - \frac{1}{\lambda-\kappa}e^{(\lambda-\kappa)s}\Big|_{s=0}^{s=t})$$

$$+ \gamma_{11}e^{-\gamma t}\frac{1}{\gamma-\kappa}(te^{(\gamma-\kappa)t} - \frac{1}{\gamma-\kappa}e^{(\gamma-\kappa)s}\Big|_{s=0}^{s=t}))$$

$$= \kappa\lambda\gamma(\frac{\kappa_{11}}{2}t^2e^{-\kappa t} + \lambda_{11}e^{-\lambda t}\frac{1}{\lambda-\kappa}(te^{(\lambda-\kappa)t} - (\frac{1}{\lambda-\kappa}(e^{(\lambda-\kappa)t}-1))$$
$$+\gamma_{11}e^{-\gamma t}\frac{1}{\gamma-\kappa}(te^{(\gamma-\kappa)t} - \frac{1}{\gamma-\kappa}(e^{(\gamma-\kappa)t}-1)))$$

$$= \kappa\lambda\gamma(\frac{\kappa_{11}}{2}t^2e^{-\kappa t} + \frac{\lambda_{11}}{\lambda-\kappa}te^{-\kappa t} - \frac{\lambda_{11}}{(\lambda-\kappa)^2}(e^{-\kappa t} - e^{-\lambda t})$$

$$+\frac{\gamma_{11}}{\gamma-\kappa}te^{-\kappa t} - \frac{\gamma_{11}}{(\gamma-\kappa)^2}(e^{-\kappa t} - e^{-\gamma t}))$$

$$= \kappa\lambda\gamma(\frac{\kappa_{11}}{2}t^2e^{-\kappa t} + (\frac{\lambda_{11}}{\lambda-\kappa} + \frac{\gamma_{11}}{\gamma-\kappa})te^{-\kappa t} - (\frac{\lambda_{11}}{(\lambda-\kappa)^2} + \frac{\gamma_{11}}{(\gamma-\kappa)^2})e^{-\kappa t}$$

$$+\frac{\lambda_{11}}{(\lambda-\kappa)^2}e^{-\lambda t} + \frac{\gamma_{11}}{(\gamma-\kappa)^2}e^{-\gamma t})$$

$L(te^{-\lambda t}) = \kappa\lambda\gamma(\int_0^t(\kappa_{11}e^{-\kappa(t-s)} + \lambda_{11}e^{-\lambda(t-s)} + \gamma_{11}e^{-\gamma(t-s)})se^{-\lambda s}ds)$ since

$E_{ij}(t) = \kappa_{ij}e^{-\kappa t} + \lambda_{ij}e^{-\lambda t} + \gamma_{ij}e^{-\gamma t}$ and $L(f(t)) = \kappa\lambda\gamma\int_0^t E_{11}(t-s)f(s)ds$

$$= \kappa\lambda\gamma(\int_0^t(\kappa_{11}se^{(\kappa-\lambda)s-\kappa t} + \lambda_{11}se^{-\lambda t} + \gamma_{11}se^{(\gamma-\lambda)s-\gamma t})ds)$$

$$= \kappa\lambda\gamma(\kappa_{11}e^{-\kappa t}\int_0^t se^{(\kappa-\lambda)s}ds + \frac{\lambda_{11}}{2}s^2e^{-\lambda t}\Big|_{s=0}^{s=t} + +\gamma_{11}e^{-\gamma t}\int_0^t se^{(\gamma-\lambda)s}ds)$$

$$= \kappa\lambda\gamma(\kappa_{11}e^{-\kappa t}\frac{1}{\kappa-\lambda}\int_0^t sde^{(\kappa-\lambda)s} + \frac{\lambda_{11}}{2}t^2e^{-\lambda t} + \gamma_{11}e^{-\gamma t}\frac{1}{\gamma-\lambda}\int_0^t sde^{(\gamma-\lambda)s})$$

$$= \kappa\lambda\gamma(\kappa_{11} e^{-\kappa t} \frac{1}{\kappa-\lambda}(s e^{(\kappa-\lambda)s}\Big|_{s=0}^{s=t} - \int_0^t e^{(\kappa-\lambda)s} ds) + \frac{\lambda_{11}}{2} t^2 e^{-\lambda t}$$

$$+ \gamma_{11} e^{-\gamma t} \frac{1}{\gamma-\lambda}(s e^{(\gamma-\lambda)s}\Big|_{s=0}^{s=t} - \int_0^t e^{(\gamma-\lambda)s} ds)) \quad \text{since } \int_a^b u(v)' dx = uv\Big|_a^b - \int_a^b (u)' v dx$$

$$= \kappa\lambda\gamma(\kappa_{11} e^{-\kappa t} \frac{1}{\kappa-\lambda}(t e^{(\kappa-\lambda)t} - \frac{1}{\kappa-\lambda} e^{(\kappa-\lambda)s}\Big|_{s=0}^{s=t}) + \frac{\lambda_{11}}{2} t^2 e^{-\lambda t} +$$

$$\gamma_{11} e^{-\gamma t} \frac{1}{\gamma-\lambda}(t e^{(\gamma-\lambda)t} - \frac{1}{\gamma-\lambda} e^{(\gamma-\lambda)s}\Big|_{s=0}^{s=t}))$$

$$= \kappa\lambda\gamma(\kappa_{11} e^{-\kappa t} \frac{1}{\kappa-\lambda}(t e^{(\kappa-\lambda)t} - \frac{1}{\kappa-\lambda}(e^{(\kappa-\lambda)t} - 1)) + \frac{\lambda_{11}}{2} t^2 e^{-\lambda t}$$

$$+ \gamma_{11} e^{-\gamma t} \frac{1}{\gamma-\lambda}(t e^{(\gamma-\lambda)t} - \frac{1}{\gamma-\lambda}(e^{(\gamma-\lambda)t} - 1)))$$

$$= \kappa\lambda\gamma(\frac{\kappa_{11}}{\kappa-\lambda} t e^{-\lambda t} - \frac{\kappa_{11}}{(\kappa-\lambda)^2}(e^{-\lambda t} - e^{-\kappa t}) + \frac{\lambda_{11}}{2} t^2 e^{-\lambda t}$$

$$+ \frac{\gamma_{11}}{\gamma-\lambda} t e^{-\lambda t} - \frac{\gamma_{11}}{(\gamma-\lambda)^2}(e^{-\lambda t} - e^{-\gamma t}))$$

$$= \kappa\lambda\gamma(\frac{\lambda_{11}}{2} t^2 e^{-\lambda t} + (\frac{\kappa_{11}}{\kappa-\lambda} + \frac{\gamma_{11}}{\gamma-\lambda}) t e^{-\lambda t} - (\frac{\kappa_{11}}{(\kappa-\lambda)^2} + \frac{\gamma_{11}}{(\gamma-\lambda)^2}) e^{-\lambda t}$$

$$+ \frac{\kappa_{11}}{(\kappa-\lambda)^2} e^{-\kappa t} + \frac{\gamma_{11}}{(\gamma-\lambda)^2} e^{-\gamma t})$$

The above formulae will be used in Chapter 6. The six transition frequency probabilities, $P_{1q}(t)$ - $P_{3e}(t)$, are graphed in Figure 5.1 for $\kappa = 1/3$, $\lambda = 1/5$, and $\gamma = 1/6$.

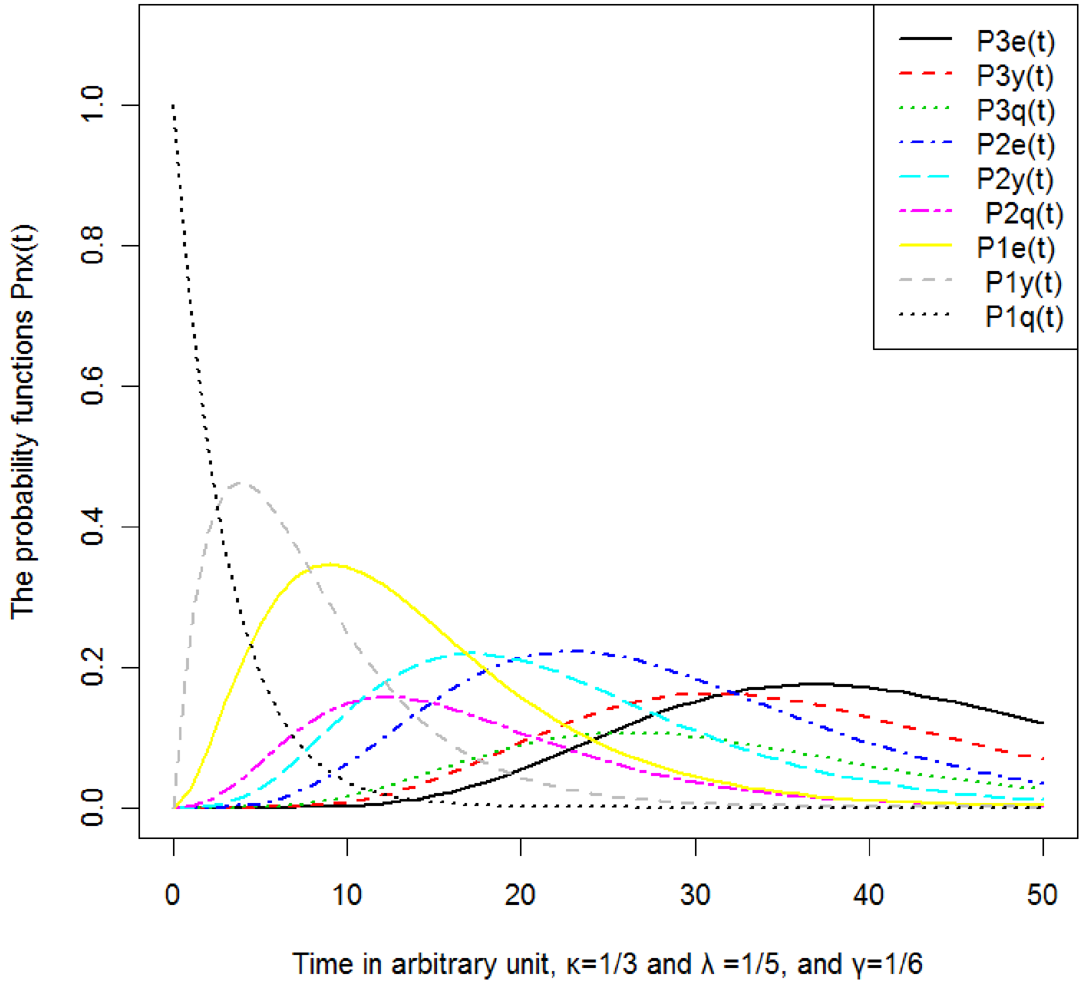

Figure 5.1 The plot of transition frequency probabilities $P_{1q}(t)$ - $P_{3e}(t)$.

5.2. Analytical properties of the transition frequencies probabilities

As shown by the examples discussed above, it is practically unfeasible to write down all transition frequency probabilities $P_{nx}(t)$ in exact form. Furthermore, even with the exact form at hand, it is not adequate to work with it directly to explore its analytical properties. For instance, it is a rather demanding elementary exercise to verify the simple fact that $P_{3e}(0) = 0$. We derive some of their important properties using the methods of mathematical analysis. And we summarize the mathematical properties of $P_{nx}(t)$ in the following section.

Theorem 5.2 Assume that the initial condition (5.2) holds, that is

$P_{1q}(0)=1$, $P_{1y}(0)=P_{1e}(0)=0$, and $P_{nq}(0)=P_{ny}(0)=P_{ne}(0)=0$ for $n>1$.

Then we have:

(1) All transition frequency probabilities $P_{nx}(t)$ are infinitely differentiable, and are positive and strictly less than 1. They all approach zero as $t \to \infty$.

(2) Let $j = j(n,x)$ indicates the position of the state (n,x) in the Markov chain

$(1,q)\xrightarrow{\kappa}(1,y)\xrightarrow{\lambda}(1,e)\xrightarrow{\gamma}(2,q)\xrightarrow{\kappa}...(n,y)\xrightarrow{\lambda}(n,e)\xrightarrow{\gamma}(n,q)\xrightarrow{\kappa}...$,

that is, $j(1,q)=1$, $j(1,y)=2$, $j(1,e)=3$, and more generally

$$j(n,q)=3n-2,\ j(n,y)=3n-1, \text{ and } j(n,e)=3n. \tag{5.20}$$

Then the local behavior of $P_{nx}(t)$ at $t=0$ is characterized by

$$P_{nx}^{'}(0)=P_{nx}^{''}(0)=...=P_{nx}^{(j-2)}(0)=0, \qquad P_{nx}^{(j-1)}(0)>0. \tag{5.21}$$

$$\text{and } P_{nq}^{(j-1)}(0)=(\kappa\lambda\gamma)^{(n-1)}, P_{ny}^{(j-1)}(0)=\kappa^{n}(\lambda\gamma)^{(n-1)}, P_{ne}^{(j-1)}(0)=(\kappa\lambda)^{n}\gamma^{(n-1)}. \tag{5.22}$$

(3) Let $j>1$. Then $P_{nx}(t)$ has a unique positive critical point, called the peak instant of the state (n,x) and denoted by T_j or T_{nx}, where it assumes its unique and absolute maximum value. The peak instant $T_j=T_{nx}$ is an increasing function of j:

$$0=T_{1q}<T_{1y}<T_{1e}<T_{2q}<...<T_{nq}<T_{ny}<T_{ne}<... \tag{5.23}$$

(4) Let $j>1$. All transition frequency probabilities $P_{\overline{n}\overline{x}}(t)$ with $j(\overline{n},\overline{x})>j(n,x)$ are increasing for $0<t<T_{nx}$, and all functions $P_{\overline{n}\overline{x}}(t)$ with $j(\overline{n},\overline{x})<j(n,x)$ are decreasing for $t>T_{nx}$.

We give a brief discussion of this theorem before the proof. Part (1) simply means that $P_{nx}(t)$ are well defined mathematically as probabilities. The vanishing asymptote agrees with the basic fact that the state (n, x) is transient for any fixed n and x. By the initial condition, $P_{1q}(0) = 1$ and all other transition frequency probabilities $P_{nx}(t)$ vanish at $t = 0$. As time goes on, $P_{1q}(t)$ decays and the value lost by $P_{1q}(t)$ is gained by the rest of $P_{nx}(t)$. For small $t > 0$, it is expected that $P_{1y}(t)$ should take up the most part, because the transcription system has to arrive at excited state the first time before shifting to other states in the Markov chain (5.1). This is justified by the fact that $P_{1y}^{'}(0) = \kappa > 0$, and $P_{1e}^{'}(0) = P_{nx}^{'}(0) = 0$ for all $n > 1$. The argument here can be generalized to each state (n, x) and $t > 0$. Consider two states (n, x) and $(\overline{n}, \overline{x})$ with $j(\overline{n}, \overline{x}) > j(n, x)$, then we expect that $P_{\overline{n}\overline{x}}(t)$ grows behind the growth of $P_{nx}(t)$, and keeps growing even when $P_{nx}(t)$ reaches its maximum value at the peak instant. Parts (2)-(4) basically quantify this observation mathematically.

To help us to further understand the above properties of $P_{nx}(t)$, three transition frequency probabilities $P_{2q}(t)$, $P_{2y}(t)$, and $P_{2e}(t)$ are graphed in Figure 5.2 for $\kappa = 1/3$, $\lambda = 1/5$, and $\gamma = 1/6$. The three maximum values of $P_{2q}(t)$ - $P_{2e}(t)$ are marked with X. It is obvious that properties (1)-(3) are valid. To verify (4), we may note that these transition frequency probabilities increase for $t \in (0, T_{2q})$, and decrease for $t > T_{2e}$. Furthermore, $P_{2y}(t)$ and $P_{2e}(t)$ increase in $(0, T_{2y})$, whereas $P_{2q}(t)$ and $P_{2y}(t)$ decrease for $t > T_{2y}$.

