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Independent Control in the Cell Cycle Created by Network Structure

—Quantitative Validation of Regulatory Modules Predicted by Mathematics

Abstract

For cells to survive, reactions with various functions must occur within the cell, and each must be appropriately regulated. It is known that all reactions within a cell form a single interconnected network, as the products of one reaction serve as substrates for another. But is it actually possible to individually regulate different substances that perform different functions within a single, interconnected dynamic system?

Yuhei Yamauchi, Specially Appointed Assistant Professor, and Atsushi Mochizuki, Professor, at the Institute for Life and Medical Sciences, Kyoto University, along with their colleagues, have previously demonstrated through mathematical theory that when a portion of a reaction network (Note 1) satisfies a certain mathematical equation, it forms a “buffering structure” (Note 2) and acquires “modularity”—the ability to be controlled independently of other parts of the network. In this latest study, through a collaborative effort with Specially Appointed Assistant Professor Hiroki Sugiyama of the Institute for Earth and Life Sciences at Institute of Science Tokyo, Associate Professor Yuhei Goto, and Professor Kazuhiro Aoki of the Graduate School of Biostudies at Kyoto University, the researchers demonstrated that this buffering structure actually exists within the “cell cycle system” (Note 3)—which governs the life stages of cells—and plays a crucial role. Analysis of the cell cycle system suggested that two types of protein complexes, which regulate cell cycle transitions, are contained within different buffering structures and may therefore be controlled independently of one another. Using the latest quantitative analysis, we confirmed that one protein complex indeed behaves independently of the other. Furthermore, by comparing theoretical predictions with experimental verification, we theoretically predicted an unknown reaction and proved its existence through verification experiments.

This research demonstrates that buffering structures—which were previously only mathematical predictions—actually perform important biological functions, thereby updating our understanding of the cell cycle system. Furthermore, a new method in systems biology was proposed, one that derives unknown information as requirements from both theory and experimentation.

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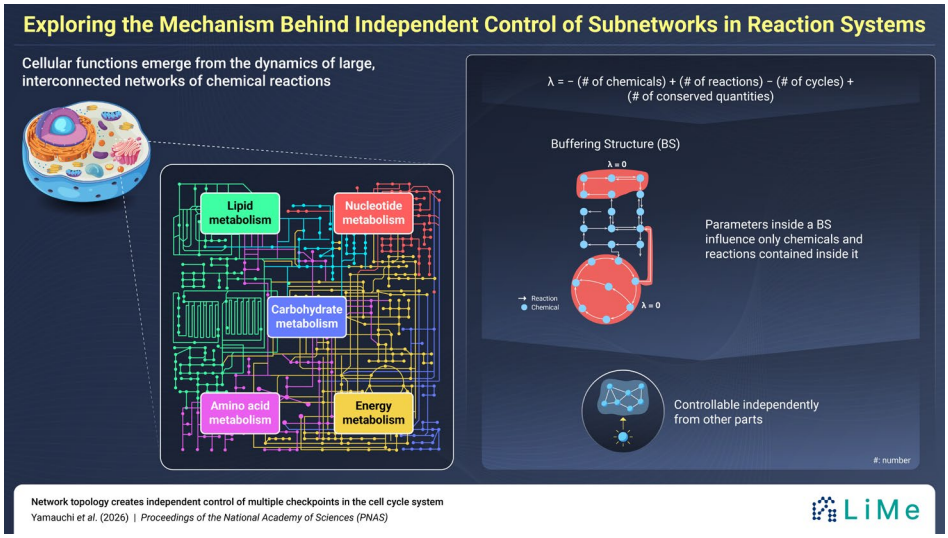

Conceptual diagram of the entire study

1. Background

There are a vast number of chemical reactions that occur within cells, but it is known that these do not operate in isolation; rather, they are interconnected by sharing substrates and products, collectively forming a single, vast reaction network. The various biological functions of cells arise from the dynamics of this interconnected network system. It is believed that cellular functions are regulated by changes in the amount and activity of the enzymes that catalyze individual reactions, which in turn alter the levels of the chemical substances responsible for biological functions. However, controlling a single factor in a dynamic system is likely to have an impact not limited to that specific substance but rather to propagate in a chain reaction to various other substances within the system. Is it truly possible to control, individually, different chemical substances that perform different biological functions within a single, interconnected chemical reaction system? This has long been a fundamental and challenging question in biology.

