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A simple comparison of the extrinsic noise in gene expression between native and foreign regulations in *Escherichia coli*

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ABSTRACT

Living cells reorganize their gene expression through regulatory machineries in response to external perturbations. The contribution of the regulation to the noise in gene expression is of great interest. In this study, we evaluate the contribution of both native and foreign regulations to the extrinsic noise in gene expression. We analyzed the gene expression data of a mini-library containing 70 genetic constructs of 136 clones into which the *gfp* gene had been chromosomally incorporated under the control of either native or foreign regulation. We found that the substitution of native by foreign regulation, *i.e.*, the insertion of the P_{tet} promoter, triggered a decrease in the extrinsic noise, which was independent of the protein abundance. The reanalyses of varied genomic data sets verified that the noisy gene expression mediated by native regulations is a common feature, regardless of the diversity in the genetic approaches used. Disturbing native regulations by a synthetic promoter reduced the extrinsic noise in gene expression in *Escherichia coli*. It indicated that the extrinsic noise in gene expression caused by the native regulation could be further repressed. These results suggest a tendency of released regulation leading to reduced noise and a linkage between noise and plasticity in the regulation of gene expression.

1. Introduction

Noise in gene expression has been considered universal in living organisms [1–4]. This stochasticity in gene expression was proposed and demonstrated to be at least partly beneficial for the adaptation and sustenance of living cells [2,5–7]. Because the biological impact of the noise in gene expression is evident, there has been an increased effort to obtain a snapshot of the global overview of protein abundance and fluctuations, particularly in yeast and bacteria [8–10]. Genome-wide studies provided evidence of the fluctuating native regulations [8–10].

Regulation of gene expression in bacteria is generally known as the operon and regulon [11–13]. Such genetic architectures may provide efficiency in the gene regulation responsive to external perturbations [11,12,14,15]. The synchronized fluctuation of co-expressed genes is hypothesized to result in an equal amount of correlated transcripts (mRNA) and is potentially beneficial for the fitness and evolution of the cells [16–20].

The native regulatory process in living cells offers both fluctuation and efficiency to gene expression. An increased level of the complexity in regulatory processes may not only improve the regulatory efficiency but also amplify the stochasticity in regulatory process. Previous studies proposed a relationship between the noise that causes the fluctuation of expression level and the plasticity that reflects the capacity of the expression changes [8,21,22]. This is somehow linked to the cellular function of the genes [23]. Regulation complexity, such as promoter architecture, has been proposed to be one of the main contributors to the noise and plasticity in gene expression [24–26]. These reports explained to a great extent why the regulation requires noise. However, whether the fluctuation of native regulation is large or small and whether the replacement of native regulation by simple regulation would amplify or reduce the noise in gene expression is still uncertain.

As known, the noise in gene expression is divided into intrinsic and extrinsic derivatives [1]. Variation in the regulation and chromosomal locations will obviously lead to different magnitudes of noise in gene expression, particularly intrinsic noise. In this study,

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we focused on extrinsically derived noise, which is independent of protein abundance and represents a general feature of fluctuating regulations. As a pilot survey, we used a previously constructed mini-library of *E. coli* strains in which the target genes, which are nonessential but required under certain conditions for cell growth, were replaced by a reporter gene, *gfp*, either with or without a foreign promoter, P_{tet} [27]. The protein abundance and the noise in the native and P_{tet} regulated *gfp* constructs reflected the gene expression that is controlled by native and foreign regulations, respectively. A reduction in the extrinsic noise was observed when the native regulations were disturbed through the insertion of the P_{tet} promoter. This reduction indicated that fluctuating native regulations largely contribute to the extrinsic noise in gene expression and that the evolved regulatory architecture in *E. coli* most likely balances between efficiency and plasticity.

2. Material and methods

2.1. FCM data analysis

The flow data sets, containing 70 genetic constructs of 136 clones [27], were used and analyzed. A narrowly gated FSC data set could be used instead of the normalised FITC/FSC values. A cross-comparison between the gated and total *E. coli* cells in FCM analyses had been performed previously [8,9,28–31]. The results showed that a reduced magnitude of extrinsic noise was observed in the gated cells compared to the total cells; this finding is supported by the work of Silander et al.'s [8,9,28–31]. Consequently, one per cent of the total number of cells (events) from both the highest and the lowest values of the green fluorescence intensity were removed to eliminate unreliable rare events, such as, the systematic error resulting from events that occurred at the extremes of the instrument's range [8,9,28–31]. After subtracting background fluorescence using the native strain (MDS42), the mean and standard deviation of the GFP concentrations were calculated using the data with positive values. The *CV* was defined as the standard deviation divided by the mean [1,8,10,30,32]. For each genetic construct, the statistical values were averaged over multiple data sets. The native and foreign regulations were analyzed separately, and data below the detection limit were removed.