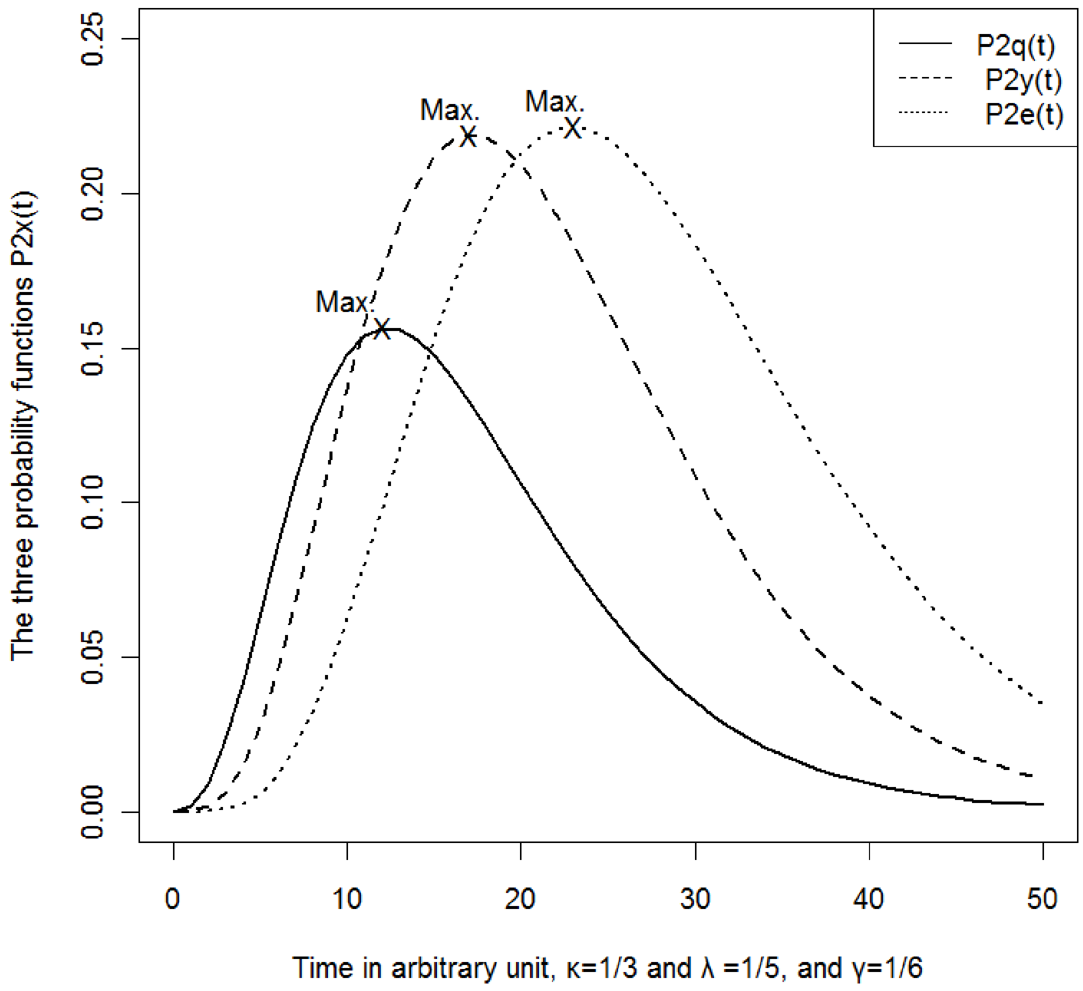

Figure 5.2 The plot of transition frequency probabilities $P_{2q}(t)$ - $P_{2e}(t)$. The three maximum values of $P_{2q}(t)$ - $P_{2e}(t)$ are marked with X. It is obvious that properties (1)-(3) are valid. To verify (4), we may note that these functions increase for $t \in (0, T_{2q})$, and decrease for $t > T_{2e}$. Furthermore, $P_{2y}(t)$ and $P_{2e}(t)$ increase in $(0, T_{2y})$, whereas $P_{2q}(t)$ and $P_{2y}(t)$ decrease for $t > T_{2y}$.

Parts of Theorem 5.2 were proved in Felmer et al. (2009) by an approach relying on the theory of linear operators. The proof we present here is self-contained and uses only elementary arguments.

Proof Theorem 5.2: (1)-1: show all transition frequency probabilities $P_{nx}(t)$ are infinitely differentiable and approaches zero as t →∞: By substituting $P_{1q}(t) = e^{-\kappa t}$ into (5.7), which is $P_{ny}(t) = \kappa\int_0^t e^{\lambda(s-t)}P_{nq}(s)ds$, and evaluating the integral of (5.7) with $n = 1$, we find that $P_{1y}(t)$ is a linear combination of $e^{-\lambda t}$, $e^{-\kappa t}$, and $te^{-\kappa t}$, as justified explicitly by (5.11) which is

$$P_{1y}(t) = \begin{cases} \dfrac{\kappa}{\lambda-\kappa}(e^{-\kappa t} - e^{-\lambda t}) & \kappa \neq \lambda \\ \kappa te^{-kt} & \kappa = \lambda \end{cases}$$

Continuing the process of substitution and integration for Equations (5.7)-(5.9), which are

$$P_{ny}(t) = \kappa\int_0^t e^{\lambda(s-t)}P_{nq}(s)ds,$$

$$P_{ne}(t) = \lambda\int_0^t e^{\gamma(s-t)}P_{ny}(s)ds,$$

$$\text{and } P_{(n+1)q}(t) = \gamma\int_0^t e^{\kappa(s-t)}P_{ne}(s)ds,$$

with increasing n we find that $P_{nx}(t)$ can be expressed as a linear combination of $t^{i_1}e^{-\kappa t}$, $t^{i_2}e^{-\lambda t}$, and $t^{i_3}e^{-\gamma t}$ where i_1, i_2, and i_3 are non-negative integers. This is also supported by the expression in (5.11)-(5.19). Since each of $t^{i_1}e^{-\kappa t}$, $t^{i_2}e^{-\lambda t}$, and $t^{i_3}e^{-\gamma t}$ is infinitely differentiable and approaches zero as t →∞, so does $P_{nx}(t)$.

(1)-2: show all $P_{nx}(t)$ are positive and strictly less than 1: Apparently, $P_{1q}(t) = e^{-\kappa t} > 0$ for all $t > 0$. It follows from (5.7), which is $P_{ny}(t) = \kappa \int_0^t e^{\lambda(s-t)} P_{nq}(s) ds$, that $P_{1y}(t) > 0$, and in turn, from (5.8), which is $P_{ne}(t) = \lambda \int_0^t e^{\gamma(s-t)} P_{ny}(s) ds$, that $P_{1e}(t) > 0$ for all $t > 0$. Applying (5.7)-(5.9) iteratively we find that $P_{nx}(t) > 0$ as long as $t > 0$. From the conservation relation (5.2), which is $\sum_{n=1}^{\infty} P_{nq}(t) + P_{ny}(t) + P_{ne}(t) = 1$ for all $t > 0$, it is clear that $P_{nx}(t) < 1$ for $t > 0$.

(2) We prove (5.21) and (5.22) by induction on n. The proof makes use of the initial condition (5.3) and the master equations (5.4)-(5.6) repeatedly, which are

$$P_{1q}(0) = 1,\ P_{1y}(0) = P_{1e}(0) = 0, \text{ and } P_{nq}(0) = P_{ny}(0) = P_{ne}(0) = 0 \text{ for } n > 1, \quad (5.3)$$

$$\frac{dP_{ny}(t)}{dt} = \kappa P_{nq}(t) - \lambda P_{ny}(t), \quad (5.4)$$

$$\frac{dP_{ne}(t)}{dt} = \lambda P_{ny}(t) - \gamma P_{ne}(t), \quad (5.5)$$

$$\text{and } \frac{dP_{(n+1)q}(t)}{dt} = -\kappa P_{(n+1)q}(t) + \gamma P_{ne}(t) \quad (5.6)$$

(2)-1 Show (5.21) and (5.22) are true for $n = 1$. To refresh our memory, equations (5.21) and (5.22) are

$$P_{nx}^{'}(0) = P_{nx}^{''}(0) = ... = P_{ny}^{(j-2)}(0) = 0, \qquad P_{nx}^{(j-1)}(0) > 0, \quad (5.21)$$

$$\text{and } P_{nq}^{(j-1)}(0) = (\kappa\lambda\gamma)^{(n-1)}, P_{ny}^{(j-1)}(0) = \kappa^n (\lambda\gamma)^{(n-1)}, P_{ne}^{(j-1)}(0) = (\kappa\lambda)^n \gamma^{(n-1)}. \quad (5.22)$$

When $n=1$, j takes values 1, 2, and 3. So we need to show that $P_{1q}(0)=1$ when $j=1$, $P_{1y}(0)=0$ and $P_{1y}^{'}(0)=\kappa$ when $j=2$, and $P_{1e}(0)=0$, $P_{1e}^{'}(0)=0$, and $P_{1e}^{''}(0)=\lambda\kappa$ when $j=3$.

$P_{1q}(0)=1$ is a part of the initial condition (5.3).

$P_{1y}(0)=0$ is also a part of the initial condition (5.3).

$P_{1y}^{'}(0)=\kappa$ because of the following argument:

$$\frac{dP_{ny}(t)}{dt}=\kappa P_{nq}(t)-\lambda P_{ny}(t) \text{ by (5.4)}$$

$$\Rightarrow P_{1y}^{'}(0)=\kappa P_{1q}(0)-\lambda P_{1y}(0)$$

$$\Rightarrow P_{1y}^{'}(0)=\kappa\times 1-\lambda\times 0=k \text{ since } P_{1q}(0)=1 \text{ and } P_{1y}(0)=0$$

$P_{1e}(0)=0$ is a part of the initial condition (5.3).

$P_{1e}^{'}(0)=0$ because of the following argument:

$$\frac{dP_{ne}(t)}{dt}=\lambda P_{ny}(t)-\gamma P_{ne}(t) \text{ by (5.5)}$$

$$\Rightarrow P_{1e}^{'}(0)=\lambda P_{1y}(0)-\gamma P_{1e}(0)$$

$$\Rightarrow P_{1e}^{'}(0)=\lambda\times 0-\gamma\times 0=0 \text{ since } P_{1y}(0)=0 \text{ and } P_{1e}(0)=0$$

$P_{1e}^{''}(0)=\lambda\kappa$ because of the following argument:

$$\frac{dP_{ne}(t)}{dt}=\lambda P_{ny}(t)-\gamma P_{ne}(t) \text{ by (5.5)}$$

$$\Rightarrow P_{1e}^{''}(0)=\lambda P_{1y}^{'}(0)-\gamma P_{1e}^{'}(0)$$

$$\Rightarrow P_{1e}^{''}(0)=\lambda\kappa-\gamma\times 0=\lambda\kappa \text{ since } P_{1y}^{'}(0)=\kappa \text{ and } P_{1e}^{'}(0)=0.$$

(2)-2 Assume that (5.21) and (5.22) are valid for $n = m - 1$ and $m > 1$. It means that when $j(m-1,q) = 3(m-1) - 2 = 3m - 5$, $P'_{(m-1)q}(0) = P''_{(m-1)q}(0) = ... = P^{(3m-7)}_{(m-1)q}(0) = 0$ and $P^{(3m-6)}_{(m-1)q}(0) = (\kappa\lambda\gamma)^{(m-2)}$,

when $j(m-1,y) = 3(m-1) - 1 = 3m - 4$, $P'_{(m-1)y}(0) = P''_{(m-1)y}(0) = ... = P^{(3m-6)}_{(m-1)y}(0) = 0$ and $P^{(3m-5)}_{(m-1)y}(0) = \kappa^{(m-1)}(\lambda\gamma)^{(m-2)}$, and

when $j(m-1,e) = 3(m-1) = 3m - 3$, $P'_{(m-1)e}(0) = P''_{(m-1)e}(0) = ... = P^{(3m-5)}_{(m-1)e}(0) = 0$ and $P^{(3m-4)}_{(m-1)e}(0) = (\kappa\lambda)^{(m-1)}\gamma^{(m-2)}$.

(2)-3 Prove that (5.21) and (5.22) are valid when $n = m$, $m > 1$, and $j = 3m - 2$, $3m - 1$, and $3m$. It means that we need to show that

when $j(m,q) = 3m - 2$, $P'_{mq}(0) = P''_{mq}(0) = ... = P^{(3m-4)}_{mq}(0) = 0$ and $P^{(3m-3)}_{mq}(0) = (\kappa\lambda\gamma)^{(m-1)}$,

when $j(m,y) = 3m - 1$, $P'_{my}(0) = P''_{my}(0) = ... = P^{(3m-3)}_{my}(0) = 0$ and $P^{(3m-2)}_{my}(0) = \kappa^{m}(\lambda\gamma)^{(m-1)}$, and when $j(m,e) = 3m$, $P'_{me}(0) = P''_{me}(0) = ... = P^{(3m-2)}_{me}(0) = 0$ and $P^{(3m-1)}_{me}(0) = (\kappa\lambda)^{m}\gamma^{(m-1)}$. The details for the proof is given below:

(2)-3-a Show $P'_{mq}(0) = P''_{mq}(0) = ... = P^{(3m-4)}_{mq}(0) = 0$ when $j = j(m,q) = 3m - 2$ and $(n,x) = (m,q)$.

$\frac{dP_{(n+1)q}(t)}{dt} = -\kappa P_{(n+1)q}(t) + \gamma P_{ne}(t)$ by (5.6)

$\Rightarrow P^{(i)}_{mq}(0) = -\kappa P^{(i-1)}_{mq}(0) + \gamma P^{(i-1)}_{(m-1)e}(0)$

$\Rightarrow P^{(i)}_{mq}(0) = -\kappa P^{(i-1)}_{mq}(0)$ for $1 \le i \le 3m - 4$ and $m > 1$ since $P_{(m-1)e}(0) = 0$ by initial condition and $P'_{(m-1)e}(0) = P''_{(m-1)e}(0) = ... = P^{(3m-5)}_{(m-1)e}(0) = 0$ by the assumption for $n = m - 1$.

$\Rightarrow P_{mq}^{(i)}(0)=0$ for $1 \le i \le 3m-4$ and $m>1$ since $P_{mq}(0)=0$, $P_{mq}^{'}(0)=0$, ..., $P_{mq}^{(i-1)}(0)=0$ for $1 \le i \le 3m-4$ and $m>1$.

As follows is the proof for $P_{mq}(0)=0$, $P_{mq}^{'}(0)=0$, ..., $P_{mq}^{(i-1)}(0)=0$ for $1 \le i \le 3m-4$ and $m>1$:

$P_{mq}(0)=0$ is due to initial condition.

$P_{mq}^{'}(0)=0$ because of the following argument:

$\frac{dP_{(n+1)q}(t)}{dt}=-\kappa P_{(n+1)q}(t)+\gamma P_{ne}(t)$ by (5.6)

$\Rightarrow P_{mq}^{'}(0)=-\kappa P_{mq}(0)+\gamma P_{(m-1)e}(0)$

$\Rightarrow P_{mq}^{'}(0)=0$ since $P_{mq}(0)=0$ and $P_{(m-1)e}(0)=0$ for $m>1$ is a part of initial condition.

$P_{mq}^{''}(0)=0$ because of the following argument:

$\frac{dP_{(n+1)q}(t)}{dt}=-\kappa P_{(n+1)q}(t)+\gamma P_{ne}(t)$ by (5.6)

$\Rightarrow P_{mq}^{''}(0)=-\kappa P_{mq}^{'}(0)+\gamma P_{(m-1)e}^{'}(0)$

$\Rightarrow P_{mq}^{'}(0)=-\kappa \times 0+\gamma \times 0$ since $P_{mq}^{'}(0)=0$ proved above and $P_{(m-1)e}^{'}=0$ by the assumption for $n=m-1$. Repeating this process until $P_{mq}^{(i-1)}(0)=0$ for $i=3m-4$ and $m>1$.

(2)-3-b Show $P_{(m-1)q}^{(3m-3)}(0)=(\kappa\lambda\gamma)^{(m-1)}$ when $j=j(m,q)=3m-2$, $m>1$, and $(n,x)=(m,q)$.

$\frac{dP_{(n+1)q}(t)}{dt}=-\kappa P_{(n+1)q}(t)+\gamma P_{ne}(t)$ by (5.6)

$\Rightarrow P_{mq}^{(i)}(0) = -\kappa P_{mq}^{(i-1)}(0) + \gamma P_{(m-1)e}^{(i-1)}(0)$

$\Rightarrow P_{mq}^{(3m-3)}(0) = -\kappa P_{mq}^{(3m-4)}(0) + \gamma P_{(m-1)e}^{(3m-4)}(0)$

$\Rightarrow P_{mq}^{(3m-3)}(0) = -\kappa \times 0 + \gamma P_{(m-1)e}^{(3m-4)}(0)$ since $P_{mq}^{(3m-4)}(0) = 0$ proved in (2)-3-a

$\Rightarrow P_{mq}^{(3m-4)}(0) = \gamma(\kappa\lambda)^{(m-1)}\gamma^{(m-2)}$ since $P_{(m-1)e}^{(3m-4)}(0) = (\kappa\lambda)^{(m-1)}\gamma^{(m-2)}$ by the assumption for $n = m-1$.

$\Rightarrow P_{mq}^{(3m-4)}(0) = (\kappa\lambda\gamma)^{(m-1)}$

(2)-3-c Show $P_{my}^{'}(0) = P_{my}^{''}(0) = ... = P_{my}^{(3m-3)}(0) = 0$ when $j = j(m,y) = 3m-1$, $m > 1$, and $(n,x) = (m,y)$.

