The \textit{all0458/lti46.2} gene encodes a low temperature-induced Dps protein homologue in the cyanobacteria \textit{Anabaena} sp. PCC 7120 and \textit{Anabaena variabilis} M3

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DNA-binding proteins from starved cells (Dps), which are encoded by many bacterial genomes, protect genomic DNA via non-specific DNA binding, as well as inhibition of free radical formation by chelating Fe(II). In the filamentous cyanobacterium \textit{Anabaena}, the second gene \textit{lti46} in the low temperature-induced gene operon \textit{lti46} in strain M3 was found to encode a homologue of Dps, but for a long time this gene remained poorly characterized. A gene cluster, \textit{all0458–all0457} was found later to be 100\% identical to the \textit{lti46} gene cluster in a closely related strain, PCC 7120. In the present study, we detected ferroxidase activity of the \textit{lti46.2}/\textit{all0458} protein, which formed a dodecamer, as found in other Dps proteins. In addition, three homologues of \textit{all0458} were found in strain PCC 7120, namely, \textit{all1173}, \textit{alr3808} and \textit{all4145}. We analysed expression of the \textit{lti46} or \textit{all0459–8-7} gene cluster in both strains, M3 and PCC 7120, and confirmed its induction by low temperature. We found that the \textit{Alr3808}–GFP fusion protein and the \textit{All1173–GFP} fusion protein were localized to the nucleoids. In the \textit{all0458} null mutant, the transcript of the \textit{alr3808} gene accumulated. These results suggest that there might be complex cooperation of various members of the \textit{dps} family in protecting the genome from environmental stresses such as changing temperature.

\textit{INTRODUCTION}

Dps (DNA-binding protein from starved cells) is a bacterial protein involved in protection and compaction of genomic DNA (Pettijohn, 1996). In \textit{Escherichia coli} (Almirón \textit{et al.}, 1992), this protein is known to act specifically in the stationary phase and, in this respect, it is different from other nucleoid proteins (Azam & Ishihama, 1999), such as HU (heat-stable protein from \textit{E. coli} U13) (Rouvière-Yaniv & Gros, 1975) and integration host factor (IHF). Historically, the Dps homologues discovered first were the TpF1/TyF1 proteins in two closely related subspecies of \textit{Treponema pallidum} (Noordhoek \textit{et al.}, 1989). We then found that the product of the second gene, \textit{lti46.2}, of the \textit{lti46} operon (GenBank accession no. D01016; cited by Almirón \textit{et al.}, 1992; Evans \textit{et al.}, 1995), which was induced at low temperature in \textit{Anabaena variabilis} M3 (Sato, 1992, 1993), was a homologue of TpF1/TyF1. Nevertheless, no functional relationship could be found between these proteins in such divergent bacteria. Various homologues of Dps were then reported, such as MrgA and MrgC (for metalloregulation) in \textit{Bacillus subtilis} (Chen \textit{et al.}, 1993), ORF1, encoded by a gene adjacent to the BPO gene in \textit{Streptomyces aureofaciens} (Pfeifer \textit{et al.}, 1992), neutrophil-activating protein (NapA) in \textit{Helicobacter pylori}, and \textit{E. coli} Dps, among others (Evans \textit{et al.}, 1995).

Although different functions were ascribed to these homologues of Dps, current understanding suggests that the Dps family is a member of the ferritin superfamily and involved in bacterial iron homeostasis (Andrews \textit{et al.}, 2003). Dps protein is characterized by a common 3D dodecameric (12-mer) structure (Haikarainen & Papageorgiou, 2010), whereas bacterioferritin forms a tetracosamer (24-mer) that covalently binds 12 haems (Andrews \textit{et al.}, 2003). Most known Dps proteins have a ferroxidase activity that oxidizes Fe(II) to Fe(III), but DNA-binding activity is found in only limited numbers of members of the Dps family. Two mechanisms are proposed for DNA protection. First, non-specific interaction with DNA mediates the formation of a crystalline structure in the stationary phase in \textit{E. coli} that

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results in hyper-condensation of nucleoids, which physically shields DNA from damage (Almirón et al., 1992; Frenkel-Krispin et al., 2001; Nair & Finkel, 2004). Second, the Fe(II)-chelating activity provided by the Dps ferroxidase centre inhibits formation of free radicals by Fenton-type reactions (Zhao et al., 2002). The level of Dps protein generally increases when cells are subjected to stresses. In E. coli, Dps is accumulated as the most abundant protein in the stationary phase. Dps protects the cell not only from oxidative stress in the narrow sense of the word, but also from damage caused by UV and gamma irradiation or from iron and copper toxicity among others (Nair & Finkel, 2004). Extracellular functions of Dps homologues are also known, such as cell adhesion and chemotaxis of neutrophils (Dundon et al., 2001).

