behavior, involves the interactions of a large number of components, and can thus be seen as an intermolecular interaction network. While the molecules involved in this reaction and the physical interactions among them have been studied extensively, contribution of inter-component interactions on protein synthesis activity remains to be elucidated. We used an *in vitro* translation system consisting of 69 defined components to quantify the contributions of inter-component interactions and those of individual components on protein synthesis activity. By varying the concentrations of 69 individual components and by carrying out combinatorial experiments, we determined the protein synthesis activity at a number of different concentrations of each of the 69 components. By analyzing the experimental results using Bahadur expansion, we quantified all inter-component interaction terms, *i.e.*, two- to 69-body interactions, and found that larger than two-body inter-component interaction can be approximated to zero to describe protein synthesis activity. Furthermore, the average ratio of the contribution of the nonadditive term to that of the additive terms was 0.09. That is, the effects of altering one of the components concentrations were reduced or increased on average by a factor of 0.09 when combined with other changes. These findings suggest that as opposed to the physical interactions among the components, the extent of functional interaction is relatively low.

3P-281　再構成無細胞翻訳系のタンパク質合成活性の予測

Prediction of the protein translation activity values by estimating Bahadur coefficients from a small number of experimental data set

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To optimize an in vitro translation system consisting of 69 defined components, the protein synthesis activities obtained with different combinations of the concentrations of 69 components were subjected to Bahadur expansion analysis, which gave the respective contributions of one- to 69-body inter-component interactions. We showed that the contributions of greater than two-body interactions could be approximated to zero. The absence of larger than two-body inter-component interactions means that activity values can be predicted from a small number of experimental data set. Here, we tested the predictability. When each of six components takes two different concentrations, there are 64 possible conditions, however, the lack of larger than two-body interactions allow to predict 42 results from at least 22 (= 64 - 42) experimental data. The predicted data sets were correlated with the experimental data sets, whose R^2-value was higher than 0.8 in 57 of 64 cases, and thus high R^2-values could be obtained with 90% probability. Such high R^2-values were not obtained with the same prediction using up to the 1^{st} order Bahadur coefficients, indicating the necessity of 2^{nd} order coefficients for accurate predictions. All biological systems are composed of large numbers of components that interact with each other, and thus the methodology presented here is likely to also be applicable for analysis and optimization of other biological systems.

3P-282　lacオペロンの二重安定性における環境応答

Adaptive response to environmental changes utilizing the bistability of the lactose operon

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Cells show different expression patterns in the same environment, even if they have identical genetic information, such as cell differentiation occurred in the development process. Multistability (e. g. bistablility) of gene expression networks is reported to contribute to the variety of the phenotypes. Recent studies indicate that cells could transit between bistable states according to environmental variation. Here, we focus on the bistability of the lactose utilization network, a well-known positive feedback loop, in *E. coli*, to investigate whether the transition between two stable states happens when the environment changes. An antibiotic resistance gene under the control of the *lac* promoter is integrated into the chromosome of *E. coli*. Quantitative analysis of the expression level of this gene is monitored by the co-expressed green fluorescent protein, using the flow cytometer. The bistability of this simple genetic network is demonstrated by adjusting the concentration of the inducer, Methyl β-D-thiogalactoside (TMG). Under the bistable condition, we find that the fluorescence intensity in a small population of cells is increased only in the presence of the additional antibiotics, which suggests that cells may transit from the low expression bistable state to the high expression one, in response to the changed environment. Additionally, genes involved in biosynthetic metabolism are evaluated as well. Further analysis and details will be reported.

