Regulatory mechanism of expression of chaperone protein genes (*hsp* genes) in cyanobacteria

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Research objective

Cyanobacteria are a large group of bacteria that have dramatically changed the earth's environment through oxygenic photosynthesis. Furthermore, the photosynthetic function of plants is also derived from the symbiosis of cyanobacteria. The photosynthetic light reaction is an electron transport system driven by light and is inherently vulnerable to changes in the external environment, thus cyanobacteria have evolved strong stress response abilities and have thrived in extremely diverse environments. However, many aspects of this control mechanism are still unclear, and the purpose of this research is to elucidate the expression regulation mechanism of the chaperone genes (*hsp* genes) that support stress tolerance in cyanobacteria. Elucidation of the control system in cyanobacteria will lead to the elucidation of the mechanism that protects photosynthesis and is expected to provide basic information for various applications of photosynthetic function and maintenance of the global environment.

Regarding the regulation of the expression of chaperone proteins in bacteria, transcriptional activation by heat shock sigma factor (σ^H) in the heat shock response of *Escherichia coli* has been analyzed in the most detail. On the other hand, σ^H does not exist in *Bacillus subtilis*, and instead it has been shown that the heat shock response is due to the release of a repressor that binds to the promoter. A heat shock response mechanism based on *Bacillus subtilis*-type disinhibition was initially discovered in cyanobacteria, but this only affected the response of a small number of genes, and the molecular mechanism of the original heat shock response remains unclear. In this context, we found in the model cyanobacterium *Synechococcus elongatus* strain PCC 7942 that the two-component regulatory system Hik2-Rre1, which is universally conserved in cyanobacteria, is directly involved in the heat shock response. That is, the response regulator Rre1 was phosphorylated by the sensory kinase Hik2 during heat shock and bound to the promoter regions of many chaperone protein genes to activate transcription [ref 1)]. Furthermore, it was revealed that temperature information is not directly recognized by Hik2 kinase, but that Hik2 is involved in the state of the photosynthetic light reaction system that changes due to heat shock, especially the reduction state of plastoquinone [ref 2)]. In this study, we advanced our understanding of this Hik2-Rre1-dependent heat shock response and analyzed a newly discovered Hik2-Rre1-independent transcriptional activation mechanism of chaperone genes.

Methods

The promoter of the *hspA* gene, which encodes a small chaperone protein, was mainly used as the regulatory target for analysis. The phosphorylation state of the response regulator Rre1 was detected by phos-tag gel electrophoresis, and the interaction between DNA-binding proteins and promoters was analyzed by chromatin immunoprecipitation.

Results

The results obtained from this study are as follows.

1) The induction of expression of chaperone genes by heat shock response rapidly peaks and continues for about 30 minutes, and the expression declines to the basal level. At this time, if a protein synthesis inhibitor such as chloramphenicol was added to the medium during the heat shock, the induction continued without decline after the heat shock induction. This means that some protein synthesized after heat shock negatively regulates the response. In this study, we hypothesized that the Hsp70-type chaperone DnaK2 is a negative regulatory factor, based on previous findings in other organisms, and conducted verification experiments. When we construct a strain that can forcefully overexpress DnaK2 and apply heat shock while overexpressing DnaK2, Rre1 phosphorylation, binding to the promoter region, and downstream *hspA* gene expression are suppressed. In addition, since *in silico* analysis using AlphaFold2 suggested an interaction between Hik2 and DnaK2, we constructed a regulatory model in which DnaK2 accumulated upon heat shock induction suppresses sensory kinase Hik2 activity [ref 3)].

2) Over-oxidation of plastoquinone in the electron transport chain is induced by the inhibitor of photosystem II as DCMU. At this time, transcriptional activation of chaperone genes was also observed, but it was unclear whether Hik2-Rre1 was involved here as well. In this study, we analyzed the phosphorylation status of Rre1 and its binding to the promoter and found that no phosphorylation or DNA binding of Rre1 was observed. This result suggests that a signal transduction system different from Hik2-Rre1 is involved in the response to plastoquinone over-oxidation (manuscript in preparation).

3) In the process of analyzing the heat shock response system, we obtained an unknown temperature-sensitive mutant strain. As a result of analyzing the whole genome sequence, a point mutation in the gene encoding leu-tRNA was identified. Since a mutation that suppresses temperature sensitivity was mapped to a specific RNA degrading enzyme gene, it was concluded that this tRNA undergoes degradation due to changes in its 3D structure at high temperatures, making cell growth temperature sensitive [ref 4)].

Conclusion

Heat shock response is a temporary response accompanying a shift to high temperature in the growth environment. In previous studies, we have elucidated the induction mechanism of the heat shock response in cyanobacteria, and in this study, we have revealed the termination

mechanism of the heat shock response. Furthermore, two additional pathways were suggested to induce the expression of chaperone genes independent of the two-component regulatory system Hik2-Rre1. In summary, in cyanobacteria, various stresses are mainly recognized as changes in the photosynthetic light reaction system, and an overall picture of inducing chaperone genes through multiple signal transduction systems was revealed.

References

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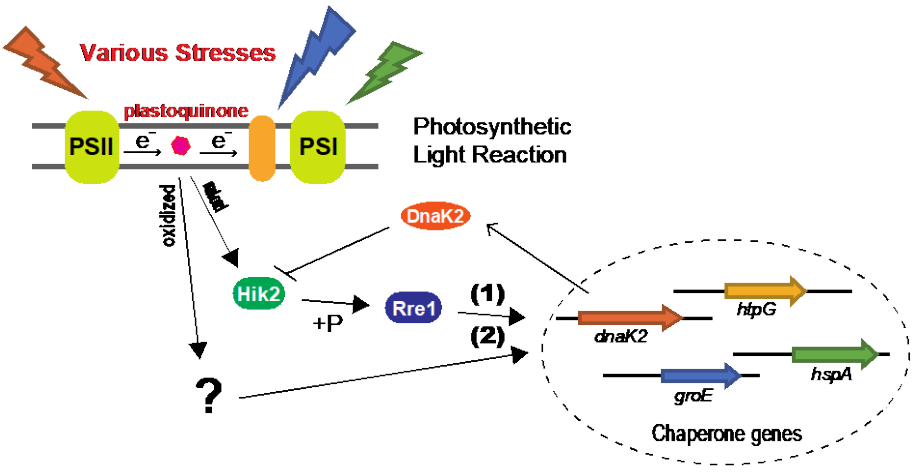

Fig. 1 Various stresses are recognized as changes of electron status in photosynthetic light reaction, where the information is transduced to modulate chaperone gene expressions through at least two independent signaling pathways.