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The two-component response regulator OrrA confers dehydration tolerance by regulating *avaKa* expression in the cyanobacterium *Anabaena* sp. strain PCC 7120

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Abstract

The cyanobacterium *Anabaena* sp. strain PCC 7120 exhibits dehydration tolerance. The regulation of gene expression in response to dehydration is crucial for the acquisition of dehydration tolerance, but the molecular mechanisms underlying dehydration responses remain unknown. In this study, the functions of the response regulator OrrA in the regulation of salt and dehydration responses were investigated. Disruption of *orrA* abolished or diminished the induction of hundreds of genes in response to salt stress and dehydration. Thus, OrrA is a principal regulator of both stress responses. In particular, OrrA plays a crucial role in dehydration tolerance because an *orrA* disruptant completely lost the ability to regrow after dehydration. Moreover, in the OrrA regulon, *avaKa* encoding a protein of unknown function was revealed to be indispensable for dehydration tolerance. OrrA and AvaK are conserved among the terrestrial cyanobacteria, suggesting their conserved functions in dehydration tolerance in cyanobacteria.

INTRODUCTION

Cyanobacteria comprise a diverse group of bacteria characterized by oxygen-evolving photosynthesis. Their photosynthetic ability enables them to inhabit almost all illuminated environments. The habitat of cyanobacteria is not limited to aquatic ecosystems, but it extends to terrestrial ecosystems including extremely arid environments such as deserts (Pointing & Belnap, 2012). Some cyanobacterial species belonging to the genera *Nostoc* and *Chroococcidiopsis* are present in dry areas, and they exhibit high tolerance to desiccation (Singh, 2018). For example, *Chroococcidiopsis* strains isolated from deserts could form colonies after 4 years of storage in a dry state, and the terrestrial *Nostoc commune* was revived via inoculation into medium after storage in a desiccated state for approximately 90 years (Fagliarone et al., 2017; Lipman, 1941).

Loss of water from cells induces various stresses and damages various cellular components including proteins, membrane lipids, and DNA (Potts, 2001; Singh, 2018). Desiccation-tolerant cyanobacteria adopt multiple strategies to survive harsh environments during desiccation. *N. commune* cells are embedded in extracellular polysaccharide (EPS), which protects cells from desiccation (Tamaru et al., 2005). In *Anabaena* sp. strain PCC 7120 (hereafter *Anabaena* PCC 7120), EPS excretion was increased by overexpression of *sigJ*, which encodes the sigma factor of RNA polymerase, and the mutant displays higher dehydration tolerance than the wild-type strain (WT) (Yoshimura et al., 2007). The accumulation of compatible solutes

such as trehalose and sucrose, which stabilize the proteins and cellular membranes of dehydrated cells (Tapia & Koshland, 2014), represents another strategy of desiccation tolerance. *N. commune* and a *Chroococcidiopsis* strain accumulate trehalose and sucrose in response to dehydration, salt and osmotic stress (Hershkovitz et al., 1991; Sakamoto et al., 2009). *Anabaena* PCC 7120 also utilizes both sugars as compatible solutes, and disruption of trehalose synthesis genes decreases dehydration tolerance (Higo et al., 2006). Moreover, *Escherichia coli* expressing the sucrose synthesis gene *spsA* from the cyanobacterium *Synechocystis* sp. strain PCC 6803 (hereafter *Synechocystis* PCC 6803) accumulates sucrose within cells and exhibits a drastic increase in survival rate after dehydration (Billi et al., 2002).

Oxidative damage caused by reactive oxygen species (ROS) is one of the most deleterious effects of dehydration (França et al., 2007). ROS generation is increased by dehydration, resulting in lipid peroxidation, denaturation of proteins through oxidative modifications, and DNA double-strand breakage. Antioxidant defence systems that suppress ROS generation and scavenge generated ROS protect cellular components from oxidation (Singh, 2018). Because most antioxidant defence systems are proteinous, avoidance of protein oxidation is a key factor for desiccation tolerance (Daly, 2009). Desiccation-resistant bacteria display lower oxidative modification of proteins than desiccation-sensitive bacteria during dehydration (Fredrickson et al., 2008). *Chroococcidiopsis* sp. strain CCMEE 029 is highly tolerant to desiccation, and it does not undergo protein oxidation after 1 year of incubation under dry conditions. Contrarily, *Synechocystis* PCC 6803 exhibits substantial protein oxidation, and it is incapable of surviving under the same conditions (Fagliarone et al., 2017).

