論文の内容の要旨

Molecular mechanisms of cyanobacteriochrome signaling via c-di-GMP

（シアノバクテリオクロムが制御する c-di-GMP を介したシグナリングの分子機構）

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Introduction

Cyanobacteria are photoautotrophic prokaryotes that carry out oxygenic photosynthesis and they occupy a wide range of ecological niches. Light is an important environmental signal for almost all of life, and it is additionally crucial for phototrophs because it is essential for their phototrophic growth. Cyanobacteria contain photoreceptors, denoted cyanobacteriochromes (CBCRs), which are distantly related to phytochromes. CBCRs show various optical properties covering entire visible spectrum and they are usually found in a large number in a single cyanobacterial genome. However, how multiple and diverse cyanobacteriochromes work together in cyanobacteria was unknown. Some of cyanobacteriochromes carry output domains that are involved in regulation of a bacterial second messenger, c-di-GMP. C-di-GMP regulates a lifestyle transition, motility, cell cycle, and virulence in other bacteria, but its role in cyanobacteria remained elusive.

To study the photobiochemical and signaling mechanisms of CBCRs and c-di-GMP, I have focused on those in the thermophilic cyanobacterium *Thermosynechococcus*. CBCRs and c-di-GMP synthesis/degradation domain proteins are relatively small in number in *Thermosynechococcus* spp. and thus easier to explore the individual function

and the overall integrated system. There are 10 c-di-GMP synthesis/degradation domain proteins, of which only SesA (Tlr0924), SesB (Tlr1999), and SesC (Tlr0911) contain a photosensory domain. Previous study in my laboratory reported that SesA from *T. elongatus* has the CBCR-GAF domain activated by blue-light irradiation, and disruption of *Thermosynechococcus vulcanus sesA* has been shown to inhibit cell aggregation. The cellulose synthase *T. vulcanus* Tll0007, which has also been shown to be essential for cell aggregation, contains a putative c-di-GMP–binding PilZ domain and may be the acceptor for SesA-produced c-di-GMP. The functions of SesB and SesC to our knowledge had not been characterized before, but the presence of a CBCR-GAF domain in these two proteins implied that they might also be involved in the light-regulated cell aggregation. In this thesis, I studied the molecular mechanisms of CBCR signaling via c-di-GMP, focusing on photobiochemical properties and physiological function of SesA, SesB and SesC.

Results #1 Photochemical properties of SesB

For some cyanobacteriochromes, in addition to the widely conserved cysteine to anchor the chromophore, its ligation with a second cysteine is responsible for a remarkable blue shift. I show that SesB exhibits reversible photoconversion between a blue-absorbing form at 418 nm (P418) and a teal-absorbing form at 498 nm (P498). Acidic denaturation suggests that P418 harbors C15-*Z* phycoviolobilin, whereas P498 harbors C15-*E* phycoviolobilin. When treated with iodoacetamide, which irreversibly modifies thiol groups, P418 is slowly converted to a green-absorbing photoinactive form denoted P552. The absorption spectrum of P498 appears to be unaffected by iodoacetamide, but when iodoacetamide modified, it is photoconverted to P552. These results suggest that a covalent bond exists between the second Cys and the phycoviolobilin in P418 but not in P498. Subsequent treatment with dithiothreitol converts P552 into P418, whereas dithiothreitol reduces P498 to yield P420, a photoinactive form. Site-directed mutagenesis shows that the second Cys is essential for assembly of the photoactive holoprotein and that the photoactivity of this inert mutant is partially rescued by β-mercaptoethanol. These results suggest that the covalent attachment and detachment of a thiol, although not necessarily that of the second Cys, is critical for the reversible spectral blue shift and the complete photocycle. I propose a thiol-based photocycle, in which the thiol-modified P552 and P420 are intermediate-like forms.

Results #2 Photobiochemical properties and physiological functions of SesA/B/C

I first characterize the photobiochemical properties of SesB and SesC. Blue/teal light-responsive SesB has only c-di-GMP phosphodiesterase (PDE) activity, which is up-regulated by teal light and GTP. Blue/green light-responsive SesC has DGC and PDE activities. Its DGC activity is enhanced by blue light, whereas its PDE activity is enhanced by green light. A Δ*sesB* mutant cannot suppress cell aggregation under teal-green light. A Δ*sesC* mutant shows a less sensitive cell-aggregation response to ambient light. Δ*sesA*/Δ*sesB*/Δ*sesC* shows partial cell aggregation, which is accompanied by the loss of color dependency, implying that a nonphotoresponsive DGC(s) producing c-di-GMP can also induce the aggregation. The results suggest that SesB enhances the light color dependency of cell aggregation by degrading c-di-GMP, is particularly effective under teal light, and, therefore, seems to counteract the induction of cell aggregation by the main trigger SesA. In addition, SesC seems to improve signaling specificity as an auxiliary backup to SesA/SesB activities. The coordinated action of these three CBCRs highlights why so many different CBCRs exist.

Results #3 Detailed analysis of the whole c-di-GMP signaling network

The cellulose synthase, Tll0007, is essential for the cell aggregation and has a putative PilZ domain, which may work as a c-di-GMP binding module. However, experimental validation of c-di-GMP binding is lacking. Here, I show that Tll0007-PilZ domain indeed binds c-di-GMP by fluorescence resonance energy transfer (FRET)-based biosensor assays. The affinity towards c-di-GMP of Tll0007-PilZ is relatively low (K_d = 63.9 ± 5.1 μM), suggesting that the high amount of c-di-GMP is needed for the activation of Tll0007. I also show that SesA, the main trigger of the cell aggregation, is subject to product feedback inhibition with high affinity (IC50 = 1.07 ± 0.13 μM). The results suggest that Tll0007 may not be a direct target of c-di-GMP produced by SesA. I systematically analyze the contributions of all of the ten c-di-GMP synthesis/degradation domain proteins of *Thermosynechococcus*, and find that *tlr0627* gene is also crucial for the cell aggregation. Tlr0627 may work as a c-di-GMP amplifier to act downstream of SesA and activate Tll0007 directly. The two step regulation of c-di-GMP signaling seems to be reasonable because the light regulation of the “first” c-di-GMP pool by SesA/B/C will be efficient in the narrow range of c-di-GMP concentration. Cellulose produced by Tll0007 may work as a matrix to enclose gathered cells at later stages of cell aggregation, after amplification of the “second” c-di-GMP pools possibly by Tlr0627.

Discussion

In this thesis, I show that the three CBCRs SesA, SesB, and SesC have distinguishable, but congruent, light color-dependent c-di-GMP synthetic and/or degrading activities. The three CBCRs work together in a light color-sensitive manner to regulate cyanobacterial cell aggregation, probably by working as the most upstream system of the whole c-di-GMP signaling network. In terms of intramolecular signaling mechanisms, all of the three CBCRs are interesting targets because SesA shows the very strict light regulation; SesB shows two independent activation by teal light and GTP; and SesC shows different regulation of two output domains by its one CBCR-GAF domain. I show that Tll0007 is the first experimentally validated c-di-GMP binding proteins in cyanobacteria, but the direct target of c-di-GMP regulated by SesA/B/C may not be Tll0007, suggesting that another c-di-GMP receptor will await identification. This study will pave the way for elucidation of how cyanobacteriochromes orchestrate the c-di-GMP signaling for light-color acclimation responses in cyanobacteria.