$\frac{dP_{ny}(t)}{dt} = \kappa P_{nq}(t) - \lambda P_{ny}(t)$ by (5.4)

$\Rightarrow P_{my}^{(i)}(0) = \kappa P_{mq}^{(i-1)}(0) - \lambda P_{my}^{(i-1)}(0)$

$\Rightarrow P_{my}^{(i)}(0) = \kappa P_{my}^{(i-1)}(0)$ for $1 \le i \le 3m-3$ and $m > 1$ since

$P_{mq}^{'}(0) = P_{mq}^{''}(0) = ... = P_{mq}^{(3m-4)}(0) = 0$ proved in (2)-3-a

$\Rightarrow P_{my}^{(i)}(0) = 0$ for $1 \le i \le 3m-3$ and $m > 1$ since $P_{my}(0) = 0$, $P_{my}^{'}(0) = 0$, ..., $P_{my}^{(i-1)}(0) = 0$ for $1 \le i \le 3m-3$ and $m > 1$.

As follows is the proof for $P_{my}(0) = 0$, $P_{my}^{'}(0) = 0$, ..., $P_{my}^{(i-1)}(0) = 0$ for $1 \le i \le 3m-3$ and $m > 1$:

$P_{my}(0) = 0$ is due to the initial condition.

$P_{my}^{'}(0) = 0$ because of the following argument:

$\frac{dP_{ny}(t)}{dt} = \kappa P_{nq}(t) - \lambda P_{ny}(t)$ by (5.4)

$\Rightarrow P_{my}^{'}(0) = \kappa P_{mq}(0) - \lambda P_{my}(0)$

$\Rightarrow P_{my}^{'}(0) = \kappa \times 0 - \lambda \times 0$ since $P_{mq}(0) = 0$ and $P_{my}(0) = 0$ for $m > 1$ is a part of initial condition.

$\Rightarrow P_{my}^{'}(0) = 0$

$P_{my}^{''}(0) = 0$ because of the following argument:

$\frac{dP_{ny}(t)}{dt} = \kappa P_{nq}(t) - \lambda P_{ny}(t)$ by (5.4)

$\Rightarrow P_{my}^{''}(0) = \kappa P_{mq}^{'}(0) - \lambda P_{my}^{'}(0)$

$\Rightarrow P_{my}^{''}(0) = \kappa \times 0 - \lambda \times 0$ since $P_{mq}^{'}(0) = 0$ proved in section (2)-3-a and $P_{my}^{'}(0) = 0$ proved above. Repeating this process until $P_{my}^{(i-1)}(0) = 0$ for $i = 3m - 3$ and $m > 1$.

(2)-3-d Show $P_{my}^{(3m-2)}(0) = \kappa^{m}(\lambda\gamma)^{(m-1)}$ when $j = j(m, y) = 3m - 1$, $m > 1$, and $(n, x) = (m, y)$.

$\frac{dP_{ny}(t)}{dt} = \kappa P_{nq}(t) - \lambda P_{ny}(t)$ by (5.4)

$\Rightarrow P_{my}^{(i)}(0) = \kappa P_{mq}^{(i-1)}(0) - \lambda P_{my}^{(i-1)}(0)$

$\Rightarrow P_{my}^{(3m-2)}(0) = \kappa P_{mq}^{(3m-3)}(0) - \gamma P_{my}^{(3m-3)}(0)$

$\Rightarrow P_{my}^{(3m-2)}(0) = \kappa P_{mq}^{(3m-3)}(0) - \gamma \times 0$ since $P_{my}^{3m-3}(0) = 0$ proved above in (2)-2-c

$\Rightarrow P_{my}^{(3m-2)}(0) = \kappa(\kappa\lambda\gamma)^{(m-1)}$ since $P_{mq}^{(3m-3)}(0) = (\kappa\lambda\gamma)^{(m-1)}$ proved above in (2)-2-b

$\Rightarrow P_{my}^{(3m-2)}(0) = \kappa^{m}(\lambda\gamma)^{(m-1)}$

(2)-3-e Show $P_{me}^{'}(0) = P_{me}^{''}(0) = ... = P_{me}^{(3m-2)}(0) = 0$ when $j = j(m, e) = 3m$, $m > 1$, and $(n, x) = (m, e)$.

$\frac{dP_{ne}(t)}{dt} = \lambda P_{ny}(t) - \gamma P_{ne}(t)$ by (5.5)

$\Rightarrow P_{me}^{(i)}(0) = \lambda P_{my}^{(i-1)}(0) - \gamma P_{me}^{(i-1)}(0)$

$\Rightarrow P_{me}^{(i)}(0) = \gamma P_{me}^{(i-1)}(0)$ for $1 \le i \le 3m-2$ and $m > 1$ since

$P_{my}^{'}(0) = P_{my}^{''}(0) = ... = P_{my}^{(3m-3)}(0) = 0$ proved above in (2)-3-c

$\Rightarrow P_{me}^{(i)}(0) = 0$ for $1 \le i \le 3m-2$ and $m > 1$ since $P_{me}(0) = 0$, $P_{me}^{'}(0) = 0$, ...,

$P_{me}^{(i-1)}(0) = 0$ for $1 \le i \le 3m-2$ and $m > 1$.

As follows is the proof for $P_{me}(0) = 0$, $P_{me}^{'}(0) = 0$, ..., $P_{me}^{(i-1)}(0) = 0$ for

$1 \le i \le 3m-2$ and $m > 1$:

$P_{me}(0) = 0$ is a part of initial condition.

$P_{me}^{'}(0) = 0$ because of the following argument:

$\frac{dP_{ne}(t)}{dt} = \lambda P_{ny}(t) - \gamma P_{ne}(t)$ by (5.5)

$\Rightarrow P_{me}^{'}(0) = \kappa P_{my}(0) - \gamma P_{me}(0)$

$\Rightarrow P_{me}^{'}(0) = \kappa \times 0 - \gamma \times 0$ since $P_{my}(0) = 0$ and $P_{me}(0) = 0$ is a part of initial condition.

$\Rightarrow P_{me}^{'}(0) = 0$

$P_{me}^{''}(0) = 0$ because of the following argument:

$\frac{dP_{ne}(t)}{dt} = \lambda P_{ny}(t) - \gamma P_{ne}(t)$ by (5.5)

$\Rightarrow P_{me}^{''}(0) = \lambda P_{my}^{'}(0) - \gamma P_{me}^{'}(0)$

$\Rightarrow P_{my}^{''}(0) = \lambda \times 0 - \gamma \times 0 = 0$ since $P_{my}^{'}(0) = 0$ proved above in section (2)-3-c and $P_{me}^{'}(0) = 0$ proved above. Repeating this process until $P_{me}^{(i-1)}(0) = 0$ for $i = 3m-2$ and $m > 1$.

(2)-3-f Show $P_{(m-1)e}^{(3m-1)}(0) = (\kappa\lambda)^m \gamma^{(m-1)}$ when $j = j(m,e) = 3m$, $m > 1$, and $(n,x) = (m,e)$.

$\frac{dP_{ne}(t)}{dt} = \lambda P_{ny}(t) - \gamma P_{ne}(t)$ by (5.5)

$\Rightarrow P_{me}^{(i)}(0) = \lambda P_{my}^{(i-1)}(0) - \gamma P_{me}^{(i-1)}(0)$

$\Rightarrow P_{me}^{(3m-1)}(0) = \lambda P_{my}^{(3m-2)}(0) - \gamma P_{me}^{(3m-2)}(0)$

$\Rightarrow P_{me}^{(3m-1)}(0) = \lambda P_{my}^{(3m-2)}(0) - \gamma \times 0$ since $P_{me}^{3m-2}(0) = 0$ proved above in (2)-3-e

$\Rightarrow P_{me}^{(3m-1)}(0) = \lambda \kappa^m (\lambda\gamma)^{(m-1)}$ since $P_{my}^{(3m-2)}(0) = \kappa^m (\lambda\gamma)^{(m-1)}$ proved above in (2)-3-d.

$\Rightarrow P_{me}^{(3m-1)}(0) = (\kappa\lambda)^m \gamma^{(m-1)}$

(3) Show (5.23). To refresh our memory, (5.23) is as follows:

Let $j > 1$, then $P_{nx}(t)$ has a unique positive critical point, called the peak instant of the state (n,x) and denoted by T_j or T_{nx}, where it assumes its unique and absolute maximum value. The peak instant $T_j = T_{nx}$ is an increasing function of j:

$$0 = T_{1q} < T_{1y} < T_{1e} < T_{2q} < ... < T_{nq} < T_{ny} < T_{ne} < ... \qquad (5.23)$$

We use mathematical induction to prove that the maximum value for each $P_{nx}(t)$ is unique and $T_{j-1} < T_j$. Here are the details for proof:

(3)-1 Show $P_{1q}(t)$ has a unique maximum value at $T = 0$ when $j = 1$ and $(n,x) = (1,q)$.

$P_{1q}(t) = e^{-\kappa t}$ is decreasing because $P_{1q}^{'}(t) = -\kappa e^{-\kappa t} < 0$, so it take the only critical point 1 at $T_1 = 0$.

(3)-2 Show $P_{1y}(t)$ has a unique maximum value at T_2 and $0 = T_1 < T_2$ when $j = 2$ and $(n, x) = (1, y)$.

Let $\overline{T}_2$ be an arbitrary positive critical point of $P_{1y}(t)$ so that $P_{1y}^{'}(\overline{T}_2) = 0$. We find that $P_{1y}^{''}(\overline{T}_2) < 0$ because of the following argument:

$\dfrac{dP_{ny}(t)}{dt} = \kappa P_{nq}(t) - \lambda P_{ny}(t)$ by master equation (5.4)

$\Rightarrow P_{1y}^{''}(\overline{T}_2) = \kappa P_{1q}^{'}(\overline{T}_2) - \lambda P_{1y}^{'}(\overline{T}_2)$

$\Rightarrow P_{1y}^{''}(\overline{T}_2) = \kappa P_{1q}^{'}(\overline{T}_2) - \lambda \times 0$ since $P_{1y}^{'}(\overline{T}_2) = 0$ by assuming $\overline{T}_2$ be an arbitrary positive critical point of $P_{1y}(t)$.

$\Rightarrow P_{1y}^{''}(\overline{T}_2) = \kappa P_{1q}^{'}(\overline{T}_2) < 0$ since $P_{1q}^{'}(\overline{T}_2) = -\kappa \exp(-\kappa \overline{T}_2) < 0$.

Therefore, $P_{1y}(t)$ must take a maximum value at $\overline{T}_2$ because $\overline{T}_2$ be an arbitrary positive critical point of $P_{1y}(t)$ and $P_{1y}^{''}(\overline{T}_2) < 0$. And furthermore, there exists exactly one positive critical point of $P_{1y}(t)$: If there were more, then $P_{1y}(t)$ should assume a minimum value somewhere, i.e. $P_{1y}^{'}(\overline{T}_2) = 0$ and $P_{1y}^{''}(\overline{T}_2) > 0$ since $\overline{T}_2$ is assumed to be an arbitrary positive critical point of $P_{1y}(t)$. $P_{1y}^{''}(\overline{T}_2) > 0$ contradicts $P_{1y}^{''}(\overline{T}_2) < 0$ showed above. Thus $P_{1y}(t)$ must take a unique maximum value at $\overline{T}_2$, i.e. $\overline{T}_2 = T_2$.

Also we have $0 = T_1 < T_2$ because $P_{1y}(t)$ must take a unique maximum value at T_2 and $P_{1y}(0) = 0$ by initial condition.

Put all together, we have $P_{1y}(t)$ has a unique maximum value at T_2 and $0 = T_1 < T_2$.

(3)-3 Assume that $P_{nx}(t)$ has a unique positive critical point T_J at which $P_{nx}''(T_J) < 0$ and $T_{J-1} < T_J$ when $j(n,x) = J > 1$. Then show $P_{nx}(t)$ has a unique positive critical point T_{J+1} and $T_J < T_{J+1}$ when $j(n,x) = J+1 > 1$.

Without loss of generality, we assume $(n,x) = (n,q)$ when $j = J$ for clarity of presentation. That is we assume

$$P_{nq}'(T_J) = 0\,,\ P_{nq}''(T_J) < 0\,, \tag{5.24}$$

and $(n,x) = (n,q)$ when $j = J$. We need to show $P_{ny}(t)$ has a unique positive critical point T_{J+1} and $T_J < T_{J+1}$ when $j(n,y) = J+1 > 1$.

(3)-3-a Define $\overline{T}_{J+1}$ be the first positive critical point of $P_{ny}(t)$ and show $P_{ny}(t)$ takes a maximum value at $\overline{T}_{J+1}$. Because $\overline{T}_{J+1}$ is the first positive critical point of $P_{ny}(t)$. Also $P_{ny}(t)$ increases for small $t > 0$ because $P_{nx}'(0) = P_{nx}''(0) = ... = P_{ny}^{(j-2)}(0) = 0$ and $P_{nx}^{(j-1)}(0) > 0$ (5.21). Then $P_{ny}(t)$ takes a maximum value at $\overline{T}_{J+1}$.

(3)-3-b Show $\overline{T}_{J+1} \ge T_J$.

$\dfrac{dP_{ny}(t)}{dt} = \kappa P_{nq}(t) - \lambda P_{ny}(t)$ by master equation (5.4)

$\Rightarrow \kappa P_{nq}'(\overline{T}_{J+1}) = P_{ny}''(\overline{T}_{J+1}) + \lambda P_{ny}'(\overline{T}_{J+1})$

$\Rightarrow \kappa P_{nq}'(\overline{T}_{J+1}) = P_{ny}''(\overline{T}_{J+1}) + \lambda \times 0$ since $P_{ny}'(\overline{T}_{J+1}) = 0$ because $\overline{T}_{J+1}$ is a positive critical point of $P_{ny}(t)$.

$\Rightarrow \kappa P'_{nq}(\overline{T}_{J+1}) = P''_{ny}(\overline{T}_{J+1}) \le 0$ because $P_{ny}(t)$ takes a maximum value at $\overline{T}_{J+1}$ proved above in (3)-3-a.

$\Rightarrow P'_{nq}(\overline{T}_{J+1}) \le 0$

$\Rightarrow \overline{T}_{J+1} \ge T_J$ because $P_{nq}(t)$ takes the unique maximum value at T_J by the assumption for $j(n,q) = J$

(3)-3-c Show that $\overline{T}_{J+1} \ge T_J$ can be strengthened to the strict inequality $\overline{T}_{J+1} > T_J$. If not, then $\overline{T}_{J+1} = T_J$ and $P_{nq}(t)$ and $P_{ny}(t)$ take the maximum values at the same instant T_J. We have $\dfrac{dP_{ny}(t)}{dt} = \kappa P_{nq}(t) - \lambda P_{ny}(t)$ by master equation (5.4)

$\Rightarrow P'''_{ny}(\overline{T}_J) = \kappa P''_{nq}(\overline{T}_J) - \lambda P''_{ny}(\overline{T}_J)$

$\Rightarrow P'''_{ny}(\overline{T}_J) = \kappa P''_{nq}(\overline{T}_J) - \lambda(\kappa P'_{nq}(\overline{T}_J) - \lambda P'_{ny}(\overline{T}_J))$ since $P''_{ny}(\overline{T}_J) = \kappa P'_{nq}(\overline{T}_J) - \lambda P'_{ny}(\overline{T}_J)$

$\Rightarrow P'''_{ny}(\overline{T}_J) = \kappa P''_{nq}(\overline{T}_J) - \lambda \times 0$ since $P'_{nq}(\overline{T}_J) = 0$ and $P'_{ny}(\overline{T}_J) = 0$ which is because we assume that $\overline{T}_{J+1} = T_J$ and $P_{nq}(t)$ and $P_{ny}(t)$ take the maximum values at the same instant T_J.

$\Rightarrow P'''_{ny}(\overline{T}_J) < 0$ because we assume $P''_{nq}(T_J) < 0$ and $(n,x) = (n,q)$ when $j = J$.

$\Rightarrow P''_{ny}(t)$ is decreasing near T_J since $f'''(x) < 0$ implies $f''(x)$ is decreasing near x.

$\Rightarrow P''_{ny}(t) > 0$ for $t < T_J$ and close to T_J because $P''_{ny}(T_J) = \kappa P'_{nq}(T_J) - \lambda P'_{ny}(T_J) = 0$ by the assumption that $\overline{T}_{J+1} = T_J$ and $P_{nq}(t)$ and $P_{ny}(t)$ take the maximum values at the same instant T_J.

