Dynamic transcriptional changes in response to rehydration in *Anabaena* sp. PCC 7120

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Global transcriptional responses to dehydration and rehydration were determined in *Anabaena* sp. PCC 7120. Nearly 300 genes were up- or downregulated during both dehydration and rehydration. While as many as 133 genes showed dehydration-specific downregulation, only 29 genes showed dehydration-specific upregulation. In contrast, while only 13 genes showed rehydration-specific downregulation, as many as 259 genes showed rehydration-specific upregulation. The genes upregulated during rehydration responded rapidly and transiently, whereas those upregulated during dehydration did so gradually and persistently. The expression of various genes involved in DNA repair, protein folding and NAD synthesis, as well as genes responding to nitrogen depletion and CO₂ limitation, was upregulated during rehydration. Although no genes for transcriptional regulators showed dehydration-specific upregulation, eight showed rehydration-specific upregulation. Among them, two genes, *ancrpB* and *alr0618*, encode putative transcriptional activators of the cAMP receptor protein (CRP) family. DNA microarray analysis using gene disruptants revealed that AnCrpB and Alr0618 regulate the genes induced by nitrogen depletion and by CO₂ limitation, respectively. We conclude that rehydration is a complex process in which the expression of certain genes, particularly those for metabolism, is dramatically induced.

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INTRODUCTION

Desiccation-tolerant organisms are able to retain their cellular components during desiccation and rapidly resume metabolism when they are rewetted after prolonged desiccation (Potts, 1994, 2001). It is widely believed that the accumulation of disaccharides, such as trehalose and sucrose, during the dehydration process confers desiccation tolerance. However, it has become increasingly clear that true desiccation tolerance is likely to involve several mechanisms working in concert. These include the scavenging of reactive oxygen species, the downregulation of metabolism, and the accumulation of certain amphiphilic solutes, proteins and polysaccharides (Oliver *et al.*, 2001). The cellular responses to desiccation and rehydration are distinct from one another and are equally complex (Shaw *et al.*, 2003; Singh *et al.*, 2005). The strictly regulated

resumption of cellular metabolic processes is required on rehydration (Potts, 1994; Scherer *et al.*, 1984). Furthermore, some damage to DNA and membranes becomes manifest upon rehydration (Potts, 1994).

The N₂-fixing filamentous cyanobacteria *Nostoc commune* can survive repeated cycles of desiccation and rehydration (Potts, 1994, 1999; Shaw *et al.*, 2003). Desiccated *N. commune* rapidly recovers its photosynthetic activity after being soaked (Satoh *et al.*, 2002; Scherer *et al.*, 1984; Tamaru *et al.*, 2005). An extracellular substance, such as exopolysaccharide (EPS), seems to function in desiccation tolerance (Helm *et al.*, 2000; Hill *et al.*, 1994a, b, 1997; Scherer & Potts, 1989; Shaw *et al.*, 2003; Shirkey *et al.*, 2000; Tamaru *et al.*, 2005; Wright *et al.*, 2005). Only a few studies of the intracellular mechanism of desiccation tolerance have been reported (Shirkey *et al.*, 2000; Xie *et al.*, 1995).

Anabaena sp. PCC 7120 (hereafter *Anabaena* PCC 7120), which is a close relative of the desiccation-tolerant *Nostoc* sp. HK-01 (Katoh *et al.*, 2003), is also tolerant to desiccation, although only weakly (Higo *et al.*, 2006; Katoh *et al.*, 2004). The genome sequence of *Anabaena* PCC 7120 has been completely determined (Kaneko *et al.*,

Abbreviations: CRP, cAMP receptor protein; ROS, reactive oxygen species; RuBisCO, ribulose biphosphate carboxylase.

The microarray data for this paper have been deposited in the KEGG expression database; accession numbers are given in the text.

Seven supplementary tables with details of the up- and downregulated genes are available with the online version of this paper.

2001) and a genetic transformation system has been developed (Elhai *et al.*, 1997). We have shown that *Anabaena* PCC 7120 accumulates large amounts of sucrose but only small amounts of trehalose (0.05–0.1% of dry weight), although trehalose is important for desiccation tolerance (Higo *et al.*, 2006). We have also previously reported microarray analysis of the genome-wide gene expression changes during dehydration (Higo *et al.*, 2006; Katoh *et al.*, 2004).

In this study, we performed DNA microarray analysis during rehydration following dehydration. We discuss the similarities and differences in transcriptional profiles during dehydration and rehydration. Whereas no genes encoding transcriptional regulators were specifically upregulated during dehydration, eight were specifically upregulated during rehydration. Of these, we focused on *ancrpB* and *alr0618*, encoding putative transcriptional regulators of the cAMP receptor protein (CRP) family.

METHODS

Strains, growth conditions, and dehydration and rehydration treatments. *Anabaena* PCC 7120 (wild-type strain) and gene disruptants were grown at 30 °C at 40 µmol photons m⁻² s⁻¹ in nitrogen-free modified Detmer's medium (MDM₀) (Watanabe, 1960) until late-exponential phase. MDM₀ medium contains, per litre: 13 mg CaCl₂·2H₂O, 0.25 g MgSO₄·7H₂O, 0.1 g NaCl, 0.25 g K₂HPO₄, 20 mg FeSO₄·7H₂O and 1 ml trace elements A₆ (Kratz & Myers, 1955). Liquid cultures were bubbled with air containing 1.0% (v/v) CO₂.

Dehydration stress was imposed on cells as described previously (Higo *et al.*, 2006). A 30 ml portion of cell culture was filtered onto cellulose acetate filter paper (0.45 µm pore size, 47 mm diameter; Advantec) and dried for 24 h at 30 °C at 30–40 µmol photons m⁻² s⁻¹ in a Petri dish. After dehydration for 24 h, cells on the filter paper were rehydrated in 30 ml MDM₀ and incubated as described above for the indicated times.

DNA microarray analysis. RNA was extracted from cells as described previously (Katoh *et al.*, 2004). DNA microarray analysis with an *Anabaena* oligonucleotide microarray (Ehira & Ohmori, 2006; Higo *et al.*, 2006) (Sigma-Aldrich) was performed according to the method described previously (Higo *et al.*, 2006) with some modifications.

