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Role of the 5′-UTR in accumulation of the *rbpA1* transcript at low temperature in the cyanobacterium *Anabaena variabilis* M3

Shigeki Ehira [a,b], Masayuki Ohmori [a,b], Naoki Sato [a,b,*]

[a] *Department of Biochemistry and Molecular Biology, Faculty of Science, Saitama University, 255 Shimo-Ohkubo, Sakura, Saitama 338-8570, Japan*
[b] *Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro, Tokyo 153-8902, Japan*

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Abstract

The expression of the *rbp* genes, which encode RNA-binding proteins with a single RNA-recognition motif and a glycine-rich sequence, is known to increase at low temperature in cyanobacteria. We previously showed that their regulation involved both transcription and mRNA stability. In the present study, various reporter constructs with deletions and mutations were used to analyze this regulation, revealing that at least the following three elements are involved. First, a putative enhancer element is located within the upstream gene. Second, the *rbpA1* transcript is dramatically stabilized by a large stem-loop structure located at the 5′ terminus. Third, the transcript is also destabilized by a downstream box located within the coding region.

Keywords: Cyanobacterial *rbp*; Low temperature; *Anabaena variabilis* M3; 5′-UTR; RNA stability; Downstream box

1. Introduction

The *rbpA1* gene encodes an RNA-binding protein, having a single RNA-recognition motif at its N-terminus and a glycine-rich sequence at its C-terminus. The genes encoding such glycine-rich RNA-binding proteins have been identified in various organisms, such as animals [1–3], flowering plants [4–6], moss [7] and cyanobacteria [8–11]. In most of them, accumulation of their transcript is elevated by temperature downshift. This suggests that the glycine-rich RNA-binding proteins play an important role in the acclimation to low temperature in various organisms. However, it remains to be determined how glycine-rich RNA-binding proteins function in acclimation to low temperature and how their expression is regulated.

A family of *rbp* genes has been identified in *Anabaena variabilis* M3, consisting of nine members: *rbpA1*, *rbpA2*, *rbpA3*, *rbpB*, *rbpC*, *rbpD*, *rbpE*, *rbpF* and *rbpG* [10–13]. Among them, *rbpA1*, *rbpA2*, *rbpA3*, *rbpB*, *rbpC*, *rbpE* and *rbpF* were upregulated by a downshift in growth temperature. An analysis of upstream sequence of these genes revealed that a 140 bp region that lies upstream of the translation start site was conserved among the cold-regulated *rbp* genes [11]. We also showed that the *rbp* genes having a conserved upstream sequence were upregulated at a low temperature in other strains of cyanobacteria, *Anabaena* PCC 7120 and *Thermosynechococcus elongatus* BP-1 [14]. These findings suggest that a mechanism of cold-regulation of *rbp* genes is common among various species of cyanobacteria and the conserved upstream sequence is involved in cold-regulation of the *rbp* genes.

* Corresponding author. Tel.: +81 3 5454 6631; fax: +81 3 5454 6998.
E-mail address: naokisat@bio.c.u-tokyo.ac.jp (N. Sato).

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In the present study, we analyzed the involvement of various sequence elements in the accumulation of the *rbpA1* transcript at different temperatures using *rbpA1–lacZ* fusion transcripts with deletions and mutations, and found that the following three elements are involved in the regulation of *rbpA1* transcript: a temperature-dependent putative enhancer element within the upstream gene, a large stem-loop structure formed at the 5′ terminus of the transcript, and a downstream sequence within the coding region that reduces the stability of the *rbpA1* transcript.

2. Materials and methods

2.1. Strains and growth conditions

A. variabilis M3 was grown at 38 °C in the medium C of Kratz and Myers [15] under continuous illumination at 50 μE m^{-2} s^{-1}. *Anabaena* sp. PCC 7120 was grown at 32 °C in the BG-11 medium [16]. The *rbpA1* deletion mutant derived from *Anabaena* PCC 7120 was generated by replacing the entire coding region (nucleotides −1 to +329 with respect to the translation start) with a neomycin-resistant cassette. Liquid culture was bubbled with air containing 1.0% (v/v) CO_2. Temperature-shift experiments were performed as described previously [17]. For the growth of *A. variabilis* M3 containing a shuttle vector with various *rbpA1–lacZ* constructs, neomycin was added to a final concentration of 30 μg ml^{-1}.

2.2. Plasmid construction

For construction of pU209, pU177, pU140, pU100, pU62, pU35, pU8 and pU+14 (Fig. 2(c)), the upstream regions of *rbpA1* were amplified by PCR with a primer R1 in combination with primers F1, UP, F7, F8, F9, F12, F10 and F11, respectively (Table 1). For construction of pD139, pD112, pD103, pD46, pD22 and pD4 (Fig. 3), a primer F1 was used in combination with primers R6, DN1, DN3, 2R, R12 and R13, respectively. The plasmid pLti26 [12] was used as a template DNA. Each PCR fragment was inserted into the *Kpn*I–*Bam*HI site of pRA201, which is a derivative of pRA101 [17] (Fig. 2(a)).

