On a stochastic gene expression with pre-mRNA, mRNA and protein contribution

Ryszard Rudnicki\textsuperscript{1} and Andrzej Tomski\textsuperscript{2}

\textsuperscript{1}Institute of Mathematics, Polish Academy of Sciences, Bankowa 14, 40-007, Katowice, Poland
ryszard.rudnicki@us.edu.pl
\textsuperscript{2}Institute of Mathematics, Jagiellonian University, Łojasiewicza 6, 30-348 Kraków, Poland
andrzej.tomski@im.uj.edu.pl

March 2, 2022

DOI: 10.1016/j.jtbi.2015.09.012
< 2015 >. This manuscript version is made available under the CC-BY-NC-ND 4.0 license

Abstract

In this paper we develop a model of stochastic gene expression, which is an extension of the model investigated in the paper [T. Lipniacki, P. Paszek, A. Marciniak-Czochra, A.R. Brasier, M. Kimmel, Transcriptional stochasticity in gene expression, J. Theor. Biol. 238 (2006) 348 – 367]. In our model, stochastic effects still originate from random fluctuations in gene activity status, but we precede mRNA production by the formation of pre-mRNA, which enriches classical transcription phase. We obtain a stochastically regulated system of ordinary differential equations (ODEs) describing evolution of pre-mRNA, mRNA and protein levels. We perform mathematical analysis of a long-time behaviour of this stochastic process, identified as a piece-wise deterministic Markov process (PDMP). We check
exact results using numerical simulations for the distributions of all three types of particles. Moreover, we investigate the deterministic (adiabatic) limit state of the process, when depending on parameters it can exhibit two specific types of behavior: bistability and the existence of the limit cycle. The latter one is not present when only two kinds of gene expression products are considered.

Keywords: Stochastic gene expression, Pre-mRNA, Piece-wise deterministic Markov process, Invariant density

1 Introduction

Gene expression and its regulation is a very complex process, which takes place in the cells of living organisms, especially in eukaryotes [24]. It is widely known that this process depends on the behaviour of crucial substances, called transcription factors (TFs) and chromatin architecture. Our investigation is based on the idea of [20], where a simplified diagram of gene expression was presented. It was mentioned there that genes fluctuate randomly between their activity or inactivity status and transcripts are produced in bursts. Stochastic effects at the initial stage are very strong compared to both the matter production and degradation processes, so we consider the noise of Markov-type origin merely at the activation stage. These claims were verified and analysed through the years [4], [11], [15], [16], [27]. The whole scheme describes expression of a single gene, assuming it has \( n \) copies, but further analyse was performed in the case of one copy only. After activation of the gene (which is initiated by binding to the promoter region some of TFs), mRNA transcription and protein translation phases follow. At first, mature mRNA is produced in the nucleus, then it is transported from the nucleus to the cytoplasm, where the second phase takes place. As a result, new proteins are born.

In the mentioned class of models, not only transcription and translation evolution were considered, but also biological degradation of both types of the particles: mRNA and protein. All the processes were recognised as continuous, so the planar system of ordinary linear differential equations were used to represent the dynamics of fluctuations in the level of certain type particles. Moreover, first equation included stochastic “switch” component, being responsible for the control of gene activity status. This system has been identified in [6] as a Piece-wise Deterministic Markov Process (PDMP),
introduced by [10]. However, after reflection on these results, an important question arises: to what extent does the two-stage model fit the current state of biological knowledge? Would adding another stage make description of the gene expression more precise? Finally, will the problem be much more complicated if we add the third stage? In the mentioned work of [20] there is a remark that translated mRNA particle must get through some further processing before a new, mature protein is formed. Beside that, plenty of thematic books; [22], [35] and publication sources; [9], [36] claiming that at least one additional phase, called primary transcript (or pre-mRNA) processing should be taken into account. Actually, in eukaryotic genes, after the activation signal, the DNA code is transformed into pre-mRNA form of transcript. Then, the non-coding sequences (introns) of transcript are cut off. This action is combined with other modifications widely known as RNA processing. Only then we get a functional form of mRNA, which is transferred into the cytoplasm, where during the third phase, translation phase, mRNA is decoded into a protein. In short, we consider three-phase model of gene expression with three main components, i.e. three variables $x_1, x_2, x_3$ describing evolution of pre-mRNA, mRNA and protein levels. Firstly, we assume that pre-mRNA molecules are produced at the rate $A_1 \gamma(t)$, where $A_1$ is a constant and we introduce a stochastic binary valued function $\gamma(t) \in \{0, 1\}$ which marks, at time $t \geq 0$, if the gene is in active ($\gamma(t) = 1$) or inactive ($\gamma(t) = 0$) state. This function will be described in detail in Sec. 2.4. The mRNA production rate is equal to $A_2 x_1(t)$, where $A_2$ is a constant and $x_1(t)$ denotes the number of pre-mRNA molecules at time $t$. Similarly, the protein translation takes the place at the rate $A_2 x_1(t)$, where $x_2(t)$ denotes the number of mRNA molecules at time $t$. Moreover, all three types of particles undergo the degradation process. The total lost of pre-mRNA particles is given by $d_1 x_1 = d_1' x_1 + A_2 x_1$, where the constant $d_1'$ is the degradation rate of pre-mRNA particles and another constant $A_2$ is the rate of converting pre-mRNA into mRNA particles. It means that $d_1 = d_1' + A_2$ should be treated as the total degradation rate of pre-mRNA particles. This concept takes into consideration that pre-mRNA is converted to mRNA [21], in contrast to mRNA which serves as a template for mRNA synthesis, but is not degraded during the synthesis. Thus, in other cases we use standard description, i.e. the constants $d_2$ and $d_3$ denote, respectively, mRNA and protein degradation rates. This expansion of the previous, simplified diagram of gene expression depicted in [20], is now presented in Fig. 1. We note that the switching between active and inactive state of the gene depends on the so-called jump
rates (activation/inactivation rates).

Fig. 1: An extended scheme of (auto-regulated) gene expression.

Introduction of the third variable to the model means, that its geometry moves unavoidably into ℝ³ space. Although, in the last few years some PDMP-based biological models were presented, they focus on the applications in planar systems: [6], [20], [25], [32]. What is worth mentioning, in the paper of [1] it was investigated that three-dimensional Lorenz system with stochastic switching “admits a robust strange attractor”, but here we concentrate on a situation, when jump rates are not necessarily constant and also on the convergence in time of the distribution of the process to the equilibrium distribution. In the proof of the main theorem, we use some results concerning asymptotic stability of Markov semigroups. The main idea is that we need to check two conditions: irredicibility of the semigroup and the existence of some estimation of the semigroup from below which can be checked by using Hörmander condition for considered process. An alternative (with no reference to Markov semigroups) approach concerning long-time behavior of PDMP is based on regularity and convergence results from the papers [3] and [2], where similar conditions for the convergence of PDMP were developed. Recently, [23] asked the question, is it possible to describe long-time qualitative properties of the process for spatial dynamics? Here we do such an analysis with the aim to include the role of primary transcript in the processes basic for eukaryotic genes.

This paper is organised as follows. First, in Section 2 we present idea of the model. Then we discuss the deterministic (adiabatic) limit state, which can exhibit two specific types of behavior: bistability and the existence of the limit cycle. Later, we present mathematical description of the process, including deterministic part and the stochastic component. Having done that, we introduce Markov semigroups and we recall how they can be generated by PDMP to describe time evolution of the densities of the process. In the
first part of Section 3 we formulate the main theorem of this paper, which says that the Markov semigroup related to the model is asymptotically stable. This means that there exists a stationary density and independently on the initial distribution the density of the process converges to the stationary density as time goes to infinity. We find a set, an “attractor”, on which this three-dimensional distribution is concentrated. The second part of Section 3 is devoted to stochastic simulations of the process. We show time-dependent and mutual dependent behaviour of levels of pre-mRNA, mRNA and protein. We also approximate the above-mentioned limit stationary density for all types of the particles. In the further part we also refer to the deterministic state behavior. In Section 4 we sum up the results of our paper and give some conclusion remarks.