3P-283　自然淘汰型進化リアクターを用いたAスタートmRNA用プロモーターの進化

Evolution of promoter for A-start mRNA in a natural selection-type evolution reactor

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Most of wild type T7RNA promoter is for G-start mRNA. But the ϕ2.5 promoter is for A-start mRNA. In the biotechnology applications, the ligation of an mRNA with an upstream functional sequence by using RNA ligase has been expected. The ligation efficiency, however, is higher in the case of A-start mRNA than in the case of G-start mRNA. We tried, therefore, to confirm whether ϕ2.5 is the optimal promoter for A-start mRNA and tried to get a better sequence. Our evolution reactor process is based on the RNA-Z isothermal amplification using HIV-reverse transcriptase and TT7 RNA polymerase in 1.4M trehalose at 50 degrees. In order to check the evolutional stability of the ϕ2.5 sequence, we perform a serial transfer experiment for the template having the ϕ2.5 promoter and the stable context sequences. We observed two kinds sequence from the 5th round and the 8th round of the transfer product. The nine-tenth of the sequence was the ϕ2.5 promoter and the other was one base deletant. Next we performed a serial transfer with the template having a random 18-base region as promoter for A-start mRNA and stable upstream and downstream context sequences which have no homology to 5'end and 3'end sequences of the T7 promoters. We got a sequence family to the ϕ2.5 promoter from the 5th round product of the transfer.We will discuss about the competition experiment between the optimal promoter we obtained and the ϕ2.5 promoter.

3P-284　大腸菌と細胞性粘菌からなる共生コロニー形成過程における大腸菌の詳細な遺伝子発現解析

Detailed and advanced analysis of Escherichia coli gene expression in the symbiotic colony with Dictyostelium discoideum

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An experimental symbiotic system composed of Eschericha coli and Dictyostelium discoideum (Todoriki et al. 2002) was useful to examine how symbiosis occurs from organisms in the non-symbiotic relationship. The two species coexist stably for several months within mucoidal colonies, which we named "Symbiotic colony", after several weeks of co-cultivation on minimal agar plates, although E.coli - D.discoideum is the prey-predator relationship. In our previous report, physiological changes in E.coli were described and this symbiotic relationship is possibly established within one month. Therefore, this period are likely important in the evolution of this new symbiotic system. We report here the changes in the global gene expressions of E. coli within one month of the co-culture by using whole-genome tilling array that contains oligonucleotide probes representing the entire genomic sequence. In the transcriptional profile of symbiotic E. coli, the expression of "response to desiccation" genes significantly decreased, and those of some amino acid biosynthesis and chorismate biosynthesis genes significantly increased within 21 days of co-culture. These gene expression changes may be associated with the development of the symbiosis. We would like to talk detailed investigation about these gene expression.

3P-285　オペロン制御を利用しない環境適応的遺伝子発現

Adaptive response to leucine starvation occurred devoid of the native leu operon

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Regulation of gene expression in cells is dominantly controlled by the signal transduction networks, such as, operons and regulons, which using the specific signals responding to the external perturbations. Such regulatory mechanisms might be formed during the evolutionary process, efficiently adjust the gene expression to the changing environments. However, it is definitely unknown that how cells adapt to the environments, while no corresponding regulatory mechanism is available. To address this question, we shuffle the structure genes involved in the *leu* operon to an artificial genetic network, and investigate the dynamics of gene expression responding to leucine starvation.The artificially constructed genetic network other than the native *leu* operon is integrated into the genome of *E. coli*. Genes involved in the leucine biosynthesis, together with the green fluorescent protein as a quantitative monitor, are employed under the same promoter, Ptet. Cells genetically carrying this network are found to autoregulate the gene expression in response to leucine starvation. Such adaptive gene expression is clearly observed devoid of the native regulatory mechanism mediated by the *leu* operon. Further analysis is going to clarify the principle involved in the adaptive response.

3P-286　生物のゆらぎは進化によっても創られる

Evolutionary restoration of phenotypic fluctuation

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Phenotypic diversity due to fluctuations among isogenic individuals is expected to be suppressed through evolution, as it prevents optimization of fitness and/or function. However, recent studies on stochastic gene expression have highlighted that cells maintain relatively large fluctuations. To discuss evolutionary origin of such large fluctuations, we carried out an artificial selection experiment for higher GFP fluorescence with Escherichia coli cells, and found that mutants possessing broad GFP fluorescence distributions with low average values emerged under strong selection pressure. These "broad mutants" appeared independently on the phylogenetic tree, while increase in variance of GFP fluorescence is originated in fluctuation in mRNA expression level, as concluded from the co-expression analysis between GFP and RFP introduced at the same plasmid. In addition to the

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