In recent years, the research group has developed a mathematical framework called structural sensitivity analysis (Note 4), which determines a reaction system's response to changes in enzyme levels or activity based solely on network information. Structural sensitivity analysis is a model-free theory (Note 5) that requires no assumptions other than network information. Based on this, we have demonstrated through mathematical theory that "modularity"—the property whereby a part of a system can be controlled independently of the rest—arises solely from the "topology" (Note 6) of the network. For any substructure of a reaction network, if the number of elements contained within it satisfies the condition $\lambda \coloneqq -(\#.\,\text{chemical species}) + (\#.\,\text{reaction species}) - (\#.\,\text{cycles}) + (\#.\,\text{conserved quantities}) = 0$, that substructure is called a "buffering structure." When the enzyme concentration or activity (parameters) of a reaction within a buffering structure changes, the effect is limited solely to the chemical concentrations and reaction rates within the buffering structure and does not extend to the chemical concentrations or reaction rates outside it. Furthermore, this kind of "independent control" is realized only when a buffering structure is present. While this theory appears to offer a solution to the question mentioned above, it remains strictly a theoretical concept, and it was unclear whether this principle actually operates in real biological systems.

2. Research Methods and Results

In this study, the research group focused on the yeast cell cycle system. From one cell division to the next, cells must progress through four phases—G_1 phase, S phase, G_2 phase, and M phase—in this order and at the appropriate times. Among these, the transitions from G_1 phase to S phase and from G_2 phase to M phase are called checkpoints; they are strictly regulated and require the activation of distinct protein complexes (referred to here as the G_1-S complex and the G_2-M complex, respectively). The G_1-S complex and the G_2-M complex are distinct complexes that must be activated during different phases of the cell cycle. However, because they share a common protein, they are connected within the cell cycle system via multiple reactions. In other words, the cell cycle system can be considered a typical model of a connected system that contains two entities with distinct functions (the G_1-S complex and the G_2-M complex).

Analysis of the network structure of known cell cycle systems revealed that these two types of complexes belong to different buffering structures (Figure 1). In other words, it was predicted that the two types of protein complexes can be regulated independently of one another, because they are contained within different buffering structures. Using actual yeast, the research group verified this prediction by accurately

quantifying the levels of the two protein complexes in both wild-type and mutant strains using the state-of-the-art methods FCS and FCCS (Note 7). The results revealed that the levels of the more important G_2-M complex are regulated independently, without being influenced by the levels of the G_1-S complex. In other words, it was confirmed that a buffering structure containing G_2-M does indeed exist.

On the other hand, we found that controlling the amount of the G_1-S complex also affects the G_2-M complex. At first glance, this might seem like an undesirable result, but it is not. The predictions of structural sensitivity analysis—a model-free theory—are behaviors that should necessarily occur if the network information is correct. In other words, the fact that the predicted behavior was not observed in the verification experiments indicates that there is missing information in the underlying reaction network. The research group comprehensively explored all possible modifications to the known network information and demonstrated that there is only one possible addition of a reaction that would account for the observed behavior. This effectively predicted the existence of an unknown reaction as a necessary condition. The research group conducted further verification experiments and confirmed that the predicted reaction does indeed exist.

This research demonstrated that buffering structures—a principle of independent control or modularity predicted mathematically—do indeed exist in actual biological systems and perform critical functions for cells. Furthermore, by using theoretical predictions and experimental verification, the research group identified a previously unknown reaction in the yeast cell cycle system, thereby updating our understanding of a system that has long been studied in molecular biology. In addition to these achievements, the proposal of a new approach to systems biology—combining model-free theory with experimental validation—is significant. Specifically, when a contradiction arises between predictions based on model-free theory and the results of quantitative experiments, we can suspect a lack of network information and predict unknown reactions or regulatory mechanisms as necessary conditions to explain the experimental results. Such powerful predictive and verifiable elucidation was made possible precisely because we employed model-free theory.

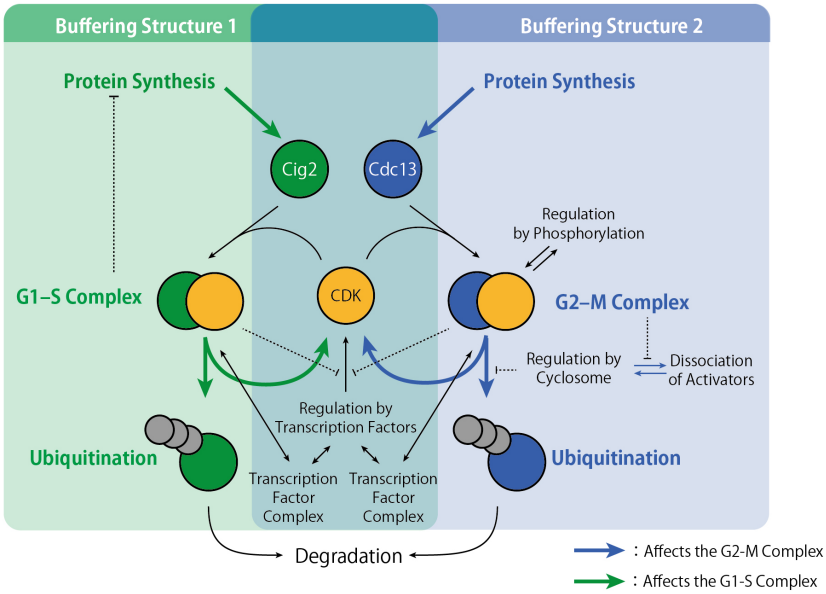

Figure 1. Analysis of the network structure of the cell cycle system.

