論文内容の要旨

論文題目　　A Study on Intracellular Stochasticity

（細胞内ゆらぎに関する研究）

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Interactions among genes and proteins in living organisms are chemical reactions. Considering a tiny volume of individual cells, intracellular chemical reactions are inevitably stochastic. This intracellular stochasticity not only gives rise to phenotypic variability in a clonal population of cells, but also fundamentally limits signaling fidelity. Even in the absence of input stimuli, cells spontaneously exert signals through internal computational systems. It has been believed that the spontaneous signaling is beneficial for foraging nutrients in prokaryote (bacteria) and keeping their heterogeneity. However, the mechanism how eukaryotic cells exert stochastic bursts of output signals spontaneously is poorly understood. On the other hand, the intracelular stochasticity is sometimes a nuisance for accurate switching of genes. The large stochasticity causes the wrong switching, which may result in fetal defects for animals. Cells thereby have to develop computational systems for filtering harmful noise. Thus, intracellular stochasticity is crucial for understanding the spontaneous and reliable signal processing.

In the first part of this thesis (Chapter 2), we take cell migration for instance of the spontaneous signal processing. Despite recent progress in the identification of the molecules involved in cell migration, the mechanism that migrating cells spontaneously coordinate the dynamics of cell shape with cell migration remain poorly understood. To determine how cells create spontaneous cell migration, we measured the stochastic dynamics of cell morphology in single *Dictyostelium discoideum* cells in the absence of external stimuli. Using quantitative analysis of fluctuations of morphological dynamics, we found that noise in morphological dynamics are not random, and that stochastic morphological dynamics are organized into 3 orderly patterns: elongating, rotating, and oscillating. In addition, we also demonstrate that PI3-kinase (PI3K) and PTEN play a more

fundamental role than hitherto thought. The mechanism, which occurs independently of chemotaxis, involves the organization of cell shape into orderly patterns without the need for external stimuli. Cells organize three orderly patterns through PI3K/PTEN/F-actin from intrinsically noisy and random deformations. Importantly, the orderly patterns are conserved in both vegetative cells where cell shape and migration are apparently random and starved cells where two processes appear to be orchestrated.

Furthermore, we found that cells direct where F-actin accumulates and steer the direction of motion assisted by orderly patterns. Thus, cells organize the orderly patterns of cell shape through PI3K/PTEN/F-actin and spontaneously coordinate cell shape and movement to forage spontaneously.

In the second part of this thesis (Chapter 3), we take the synthetic gene network with positive auto-feedback regulation for instance of reliable signal processing. Proteins are the functional machinery in living cells. Proteins interact with each other and bind to DNA to form regulatory networks and regulate the level, location and timing of expression of functional proteins. In this way, undifferentiated cells differentiate at right position and at right timing, and differentiated cell in a multicellular organism remembers its expression profile throughout its life. However, how do cells accomplish precise differentiation against intracellular stochasticity? Suppressing the stochastic noise is essential for the precise differentiation. Recent works have found that large transcriptional networks are buildings of simple regulatory circuits, which are called network motifs. Network motifs thought to be important for reliable signal processing against intracellular stochasticity. In the Chapter 3, we demonstrate the way how the timing of gene expression is regulated by positive auto-regulation, which is a network motif recurring in differentiation. The results provide the first experimental evidence that the dynamics of gene expression slow down by positive auto-regulation. We also provide the mathematical model that explains the dynamics of gene expression, and reveals the response delay from positive feedback systems.

In addition, we show the experimental result that positive auto-regulation creates two distinct stable gene expression states. The multistable response of synthetic gene network is experimentally converted to a graded response by altering promoter activity. Our results suggest that positive auto-regulation creates the temporal order in gene expression due the response delay and endows cells to have a lasting memory of gene expression due to multistability.

In the third part (Chapter 4), we summarize the results obtained from our studies and then present perspectives on a next step to be taken.