Anabaena PCC 7120 is a model organism of bacterial cellular differentiation (Flores et al., 2019). It has been also used to study the molecular mechanisms of responses to stresses including dehydration (Ohmori et al., 2001; Wang et al., 2002). *Anabaena* PCC 7120 displays moderate dehydration tolerance and shows drastic changes in gene expression by dehydration (Higo et al., 2006; Singh et al., 2013). However, the molecular mechanisms of regulation of gene expression in response to dehydration have not been elucidated. In *Anabaena* PCC 7120, *orrA*, which encodes a response regulator of the bacterial two-component regulatory system, was identified as a regulator of *lti2* in response to salt and osmotic stresses (Schwartz et al., 1998). We previously indicated that OrrA regulates the expression of *spsA*, *susA*, and *susB*, which participate sucrose synthesis, in response to salt stress (Ehira et al., 2014). Sucrose accumulation under salt stress conditions is lowered by the *orrA* disruption, and intracellular sucrose levels are increased by *orrA* overexpression (Ehira et al., 2014). In this study, we analysed the global effects of *orrA* disruption on gene expression, observing that OrrA is a master regulator of dehydration and salt stress responses in *Anabaena* PCC 7120. Moreover, among hundreds of genes regulated by OrrA, *avaKa*, which encodes a protein of unknown function, proved essential for dehydration tolerance.

EXPERIMENTAL PROCEDURES

Bacterial strains and culture conditions

Anabaena sp. strain PCC 7120 and its derivatives were grown at 30°C under continuous illumination provided by a fluorescent lamp at 30 μE m^{-2} s^{-1} in the nitrogen-free modified Detmer's medium (MDM_0) (Watanabe, 1960). Liquid cultures were bubbled with air containing 1% (v/v) CO_2. Salt stress conditions were generated by adding 50 mM NaCl to cultures in the late-logarithmic phase (OD_{750} = 0.5–0.7). Dehydration stress was imposed on *Anabaena* PCC 7120 filaments as described previously (Higo et al., 2006). A 25-ml portion of cultures in the late-logarithmic phase was filtered through 47-mm, 0.45-μm pore size mixed cellulose ester filters (Tokyo Roshi Kaisha, Tokyo, Japan) and dried for the indicated times at 30°C under continuous illumination provided by a fluorescent lamp at 10 μE m^{-2} s^{-1} in a Petri dish.

Mutant construction

All primers used in this study (Table S2) were designed based on genome data from CyanoBase (Fujisawa et al., 2017). DRorrAS was used as an *orrA* disruptant in this study (Ehira et al., 2014). DRavaKaK, DRavaKbK, DRavaKcS, and DRavaKdS, in which *avaKa* (*all4050*), *avaKb* (*all5315*), *avaKc* (*all4051*), and *avaKd* (*alr5332*), respectively, were disrupted, were constructed by replacing each gene with an antibiotic-resistant gene in the same manner as performed for DRorrAS using primer pairs specific for each gene (Ehira et al., 2014).

DNA microarray analysis

Total RNA was extracted from whole filaments according to Pinto et al. (Pinto et al., 2009) and was treated with DNase I (Takara Bio, Shiga, Japan). Global gene expression was analysed using the *Anabaena* oligonucleotide microarray as described previously (Ehira & Ohmori, 2006). Microarray analyses were conducted using three sets of RNA samples isolated from independently grown cultures. Two hybridization reactions

were performed with different combination of Cy-dyes for each set of RNA samples. Thus, six replicates were available per gene to determine changes in gene expression. Genes with at least twofold differences in transcript levels ($p < 0.05$; Student's *t*-test) were identified. The microarray data were deposited in the KEGG Expression Database (accession numbers ex0001979 to ex0002002).

Quantitative reverse transcription-PCR

cDNAs were synthesized from 1 μg of total RNA with random hexamer primers using a PrimeScript first strand cDNA synthesis kit (Takara Bio). qRT-PCR was performed using the Thermal Cycler Dice Real Time System II (TP900; Takara Bio) in a 20-μl reaction mixture containing 10 μl of THUNDERBIRD SYBR qPCR Mix (Toyobo, Osaka, Japan), 0.2 μM each of gene-specific forward and reverse primers, and cDNA. Relative transcript levels were normalized to the value for 16S rRNA and represented as means of duplicate measurements.

Dehydration tolerance assay

Cells exposed for dehydration stress as described above were suspended in MDM_0 at the indicated time. The suspended cultures were diluted with MDM_0 to OD_{750} of 0.1 and incubated at 30°C at 10 μE m^{-2} s^{-1}. After 72 h of incubation, OD_{750} of the cultures was measured.

Determination of sucrose contents

The low-molecular-mass compounds of cells dehydrated for the indicated time were extracted with 80% ethanol for 3 h at 65°C (Higo et al., 2006). After centrifugation, supernatants were vacuum dried, and the residue was dissolved in 0.5 ml of water. The samples were treated with 3 U of invertase (Sigma) in 50 mM sodium acetate (pH 4.5) for 2 h at 37°C, and the concentration of released glucose was determined using a Glucose Assay Kit (Biovision). The chlorophyll *a* content of cultures was determined as described previously (Mackinney, 1941).