$\Rightarrow P_{ny}^{'}(t)$ is increasing for $t < T_J$ and close to T_J since $f^{''}(x) > 0$ implies $f^{'}(x)$ is increasing near x.

$\Rightarrow P_{ny}^{'}(t) < 0$ when $t < T_J$ and t is close to $\overline{T}$ since $P_{ny}^{'}(T_J) = 0$ by the assumption that $\overline{T}_{J+1} = T_J$ and $P_{nq}(t)$ and $P_{ny}(t)$ take the maximum values at the same instant T_J.

$\Rightarrow P_{ny}^{'}(t) < 0$ when $t < \overline{T}_{J+1}$ and t is close to $\overline{T}_{J+1}$ because we assume $\overline{T}_{J+1} = T_J$. On the other hand we have $P_{ny}^{'}(t) > 0$ when $t < \overline{T}_{J+1}$ and t is close to $\overline{T}_{J+1}$ because $\overline{T}_{J+1}$ is defined to be the first positive critical point of $P_{ny}(t)$ and $P_{ny}(t)$ takes a maximum value at $\overline{T}_{J+1}$. Assuming $\overline{T}_{J+1} = T_J$ gives a contradiction, so $\overline{T}_{J+1} > T_J$.

(3)-3-d Show $P_{ny}(t)$ can have only one critical point. If $P_{ny}(t)$ has more than one critical point, and let S_{J+1} be the smallest critical point of $P_{ny}(t)$ such that $S_{J+1} > \overline{T}_{J+1}$, then $P_{ny}(t)$ should take a minimum value at S_{J+1} because $P_{ny}(t)$ takes a maximum value at $\overline{T}_{J+1}$ showed in (3)-3-a. It gives $P_{ny}^{''}(S_{J+1}) \geq 0$.

On the other hand, $\dfrac{dP_{ny}(t)}{dt} = \kappa P_{nq}(t) - \lambda P_{ny}(t)$ by master equation (5.4)

$\Rightarrow P_{ny}^{''}(S_{J+1}) = \kappa P_{nq}^{'}(S_{J+1}) - \lambda P_{ny}^{'}(S_{J+1})$.

$\Rightarrow P_{ny}^{''}(S_{J+1}) = \kappa P_{nq}^{'}(S_{J+1})$ since $P_{ny}^{'}(S_{J+1}) = 0$ because of $P_{ny}(t)$ takes a critical value at S_{J+1}.

$\Rightarrow P_{ny}^{''}(S_{J+1}) = \kappa P_{nq}^{'}(S_{J+1}) < 0$ since $P_{nq}(t)$ takes the unique maximum value at T_J, and $S_{J+1} > T_J$.

$\Rightarrow P_{ny}''(S_{J+1}) < 0$, which gives a contradiction. Therefore, $P_{ny}(t)$ can have only one critical point.

Put 3)-3-a to 3)-3-d together, there is exactly one positive critical point of $P_{ny}(t)$, and $\overline{T}_{J+1} = T_{J+1}$, and $T_{J+1} > T_J$. By mathematical induction, property (3) is established for all $j \geq 1$.

(4) This is an immediate consequence of (3).

Chapter 6

The Burst Frequency in the Three State Model

In this chapter, we define and compute the distribution of the burst frequency in the Three State Model, then discuss the properties of the burst frequency. Finally, we exemplify the mathematical results by the experimental data and simulation.

6.1 The distribution of the burst frequency in the Three State Model

For each integer $n > 0$, we define $P_n(t)$ to be the probability that exactly n transcription bursts have occurred during the time period $(0, t]$, and call it the burst frequency probability. It gives the likelihood of n transcription bursts in a period of observation. It also give the likelihood of n transcript synthesis cycle in a period of observation because one burst indicates one synthesis cycles . It can be used to compute the percentage of cells with the given number of bursts or synthesis cycles within a cell population. In particular, $P_0(t)$ is the probability that the system has not reached the engaged state in $(0, t]$, which occurs if the system is in the ground state or the excited state the first time at time t. In chapter 3, we obtained

$$P_0(t) = \begin{cases} \dfrac{\lambda}{\lambda - \kappa} e^{-\kappa t} + \dfrac{\kappa}{\kappa - \lambda} e^{-\lambda t}, & \kappa \neq \lambda \\ (1 + kt) e^{-kt} & k = \lambda \end{cases} \tag{3.1}$$

In general, let $A_n(t)$ denote the event that n transcription bursts have occurred during the time interval $(0, t]$. Then

$$P_n(t) = \Pr ob\{A_n(t)\}.$$

For n > 0, $A_n(t)$ corresponds to the three transient states (n,e), $(n+1,q)$ or $(n+1,y)$ in the chain (5.1). Hence burst frequency probability $P_n(t)$ and the transition frequency probability $P_{nx}(t)$ are related by

$$P_n(t) = P_{ne}(t) + P_{(n+1)q}(t) + P_{(n+1)y}(t), \qquad n > 0. \tag{6.1}$$

6.2. Analytical properties of the burst frequency probability in the Three State Model

The mathematical properties of transition frequency probabilities $P_n(t)$ are given in the following Theorem:

Theorem 6.2 Assume that $n \geq 1$ and the initial condition (5.3) holds, which is $P_{1q}(0) = 1$, $P_{1y}(0) = P_{1e}(0) = 0$, and $P_{nq}(0) = P_{ny}(0) = P_{ne}(0) = 0$ for $n > 1$.

(1) All transition frequency probabilities $P_n(t)$ are infinitely differentiable, positive, and strictly less than 1. They all approach zero as $t \to \infty$.

(2) The local behavior of $P_n(t)$ at $t = 0$ is characterized by

$$P_n^{'}(0) = P_n^{''}(0) = ... = P_n^{(3n-2)}(0) = 0, \qquad P_n^{(3n-1)}(0) = (\kappa\lambda)^n \gamma^{n-1} > 0. \tag{6.2}$$

(3) $P_n(t)$ has a unique positive critical point, called the peak instant of n transcription bursts and denoted by S_n, where it takes its absolute maximum value. Furthermore

$$S_n > T_{ny}, \tag{6.3}$$

where T_{ny} is the peak instant of the state (n,y).

To help us understand the properties of $P_n(t)$, three burst frequency probabilities $P_0(t)$, $P_1(t)$, and $P_2(t)$ are graphed in Figure 6.1 for $\kappa = 5$, $\lambda = 1/5$, and $\gamma = 1/5.8$. The three maximum values of $P_0(t)$, $P_1(t)$, and $P_2(t)$ are marked with X. It is obvious that properties (1)-(3) are valid.

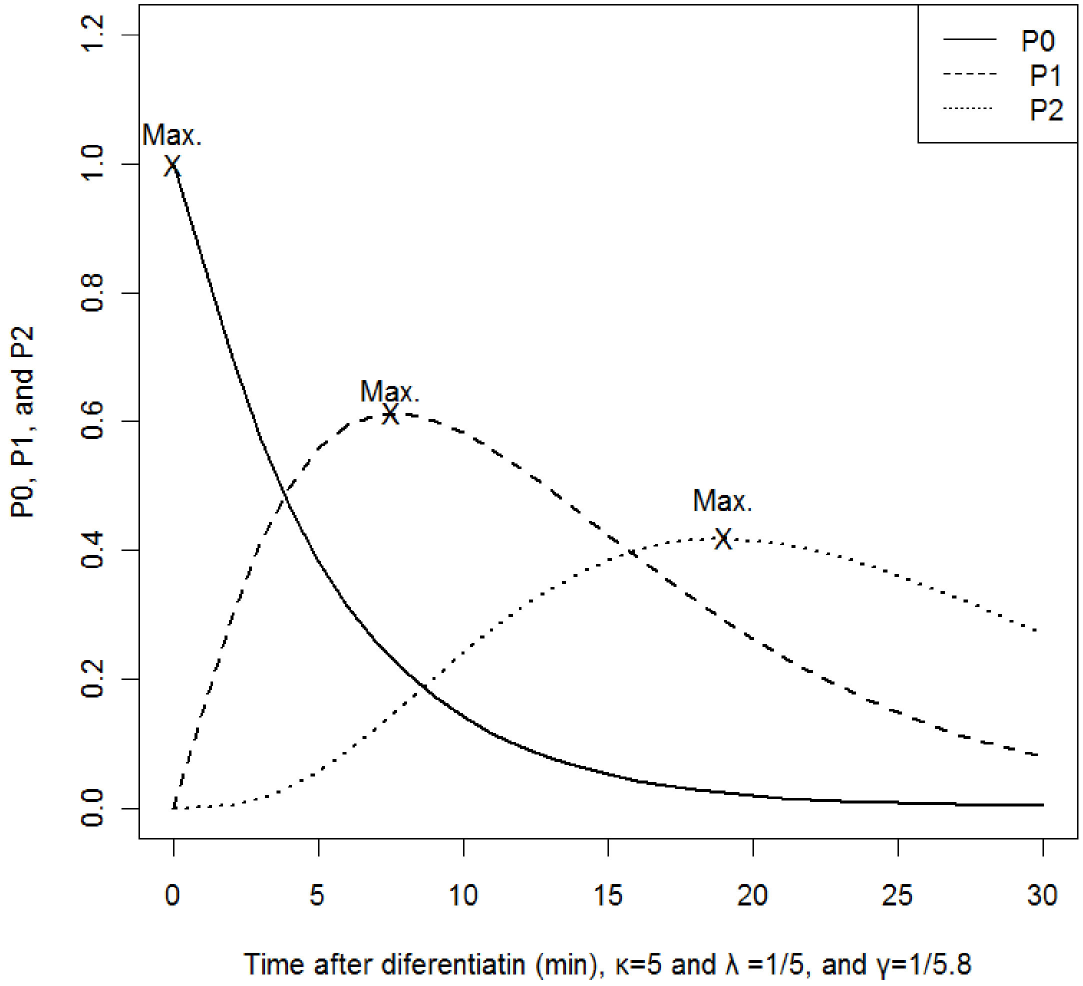

Figure 6.1 The plot of three bursting frequency probabilities $P_0(t)$, $P_1(t)$, and $P_2(t)$. The three maximum values of $P_0(t)$, $P_1(t)$, and $P_2(t)$ are marked with X.

Proving Part (3) of theorem 6. 2 requires two technical lemmas (proof of theorem 6. 2 requires lemma 6.3 and proof of lemma 6.3 requires lemma 6.2). The lemmas are given here, but the proof of lemmas is given immediately after we prove Theorem 6. 2.

Lemma 6.2 Assume that $n \geq 1$ and the initial condition (5.3) holds, which is $P_{1q}(0) = 1$, $P_{1y}(0) = P_{1e}(0) = 0$, and $P_{nq}(0) = P_{ny}(0) = P_{ne}(0) = 0$ for $n > 1$, then

$P_{(n+1)x}(t) < P_{nx}(t)$ and $P'_{(n+1)x}(t) > 0$ for all $0 < t \leq T_{nx}$, $x = q, y, e.$

Lemma 6.3 Assume that the initial condition (5.3) holds and $n \geq 1$. Then $P_{nx}(t)$ and $P_{(n+1)x}(t)$ intersect exactly once over $(0, \infty)$. More precisely, there is a time $S_{nx} > T_{nx}$ such that

$$P_{nx}(t) > P_{(n+1)x}(t) \text{ for } 0 < t < S_{nx}, \; P_{nx}(t) < P_{(n+1)x}(t) \text{ for } t > S_{nx}, \tag{6.4}$$

and

$$P'_{nx}(S_{nx}) < P'_{(n+1)x}(S_{nx}) . \tag{6.5}$$

6.2.1 Proof of Theorem 6.2

(1) Show part (1), that is $P_n(t)$ are infinitely differentiable, positive, and strictly less than 1. They all approach zero as $t \to \infty$.

We know $P_n(t) = P_{ne}(t) + P_{(n+1)q}(t) + P_{(n+1)y}(t)$. And all $P_{nx}(t)$ are infinitely differentiable and positive, and they all approach zero as $t \to \infty$ by Theorem 5.2. Also $\sum_{n=1}^{\infty} P_{nq}(t) + P_{ny}(t) + P_{ne}(t) = 1$ for all $t > 0$ because of conservation relations (5.2). So we have all functions $P_n(t)$ are infinitely differentiable, positive, and strictly less than 1. They all approach zero as $t \to \infty$.

(2) Show part (2), i.e. $P'_n(0) = P''_n(0) = ... = P_n^{(3n-2)}(0) = 0$ and $P_n^{(3n-1)}(0) = (\kappa\lambda)^n \gamma^{n-1} > 0.$

We can get $P_n^{(i)}(0) = P_{ne}^{(i)}(0) + P_{(n+1)q}^{(i)}(0) + P_{(n+1)y}^{(i)}(0)$ by differentiating

$P_n(t) = P_{ne}(t) + P_{(n+1)q}(t) + P_{(n+1)y}(t)$ (6.1). We also have $j(n,e) = 3n$, $j(n+1,q) = 3n+1$, and $j(n+1,y) = 3n+2$ by definition of $j = j(n,x)$ given in (5.20) .

If $i \le 3n-2$, then $i \le j-2$ for $j(n,e)$, $j(n+1,q)$, and $j(n+1,y)$, so $P_n^{(i)}(0) = P_{ne}^{(i)}(0) + P_{(n+1)q}^{(i)}(0) + P_{(n+1)y}^{(i)}(0) = 0$ because the three derivatives $P_{ne}^{(i)}(0)$, $P_{(n+1)q}^{(i)}(0)$, and $P_{(n+1)y}^{(i)}(0)$ are all zeros by $P_{nx}^{'}(0) = \ldots = P_{nx}^{(j-2)}(0) = 0$ in (5.21).

If $i = 3n-1$, then $P_n^{(i)}(0) = P_{ne}^{(i)}(0) + P_{(n+1)q}^{(i)}(0) + P_{(n+1)y}^{(i)}(0)$

$= P_{ne}^{(3n-1)}(0) + P_{(n+1)q}^{(3n-1)}(0) + P_{(n+1)y}^{(3n-1)}(0)$

$= P_{ne}^{(j-1)}(0) + P_{(n+1)q}^{(j-2)}(0) + P_{(n+1)y}^{(j-3)}(0)$ since $j(n,e)-1 = 3n-1$, $j(n+1,q)-2 = 3n-1$, and $j(n+1,y)-3 = 3n-1$

$= P_{ne}^{(j-1)}(0)$ since $P_{(n+1)q}^{(j-2)}(0)$ and $P_{(n+1)y}^{(j-3)}(0)$ are both zeros due to $P_{nx}^{'}(0) = \ldots = P_{nx}^{(j-2)}(0) = 0$ in (5.21)

$= (\kappa\lambda)^n \gamma^{(n-1)}$ since $P_{ne}^{(j-1)}(0) = (\kappa\lambda)^n \gamma^{(n-1)}$ in (5.22)

(3) Show part (3), i.e. $P_n(t)$ has a unique positive critical point, called the peak instant of n transcription bursts and denoted by S_n, where it takes its absolute maximum value. Furthermore

$$S_n > T_{ny} \tag{6.3}$$

where T_{ny} is the peak instant of the state (n,y).

(3)-1 Show that $P_n(t)$ has a unique positive critical point, S_{ny}, where it takes its absolute maximum value, and $S_{ny} > T_{ny}$ (6.3) where T_{ny} is the peak instant of the state (n,y). Here are the details for the proof:

$P_n'(S_{ny}) = P_{ne}'(S_{ny}) + P_{(n+1)q}'(S_{ny}) + P_{(n+1)y}'(S_{ny})$ since $P_n(t) = P_{ne}(t) + P_{(n+1)q}(t) + P_{(n+1)y}(t)$.

$\Rightarrow P_n'(S_{ny}) = \lambda P_{ny}(S_{ny}) - \gamma P_{ne}(S_{ny}) - \kappa P_{(n+1)q}(S_{ny}) + \gamma P_{ne}(S_{ny})$

$+ \kappa P_{(n+1)q}(S_{ny}) - \lambda P_{(n+1)y}(S_{ny})$ by master equations (5.4)-(5.6)

$\Rightarrow P_n'(S_{ny}) = \lambda P_{ny}(S_{ny}) - \lambda P_{(n+1)y}(S_{ny})$

$$\Rightarrow \begin{cases} P_n'(S_{ny}) = 0 & \text{Since } P_{ny}(t) \text{ and } P_{(n+1)y}(t) \text{ intersect exactly once at t} = S_{ny} > T_{nx} \text{ by Lemma 6.3} \\ P_n''(S_{ny}) = \lambda P_{ny}'(S_{ny}) - \lambda P_{(n+1)y}'(S_{ny}) < 0 & \text{Since Lemma 6.3 asserts that } P_{ny}'(S_{ny}) < \lambda P_{(n+1)y}'(S_{ny}) \end{cases}$$

$\Rightarrow P_n(t)$ has a unique positive critical point S_{ny}, where it takes its absolute maximum value. Furthermore, $S_{ny} > T_{ny}$.