2 The model

2.1 Construction

Let \( x_1, x_2, x_3 \) denote three non-negative variables, which describe time-evolving levels of pre-mRNA, mRNA and protein, respectively. In accordance with the current surveys, we consider that the activation and inactivation rate functions \( q_0(x_1, x_2, x_3) \) and \( q_1(x_1, x_2, x_3) \) can be constant \cite{1}, \cite{26} or can depend on the number of the particles of one type, usually the proteins \cite{8}, \cite{20}. Briefly speaking, the gene is activated with the rate \( q_0(x_1, x_2, x_3) \) and inactivated with the rate \( q_1(x_1, x_2, x_3) \). The minimal mathematical assumptions about \( q_0 \) and \( q_1 \) in the case of two variables are discussed by \cite{6}. In line with the approach of \cite{20}, we study evolution of the following system of ODEs with a stochastic component:

\[
\begin{align*}
0 \cdot q_0(x_1, x_2, x_3) &\rightarrow 1, \quad 0 \cdot q_1(x_1, x_2, x_3) \\
\frac{dx_1}{dt} &= A_1 \gamma(t) - d_1 x_1 \\
\frac{dx_2}{dt} &= A_2 x_1 - d_2 x_2 \\
\frac{dx_3}{dt} &= A_3 x_2 - d_3 x_3,
\end{align*}
\]

(1)

where \( A_i \) and \( d_i \) are positive constants.

Remark 1. We pay attention to the fact that a standard three-dimensional Goodwin model of an oscillatory gene regulation loop \cite{13} was developed in
a similar manner to ours and it can be interpreted even in the same way [34]. However, instead of the presence of the stochastic process \( \gamma(t) \), Goodwin model contains a non-linear term describing the production rate of mRNA. The source of nonlinearity is the dependence of this rate from the protein level. In our work we take this fact into account by making the intensity functions \( q_0 \) and \( q_1 \) possibly dependent from the level of any type of particles, especially the proteins.

If \( q_0 \) and \( q_1 \) are constant, we calculate the expected levels of pre-mRNA, mRNA and protein in the molecular population:

\[
E(x_1) = \frac{A_1 q_0}{d_1(q_0 + q_1)}, \\
E(x_2) = \frac{A_1 A_2 q_0}{d_1 d_2(q_0 + q_1)}, \\
E(x_3) = \frac{A_1 A_2 A_3 q_0}{d_1 d_2 d_3(q_0 + q_1)},
\]

despite the fact that these levels oscillate in time (see Sec. 3.2 for details). Using standard rescaling techniques known from investigation of the planar model in [6], we obtain the system:

\[
\begin{align*}
0 \frac{q_0(x_1,x_2,x_3)}{d_1(q_0 + q_1)} & \rightarrow 1, & 0 \frac{q_1(x_1,x_2,x_3)}{d_1(q_0 + q_1)} & \leftarrow 1 \\
\frac{dx_1}{dt} & = \gamma(t) - x_1 \\
\frac{dx_2}{dt} & = a(x_1 - x_2) \\
\frac{dx_3}{dt} & = b(x_2 - x_3),
\end{align*}
\]

(2)

a, b > 0, in addition \( a \neq b, a \neq 1 \) and \( b \neq 1 \). We investigate this system in the next sections of the paper. Our results remain true also if \( a = b \) or \( a = 1 \) or \( b = 1 \) (see Remark 3.)

Remark 2. We can consider a more complicated process containing larger number of intermediate steps which lead to equations like in the system [2] and the approach presented below will not change. However, we analyse the system [2] with three equations for the brevity of notation. Larger number of intermediate steps introduce time delay, which in the case of negative feedback makes the system oscillatory. We have observed such oscillatory behaviour even in the three-dimensional case but for very special rate functions \( q_0 \) and \( q_1 \) (see Fig. 12).
2.2 The adiabatic limit

We shall consider particularly interesting behavior of our model, when both of the jump rates $q_0$ and $q_1$ tend to infinity, unlike their ratio. In this case, we can replace the stochastic process $\gamma(t)$ by its expected value $\Gamma := E\gamma = \frac{q_0}{q_0+q_1}$ to obtain a state called deterministic or adiabatic limit. Hence, the system transforms to deterministic system of three ODEs:

$$\begin{align*}
\frac{dx_1}{dt} &= \Gamma(x_3) - x_1 \\
\frac{dx_2}{dt} &= a(x_1 - x_2) \\
\frac{dx_3}{dt} &= b(x_2 - x_3).
\end{align*}$$

(3)

Depending on the values of the parameters $a, b$ we investigate some specific types of behavior. Firstly, we consider the case of the positive autoregulation, i.e. when $\Gamma$ is an increasing function of $x_3$. Assume that the equation $\Gamma(c) = c$ has three roots $c_1 < c_2 < c_3$ in the interval $(0, 1)$ and $\Gamma'(c_1) < 1$, $\Gamma'(c_2) > 1$, $\Gamma'(c_3) < 1$. Then the system (3) has three stationary points $x_i = (c_i, c_i, c_i)$, $i = 1, 2, 3$. The linearization of (3) at $x_i$ leads to the following characteristic polynomial

$$P(\lambda) = (1 + \lambda)(a + \lambda)(b + \lambda) - \Gamma'(c_i)ab.$$  

Since $\Gamma'(c_i) > 1$ we have $P(0) < 0$, and $P(\lambda) > 0$ for sufficiently large, which means that the polynomial $P$ has a positive root and, therefore, the point $x_2$ is unstable. Now we check that stationary points are $x_1$ and $x_3$ are asymptotically stable, i.e. all roots of $P$ have negative real parts. Indeed, if $a = b = 1$ then $P$ has all roots with negative real parts:

$$\lambda_1 = \Gamma'(c_i)^{1/3} - 1, \quad \lambda_2 = -1 + (-\frac{1}{2} + \frac{\sqrt{3}i}{2})\Gamma'(c_i)^{1/3}, \quad \lambda_3 = -1 + (-\frac{1}{2} - \frac{\sqrt{3}i}{2})\Gamma'(c_i)^{1/3}.$$  

If we find some coefficients $a, b$ such that $P$ has a root with a nonnegative real part, then we find some coefficients $a, b$ such that $P$ has a root with a zero real part, i.e., $\lambda = \alpha i, \alpha \in \mathbb{R}$. But then $\alpha = 0$ or

$$(a + b + ab) = \alpha^2 \text{ and } (1 + a + b)\alpha^2 = (1 - \Gamma'(c_i))ab.$$  

Both cases are impossible if $\Gamma'(c_i) < 1$, hence we obtain a bistable state (see [14]). In such a case one can expect that the stationary density of the
stochastic process will be bimodal provided that \(q_0\) and \(q_1\) are finite, but sufficiently large. We check this by performing extensive numerical simulations presented in Sec. 3.2 In the case of the negative autoregulation, i.e. when \(\Gamma\) is a decreasing function of \(x_3\), we have only one stationary point \(x = (c, c, c)\), where \(c\) is the unique solution of the equation \(\Gamma(c) = c\). Observe that in the case \(a = b = 1\) the polynomial \(P\) has one negative real root, and two complex roots with positive real parts if \(\Gamma'(c) < -8\). This suggest that in this case the limit cycle can appear. We check its existence by simulating the system in Sec. 3.2. This case is especially interesting, since from the standard Bendixson–Dulac theorem it follows that such limit cycle oscillations are not observed in the two-dimensional system studied before. Again, one can expect that for sufficiently large \(q_0\) and \(q_1\) the stationary density for the process will be distributed close to the limit cycle trajectory.