Quantification of eluted phycobiliproteins

Dehydrated cells were resuspended in MDM_0 and statically incubated for 5 min at room temperature. Cells were collected by centrifugation, and the absorbance of the supernatants at 620 nm was measured. A_{620} was normalized by OD_{750} measured prior to centrifugation.

RESULTS

OrrA is a major regulator of salt stress response

Because sucrose synthesis under salt stress conditions is regulated by OrrA in *Anabaena* PCC 7120 (Ehira et al., 2014), the functions of OrrA in global salt stress response regulation were investigated by transcriptome analysis. The gene expression profiles of WT and the *orrA* mutant strain DRorrAS were compared between cells subjected to salt stress (50 mM NaCl) for 3 h and cells before stress treatment. The transcript levels of 298 genes were increased at least twofold by salt stress in WT, while those of another 1358 genes were decreased (Table S1). Three genes involved in sucrose metabolism, namely *spsA*, *susA* and *susB*, were upregulated by salt stress as previously reported (Ehira et al., 2014). The upregulated genes included *mth* encoding an enzyme for trehalose synthesis (Higo et al., 2006) and *avaK* genes (all4050, all4051, and all5315), which are predominantly expressed in cyanobacterial resting cells called akinetes in *Anabaena variabilis* ATCC 29413 (Zhou & Wolk, 2002). Genes encoding proteins that prevent ROS generation, such as *hli/scp* genes (asl0514, asl0873, asr3042, and asr3043) and *dps* genes (all0458 and all1173), and genes that detoxify ROS, such as *katA* and *katB*, were also upregulated (Howe et al., 2018; Tibiletti et al., 2018). The downregulated genes included genes involved in photosynthesis (*psa* and *psb* genes) and carbon fixation (*rbcLXS*, *prk*, *ccmNMLK*, *ecaA*, and *cmpABCD*), and genes encoding components of the phycobilisome (*apc*, *cpc*, and *pec* genes). Moreover, the transcript levels of genes involved in transcription and translation (*rpl* and *rps* genes, *rpoA*, *rpoC1*, and *rpoC2*), and ATP synthesis (*atp* genes) were decreased. The suppression of cell growth under salt stress conditions leads to downregulation of these genes (Ehira et al., 2014).

In DRorrAS, the numbers of genes upregulated and downregulated by salt stress were decreased to 71 and 51, respectively (Table S1). Meanwhile, 37 genes were upregulated in both WT and DRorrAS (Figure 1), but the degree of upregulation was higher in WT than in DRorrAS. Temporal changes in the expression of salt-induced genes were examined by quantitative reverse transcription (qRT)-PCR (Figure 2). In WT, *mth* expression was induced within 30 min after NaCl addition, with the *mth* transcript level increasing approximately 800-fold after 60 min, but its induction was completely abolished in DRorrAS (Figure 2A). The induction patterns of two *dps* genes (all0458 and all1173) differed from each other, but both genes lost salt-responsive induction by *orrA* disruption (Figure 2B). There are four paralogs of *avaK* (*avaKa*, *avaKb*, *avaKc*, and *avaKd*) in *Anabaena* PCC 7120. All *avaK* genes were induced by salt stress, which depended on *orrA* (Figure 2C). These

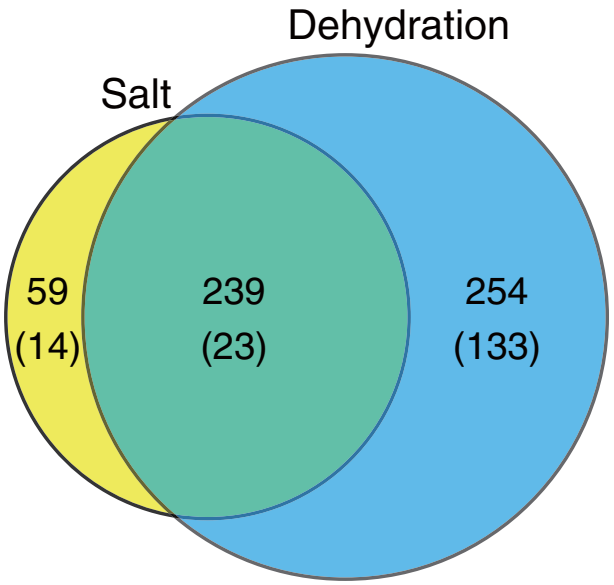

FIGURE 1 Venn diagrams of genes upregulated by salt stress and dehydration in wild-type *Anabaena* sp. strain PCC 7120 (WT) and the *orrA* disruptant DRorrAS. The numbers in parenthesis indicate genes upregulated in both WT and DRorrAS.

results indicate that OrrA is a major regulator of salt stress response in *Anabaena* PCC 7120.

