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Adaptive gene expression beyond operator-repressor molecular regulatory system

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Abstract: In wild nature, living systems obtain various regulatory machineries corresponding to specific environmental changes, during the evolutionary process. These regulatory machineries exhibit the same framework as that of the IF THEN ELSE type of conditional statement in computing system. Repressor-operator molecular regulatory networks, which are the fundamental systems for regulatory gene expression in cells, work similarly as the IF THEN ELSE commands in computational programs. Such regulatory systems work efficiently, when cells encounter the specific environmental changes as programmed in the regulatory networks. However, the IF THEN ELSE type network lose its efficiency against the environmental alterations that out of the programmed regulato ry mechanisms. As the space of environmental conditions is much larger than that of cellular response programs, there isn't corresponding program for each condition. To achieve the adaptive response, cells may use other paradigms tha n the IF THEN ELSE type. Here, we employed an artificial gene expression network in bacterial cells, and obser ved that the gene expression was auto-regulated adapted to the changing environment, without using the IF THEN ELSE type of regulatory system. The role of such adaptive response of living systems allow us to find a de sign principle of constructing an auto-regulatory network against unexpected perturbations.

Keywords: environmental change, adaptive response, gene expression, systems biology

I. INTRODUCTION

Generally, cells regulate the level of gene expression, in response to the environmental changes or external perturbations, to reach an appropriate phenotypic state. Such adaptive cellular response is vital for living systems. What is the design principle of the adaptive response to environmental changes? According to the principles explained by the molecular biologists, the mechanism of adaptive responses to environmental changes is clarified as following: cells use molecular regulatory systems (*e.g.* repressor-operator regulatory networks), which are preliminarily programmed as IF THEN ELSE type of conditional statement [1], to manage the varied situations. In biological systems, for instance, if the nutritional condition is altered, the expression level of the required gene is increased, which is regulated by the intrinsic networks programmed for the environmental changes of the corresponding nutrient. A classical example of such working system in bacteria is the utilization of lactose, which is subject to the repressor–operator molecular regulatory network, called Lac Operon [2, 3]. The active repressor specifically binds to the regulatory sequence (operator) upstream of the target gene for expression. The association between repressor and operator prevent the initiation of the transcription and translation procedure, leading to the inhibition of the expression of the downstream target gene. As an IF THEN ELSE type of conditional statement programmed preliminarily in cells, the repressor-operator mechanism is one of the design principles of regulatory systems, responding to the specific environmental changes [4].

Such regulatory systems work efficiently, if the cells encounter the specific environmental changes of corresponding cellular regulatory machineries. However,

when the cells meet the unpredicted environmental perturbations, those IF THEN ELSE type of cellular regulatory machineries are unable to respond functionally, as there is no corresponding program for these unexpected environmental changes. As the space of environmental conditions is much larger than that of cellular response programs, there are not enough programs for each condition. Cells need to choose the appropriate program for a given condition. Lacking the corresponding IF THEN ELSE type of regulatory system, how do cells respond and survive themselves, when the unexpected environmental changes happened?

Here, we constructed an artificial gene expression network, which is out of the control under the original repressor-operator molecular regulatory system. Subsequently, we investigated the bacteria cells genetically embedded with the constructed network, and found that the adaptive gene expression occurred, without using the IF THEN ELSE type of regulatory system.

II. RESULTS

In the absence of the corresponding repressor-operator regulatory machinery, how is the adaptive response to environmental changes happened in cells? To address the question, we designed a synthetic gene regulatory module, and inserted it into the bacteria (*Escherichia coli*) genome, illustrated in Fig. 1. In this module, the target gene, *leuD*, encoding the leucine synthetase, was constructed under the regulation of the artificial promoter Ptet, which is activated by the chemical, doxycycline, as an inducer. The expression of the *leuD* gene is quantitatively monitored by the upstream fluorescent protein (gene), green fluorescent protein (GFP), which is under the regulation of the same promoter. Moreover, as the inherent *leuD* gene was deleted from the original repressor-operator regulatory machinery (leu operon) [5], the leuD gene employed in the module is out of the control under the original cellular regulatory system. As leucine is one of the essential amino acids for cells to grow and proliferate, addition or removal of leucine in or from the medium result in the environmental changes (perturbation) for the cells. Consequently, the expression of the *leuD* gene is supposed to be varied accordingly, toward the changes in the environment (medium).