A mixture of RNA and *Anabaena* primer mix (Ehira & Ohmori, 2006; Higo *et al.*, 2006) (Sigma-Aldrich) in an 11 µl volume was heated to 90 °C for 5 min, and then gradually cooled to 42 °C for 20 min. Reverse transcription was performed at 42 °C for 90 min in a 20 µl volume containing 5 µg RNA, *Anabaena* primer mix (3.44 nM each ORF), 1 × first-strand buffer (50 mM Tris/HCl, 75 mM KCl, 3 mM MgCl₂, pH 8.3), 10 mM DTT, 250 µM each dATP, dGTP and dCTP, 200 µM dTTP, 50 µM Cy3-dUTP or Cy5-dUTP, 200 U SuperScript II Reverse Transcriptase (Invitrogen) and 20 U RNase inhibitor (Takara). The RNA in the reaction mixtures was hydrolysed by incubation at 70 °C after the addition of 10 µl 0.1 M NaOH, after which the mixtures were neutralized by the addition of 15 µl 1 M Tris/HCl (pH 7.5). Two reaction mixtures (one labelled with Cy3 and the other labelled with Cy5) were combined and purified with a QIAquick PCR Purification kit (Qiagen) to remove unincorporated fluorescent nucleotides. The purified cDNA solution was vacuum-concentrated to about 10 µl. The cDNA solution (9.4 µl) and 3.3 µl

20 × saline sodium citrate (SSC) were combined and denatured at 95 °C for 5 min. The mixture was cooled at room temperature, then 0.65 µl 10% (w/v) SDS was added.

The microarrays were hybridized, washed and scanned as described previously (Ehira *et al.*, 2003). The relative expression ratio for each oligoDNA spot was defined as the normalized fluorescence intensity for each DNA spot at each time point relative to that at time zero (before dehydration). The experiments were performed twice independently, with different combinations of Cy dyes. Thus, the relative ratio for each DNA spot is represented by four measurements.

The means and standard deviations of the ratios of the transcript levels at each time point relative to those of the control were calculated as base-2 logarithms. A gene was judged to be upregulated when its mean relative ratio was greater than 1.6 (equivalent to threefold induction) with *P* value (Student's one-sample *t* test against 0) of less than 0.01. A gene was judged to be downregulated when its mean relative ratio was less than -1.6 (equivalent to threefold repression) with *P* value (Student's one-sample *t* test against 0) less than 0.01.

The microarray data have been deposited in the KEGG expression database (<http://www.genome.jp/kegg/expression/>), with the following accession numbers. *Anabaena* PCC 7120 (wild type strain): dehydration for 3 h, ex0001765-0001768; dehydration for 9 h, ex0001769-0001772; dehydration for 24 h, ex0001773-0001776; rehydration for 20 min, ex0001777-0001780; rehydration for 1 h, ex0001781-0001784; rehydration for 3 h, ex0001785-0001788. *Anabaena ancrpB* disruption mutant: dehydration for 24 h, ex0001789-0001792; rehydration for 20 min, ex0001793-0001796; rehydration for 1 h, ex0001797-0001800; rehydration for 3 h, ex0001801-0001804. *Anabaena alr0618* disruption mutant: dehydration for 24 h, ex0001805-0001808; rehydration for 20 min, ex0001809-0001812; rehydration for 1 h, ex0001813-0001816; rehydration for 3 h, ex0001817-0001820.

Inactivation of *ancrpB* and *alr0618*. To inactivate the gene *ancrpB* (alr2325) (Suzuki *et al.*, 2004), a DNA fragment from the *ancrpB* region of *Anabaena* PCC 7120 was amplified by PCR using primers 5'-ACTACTCAATTTACCCTC-3' and 5'-CCCGGGCGTTCTCTAG-AAATACTG-3' or 5'-CCCGGGGCATCATAGAATGCTGCA-3' and 5'-GTCAAGAATTGGTGATGA-3'. Their respective PCR products were cloned into the pGEM-T Easy vector (Promega). Both plasmids were digested with *ScaI* and *SmaI*, and were ligated together, generating a plasmid with a new *SmaI* restriction site at the centre of *ancrpB*. The ΩSp^r-Sm^r cassette excised with *SmaI* was inserted into the *SmaI* site present in the inserted DNA fragment of *ancrpB*. The *EcoRI* fragment from this plasmid was blunted with Klenow fragment and then ligated into vector pRL271 (Cai & Wolk, 1990) digested with *NaeI*.

To inactivate the gene *alr0618*, a DNA fragment from the *alr0618* region of *Anabaena* PCC 7120 was amplified by PCR using primers 5'-AAGCTGCGAGGATTGGTCTA-3' and 5'-GGATCCCGTGAAA-TTTGTTCCAGGG-3' or 5'-GGATCCGCCGAGAAATTGAATGC-GAT-3' and 5'-TCTGAGCATACTTCCACTCG-3'. Their respective PCR products were cloned into the pGEM-T Easy vector. Both plasmids were digested with *PstI* and *BamHI*, and were ligated together, generating a plasmid with a new *BamHI* restriction site at the centre of *alr0618*. The ΩSp^r-Sm^r cassette excised with *BamHI* was inserted into the *BamHI* site present in the inserted DNA fragment of *alr0618*. The fragment digested from this plasmid with *PstI* and *NsiI* was ligated into vector pRL271 (Cai & Wolk, 1990) digested with *PstI*.

ancrpB and *alr0618* were inactivated with *sacB*-mediated positive selection for double recombination (Cai & Wolk, 1990). Transformation of *Anabaena* PCC 7120 was performed by a conjugation method (Elhai & Wolk, 1988), and both single and

double recombinations were confirmed by PCR. Completely segregated clones of the mutants were used for the study.

Northern blotting analysis. This was carried out as described previously (Higo *et al.*, 2006). The *cmpA* probe was synthesized with a DIG PCR synthesis kit (Roche Molecular Biochemicals) using primers 5'-CCCTTCACAGCTGCTCAAAC-3' and 5'-GCCATGAAACCG-ACTTTGTC-3'.

