ELSEVIER

Institut Pasteur

Research in Microbiology xx (2016) 1–11

www.elsevier.com/locate/resmic

Original article

Phase-dependent dynamics of the *lac* promoter under nutrient stress

Anisha Viswanathan [a], Olga Anufrieva [a], Adrien Sala [a], Olli Yli-Harja [a,b], Meenakshisundaram Kandhavelu [a,*]

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

[b] *Institute for Systems Biology, 1441N 34th Street, Seattle, WA, 98103-8904, USA*

Received 23 January 2016; accepted 4 April 2016

Available online ■ ■ ■

Abstract

To survive, a bacterial population must sense nutrient availability and adjust its growth phase accordingly. Few studies have quantitatively analyzed the single-cell behavior of stress and growth phase-related transcriptional changes in *Escherichia coli*. To investigate the dynamic changes in transcription during different growth phases and starvation, we analyzed the single-cell transcriptional dynamics of the *E. coli lac* promoter. Cells were grown under different starvation conditions, including glucose, magnesium, phosphate and thiamine limitations, and transcription dynamics was quantified using a single RNA detection method at different phases. Differences in gene expression over conditions and phases indicate that stochasticity in transcription dynamics is directly connected to cell phase and availability of nutrients. Except for glucose, the pattern of transcription dynamics under all starvation conditions appears to be similar. Transcriptional bursts were more prominent in lag and stationary phase cells starved for energy sources. Identical behavior was observed in exponential phase cells starved for phosphate and thiamine. Noise measurements under all nutrient exhaustion conditions indicate that intrinsic noise is higher than extrinsic noise. Our results, obtained in a *relA*1 mutational background, which led to suboptimal production of ppGpp, suggest that the single-cell transcriptional changes we observed were largely ppGpp-independent. Taken together, we propose that, under different starvation conditions, cells are able to decrease the trend in cell-to-cell variability in transcription as a common means of adaptation.

Keywords: Nutrient stress; Single-cell analysis; mRNA variation; Cell phase; Signal processing

1. Introduction

Bacterial cells are constantly exposed to varying environmental changes wherein essential nutrients, including carbon, nitrogen, phosphorus, vitamins or magnesium, are depleted individually or in combination. Growth transitions associated with various nutrient deprivations can have a great impact on microbial physiology and stress response [1]. One of the main strategies used by bacteria during nutrient deprivation is modulation of its transcriptional program [1]. A series of events, resulting in induction of selective genes and shutdown of most of the genes, favors survival under starvation conditions. A thorough investigation of the means by which bacteria deal with stressful environments is necessary from both clinical and industrial viewpoints.

Gene expression dynamics in *Escherichia coli* are mainly controlled at the level of transcription [2]. Within this multistep process of transcription, the initiation step controls RNA turnover [3–6]. Current models suggest that the dynamics of RNA production follows a sub-Poissonian fit for two or more steps, which are then exponentially distributed in duration [6,7]. Moreover, the kinetics of transcription are modulated by several factors including regulatory molecules [8,9], environmental cues [10], and promoter sequences [11].

Investigation of single-cell response tactics, consistent with physiological needs, is of high significance, as characterized

* Corresponding author. Tel.: +358 417488772.
E-mail address: meenakshisundaram.kandhavelu@tut.fi (M. Kandhavelu).

http://dx.doi.org/10.1016/j.resmic.2016.04.002
0923-2508/

by distinct transcriptional mechanisms and high levels of noise in their expression patterns. In unstable environments, genotypes that display higher adaptive gene expression variation are suggested to exhibit more survival potential than others [12–14]. Even under stable environmental conditions, single cells can show variant phenotypes, including differences in enzymatic and transporter functions. Such changes are observed mainly due to the stochastic nature of gene expression [15–20].

In stressful environments, transcriptional noise can be favorable for bacterial adaptation. By acting as a 'bet-hedging strategy', transcriptional noise increases the survival potential of a subpopulation of cells [24]. This has been largely attributed to the phenomenon of 'persistence' where a selective subpopulation of bacteria, called persisters, tolerate antibiotic exposure mainly due to phenotypic changes that result in an inactive state of growth [21]. Transcriptional noise arises due to intrinsic factors, including inherent stochasticity of transcription and extrinsic factors, such as unequal partitioning of transcription factors and regulatory molecules during cell division [22]. Studies have reported that changes in the transcription level as a result of stress-induced growth arrest significantly correlate with changes observed when cell growth ceases during the stationary phase [23,24]. A central molecule that modifies the transcriptional program, both during stationary phase as well as during stress-induced growth arrest, is an unusual guanine analog, GDP 3′-diphosphate or GTP 3′-diphosphate, collectively called (p)ppGpp. In *E. coli*, (p)ppGpp is synthesized mainly by RelA, while another enzyme, SpoT, has mild (p)ppGpp-synthase activity, but strong degradation effects [25]. During nutrient deprivation, particularly when amino acids are starved, RelA is activated by stalled ribosomes resulting in induction of (p)ppGpp synthesis. (p)ppGpp directly binds to RNA polymerase and destabilizes the short-lived open complexes that are formed in certain promoters, including rRNA genes. Under conditions of stress, DksA acts as an important transcription factor that co-operates with (p)ppGpp for transcriptional programming [26]. Thus, during nutrient depletion conditions, both (p)ppGpp and DksA modify the transcriptional program, resulting in induction of stress-tolerant and amino acid synthetic genes while shutting down rRNA synthesis [26–28]. Recent studies indicate that stochastic induction of (p)ppGpp in subpopulations of cells is a main factor that contributes to gene expression heterogeneity in a population [29,30]. While significant understanding has been gained on transcriptional reprogramming that occurs during nutrient deprivation, only a few quantitative studies have tried to elucidate the dynamics of transcriptional changes under different stress conditions.