2.2. Noise calculation and theoretical fitting

The CV^2 of the intrinsic and extrinsic noise (CV^2_{int} and CV^2_{ext}, respectively) were analytically obtained based on the total noise (CV^2_{tot}) by fitting the data to the following equation.

$$\mathrm{CV}^2_{\mathrm{tot}} = \mathrm{CV}^2_{\mathrm{int}} + \mathrm{CV}^2_{\mathrm{ext}} = \mathrm{a}/\langle \mathrm{GFP} \rangle + \mathrm{b}$$

where *a* represents the coefficient for the intrinsic noise, *b* represents the magnitude of the extrinsic noise, and <GFP> represents the mean GFP concentration. This relationship has been widely demonstrated and has been commonly used in the studies on biological noise [8–10,32,33]. Good regressions, which were accompanied by a 95% confidence level, were achieved for the three collections. The details on the theoretical background have been largely described elsewhere [8,9,28–31]. In short, the coefficient for the intrinsic noise, *a*, is based on the Poisson statistics of the chemical reactions in gene expression, whereas the magnitude of the extrinsic noise, *b*, is based on a constant independent of the protein expression levels. Thus, the total noise decreases with decreasing expression level until a certain level of intrinsic noise is reached; subsequently, the total noise becomes constant due to the extrinsic noise. This estimation agrees well with standard dual-color experiments [8]. We used custom software with nonlinear regression functions (nls) written in R for the curve fitting. We used the mean GFP concentration, the mean GFP expression and the mean GFP abundance as the values of <GFP> in our data sets, the data set published by Silander et al. and the data set published by Taniguchi et al., respectively.

2.3. Transcriptome and proteome data sets

The transcriptome data sets for MDS42 and MG1655 growing in minimal medium were acquired from the raw data deposited in NCBI's Gene Expression Omnibus under the GEO Series accession number GSE33212. The proteome data sets were obtained from other publications: the mean values and noise (or SD) of the protein abundance were obtained from Table S6 (1018 genes) of the report by Taniguchi et al. [10] and the online data file (1832 genes) from the report by Silander et al. [34].

2.4. Genome information and gene categories

The gene names and chromosomal positions (distance from *oriC*) were based on the genome information for MDS42 that has been deposited in the DNA Data Bank of Japan under the DDBJ accession number AP012306. The genes in common (overlaps) among the different data sets were determined based on the genome information of the strains W3110 and MG1655 strains in GenoBase, Japan. The functional essentiality gene categories were determined according to the open access data banks and the related publications, as follows. First, the essential genes were identified through single gene deletion experiments [35] and the PEC data bank (Profiling of *E. coli* Chromosome). Second, the conditionally required genes were selected according to the information on selectable phenotypes from the National BioResource Project of the National Institute of Genetics, Japan (NBRP *E. coli* Strain). A total of 770 genes were deposited as selection markers to determine when mutations occurred. Genes for which phenotypic changes were unknown or non-existent and genes showing phenotypic changes in colony formation were excluded. Only the mutations or deletions of genes that directly influenced the cell growth rate were selected and defined as conditionally required genes. As a result, 339 genes with locus tags in MG1655 (equivalent to 343 locus tags in W3110), which largely contained the genes responsive to the nutritional state or environmental temperature, were identified (Table S1). The rest of the genes were categorized as belonging to the "others" category.

3. Results and discussion

3.1. A summary of genetic constructs reporting native and foreign regulations

A previously constructed mini-library that targeted 37 genes was used (Fig. S1). Most of these genes are related to amino acid biosynthesis and are located at various distances from *oriC*. These genes were replaced with a reporter gene (*i.e.*, *gfp*) that either remained under the control of the native regulation or was inserted with a foreign promoter, P_{tet}, as previously described [27]. The *galK::*P_{tet}*-gfp* construct was used as a control because this chromosomal site has been widely studied [29,31,36–39]. Since there is no repressor, *i.e.*, *tetR*, in the genome, the expression of the P_{tet} controlled constructs represented the steady expression capacity of the corresponding chromosomal location. A comparison between these two genetic formats allowed us to determine whether and how the noise in gene expression changed when the native regulation was replaced by a foreign promoter with steady expression.

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