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LitR-mediated Photoresponse in Non-phototrophic Bacteria: Studies on Its Molecular Mechanism and Potential Diversity

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Research aims

Light is an environmental stimulus that affects many living organisms; such is also the case for prokaryotes[1]. Light-inducible transcriptional regulator (LitR), a MerR-family transcriptional regulatory protein is a common central regulator for light-dependent carotenoid (Crt) production in *Streptomyces coelicolor*, a gram-positive soil bacterium, and *Thermus thermophilus*, an extremely thermophilic bacterium[3]. LitR may also serve as a photosensor by receiving blue light through cobalamin (Cbl; vitamin B12), a putative ligand for LitR[2]. To understand the molecular mechanism underlying LitR-mediated photosensing in non-phototrophic bacteria, we here examined biochemical properties of the *T. thermophilus* LitR and TTP55 proteins, which are putative central regulators for light-dependent transcription in this non-phototrophic bacterium. We also performed transcriptome analysis to identify novel light-inducible genes in *T. thermophilus*. In addition, we newly isolated light-responsive microorganisms from environmental samples with the aim of discovering proteins with novel function.

Methods

Glutathione S-transferase (GST)-tagged LitR and TTP55 proteins were prepared by an *E. coli* expression system using BL21(DE3) strain and pGEX-6P-2 vector. These GST fusion proteins were purified using a GST column, and the GST tag was removed by treatment with Precision protease. Using the LitR and TTP55 proteins thus prepared, DNase I footprinting analysis and *in vitro* run-off transcription assay were carried out. DNA microarray analysis of light-inducible genes in *T. thermophilus* was performed using GeneChip (Affymetrix), which contained probe sets of 25-mer oligonucleotides representing 2,238 open reading frames (ORFs), as well as 1,096 intergenic regions. Total RNA was isolated from cells cultured under the dark or light conditions.

We isolated approximately 2,300 bacterial strains from soil. Bacteria showing photo-dependent phenotypes were selected by growing the bacteria in an illuminating incubator (BR-180LF; Taitech). Phylogenetic analysis for bacteria thus selected was performed on the basis of the DNA sequences encoding 16S rRNA.

Results

1. Elucidation of the molecular mechanism for photoresponse in *T. thermophilus*.

Light-dependent transcriptional regulation of the *crt* biosynthesis gene cluster is primarily regulated by two transcriptional regulators: LitR and a CRP homolog (TTP55) encoded by a gene located downstream of the *litR* gene (Fig. 1). To observe the effect of LitR and TTP55 on the transcriptional activity of *crtB* (phytoene synthase gene), an *in vitro* transcription analysis was carried out. The *in vitro* transcription analysis showed that RNA polymerase holoenzyme and the recombinant TTP55 were sufficient for generation of a specific transcript exhibiting the length corresponding to that for *crtB*. This activation by TTP55 at least required the position -56 with respect to the transcriptional start site of *crtB*. Furthermore, the assay revealed that recombinant LitR inhibits TTP55-dependent transcription in a dose-response manner. DNase I footprinting assay showed that the LitR binding site is located at the positions between -55 and -89 on the *crtB* promoter. Transcriptome analysis using DNA microarray revealed the existence of twenty-three novel light-induced genes, including *TTHB89-91*, *TTHB94-97* and *TTHB112-113*, which are located in the neighborhood of the *litR* and *crt* genes. The function of most of these gene products is unknown. This indicates that further studies may lead to the discovery of proteins with novel functions.

2. Screening for light-dependent microorganisms and their function.

From environmental soil samples, we isolated 80 bacterial strains which produce, in response to illumination, yellow or orange pigments that are most likely carotenoids. Analysis of 16S rRNA sequences indicated that the light-responsive bacteria isolated in this study belong to the following genera: Group 1 contained *Bacillus*, *Lysinibacillus*, *Staphylococcus*, *Halomonas*, *Pseudomonas*, *Ralstonia*, and *Sphingomonas*; Group 2 contained *Arthrobacter*, *Brevibacterium*, and *Microbacteria*. The bacteria were classified into two groups based on the presence or absence of the *litR* homologue (or known photosensor genes) on the genomes of closely-related bacteria. Bacteria belonging to the group 1 have *litR* homologue(s) in their genome. In contrast, bacteria belonging to the group 2 do not retain *litR* or any other known photosensor genes. This result indicated the possible presence of novel light-sensing proteins in the group 2 bac-

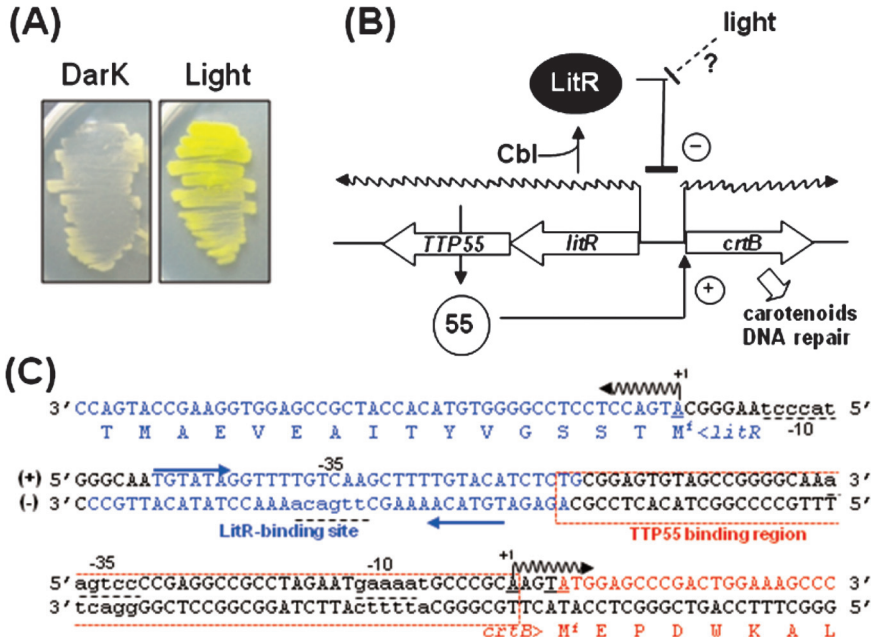

Fig. 1. Light-dependent carotenoid production (A) and its regulatory mechanism (B) in *T. thermophilus*. LitR protein binds to the promoter region of *crtB* (C) to repress its transcriptional initiation under dark conditions. Absorption of blue light by the LitR-cobalamin (Cb1) complex may cause its inactivation followed by a conformational change in protein structure, which in turn allows TTP55, a cAMP receptor-like activator protein, to promote the transcription of *crtB*.

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Conclusion

The lines of evidence obtained in this study indicates that TTP55 is a positive regulator essential for transcriptional initiation of the *crt* gene cluster and that LitR confers photo-dependency to the TTP55-dependent transcription via its negative regulatory action. Moreover, novel light-responsive genes related to LitR and TTP55 were identified via transcriptome analysis. Our screening for non-phototrophic bacteria exhibiting light-responsive phenotype showed the diversity of photoresponse in these bacteria and implied the existence of an as-yet-unidentified photosensor in Actinobacteria. These findings provide new insights into the physiology and ecology of non-phototrophic bacteria.

References

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