In this study, we explored how transcriptional dynamics is modulated during stress and how transcription noise contributes to phenotypic differences at the single-cell level. By following the expression pattern of the *E. coli lac* promoter, using the MS2-GFP single RNA detection method [31,32], we provide quantitative insight into the dynamic changes that occur in transcription initiation during different starvation conditions. Our findings indicate that the transcriptional dynamics observed between different starvation conditions are comparable, suggesting the possibility of cross-protection among different stress conditions as a survival strategy. Finally, our results, which were observed in a strain largely depleted of (p)ppGpp, indicate that significant changes in transcription dynamics can occur in the absence of a stringent response.

2. Materials and methods

2.1. Strains, plasmids and media

The *E. coli* K12 (DH5α-PRO) strain was used as a host to study starvation-dependent gene expression throughout the phases [6]. This strain genotype information is as follows: deoR, endA1, gyrA96, hsdR17($r_{k-}m_{k+}$), recA1, relA1, supE44, thi-1, Δ(lacZYA-argF)U169, Φ80δlacZΔM15, F-, λ-, P_{N25}/tetR, P_{lacIq}/lacI, and Sp^R. The relA1 allele has an IS2 insertion between the 85th and 86th codon, making it similar to a strain completely deleted for relA [33]. Thi-1 mutation makes the strain auxotroph for thiamine. This is important for maintaining strict thiamine starvation in our nutrient depletion experiments. Frag1A: F-, rha-, thi, gal, $lacZ_{am}$, ΔacrAB::kan^R, P_{N25}/tetR, P_{lacIq}/lacI, and Sp^R. Frag1B: F-, rha-, thi, gal, $lacZ_{am}$, P_{N25}/tetR, P_{laciq}/lacI, and Sp^R. The P_{N25}/tetR, P_{laciq}/lacI, Sp^R cassette was transduced from DH5αPRO to Frag1 to generate Frag1B by P1 transduction. The Δ*acrAB*::kan^R cassette was transferred from KZM120 to Frag1B to generate Frag1A. This strain is the most commonly used for studying the transcriptional dynamics of *lac* promoters and environmental stress responses [34–36].

To study the dynamics of transcription at the single-cell level, we transformed two vectors in *E. coli*. The first was pTRUEBLUE-BAC2, a single-copy bacterial artificial chromosome that has 96 target binding sites (96xbs). It is under the control of the wild-type *lac* promoter with the catabolic activator protein-CAP site upstream, for binding of CAP, and operator 1 site downstream for binding of the *lac* repressor [37]. The CAP and *lac* repressors are inducible by cyclic AMP (cAMP) and isopropyl-D-1-thiogalactopyranoside (IPTG) respectively. The second vector was pPROTET.E, a medium-copy plasmid that expresses bacteriophage MS2 coat protein, tagged with a fluorescent protein coding gene (GFP-mut3), located under the control of promoter tet-O1 [37] (generously provided by Prof. Ido Golding, University of Illinois), and also used in our previous studies [35].

To grow the cells, M63 base media [$(NH_4)_2SO_4$ 15 mM, $FeSO_4$ 3 μM, casamino acids 0.1%, KH_2PO_4 0.1 M, $MgSO_4$ 1 mM, thiamine ($C_{12}H_{17}ClN_4OS$) 16.6 mM and glucose 20 g/L] were used. In case of depleted media, the concentration of either KH_2PO_4, $MgSO_4$, thiamine or glucose was reduced to 0.066 mM, 0.01 mM, 0.01 mM and 0.2 g/L, respectively. pH was adjusted to 7.5 using 1 M NaOH. Antibiotics were also used to grow the cells according to the respective antimicrobial resistance.

Please cite this article in press as: Viswanathan A, et al., Phase-dependent dynamics of the *lac* promoter under nutrient stress, Research in Microbiology (2016), http://dx.doi.org/10.1016/j.resmic.2016.04.002