(3)-2 Let $S_n = S_{ny}$, then $P_n(t)$ has a unique positive critical point S_n, where it takes its absolute maximum value. Furthermore, $S_n > T_{ny}$.

We conjecture that $S_n < T_{(n+1)y}$. Although it is similar to (6.3), we are unable to prove or disprove it by a similar argument. If it could be proved, then it would follow that S_n increases with n.

6.2.2 Proof of Lemma 6.2

Lemma 6.2 Assume that $n \geq 1$ and the initial condition (5.3) hold, which is $P_{1q}(0) = 1$, $P_{1y}(0) = P_{1e}(0) = 0$, and $P_{nq}(0) = P_{ny}(0) = P_{ne}(0) = 0$, then

$P_{(n+1)x}(t) < P_{nx}(t)$ and $P_{(n+1)x}'(t) > 0$ for all $0 < t \leq T_{nx}$, $x = q, y, e.$

Proof of Lemma 6.2: 1) Show $P_{(n+1)x}(t) < P_{nx}(t)$ for $0 < t \leq T_{nx}$.

$P_{(n+1)x}(t) = \kappa\lambda\gamma \int_0^t E_{11}(t-s) P_{nx}(s)\,ds$ by master operator

$L(f(t)) = \kappa\lambda\gamma\int_0^t E_{11}(t-s)f(s)ds$ (5.10) and $P_{nx}(t) = L(P_{(n-1)x}(t))$

$\Rightarrow P_{(n+1)x}(t) = \kappa\lambda\gamma\int_0^t E_{11}(s)P_{nx}(t-s)ds$ since $\int_0^t f(t-s)g(s)ds = \int_0^t f(s)g(t-s)ds$

$\Rightarrow P_{(n+1)x}(t) < \kappa\lambda\gamma\int_0^t E_{11}(s)P_{nx}(t)ds$ for $0 < t \le T_{nx}$ since $E_{11} \ge 0$, $P_{nx}(t)$ is strictly increasing over $(0, T_{nx})$ by part (3) of Theorem 5.2, and $\int_a^b f(x)dx < \int_a^b g(x)dx$, $a < b$ if $f(x) < g(x)$.

$\Rightarrow P_{(n+1)x}(t) < \kappa\lambda\gamma\int_0^\infty E_{11}(s)P_{nx}(t)ds$ for $0 < t \le T_{nx}$ since $\int_0^t f(s)ds < \int_0^\infty f(s)ds$ when $f(s) > 0$ over $(0, \infty)$.

$\Rightarrow P_{(n+1)x}(t) < P_{nx}(t)\kappa\lambda\gamma\int_0^\infty E_{11}(s)ds$ for $0 < t \le T_{nx}$

$\Rightarrow P_{(n+1)x}(t) < P_{nx}(t)$ for $0 < t \le T_{nx}$ since $\kappa\lambda\gamma\int_0^\infty E_{11}(s)ds = 1$, and the proof of $\kappa\lambda\gamma\int_0^\infty E_{11}(s)ds = 1$ is in Tang, 2010.

2) Show $P'_{(n+1)x}(t) > 0$ for $n \ge 1$ and $0 < t \le T_{nx}$.

$P_{(n+1)x}(t) = \kappa\lambda\gamma\int_0^t E_{11}(t-s)P_{nx}(s)ds$ by master operator

$L(f(t)) = \kappa\lambda\gamma\int_0^t E_{11}(t-s)f(s)ds$ (5.10) and $P_{nx}(t) = L(P_{(n-1)x}(t))$

$\Rightarrow P_{(n+1)x}(t) = \kappa\lambda\gamma\int_0^t E_{11}(s)P_{nx}(t-s)ds$ since $\int_0^t f(t-s)g(s)ds = \int_0^t f(s)g(t-s)ds$

$\Rightarrow P'_{(n+1)x}(t) = \frac{d}{dt}(\kappa\lambda\gamma\int_0^t E_{11}(s)P_{nx}(t-s)ds)$

$\Rightarrow P'_{(n+1)x}(t) = \kappa\lambda\gamma E_{11}(t)P_{nx}(t-t) + \kappa\lambda\gamma\int_0^t E_{11}(s)\frac{d}{dt}(P_{nx}(t-s))ds$ since

$(\int_0^t g(s,t)ds)' = g(t,t) + \int_0^t (g(s,t))' ds$

$\Rightarrow P'_{(n+1)x}(t) \geq \kappa\lambda\gamma E_{11}(t)\times 0 + \kappa\lambda\gamma\int_0^t E_{11}(s)P'_{nx}(t-s)ds$ since $P_{nx}(0) \geq 0$ for $n \geq 1$

by the initial condition $P_{1q}(0) = 1$, $P_{1y}(0) = P_{1e}(0) = 0$, and

$P_{nq}(0) = P_{ny}(0) = P_{ne}(0) = 0$ for $n > 1$.

$\Rightarrow P'_{(n+1)x}(t) \geq \kappa\lambda\gamma\int_0^t E_{11}(s)P'_{nx}(t-s)ds$ for $n \geq 1$

$\Rightarrow P'_{(n+1)x}(t) > 0$ for $n \geq 1$ and $0 < t \leq T_{nx}$ since $\kappa\lambda\gamma\int_0^t E_{11}(s)P'_{nx}(t-s)ds > 0$ for

$n \geq 1$ and $0 < t \leq T_{nx}$, which is because $\int_0^t f(s)g(t-s)ds > 0$ as long as $f(s)g(t-s) > 0$,

and $E_{11}(s) > 0$ by definition of $E_{11}(s)$ introduced in section 5.1, and $P'_{nx}(t-s) > 0$

because $P_{nx}(t)$ is strictly increasing over $(0, T_{nx})$ by part (3) of Theorem 5.2.

6.2.3 Proof of Lemma 6.3

Lemma 6.3 Assume that $n \geq 1$ and the initial condition (5.3) holds, which is $P_{1q}(0) = 1$, $P_{1y}(0) = P_{1e}(0) = 0$, and $P_{nq}(0) = P_{ny}(0) = P_{ne}(0) = 0$, then $P_{nx}(t)$ and $P_{(n+1)x}(t)$ intersect exactly once over $(0,\infty)$. More precisely, there is a time $S_{nx} > T_{nx}$ such that

$$P_{nx}(t) > P_{(n+1)x}(t) \text{ for } 0 < t < S_{nx},\ P_{nx}(t) < P_{(n+1)x}(t) \text{ for } t > S_{nx}, \tag{6.4}$$

and

$$P'_{nx}(S_{nx}) < P'_{(n+1)x}(S_{nx}). \tag{6.5}$$

Proof of Lemma 6.3:

We prove Lemma 6.3 by induction on $(0,\infty)$ in the order of chain

$$(1,q) \xrightarrow{\kappa} (1,y) \xrightarrow{\lambda} (1,e) \xrightarrow{\gamma} (2,q) \xrightarrow{\kappa} \ldots (n,y) \xrightarrow{\lambda} (n,e) \xrightarrow{\gamma} (n,q) \xrightarrow{\kappa} \ldots$$

(5.1).

(1) Let $(n,x) = (1,q)$, show $P_{1q}(t)$ and $P_{2q}(t)$ intersect exactly once over $(0,\infty)$. More precisely, show there is a time $S_{nx} > T_{nx}$ such that $P_{1q}(t) > P_{2q}(t)$ for $0 < t < S_{1q}$, $P_{1q}(t) < P_{2q}(t)$ for $t > S_{1q}$, and $P'_{1q}(S_{1q}) < P'_{2q}(S_{1q})$.

$$\frac{d}{dt}\left(\frac{P_{2q}(t)}{\kappa\lambda\gamma P_{1q}(t)}\right) = \frac{d}{dt}\left(\frac{\kappa\lambda\gamma\int_0^t E_{11}(t-s)P_{1q}(s)ds}{\kappa\lambda\gamma P_{1q}(t)}\right) \text{ by master operator}$$

$$L(f(t)) = \kappa\lambda\gamma\int_0^t E_{11}(t-s)f(s)ds \text{ (5.10) and } P_{nx}(t) = L(P_{(n-1)x}(t))$$

$$\Rightarrow \frac{d}{dt}\left(\frac{P_{2q}(t)}{\kappa\lambda\gamma P_{1q}(t)}\right) = \frac{d}{dt}\left(\frac{\int_0^t E_{11}(t-s)P_{1q}(s)ds}{P_{1q}(t)}\right)$$

$$\Rightarrow \frac{d}{dt}\left(\frac{P_{2q}(t)}{\kappa\lambda\gamma P_{1q}(t)}\right) = \frac{d}{dt}\left(\frac{\int_0^t E_{11}(s)P_{1q}(t-s)ds}{P_{1q}(t)}\right) \text{ since } \int_0^t f(t-s)g(s)ds = \int_0^t f(s)g(t-s)ds$$

$$\Rightarrow \frac{d}{dt}\left(\frac{P_{2q}(t)}{\kappa\lambda\gamma P_{1q}(t)}\right) = \frac{d}{dt}\left(\frac{\int_0^t E_{11}(s)P_{1q}(t)P_{1q}(-s)ds}{P_{1q}(t)}\right) \text{ since}$$

$$P_{1q}(t-s) = e^{-\kappa(t-s)} = e^{-\kappa t}e^{-\kappa(-s)} = P_{1q}(t)P_{1q}(-s)$$

$$\Rightarrow \frac{d}{dt}\left(\frac{P_{2q}(t)}{\kappa\lambda\gamma P_{1q}(t)}\right) = \frac{d}{dt}\int_0^t E_{11}(s)P_{1q}(-s)ds$$

$$\Rightarrow \frac{d}{dt}\left(\frac{P_{2q}(t)}{\kappa\lambda\gamma P_{1q}(t)}\right) = E_{11}(t)P_{1q}(-t) \text{ since } \frac{d}{dt}\int_0^t f(s)ds = f(t)$$

$$\Rightarrow \frac{d}{dt}\left(\frac{P_{2q}(t)}{\kappa\lambda\gamma P_{1q}(t)}\right) > 0 \text{ since } E_{11}(t) > 0 \text{ and } P_{1q}(-t) > 0$$

$\Rightarrow {P_{2q}(t)}/{P_{1q}(t)}$ is a strictly increasing function

$\Rightarrow P_{1q}(t)$ and $P_{2q}(t)$ intersect exactly once at S_{1q} over $(0,\infty)$ because $P_{1q}(0) = 1$ and $P_{2q}(0) = 0$ by the initial condition (5.3)

$\Rightarrow P_{1q}(t) > P_{2q}(t)$ for $0 < t < S_{1q}$, $P_{1q}(t) < P_{2q}(t)$ for $t > S_{1q}$, and $P_{1q}^{'}(S_{1q}) < P_{2q}^{'}(S_{1q})$.

Also $S_{nx} > T_{nx}$ because $P_{2q}(t) < P_{1q}(t)$ for all $0 < t \le T_{1q}$ from Lemma 6.2, and $P_{2q}(t) < P_{1q}(t)$ for $t > S_{1q}$, $P_{1q}(t) > P_{2q}(t)$ for $0 < t < S_{1q}$ from argument above.

(2) Let $(n,x) = (n,q)$, assume it's true that $P_{nq}(t)$ and $P_{(n+1)q}(t)$ intersect exactly once over $(0,\infty)$. More precisely, there is a time $S_{nq} > T_{nq}$ such that $P_{nq}(t) > P_{(n+1)q}(t)$ for $0 < t < S_{nq}$, $P_{nq}(t) < P_{nq}(t)$ for $t > S_{nq}$, and $P_{nq}^{'}(S_{nq}) < P_{(n+1)q}^{'}(S_{nq})$.

(3) Let $(n,x)=(n,y)$, we need to show it is true that $P_{ny}(t)$ and $P_{(n+1)y}(t)$ intersect exactly once over $(0,\infty)$. More precisely, there is a time $S_{ny}>T_{ny}$ such that

$$P_{ny}(t)>P_{(n+1)y}(t) \text{ for } 0<t<S_{ny},\; P_{ny}(t)<P_{(n+1)y}(t) \text{ for } t>S_{ny}, \tag{6.4}$$

and

$$P_{ny}^{'}(S_{ny})<P_{(n+1)y}^{'}(S_{ny}). \tag{6.5}$$

We define s_{ny} as the smallest positive point where $P_{ny}(t)$ and $P_{(n+1)y}(t)$ meet, then show in sequential order that $s_{ny}>T_{ny}$, $P_{(n+1)y}^{'}(s_{ny})>P_{ny}^{'}(s_{ny})$, $P_{ny}(t)$ and $P_{(n+1)y}(t)$ can intersect only once over $(0,\infty)$ at s_{ny}, indeed $s_{ny}=S_{ny}$, and $P_{ny}(t)>P_{(n+1)y}(t)$ for $0<t<S_{ny}$, $P_{ny}(t)<P_{(n+1)y}(t)$ for $t>S_{nx}$ (6.4).

(3)-1 Show $s_{ny}>T_{ny}$ and $P_{(n+1)y}^{'}(s_{ny})>P_{ny}^{'}(s_{ny})$. We have $P_{(n+1)y}(s_{ny})=P_{ny}(s_{ny})$ because s_{ny} denote the smallest positive point where $P_{ny}(t)$ and $P_{(n+1)y}(t)$ meet. Also by Lemma 6.2.2, we have

$$s_{ny}>T_{ny} \text{ and } P_{(n+1)y}(t)<P_{ny}(t) \text{ for } 0<t<s_{ny} \tag{6.6}$$

It follows that

$$P_{(n+1)y}^{'}(s_{ny})\geq P_{ny}^{'}(s_{ny}). \tag{6.7}$$

Next we show $P_{(n+1)y}^{'}(s_{ny})\geq P_{ny}^{'}(s_{ny})$ can be strengthened to the strict inequality $P_{(n+1)y}^{'}(s_{ny})>P_{ny}^{'}(s_{ny})$ because assuming $P_{(n+1)y}^{'}(s_{ny})=P_{ny}^{'}(s_{ny})$ can lead to contradiction:

$P_{(n+1)y}^{'}(s_{ny})=P_{ny}^{'}(s_{ny})$

$\Rightarrow P_{(n+1)y}^{'}(s_{ny})=\kappa P_{nq}(s_{ny})-\lambda P_{ny}(s_{ny})$ since $P_{ny}^{'}(s_{ny})=\kappa P_{nq}(s_{ny})-\lambda P_{ny}(s_{ny})$ by equation (5.4).

$\Rightarrow \kappa P_{nq}(s_{ny}) = P'_{(n+1)y}(s_{ny}) + \lambda P_{ny}(s_{ny})$

$\Rightarrow \kappa P_{nq}(s_{ny}) = P'_{(n+1)y}(s_{ny}) + \lambda P_{(n+1)y}(s_{ny})$ since $P_{(n+1)y}(s_{ny}) = P_{ny}(s_{ny})$ because s_{ny} is the point where $P_{ny}(t)$ and $P_{(n+1)y}(t)$ meet.

$\Rightarrow \kappa P_{nq}(s_{ny}) = \kappa P_{(n+1)q}(s_{ny})$ since $\kappa P_{(n+1)q}(s_{ny}) = P'_{(n+1)y}(s_{ny}) + \lambda P_{(n+1)y}(s_{ny})$ because of $P'_{(n+1)y}(s_{ny}) = \kappa P_{(n+1)q}(s_{ny}) - \lambda P_{(n+1)y}(s_{ny})$ by equation (5.4).

$\Rightarrow$ $P_{nq}(t)$ and $P_{(n+1)q}(t)$ also meet at s_{ny}.

$\Rightarrow S_{nq} = s_{ny}$ and $P'_{nq}(s_{ny}) < P'_{(n+1)q}(s_{ny})$ since we assume that $P_{nq}(t)$ and $P_{(n+1)q}(t)$ intersect exactly once over $(0,\infty)$. More precisely, we assume that there is a time $S_{nq} > T_{nq}$ such that $P_{nq}(t) > P_{(n+1)q}(t)$ for $0 < t < S_{nq}$, $P_{nq}(t) < P_{nq}(t)$ for $t > S_{nq}$, and $P'_{nq}(S_{nq}) < P'_{nq}(S_{nq})$.