orrA is necessary for dehydration tolerance

OrrA regulates the expression of most salt-responsive genes, whereas its disruption does not affect growth under salt stress conditions (Ehira et al., 2014). Expression of *orrA* is induced by not only salt stress but also dehydration (Higo et al., 2006). Moreover, many genes upregulated by salt stress, such as *mth*, *hli/scp* genes, and *dps* genes, are also upregulated by dehydration (Higo et al., 2006). To investigate the effect of the *orrA* disruption on gene expression in response to dehydration, gene expression profiles were compared between WT and DRorrAS after 3 h of dehydration. In WT, 493 genes were upregulated by dehydration, while 1307 genes were downregulated (Table S1). Among the upregulated genes in WT, 156 genes were also upregulated in DRorrAS, but the extent of upregulation was significantly lower in DRorrAS than in WT (Figure 1

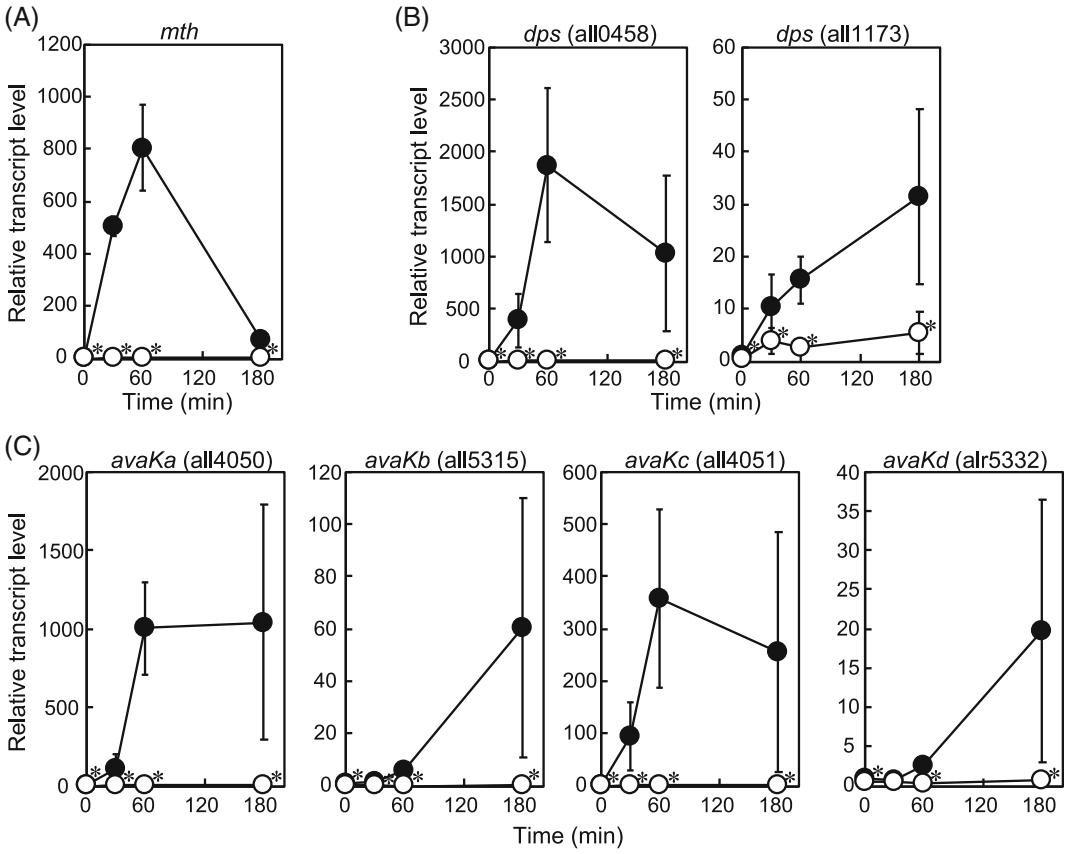

FIGURE 2 Temporal changes in the transcript levels of genes upregulated by salt stress. The transcript levels of *mth* (A), *dps* (B), and *anaK* (C) after the addition of 50 mM NaCl were determined by qRT-PCR in WT (black circles) and the *orrA* disruptant (white circles). The transcript levels were determined in duplicated measurements using three independently grown cultures. The transcript level at 0 min in WT was taken as 1. Data are presented as the mean ± SD. Data that represent a significant difference ($p < 0.05$; Student's *t*-test) between WT and the *orrA* disruptant are marked with asterisks.

TABLE 1 Sucrose contents after dehydration

	Sucrose (μmol/mg chla)	
Strain	**0 h**	**9 h**
WT	0.06 ± 0.06	5.00 ± 0.39
DRorrAS	0.04 ± 0.04	2.26 ± 0.29

Abbreviations: chla, chlorophyll *a*; DRorrAS, *orrA* mutant *Anabaena* sp. strain PCC 7120; WT, wild-type *Anabaena* sp. strain PCC 7120.

and Table S1). Salt stress and dehydration increased the expression of the same 239 genes, only 23 of which were upregulated by both stresses in DRorrS (Figure 1). *spsA*, *susA*, and *susB* were also induced by dehydration under the control of OrrA (Table S1). The intracellular sucrose level in WT was increased more than 80-fold after 9 h of dehydration, whereas the level was twofold lower in DRorrAS than in WT (Table 1). Thus, OrrA plays a principal role in regulating gene expression during dehydration in *Anabaena* PCC 7120.