Measurement of oxygen-evolving activity. Oxygen-evolving activity was measured in intact cells with or without 5 mM NaHCO₃, 10 mM HEPES (pH 7.5), using a Clark-type oxygen electrode (Rank Brothers) at a light intensity of 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 30 °C. Light was provided by a halogen lamp. The absorbance of chlorophyll extracted by methanol was measured at 665 nm and the chlorophyll concentration was calculated from the equation 1 A_{665} unit = 13.42 $\mu\text{g chlorophyll ml}^{-1}$ (Mackinney, 1941).

RESULTS

Genes up- or downregulated during both dehydration and rehydration

RNA was extracted after 24 h of dehydration and after 20 min, 1 h and 3 h of rehydration. The expression profiles at each time point were compared with that before dehydration with the *Anabaena* oligonucleotide microarray. The DNA microarray data were analysed in reference to the data for dehydration for 3 h and 9 h (Higo *et al.*, 2006).

We found that the expression of 399 genes was upregulated during dehydration and the expression of 769 genes was upregulated during rehydration when compared with their expression levels before dehydration. Among those upregulated genes, 292 genes overlapped (Fig. 1a and Supplementary Table S1, available with the online version of this paper). The genes induced under both conditions were classified into two patterns in terms of the time of maximum gene expression. (1) More than half of them

showed maximum gene expression not only in 3 h or 3–24 h of dehydration but also in 1–3 h of rehydration. Many of these genes encode hypothetical and unknown proteins, while genes for trehalose metabolism were also included in this category (Higo *et al.*, 2006). (2) The rest of the genes showed maximum expression between late dehydration and early rehydration. Among these genes, those for chaperones and proteases were markedly upregulated upon rehydration, suggesting that the control of protein quality is important after rehydration.

The expression of 583 genes was downregulated during dehydration and 492 genes were downregulated during rehydration. Among these, 289 genes overlapped (Fig. 1b and Supplementary Table S2). Most of these genes are involved in various cellular processes, such as photosynthesis, ATP synthesis, nitrogen fixation, cell wall synthesis and translation, which are essential for growth. The transcription levels of the downregulated genes were restored gradually during the rehydration period (1–3 h). Genes for ATP synthesis, photosystem II and ribosomal proteins recovered early, whereas genes for photosystem I and nitrogen fixation recovered later.

Dehydration-specific gene expression change

To exclude the genes with obscure peaks of up- or downregulation, the mean ratios at dehydration were compared with those at rehydration. We assessed whether they differed significantly using Student's two-sample *t* test. Of the 107 genes that were judged to be upregulated during dehydration (Fig. 1a), only 29 clearly showed maximum transcriptional level during dehydration (Supplementary Table S3). Many of these showed maximum transcriptional level during later dehydration (9–24 h). Of these, 26 genes encode hypothetical or unknown proteins. The rest encode thioredoxin, alcohol dehydrogenase and transglycosylase. In contrast, of the 299 genes downregulated during

Fig. 1. Number of genes upregulated (a) or downregulated (b) during dehydration (top) and rehydration (bottom). Black bars, number of genes up- or downregulated in both phases; grey bars, number of genes showing a clear maximum (a) or minimum (b) transcriptional level in only one phase; white bars, number of genes which did not show a clear peak in one phase.

dehydration (Fig. 1b), 133 clearly showed minimum transcriptional levels during dehydration (Supplementary Table S4). Gene expression of many of those that had been downregulated after 3 h of dehydration were restored immediately after rehydration. Representatives of these include genes encoding ribulose biphosphate carboxylase (RuBisCO), FtsZ and some ribosomal proteins. Expression of genes involved in central cellular energy metabolism was restored in a stepwise manner.

Dynamic transcriptional response to rehydration

Of the 477 genes that were upregulated during rehydration (Fig. 1a), 259 clearly showed maximum transcriptional level in the rehydration phase (Supplementary Table S5). Many of these genes showed rapid and transient upregulation with a maximum transcriptional level within 1 h of rehydration. These 259 genes were classified into the following functional groups: (a) protein folding and degradation; (b) DNA repair; (c) metabolism; (d) signal transduction; and (e) other, hypothetical and unknown function. It was noted that expression of a gene orthologue to *Synechocystis* sp. PCC 6803 *hik34*, the product of which regulates genes for chaperones and proteases (Paithoonrangsarid *et al.*, 2004; Suzuki *et al.*, 2005), was upregulated upon rehydration.

Forty genes upregulated on rehydration were also upregulated by nitrogen depletion (Ehira & Ohmori, 2006). Nitrogen metabolism in cyanobacteria is generally regulated by NtcA, a global nitrogen-responsive transcriptional regulator protein (Herrero *et al.*, 2001), and *ntcA* was downregulated during dehydration and was also slightly downregulated during rehydration (data not shown). The gene expression patterns of many NtcA regulons and the genes involved in heterocyst formation were similar to that of *ntcA*. This suggests that genes upregulated during both rehydration and nitrogen depletion are independent of NtcA regulation.

As many as 57 genes related to various cellular metabolic reactions were sequentially upregulated during rehydration (Fig. 2). Quinolinate synthase (NadA) encoded by *all4673*, PncB encoded by *alr2482*, and NadD encoded by *alr2483* are required for NAD biosynthesis (Gerdes *et al.*, 2006), suggesting the depletion of the NAD pool during rehydration. Other genes markedly upregulated during rehydration were the *suf* operon (*alr2492–alr2495*), which has been shown to function in the assembly of iron–sulfur clusters under conditions of oxidative stress (Nachin *et al.*, 2001). Rewetting the cells would cause oxidative stress (Potts, 1994). The remaining genes are involved in respiration (cytochrome *c* oxidase), the pentose phosphate pathway (ribulose-phosphate 3-epimerase), lipid synthesis (diacylglycerol kinase), and nitrogen and amino acid metabolism. Of the 203 genes that were downregulated during rehydration (Fig. 1b), only 13 showed minimum transcriptional level in the rehydration phase (Supplementary Table S6).

Fig. 2. Changes in the transcript levels of representative metabolic genes upregulated during rehydration. *sufB* and *sufC*, iron–sulfur cluster assembly protein; *coxA*, cytochrome *c* oxidase subunit I; *coxB*, cytochrome *c* oxidase subunit II; *nadA*, quinolinate synthase; *ndhD2*, NADH dehydrogenase subunit 4; *rpe*, pentose-5-phosphate 3-epimerase; *all4422*, UDP-*N*-acetyl-D-mannosamine transferase; *all4342*, mannose-6-phosphate isomerase.