The lti46.2 gene in A. variabilis was found to be identical to the all0458 gene in a closely related Anabaena sp. PCC 7120 (also referred to as Nostoc sp. PCC 7120), in which the complete genome has been determined (Kaneko et al., 2001). Strain M3 is also known as Anabaena sp. PCC 7118, and is defective in heterocyst formation due to an unknown mutation (Sato & Wada, 1996). However, both M3 and PCC 7118 are essentially identical to PCC 7120 in genomic sequence according to large-scale sequencing (N. Sato and N. Tajima, unpublished results). A clearly defined difference between PCC 7120 and M3 is the absence of the rbpA3 gene in the former (Sato & Maruyama, 1997). In Anabaena sp. PCC 7120, there are four genes encoding Dps (all0458, all1173, alr3808 and all4145). M3 has identical copies of these genes without a mutation. The alr3808 gene is regulated by ferric uptake regulator (Fur) (Hernández et al., 2007), which is known to act as an Fe(II)-dependent transcriptional repressor of bacterial promoters. All1173 protein has DNA-binding activity (Wei et al., 2007). However, the function of all0458 and all4145 has not been reported. Cyanobacterial Dps has been studied in the light of protection from stress-induced DNA damage in Synechococcus sp. PCC 7942 (Peña & Bullerjahn, 1995; Peña et al., 1995; Dwivedi et al., 1997; Sen et al., 2000) and Thermosynechococcus elongatus (Alaleona et al., 2010).

In the present study, we first identified ferroxidase activity, multimer formation and nucleoid localization of the lti46.2/all0458 protein. Then we analysed the expression of the lti46.2/all0458 gene under low-temperature conditions. Effects of deletion of this gene on the expression of other dps homologues were also analysed. The results suggest that the lti46.2/all0458 protein is involved in DNA protection under stress conditions.

**METHODS**

**Growth of the organism.** A. variabilis M3 was grown at 38 °C as described previously (Sato, 1994). In temperature shift experiments, growth temperature was shifted from 38 to 22 °C. Anabaena sp. PCC 7120 and its derivatives were grown photoautotrophically in BG-11 medium (Rippka et al., 1979) buffered with 5 mM HEPES-NaOH (pH 7.5) at 32 °C under continuous illumination provided by fluorescent lamps at a fluence rate of 50 μE m⁻² s⁻¹, with bubbling with air containing 1.0% (v/v) CO₂. Young culture (exponential or linear phase) cells were harvested when OD₇₅₀ was 0.4–0.5, whereas old culture (stationary phase) cells were harvested when OD₇₅₀ was over 3.0. In temperature shift experiments, cells grown at 32 °C were transferred to 22 °C. The temperature of the culture medium reached 22 °C within a few minutes (Sato & Nakamura, 1998).

**Strain construction.** The all0458-gfp strain SR0458GS was constructed as follows. A DNA fragment containing the all0458 gene was amplified by PCR using primers 0458-Sall, containing a Sall site, and 0458-Nocl, containing an Nocl site, corresponding to nucleotides +500 to +538 with respect to the translational start site of the all0458 gene. The resulting PCR product was cloned into the Sall–Nocl site of SgFP (S65T) to construct p0458G. A Sall–EcoRI fragment from p0458G was cloned between the Sall and EcoRI sites of pBluescriptII SK + (Stratagene), and then a BamHI fragment containing a spectinomycin resistance cassette derived from pDW9 (Golden & Wiest, 1988) was inserted into the BamHI site to construct p0458GS. An Xhol–SpeI fragment containing this whole construct was cloned between the Xhol and SpeI sites of pBRBSII (Toyoshima et al., 2010) to construct pB0458GS. pB0458GS was transferred by conjugation into PCC 7120 cells according to the method of Elhai & Wolk (1988a) and a single recombinant, SR0458GS, was selected on a BG-11 plate containing spectinomycin (30 μg ml⁻¹). The strains alr3838-gfp and all1173-gfp were also constructed in a similar way, using the primers listed in Table S1.