### 2.3 Two deterministic systems

For a fixed state of the gene, which determines the value of \(\gamma(t) \equiv i, i \in \{0, 1\}\), the process is purely deterministic and we get the system of the first order differential equations

\[
\begin{align*}
\frac{dx_1}{dt} &= i - x_1 \\
\frac{dx_2}{dt} &= a(x_1 - x_2) \\
\frac{dx_3}{dt} &= b(x_2 - x_3),
\end{align*}
\]

with the initial condition \(x_0 = (x_0^1, x_0^2, x_0^3) \in \mathbb{R}_+^3\) with \(a, b > 0, a \neq b, a \neq 1\) and \(b \neq 1\). The solution \(\pi_i^t(x_0)\) of this system is

\[
\pi_i^t(x_0) = i\mathbf{1} + \exp(Mt)(x_0 - i\mathbf{1}),
\]

where \(\mathbf{1} = [1, 1, 1]\) and

\[
M = \begin{bmatrix}
-1 & 0 & 0 \\
a & -a & 0 \\
0 & b & -b
\end{bmatrix}.
\]

Moreover, with a similarity to the two-dimensional case, we have:

\[
\pi_i^1(x_0) = 1 - \pi_i^0(\mathbf{1}) + \pi_i^0(x_0).
\]
In Fig. 2 phase portraits of the system (4) for both values of \( i \in \{0, 1\} \) are shown. Each time, there exists one stationary solution: for \( i = 0 \); a point \((0, 0, 0)\) is asymptotically stable steady state, as is a point \((1, 1, 1)\) for \( i = 1 \). Looking at the right-hand sides of system \( \mathcal{F} \) we state that if \( x_1 > 1 \), then (no matter what the value of \( i \) is), \( x_1 \) decreases, moreover \( x_2 \) as well \( x_3 \) follow \( x_1 \). On the other hand, if \( x_1 < 1 \), then it stays in the interval \([0, 1]\) forever, oscillating between 0 and 1, the same happens with \( x_2 \) and \( x_3 \). Hence, we can reduce the phase space of the process to a cube \( X = [0, 1]^3 \).

![Fig. 2: A sample solutions of Eq. (4) for \( a = 2, b = 3, i = 0 \) (left) and \( a = 2, b = 3, i = 1 \) (right).](image)

### 2.4 PDMP: a definition

We will briefly mention an idea behind PDMP introduced in [10]. We consider \( q_0(x_1, x_2, x_3) \) and \( q_1(x_1, x_2, x_3) \) as two continuous and non-negative functions on \( \mathbb{R}^3 \) such that:

\[
 q_0(0,0,0) \neq 0 \text{ and } q_1(1,1,1) \neq 0.
\]
Let \( i_0 \in \{0,1\}, \ T_0 = 0, \ x_0 \in \mathbb{R}^3_+ \) and we define a (random) function \( \gamma: [0, \infty) \to \{0,1\} \) satisfying \( \gamma(0) = i_0 \) and

\[
\gamma(t) := \begin{cases} i, & \text{if } T_{n-1} \leq t < T_n, \\ 1 - i, & \text{if } t = T_n, \end{cases}
\]

where for \( n \geq 1, \ T_n \) is a positive random variable satisfying:

\[
F_{x_s}(t) = \text{Prob}(T_n - T_{n-1} \leq t | \gamma(T_{n-1}) = i) = 1 - \exp \left( - \int_0^t q_i(\pi_i(s,x_s))ds \right),
\]

\[
x_s := \begin{cases} \pi_i^{s-T_{n-1}}(x_{n-1}), & \text{if } T_{n-1} \leq s < T_n, \\ x_n, & \text{if } s = T_n, \end{cases}
\]

where

\[
x_n = \pi_i^{T_n-T_{n-1}}(x_{n-1}).
\]

In consequence, replacing a constant value \( i \in \{0,1\} \) in the system (4) by a stochastic process \( \gamma(t) \):

\[
\begin{align*}
\frac{dx_1}{dt} &= \gamma(t) - x_1 \\
\frac{dx_2}{dt} &= a(x_1 - x_2) \\
\frac{dx_3}{dt} &= b(x_2 - x_3),
\end{align*}
\]

gives a definition of a Markov process \( \zeta(t) \) called a piece-wise deterministic Markov process, described by the quartet:

\[
\zeta(t) := (x_1(t), x_2(t), x_3(t), \gamma(t)) = (x(t), \gamma(t)).
\]

The state space of this process is \( X = X \times \{0,1\} \). The remaining characteristics are the jump rates \( q_i \) and the jump distribution \( \mathbb{J}((x,i), \cdot) \) being the Dirac measure \( \delta_{(x,1-i)} \) such that

\[
\mathbb{J}((x,i), X) = 1.
\]

A random variable \( T_n \) is called a time of the \( n-th \) jump of the process. In [6] it was shown that in such a case \( \Delta_k = T_k - T_{k-1} > 0, \) where \( k \geq 1, \ \Delta_k < \infty \) and

\[
\lim_{k \to \infty} T_k = \infty,
\]

which means that the process is well-defined for all times \( t \geq 0. \)
2.5 Markov semigroups and their link with PDMP

Now we will recall some definitions about Markov semigroups. We use them to describe the evolution of distributions of the process given by the system (2). Detailed information about some connections between semigroup theory and stochastic processes can be found in [19] or [30]. Let \((\mathbb{X}, \Sigma, m)\) be a \(\sigma\)-finite measure space and let \(D \subset L^1 = L^1(\mathbb{X}, \Sigma, m)\) be the set of the densities, i.e.

\[ D = \{ f \in L^1 : f \geq 0, \| f \| = 1 \}. \]

**Definition 1** A linear \(D\) preserving mapping \(P : L^1 \rightarrow L^1\) is called a Markov (or stochastic) operator.

**Definition 2** A family \(\{P(t)\}_{t \geq 0}\) of Markov operators, which satisfies the following conditions:

- \(P(0) = \text{Id} \) (identity condition),
- \(P(t+s) = P(t)P(s) \) for \(s, t \geq 0\) \(\) (semigroup condition),
- for each \(f \in L^1\) the function \(t \rightarrow P(t)f\) is continuous with respect to the \(L^1\) norm \(\) (strong continuity),

is called a Markov semigroup.

**Definition 3** A Markov semigroup \(\{P(t)\}_{t \geq 0}\) is partially integral if there exist \(t_0 > 0\) and a measurable function \(k : \mathbb{X} \times \mathbb{X} \rightarrow \mathbb{R}^+\), such that for every \(f \in D\) :

\[
\int_{\mathbb{X}} \int_{\mathbb{X}} k(p,q) m(dp) m(dq) > 0 \tag{15}
\]

and

\[
P(t_0)f(p) \geq \int_{\mathbb{X}} k(p,q) f(q) m(dq). \tag{16}
\]

**Definition 4** A Markov semigroup \(\{P(t)\}_{t \geq 0}\) is asymptotically stable if

- there exists an invariant density for \(\{P(t)\}_{t \geq 0}\), i.e. \(f^* \in D\) such that \(P(t)f^* = f^*\) for all \(t > 0\),
- for every density \(f \in D\) :
\[
\lim_{t \to \infty} \| P(t)f - f^* \| = 0.
\]

(17)

Below we define a property, which is in some sense “opposite” to asymptotic stability, introduced in [18].