To investigate the physiological role of OrrA in dehydration tolerance, growth ability after dehydration was analysed. Filaments of *Anabaena* PCC 7120 grown in the liquid medium were collected by filtration, and the filaments collected on the filter were dehydrated in a Petri dish. The weight of the filters decreased during the first 8 h of incubation before reaching a plateau, indicating that all water had evaporated at 8 h (Figure 3A). To determine growth ability after dehydration, filaments that had been dehydrated for the indicated times were resuspended in liquid medium. After adjusting the optical density at 750 nm (OD_{750}) of the suspended filaments to 0.1, the filaments were incubated under normal growth conditions, and the OD_{750} was measured after 3 days incubation (Figure 3B). In WT, filaments were able to resume growth even after 24 h of dehydration on the filters (Figure 3B). DRorrAS retained its ability to grow after 6 h of dehydration on the filters, but their growth was severely impaired after 7 and 8 h (Figure 3B). Moreover, DRorrAS completely lost its growth ability after 24 h of dehydration. Thus, *orrA* is necessary for survival under dehydration conditions.

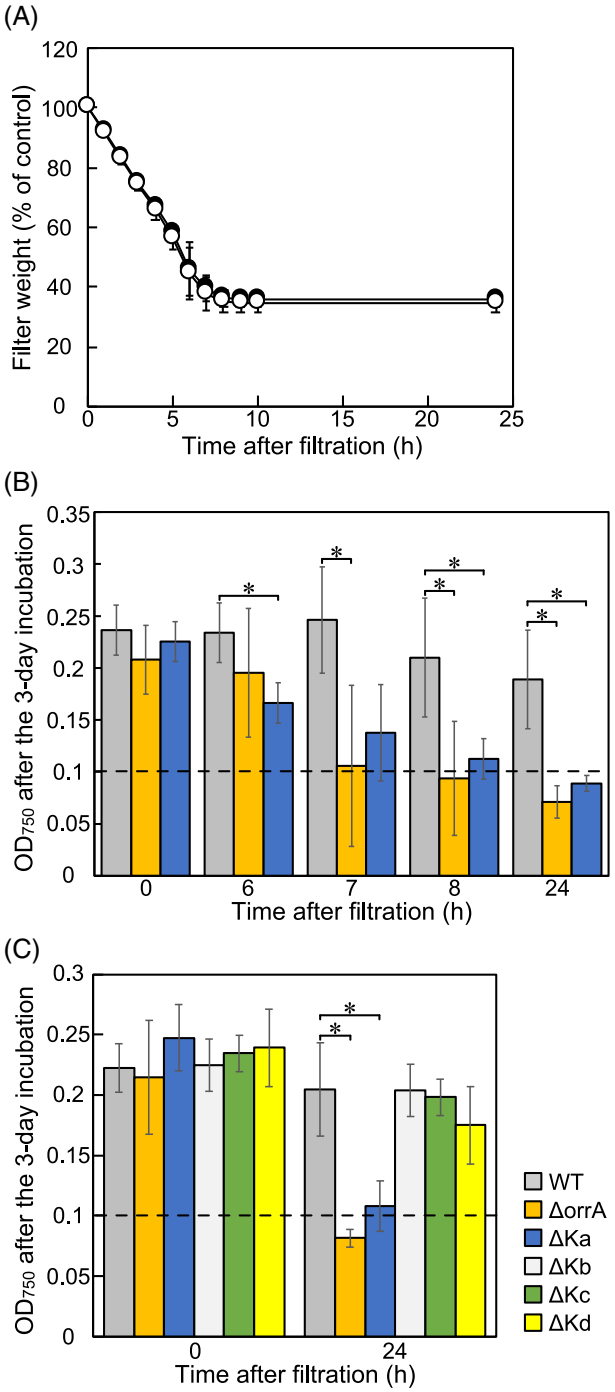

FIGURE 3 Dehydration tolerance of *Anabaena* PCC 7120. (A) Changes in weight of filters during 24 h of incubation. Filaments of WT (black circles) and the *orrA* disruptant (white circles) were collected on filters and the filters dried for 24 h. The weight of each filter was measured during the 24-h incubation and presented as a ratio to the control (0 h). (B) Growth ability of disruptants of *orrA* and *avaKa* after dehydration. Filaments on the filters were resuspended in liquid medium at the indicated time, the optical density at 750 nm (OD_{750}) of the suspension was adjusted to 0.1, and then filaments were incubated for 3 days in liquid medium. OD_{750} after the 3-day incubation was determined for WT (grey bars), the *orrA* disruptant (orange bars), and the *avaKa* disruptant (blue bars). The experiments were repeated using at least three independently grown cultures. Data are presented as the mean ± SD. Significance testing was performed by Student's *t*-test corrected by Bonferroni method (*$p < 0.05$). (C) Dehydration tolerance of disruptants of the *avaK* genes. Filaments of WT (grey bars), the *orrA* disruptant (orange bars), and disruptants of *avaKa* (blue bars), *avaKb* (light grey bars), *avaKc* (green bars), and *avaKd* (yellow bars) were exposed to dehydration for 0 or 24 h, and then their growth ability was evaluated as described in Figure 3B. The experiments were repeated using three independently grown cultures. Data are presented as the mean ± SD. Significance testing was performed by Student's *t*-test corrected by Bonferroni method (*$p < 0.05$).