The all0458 deletion strain DR0458N was constructed as follows. The DNA fragments upstream and downstream of the all0458 gene were amplified by PCR using primer set 0458upXhol, containing a Xhol site, and 0458upBamHI, containing a BamHI site (for nucleotides −1450 to −8 with respect to the translational start site); and primer set 0458downBamHI, containing a BamHI site, and 0458downSpeI, containing a SpeI site (for nucleotides +12 to +1496 with respect to the translational stop site), using genomic DNA of strain PCC 7120 as template. The upstream fragment was cloned between the Xhol and BamHI sites of pBluescriptII SK +, and the downstream fragment was then cloned between the BamHI and SpeI sites of the resulting plasmid to finally construct p0458. A BamHI fragment containing a neomycin resistance cassette derived from pRL161 (from C.P. Wolk; the plasmid corresponds to structure S.AI/L.HEHI [BamHI]/C.K1 according to the nomenclature of Elhai & Wolk, 1988b) was inserted into the BamHI site of p0458 to construct p0458N. The Xhol–SpeI fragment from p0458N was cloned between the Xhol and SpeI sites of the sacB vector pRL271 (Maldener et al., 1991) to construct pR0458N. pR0458N was transferred by conjugation into the cells of strain PCC 7120 according to the method of Elhai & Wolk (1988a), and double recombinants were selected for resistance to neomycin and sucrose as described by Cai & Wolk (1990). Segregation of mutant chromosomes was tested by PCR and completely segregated clone DR0458N was selected. In DR0458N, the region from −9 to +551 with respect to the translation start site of the all0458 gene (540 bp) was replaced by the neomycin resistance cassette.

**Cloning of the lti46 gene cluster.** Differential hybridization of a lambda EMBL4 library containing partially digested EcoRI genomic DNA fragments of strain M3 was performed as described previously (Sato, 1992). Clone pLti46E10 was a 10 kb subclone of a selected lambda clone, Lti46, in pBluescript SK +. An overlapping clone, pLti46H12, containing a 12 kb HindIII fragment was obtained by screening a mini-library in the plasmid using a 2 kb HindIII–EcoRI fragment of pLti46E10 containing the lti46.1 gene region as a probe. Nucleotide sequences of appropriate DNA regions were determined by the chemical method using end-labelled DNA fragments (Sato, 1992) and deposited in the database under accession number D01016.
S1 nuclease mapping of the transcribed regions was performed using an end-labelled DNA probe (RV) and *in vitro*-synthesized RNA probes (A, B, EH and H; see Fig. 1) as described previously (Sato, 1992).

**Promoter analysis.** A 3.5 kb EcoRV DNA fragment containing the first half of the coding region and the upstream region of the *lti46.1* gene was inserted in the promoter-selection vector pRL576 containing promoterless *lacZ* (Elhai & Wolk, 1988b) and mobilized into *Anabaena* M3 cells by triparental conjugation (Elhai & Wolk, 1988a). Since this plasmid cannot replicate itself in the cyanobacterial cells, exconjugants that were selected by neomycin (30 μg ml⁻¹) were expected to contain the chimeric plasmid integrated into the genome by a single recombination event, which was confirmed by Southern analysis.

The transgenic cyanobacteria were grown as described above in the presence of neomycin. The activity of β-galactosidase was determined using ONPG as a substrate, essentially according to the method used in *E. coli* (Miller, 1972), except that the reaction mixture was centrifuged at 15 000 g for 2 min before reading the A₄₂₀ and that the activity unit was defined as A₄₂₀/(time in min) × (volume in ml) × OD₇₅₀.

**Preparation of antibody against GST–Lti46.2/All0458 fusion protein.** The coding region of the *lti46.2* gene was amplified by PCR using the primers gst-1 and gst-2. The PCR product was digested with EcoRI and SalI, and inserted into a GST fusion vector pGEX-4T-1 (GE-Healthcare Bio-Sciences). The overproduction of GST–Lti46.2/All0458 fusion protein in *E. coli* XL-1 blue cells harbouring the recombinant plasmid and its purification were performed according to the manufacturer’s protocol. Antiserum against the GST–Lti46.2/All0458 protein was raised in guinea pigs using Freund’s adjuvant.