**Definition 5** A Markov semigroup is sweeping (or zero-type) with respect to a set \( A \in \Sigma \) if for every \( f \in D \):

\[
\lim_{t \to \infty} \int_A P(t)f(x)m(dx) = 0.
\]

(18)

A precise instruction on how to construct Markov semigroup for PDMP is given by [6]. Using the analogy with the two-dimensional model, we write Fokker-Planck system of equations for the partial densities \( f_0, f_1 \) of the process

\[
\begin{align*}
\frac{\partial f_0}{\partial t} + \frac{\partial}{\partial x_1}(-x_1 f_0) + a \frac{\partial}{\partial x_2} ((x_1 - x_2) f_0) + b \frac{\partial}{\partial x_3} ((x_2 - x_3) f_0) &= q_1 f_1 - q_0 f_0 \\
\frac{\partial f_1}{\partial t} + \frac{\partial}{\partial x_1}((1 - x_1) f_1) + a \frac{\partial}{\partial x_2} ((x_1 - x_2) f_1) + b \frac{\partial}{\partial x_3} ((x_2 - x_3) f_1) &= q_0 f_0 - q_1 f_1,
\end{align*}
\]

(19)

where \( f_0, f_1 \) are the functions defined on \([0, \infty) \times [0, 1]^3\) such that for any Borel set \( \mathcal{B} \subset \mathbb{R}^+ \times \mathbb{R}^+ \times \mathbb{R}^+ \)

\[
\text{Prob}(x(t) \in \mathcal{B}, \gamma(t) = i) = \int \int \int_{\mathcal{B}} f_i(t, x_1, x_2, x_3) \, dx_1 \, dx_2 \, dx_3, \quad i = 0, 1.
\]

(20)

For the reason of the presence of three spatial variables and a wide range of possible jump rates, system (19) is difficult to be solved analytically. However, we will use Markov semigroup \( \{ P(t) \}_{t \geq 0} \) generated by this process to prove that it has stationary density, which is an equilibrium with respect to time evolution of the distributions.

## 3 Results

### 3.1 Asymptotic stability

In this section we present main result of this paper. We consider two particular solutions of the system (2). The first, \( \phi(t) = (\phi_1(t), \phi_2(t), \phi_3(t)) \) is
the solution of (4) with $i = 0$ and the initial condition $(\phi_1(0), \phi_2(0), \phi_3(0)) = (1, 1, 1)$. The second $\psi(t) = (\psi_1(t), \psi_2(t), \psi_3(t))$ is the solution of (2) with $i = 1$ and the initial condition $(\psi_1(0), \psi_2(0), \psi_3(0)) = (0, 0, 0)$. We conclude that $\phi$ and $\psi$ are two solutions of the system (2) with $i = 0$ and $i = 1$, respectively, which join the asymptotically stable points $(0, 0, 0)$ and $(1, 1, 1)$.

We construct the set $A$ in the following way. Let $A_0$ be the surface made of all solutions of the system (2) with $i = 1$, which start from any point lying on $\phi$. This is also the case with $A_1$, being the surface made of all the solutions of the system (2) with $i = 0$, which start from any point lying on $\psi$. We derive algebraic formulas describing $A_0$ as well as $A_1$ in Appendix A. Having done that, we define $A$ as a subset of $[0, 1]^3$, bounded by $A_0$ and $A_1$. In Fig. 3 we show geometric visualisation of $A$. For comparison, in Fig. 8 we portray the sketch of this set, obtained by numerical simulations of the trajectories of the process (see Sec. 3.2).

Now we can formulate the main result of the paper.
Theorem 1 Let $q_i(x) > 0$, $i = 0, 1$ for $x \in [0, 1]^3$. Then, the Markov semi-group $\{P(t)\}_{t \geq 0}$ is asymptotically stable and the support of the invariant density is the set $A = A \times \{0, 1\}$, where $A$ is expressed in the basis $(22)$ by

$$A = \{(x - y + z, x^a - y^a + z^a, x^b - y^b + z^b) : 1 \geq x \geq y \geq z \geq 0\}. \quad (21)$$

A general idea beyond the strict proof of this theorem is provided by [6]. However, the proof in the two-dimensional model is simpler, because all the properties of the attractor are easy to deduce using geometrical arguments. For example, in this case the proof that $A$ is an invariant set for the process follows immediately from the Müller theorem [33] and the communication between states inside $A$ follows from the Darboux property. Since the geometric arguments in the three-dimensional model are not obvious, we need to use precise formula to define the set $A$ and to prove its properties. Namely, we follow [31] and prove that $A$ is a set such that

- $A$ is invariant for the process, i.e. if $(x_1(0), x_2(0), x_3(0), \gamma(0)) \in A$, then $(x_1(t), x_2(t), x_3(t), \gamma(t)) \in A$ for any $t > 0$,
- trajectories $(x_1(t), x_2(t), x_3(t), \gamma(t))$ of the process starting from any arbitrary point from $[0, 1]^3 \times \{0, 1\}$ converge to $A$ when time goes to infinity,
- there is no smaller set satisfying these two conditions above.

Detailed mathematical proofs of these claims are long and provided in Appendix B. In Fig. 4 we show two-dimensional projections of $A$ onto the 2D plane, looking exactly the same as the set proposed by [20].

3.2 Stochastic simulations

Although it is difficult to solve Fokker-Planck equations (19) analytically, here we discuss stochastic simulations of the trajectories and distributions of the system (11), made to check the accuracy of our statements. Such an approach, based on the [12] algorithm, was used by [25] to visualize the evolution of the trajectories in the model of self-renewal cells differentiation. For our model a similar code in Wolfram Mathematica environment was generated and run.

We have compared the trajectories of the system (11) for selected values of the parameters $a, b$ and jump rates $q_0(x_1, x_2, x_3), q_1(x_1, x_2, x_3)$ up to the final
time moment $T = 150$ or at least 1300 jumps were performed. Afterwards, we depicted time evolution of the levels of all three kinds of gene expression products for $a = \frac{1}{2}, b = \frac{1}{3}, q_0 = 3$ and $q_1 = 6$ with the initial condition $x(0) = y(0) = z(0) = \frac{1}{3}$, separately in Fig. 5 and pairwise in Fig. 7 (left). In Fig. 6 and in Fig. 7 (right), where we use the same parameters as in the previous case, we assume that the inactivation rate depends on the protein level, i.e. $q_1(x_1, x_2, x_3) = q_1 x_3$.

We notice that in both cases, as it was expected, fluctuation in pre-mRNA level is much stronger than it is in mRNA or protein level. However, when the jump rates are constant, then all three levels seem to vary in a more limited range than in the case when the jump rates are protein-mediated: the values of standard deviation for consecutive phases are $0.151, 0.0887, 0.0609$ and $0.182, 0.099, 0.0617$, respectively. Moreover, we empirically calculated correlation level between each two phases. While pre-mRNA and mRNA levels (with the values of the coefficient $0.555$ for constant and $0.573$ for protein-mediated jump rates), as well as mRNA and protein ($0.685$ and $0.668$) levels were significantly correlated, pre-mRNA and protein levels were poorly related to each other ($0.07$ and $0.058$).
Fig. 5: Trajectories of the stochastic process (11). The initial condition is $x(0) = \frac{1}{2}, y(0) = \frac{1}{2}, z(0) = \frac{1}{2}, \gamma(0) = 0$ and $a = \frac{1}{2}, b = \frac{1}{3}, q_0 = 3, q_1 = 6$ are set to show the level of pre-mRNA (top), mRNA (center) and protein (bottom).
Fig. 6: Trajectories of the stochastic process (11). The initial condition is $x(0) = \frac{1}{2}, y(0) = \frac{1}{2}, z(0) = \frac{1}{2}, \gamma(0) = 0$ and $a = \frac{1}{2}, b = \frac{1}{3}, q_0 = 3, q_1 = 6 z(t)$ are set to show the level of pre-mRNA (top), mRNA (center) and protein (bottom).
Fig. 7: Two-dimensional trajectories of the stochastic process (11). The initial condition is $x(0) = \frac{1}{2}, y(0) = \frac{1}{2}, z(0) = \frac{1}{2}, \gamma(0) = 0$ and $a = \frac{1}{2}, b = \frac{1}{3}, q_0 = 3$ are set to show the dependence between pairs of the variables, for constant ($q_1 = 6$, left) and protein-mediated ($q_1 = 6x_3$, right) jump rates.
Leaving all parameters unchanged, we analysed the distributions obtained by the simulations of system (11) with constant and linearly dependent inactivation rate function $q_1(x_1, x_2, x_3)$, respectively; see Fig. [9]. To follow the behaviour of a gene, we pictured two-phase marginal distributions, i.e. $\rho(t, x_1, x_2)$, $\rho(t, x_2, x_3)$, $\rho(t, x_1, x_3)$, where $\rho(t, x_k, x_j) = f_0(t, x_k, x_j) + f_1(t, x_k, x_j)$. The graphs were made by simulating the system (11) up to $T_F = 15$, repeated 5000 times and hence it is expected (see also [20]) that the points $(x(T_F), y(T_F), z(T_F), i)$ obtained this way approximate stationary distributions $f_i(x_1, x_2, x_3)$. Fluctuation strength, described by the jump rates, decides about the broadness of $\rho$: stronger fluctuations give the broader distribution. However, on the left hand side of Fig. [9] the jump inactivation rate is twice as large as the jump activation rate, so the inactive state becomes dominant. As a result, the distribution much more significantly points into the zero direction.