FIGURE 3 Legend on next page.

avaKa is a determinant of dehydration tolerance

To further reveal the mechanisms of dehydration tolerance, the involvement of genes regulated by OrrA in dehydration tolerance was investigated. We focused on the *avaK* genes. *avaK* was originally identified as a gene that is predominantly expressed in akinetes in *A. variabilis* ATCC 29413 (Zhou & Wolk, 2002). Akinetes are extremely resistant to stresses such as cold and desiccation (Adams & Duggan, 1999). Although *Anabaena* PCC 7120 does not form akinetes, there are four homologues of the *avaK* genes; *avaKa* (*all4050*), *avaKb* (*all5315*), *avaKc* (*all4051*), and *avaKd* (*alr5332*). All *avaK* genes were induced by salt stress and dehydration, which depended on *orrA* (Figure 2C and Table S1). Each *avaK* paralog was inactivated, and the growth of the disruptants after dehydration was determined (Figure 3C). The *avaKa* disruptant DRavaKaK could not grow after 24 h of dehydration on the filters, whereas the other *avaK* disruptants retained similar growth ability as WT (Figure 3C). Time-course experiments illustrated that DRavaKaK lost growth ability at 8 h (Figure 3B), when water had completely evaporated from filters (Figure 3A). This indicated that *avaKa* disruption has a fatal effect on survival under dehydration.

Disruption of *avaKa* alone resulted in the loss of dehydration tolerance. To examine the involvement of other genes regulated by OrrA in dehydration tolerance, damage to DRorrAS and DRavaKaK cells after dehydration was assessed by quantifying phycocyanin elution from the cells (Figure 4). Phycocyanin is a water-soluble pigment protein and is released extracellularly upon cell lysis (Arii et al., 2015). In WT, phycocyanin elution was not enhanced by dehydration. In DRorrAS and DRavaKaK, eluted phycocyanin levels were comparable to WT levels before dehydration, but phycocyanin elution was increased by dehydration. The amount of eluted phycocyanin was more than two-fold higher in DRorrAS than in DRavaKaK, indicating greater cell membrane damage in DRorrAS. Thus, the *orrA* disruption is more detrimental than the *avaKa* disruption.

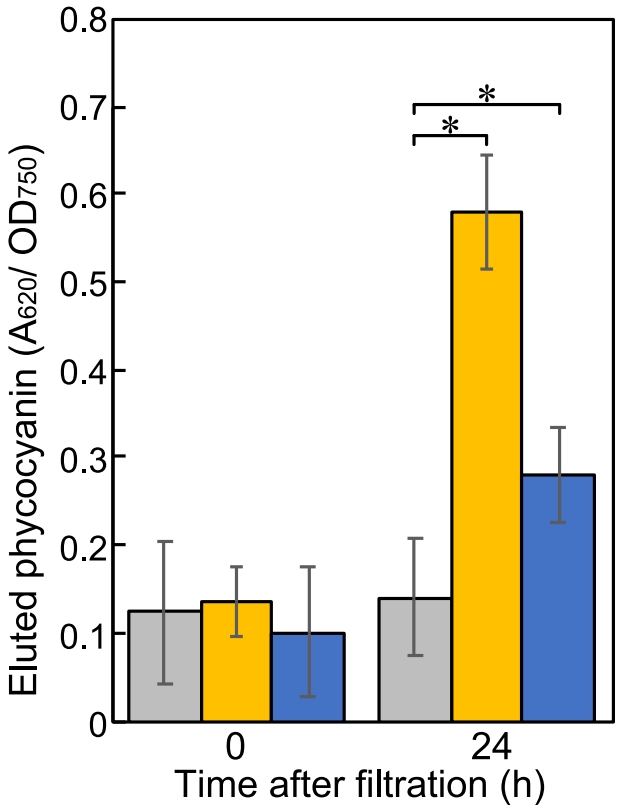

FIGURE 4 Elution of phycocyanin after dehydration. Phycocyanin eluted into the medium from cells of WT (grey bars), the *orrA* disruptant (orange bars), and the *avaKa* disruptant (blue bars) that were dehydrated on filters for 0 or 24 h was determined by measuring the absorbance at 620 nm. Significance testing was performed by Student's *t*-test corrected by Bonferroni method (*$p < 0.05$)

