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Analysis of fluctuation in protein abundance without promoter regulation based on *Escherichia coli* continuous culture

Tadashi Yamada [a], Chikara Furusawa [a,b], Keisuke Nagahisa [a], Akiko Kashiwagi [a], Tetsuya Yomo [a,b], Hiroshi Shimizu [a,*]

[a] *Department of Bioinformatic Engineering, Graduate School of Information Science and Technology, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan*
[b] *Complex Systems Biology Project, ERATO JST, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan*

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Abstract

Fluctuation of protein abundance of isogenic *Escherichia coli* cells in uniform environment was studied. Based on a continuous culture system, which provides homogeneous culture environment, we investigated the fluctuation in GlnA protein abundance regardless of known *glnALG* promoter regulation. As results by flow cytometer, we found that the GlnA protein abundance in the cells exhibit a large fluctuation, even though GlnA protein is an essential factor for cell growth and the environment is homogeneous. Furthermore, among several steady states, transient processes of such heterogeneous cell population were investigated, by changing the environmental conditions. The results showed that the expression of GlnA protein can be controlled, depending on its necessity, even though there is no known regulatory machinery. These results may provide a clue to understand the nature of regulation of protein expression dynamics with the stochastic fluctuation.

Keywords: Stochastic fluctuation; Flow cytometer; Continuous culture; Cell population

1. Introduction

In studies of the biological systems, it is generally assumed that the intracellular state of the isogenic cells in a fixed environment is homogeneous, and the analyses of the systems are based on this assumption. For example, cell concentration is usually measured at the macroscopic level, such as measurement of turbidity of culture medium, which relies on the homogeneity of the cells. However, recent developments of experimental techniques such as flow cytometer enable us to measure the intracellular state such as protein abundance at the single cell level, and such measurements indicates that the intracellular states, such as a protein abundance in a cell, generally show fluctuations, which are maintained by the stochastic nature of chemical reactions (Elowitz et al., 2002; Becskei et al., 2005; Furusawa et al., 2005; Bar-Even et al., 2006; Suzuki et al., 2006).

In this study, we focus on the regulation of the protein expression in such fluctuating dynamics. In molecular biology, dynamics of protein expressions is described to be regulated precisely by several chemical components, including other proteins, metabolites, and so forth. The input output relationships of these regulations are explained by if-then type logic like computer programs.

* Corresponding author. Tel.: +81 6 6879 7446;
‘ fax: +81 6 6879 7446.
E-mail address: shimizu@ist.osaka-u.ac.jp (H. Shimizu).

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Networks organized by such regulations enable protein expression to respond correctly to changes of environmental conditions. However, since dynamical change of such regulatory components are accompanied by large fluctuations, there is possibility that precise controls of the intracellular states with respect to environmental changes are disturbed by noise. For example, in the case of signal transduction, signal from the second messenger such as cAMP is amplified through the cascade reaction, and the amplified signal induces many reactions (Walsh and van Patten, 1994). In this case, the fluctuation of initial signal amplified through the cascade reaction process can finally become significant level. Nevertheless, cellular systems seem to respond to a large number of environmental conditions correctly. This suggests that there is some machinery in the protein expression dynamics, by which the intracellular expression dynamics can respond to environmental changes correctly even under a certain magnitude of molecular fluctuations. To understand such robust response with respect to molecular fluctuation, we investigated the fluctuated expression dynamics of a protein, which are not regulated by any known regulatory machinery, in adaptation processes to environmental changes in a continuous culture of *Escherichia coli* cells (Fig. 1).

In the system, a target protein GlnA (Chen et al., 1982) catalyzing the conversion reaction from glutamate to glutamine is an essential factor for cell growth of *E. coli* under basal medium composition (B medium; see Section 2). The B medium contains glutamate as a sole nitrogen source, so cells must produce the other amino acids using uptaken glutamate through their own metabolic pathways to grow in this medium condition. Because glutamine can be produced via only GlnA catalyzing pathway, the expression of GlnA is essential for cell growth in the B medium. To quantify the expression level of GlnA, *glnA* gene is fused with *gfp* gene (Chalfie et al., 1994). To observe the nature of protein expression dynamics such as the change of the protein abundance and the fluctuation, the fluorescent intensity of the fused protein is measured by flow cytometer. In the system, the regulatory region of the target protein is replaced from the inherent *glnALG* promoter (Ueno-Nishio et al., 1984) to the artificial-synthesized *tac* promoter. Our strains do not code the any regulator genes about *lac* system such as the repressor *lacI* on the genome or plasmid, so *tac* promoter (de Boar et al., 1983) expresses constantly. Therefore, this replacement removes the change of protein abundance by the promoter regulation and the propagation of the upstream fluctuations by the inherent regulators. We expect that use of this promoter allows us to observe the nature of expression dynamics of a protein regardless of inherent regulations. Such a strain is cultured in chemostat. At the steady state in chemostat, cell and substrates concentrations become constant. This system allows us to investigate expression dynamics in a uniform environment. Also, we can easily perturb the target protein necessity by changing the feeding medium composition. For example, the expression of GlnA is necessary when the feeding medium contains glutamate as a sole nitrogen source, while the expression is not necessary in the feeding medium containing glutamine.

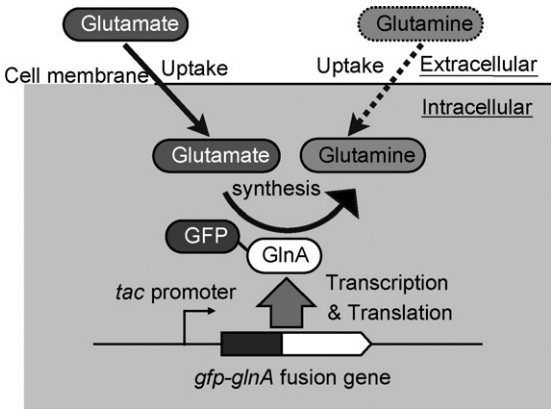

Fig. 1. Schematic illustration of the system constructed in this study. Glutamine is an essential factor for cell growth. Here, the difficulty of acquisition (uptaking and self-production) of glutamine can be treated as the necessity of the target GlnA protein. This necessity can be perturbed by the change of the extracellular environment such as the change of the glutamate concentration and glutamine addition. We investigated the behavior of the GlnA protein abundance regardless of inherent promoter at a single cell level when its necessity was changed.

Using this system, we investigated the dynamics of GlnA abundance without sophisticated regulatory machinery in the continuous culture system. As results, first we found that even at the steady state of the continuous culture using basal medium, GlnA protein abundance in the cells showed a broad distribution (with two orders of magnitude of protein abundance), even though the reaction catalyzed by GlnA is essential for cell growth in the environmental condition. Second, it was shown that the expression of *glnA* gene could respond to environmental changes even though there is no regulatory machinery. We found that the GlnA protein abundance increased when the importance of the protein increased in the environmental conditions, and vice versa. This suggests that there is machinery by which protein expression is controlled depending on its necessity, without known regulatory mechanisms. This may provide a clue to understand the nature of protein expression dynamics with stochastic fluctuation.