Transcriptional regulator genes upregulated during rehydration

No genes involved in signal transduction displayed dehydration-specific upregulation (Supplementary Table S3). Among genes showing rehydration-specific upregulation (Supplementary Table S5), eight encode transcriptional regulators (Table 1) and six belong to a transcriptional repressor family. The remaining two genes, *ancrpB* and *alr0618*, encode CRP family transcriptional activators, and the former gene product binds to cAMP (Suzuki *et al.*, 2004).

Table 1. Transcription factor genes upregulated during rehydration

Gene	Family	Dehydration*	Rehydration*			
		24 h	20 min	1 h	3 h	
<i>alr2325</i> (<i>ancrpB</i>)	CRP	1.0 ± 0.5	2.2 ± 0.7	2.9 ± 0.4	0.6 ± 1.5	
<i>alr0618</i>	CRP	−1.3 ± 0.5	3.1 ± 0.5	1.6 ± 0.3	−1.2 ± 0.6	
<i>alr0810</i>	TetR	0.0 ± 0.9	3.4 ± 1.1	2.9 ± 0.4	−0.2 ± 0.4	
<i>alr1721</i>	TetR	0.7 ± 0.4	3.3 ± 0.9	1.6 ± 0.4	1.1 ± 0.3	
<i>alr4279</i>	TetR	0.2 ± 0.5	2.9 ± 0.9	2.5 ± 0.4	0.7 ± 0.8	
<i>alr0957</i> (<i>furC</i>)	FUR	0.6 ± 0.3	2.5 ± 0.4	0.8 ± 0.3	0.4 ± 0.4	
<i>all2491</i> (<i>sufR</i>)	DeoR	1.0 ± 0.4	2.9 ± 0.4	0.6 ± 0.3	0.4 ± 0.2	
<i>alr4908</i> (<i>lexA</i>)	LexA	−1.4 ± 0.6	−0.7 ± 0.7	0.8 ± 0.2	2.6 ± 0.2	

*Means ± SD of ratio in log₂.

Anabaena AnCrpB binds to the consensus DNA sequences of *Escherichia coli* CRP. However, AnCrpB seems to lack the ordinary DNA-binding domain (Suzuki *et al.*, 2004). An HMMSEARCH with the HMMER2.2 software package (Eddy, 1998) against the C-terminal sequence of AnCrpB using the CRP Pfam profile (PF00325) resulted in a negative bit score, but in an *E*-value of 10^{-3} . This means that the C-terminal sequence of AnCrpB is a distant homologue of the DNA-binding CRP family.

As regards the N-terminal sequence of both AnCrpB and Alr0618, they both possess a cNMP-binding domain. AnCrpB has a cAMP-binding motif and it binds cAMP (Suzuki *et al.*, 2004). In contrast, Alr0618 does not have the motif, suggesting that this protein does not bind cAMP.

Effect of *ancrpB* and *alr0618* disruption on gene expression during rehydration

To identify the genes regulated by AnCrpB and Alr0618, DNA microarray analysis was performed using disruptants of the corresponding genes. The genes that were upregulated by rehydration in the wild-type strain, but not in the *ancrpB* disruptant, are shown in Table 2. Twenty-two of 33 genes were upregulated after 20 min of rehydration only in the wild-type strain (Table 2), suggesting that *ancrpB* is involved in gene expression at early rehydration. The expression of five genes was upregulated by nitrogen deprivation (Table 2) and that of another five genes was also slightly upregulated by nitrogen deprivation (Ehira & Ohmori, 2006). This suggests that AnCrpB regulates the expression of genes related to nitrogen depletion.

Table 3 shows that the expression of 20 genes was upregulated by rehydration in the wild-type strain, but not in the *alr0618* disruptant. The expression patterns of *alr4157* (*ndhD3*)–*alr4158* (*cupA*), and *alr2877* (*cmpA*)–*alr2878* (*cmpB*), encoding components of the NdhD3/NdhF3/CupA NAD(P)H dehydrogenase complex essential for growth under low-CO₂ conditions and components of the high-affinity bicarbonate transporter, respectively, were similar to that of *alr4156* (*ndhF3*), although the standard deviations were rather high (Supplementary Table S7). Northern blotting analysis using a *cmpA* probe confirmed that the entire *cmp* operon (*alr28–77–alr2880*) is upregulated by rehydration in the wild-type strain, but not in the *alr0618* disruptant (Fig. 3). These genes in two gene clusters are orthologues of genes induced under inorganic carbon limitation in *Synechocystis* sp. PCC 6803 (Wang *et al.*, 2004). It is suggested that Alr0618 regulates the expression of genes related to inorganic carbon uptake.

Contribution of *ancrpB* and *alr0618* to the recovery of photosynthesis during rehydration

Oxygen-evolving activities were measured during rehydration as an indicator of the recovery of the cells (Fig. 4). In the wild-type strain, approximately 30 % of the oxygen-evolving activity was restored within 30 min of rehydration. This

activity gradually returned to the level before rehydration within 24 h (data not shown).

To determine the physiological function of *ancrpB* and *alr0618* during rehydration, oxygen-evolving activities were measured in the wild-type strain and the *ancrpB* and *alr0618* disruptants (Fig. 4). Before dehydration, this activity was the same amongst all these strains. In the absence of bicarbonate, during the first 2 h, oxygen-evolving activity of the *ancrpB* disruptant was only slightly lower than the wild-type strain, whilst that of the *alr0618* disruptant was similar to the wild-type (Fig. 4a). However, the oxygen-evolving activities in the *ancrpB* and the *alr0618* disruptants were 60–75 % of that in the wild-type strain after 2 h of rehydration. In the presence of bicarbonate, there was no difference in oxygen-evolving activity between the wild-type and the *alr0618* disruptant, while the activity of the *ancrpB* disruptant was lower than in the wild-type strain (Fig. 4b). These results suggest that these CRP family transcription factors are required for the recovery of photosynthetic activity during late rehydration. *alr0618* is related to inorganic carbon uptake in particular.

