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著者(和文)	長谷川葉月
Author(English)	Hazuki Hasegawa
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論 文 要 旨

THESIS SUMMARY

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学生氏名： Student's Name	長谷川 葉月	指導教員（主）： Academic Supervisor(main)	田中 寛
		指導教員（副）： Academic Supervisor(sub)	吉田 啓亮

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

The mechanisms of high temperature acclimation and high temperature tolerance are essential and are conserved in all organisms to survive temperature changes and high temperatures in their living environments. While highly conserved factors function in these mechanisms, species-specific factors and regulatory systems are also considered to be involved in each species, and their understanding has been elusive. In recent years, there has been concern that high temperature environments caused by global warming may have adverse effects on various biological processes involved in maintaining the global environment. Photosynthesis is a typical example, and it is urgent to elucidate the mechanisms of survival and growth at high temperatures in photosynthetic organisms. Thus, I decided to use an oxygen-evolving photosynthetic cyanobacterium *Synechococcus elongatus* PCC 7942 (hereafter *S. elongatus*) as experimental material. I investigated to the regulatory factors of high temperature acclimation and high temperature tolerance in *S. elongatus*. In this research, I identified two proteins which involved in the regulation of high temperature acclimation, especially in heat shock response, and one gene involved in growth at high temperature in *S. elongatus*.

Heat shock response induces transient expression of genes encoding heat shock proteins such as chaperones after sudden temperature upshift in order to acclimate to high temperatures, which is the initial stage of high temperature acclimation. Previous studies in our laboratory have shown that a two-component system, Hik2-Rre1, is involved in the heat shock response in *S. elongatus*, but the detailed regulatory mechanism has been elusive. I found a gene of unknown function that is highly conserved in the genome as a set with *hik2*, and named it *sinA* (<u>S</u>igma <u>In</u>teracting Protein <u>A</u>) because this gene product bound an RNA polymerase major sigma factor (RpoD1). Since the *sinA*-deficient strain showed a marked high temperature sensitivity, I hypothesized that SinA was involved in the Hik2-Rre1 mediated heat shock response. My results suggested that SinA indeed enhanced the Hik2-Rre1-dependent transcriptional activation of the heat shock response, thereby promoting high temperature acclimation. Furthermore, since Rre1 stability was markedly increased in *sinA*-deficient strain, I observed temperature-dependent growth in *rre1*-overexpressing strain. The results showed that *rre1* overexpression did not affect growth at normal growth temperature (30 ˚C), but inhibited growth at high temperature (40 ˚C). Therefore, it was suggested that quantitative control of Rre1 was required for high temperature acclimation and SinA was essential for high temperature acclimation. Previous study also suggested that the Hik2-Rre1-dependent heat shock response in *S. elongatus* is transient due to repression by newly synthesized protein(s) in heat shock response. However, the negative regulator(s) of heat shock response in *S. elongatus* has not yet been identified. Since Hsp70-type chaperone (DnaK) is known as a negative regulator of heat shock response in *E. coli* and other bacteria, we focused on one type of chaperone in *S. elongatus* that is an *E. coli* DnaK ortholog and an Rre1-dependent gene. I found that overexpression of the DnaK ortholog repressed Rre1 phosphorylation and expression of an Rre1-regulated heat shock response gene in heat shock response. The Hik2-Rre1 pathway is also known to be activated by the addition of DBMIB, a photosynthesis inhibitor, The overexpression of the DnaK ortholog also repressed Rre1 phosphorylation by DBMIB addition, similar to that observed in the heat shock response. In contrast, overexpression of another Rre1-regulated chaperone did not repress Rre1 phosphorylation in heat shock response. In addition, *in silico* analysis indicated the interaction between Hik2 and the DnaK ortholog. Thus, this DnaK ortholog in *S. elongatus* is considered to be a specific negative feedback regulator of Hik2-Rre1-dependent transcription via interaction with Hik2. In conclusion, I propose a model in which high temperature acclimation is induced by SinA-dependent gene expression and quantitative regulation of Rre1 in heat shock response, and the heat shock response is quenched by the DnaK ortholog-dependent negative feedback in *S. elongatus*.

One laboratory-stored *S. elongatus* strain (hereafter H1 strain) coincidentally showed a high temperature-sensitive growth phenotype. Here, I aimed to identify the genetic mutation(s) which caused the high temperature sensitivity of H1 strain. Whole genome sequencing of H1 strain identified a single nucleotide substitution mutation (G60T) in synpcc7942_R0040 encoding tRNA-Leu(CAA) was the cause of high temperature sensitivity of H1 strain. Secondary structure prediction of the tRNA indicated that this mutation decreased the length of the t-arm of tRNA-Leu(CAA) from 5 base pairs to 4 base pairs. Introduction of an additional wild-type gene copy encoding tRNA-Leu(CAA) with 5 base pairs of t-arm into the H1 strain restored growth of the H1 at high temperature. Interestingly, introduction of a mutated gene copy encoding tRNA-Leu(CAA) with 4 base pairs of t-arm into H1 strain also somewhat restored growth of H1 strain at high temperature. Moreover, suppressor mutant strains with suppressed high temperature susceptibility of H1 strain were isolated and whole genome sequencing of these strains revealed that secondary mutations in synpcc7942_1640 suppressed the high temperature-sensitive phenotype of H1 strain. synpcc7942_1640 putatively encodes a NYN domain-containing protein (*nynA*). The NYN domain is considered to be involved in tRNA/rRNA degradation. Thus, tRNA-Leu(CAA) with 4 base pairs of t-arm is likely unstable at high temperature and degraded by NynA in H1 strain. The quantitative deficiency of functional tRNA-Leu(CAA) inhibited translation, thereby inhibiting growth of H1 strain at high temperatures. The structural stability of tRNA-Leu is critical for growth at high temperatures in *S. elongatus*. In summary, this thesis discusses a regulatory model of the heat shock response and the importance of tRNAs in high temperature tolerance in the oxygen-evolving photosynthetic cyanobacterium *S. elongatus*.