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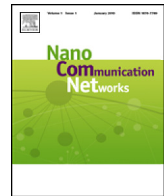

Real-time observation of bacterial gene expression noise

Olga Anufrieva, Adrien Sala, Olli Yli-Harja, Meenakshisundaram Kandhavelu *

Molecular Signaling Lab, Computational Systems Biology Research Group, Signal Processing Department, Tampere University of Technology, P.O.Box 553, 33101, Tampere, Finland

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ABSTRACT

The mRNA variability in the single cells attributes to random periods of transcription bursts. The cell cycle possesses a global role in affecting transcriptional output. By expressing this process as a communication system at the nanoscale, this transcription output often accounts for the gene expression noise, and this quantifies the promoter activity. However, current stochastic models ignore the fact that gene expression noise is affected not only by the cell cycle and promoter activity, but, also, by the time during which new mRNAs are produced. Here we show that noise from the *lac* promoter in *E.coli* is dominated by intrinsic and extrinsic noises in slow and fast dividing cells, respectively. Such domination of the noise components in phases with different generation times is achieved through distinct and combinatorial interactions between the upstream/downstream regulatory elements. These mechanisms serve as an adaptive strategy for increasing population homogeneity under changing environments.

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1. Introduction

Inter and intracellular communications are essential for the cell survival. This communication process at the nanoscale are quite often disrupted by the external and internal environmental condition of the cell. In recent years, numerous research have been conducted at modeling nano communication systems. From an artificial communication system perspective, research has been conducted on utilizing bacteria as information carrier between different nanomachines [1]. However, from the perspective of natural communication at the nanoscale, considerable effort has also gone into understanding how cells perform communication to achieve specific functionalities. An example of natural inter cellular nano communication process is quorum sensing which is influenced by the external environmental where the bacterial gene expression is synchronized to produce signaling molecules [2]. This process occurs where synthesized molecules are shared between the cells to transfer the signals. If the concentration of these molecules is decreased beyond the threshold, cells start to produce more signaling molecule using intracellular gene regulatory mechanism [3]. However, in this paper we focus on intracellular communication process, and this is regulated by signaling proteins. Lac operon is one of well-studied gene expression system in the last decades, regulated by LacI protein, 4.9 nanometer (nM) in size [4,5]. Binding and unbinding rate of this nanosized molecule regulate the frequency of the gene expression to determine the intracellular communication network [6,7]. The concentration of protein numbers is determined by mRNA numbers (Fig. 1) [8,9]. Hence, we aimed to quantify the mRNA number variation of the lac promoter to understand the role of the nanosized molecules, in a single cell.

Natural to all communication systems, there will exist noise that result from variability in the environment. At the single cell level, mRNA number of regulable genes is often highly variable. The resulting long-tailed mRNA distribution is well-described by the transcriptional bursting, where promoter undergoes random periods of high

* Corresponding author.
E-mail address: meenakshisundaram.kandhavelu@tut.fi (M. Kandhavelu).

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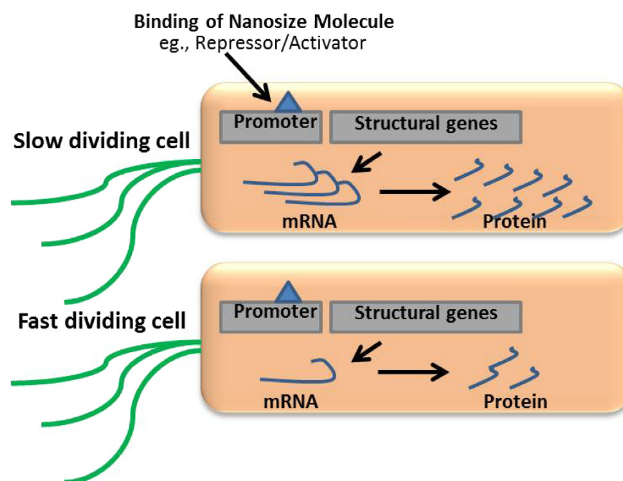