DISCUSSION

Similarities and differences in transcriptional responses to dehydration and rehydration

We performed a comprehensive analysis of transcriptional profiles during dehydration and rehydration in *Anabaena* PCC 7120 and found that the expression of as many as 292 genes was upregulated during both dehydration and rehydration (Fig. 1a). These genes are thought to be general stress-responsive genes, involved in switching from a metabolism directed toward maximal growth to a maintenance metabolism (Hengge-Aronis, 2002). In dry yeast, 'stationary-phase-essential genes' and those induced during both desiccation and rehydration are similar (Singh *et al.*, 2005). These genes might not be stimulated by a specific environmental stress, but rather by reduced metabolic states (Singh *et al.*, 2005).

The results of the present work show that the down-regulation of genes is marked during dehydration, whereas the upregulation of genes is marked during rehydration. This suggests that endurance of a stress is important during dehydration, whereas the rapid repair of damage and the resumption of metabolism are important during rehydration. A striking feature of the upregulation of gene expression during rehydration is the dynamic changes, a response that is markedly different from the upregulation of genes during dehydration. A feature of the latter was a gradual change and persistence (Katoh *et al.*, 2004). These results reflect a difference in the dehydration and rehydration processes, the former being gradual, and the latter rapid.

In this study, the *Anabaena* cells were dehydrated for 24 h and then rehydrated. Previous data showed that the wet

Table 2. Effects of the disruption of *ancrpB* on the upregulation of gene expression during rehydration

Results in bold represent significant upregulation of genes at the time points compared to dehydration phase in the wild-type strain, but not in the gene disruptant strains.

Gene	Description	Wild-type strain*				<i>ancrpB</i> disruptant*			
		Dehydration	Rehydration			Dehydration	Rehydration		
		24 h	20 min	1 h	3 h	24 h	20 min	1 h	3 h
<i>asl0171</i>	Hypothetical protein	0.3 ± 0.3	2.0 ± 0.3	1.4 ± 0.1	0.8 ± 0.2	1.6 ± 0.9	0.9 ± 0.6	-0.1 ± 0.7	0.0 ± 0.5
<i>all0334</i>	Hypothetical protein	-0.2 ± 0.4	1.8 ± 0.3	1.1 ± 0.4	0.4 ± 0.5	0.5 ± 0.6	1.2 ± 0.6	0.8 ± 0.3	0.4 ± 0.3
<i>alr0774</i>	Two-component response regulator	-1.0 ± 0.3	2.0 ± 0.7	0.4 ± 0.2	0.1 ± 0.4	0.0 ± 0.8	-0.1 ± 0.9	0.2 ± 0.6	0.1 ± 0.9
<i>alr0965</i>	Unknown protein	-0.1 ± 0.1	1.7 ± 0.6	1.0 ± 0.3	0.6 ± 0.1	0.8 ± 0.2	1.2 ± 0.8	0.9 ± 0.3	0.6 ± 0.5
<i>all1163</i>	Hypothetical protein	0.4 ± 0.4	2.3 ± 0.4	1.1 ± 0.1	0.4 ± 0.2	1.0 ± 0.4	1.2 ± 0.3	0.5 ± 0.5	0.1 ± 0.2
<i>asl1274</i>	Unknown protein	0.2 ± 0.5	2.5 ± 0.8	1.7 ± 0.2	0.3 ± 0.5	0.1 ± 0.4	0.6 ± 0.4	0.2 ± 0.6	-0.2 ± 0.2
<i>alr1721</i>	Transcriptional regulator	0.7 ± 0.4	3.3 ± 0.9	1.6 ± 0.4	1.1 ± 0.3	1.7 ± 0.5	1.2 ± 0.6	1.0 ± 0.3	0.6 ± 0.1
<i>alr2310</i>	Similar to agmatinase	-0.3 ± 0.2	1.7 ± 0.4	1.5 ± 0.3	0.7 ± 0.2	0.5 ± 0.3	0.7 ± 0.2	0.9 ± 0.7	-0.1 ± 0.3
