「ライフサイエンス統合データベース開発運用」 (統合データベース開発:植物及び植物関連微生物の ゲノム情報データベース統合と高度化)

実施機関:かずさディー・エヌ・エー研究所 担当責任者:中村保一









成果報告

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デノムアノテーション情報の蓄積・公開

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- **~**蓄積した情報を閲覧する方法の開発



論文からの "Gene Indexing"

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JOURNAL OF BACTERIOLOGY, Apr. 2007, p. 2750–2758 0021-9193/07/\$08.00+0 doi:10.1128/JB.01903-06 Copyright © 2007, American Society for Microbiology. All Rights Reserved.

Coordinated High-Light Response of Genes Encoding Subunits of Photosystem I Is Achieved by AT-Rich Upstream Sequences in the Cyanobacterium *Synechocystis* sp. Strain PCC 6803[∇]

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Genes encoding subunits of photosystem I (PSI genes) in the cyanobacterium Synechocystis sp. strain PCC 6803 are actively transcribed under low-light conditions, whereas their transcription is coordinately and rapidly down-regulated upon the shift to high-light conditions. In order to identify the molecular mechanism of the coordinated high-light response, we searched for common light-responsive elements in the promoter region of PSI genes. First, the precise architecture of the *psaD* promoter was determined and compared with the previously identified structure of the *psaAB* promoter. One of two promoters of the *psaAB* genes (P1) and of the *psaD* gene (P2) possessed an AT-rich light-responsive element located just upstream of the basal promoter region. These sequences enhanced the basal promoter activity under low-light conditions, and their activity was transiently suppressed upon the shift to high-light conditions. Subsequent analysis of *psaC*, *psaE*, *psaKI*, and *psaLI* promoters revealed that their light response was also achieved by AT-rich sequences located at the -70 to -46 region. These results clearly show that AT-rich upstream elements are responsible for the coordinated high-light response of PSI genes dispersed throughout Synechocystis genome.

Photosynthetic organisms have ability to cope with the changes in light environment by modulating both the structure and the function of the photosynthetic machinery (31, 59). A typical example is the flexible control of the amounts of photosystem (PS) and light-harvesting antenna complexes depending on the availability of light energy (4, 27, 38). Under light-limiting conditions, the amount of these complexes is maintained at high level, because maximal capture of light energy is required to fulfill the energy demand of cells. Under light-saturating conditions, on the other hand, they are largely downregulated since absorption of excess light energy tends to cause the generation of harmful reactive oxygen species (6).

The dynamics of reaction center complexes during the process of high-light (HL) acclimation have been well characterized in cyanobacteria. Amount of PSI is more strictly downregulated than that of PSII upon the exposure to HL (28, 40). The analysis of the *pmgA* mutant deficient in down-regulation of PSI content revealed that the selective repression of PSI is essential for growth under continuous HL conditions (28, 54). Although the primary determinant of PSI content under HL conditions has not been identified, transcriptional regulation is likely to be one of the important factors. The cyanobacterial PSI complex is comprised of about 11 subunits, with some exceptions (23), and genes encoding these subunits (PSI genes) are dispersed throughout the genome. In *Synechocystis* sp. dinately and rapidly down-regulated upon the shift to HL conditions (26, 29, 30, 42, 57), except for the *psaK2* gene encoding an HL-inducible isoform of the PsaK subunit (19). PSI transcripts become barely detectable within 1 h of HL exposure and then gradually reaccumulate after 3 h. The change in promoter activities of PSI genes is well coincident with the change in transcript levels (42, 43), suggesting that the coordinated light response of PSI genes is achieved at the level of transcriptional regulation. In the course of HL acclimation, cells need to activate genes related to several processes such as CO₂ fixation, protection from photoinhibition, and general stress management (29). The down-regulation of high promoter activities of PSI genes upon the shift to HL conditions may be important not only for the repression of PSI content, but also for the recruitment of RNA polymerases to active transcription of such HL-inducible genes.