As shown in Fig. 2a, the fluorescence density, represented the expression level of the *leuD* gene, was gradually increased in the presence of the additional doxycycline. If the activity of the Ptet promoter was repressed tightly, the cellular growth was hard to detect, as the concentration of expressed LeuD protein (leucine synthetase) was too low to produce enough leucine for cellular proliferation (Fig. 2b, circle). When the Ptet promoter was activated by adding the inducer, doxycycline, the LeuD protein was greatly produced, which allowed cells to proliferate without the nutrient supply (leucine) in the medium (Fig. 2b, black cross, square and triangle).

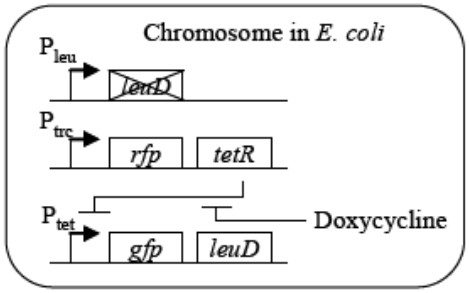

Fig. 1 Regulatory gene expression network. leuD gene was removed from the original leu operon regulatory system and placed under the control of the synthetic promoter, Ptet, which is inhibited by the Tet repressor and is activated by the inducer, doxycycline.

In addition, the growth rate was relied on the expression level of *leuD*. The higher level of the gene expression resulted in the faster growth of the cells, presented the adaptive phenotypic states. On the other hand, the doxycycline-dependent cellular growth was unobserved, when leucine (1mM) was supplied externally. Thus, the cells carried the synthetic module exhibited the adaptive behavior toward nutrient depletion condition, by means of regulating the gene expression. The function of the synthetic module genetically inserted in the cells is similar as that of the original repressor-operator regulatory system, though their cellular processing ways are different.

Subsequently, we studied temporal changes of the adaptive expression of the *LeuD* gene. The distribution graphs in Fig. 3 display the temporal changes (from 2 h to 12 h) of the fluorescent density (GFP) stood for the expression level of *leuD*, after removing leucine from the environment. Nevertheless, there was no

programmed regulatory system specific for the leucine depletion (environmental perturbation), the distribution of gene expression shifted to the adaptive state (Fig. 3a). This movement was the same as that of the adaptive response observed in wild-type strain which utilize its original regulatory machinery. No shift was detected when the concentration of leucine in the environment was constantly enough (Fig. 3b). It indicated that cells were able to adjust the expression level to the adaptive status, without using the original repressor-operator regulatory system.

Additionally, as the expression level shifted to the adaptive status, the cellular growth rate increased gradually, up to the optimal growth rate that refers to the cellular growth rate in the medium rich in leucine (1 mM). Similar adaptive responses were observed as well, under the various concentrations of doxycycline (data not shown).

III. CONCLUSION

In this study, we constructed a regulatory gene expression network, out of the control of the original repressor-operator molecular mechanism. When the essential nutrient, leucine was depleted from the environment, the expression level of the corresponding gene (leuD), shift toward the more adaptive states, in the absence of the IF THEN ELSE type of regulatory systems (Fig.3). In addition, the cellular growth rate was optimized under the new condition (Fig. 2b). Taken together, it indicated that the changes of the expression level (shifts in distribution) contributed to the optimization of cellular growth rate. In a word, the effective cellular response to an alternative environment resulted from the adaptive gene expression but not from the programmed regulatory processing.