Although the network is interconnected, the G_1-S complex and the G_2-M complex can be controlled independently.

3. Implications and Future Plans

Cells possess various other functions, and it is not yet fully understood how these are individually regulated. The research group believes that the vast reaction networks within cells contain many other buffering structures, which enable the independent regulation of different biological functions. One of the future goals of the group is to identify such buffering structures within the cell's vast reaction network and elucidate their functional significance. Understanding the mechanisms governing the regulation of cells will enable the rational selection of cells capable of high-yield production of useful substances, thereby facilitating effective progress in breed improvement and drug discovery.

We also believe that buffering structures have evolutionary significance. In other words, as long as a substructure within a reaction system satisfies the conditions (even if by chance) for becoming a buffering structure, that substructure acquires the function of a regulatory module. If it is adaptive for the substances contained within it to be controlled independently of others, the buffering structure will be maintained through evolution. The research group believes that such events occurred repeatedly during evolution, leading to the complexity of reaction networks.

Furthermore, structural sensitivity analysis is a method that can be generally applied to the dynamics generated by state transitions, not limited to intracellular reaction systems. We hope to widely promote this method as a way to elucidate the behavior of various dynamic systems—whether biological or non-biological—in a model-independent manner.

4. About the Research Project

This research was supported by the JST Strategic Basic Research Program (CREST) (JPMJCR1922, JPMJCR24Q4), JST ACT-X (JPMJAX22B8), the JSPS Grant-in-Aid for Scientific Research (JP24H00566, JP25K24467, JP19K16050, JP22K15110,JP22K15115, JP22H02625, JP23K14156, JP21J01354), the Takeda Science Foundation, the Dr. Yoshifumi Jigami Memorial Fund, a collaborative research project with the Center for the Exploration of Life Origins (ExCELLS) at the National Institutes of Natural Sciences, and the Collaborative Research Center at the Institute for Medical and Biological Research, Kyoto University, among others.

<**Glossary**>

Note 1 **Reaction network:** A structure composed of chemical species and reaction species, formed by the interconnection of numerous reactions occurring within a cell. It is estimated that the number of different reactions occurring within a cell ranges from several thousand to several ten thousand, and it is believed that all of these are interconnected to form a single network.

Note 2 **Buffering structure:** A substructure that defines the range of a response, as demonstrated by the "law of localization"—a theory mathematically proving that responses to changes in reaction system parameters are localized, based on structural sensitivity analysis (Note 4). This concept was discovered by Okada and Mochizuki in 2016. Buffering structures are fundamentally determined by local information

within the reaction network.

Note 3 **Cell cycle system:** A reaction network that determines the reaction dynamics of proteins and protein complexes governing changes in a cell's phase from one division to the next, particularly the critical transitions known as checkpoints.

Note 4 **Structural Sensitivity Analysis:** A mathematical analytical method that determines, based solely on the network structure, the changes in the concentration of each chemical and the reaction rates when various parameters (such as the amount or activity of enzyme) vary, with respect to the steady state of a reaction system's dynamics. It was developed by Mochizuki et al. in 2015. It is a model-free theory (Note 5) that uses only network information and does not require assumptions about reaction rate functions.

Note 5 **Model-free theory:** A theory that makes no assumptions about unknown information. Here, it refers to structural sensitivity analysis, which uses only the known reaction network and makes no assumptions about functions or parameters.

Note 6 **Topology:** A method of studying form by focusing solely on how components are connected, or the manner in which components are connected. Here, it refers to the connections between nodes (chemical substances) and arrows (reactions) in a reaction network.

Note 7: **FCS (Fluorescence Correlation Spectroscopy), FCCS (Fluorescence Cross-Correlation Spectroscopy):** FCS is a technique for measuring the concentration of molecules within living cells by analyzing fluctuations in fluorescence intensity associated with the entry and exit of fluorescently labeled molecules into the observation field (Brownian motion).FCCS is a type of FCS that quantifies the concentration of a complex formed by two molecules by analyzing the extent to which two types of molecules, labeled with different fluorescent colors, move together.

<Researcher's Comment>

Biological phenomena are complex, and it is difficult to understand the overall mechanisms simply by examining individual molecules one by one. Therefore, it is important to grasp the entire biological system theoretically, but this is no easy task. In this study, we elucidated the principles governing the cell cycle using a theory that explains biological phenomena based solely on network structure. We were also involved in developing a new measurement technique to verify this theory. I am very pleased that tackling a single challenge from both theoretical and experimental perspectives led to these results.(Yamauchi)

We have long been engaged in research aimed at understanding the dynamics of complex systems based on network information. In addition to developing what we call "structural theory"—a model-free theory—our goal has been to use these theories to elucidate actual biological phenomena and contribute to biology. Through this research, we have been able to achieve that goal in an ideal way. (Mochizuki)

<Paper Title and Authors>

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