**Immunoblot analysis.** Protein was analysed by SDS-PAGE using a 12 % gel in a Mini-Protean II apparatus (Bio-Rad). Separated proteins were transferred to a PVDF membrane (Immobilon P, Millipore) and then probed with the guinea pig anti-GST–Lti46.2/All0458 antiserum.

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**Fig. 1.** Summary of mapping of transcripts for the *lti46* gene in strain M3. A schematic gene arrangement is shown at the top, with putative promoters (arrows) and terminators (inverted L-shaped marks). A hybridization probe as shown was used for the RNA blot hybridization in Fig. 4. The transcripts (monogenic, digenic and trigenic) that were estimated from the S1 mapping (lower part) are shown in the middle. The fragments detected by S1 mapping in Fig. S1 are shown in the lower part. Each detected fragment is marked by a letter, a–l. The same letters are used to label the detected bands in Fig. S1. The S1 probe RV was a 5′-labelled probe, whereas probes A, B, EH and H were labelled internally (see Methods).
All0458 antibody. The secondary antibody was a rabbit anti-guinea pig IgG antibody conjugated with alkaline phosphatase (Zymed). Detection was performed with NBT and BCIP (Promega).

Ferroxidase assay. A 5 μl volume of freshly prepared 8 mM FeSO₄ in distilled water was added to a solution of Lti46.2/All0458 protein [9.5 μg in 500 μl of 10% (v/v) glycerol in PBS, 0.08 μM] in an optical cuvette. A₃₁₀ was measured in a spectrophotometer (Shimadzu model UV-160A) to monitor oxidation of Fe(II) ions (van Wuytswinkel & Briat, 1995).

Gel filtration. A Superdex 200HR (10/30) column (GE-Healthcare) was used in an HPLC apparatus (Shimadzu LC-4A). Elution buffer was 50 mM sodium/potassium phosphate (pH 7.0), 150 mM NaCl. Molecular mass markers (thyroglobulin, ferritin, catalase, aldolase, BSA and ovalbumin) were obtained from GE-Healthcare.

Microscopy. Cells were viewed under an upright microscope model BX-60 (Olympus) using a 63×100 objective, and images were captured with an Olympus DP70 digital camera. For DAPI-stained DNA images, Anabaena cells were stained with 1 μg DAPI ml⁻¹ in TAN buffer (20 mM Tris/HCl, pH 7.5, 0.5 mM EDTA, 0.5 M sucrose, 7 mM 2-mercaptoethanol, 0.4 mM PMSF, 1.2 mM spermidine). The cells were examined using a U-MWU mirror unit (Olympus). For GFP fluorescence images, a fluorescence mirror unit, U-MNIBA3 (Olympus), allowing excitation at 470–495 nm and emission at 510–550 nm, was used.

RNA isolation and gel blot analysis. Isolation of total RNA from the whole filaments and RNA gel blot analysis were performed essentially as described previously with digoxigenin-labelled DNA probes (Sato, 1995). The DNA probe for the RNA blot analysis in Fig. 4 was an EcoRI–HindIII fragment containing nearly all the lti46 region (Fig. 1). For other analyses, each DNA probe was prepared by PCR with a set of specific primers (see Table S1 available with the online version of this paper). The coding region 19–515 of the all0458 gene was amplified with all0458-1 and all0458-2. The coding region 16–508 of the all1173 gene was amplified using all1173-1 and all1173-2/mk-2. The coding region 39–549 of the allr3808 gene was amplified using alrr3808-1 and alrr3808-2. The coding region 53–487 of the all4145 gene was amplified using all4145-1 and all4145-2.

Quantitative RT-PCR analysis. Total RNA was prepared by phenol/chloroform extraction, and then precipitated with 2.5 M LiCl. RNA was further purified with an RNeasy Plant Mini kit and an RNase-Free DNase Set (Qiagen). First-strand cDNA was prepared using 0.2 μg total RNA and random primers by SuperScript III reverse transcriptase (Invitrogen) and Recombinant RNase Inhibitor (Takara). Quantitative PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems) and appropriate primers in a Real-Time PCR system (model 7300, Applied Biosystems). The primers are listed in Table S1. The 16S rRNA gene was used to normalize transcript abundance.