<i>all2371</i>	Unknown protein	-0.7 ± 0.6	2.0 ± 0.5	1.3 ± 0.2	0.1 ± 0.6	0.6 ± 0.2	1.3 ± 0.9	0.6 ± 0.7	
<i>alr2412</i>	Serine/threonine kinase	-2.0 ± 0.3	2.0 ± 0.2	0.8 ± 0.3	0.5 ± 0.1	-0.5 ± 1.0	0.6 ± 0.6	0.3 ± 0.9	0.3 ± 0.7
<i>alr2478†</i>	Hypothetical protein	-0.7 ± 0.1	2.4 ± 0.4	1.7 ± 0.3	1.3 ± 0.5	-0.4 ± 0.7	0.5 ± 0.7	0.8 ± 0.4	1.0 ± 0.8
<i>alr2483†</i>	Nicotinate-nucleotide adenylyltransferase	-0.3 ± 0.7	1.6 ± 0.4	0.4 ± 0.1	0.2 ± 0.3	0.1 ± 0.1	0.3 ± 0.4	0.0 ± 0.3	-0.2 ± 0.3
<i>alr2488</i>	Hypothetical protein	0.1 ± 0.3	2.3 ± 0.5	1.4 ± 0.1	1.3 ± 0.4	-0.2 ± 0.6	0.6 ± 0.3	0.5 ± 0.6	0.4 ± 0.3
<i>asr3796</i>	Stress-induced hydrophobic peptide	-0.9 ± 0.4	2.3 ± 0.3	1.5 ± 0.2	0.0 ± 0.2	-0.2 ± 0.4	0.7 ± 0.5	0.4 ± 0.7	-0.6 ± 0.3
<i>alr3900</i>	Hypothetical protein	0.0 ± 0.2	1.7 ± 0.3	1.2 ± 0.2	0.9 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	0.5 ± 0.4	0.9 ± 0.4
<i>all4040</i>	Unknown protein	-0.7 ± 0.1	2.2 ± 0.6	1.3 ± 0.2	0.9 ± 0.3	-0.3 ± 0.3	0.4 ± 0.7	-0.5 ± 1.0	0.1 ± 0.9
<i>all4333</i>	Hypothetical protein	-1.1 ± 0.3	2.3 ± 0.3	1.8 ± 0.2	1.0 ± 0.2	-1.2 ± 0.3	0.0 ± 0.4	0.7 ± 0.5	0.2 ± 0.5
<i>asr4352</i>	Unknown protein	-1.3 ± 0.4	1.7 ± 0.4	1.3 ± 0.2	0.3 ± 0.4	-0.8 ± 0.6	0.3 ± 0.5	0.0 ± 0.8	-0.5 ± 0.8
<i>all4590</i>	Protease IV	-0.7 ± 0.4	1.9 ± 0.3	1.5 ± 0.4	1.2 ± 0.2	0.2 ± 0.4	1.2 ± 0.1	0.4 ± 0.5	0.3 ± 0.3
<i>all4759</i>	WD-repeat protein	-0.5 ± 0.1	2.7 ± 1.0	1.1 ± 0.2	0.6 ± 0.4	0.2 ± 0.3	0.9 ± 0.7	-0.1 ± 1.0	0.3 ± 0.3
<i>all5173†</i>	Two-component hybrid sensor and regulator	1.1 ± 0.4	3.3 ± 0.1	1.6 ± 0.3	0.7 ± 1.0	0.7 ± 0.8	0.3 ± 0.5		
<i>all5174</i>	Two-component response regulator	-0.1 ± 1.0	3.7 ± 0.6	2.0 ± 0.2	0.5 ± 0.2	-0.4 ± 0.6	1.0 ± 0.3	0.4 ± 0.7	
<i>alr0540</i>	1-Pyrroline-5-carboxylate dehydrogenase	-0.5 ± 1.0	1.2 ± 0.8	1.9 ± 0.3	0.9 ± 0.3	1.1 ± 0.7	1.2 ± 0.3	1.4 ± 0.5	0.5 ± 0.5
<i>all1411</i>	Unknown protein	1.0 ± 0.6	0.3 ± 0.5	2.6 ± 0.4	2.6 ± 0.8	0.2 ± 0.7	0.7 ± 0.4	0.2 ± 0.9	0.3 ± 0.5
<i>alr1594</i>	RNA polymerase β subunit	-1.2 ± 0.1	1.1 ± 0.4	1.6 ± 0.2	0.9 ± 0.3	-0.2 ± 0.1	-0.4 ± 0.1	0.3 ± 0.4	0.1 ± 0.1
<i>alr3815</i>	Unknown protein	-0.3 ± 0.4	1.9 ± 0.3	1.9 ± 0.3	0.3 ± 0.2	-0.5 ± 0.5	0.6 ± 0.8	0.1 ± 0.7	-0.3 ± 0.8
<i>alr4281</i>	DevC homologue	-0.8 ± 0.5	1.5 ± 1.6	3.1 ± 0.3	-0.7 ± 0.4	-0.5 ± 0.9	0.2 ± 0.5	-0.5 ± 1.2	-1.3 ± 0.9
<i>alr4974</i>	DevC homologue	0.9 ± 0.3	1.6 ± 1.8	3.9 ± 0.4	0.4 ± 0.7	0.3 ± 1.5	0.5 ± 0.2	0.5 ± 1.0	-0.2 ± 0.8
<i>all5265</i>	DNA gyrase B subunit	-1.4 ± 0.3	1.4 ± 0.4	2.0 ± 0.5	0.7 ± 0.2	0.2 ± 0.9	1.0 ± 0.3	0.7 ± 0.7	-0.5 ± 0.2
<i>all4422†</i>	UDP-N-acetyl-D-mannosamine transferase	-0.5 ± 0.6	0.6 ± 0.9	2.4 ± 0.2	2.6 ± 0.6	0.3 ± 1.0	0.9 ± 0.0	0.8 ± 0.5	0.8 ± 0.2
<i>alr4057†</i>	Similar to peptide synthetase	-0.5 ± 0.4	0.1 ± 0.3	0.6 ± 0.6	1.7 ± 0.4	-0.2 ± 0.7	0.6 ± 0.4	0.7 ± 0.3	0.1 ± 0.5
<i>alr4576</i>	Hypothetical protein	-0.2 ± 0.4	0.6 ± 0.7	-0.1 ± 0.6	1.6 ± 0.6	-0.2 ± 0.5	-0.4 ± 0.3	-0.1 ± 1.0	0.1 ± 0.6
<i>alr5223</i>	Glycosyltransferase	0.5 ± 0.2	-0.8 ± 0.7	0.4 ± 0.3	2.2 ± 0.5	0.6 ± 1.0	-0.2 ± 0.8	0.9 ± 0.2	0.8 ± 0.6