$\Rightarrow \kappa P'_{nq}(s_{ny}) < \kappa P'_{(n+1)q}(s_{ny})$

$\Rightarrow \kappa P'_{nq}(s_{ny}) - \lambda P'_{ny}(s_{ny}) < \kappa P'_{(n+1)q}(s_{ny}) - \lambda P'_{ny}(s_{ny})$

$\Rightarrow \kappa P'_{nq}(s_{ny}) - \lambda P'_{ny}(s_{ny}) < \kappa P'_{(n+1)q}(s_{ny}) - \lambda P'_{(n+1)y}(s_{ny})$ since we assume $P'_{(n+1)y}(s_{ny}) = P'_{ny}(s_{ny})$ above

$\Rightarrow P''_{ny}(s_{ny}) < P''_{(n+1)y}(s_{ny})$ since $P''_{ny}(s_{ny}) = \kappa P'_{nq}(s_{ny}) - \lambda P'_{ny}(s_{ny})$ and $P''_{(n+1)y}(s_{ny}) = \kappa P'_{(n+1)q}(s_{ny}) - \lambda P'_{(n+1)y}(s_{ny})$ by equation (5.4).

$\Rightarrow P''_{ny}(s_{ny}) - P''_{(n+1)y}(s_{ny}) < 0$

$\Rightarrow P_{ny}(t) - P_{(n+1)y}(t)$ takes a maximum value at $t = s_{ny}$ because we assume that $P'_{(n+1)y}(s_{ny}) = P'_{ny}(s_{ny})$ above. And this maximum value is zero since $P_{ny}(t)$ and $P_{(n+1)y}(t)$

meet at $t = s_{ny}$ by our definition of s_{ny}

$\Rightarrow P_{ny}(t) - P_{(n+1)y}(t) < 0$ for some $t < s_{ny}$, leading to a contradiction of $P_{(n+1)y}(t) < P_{ny}(t)$ for $0 < t < s_{ny}$ in (6.6).

Taken together, we prove that $P^{'}_{(n+1)y}(s_{ny}) > P^{'}_{ny}(s_{ny})$.

(3)-2 We need $P_{nq}(s_{ny}) < P_{(n+1)q}(s_{ny})$ to show $P_{ny}(t)$ and $P_{(n+1)y}(t)$ can intersect only once over $(0, \infty)$ at s_{ny}. So we prove it first. Here are the details for the proof:

$P^{'}_{ny}(s_{ny}) = \kappa P_{nq}(s_{ny}) - \lambda P_{ny}(s_{ny})$ by equation (5.4)

$\Rightarrow \kappa P_{nq}(s_{ny}) = P^{'}_{ny}(s_{ny}) + \lambda P_{ny}(s_{ny})$

$\Rightarrow \kappa P_{nq}(s_{ny}) < P^{'}_{(n+1)y}(s_{ny}) + \lambda P_{ny}(s_{ny})$ for $s_{ny} > T_{ny}$ since $P^{'}_{(n+1)y}(s_{ny}) > P^{'}_{ny}(s_{ny})$ proved in (3)-1.

$\Rightarrow \kappa P_{nq}(s_{ny}) < P^{'}_{(n+1)y}(s_{ny}) + \lambda P_{(n+1)y}(s_{ny})$ for $s_{ny} > T_{ny}$ since $P_{(n+1)y}(s_{ny}) = P_{ny}(s_{ny})$ because s_{ny} is the point where $P_{ny}(t)$ and $P_{(n+1)y}(t)$ meet.

$\Rightarrow \kappa P_{nq}(s_{ny}) < \kappa P_{(n+1)q}(s_{ny})$ for $s_{ny} > T_{ny}$ since $P^{'}_{(n+1)y}(s_{ny}) + \lambda P_{(n+1)y}(s_{ny}) = \kappa P_{(n+1)q}(s_{ny})$ because of $P^{'}_{(n+1)y}(s_{ny}) = \kappa P_{(n+1)q}(s_{ny}) - \lambda P_{(n+1)y}(s_{ny})$ in equation (5.4)

$\Rightarrow P_{nq}(s_{ny}) < P_{(n+1)q}(s_{ny})$ for $s_{ny} > T_{ny}$ (6.8)

(3)-3 Show $P_{ny}(t)$ and $P_{(n+1)y}(t)$ can intersect only once over $(0, \infty)$ at s_{ny}, indeed $s_{ny} = S_{ny}$. Suppose for contradiction that $P_{ny}(t)$ and $P_{(n+1)y}(t)$ intersect more than once. Then we can find a time $t_{ny} > s_{ny}$ such that

$$P_{ny}(t) < P_{(n+1)y}(t) \text{ for } s_{ny} < t < t_{ny}, \text{ and } P_{ny}(t_{ny}) = P_{(n+1)y}(t_{ny}) \qquad (6.9)$$

It follows that $P^{'}_{ny}(t_{ny}) \geq P^{'}_{(n+1)y}(t_{ny})$. We show both $P^{'}_{ny}(t_{ny}) = P^{'}_{(n+1)y}(t_{ny})$ and

$P'_{ny}(t_{ny}) > P'_{(n+1)y}(t_{ny})$ could lead to contradiction in the following sections.

First, show $P'_{ny}(t_{ny}) = P'_{(n+1)y}(t_{ny})$ can lead to contradiction: $P'_{ny}(t_{ny}) = P'_{(n+1)y}(t_{ny})$,

$\Rightarrow \kappa P_{nq}(t_{ny}) - \lambda P_{ny}(t_{ny}) = P'_{(n+1)y}(t_{ny})$ since $P'_{ny}(t_{ny}) = \kappa P_{nq}(t_{ny}) - \lambda P_{ny}(t_{ny})$ by equation (5.4).

$\Rightarrow \kappa P_{nq}(t_{ny}) = P'_{(n+1)y}(t_{ny}) + \lambda P_{ny}(t_{ny})$

$\Rightarrow \kappa P_{nq}(t_{ny}) = P'_{(n+1)y}(t_{ny}) + \lambda P_{(n+1)y}(t_{ny})$ since $P_{(n+1)y}(t_{ny}) = P_{ny}(t_{ny})$ by assuming that $P_{ny}(t)$ and $P_{(n+1)y}(t)$ also intersect at t_{ny} in (6.9)

$\Rightarrow \kappa P_{nq}(t_{ny}) = \kappa P_{(n+1)q}(t_{ny})$ since $\kappa P_{(n+1)q}(t_{ny}) = P'_{(n+1)y}(t_{ny}) + \lambda P_{(n+1)y}(t_{ny})$ because of $P'_{(n+1)y}(t_{ny}) = \kappa P_{(n+1)q}(t_{ny}) - \lambda P_{(n+1)y}(t_{ny})$ in equation (5.4).

$\Rightarrow$ $P_{nq}(t)$ and $P_{(n+1)q}(t)$ also meet at t_{ny}, which contradicts our assumption that $P_{nq}(t)$ and $P_{(n+1)q}(t)$ intersect uniquely at S_{nq} in section (2).

Second, show $P'_{ny}(t_{ny}) > P'_{(n+1)y}(t_{ny})$ can lead to contradiction. If $P'_{ny}(t_{ny}) > P'_{(n+1)y}(t_{ny})$, we can have $\kappa P_{nq}(t_{ny}) > \kappa P_{(n+1)q}(t_{ny})$ by similar argument to above. Combining with $P_{nq}(s_{ny}) < P_{(n+1)q}(s_{ny})$ (6.8), it implies that $P_{nq}(t)$ and $P_{(n+1)q}(t)$ intersect at least one time in the interval (s_{ny}, t_{ny}), again leading to a contradiction to our assumption that $P_{nq}(t)$ and $P_{(n+1)q}(t)$ intersect uniquely at S_{nq} in section (2).

Put all together, we prove that $P_{ny}(t)$ and $P_{(n+1)y}(t)$ intersect exactly once, and indeed $s_{ny} = S_{ny}$.

(3)-4 Show $P_{ny}(t) > P_{(n+1)y}(t)$ for $0 < t < S_{ny}$, $P_{ny}(t) < P_{(n+1)y}(t)$ for $t > S_{nx}$ (6.4). We have proved $P_{ny}(t)$ and $P_{(n+1)y}(t)$ intersect exactly once at S_{ny}. Combing it with

$P_{ny}(t) > P_{(n+1)y}(t)$ for $0 < t \le T_{ny}$ from Lemma 6.2, we have $P_{ny}(t) > P_{(n+1)y}(t)$ for $0 < t < S_{ny}$, $P_{ny}(t) < P_{(n+1)y}(t)$ for $t > S_{ny}$, and $S_{ny} > T_{ny}$.

Put (1) to (3)-4 together, we prove Lemma 3 by induction.

6.3 Example for burst frequency probability in the Three State Model

In this section we demonstrate how the functions $P_n(t)$ could be used to estimate the frequency of gene transcription in eukaryotes by the transcription of an endogenous developmental gene *dscA* in the social amoeba *Dictyostelium* studied by Chubb et al. (2006). The detection system is similar to that of Golding et al. (2005), which followed the approach pioneered by Singer and colleagues (Bertrand et al., 1998). In the system, twenty-four MS2 stem loops were integrated at 6 bp downstream of the ATG start codon of the *dscA* gene. Upon transcription, the MS2 stem loops were read into nascent RNA and detected as a well-resolved fluorescent nuclear spot at the site of transcription, by the rapid binding of MS2-GFP stably expressed in the cells.

The number of transcriptional bursts of the *dscA* gene was directly counted during each 30 min capture period by Chubb and his colleagues, which enabled them to calculate accurately the percentage of the cells with given number of bursts.

This percentage of the cells with given number of bursts can be estimated by the following equation:

$$p_n(t) = \frac{P_n(t)}{1 - P_0(t)} = \frac{P_{ne}(t) + P_{(n+1)q}(t) + P_{(n+1)y}(t)}{1 - P_0(t)}, \ t > 0, \qquad (6.7)$$

Here, $P_n(t)$ equals the probability that exactly n transcriptional bursts have occurred since time zero. $P_0(t)$ is portion of silent cells. We also know $\sum_{n=0}^{\infty} P_n(t) = 1$ due to conservation property (5.2). Hence the percentage of the cells that have 3 or more bursts *among expressed cells* in $(0,t)$ is given by

$$p_{n\geq 3}(t) = \frac{1-P_0(t)-P_1(t)-P_2(t)}{1-P_0(t)} = 1-\frac{P_1(t)+P_2(t)}{1-P_0(t)} \qquad (6.8)$$

Our estimated percentages of the cells among all expressing cells with 1, 2, or 3 and more transcriptional bursts within the first 30 min are 8%, 27% and 65%, respectively, assuming that $\gamma = 1/5.8\ \text{min}^{-1}$, $\kappa = 5\ \text{min}^{-1}$, and $\lambda = 1/5\ \text{min}^{-1}$. They are close to the measured data of Chubb et al. (2006), where these values were estimated as about 12%, 26% and 62% respectively, even though they did not exactly match.

The percentage of the cells with 1, 2, and 3 and plus transitional bursts ($p_1(t)$, $p_2(t)$ and $p_{n\geq 3}(t)$) based on our model were graphed in Figure 6.2. As shown in Figure 6.2, $p_1(t)$ increases for a short time period (not shown in the Figure 6.2) and then decreases, $p_2(t)$ increases in the first 19 min, and then decreases. Although $p_{n\geq 3}(t)$ keeps increasing in the time frame of the graph, it decreases sometime later. We took $\gamma^{-1} = 5.8\ \text{min}$, $\kappa^{-1}+\lambda^{-1}$=5.2 min since the mean durations of gene on and off periods were estimated to be 5.8 min and 5.2 min, respectively. Although no further information is currently available to estimate κ and λ separately, we assume that κ is much bigger than λ, because HL5 media was added at optimal amounts and the promoter was observed to be fully activated when the data were collected. This consideration suggests us to take κ=5 min^{-1} and $\lambda = 1/5\ \text{min}^{-1}$ in our simulation.

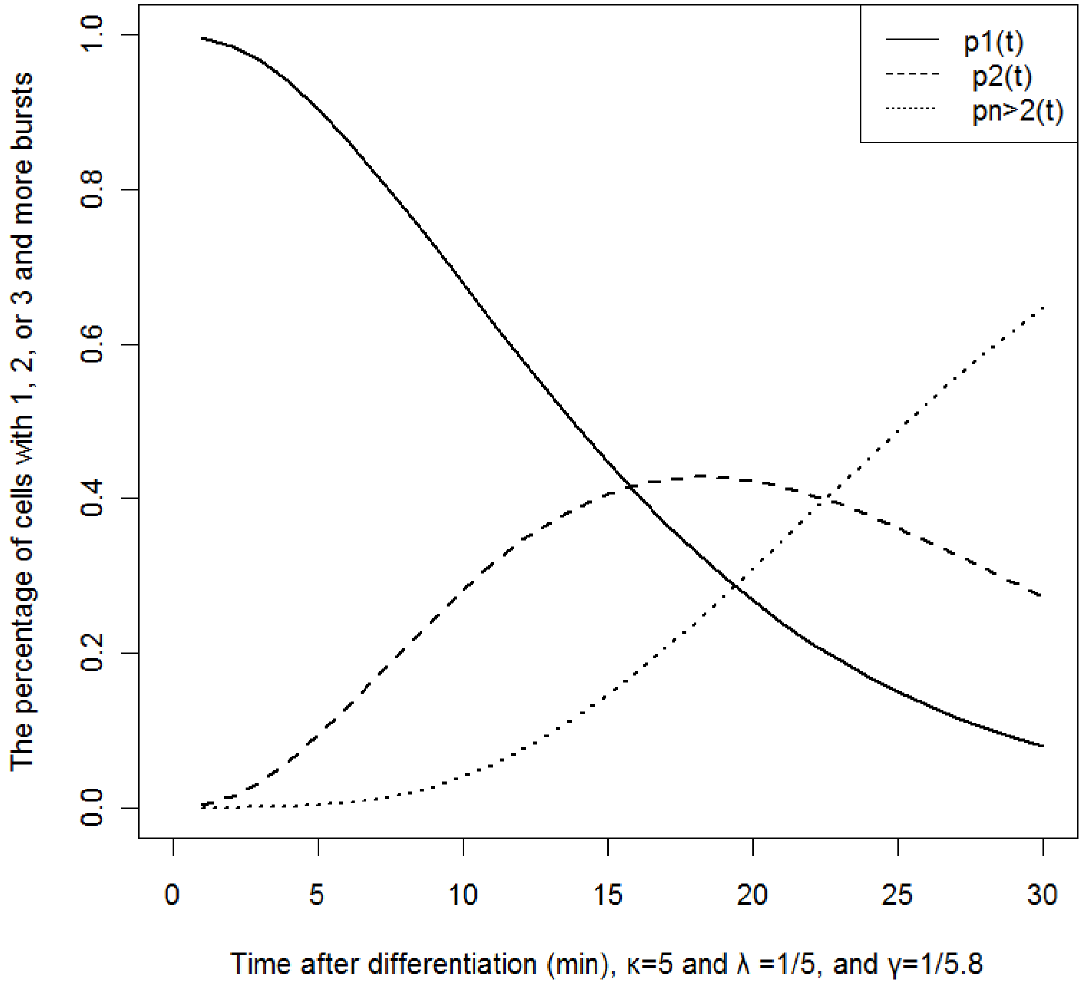

Figure 6.2 The percentages of the cells with 1, 2, or 3 and more transcriptional bursts during the time period $(0,t)$. Here $p_1(t)$, $p_2(t)$ and $p_{n\geq3}(t)$ give the percentages of the cells with 1, 2, or 3 and more transcriptional bursts during the time period $(0,t)$, among the expressing cells only. $p_1(t)$ increases for a short time period (not shown in the Fig.) and then decreases. $p_2(t)$ increases in the first 19 min, and then decreases. Although $p_{n\geq3}(t)$ keeps increasing in the time frame of the graph, it decreases sometime later. The

percentages of cells with 1, 2, or 3 and more transcriptional bursts within the first 30 min are 8%, 27% and 65%, respectively.

Our simulation suggests considerably more cells with multiple transcriptional bursts. It is unclear what caused the slight discrepancy. We offer two arguments here, not necessarily exclusive each other. First, the induction signals were not stably applied, and so κ needed to be replaced by a time and space dependent function. Second, and more directly, the measurement of the durations in gene on and off periods could artificially cut off some transcriptional activities, because the gene activities were monitored within 30 min blindly captured periods in different developmental stages of a total of 5 hours. Therefore, the real durations could be substantially longer, and the values κ, λ, and γ could be much less. When smaller values of κ, λ, and γ are substituted, the number of cells with multiple bursts will decrease, and the number of cells with only one burst will increase. This could yield a robust fit with the experimental data by the same simulation procedure.