RESULTS

Characterization of the lti46 gene cluster

The lti46.2/all0458 gene is a member of an operon consisting of three genes, all0459—all0458—all0457, in Anabaena sp. PCC 7120. The nucleotide sequence of this region is 100% identical to the sequence of the lti46 operon (lti46.1–lti46.2–lti46.3), which was isolated as a low temperature-responsive gene cluster in a closely related strain, Anabaena variabilis M3 (GenBank accession no. D01016). Comparative genomic analysis using the CyanoClust database (http://cyanoclust.c.u-tokyo.ac.jp/) (Sasaki & Sato, 2010) suggested that the N-terminal amino acid residues of the third gene product, Lti46.3/All0457, were missing with respect to its...
homologues. The initiation site of the lti46.3/all0457 gene should be a GTG codon located 120 bp upstream. This is true for both strain M3 and PCC 7120.

Based on detailed transcript mapping in strain M3 (Figs 1 and S1), various transcripts were identified: monogenic transcript (meaning a transcript containing only a single gene, but not necessarily meaning that the gene is transcribed monocistronically) lti46.1, monogenic transcript lti46.2, digenic transcript lti46.1–lti46.2, and trigenic transcript lti46.1–lti46.2–lti46.3. Using these results together with sequence analysis, we identified two promoters in the lti46 region: one located upstream of the lti46.1 gene and the other located upstream of the lti46.2 gene (Fig. S2). The results also suggested that all transcripts accumulated at lower growth temperatures.

Homologues of the three genes were searched in the Gclust database at http://gclust.c.u-tokyo.ac.jp/ (Sato, 2009). For a comprehensive search, a special dataset, AllBact2010, including 1196 prokaryotic genomes as of November 2010, was used. The results suggest that only a limited number of species of cyanobacteria possess lti46.1/all0459 genes. In contrast, as many as 924 dps homologues were detected in 736 bacteria. A complete analysis of all these dps homologues will be presented in a future publication. Here, a limited version of the phylogenetic tree of dps homologues (using the dataset Gclust2010e29b; data available from the URL above) is shown in Fig. S3. Cluster 1281 contained three Dps homologues, All0458, All4145 and All1173, and each belonged to different clades in the phylogenetic tree. Another homologue, Alr3808, is included in cluster 8141. Homologues of Lti46.3/All0457 were found in various bacteria in the AllBact2010 dataset, mostly in firmicutes, α-proteobacteria and cyanobacteria (Fig. S4). The functions of the protein families Lti46.1 and Lti46.3 are still not known.

**Detection, purification and activity of the Lti46.2/All0458 protein in strain M3**

Lti46.2/All0458 protein was purified from the soluble protein fraction of M3 cells by single-step immunoaffinity column chromatography (Fig. 2a). A prominent band was detected with an apparent molecular mass of 23.5 kDa, which was roughly consistent with the calculated value, 20.2 kDa, of the Lti46.2/All0458 polypeptide. The resultant protein was judged nearly homogeneous upon Coomassie staining, although a small amount of a 43 kDa band was detected by immunoblotting. The native protein was eluted as a 250 kDa protein in gel filtration (not shown, but similar to Fig. 2c). The molecular mass was consistent with the estimated size of a dodecamer (242 kDa).

The purified Lti46.2/All0458 protein showed no notable absorption above 300 nm [Fig. 2b, inset (i)]. This indicates that the protein was free of non-haem iron or protohaem, which have been found in typical bacterioferritins. When FeSO4 (1000-fold molar excess over the 250 kDa protein) was added to the protein, a dramatic increase in the absorbance at 310 nm was found (Fig. 2b). The absorption spectrum showed a broad band in the 280–400 nm region [Fig. 2b, inset (ii)]. The absorbance change attained a plateau after about 10 min. This saturable increase in the absorbance at 310 nm is a characteristic of ferritins (van Wuytswinkel & Briat, 1995), which oxidize Fe(II) to Fe(III) and incorporate the latter into the core.
enclosed by the protein shell. The oxidized iron was tightly bound to the protein, as evidenced by the results of gel filtration of the reaction product (Fig. 2c): the peak detected by $A_{310}$ coincided with the peak detected by $A_{280}$. The apparent molecular mass of the protein–iron complex did not differ significantly from that of the apoprotein.