strain PCC 6803, PSI genes are actively transcribed under

low-light (LL) conditions, whereas their transcription is coor-

As the first step for the elucidation of the molecular mechanism of coordinated HL response of PSI genes in Synechocystis sp. strain PCC 6803, we recently dissected the promoter architecture of the psaAB genes encoding reaction center subunits (43). The *psaAB* genes have two promoters, P1 and P2, both of which are responsible for the photon flux densitydependent transcription. Deletion analysis of the upstream region of *psaAB* fused to bacterial luciferase reporter genes (luxAB) indicated that the light responses of P1 and P2 are achieved in different manners. The cis element required for the light response of P1, designated as PE1, was located just upstream of the -35 element of P1 and was comprised of AT-rich sequence. PE1 activated P1 under LL conditions, and the down-regulation of P1 was achieved by rapid inactivation of PE1 upon the shift to HL conditions. On the other hand, the cis element required for the light response of P2, designated as



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FIG. 3. Mapping of the 5' ends of PSI transcripts. (A) Total RNA was isolated from the wild-type cells incubated under HL conditions for 0, 1, 3, and 6 h and used for primer extension analysis of *psaC*, *psaE*, *psaFl*, *and psaLl*. Detected 5' ends of the major transcripts are indicated by asterisks, and those of minor ones are indicated by dots. (B) Nucleotide sequences of the core promoter and its upstream region of PSI promoters. Transcriptional start points are shown in boldface letters. The promoters are aligned according to the major transcriptional start point noted as +1. Putative -35 and -10 hexamers are boxed. Light-responsive positive elements identified in *psaAB* and *psaD* promoters are shaded in gray. The nucleotide shown to be critical for the light response of *psaAB* promoter (43) are underlined. The numbers in parentheses shown above the nucleotide sequence of the P2 promoter of *psaFJ* indicate the position according to the major transcriptional start point of the P1 promoter.

the analysis since the arrangement of regulatory elements for two overlapping promoter is difficult to predict without precise promoter analysis.

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Effect of the -70 to -46 region on the promoter activity of PSI genes. Figure 4A shows the bioluminescence level of Synechocystis cells harboring PSI promoter-luxAB reporter genes with or without the upstream region under LL conditions. The reporter activities of the downstream promoter fragments alone were generally low, but there existed some differences among them. For example, the promoter activity of the downstream region was low in the case of psaD [(8.2 \pm 0.2) \times 10⁵ relative units/OD₇₃₀] and *psaK1* [(2.3 ± 0.4) × 10⁵ relative units/OD₇₃₀], whereas that of *psaLI* was significantly high [(1.1 \pm 0.2) $\times 10^7$ relative units/OD₇₃₀]. It is possible that the high activity of the *psaLI* promoter is brought about by a positive regulatory element located within the downstream region. When the AT-rich upstream region was added, each promoter displayed much higher activity compared with the corresponding derivative containing only the downstream region. This demonstrates that the -70 to -46 region can work as a positive regulatory element for every PSI gene examined here. The low activity of *psaD* and *psaK1* promoters was largely upregulated in the presence of the upstream region by 40.1- and 99.9-fold, respectively. On the other hand, strong promoter activity of *psaL1* was not enhanced as much by the upstream region (5.1-fold). As a result, similar promoter activity (around 5.0×10^7 relative units/OD₇₃₀) was attained among PSI genes irrespective of the activity of the downstream promoter region.

Next, we transformed *E. coli* cells with the above mentioned reporter constructs and measured the level of bioluminescence to see whether the upstream region can work as a positive regulatory element in *E. coli* cells (Fig. 4B). In all strains harboring PSI promoter-*luxAB* constructs, the luminescence level was higher than that of the control cells having promotereless *luxAB* genes [$(1.4 \pm 0.5) \times 10^8$ relative units/OD₆₀₀], showing that PSI promoters can be recognized by RNA polymerase of *E. coli*. The rank orders of promoter strength are similar in both *Synechocystis* and *E. coli* cells. Namely, the activities of the downstream promoter fragments of *psaD* and *psaK1* were low [$(2.4 \pm 1.0) \times 10^8$ relative units/OD₆₀₀ and

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⁷ Published ahead of print on 2 February 2007.