*Means ± SD of ratio in log₂.

†Genes induced by nitrogen depletion (Ehira & Ohmori, 2006).

weight of the cells was constant after 9 h of dehydration (Kato et al., 2004) and the viability of the wild-type cells was 64 % at 24 h (Higo et al., 2006). In the wild-type strain, approximately 30 % of the oxygen-evolving activity recovered within 30 min of rehydration, then there was further gradual recovery during rehydration. The activity returned to the level before rehydration within 24 h (data not shown). Tamaru et al. (2005) reported that while *N.*

commune colonies with EPS recovered 100 % of oxygen-evolving activity rapidly after rehydration following overnight air-drying, EPS-depleted cells recovered only 30 % of the activity, a level similar to that in *Anabaena* PCC 7120, which contains little EPS. These results suggest that desiccation tolerance attributed to intracellular mechanisms is similar between *Anabaena* PCC 7120 and *N. commune*.

Table 3. Effects of the disruption of *alr0618* on the upregulation of gene expression during rehydration

Results in bold represent significant upregulation of genes at the time points compared to dehydration phase in the wild-type strain, but not in the gene disruptant strains.

Gene	Description	Wild-type strain*				<i>alr0618</i> disruptant*			
		Dehydration	Rehydration			Dehydration	Rehydration		
		24 h	20 min	1 h	3 h	24 h	20 min	1 h	3 h
<i>alr0965</i>	Unknown protein	-0.1 ± 0.1	1.7 ± 0.6	1.0 ± 0.3	0.6 ± 0.1	0.4 ± 1.7	0.6 ± 0.8	0.0 ± 0.7	-0.4 ± 1.3
<i>alr1054</i>	DNA polymerase III α subunit	-0.1 ± 0.4	1.6 ± 0.2	1.0 ± 0.3	0.2 ± 0.2	0.2 ± 0.7	0.7 ± 0.5	0.2 ± 0.5	-0.2 ± 1.0
<i>all3107</i>	Probable integrase	-1.0 ± 1.3	2.6 ± 0.8	-0.2 ± 0.7	-0.7 ± 0.4	0.6 ± 0.4	1.1 ± 0.9	-0.8 ± 1.4	0.5 ± 0.7
<i>alr4156</i>	NADH dehydrogenase subunit 5	-0.9 ± 0.9	2.3 ± 0.3	-0.9 ± 0.1	0.4 ± 0.3	-0.2 ± 0.7	0.5 ± 0.5	-1.4 ± 1.0	-0.5 ± 1.1
<i>all4590</i>	Protease IV	-0.7 ± 0.4	1.9 ± 0.3	1.5 ± 0.4	1.2 ± 0.2	-0.2 ± 1.0	0.5 ± 0.5	0.6 ± 0.9	0.5 ± 0.2
<i>all4983</i>	Unknown protein	-0.9 ± 0.9	2.0 ± 0.6	0.5 ± 0.1	-0.4 ± 0.3	-0.5 ± 0.9	-0.3 ± 0.4	-0.5 ± 1.1	-0.8 ± 0.4
<i>alr2554</i>	Unknown protein	-0.4 ± 0.5	1.8 ± 0.6	1.0 ± 0.2	2.3 ± 0.8	0.8 ± 0.4	1.2 ± 0.6	0.0 ± 0.8	0.4 ± 0.8
<i>alr0030</i>	Similar to Mg-protoporphyrin IX monomethyl ester oxidative cyclase	-0.1 ± 0.3	0.1 ± 1.1	1.7 ± 0.2	1.3 ± 0.6	0.0 ± 0.4	0.4 ± 0.3	0.1 ± 0.0	0.6 ± 0.6
<i>alr2449</i>	Hypothetical protein	1.5 ± 0.4	2.7 ± 0.6	3.1 ± 0.4	0.4 ± 0.2	1.5 ± 0.4	2.2 ± 0.9	2.0 ± 0.7	1.1 ± 0.6
<i>alr0088</i>	Single-stranded DNA-binding protein	-0.1 ± 0.3	-0.5 ± 0.2	0.4 ± 0.1	1.6 ± 0.4	0.3 ± 1.0	-0.5 ± 0.7	-0.2 ± 0.6	0.7 ± 0.8
<i>all0187</i>	Transcriptional regulator	-2.0 ± 0.4	-1.0 ± 0.2	0.3 ± 0.2	1.8 ± 0.3	-0.5 ± 0.5	-0.2 ± 0.3	0.6 ± 0.3	0.8 ± 0.8
<i>alr0607</i>	Nitrite reductase NirA	-0.1 ± 0.2	0.4 ± 0.3	-0.4 ± 1.1	1.6 ± 0.5	-1.2 ± 0.4	-0.9 ± 0.4	-1.0 ± 0.4	-0.7 ± 0.6
<i>alr0608</i>	Nitrate transport nitrate-binding protein NrtA	-0.4 ± 0.5	-0.2 ± 0.2	0.3 ± 0.9	1.6 ± 0.3	-1.3 ± 0.7	-1.3 ± 1.0	-1.0 ± 1.2	-1.2 ± 1.4
<i>alr0611</i>	Nitrate transport ATP-binding protein NrtD	-0.3 ± 0.4	0.6 ± 0.6	1.4 ± 1.8	2.0 ± 0.3	0.4 ± 0.9	-1.2 ± 1.2	-0.5 ± 1.6	-1.3 ± 1.1
<i>all1848</i>	Similar to methyltransferase	0.9 ± 0.4	0.3 ± 0.4	1.0 ± 0.2	2.5 ± 0.5	0.7 ± 0.3	0.2 ± 0.2	0.6 ± 0.6	1.3 ± 0.6
<i>alr3720</i>	Unknown protein	0.3 ± 0.3	0.0 ± 0.4	0.8 ± 0.5	2.4 ± 0.5	0.5 ± 1.5	0.7 ± 1.6	0.7 ± 1.1	1.0 ± 1.0
<i>all3797</i>	Hypothetical protein	-0.8 ± 0.2	0.4 ± 0.3	1.2 ± 0.7	1.8 ± 0.4	-0.1 ± 0.8	0.6 ± 0.4	0.4 ± 0.1	0.6 ± 0.5
<i>alr4057</i>	Similar to peptide synthetase	-0.5 ± 0.4	0.1 ± 0.3	0.6 ± 0.6	1.7 ± 0.4	-0.4 ± 0.4	-0.9 ± 0.4	-0.3 ± 0.5	0.0 ± 0.5
<i>all4989</i>	Hypothetical protein	-0.9 ± 0.9		1.1 ± 0.8	2.1 ± 0.6	0.2 ± 1.3	-0.3 ± 0.6	0.1 ± 0.3	0.7 ± 0.6
<i>alr5223</i>	Glycosyltransferase	0.5 ± 0.2	-0.8 ± 0.7	0.4 ± 0.3	2.2 ± 0.5	0.2 ± 0.6	-0.8 ± 0.7	0.6 ± 0.9	0.3 ± 0.8

*Means $\pm$ SD of ratio in log₂.

Fig. 3. Northern blotting analysis of *cmpA*. RNA was extracted from cells dehydrated for 24 h (lanes 1 and 4), and from cells rehydrated for 20 min (lanes 2 and 5) and 1 h (lane 3 and 6) following dehydration for 24 h. Lanes 1–3, wild-type strain; lanes 4–6, *alr0618* disruptant.

Repression of cellular metabolism during dehydration and resumption of it following rehydration

The repression of cellular metabolism during desiccation is an essential mechanism for desiccation tolerance based on the avoidance of reactive oxygen species (ROS) formation, and is not just an effect of water removal (Oliver *et al.*, 2001). Indeed, expression of genes involved in central cellular energy metabolism was downregulated during dehydration in *Anabaena* PCC 7120. First, genes encoding components of photosystem II, ATP synthesis and RuBisCo were downregulated. Then, expression of genes encoding components of photosystem I and nitrogen fixation were downregulated before drying of cells was complete (Fig. 5). It would thus be reasonable to suggest that transcription of genes involved in electron-transfer processes such as photosystem II, which is a main source of ROS, were downregulated first. Downregulation of genes

Fig. 4. Recovery of oxygen-evolving activity during rehydration. After 24 h of dehydration, cells were rehydrated in MDM₀ medium and grown as before dehydration. The oxygen-evolving activity before dehydration was at the same level amongst the three strains. The oxygen-evolving activity was measured (a) without or (b) with 5 mM NaHCO₃, 10 mM HEPES (pH 7.5). ●, *Anabaena* PCC 7120; ▲, *ancrpB* disruptant; ■, *alr0618* disruptant. The results are means ± SD (*n*=3).

involved in nitrogen fixation would follow after a decrease in ATP and reducing power.