In Sec. 2.2 we discussed and justified two specific types of behavior of the process in the deterministic (adiabatic) limit. In the first case it is expected that when $q_0$ and $q_1$ are sufficiently large and $\mathbb{E}\gamma$ is an increasing function of the protein level $x_3$, we should obtain that the stationary density is bimodal, see Fig. [10]. On the other hand, when both of the jump rates are still large, but this time $\mathbb{E}\gamma$ is a decreasing function of the protein level $x_3$, we observe the existence of the limit cycle in the deterministic limit. However, after simulating the process for a sufficiently long time, we should obtain its density distributed close to this limit cycle. A comparison of the distribution.
Fig. 9: Marginal distributions \( \rho(t, x_k, x_j) = f_0(t, x_k, x_j) + f_1(t, x_k, x_j) \) calculated for time \( t = 15 \) with simulations of the system (11) repeated 5000 times for constant (left) and protein-mediated (right) jump rates.
Fig. 10: Marginal bimodal distributions $\rho(t, x_k, x_j) = f_0(t, x_k, x_j) + f_1(t, x_k, x_j)$ calculated for $t = 15$ with simulations of the system \([\text{[11]}]\) with $a = b = 1$ and jump rate functions $q_0(x_3) = 10(0.01 + x_3^2), q_1 = 10 \cdot 0.2$. 
Fig. 11: Marginal distributions $\rho(t, x_k, x_j) = f_0(t, x_k, x_j) + f_1(t, x_k, x_j)$ calculated for $t = 15$ with simulations of the system (11) with $a = b = 1$ and jump rate functions $q_0 = n, q_1(x_3) = 9 \cdot 10^{10} n x_3^{10}$ with $n = 10$. 

22
Fig. 12: The limit cycle for $a = b = 1$ and jump rate functions $q_0^n \equiv n$ and $q_1^n(x_3) = 9 \cdot 10^{10} n x_3^{10}$ in the adiabatic limit.

of the stochastic process (Fig. 11) and its deterministic approximation (Fig. 12) is provided. The latter case pays particular attention, because it is a kind of behavior which is not present in the two-dimensional system from [20].

4 Conclusion

We have studied a model of stochastic gene expression with the contribution of three main phases. Our investigation is based on the two-dimensional model introduced by [20], including: activation of the gene, mRNA transcription and protein translation. Activity of the gene is regulated stochastically, namely by a Piece-wise Deterministic Markov Process, [6]. In eukaryotes, where the transcript is produced in bursts, we can neglect other sources of stochasticity. However, many reports: [9], [22], [35], [36] suggest that at least one additional phase, i.e. pre-mRNA level regulation should be considered as well. This moves the state space of deterministic part of the process into $\mathbb{R}^3$. We have analysed long-time behaviour of densities of the process. Using Markov semigroup techniques, we have shown that its distribution converges to equilibrium, i.e. there exists a stationary density, such that independently on the initial distribution its evolution is being stabilised with this density, when time goes to infinity. Moreover, we have found a set, an “attractor”, which is a support for the equilibrium. Using statistical approach, we visualised the trajectories of the process and approximated stationary distribu-
tions in the cases of constant and protein-mediated jump rates. Moreover, we discussed two specific types of behavior of the process: bistability and the existence of limit cycle trajectory. To summarize, we obtained qualitative and statistical results for the long-time evolution of three-phase kinetics of the eukaryotic gene. We notice that the main result is in agreement with the one from the two-dimensional model. This suggests that a sequence of gene transformations described by equations including stochastic activation and only production and degradation processes, does not have a significant influence on stabilizing long-time distribution of the product levels. However, our analysis has also shown that it is not entirely true that the three-dimensional system has necessarily analogous dynamics to the two-dimensional one studied before: the appearance of the limit cycle in our system, is not possible in the planar model. Thus, larger number of intermediate steps in the case of negative feedback can make the system oscillatory.

Nonetheless, another question is how to formulate more general approach, when there is a need to analyze phases which cannot be described by linear ODEs and how their limit distribution and the behavior of the trajectories will change.

Acknowledgements

We thank P.R. Paździorek for discussion and support. We are also grateful to the reviewers for their invaluable suggestions in improving the paper. This paper was partially supported by the State Committee for Scientific Research (Poland) Grant No. 2014/13/B/ST1/00224 (RR).

Appendix A. Simplifying the system of ODEs and derivation of the formula for the attractor

Let us consider the system (2) with a given value of \( i, i = 0, 1 \). Equivalently we can rewrite it as \( \mathbf{x}' = M\mathbf{x} + c \), where

\[
M = \begin{bmatrix} -1 & 0 & 0 \\ a & -a & 0 \\ 0 & b & -b \end{bmatrix} \quad \text{and} \quad c = \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix}.
\]
We notice that $M$ has three distinct eigenvalues: $-1, -a, -b$ and we can choose the eigenvectors $v_1, v_2, v_3$ in such a way that the vector $1 = [1, 1, 1]$ has also the coordinates $[1, 1, 1]$ in the basis of the eigenvectors. Precisely, the eigenvectors $v_1, v_2, v_3$ are given by the formulas:

$$
\begin{align*}
\mathbf{v}_1 &= \left[ 1, \frac{a}{a-1}, \frac{ab}{(a-1)(b-1)} \right], \\
\mathbf{v}_2 &= \left[ 0, -1, \frac{b}{(a-1)(a-1)} \right], \\
\mathbf{v}_3 &= \left[ 0, 0, 1 - \frac{ab}{(a-1)(b-1)} - \frac{b}{(a-1)(a-b)} \right].
\end{align*}
$$

We transform (4) by rewriting it in the new basis and we obtain new formulas for the system (4):

$$
\begin{align*}
\frac{dx_1}{dt} &= -x_1 + i \\
\frac{dx_2}{dt} &= -ax_2 + ai \\
\frac{dx_3}{dt} &= -bx_3 + bi
\end{align*}
$$

for $i = 0, 1$. Let $\mathbf{x} = [x_1, x_2, x_3]^T$ be a column vector and let $\pi_i^t(\mathbf{x})$ denote the solution of (2) at time $t$ with the initial condition $\mathbf{x}$. We get

$$
\pi_i^0(\mathbf{x}) = (e^{-tx_1}, e^{-at}x_2, e^{-bt}x_3)
$$

and

$$
\pi_i^1(\mathbf{x}) = 1 + \pi_i^0(\mathbf{x}) - \pi_i^0(1),
$$

where $1$ denotes now a column vector $[1, 1, 1]^T$. By alternate compositing of these functions we have the formulas

$$
\pi_{t_2}^0 \pi_{t_1}^0(\mathbf{x}) = 1 + \pi_{t_1+t_2}^0(\mathbf{x}) - \pi_{t_2}^0 1
$$

as well

$$
\pi_{t_2}^0 \pi_{t_1}^1(\mathbf{x}) = \pi_{t_2}^0 1 + \pi_{t_1+t_2}^0(\mathbf{x}) - \pi_{t_1+t_2}^0 1
$$