Fig. 1. Molecular communication: Schematic diagram represents the fluctuation in mRNA and protein numbers in fast and slow dividing cells.

activity. Resulting cellular diversity accounts for the stochastic gene expression that is often quantified by the cell-to-cell variability or total noise [10–13]. The noise, in both prokaryotes and eukaryotes, is inherently stochastic. Stochasticity of gene expression is controlled by cells and, thus, must be included into the models describing genetic networks. Usually, these models include intrinsic and extrinsic components for quantification of the gene expression noise. An intrinsic component arises from frequency of the transcription factor binding and unbinding, mRNA partitioning, mRNA degradation and transcription initiation. An extrinsic component originates from the rate of the repressor binding, variability in RNAP copy numbers and gene copy number. Fluctuation of intrinsic and extrinsic components depends on the cell cycle [14]. However, understanding the noise emanating from gene expression has not been investigated through time and phases of cell development.

Here we focus on describing fluctuation of both noise components over the time of bacterial cell phases. For this, we propose a general, although approximate, approach for estimating the gene expression in *lac* promoter of *E. coli* cells. An activity of *lac* promoter is generalized by the synthetic—*lac-ara*1 promoter. The activities of both promoters are measured by the expression of GFP signal per single cell, using MS2-GFP method. The GFP signal is converted into the mRNA number per single cell [15]. Based on previous approaches, we use time-averaging for the evaluation of orthogonal contributions of both noise components to the cell-to-cell variability [4,6,16]. Though, an approach has been initially established by the Sala et al. [15], we extend these observations by evaluating gene expression noise over the time of *E.coli* growth phases. In addition to this, we examine how independent induction (activation of the promoter either from the upstream promoter region or downstream promoter region), affects the gene expression of *lac* promoter.

Observation of upstream/downstream promoter region mediated stochastic gene expression in the bacterial cells, over the time, in different cell growth phases is not well established. They allow for predicting how the cell population keeps the stability by modulating levels of the cell-to-cell variability and both noise. Modulation of the gene expression noise, described over the time of *E.coli* growth of the phases, expands our understanding about the bacterial cellular activity. In perspective, these results could be compared with the level of stochastic gene expression at translation, over time, to describe how bacteria behave during the cell cycle, under various experimental conditions.

2. Materials and methods

2.1. Plasmids, strains and media

To visualize the production of single mRNA molecules and its variation in a population of cell, standard molecular techniques are applied. Specifically, RNA–protein interaction method, MS2-GFP, was used to observe mRNA statistics. We used, Dh5α-PRO (*E. coli K*12) strain in order to study gene expression over the phases. We use two constructed strains to investigate the production of mRNAs (Fig. S1). In detail, one of the constructed strains is co-transformed with two plasmids. The first plasmid is single copy bacterial artificial chromosome, pTRUEBLUE-BAC2 (resistant to Chloramphenicol) tagged with 96 target binding sites (96xbs) for MS2 protein [5]. It is controlled by wild-type *lac* promoter with upstream Catabolic Activator Protein-CAP site for the binding downstream CAP and Operator 1 site for the binding of nanosized molecule binding, *lac* repressor (generously provided by Prof., Ido Golding, University of Illinois). Cyclic AMP (cAMP) and Isopropyl-D-1-thiogalactopyranoside (IPTG) are used to induce CAP and *Lac* repressor, respectively. The second plasmid is, medium-copy reporter, pPROTET.E (resistant to Kanamycin). It expresses bacteriophage MS2 coat protein, which is tagged with fluorescent protein coding gene (GFPmut3), under the control of $P_{LtetO-1}$.

Another strain used to study mRNA production also consists of two plasmids. The reporter system is identical to the first approach discussed above. The target plasmid expressing mRFP1 gene and expresses 96xbs for MS2

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