We focus on this time period of 30 minutes for two reasons: First, when gene activities were imaged by fluorescent microscopy, fields of cells were blind-captured every 2.5 min for a total of 30 min during different developmental stages (Chubb et al., 2006). Second, the proportion of cells with detectable transcription elevated from zero during the first 30 min of differentiation, suggesting that the initial condition (5.3), $P_{1q}(0)=1$, and $P_{nx}(0)=0$ otherwise, can be used. (For studies in other 30 min capture periods, (5.3) needs to be changed appropriately). The choice of κ here is not experimentally supported. Indeed, as suggested by the discussion above and further emphasized below, it is more appropriate to treat κ as a function of time and the spatial variables. In this case, (5.2) and (5.3) remain the same, but $P_{nx}(t)$ is obtained by more advanced techniques not covered in the current study.

We note that the Three State Model not only supports the existence of transcriptional bursts, but also predicts other types of transcriptional dynamics. A transcriptional burst is characterized by the production of many transcripts in a quick succession after a unambiguous period of zero transcription. It occurs if (i) the average duration of gene off period $\kappa^{-1} + \lambda^{-1}$ and the average duration of gene on period γ^{-1} are within a reasonable range, neither too small nor too large; and (ii) when the promoter is on the engaged state, mRNA is produced efficiently. These conditions were fulfilled by the examples discussed in this Section. If $\kappa^{-1} + \lambda^{-1}$ is very small, then there are only brief off periods and transcripts can be produced in a nearly continuous fashion. The same could occur if γ^{-1} is very small. If condition (ii) does not hold, then either there can be few mRNA synthesized, or the elongation activity is repeatedly interrupted. In the first case, there could be insufficient amounts of transcripts to display a burst; in the latter case, the elongation interruption could generate several "pseudo-bursts" within one round of engaged state. The distribution of the "pseudo-bursts" may not follow an exponential decay and its average duration could be much less than γ^{-1}.

Chapter 7

Conclusion and Future Work

In this chapter, I summarize our conclusions and propose future related work.

7.1 Conclusion

The analysis reveals that no promoters will be definitely turned on to transcribe within a finite time period, no matter how strong the induction signals, or how abundant the activators. Although stronger extrinsic signals could enhance promoter activation rate, there's an upper limit that no signals could cross over it in a finite time. Consequently, among a large population of isogenic cells, only a portion of the cells, but not the whole population, could be induced by environmental signals to express a particular gene within a finite time period.

We prove that the gene on duration follows an exponential distribution, and the gene off intervals show a local maximum that is best described by assuming two sequential exponential process.

The transition frequencies are determined by a system of stochastic differential equations, or equivalently, an iterative scheme of integral operators. We prove that for each positive integer n, there associates a unique time, called the peak instant, at which the system is residing on the state n^{th} time since time zero most likely. These moments constitute a time series preserving the nature order of n.

A transcriptional burst indicates a transcript synthesis cycle. We prove that for each positive integer n, there associates a unique time, called the peak instant, at which the n^{th} transcript synthesis cycle proceeds most likely. These moments constitute a time series preserving the nature order of n.

7.2 Future work

If the expression profile of the gene is sensitive to stress or developmental signals, then the induction strength κ may inherit the heterogeneity of the signal distribution in time and space. The transcription of the developmental gene *dscA* in the social amoeba provides a convincing example (Chubb et al., 2006): The histogram depicting the percentage of expressing cells during a period of more than 5 hours fits into an oscillatory curve, which is dramatically distinct from the growth curve predicted by the Three State Model for a constant κ. The observation that expressing cells were more frequently seen in clusters further indicates that induction signals have a inhomogeneous spatial distribution (Chubb et al., 2006). To gain a better understanding of the global stochastic dynamics in this case, we need a more detailed understanding of the signal transduction pathways impinging on the transcription system. The induction strength κ becomes a function of time and spatial variables, and the corresponding theoretical study requires integration of partial differential equations, which might be one topic of our future study.

In contrast, we take λ and γ as constants because they are mostly determined by the biochemical property of the gene promoter, the TF, and the basal transcription machinery. These properties may not differ significantly from cell to cell. We define the pair (λ, γ) as the transcription mode associated with the system. For most synthetic reporter gene constructs and many genes in prokaryotic cells or simple eukaryotes, we hypothesize that this mode could be evolutionary conserved and would not vary notably among cells of closely related organisms. In general, if a gene has multiple binding sites in the core promoter region, and each site is targeted by one or several types of transcription factors with considerably different functions, then it could correspond to a discrete set of different modes. If this is the case, the transcription system may transit among a cluster of functional states bifurcating from the same ground state. The treatment of this case relies on a nontrivial extension of the Three State Model, whose technical steps will be presented in our future work.