We supposed that biological systems have a design principle for adaptive response without using repressor-operator regulatory systems. This principle may allow cells to alter their phenotypic state adaptively response to unexpected environmental changes in order to

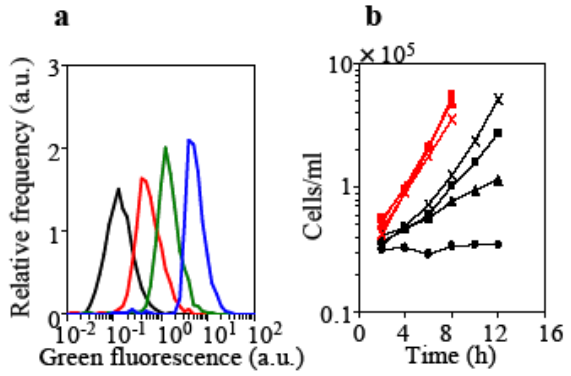

Fig. 2 Relationship between gene expression and cellular growth. **a.** Distribution of gene expression induced by doxycycline. The final concentrations of doxycycline were 0 nM (black), 20 nM (red), 30 nM (green) and 100 nM (blue). Black line represents autofluorescence. **b.** Growth curves under various medium conditions. Cells of different expression levels (a) grew equivalently, in the presence of leucine (1 mM) in the environment (red curves). Depletion of leucine from the medium showed the correlation between growth rate and expression level (black curves). The final concentrations of doxycycline in the medium were 0 nM (circle), 20 nM (triangle), 30 nM (square) and 100 nM (cross).

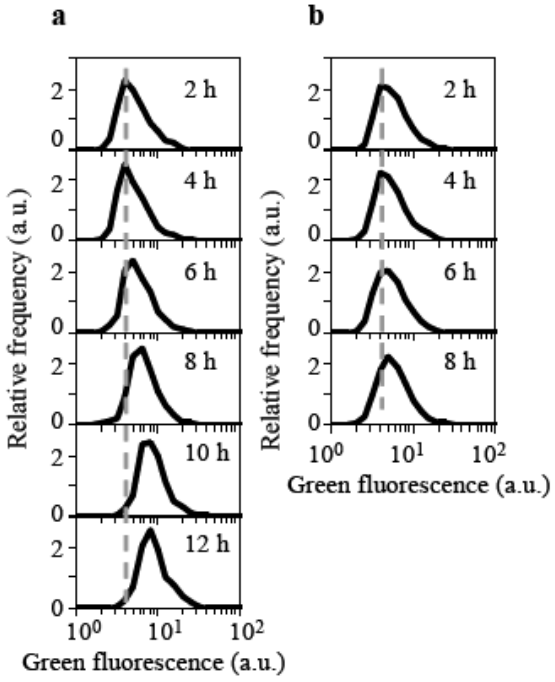

Fig. 3 Adaptive gene expression in response to the environmental changes. Distribution of the gene expression level (fluorescent density of GFP) shifted along with the time (2h, 4h, 6h, 8h, 10h and 12h), when removing leucine from the environment (a), while no significant shift was observed under the condition rich in leucine (b). Grey broken lines represent the position of the peak position of the distribution at 2h. The final concentration of the additional doxycycline was 100 nM.

maintain themselves and to proliferate.

How is the adaptive response achieved beyond the repressor-operator molecular regulatory system? Here, we propose two possible scenarios to explain our experimental observation.

Scenario 1: external signals cause all the cells to alter their gene expression toward the adaptive level, resulting in the shift of the total population toward adaptive phenotypic state. For instance, starvation of environmental nutrition may perturb the metabolic or genetic networks in cells, which potentially promote such network play a crucial role on environmental adaptation beyond the repressor-operator regulatory system. The high level of gene expression (e.g. leuD) is compensative for the depletion of the essential nutrient (leucine) in the environment, so that the cells are capable to against the changing environment and reach the adaptive state.

Scenario 2: noise in gene expression causes a portion of cells to reach the adaptive state, stochastically. In the changed environment, these adapted cells grow faster than the rest do. This stochastic nature, influenced by the fitness gradient among the cells, led to the shift of cell population [6]. Due to the huge noise in gene expression [7, 8], the distribution of the expression level is possibly influenced by the fitness gradient, which is caused by the depletion of the nutrient in the environment [9].

Further detailed study is going to show the design principle of the adaptive response observed here, and to provide the guidance for the reconstruction of the networks that can auto-regulate to the optimal state even if perturbed by unpredicted environmental changes.

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