for any $t_1, t_2 > 0$. Now, substituting $e^{-t} := \alpha$, $e^{-(t_1+t_2)} := \beta$ we get

$$
\pi_{t_2}^0 \pi_{t_1}^1(\mathbf{x}) = (\alpha + \beta(x_1 - 1), \alpha^a + \beta^a(x_2 - 1), \alpha^b + \beta^b(x_3 - 1)),
$$

and

$$
\pi_{t_2}^1 \pi_{t_1}^0(\mathbf{x}) = (1 - \alpha + \beta x_1, 1 - \alpha^a + \beta^a x_2, 1 - \alpha^b + \beta^b x_3)
$$
where \(1 \geq \alpha \geq \beta \geq 0\). Taking as the initial points \(x = (0, 0, 0)\) in the formula [28] and \(x = (1, 1, 1)\) in the formula [29], we get parametric equations for the surfaces \(A_0\) and \(A_1\) which are the boundaries of \(A\) (see Sec. 3):

\[
A_0 = \{(\alpha - \beta, \alpha^a - \beta^a, \alpha^b - \beta^b) : 1 \geq \alpha \geq \beta \geq 0\},
\]

\[
A_1 = \{(1 - \alpha + \beta, 1 - \alpha^a + \beta^a, 1 - \alpha^b + \beta^b) : 1 \geq \alpha \geq \beta \geq 0\}.
\]

Similarly:

\[
\pi_{t_3}^0 \pi_{t_2}^1 \pi_{t_1}^0(x) = (\alpha - \beta + \gamma x_1, \alpha^a - \beta^a + \gamma x_2, \alpha^b - \beta^b + \gamma x_3),
\]

\[
\pi_{t_3}^1 \pi_{t_2}^0 \pi_{t_1}^1(x) = (1 - \alpha + \beta + \gamma (x_1 - 1), 1 - \alpha^a + \beta^a + \gamma (x_2 - 1), 1 - \alpha^b + \beta^b + \gamma (x_3 - 1)),
\]

where \(1 \geq \alpha \geq \beta \geq \gamma \geq 0\).

Now, let \(V = \{(x, y, z) : 0 < z < y < x < 1\}\) and we define the function \(f : V \rightarrow \mathbb{R}^3\) by the formula

\[
f(x, y, z) = (x - y + z, x^a - y^a + z^a, x^b - y^b + z^b). \quad (30)
\]

It is easy to check that \(f\) is a local diffeomorphism. In particular,

\[
f(V) = \{x = \pi_{t_3}^0 \pi_{t_2}^1 \pi_{t_1}^0 1 : t_1 > 0, t_2 > 0, t_3 > 0\} \quad (31)
\]

is an open set and \(f(V)\) is the interior of \(A\). Hence

\[
A = \{(x - y + z, x^a - y^a + z^a, x^b - y^b + z^b) : 1 \geq x \geq y \geq z \geq 0\} \quad (32)
\]

and \(A_0 \cup A_1\) are indeed the boundaries of \(A\). Moreover \(A_1\) is the symmetrical image of \(A_0\) (and vice versa) with respect to a point \((\frac{1}{2}, \frac{1}{2}, \frac{1}{2})\). From this property we have the equivalent definition of \(A\), i.e.:

\[
A = \{(1 - (x - y + z), 1 - (x^a - y^a + z^a), 1 - (x^b - y^b + z^b) : 1 \geq x \geq y \geq z \geq 0\} \quad (33)
\]

**Appendix B. The proof of asymptotic stability**

Now we will prove the main result of this paper. We use the following theorem.
Theorem 2  Let $X$ be a compact metric space and $\Sigma$ be the Borel $\sigma$–algebra. If a Markov semigroup $\{P(t)\}_{t \geq 0}$ satisfies two conditions:

(a) for every density $f$ we have $\int_0^\infty P(t)f \, dt > 0$ a.e.,

(b) for every $q_0 \in X$ there exist $\kappa > 0$, $t > 0$ and a measurable function $\eta \geq 0$ such that $\int \eta(p) \, m(dp) > 0$ and

$$P(t)f(p) \geq \eta(p) \int_{B(q_0,\kappa)} f(q) \, m(dq),$$

for $p \in X$, where $B(q_0,\kappa)$ is the open ball with center $q_0$ and radius $\kappa$,

then the semigroup $\{P(t)\}_{t \geq 0}$ is asymptotically stable.

Theorem 2 was formulated as Corollary 1 in [6] and follows from two earlier results:

Theorem 3  If $\{P(t)\}_{t \geq 0}$ is a partially integral Markov semigroup and has a unique invariant density $f_\ast > 0$, then the semigroup $\{P(t)\}_{t \geq 0}$ is asymptotically stable.

Theorem 4  If $\{P(t)\}_{t \geq 0}$ is a Markov semigroup on a metric space, satisfies (a) and (b), and has no invariant density, then it is sweeping from compact sets.

From Theorem 4 it follows that if the space $X$ is compact and the semigroup satisfies (a) and (b), then it has a unique and positive invariant density. Now Theorem 2 follows immediately from Theorem 3.

Hence, the idea of the proof is as follows. First, we check that all trajectories enter the set $\mathbb{A}$ and this set is invariant. This allows us to reduce the proof of asymptotic stability only to the semigroup $\{P(t)\}_{t \geq 0}$ restricted to the set $\mathbb{A}$. Then we check that conditions (a) and (b) are satisfied on $\mathbb{A}$. Since $\mathbb{A}$ is compact the semigroup $\{P(t)\}_{t \geq 0}$ is asymptotically stable.

Firstly, we introduce some necessary definitions.

Definition 6  Let $V(M)$ be the set of real smooth vector fields on the manifold $M$ on $\mathbb{R}^d$ and let $C^\infty(M)$ denote the set of a real-valued smooth functions on $V(M)$. A Lie bracket of two vector fields $a, b \in V(M)$ is a vector field given by the formula:

$$[a, b]_j(x) = \sum_{k=1}^d \left( a_k \frac{\partial b_j}{\partial x_k}(x) - b_k \frac{\partial a_j}{\partial x_k}(x) \right).$$
Definition 7 Let a PDMP be defined by the systems of differential equations $x' = g_i(x)$, $i \in I = \{0, 1, ..., k\}$, $k \in \mathbb{N}$. We say that the Hörmander’s condition holds at a point $x$ if vectors

$$g_2(x) - g_1(x), \ldots, g_k(x) - g_1(x), [g_i, g_j](x)_{1 \leq i, j \leq k}, [g_i, [g_j, g_l]](x)_{1 \leq i, j, l \leq k}, \ldots$$

span the space $\mathbb{R}^d$.

Definition 8 Let $n \in \mathbb{N}$, $t > 0$, $\tau = (\tau_1, \tau_2, \ldots, t - \tau_{n-1} - \cdots - \tau_1)$ and $i = (i_1, \ldots, i_n)$ such that for all $k \in \{1, \ldots, n-1\}$ we have $\tau_k > 0$, $i_k \neq i_{k+1}$ and $i_k \in \{0, 1\}$. A function

$$\psi_{x,t,i}(\tau) := \pi_i^{\tau_n - \tau_{n-1} - \cdots - \tau_1} \circ \pi_{i_{n-1}}^{\tau_{n-1} - \cdots - \tau_2} \circ \cdots \circ \pi_{i_1}^{\tau_1}$$

is called a cumulative flow along the trajectories of the flows $\pi_i^{\tau_1}, \ldots, \pi_i^{\tau_n}$ with starting point $x$.

Definition 9 We say that a point $x \in X$ communicates with $y \in X$ if there exist $n \in \mathbb{N}$, $t > 0$, $\tau = (\tau_1, \tau_2, \ldots, t - \tau_{n-1} - \cdots - \tau_1)$ and $i = (i_1, \ldots, i_n)$ such that $\psi_{x,t,i}(\tau) = y$.