Appendix

R Code for Figures

```
############################################################
## Figure 3.1, Probability of zero transcriptions since induction (fraction of cells
having no tagged RNA (P0) as a function of time after induction t).
############################################################
k=1/5
m=1/32
t=c(1:100)
Pz=(m/(m-k))*exp(-k*t)+(k/(k-m))*exp(-m*t)
Pzz=log(Pz)
Pgolding=-0.14*t
plot(t, Pzz, type="l", col = "black", lwd=2,
xlab = "Time after induction (min), κ=1/5 and λ =1/32", ylab = "ln(P0(t))")
text(10,-0.3,'+');text(20,-0.7,'+');text(30,-1.05,'+');text(40,-1.4,'+');text(50,-
1.85,'+');text(60,-1.5,'+');text(80,-1.85,'+');text(100,-2.25,'+')
text(15,-0.625,'O'); text(30,-0.8,'O'); text(45,-1.25,'O'); text(60,-1.625,'O'); text(90,-
2.3,'O')
lines(t, Pgolding, lty = 2, lwd=2, lheight=1)


############################################################

## Figure 4.1, Plot of duration of gene off period versus density distribution $f_{off}(t)$.
############################################################
k=70
la=0.7
t=c(0:30)
Poff=k*la/(la-k)*(exp(-k*t)-exp(-la*t))
plot(t, Poff, type="l", col = " black", lwd=2, xlab = "Duration of gene off period in
arbitrary unit", ylab = "Density distribution")
Poff02_20=.20*20/(20-.2)*(exp(-.2*t)-exp(-20*t))
Poff03_04=.3*.4/(.4-.3)*(exp(-.3*t)-exp(-.4*t))
Poff05=.05^2*t*exp(.05*t)
lines(t, Poff02_20, lty = 2, lwd=2, lheight=1)
lines(t, Poff03_04, lty = 3, lwd=2, lheight=1)
lines(t, Poff05, lty = 4, lwd=2, lheight=1)
legend("topright", c("κ=70 >> λ=0.7", "κ=0.2 << λ=20", "κ is close to λ, κ=0.3 and
λ=0.4", "κ=λ=0.5 "), lty=1:4, lwd=2)

############################################################

## Figure 4.2, Plot of duration of gene off period versus logarithm of density distribution
$\log(f_{off}(t))$.
############################################################
```

```
lPoff=log(Poff)
lPoff02_20=log( Poff02_20)
lPoff03_04= log(Poff03_04)
lPoff05= log(Poff05)
plot(t, lPoff, type="l", col = " black", lwd=2, xlab = "Duration of gene off period in
arbitrary unit", ylab = "Logarithm of Density distribution")
lines(t, lPoff02_20, lty = 2, lwd=2, lheight=1)
lines(t, lPoff03_04, lty = 3, lwd=2, lheight=1)
lines(t, lPoff05, lty = 4, lwd=2, lheight=1)
legend("bottomleft", c("κ=70 >> λ=0.7", "κ=0.2 << λ=20", "κ is close to λ, κ=0.3 and
λ=0.4", "κ=λ=0.5 "), lty=1:4, lwd=2)

############################################################

## Figure 5.1, The plot of transition frequency probabilities $P_{1q}(t)$- $P_{3e}(t)$ .
############################################################
k=1/3; la=1/5; g=1/6
t=c(0:50)
# define kij;
k11=1/((k-la)*(k-g))
k12=1/( (k-la)*((k-g)^2) )
k13=1/( (k-la)*((k-g)^3) )
k21=1/( ((k-la)^2)*(k-g) )
k22=1/( ((k-la)^2)*((k-g)^2) )
k23=1/( ((k-la)^2)*((k-g)^3) )
k24=1/( ((k-la)^2)*((k-g)^4) )
k31=1/( ((k-la)^3)*(k-g) )
k32=1/( ((k-la)^3)*((k-g)^2) )
k33=1/( ((k-la)^3)*((k-g)^3) )
k34=1/( ((k-la)^3)*((k-g)^4) )
k35=1/( ((k-la)^3)*((k-g)^5) )
k42=1/( ((k-la)^4)*((k-g)^2) )
k43=1/( ((k-la)^4)*((k-g)^3) )
k44=1/( ((k-la)^4)*((k-g)^4) )
k53=1/( ((k-la)^5)*((k-g)^3) )
# define lamdaij;
la11=1/( (la-g)*(la-k) )
la12=1/( (la-g)*((la-k)^2) )
la13=1/( (la-g)*((la-k)^3) )
la21=1/( ((la-g)^2)*(la-k) )
la22=1/( ((la-g)^2)*((la-k)^2) )
la23=1/( ((la-g)^2)*((la-k)^3) )
la24=1/( ((la-g)^2)*((la-k)^4) )
la31=1/( ((la-g)^3)*(la-k) )
la32=1/( ((la-g)^3)*((la-k)^2) )
la33=1/( ((la-g)^3)*((la-k)^3) )
la34=1/( ((la-g)^3)*((la-k)^4) )
```

```
la35=1/( ((la-g)^3)*((la-k)^5) )
la42=1/( ((la-g)^4)*((la-k)^2) )
la43=1/( ((la-g)^4)*((la-k)^3) )
la44=1/( ((la-g)^4)*((la-k)^4) )
la53=1/( ((la-g)^5)*((la-k)^3) )
# define gamaij;
g11=1/( (g-k)*(g-la) )
g12=1/( (g-k)*((g-la)^2) )
g13=1/( (g-k)*((g-la)^3) )
g21=1/( ((g-k)^2)*( g-la) )
g22=1/( ((g-k)^2)*(( g-la)^2))
g23=1/( ((g-k)^2)*(( g-la)^3))
g24=1/( ((g-k)^2)*(( g-la)^4))
g31=1/( ((g-k)^3)*( g-la))
g32=1/( ((g-k)^3)*(( g-la)^2))
g33=1/( ((g-k)^3)*(( g-la)^3))
g34=1/( ((g-k)^3)*(( g-la)^4))
g35=1/( ((g-k)^3)*(( g-la)^5))
g42=1/( ((g-k)^4)*(( g-la)^2))
g43=1/( ((g-k)^4)*(( g-la)^3))
g44=1/( ((g-k)^4)*(( g-la)^4))
g53=1/( ((g-k)^5)*(( g-la)^3))
# define eij;
e11=k11*exp(-k*t)+la11*exp(-la*t)+g11*exp(-g*t)
e22=k22*exp(-k*t)+la22*exp(-la*t)+g22*exp(-g*t)
e23=k23*exp(-k*t)+la23*exp(-la*t)+g23*exp(-g*t)
e32=k32*exp(-k*t)+la32*exp(-la*t)+g32*exp(-g*t)
e33= k33*exp(-k*t)+la33*exp(-la*t)+g33*exp(-g*t)
e34= k34*exp(-k*t)+la34*exp(-la*t)+g34*exp(-g*t)
e43= k43*exp(-k*t)+la43*exp(-la*t)+g43*exp(-g*t)
e35= k35*exp(-k*t)+la35*exp(-la*t)+g35*exp(-g*t)
e44= k44*exp(-k*t)+la44*exp(-la*t)+g44*exp(-g*t)
e53= k53*exp(-k*t)+la53*exp(-la*t)+g53*exp(-g*t)
#get p1q-p2e;
p1q=exp(-k*t)
p1y=k/(la-k)*(exp(-k*t)-exp(-la*t))
p1e=k*la*e11
p2q=k*la*g*(k11*t*exp(-k*t) +(k12+k21)*exp(-k*t)-la12*exp(-la*t)-g21*exp(-g*t))
p2y=-(k^2)*la*g*(k21*t*exp(-k*t) +la12*t*exp(-la*t)+e22+2*k31*exp(-
k*t)+2*la13*exp(-la*t)- 2*g22*exp(-g*t))
p2e=(k^2)*(la^2)*g*((t*e22+2*e23+2*e32))
# get p3q, p3y and p3e
lk= k*la*g*(k11*t*exp(-k*t)+(k12+k21)*exp(-k*t)-la12*exp(-la*t)-g21*exp(-g*t))
lg= k*la*g*(g11*t*exp(-g*t)+(g12+g21)*exp(-g*t)-k12*exp(-k*t)-la21*exp(-la*t))
lla= k*la*g*(la11*t*exp(-la*t)+(la12+la21)*exp(-la*t)-g12*exp(-g*t)-k21*exp(-k*t))
ltk= k*la*g*(k11/2*(t^2)*exp(-k*t)+
```

```
(la11/(la-k)+g11/(g-k))*t*exp(-k*t)-
(la11/(la-k)^2+g11/(g-k)^2)*exp(-k*t) +la11/(la-k)^2*exp(-la*t)+g11/(g-k)^2*exp(-g*t))
ltla=k*la*g*(la11/2*(t^2)*exp(-la*t)+
(k11/(k-la)+g11/(g-la))*t*exp(-la*t)-
(k11/(k-la)^2+g11/(g-la)^2)*exp(-la*t) +k11/(k-la)^2*exp(-k*t)+g11/(g-la)^2*exp(-g*t))
p3q=k*la*g*(k11*ltk+(k12+k21)*lk-la12*lla-g21*lg)
p3y=-k^2*la*g*(k21*ltk+(k22+2*k31)*lk
+la12*ltla+(la22+2*la13)*lla-g22*lg)
p3e=k^3*la^3*g^2*(
t^2*e33/2+3*t*(e34+e43)+3*e35+5*e44+3*e53
+(la22*k31+g22*k13)*exp(-k*t)+(k22*la13+g22*la31)*exp(-la*t)+
(k22*g31+la22*g13)*exp(-g*t)
+2*k11*(la24+g33)*exp(-k*t)+2*la11*(g24+k33)*exp(-la*t)+
2*g11*(k24+la33)*exp(-g*t)
+2*k11*(la33+g42)*exp(-k*t)+2*la11*(g33+k42)*exp(-la*t)+
2*g11*(k33+la42)*exp(-g*t))
# plot;
plot(t, p3e, ylim=c(0,1.1), type="l", lty = 1, col = 1, lwd=2,
xlab = "Time in arbitrary unit, κ=1/3 and λ =1/5, and γ=1/6", ylab = "The probability
functions Pnx(t)")
lines(t, p3y, col =2, lty = 2, lwd=2, lheight=1)
lines(t, p3q, col =3, lty = 3, lwd=2, lheight=1)
lines(t, p2e, col =4, lty = 4, lwd=2, lheight=1)
lines(t, p2y, col =5, lty = 5, lwd=2, lheight=1)
lines(t, p2q, col =6, lty = 6, lwd=2, lheight=1)
lines(t, p1e, col =7, lty = 7, lwd=2, lheight=1)
lines(t, p1y, col =8, lty = 8, lwd=2, lheight=1)
lines(t, p1q, col =9, lty = 9, lwd=2, lheight=1)
legend("topright", c("P3e(t)", "P3y(t)", "P3q(t)", "P2e(t)",
 "P2y(t)", " P2q(t)" ,"P1e(t)", " P1y(t)", " P1q(t)"), lty=1:9,col=1:9, lwd=2)

############################################################
## Figure 5.2, The plot of transition frequency probabilities P2q(t) - P2e(t).
############################################################
k=1/3; la=1/5; g=1/6
t=c(0:50)
# define kij;
k11=1/((k-la)*(k-g))
k12=1/( (k-la)*((k-g)^2) )
k21=1/( ((k-la)^2)*(k-g) )
k22=1/( ((k-la)^2)*((k-g)^2) )
k23=1/( ((k-la)^2)*((k-g)^3) )
k31=1/( ((k-la)^3)*(k-g) )
k32=1/( ((k-la)^3)*((k-g)^2) )
# define lamdaij;
```

```
la12=1/( (la-g)*((la-k)^2) )
la13=1/( (la-g)*((la-k)^3) )
la22=1/( ((la-g)^2)*((la-k)^2) )
la23=1/( ((la-g)^2)*((la-k)^3) )
la32=1/( ((la-g)^3)*((la-k)^2) )
# define gamaij;
g13=1/( (g-k)*((g-la)^3) )
g21=1/( ((g-k)^2)*( g-la) )
g22=1/( ((g-k)^2)*(( g-la)^2))
g23=1/( ((g-k)^2)*(( g-la)^3))
g32=1/( ((g-k)^3)*(( g-la)^2))
# define eij;
e22=k22*exp(-k*t)+la22*exp(-la*t)+g22*exp(-g*t)
e23=k23*exp(-k*t)+la23*exp(-la*t)+g23*exp(-g*t)
e32=k32*exp(-k*t)+la32*exp(-la*t)+g32*exp(-g*t)
# get p2q-p2e;
p2q=k*la*g*(k11*t*exp(-k*t) +(k12+k21)*exp(-k*t)-la12*exp(-la*t)-g21*exp(-g*t))
p2y=-(k^2)*la*g*(k21*t*exp(-k*t) +la12*t*exp(-la*t)+e22+2*k31*exp(-
k*t)+2*la13*exp(-la*t)- 2*g22*exp(-g*t))
p2e=(k^2)*(la^2)*g*((t*e22+2*e23+2*e32))
# plot;
plot(t, p2e, ylim=c(0,0.25),type="l", lty = 3, col = "black", lwd=2,
xlab = "Time in arbitrary unit, κ=1/3 and λ =1/5, and γ=1/6", ylab = "The three
probability functions P2x(t)")
text(10,0.1656,'Max.');text(16,0.228,'Max.');text(22,0.2307,'Max.')
text(12,0.1566,'X');text(17,0.219,'X');text(23,0.2217,'X')
lines(t, p2q, col = "black", lty = 1, lwd=2, lheight=1)
lines(t, p2y, col = "black",lty = 2, lwd=2, lheight=1)
legend("topright", c("P2q(t)", " P2y(t)", " P2e(t)"), lty=1:3)

#######################################################
## Figure 6.1 The plot of three burst frequency probabilities P0(t), P1(t), and P2(t).
#######################################################
k=5; la=1/5; g=1/5.8
t=c(0:30)
# define kij;
k11=1/((k-la)*(k-g))
k12=1/( (k-la)*((k-g)^2) )
k13=1/( (k-la)*((k-g)^3) )
k21=1/( ((k-la)^2)*(k-g) )
k22=1/( ((k-la)^2)*((k-g)^2) )
k23=1/( ((k-la)^2)*((k-g)^3) )
k24=1/( ((k-la)^2)*((k-g)^4) )
k31=1/( ((k-la)^3)*(k-g) )
k32=1/( ((k-la)^3)*((k-g)^2) )
k33=1/( ((k-la)^3)*((k-g)^3) )
```

```
k34=1/( ((k-la)^3)*((k-g)^4) )
k35=1/( ((k-la)^3)*((k-g)^5) )
k42=1/( ((k-la)^4)*((k-g)^2) )
k43=1/( ((k-la)^4)*((k-g)^3) )
k44=1/( ((k-la)^4)*((k-g)^4) )
k53=1/( ((k-la)^5)*((k-g)^3) )
# define lamdaij;
la11=1/( (la-g)*(la-k) )
la12=1/( (la-g)*((la-k)^2) )
la13=1/( (la-g)*((la-k)^3) )
la21=1/( ((la-g)^2)*(la-k) )
la22=1/( ((la-g)^2)*((la-k)^2) )
la23=1/( ((la-g)^2)*((la-k)^3) )
la24=1/( ((la-g)^2)*((la-k)^4) )
la31=1/( ((la-g)^3)*(la-k) )
la32=1/( ((la-g)^3)*((la-k)^2) )
la33=1/( ((la-g)^3)*((la-k)^3) )
la34=1/( ((la-g)^3)*((la-k)^4) )
la35=1/( ((la-g)^3)*((la-k)^5) )
la42=1/( ((la-g)^4)*((la-k)^2) )
la43=1/( ((la-g)^4)*((la-k)^3) )
la44=1/( ((la-g)^4)*((la-k)^4) )
la53=1/( ((la-g)^5)*((la-k)^3) )
# define gamaij;
g11=1/( (g-k)*(g-la) )
g12=1/( (g-k)*((g-la)^2) )
g13=1/( (g-k)*((g-la)^3) )
g21=1/( ((g-k)^2)*( g-la) )
g22=1/( ((g-k)^2)*(( g-la)^2))
g23=1/( ((g-k)^2)*(( g-la)^3))
g24=1/( ((g-k)^2)*(( g-la)^4))
g31=1/( ((g-k)^3)*( g-la))
g32=1/( ((g-k)^3)*(( g-la)^2))
g33=1/( ((g-k)^3)*(( g-la)^3))
g34=1/( ((g-k)^3)*(( g-la)^4))
g35=1/( ((g-k)^3)*(( g-la)^5))
g42=1/( ((g-k)^4)*(( g-la)^2))
g43=1/( ((g-k)^4)*(( g-la)^3))
g44=1/( ((g-k)^4)*(( g-la)^4))
g53=1/( ((g-k)^5)*(( g-la)^3))
# define eij;
e11=k11*exp(-k*t)+la11*exp(-la*t)+g11*exp(-g*t)
e22=k22*exp(-k*t)+la22*exp(-la*t)+g22*exp(-g*t)
e23=k23*exp(-k*t)+la23*exp(-la*t)+g23*exp(-g*t)
e32=k32*exp(-k*t)+la32*exp(-la*t)+g32*exp(-g*t)
e33= k33*exp(-k*t)+la33*exp(-la*t)+g33*exp(-g*t)
```

```
e34= k34*exp(-k*t)+la34*exp(-la*t)+g34*exp(-g*t)
e43= k43*exp(-k*t)+la43*exp(-la*t)+g43*exp(-g*t)
e35= k35*exp(-k*t)+la35*exp(-la*t)+g35*exp(-g*t)
e44= k44*exp(-k*t)+la44*exp(-la*t)+g44*exp(-g*t)
e53= k53*exp(-k*t)+la53*exp(-la*t)+g53*exp(-g*t)
# get p1q-p2e;
p1q=exp(-k*t)
p1y=k/(la-k)*(exp(-k*t)-exp(-la*t))
p1e=k*la*e11
p2q=k*la*g*(k11*t*exp(-k*t) +(k12+k21)*exp(-k*t)-la12*exp(-la*t)-g21*exp(-g*t))
p2y=-(k^2)*la*g*(k21*t*exp(-k*t) +la12*t*exp(-la*t)+e22+2*k31*exp(-
k*t)+2*la13*exp(-la*t)- 2*g22*exp(-g*t))
p2e=(k^2)*(la^2)*g*((t*e22+2*e23+2*e32))
# for p3q, and p3y
lk= k*la*g*(k11*t*exp(-k*t)+(k12+k21)*exp(-k*t)-la12*exp(-la*t)-g21*exp(-g*t))
lg= k*la*g*(g11*t*exp(-g*t)+(g12+g21)*exp(-g*t)-k12*exp(-k*t)-la21*exp(-la*t))
lla= k*la*g*(la11*t*exp(-la*t)+(la12+la21)*exp(-la*t)-g12*exp(-g*t)-k21*exp(-k*t))
ltk= k*la*g*(k11/2*(t^2)*exp(-k*t)+
(la11/(la-k)+g11/(g-k))*t*exp(-k*t)-
(la11/(la-k)^2+g11/(g-k)^2)*exp(-k*t) +la11/(la-k)^2*exp(-la*t)+g11/(g-k)^2*exp(-g*t))
ltla=k*la*g*(la11/2*(t^2)*exp(-la*t)+
(k11/(k-la)+g11/(g-la))*t*exp(-la*t)-
(k11/(k-la)^2+g11/(g-la)^2)*exp(-la*t) +k11/(k-la)^2*exp(-k*t)+g11/(g-la)^2*exp(-g*t))
# get p3q-p3e;
p3q=k*la*g*(k11*ltk+(k12+k21)*lk-la12*lla-g21*lg)
p3y=-k^2*la*g*(k21*ltk+(k22+2*k31)*lk
+la12*ltla+(la22+2*la13)*lla-g22*lg)
p3e=k^3*la^3*g^2*(
t^2*e33/2+3*t*(e34+e43)+3*e35+5*e44+3*e53
+(la22*k31+g22*k13)*exp(-k*t)+(k22*la13+g22*la31)*exp(-la*t)+
(k22*g31+la22*g13)*exp(-g*t)
+2*k11*(la24+g33)*exp(-k*t)+2*la11*(g24+k33)*exp(-la*t)+
2*g11*(k24+la33)*exp(-g*t)
+2*k11*(la33+g42)*exp(-k*t)+2*la11*(g33+k42)*exp(-la*t)+
2*g11*(k33+la42)*exp(-g*t))
# get p0t- pge3;
p0=p1q+p1y
p1=p1e+p2q+p2y
p2=p2e+p3q+p3y
# plot;
plot(t, p0, ylim=c(0,1.2), type="l", lty =1, col = 1, lwd=2, xlab = "Time after diferentiatin
(min), κ=5 and λ =1/5, and γ=1/5.8", ylab = "P0, P1, and P2")
lines(t, p1, col =1, lty = 2, lwd=2, lheight=1)
lines(t, p2, col =1, lty = 3, lwd=2, lheight=1)
legend("topright", c("P0", " P1", " P2"), lty=1:3)
text(0,1.05,'Max.');text(7.5,0.65,'Max.');text(19,0.48,'Max.')
```

```
text(0,1,'X');text(7.5,0.615,'X');text(19,0.42,'X')

############################################################
## Figure 6.2 The percentages of the cells with 1, 2, or 3 and more transcriptional bursts
during the time period (0,t).
############################################################
k=5; la=1/5; g=1/5.8
#k=5;
#la=0.8;
t=c(0:30)
# define kij;
k11=1/((k-la)*(k-g))
k12=1/( (k-la)*((k-g)^2) )
k13=1/( (k-la)*((k-g)^3) )
k21=1/( ((k-la)^2)*(k-g) )
k22=1/( ((k-la)^2)*((k-g)^2) )
k23=1/( ((k-la)^2)*((k-g)^3) )
k24=1/( ((k-la)^2)*((k-g)^4) )
k31=1/( ((k-la)^3)*(k-g) )
k32=1/( ((k-la)^3)*((k-g)^2) )
k33=1/( ((k-la)^3)*((k-g)^3) )
k34=1/( ((k-la)^3)*((k-g)^4) )
k35=1/( ((k-la)^3)*((k-g)^5) )
k42=1/( ((k-la)^4)*((k-g)^2) )
k43=1/( ((k-la)^4)*((k-g)^3) )
k44=1/( ((k-la)^4)*((k-g)^4) )
k53=1/( ((k-la)^5)*((k-g)^3) )
# define lamdaij;
la11=1/( (la-g)*(la-k) )
la12=1/( (la-g)*((la-k)^2) )
la13=1/( (la-g)*((la-k)^3) )
la21=1/( ((la-g)^2)*(la-k) )
la22=1/( ((la-g)^2)*((la-k)^2) )
la23=1/( ((la-g)^2)*((la-k)^3) )
la24=1/( ((la-g)^2)*((la-k)^4) )
la31=1/( ((la-g)^3)*(la-k) )
la32=1/( ((la-g)^3)*((la-k)^2) )
la33=1/( ((la-g)^3)*((la-k)^3) )
la34=1/( ((la-g)^3)*((la-k)^4) )
la35=1/( ((la-g)^3)*((la-k)^5) )
la42=1/( ((la-g)^4)*((la-k)^2) )
la43=1/( ((la-g)^4)*((la-k)^3) )
la44=1/( ((la-g)^4)*((la-k)^4) )
la53=1/( ((la-g)^5)*((la-k)^3) )
# define gamaij;
g11=1/( (g-k)*(g-la) )
```

```
g12=1/( (g-k)*((g-la)^2) )
g13=1/( (g-k)*((g-la)^3) )
g21=1/( ((g-k)^2)*( g-la) )
g22=1/( ((g-k)^2)*(( g-la)^2))
g23=1/( ((g-k)^2)*(( g-la)^3))
g24=1/( ((g-k)^2)*(( g-la)^4))
g31=1/( ((g-k)^3)*( g-la))
g32=1/( ((g-k)^3)*(( g-la)^2))
g33=1/( ((g-k)^3)*(( g-la)^3))
g34=1/( ((g-k)^3)*(( g-la)^4))
g35=1/( ((g-k)^3)*(( g-la)^5))
g42=1/( ((g-k)^4)*(( g-la)^2))
g43=1/( ((g-k)^4)*(( g-la)^3))
g44=1/( ((g-k)^4)*(( g-la)^4))
g53=1/( ((g-k)^5)*(( g-la)^3))
# define eij;
e11=k11*exp(-k*t)+la11*exp(-la*t)+g11*exp(-g*t)
e22=k22*exp(-k*t)+la22*exp(-la*t)+g22*exp(-g*t)
e23=k23*exp(-k*t)+la23*exp(-la*t)+g23*exp(-g*t)
e32=k32*exp(-k*t)+la32*exp(-la*t)+g32*exp(-g*t)
e33= k33*exp(-k*t)+la33*exp(-la*t)+g33*exp(-g*t)
e34= k34*exp(-k*t)+la34*exp(-la*t)+g34*exp(-g*t)
e43= k43*exp(-k*t)+la43*exp(-la*t)+g43*exp(-g*t)
e35= k35*exp(-k*t)+la35*exp(-la*t)+g35*exp(-g*t)
e44= k44*exp(-k*t)+la44*exp(-la*t)+g44*exp(-g*t)
e53= k53*exp(-k*t)+la53*exp(-la*t)+g53*exp(-g*t)
# get p1q-p2e;
p1q=exp(-k*t)
p1y=k/(la-k)*(exp(-k*t)-exp(-la*t))
p1e=k*la*e11
p2q=k*la*g*(k11*t*exp(-k*t) +(k12+k21)*exp(-k*t)-la12*exp(-la*t)-g21*exp(-g*t))
p2y=-(k^2)*la*g*(k21*t*exp(-k*t) +la12*t*exp(-la*t)+e22+2*k31*exp(-
k*t)+2*la13*exp(-la*t)- 2*g22*exp(-g*t))
p2e=(k^2)*(la^2)*g*((t*e22+2*e23+2*e32))
# for p3q, and p3y;
lk= k*la*g*(k11*t*exp(-k*t)+(k12+k21)*exp(-k*t)-la12*exp(-la*t)-g21*exp(-g*t))
lg= k*la*g*(g11*t*exp(-g*t)+(g12+g21)*exp(-g*t)-k12*exp(-k*t)-la21*exp(-la*t))
lla= k*la*g*(la11*t*exp(-la*t)+(la12+la21)*exp(-la*t)-g12*exp(-g*t)-k21*exp(-k*t))
ltk= k*la*g*(k11/2*(t^2)*exp(-k*t)+
(la11/(la-k)+g11/(g-k))*t*exp(-k*t)-
(la11/(la-k)^2+g11/(g-k)^2)*exp(-k*t) +la11/(la-k)^2*exp(-la*t)+g11/(g-k)^2*exp(-g*t))
ltla=k*la*g*(la11/2*(t^2)*exp(-la*t)+
(k11/(k-la)+g11/(g-la))*t*exp(-la*t)-
(k11/(k-la)^2+g11/(g-la)^2)*exp(-la*t) +k11/(k-la)^2*exp(-k*t)+g11/(g-la)^2*exp(-g*t))
# get p3q, and p3y;
p3q=k*la*g*(k11*ltk+(k12+k21)*lk-la12*lla-g21*lg)
```

```
p3y=-k^2*la*g*(k21*ltk+(k22+2*k31)*lk
+la12*ltla+(la22+2*la13)*lla-g22*lg)
p3e=k^3*la^3*g^2*(
t^2*e33/2+3*t*(e34+e43)+3*e35+5*e44+3*e53
+(la22*k31+g22*k13)*exp(-k*t)+(k22*la13+g22*la31)*exp(-la*t)+
(k22*g31+la22*g13)*exp(-g*t)
+2*k11*(la24+g33)*exp(-k*t)+2*la11*(g24+k33)*exp(-la*t)+
2*g11*(k24+la33)*exp(-g*t)
+2*k11*(la33+g42)*exp(-k*t)+2*la11*(g33+k42)*exp(-la*t)+
2*g11*(k33+la42)*exp(-g*t))
# get p0, p1, p2, and pge3;
p0t=la/(la-k)*exp(-k*t)+k/(k-la)*exp(-la*t)
p0=p1q+p1y
p1=p1e+p2q+p2y
p2=p2e+p3q+p3y
pge3=1-(p0+p1+p2)
# get pp1, pp2, and ppge3;
pp1=p1/(1-p0)
pp2=p2/(1-p0)
ppge3=1-(p1+p2)/(1-p0)
# plot;
plot(t, pp1, ylim=c(0,1), type="l", lty =1, col = 1, lwd=2,
xlab = "Time after diferentiatin (min), κ=5 and λ =1/5, and γ=1/5.8", ylab = "The
percentage of cells with 1, 2, or 3 and more bursts")
lines(t, pp2, col =1, lty = 2, lwd=2, lheight=1)
lines(t, ppge3, col =1, lty = 3, lwd=2, lheight=1)
legend("topright", c("p1(t)", " p2(t)", " pn>2(t)"), lty=1:3)
```

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