**Localization of Lti46.2/All0458–GFP to nucleoids**

The localization of Lti46.2/All0458 protein within the cells was investigated by microscopy using GFP as a reporter (Fig. 3). In strain SR0458GS, an all0458–gfp translational fusion was integrated into the chromosome of PCC 7120 cells by a single recombination, so that the all0458–gfp gene was transcribed in its natural gene context. Intense GFP fluorescence was co-localized with the nucleoids, as detected by DAPI fluorescence. This suggests that Lti46.2/All0458 protein is localized to nucleoids, and might bind to DNA directly or indirectly. However, direct binding of the protein to DNA was barely detected by gel mobility shift analysis (results not shown). Therefore, the interaction of Lti46.2/All0458 protein with DNA might be indirect or require other factors.

Localization of Alr3808 and All1173 proteins was also observed using gfp fusion constructs (Fig. 3). The localization of the Alr3808 protein was diffuse over the entire cell, whereas the All1173 protein was clearly localized to the nucleoids. In addition, the overexpression of All1173–GFP resulted in compaction of the nucleoids and aberrant cell structures. This is consistent with the reported tight binding of All1173 with DNA (Wei et al., 2007). No significant differences were found at 32 and 22 °C. Unfortunately, we were not able to prepare an all4145–gfp fusion construct.

**Expression analysis of the lti46/all0459 operon**

Expression of the lti46 gene cluster was analysed in M3 cells (Fig. 4a). Temperature shift from 38 to 22 °C resulted in accumulation of three transcripts, namely, the lti46.1 monogenic transcript (0.7 knt), the lti46.1–lti46.2 digenic transcript (1.6 knt) and the trigenic transcript (2.5 knt). Although quantitative comparison was difficult, the 0.7 knt transcript was very abundant. S1 mapping analysis showed clearer results (Fig. 4b). The 212 nt band representing the 0.6 knt transcript became more intense after the temperature shift, although the final level was lower. The 1034 nt band representing the 2.5 knt transcript also became intense after the temperature shift, and then became faint. The results indicate that the expression of the lti46 operon is induced by low temperature, although the induction is transient.

To confirm that the induction is caused by the promoter activity, the first lti46 promoter was fused to a lacZ reporter and expression was analysed in M3 cells (Fig. 5). The promoter activity as measured by β-galactosidase activity increased after the temperature shift from 38 to 22 °C. As a
control, incubation at 38 °C for 24 h resulted in a twofold increase, which might represent effects of cell density. A control experiment using the inverted promoter showed no activity (data not shown). These results were consistent with those (Fig. 4) of transcript analysis, and suggest that the transcript accumulation at low temperature was mostly due to the induced promoter activity. We still do not know whether the second promoter just in front of the lti46.2 gene is active. The regulation of transcript level can be explained by the strong activity of the first promoter.

We analysed the level of the Lti46.2/All0458 protein in M3 cells (Fig. S5), but the protein level was not significantly affected by growth temperature. In old cultures, the protein level seemed to increase. This suggests that the protein level changes little even though the lti46 transcript accumulates transiently after the temperature shift.

Expression analysis of the dps homologues in strain PCC 7120

Fig. 6 shows the results of quantitative RT-PCR analysis of the all0458 gene and other dps homologues in PCC 7120 cells. In this case, temperature was shifted from 32 to 22 °C, because the PCC 7120 cells did not grow at 38 °C. The level of all0458 increased twofold after the temperature shift. The induction of the upstream gene all0459 was more pronounced. The expression of the alr3808 gene was also increased after the temperature shift. The results for all0458 and all0459 were essentially consistent with the results obtained in M3 cells, except that the increase in expression was modest in PCC 7120 cells. The expression of other dps homologues, all1173 and all4145, and the only bfr gene, all3940, was not affected markedly by the temperature shift.

Effects of all0458 inactivation on the expression of other dps genes

The all0458 gene was deleted from the chromosome of strain PCC 7120. Segregation of the deleted chromosome was confirmed by PCR and RNA gel blot analysis (results not shown). The resultant all0458 deletion mutant DR0458N grew at the same rate as the wild-type strain in the presence of nitrate as a nitrogen source, even after the temperature shift (data not shown). Another mutant bearing a deletion in the whole lti46 region (from the HindIII site to the EcoRI site in Fig. 1) in strain M3 was also viable without notable signs of defects at both 38 and 22 °C (data not shown).