If every two points from the interior of $X$ communicate, we call this property communication between states of the process. If for $q_0 \in X$ there exists $p \in X$ such that $q_0$ communicates with $p$ and the Hörmander’s condition holds at the point $p$, then $q_0$ satisfies condition (b). This fact is a simple consequence of [1, Theorem 4].

Let us denote by $a_i(x)$ a vector field representing the system (2) with a fixed value of $i \in \{0, 1\}$ at a point $x \in [0, 1]^3$. After short calculation of the following expressions:

$$a_1 - a_0 = (1, 0, 0), [a_0, a_1] = (1, -a, 0), [a_0, [a_0, a_1]] = (1, -(a^2 + a), ab);$$

we obtain three linear independent vectors in $\mathbb{R}^3$ space. Hence, these vectors span $\mathbb{R}^3$ and condition (b) of Theorem holds. However, it gets more difficult to check condition (a), because it does not hold on the whole space $[0, 1]^3 \times \{0, 1\}$. We will prove that (a) holds on $A$. Moreover, $A$ is a stochastic attractor, i.e. a measurable subset of $[0, 1]^3$ such that for every density $f \in L^1(A)$ we have

$$\lim_{t \to \infty} \int_A P(t)f(x, i) \, dx \, di = \lim_{t \to \infty} \mathbb{P}(\zeta(t) \in A) = 1. \quad (34)$$

28
First, we show that $A$ is an invariant set for the process. It follows from the fact that if we take any $\mathbf{x} \in A$, then we stay in $A$ under the action of both semi-flows given by (2) with the initial condition $\mathbf{x}$. In other words, we check that if we take any $\mathbf{x} \in A$ and $t > 0$, then both $\pi^t_0(\mathbf{x})$ and $\pi^t_1(\mathbf{x})$ stay in $A$. Since the process switches between these two flows, its trajectories cannot leave $A$.

Let us define the set:

$$D = \{(x-y+z-w, x^a-y^a+z^a-w^a, x^b-y^b+z^b-w^b) : 1 \geq x \geq y \geq z \geq w \geq 0\}.$$

We prove that $D$ is the same set as $A$. For $s \in \{1, a, b\}$ and any

$$d_0 = (x_0 - y_0 + z_0 - w_0, x_0^a - y_0^a + z_0^a - w_0^a, x_0^b - y_0^b + z_0^b - w_0^b) \in D$$

we have

$$\begin{align*}
x_0^s - y_0^s + z_0^s - w_0^s &= x_0^s \left(1 - \frac{y_0^s}{x_0^s} + \frac{z_0^s}{x_0^s} - \frac{w_0^s}{x_0^s}\right) = x_0^s(x_0^s - y_0^s + z_0^s) \\
&= (x_0 x_0')^s - (x_0 y_0')^s + (x_0 z_0')^s,
\end{align*}$$

where the second equality follows from the equivalence of two definitions of the set $A$ (see [33]). Therefore, $A = D$. Since it is easy to check that for any $t > 0$ and $i \in \{0, 1\}$ we have $\pi^i_t(A) \subset D$, $A$ is an invariant set for our process.

Using the formula [31], there exist $t^0_1 > 0$, $t^0_2 > 0$, and $t^0_3 > 0$ such that we have $\pi^0_{t^0_1}, \pi^1_{t^0_2}, \pi^1_{t^0_3} \mathbf{1} = (\frac{1}{2}, \frac{1}{2}, \frac{1}{2})$. From continuous dependence of the solutions on the initial condition, we can find $\delta > 0$ and $\varepsilon > 0$ such that for every $\mathbf{y} \in \mathbb{R}^3$ with $\|\mathbf{y} - \mathbf{1}\| < \delta$ and $t = (t_1, t_2, t_3)$ with $|t_i - t^0_i| < \varepsilon$ for $i = 1, 2, 3$ we have $\pi_{t^0_i} \pi_{t^0_2} \pi_{t^0_3} (\mathbf{y}) \in \text{Int} A$. Moreover, there exists $t_0 > 0$ such that $\|\pi^1_1(\mathbf{x}) - \mathbf{1}\| < \delta$ for each point $\mathbf{x} \in [0, 1]^3$ and $t > t_0$. Thus $\pi^0_{t_5} \pi^1_{t_2} \pi^1_{t_3} \pi^1_{t_0}(\mathbf{x}) \in \text{Int} A$ if $\mathbf{x} \in [0, 1]^3$ and $|t_i - t^0_i| < \varepsilon$ for $i = 1, 2, 3$ and $t > t_0$. The probability that the sequence of the consecutive five jump moments $T_n, \ldots, T_{n+4}$ has the properties: $T_{n+1} - T_n > t_0$ and $T_{n+i+1} - T_{n+i} \in (t^0_i - \varepsilon, t^0_i + \varepsilon)$ for $i = 1, 2, 3$; is bounded from below (see [6]) by some positive number $\eta > 0$. Since our PDMP enters the cube $[0, 1]^3$ (see the discussion under the formula [6]) we have also that it enters the interior of the attractor with probability one, which completes the proof of [34].

To prove the remaining condition (a), we use the fact that it is equivalent to communication between states for $\mathbf{x}, \mathbf{y} \in \text{Int} A$ and fixed $i \in \{0, 1\}$. In
other words, we show that the cumulative flow consisting the flows of \( \pi^0 \) and \( \pi^1 \) with the initial condition \( x \in \text{int} \ A \) generates whole \( A \). The question to face is: does the total control of the system (11) between two arbitrary points in the interior of \( A \) exist? The problem of control for linear dynamical systems has been extensively studied in the past (\[7\], \[17\]), but this special case appears to be relatively far from classical results of the controllability theory and seems to not undergo any of those procedures. However, the proof of the communication between states property in this special case is surprisingly simple. Due to symmetry of \( A \), we consider only these cumulative flows which begin from \( \pi^0 \). The case when we start from \( \pi^1 \) is analogous.

Fix a point \( x \in A \). After compositing four transformations we obtain

\[
\psi_{x,t,0} := \pi^1_{t_4} \pi^0_{t_3} \pi^1_{t_2} \pi^0_{t_1}(x) = 1 - \pi^0_{t_4} 1 + \pi^0_{t_3+t_4} 1 - \pi^0_{t_2+t_3+t_4} 1 + \pi^0_{t_1+t_2+t_3+t_4}(x).
\]

Fix \( \varepsilon > 0 \) and take any points \( x, y, z \) such that \( \varepsilon \leq z \leq y \leq x \leq 1 \). Then we can find \( t_i \geq 0 \), for \( i = 1, 2, 3, 4 \), such that \( x = e^{-t_4} \), \( y = e^{-t_3-t_4} \), \( z = e^{-t_2-t_3-t_4} \), and \( \varepsilon = e^{-t_1} z \). Thus

\[
\psi_{x,t,0} = (1 - x + y - z + \varepsilon x_1, 1 - x^a + y^a - z^a + \varepsilon^a x_2, 1 - x^b + y^b - z^b + \varepsilon^b x_3).
\]

Let

\[
A_\varepsilon = \{(1 - x + y - z, 1 - x^a + y^a - z^a, 1 - x^b + y^b - z^b): \varepsilon \leq z \leq y \leq x \leq 1\}
\]

and \( v_\varepsilon = (\varepsilon x_1, \varepsilon^a x_2, \varepsilon^b x_3) \). Then, starting from the point \( x \) and using a composition of four transformations we communicate with each point from the set

\[
A_\varepsilon + v_\varepsilon := \{y + v_\varepsilon: y \in A_\varepsilon\}.
\]

Since \( \text{Int} \ A \subset \bigcup_{\varepsilon > 0} A_\varepsilon \), we conclude that we can join \( x \) and any interior point of \( A \) by \( \psi_{x,t,0} \) (note that this property may not hold for the boundary points of \( A \)). As a result, condition (a) from Theorem 2 is satisfied and the semigroup \( \{P(t)\}_{t \geq 0} \) is asymptotically stable.