On the other hand, the stepwise and coordinated resumption of cellular metabolism during rehydration is also considered to be necessary to reduce ROS formation. In fact, the upregulation of the *suf* cluster during rehydration (Supplementary Table S5) indicates that oxidative stress occurs within cells during rehydration. In *Anabaena* PCC 7120, the transcriptional levels of genes involved in ATP synthesis, photosynthesis and nitrogen fixation, which were downregulated during dehydration, recovered after rehydration. The expression of genes for the components of RuBisCO recovered first (20 min); then the expression of genes for ATP synthesis and photosystem II recovered (1 h); and finally, the expression of the genes for photosystem I and nitrogen fixation (3 h). Expression of the genes for cytochrome *c* oxidase was upregulated at 20 min and 1 h of rehydration (Supplementary Table S5, Fig. 5). Respiratory activity recovered consistently within 1 h of rehydration (data not shown), whereas the recovery of net photosynthetic activity required more than 5 h of rehydration (Fig. 4). It has been reported that after rehydration, cells of *N. commune* resume respiration, photosynthesis and nitrogen fixation in a stepwise,

Fig. 5. Schematic model of the transcriptional changes in metabolic gene expression during dehydration and rehydration.

coordinated manner (Scherer *et al.*, 1984), as well as ATP synthesis (Scherer *et al.*, 1986) and lipid biosynthesis (Taranto *et al.*, 1993). *Anabaena* PCC 7120 cells should also recover their cellular activities in a stepwise, coordinated manner after rehydration, like *N. commune*. ATP supplied by the respiratory chain would be necessary for the recovery of fundamental cellular mechanisms including photosynthesis immediately after rehydration. Then photosystem I would supply ATP and reducing power for the resumption of nitrogen fixation. With regard to lipid biosynthesis, the expression of *all0270*, encoding diacylglycerol kinase (DAGK) is upregulated upon rehydration (Supplementary Table S5). DAGK catalyses the formation of phosphatidic acid from diacylglycerol and ATP, an important step in phospholipid biosynthesis. DAGK would be involved in the rapid biosynthesis of lipids induced upon rehydration, as in *N. commune* (Taranto *et al.*, 1993).

The expression of many genes involved in cellular metabolism is also upregulated in response to rehydration (Supplementary Table S5). Among these, the expression of the genes of the NAD biosynthetic pathway, *alr2482*, *alr2483* and *all4673*, was markedly upregulated upon rehydration (Fig. 2). NAD is used by DNA ligase, which is required for DNA repair (Eisen & Hanawalt, 1999). The expression of genes for DNA repair was upregulated after rehydration (Supplementary Table S5), suggesting that NAD is consumed to repair damaged DNA, and must be supplied via the NAD biosynthetic pathway.

Role of the two CRP family transcriptional regulators during rehydration

It has been suggested that AnCrpB regulates the expression of some genes induced by nitrogen depletion (Table 2).

Heterocysts are more sensitive to desiccation than are vegetative cells (Potts & Bowman, 1985) and the recovery of nitrogenase activity during rehydration is slow in *N. commune* (Scherer *et al.*, 1984). Accordingly, a shortage of nitrogen supply should result in nitrogen depletion in *Anabaena* cells at early rehydration. Considering that the expression of *ntcA*, a global nitrogen-responsive transcriptional regulator gene, is downregulated during rehydration and that there was no NtcA consensus sequence (Herrero *et al.*, 2001) upstream of the genes listed in Table 2, AnCrpB may perform a regulatory role under nitrogen depletion, independent of NtcA. Otherwise, AnCrpB may be involved in a stress response common to both nitrogen depletion and rehydration.

The expression of *alr4156*, a gene induced by CO₂ limitation (Wang *et al.*, 2004), is upregulated after rehydration in the wild-type strain, while it is not upregulated in the *alr0618* disruptant. The expression patterns of *alr4157* and *alr4158*, which are also induced by CO₂ limitation, were similar to that of *alr4156* (Supplementary Table S7). Northern blotting clarified that expression of the *cnp* operon is upregulated after rehydration in the wild-type strain while it is not upregulated in the *alr0618* disruptant (Fig. 3). These genes in two gene clusters encode a component of NAD(P)H dehydrogenase essential for low-CO₂ growth and high-affinity bicarbonate uptake, respectively (Shibata *et al.*, 2001). In addition, oxygen-evolving activity during rehydration in the *alr0618* disruptant was lower than that in the wild-type strain only when bicarbonate was not added (Fig. 4). These results suggest that *alr0618* is involved in inorganic carbon uptake.

The upregulation of *alr0965*, *all4590*, *alr4057* and *alr5223* expression during rehydration was abolished in the *alr0618* disruptant, as well as in the *ancrpB* disruptant. Up-regulation of *nirA*, encoding nitrate reductase, and *nrtA* and *nrtD*, encoding components of the nitrate transporter, was also abolished in the *alr0618* disruptant. These results suggest the co-regulation of genes involved in nitrogen and carbon metabolism.

In conclusion, the gene expression response during rehydration is dynamic, whereas that during dehydration is not. Fig. 5 shows a schematic model of the transcriptional changes in the expression of metabolic genes during dehydration and rehydration. During rehydration, the expression of genes downregulated during dehydration is restored in *Anabaena* PCC 7120 cells. At the same time, the expression of various metabolic genes involved in NAD depletion, nitrogen depletion and CO₂ limitation, as well as genes for the repair of DNA and proteins, is upregulated. It is suggested that two CRP family transcriptional regulators, AnCrpB and Alr0618, are required for the coordinated resumption of nitrogen and inorganic carbon metabolism during rehydration.

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