## Responses to temperature

### 073(1aC01)

STRUCTURAL ANALYSIS OF A RNA-BINDING PROTEIN, RbpA1, IN *Anabaena variabilis* M3 WITH HETERONUCLEAR MULTI-DIMENSIONAL NMR SPECTROSCOPY

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RbpA1 is a low-temperature induced protein which binds to RNA. It recognizes and unfolds the locally double-stranded RNA, which inhibits the translation of genetic information to protein, to single-stranded RNA. RbpA1 is thought to be an evolutionary fundamental RNA-binding protein because of containing two functional domains, an RNA recognition motif (RRM) and a glycine-rich domain, found in eukaryotes. To understand the structure-function relationships, we have analyzed the solution structure of each domain in RbpA1 with the heteronuclear multi-dimensional NMR spectroscopy.

We have prepared the $^{15}N$-labelled or $^{13}C$- and $^{15}N$- labelled RbpA1 sample expressed in *E.coli* and measured the $^{1}H$-$^{15}N$ HSQC, CBCA(CO)NH, HNCACB, and $^{1}H$-$^{15}N$ HSQC-NOESY spectra. In the $^{1}H$-$^{15}N$ HSQC spectrum, all peaks corresponding to each amino acid residue in RbpA1 were found clearly as well-resolved signals, and this indicates that RbpA1 has the stable solution structure, and each functional domain has the specific higher-ordered structure in solution. On the basis of the analysis of other spectra, we have assigned each signal in the $^{1}H$-$^{15}N$ HSQC spectrum and analyzed the secondary structure of functional domains.

### 074(1aC02)

ANALYSIS OF TRANSCRPTIONAL REGULATION OF COLD INDUCED *rbpA1* GENE

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The cyanobacterium *Anabaena variabilis* strain M3 contains 8 *rbp* genes that encode RNA-binding protein with an RNA recognition motif. The expression of the 7 members of *rbp* genes is induced by low temperature. These 7 *rbp* genes have 4 conserved sequences in the 5'-untranslated region (5'-UTR): namely, ribosome-binding site RBS (5'-TTYGGAGA-3'), BOX1 (TCTCCGAA), BOX2 (TTGTTTNNAGT) and BOX3 (TTCGGYGA). The DNA-binding proteins that bind to the 5'-UTR were detected in the extract from cells grown at high temperature (38°C). Then, we performed gel mobility shift analysis using mutated probes of the conserved sequences. The result suggested that the conserved sequences in the 5'-UTR play an important role in the binding to proteins. We tried to identify proteins that bind to the 5'-UTR of *rbpA1* gene. The proteins that bind to the 5'-UTR were purified by ammonium sulfate fractionation and affinity technique using magnetic particles. We purified 2 single-stranded DNA-binding proteins. There are a few more proteins that binds to the 5'-UTR.

### 075(1aC03)

LOW-TEMPERATURE SIGNAL TRANSDUCTION PATHWAYS WHICH REGULATE THE EXPRESSION OF *rbp*1 AND *crh* GENES IN *Synechocystis*.

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Genes encoding cold-inducible proteins are regarded to be essential for microorganisms and plants to acclimate to low-temperature stress. In this study, we investigated the low-temperature-induced expression of the *rbp*1 gene for an RNA-binding protein and the *crh* gene for RNA helicase in *Synechocystis* sp. PCC 6803.

We found that kinetics of induction of these two genes were different. Moreover, inactivation of Hik33 and Hik19 by targeted mutagenesis suppressed the cold-inducible expression of the *crh* gene, but not the *rbp*1 gene. These results suggest that there are two cold-signal transduction pathways in Synechocystis; Hik33 and Hik19 are involved in one of the pathways but not in other.

### 076(1aC04)

CHARACTERIZATION OF A MUTANT OF HISTIDINE KINASE-33, A PUTATIVE SENSOR OF LOW TEMPERATURE SIGNALS IN *Synechocystis* PCC6803.

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Upon exposure to low temperature, cells of *Synechocystis* PCC6803 regulate the expression of certain genes. We have demonstrated that a histidine kinase, Hik33, may be involved in the perception of the low temperature signals to up-regulate some of the low-temperature inducible genes. To investigate the function of Hik33, we mutated the gene for Hik33 and monitored changes in the gene expression by DNA microarray technique. The results indicated that some genes coding for components of transcription and translation were up-regulated and some genes coding for components of photosystems were down-regulated in wild-type cells, and that changes in levels of expression of these genes were smaller in cells of Hik33 mutant. These findings suggeted that Hik33 may be essential for the up-regulation and down-regulation of expression of the genes at low temperature.