**Remark 3.** The proof in the general case (i.e. including the subcases \( a = b, a = 1 \) and \( b = 1 \)) is similar to the presented above, but technically more difficult. We do not change the variables in the system (2) (see Appendix A), but we define the attractor for the process as the closure of the set:

\[
A_1 = \{x = \pi^0_{s_1} 1 - \pi^0_{s_2} 1 + \pi^0_{s_3} 1: 0 \leq s_1 \leq s_2 \leq s_3\} \quad (35)
\]
or equivalently as the closure of the set:

\[ A_2 = \{ x = 1 - \pi_{s_1}^0 1 + \pi_{s_2}^0 1 - \pi_{s_3}^0 1 : 0 \leq s_1 \leq s_2 \leq s_3 \}. \]  

We have \( \text{cl} \ A_1 = \text{cl} \ A_2 \), because both sets \( A_1 \) and \( A_2 \) have the same boundaries:

\[ \text{cl} \{ x = \pi_{s_1}^0 1 - \pi_{s_2}^0 1 : 0 \leq s_1 \leq s_2 \} \cup \text{cl} \{ x = 1 - \pi_{s_1}^0 1 + \pi_{s_2}^0 1 : 0 \leq s_1 \leq s_2 \}. \]

Having these formulas for the attractor one can prove that indeed this set is an attractor and that any two points in the interior of the attractor can communicate.

References

[1] Y Bakhtin and T Hurth. Invariant densities for dynamical systems with random switching. *Nonlinearity*, 25:2937–2952, 2012.

[2] M Benaïm, S Le Borgne, F Malrieu, and P A Zitt. Quantitative ergodicity for some switched dynamical systems. *Electron. Comm. Probab.*, 17(56):1–14, 2012.

[3] M Benaïm, S Le Borgne, F Malrieu, and P A Zitt. Qualitative properties of certain piecewise deterministic Markov processes. *Ann. Inst. H. Poincaré Probab. Statist.*, 51(3):1040–1075, 2015.

[4] W.J Blake, M Kaern, C.R Cantor, and J.J Collins. Noise in eucaryotic gene expression. *Nature*, 422:633–637, 2003.

[5] A Bobrowski. Degenerate Convergence of Semigroups Related to a Model of Stochastic Gene Expression. *J. Math. Anal. Appl.*, 73(3):345–366, 2006.

[6] A Bobrowski, K Pichór, T Lipniacki, and R Rudnicki. Asymptotic behavior of distribution of mRNA and protein levels in a model of stochastic gene expression. *J. Math. Anal. Appl.*, 333:753–769, 2007.

[7] F Colonius and W Kliemann. *The Dynamics of Control*. Springer Science & Business Media, New York, 2000.
[8] A Crudu, A Debussche, A Muller, and O Radulescu. Convergence of stochastic gene networks to hybrid piecewise deterministic processes. *Ann. Appl. Probab.*, 22(5):1822–1859, 2012.

[9] P Cui, S Zhang, F Ding, S Ali, and L Xiong. Dynamic regulation of genome-wide pre-mRNA splicing and stress tolerance by the Sm-like protein LSm5 in Arabidopsis. *Genome Biol.*, 15:R1, 2014.

[10] M.H.A Davis. Piece-wise deterministic Markov processes: a general class of non-diffusion stochastic processes. *J.R Stat. Soc. B*, 46:353–388, 1984.

[11] N Friedman, Long Cai, and X. S Xie. Linking stochastic dynamics to population distribution: An analytical framework of gene expression. *Phys. Rev. Lett.*, 97:168302, 2006.

[12] D.T Gillespie. Exact stochastic simulation of coupled chemical reactions. *J. Phys. Chem.*, 81(25):2340–2361, 1977.

[13] B. C Goodwin. Oscillatory behavior in enzymatic control processes. *Adv. Enzyme Regul.*, 3:425–438, 1965.

[14] J Jaruszewicz, P.J Zuk, and T Lipniacki. Type of noise defines global attractors in bistable molecular regulatory systems. *J. Theor. Biol.*, 317:140–151, 2013.

[15] T.B Kepler and T.C Elston. Stochasticity in transcriptional regulation: origins, consequences, and mathematical representations. *Biophys. J.*, 81:3116–3136, 2001.

[16] J.K Kim and J.C Marioni. Inferring the kinetics of stochastic gene expression from single-cell RNA-sequencing data. *Genome Biol.*, 14:R7, 2013.

[17] J Klamka. Controllability of dynamical systems - a survey. *Arch.Contr.Sci.*, 2:281–307, 1993.

[18] T Komorowski and J Tyrcha. Asymptotic properties of some Markov operators. *Bull. Polish Acad. Sci. Math.*, 43:221–228, 1989.

[19] A Lasota and M.C Mackey. *Chaos, Fractals and Noise. Stochastic Aspects of Dynamics*. Springer, New York, 1994.
[20] T Lipniacki, P Paszek, A Marciniak-Czochra, A R Brasier, and M Kimmel. Transcriptional stochasticity in gene expression. *J. Theor. Biol.*, 238:348–367, 2006.

[21] T Lipniacki, K Pruszynski, P Paszek, A R Brasier, and M Kimmel. Single TNFα trimers mediating NF-κB activation: stochastic robustness of NF-κ signalling. *BMC Bioinformatics*, 8:376, 2007.

[22] H Lodish, A Berk, C.A Kaiser, M Krieger, A Bretscher, H Ploegh, A Amon, and M P. Scott. *Molecular Cell Biology*. Freeman, W. H. and Company, seventh edition, 2012.

[23] F Malrieu. Some simple but challenging Markov processes. *Ann. Fac. Sci. Toulouse Math. accepted*, 2014.

[24] T Maniatis and R Reed. An extensive network of coupling among gene expression machines. *Nature*, 416:499–506, 2002.

[25] P Paździorek. A stochastic perturbation of the fraction of self-renewal in the model of stem cells differentiation. *Math Methods Appl Sci.*, submitted, 2013.

[26] J Peccoud and B Ycart. Markovian modeling of gene-product synthesis. *Theor Popul Biol*, 48(2):222–234, 1995.

[27] J.M Pedraza and J Paulsson. Effects of molecular memory and bursting on fluctuations in gene expression. *Science*, 319:339–343, 2008.

[28] K Pichór and R Rudnicki. Continuous markov semigroups and stability of transport equations. *J. Math. Anal. Appl.*, 249:668–685, 2000.

[29] R Rudnicki. On asymptotic stability and sweeping for Markov operators. *Bull. Polish Acad. Sci. Math.*, 43:245–262, 1995.

[30] R Rudnicki. Markov operators: Applications to diffusion processes and population dynamics. *Appl. Math.*, 27,1:67–238, 2000.

[31] R Rudnicki and M Tyran. Piecewise deterministic markov process in biological models. *Springer Proceedings in Mathematics and Statistics: Semigroups of Operators - Theory and Applications*, 113:235–255, 2015.
[32] A Tomski. The dynamics of enzyme inhibition controlled by piece-wise deterministic markov process. *Springer Proceedings in Mathematics and Statistics: Semigroups of Operators - Theory and Applications*, 113:299–316, 2015.

[33] W Walter. *Differential and Integral Inequalities*. Springer, New York, 1970.

[34] Y. Wang, Y. Hori, S. Hara, and F. J. Doyle III. Collective oscillation period of inter-coupled biological negative cyclic feedback oscillators. *IEEE Transactions on Automatic Control*, 60(5):1392–1397, 2015.

[35] J.D Watson, T.A Baker, S.P Bell, A Gann, M Levine, and R Losick. *Molecular Biology of the Gene*. Benjamin Cummings, seventh edition, 2013.

[36] K Yap and E.V Makeyev. Regulation of gene expression in mammalian nervous system through alternative pre-mRNA splicing coupled with RNA quality control mechanisms. *Mol Cell Neurosci.*, 56:420–428, 2013.