orrA is also involved in cold acclimation

OrrA was originally identified as a regulator of the salt and osmolyte responses of *Iti2* (Schwartz et al., 1998). *Iti2* is also known to be upregulated under low temperatures (Ehira et al., 2005). In addition, low temperature-responsive genes, such as alr0169, alr0803, alr0804, and alr1819, were under the control of OrrA (Table S1). Hence, we analysed the effects of *orrA* disruption on cold acclimation. *orrA* expression increased within 30 min after the temperature was decreased from 30 to 22°C (Figure 5A). Growth of DRorrAS at 30°C is comparable to that of WT (Ehira et al., 2014). When the growth temperature was lowered from 30 to 22°C, growth was first delayed and then recovered (Figure 5B). However, the recovery in DRorrAS took longer than that in WT (Figure 5B). Thus, OrrA also influences cold acclimation.

DISCUSSION

In this study, we revealed that OrrA played principal roles in the dehydration response and tolerance as well as cold acclimation in *Anabaena* PCC 7120 (Figures 1, 3, and 5). *orrA* disruption abolished or diminished the induction of approximately 400 genes by dehydration (Figure 1 and Table S1). Especially, induction of *avaKa* by OrrA was crucial for dehydration tolerance. Disruption of only *avaKa* among hundreds of genes included in the OrrA regulon resulted in the loss of dehydration tolerance (Figure 3). Meanwhile, diverse genes of the OrrA regulon were likely to be involved in dehydration tolerance, because dehydration had more deleterious

effects on the cells of the *orrA* disruptant than on those of the *avaKa* disruptant (Figure 4). As previously indicated in response to salt stress (Ehira et al., 2014), OrrA regulated sucrose synthesis under dehydration conditions (Table 1). In addition, the trehalose synthesis genes *mth* and *mts*, which encode maltooligosyl trehalose hydrolase and maltooligosyl trehalose synthase, respectively, were regulated by OrrA (Table S1). Because disruption of *mth* and *mts* results in decreased dehydration tolerance (Higo et al., 2006), the depression of compatible solutes synthesis partly accounts for the susceptibility of the *orrA* disruptant to dehydration. Oxidative damage caused by ROS is one of the most deleterious effects of dehydration (França et al., 2007). ROS generation is increased by dehydration, resulting in lipid peroxidation, denaturation of proteins through oxidative modifications, and DNA double-strand breakage. The upregulation of the ROS defence systems by OrrA would also contribute to dehydration tolerance.

avaKa encodes a protein of unknown function with a PRC-barrel domain at its N-terminus and a DUF2382 domain at its C-terminus. Although *avaKb*, *avaKc*, and *avaKd* were also induced by dehydration in an OrrA-dependent manner, their disruption did not affect dehydration tolerance. *avaKa* was most strongly induced among the *avaK* genes (Figure 2, Table S1). Differences in expression levels of AvaK proteins could explain the different phenotypes among the *avaK* mutants. Meanwhile, most cyanobacteria belong to the order of *Nostocales* have multiple *avaK* genes in their genomes, implying functional differences among *avaK* paralogues. AvaK and OrrA homologues are highly expressed in desiccated samples in *N. flagelliforme* (Shang et al., 2019), and an AvaK homologue is induced by dehydration in *Nostoc* sp. HK-01 (Yoshimura et al., 2006). Moreover, it is indicated that *avaK* genes are specifically enriched in the genomes of terrestrial cyanobacteria compared to marine and freshwater cyanobacteria (Chen et al., 2021). These results suggest the conserved function of *avaK* in desiccation tolerance of cyanobacteria.

The function of the PRC-barrel domain is unknown. It is found in the H subunit of photosystem reaction centre (PRC) of the purple bacteria (Anantharaman & Aravind, 2002). The H subunit of PRC is necessary for functional PRC assembly, and it regulates electron transfer in PRC (Lupo & Ghosh, 2004; Takahashi & Wraight, 1996). The PRC-barrel domain is also found at the C-terminus of RimM, which is involved in RNA processing and ribosome assembly (Anantharaman & Aravind, 2002). The PRC-barrel domain of AvaKa is phylogenetically most closely related to that of the H subunit of PRC (Anantharaman & Aravind, 2002). AvaKa homologues are present in the highly radiation- and desiccation-resistant bacterium *Deinococcus radiodurans* and some species of the genus *Psychrobacter*, which are psychro- and osmo-tolerant bacteria