ソーシャルブックマーク(SBM)による 遺伝子=URLへの文献情報蓄積

slr0473 Cph1, phytochrome

http://bacteria.kazusa.or.jp/cyanobase/Synechocystis/cgi-bin/orfinfo.cgi?title=Chr&name=slr0473

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[pmid: 9278513] [table1]
[pmid: 9278513] [fig3]



Gene Indexing 入力・公開状況

Organisms	Papers
Lotus japonicus	297
Mesorhizobium. loti MAFF303099	178
Sinorhizobium. meliloti st.1021	163
Bradyrhizobium japonicum USDAIIO	933
Rhizobium sp. NGR234	125
Rhizobium leguminosarum bv. viciae 3841	1,162
Anabaena sp. PCC 7120	588
Synechocuccus elongatus PCC 7942	449
Chlorobium tepidum TLS	174
Synechocystis sp. PCC 6803	I,490
Nostoc punctiforme ATCC 29133	54
Total	5,613

Data entries	145,890

9, Dec., 2009

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249,082	26,764	36,9	968		
最近のアノテーション					
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蓄積した情報の利用・閲覧方法の開発

CyanoBase

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cyanobase » Synechocystis sp. PCC 6803 » genes » slr0473

GeneView: slr0473

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Summary | Genomic context | Transcript | Peptide | Protein domains | Mu

ID	slr0473
Туре	Protein Coding
Gene Symbol	cph1, hik35
Gene Indexing gname	cph1, hik35, phy
Definition	cyanobacterial phytochrome 1, two-compo
Functional category	Regulatory functions
Genes and Mutants	Gene: by Teruo Ogawa (1998-04-15) Gene: phy by Masahiko Ikeuchi (1999-09- Gene: phy by Masahiko Ikeuchi (1999-09- Gene: Hik35 by Iwane Suzuki (2000-05-25

Gene: phy by Masaniko Ikeuchi (1999-09-Gene: Hik35 by Iwane Suzuki (2000-05-25 Mutant: Δphytochrome by Teruo Ogawa (Mutant: SK35 by Teruo Ogawa (2003-05-Mutant: Δhik35 by Iwane Suzuki (2007-11)

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ゲノムデータベースに "Gene Indexing" した情報を 即時公開

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1. Heyne, K. Herbst, J. Stehlik, D. Esteban, B. Lamparter, T. Hughes, J. Diller, R. Ultrafast dynamics of phytochrome from the cyanobacterium synechocystis, reconstituted with phycocyanobilin and phycoerythrobilin. Biophys J. 2002 Feb;82(2):1004-16. PMID:11806940 | Abstract | MeSH | Related Articles | Sections | Gene Index | Ab In Mm Re Di Co Fi Ta I abstract Introduction materials and methods results discussion table1 fig2 fig3 fig4 fig5 fig6 fig7 fig8 fig9 fig10 fig11 fig12

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Formation of the early photoproduct lumi-R of cyanobacterial phytochrome cph1 observed by ultrafast mid-infrared spectroscopy.
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Pleiotropic effect of a histidine kinase on carbohydrate metabolism in Synechocystis sp. strain PCC 6803 and its requirement for heterotrophic growth.

J Bacteriol. 2005 Apr;187(7):2368-76. PMID:15774880 | Abstract | MeSH | Related Articles | Sections | Gene Index | Ab In Mm Re Di Co Fi Ta | gname:cph1 abstract introduction materials and methods results discussion table1 table2 fig2 fig3 fig5

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The structure of a complete phytochrome sensory module in the Pr ground state.

Proc Natl Acad Sci U S A. 2008 Sep 23;105(38):14709-14. Epub 2008 Sep 17. PMID:18799745 | Abstract | MeSH | Related Articles | Sections | Gene Index |

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4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 hr

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2.4FPS

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H22 (2010)年度の重点は新規開発から 運用・改善のための開発と蓄積公開へ



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 - ・シロイヌナズナ (植物の統合)

- 集積した情報から生物学的な意味
 を取り出すための情報表現技術
- ●ビューワの改善と提供
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- •アノテーション手法の改善



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≫修士: 谷中(つくば) <u>阿久津(木更津)</u>鐘ケ江(東京) 笠井(つくば)