Mutation constructs were made by PCR using specific primers in which the conserved sequence was replaced by a restriction enzyme recognition sequence (Fig. 3). One of the combinations of two pairs of primers, F1:R3 and F3:R6, F1:R4-1 and F4-1:R6, or F1:R5 and F5:R6, were used for construction of pMBI, pMBII and pMBIII, respectively. pMR was constructed as follows: PCR fragments amplified with pairs of primers, F1:R2 and F2:R1, were cloned into pBluescript II SK+ (Stratagene, CA, USA). The resultant plasmid was used as a template for PCR with primers F1 and R6. For construction of pCR-BI, pMR-BI, pMR-M1 and pMR-M2, the pMR was used as a template. One of the combinations of two pairs of primers, F1:R3 and F3:R6, or F1:R3-1 and F3-1:R6, were used for construction of pCR-BI and pMR-BI, respectively. Two pairs of primers, F1:R6-2 or F1:R6-3, were used for construction of pMR-M1 or pMR-M2, respectively. The upstream region of *rbpA1* with mutation was reconstructed on pRA201.

For construction of p*tac*UTR and p*tac*R-BI, PCR fragments amplified with one of the pairs of primers F13:R6-1 and F6:R6-1, respectively, were cloned into the *Bam*HI–*Kpn*I site of pRL191*tac* [18].

The DNA sequences of all the constructs were confirmed by the dye-terminator method using the BigDye Terminator Cycle Sequencing Ready Reaction Kit and the sequencer model 310 (Applied Biosystems Japan, Tokyo, Japan).

Table 1
Primers used in this study

Primer	Sequence (5′–3′)[a]
F1	atggtaCCGTAGCCTTGCGTTTGAAGGAGTTGG
F2	ataagcttACCATCAATGTCAATTTACGTAGGCA
F3	ataagcttAATTTACATCTCTAGACAGTAACAAT
F3-1	atgaattcAATTTACATCTCTAGACAGTAACAAT
F4-1	atgatatcTTAGTATTGACGGGCTTTTCCCTTTT
F5	ataagcttAGTATCTTTGTTTTTAGTATTGACGG
F6	atggaTCCCTTTTAGTATTTACAGGC
F7	atggtaccAATGGCAGCTTGGACAGCAT
F8	atggtaccTTAATCCCAATTAAAAGCTC
F9	atggtaccATCCTACGTAACTAATTATC
F10	atggtaCCTAGAAAAGGAGAGTTAATTC
F11	atggtaccGGTGAGTATCTTTGTTTTTAG
F12	atggtaccGATGCAGAGAATTGGCTTCT
F13	taggatccGGAGAGTTAATTCGGTGAGT
UP	gaggtaccAGAGCGAATTGCTGAGAA
R1	atggatccGAACACGTTTTACAGTACCGTATTC
R2	ataagcttAATTGTTACTGTCTAGAGATGTAAAT
R3	ataagcttAAAAGCCTGTAAATACTAAAAGGGAA
R3-1	atgaattcAAAAGCCTGTAAATACTAAAAGGGAA
R4-1	atgatatcAGATACTCACCGAATTAACTCTCCTT
R5	ataagcttATTAACTCTCCTTTTCTAGGATGACA
R6	atggatccTGCCTACGTAAATTGACATTGATGG
R6-1	atggtaccTGCCTACGTAAATTGACATTG
R6-2[b]	atggtaccTG**ATCTGA**TAAATTGACATTGATG
R6-3[b]	atggtaccTGCCTACGT**CGTCGT**ACATTGATG
R12	atggatccTACTCACCGAATTAACTCTCC
R13	atggaTCCTTTTCTAGGATGACATAG
2R	atggatccGCCCGTCAATACTAAAAACAAAGATACTCACCG
DN1	tgggaTCCAAAATTGTTACTGTCTAGAG
DN3	atggatccGTTACTGTCTAGAGATGTAAAT
flzF	atctcgaggatccccGTCGTTTTACAACGTCGTGACTG
lzR	TCAGCTGGAATTCCGCCGATACTG
D-A1	GCGCTCAAGGTGTCTTGAGTAAC
D-trpA	AGCACCGGCTTCAATTAGCGTATCG

[a] Additional sequence at the 5′-end is shown in lowercase, and added restriction site is underlined.
[b] The original nucleotides were replaced by the nucleotides shown in bold letters to replace the sequence.