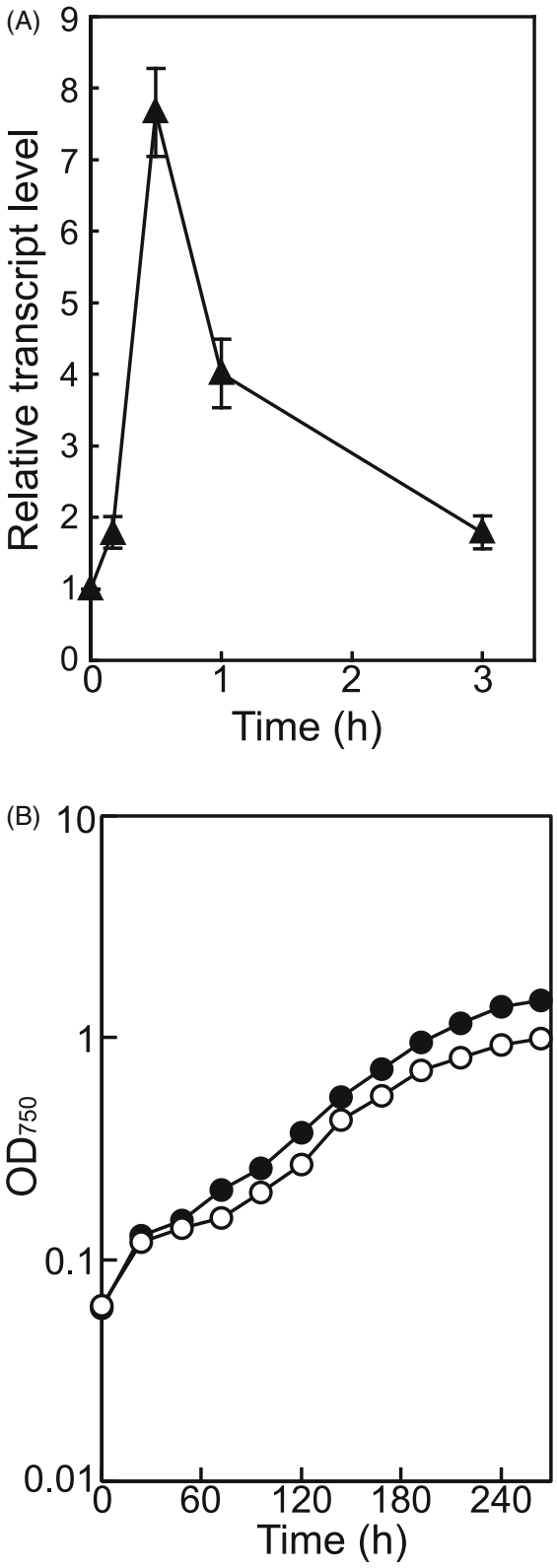

FIGURE 5 Involvement of OrrA in cold acclimation. (A) Changes in the *orrA* transcript levels after a shift to low temperature (22°C). The transcript level at 0 h was taken as 1. Data are presented as the mean ± SD. (B) Growth of WT (black circles) and the *orrA* disruptant (white circles) at 22°C. Growth was monitored by measuring OD_{750}. The experiments were repeated with three independently grown cultures. Data are presented as the mean ± SD.

(Kim et al., 2012). In *D. radiodurans*, the expression of the *avaKa* homologue DR1314 is regulated by the extracytoplasmic function sigma factor Sig1 and the response regulator DrRRA, which is essential for radioresistance in *D. radiodurans* (Schmid et al., 2005; Wang et al., 2016). The DR1314 disruptant is sensitive to hydrogen peroxide exposure and heat shock. Thus, AvaK homologues play important roles in the stress resistance of extremophiles.

OrrA was demonstrated as a master regulator of the response to salt stress and dehydration and was also involved in the response to low temperature (Figures 1 and 5). OrrA was originally identified as the regulator of *lti2* in response to salt and osmotic response (Schwartz et al., 1998), implying that OrrA plays an important role in the osmotic stress response. Moreover, OrrA is shown to be regulate expression of genes for a sunscreen compound in response to ultraviolet in *N. flagelliforme* (Shang et al., 2018). Evaporation of water from the medium surrounding cells increases external solute concentrations, resulting in salt and osmotic stresses. Therefore, a common signalling pathway could activate OrrA in response to dehydration, salt, and osmotic stresses. Because OrrA is a response regulator, its activity is regulated through the phosphorylation by a histidine kinase (Hik). Genes encoding Hik are located upstream of *orrA* in the genome of some *Anabaena* and *Nostoc* species, and these are candidates of cognate Hiks for OrrA (Ehira et al., 2014). Identification of a cognate Hik for OrrA would reveal how diverse signals are sensed and activate OrrA.

AUTHOR CONTRIBUTIONS

Masayuki Ohmori and Shigeki Ehira conceived the study. Satoshi Kimura and Shigeki Ehira designed the experiments. Satoshi Kimura, Miho Sato, Xingyan Fan, and Shigeki Ehira conducted the experiments and analysed the data. Shigeki Ehira wrote the manuscript, and all authors edited and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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