Fig. 7. RNA gel blot analysis of the three dps genes in the wild-type and all0458 deletion mutant. (a) Comparison of young and old cultures. (b) Temperature shift from 32 to 22 °C. The filters were hybridized with an appropriate probe. The 16S rRNA band stained with methylene blue is shown as a loading control. The sizes of transcripts are shown on the right. L, exponential phase; S, stationary phase.
Expression of the *dps* homologues was examined in strain DR0458N (Fig. 7). The level of the 0.8 kb *all1173* transcript was similar in young and old cultures in the wild-type, whereas it was more abundant in the old culture than in the young culture in the mutant. This is interesting, because All1173 belongs to the clade harbouring *E. coli* Dps (Fig. S3). In temperature shift experiments, the *all1173* transcript began to accumulate at 12 h and continued to increase until 24 h after the shift, in both the wild-type and mutant.

The 0.8 kb *alr3808* transcript was abundant in both young and old cultures, which was similar in both the wild-type and the mutant. After the temperature shift, the *alr3808* transcript began to increase in both the wild-type and the mutant. In the mutant, the accumulation of the transcript continued until 24 h, whereas in the wild-type, the level of transcript decreased at this time.

Two transcripts (1.2 and 0.8 kb) were found for the *all4145* gene. Neither transcript was detected in the old culture, in both the wild-type and the mutant. After the temperature shift, a temporary accumulation of the *all4145* transcript was evident at 12 h.

Curiously, the results shown in Figs 6 and 7 are qualitatively consistent, but quantitatively different. The changes detected by RNA blotting in Fig. 7 were larger than those detected by quantitative PCR in Fig. 6. The discrepancy could reflect the fact that quantitative PCR measures only a short fragment of transcript, and thus might measure transcripts under degradation. This can occur when stability is the major determinant of transcript level, e.g. transcript accumulation at low temperature (Ehira et al., 2005).

**DISCUSSION**

We report here characterization of a Dps homologue protein, Lti46.2/All0458, in two *Anabaena* strains. The protein is encoded by the second gene within a three-gene cluster, which yields a complex mixture of monogenic, digenic and trigenic transcripts. The Lti46.2/All0458 protein is a bona fide Dps protein, because it forms a dodecamer with ferroxidase activity. Although DNA-binding activity was not evident in experiments *in vitro* (data not shown), accumulation of the All0458 protein in the nucleoids of PCC 7120 cells was a good indication that the Lti46.2/All0458 protein acts as a component of the nucleoids (Fig. 3).

The expression of the *lti46.2/all0458* gene is transiently induced by low temperature in both strain M3 and PCC 7120 (Figs 4, 5 and 6). The observed changes were greater in strain M3 than in strain PCC 7120, but this discrepancy seems to reflect the range of temperature shift, as strain PCC 7120 does not grow at 38°C. The protein level of Lti46.2/All0458 protein does not significantly respond to growth temperature. The induction of transcription was not very large (Fig. 5). These results suggest that the large transient increase in the transcript level as seen in Fig. 4 could reflect the stability of the transcript, as in the case of the *rbpA1* transcript (Ehira et al., 2005).

The *lti46.2* gene was initially identified as part of a low temperature-induced gene cluster, *lti46*, although the physiological significance of the gene in cold acclimation remains unclear. The gene can be deleted completely without affecting cellular growth at various temperatures. However, this could be explained by compensatory functions of other *dps* homologues present in *Anabaena*. Information on homologous protein clusters (Gclust database) revealed that there are three other *dps* homologues, *all1173*, *alr3808* and *all4145*. We also found a *bfr* gene, *all3940*, encoding a bacterioferritin. All these act as iron chelators, which might be important in stress responses. The results of quantitative RT-PCR analysis (Fig. 6) suggest that *alr3808* also accumulates at low temperatures. The fact that the expression of *alr3808* remained high after the temperature shift in the mutant lacking *all0458* provides evidence that *alr3808* and *all0458* share a common function in the response to low temperature. However, the differences in the localization of All0458–GFP and Alr3808–GFP (Fig. 3) might make this hypothesis unlikely. We tried to construct a double mutant in these two genes, but we did not obtain a complete segregant. This should be seriously examined in a future study.

Cyanobacteria, having multiple copies of *dps* genes (Fig. 6) and a *bfr* gene, are unique among prokaryotes. These iron-related proteins could function in alleviating oxidative stress caused by various environmental changes, such as temperature and light. Photosynthetic organisms inevitably suffer from these stresses, and this is certainly the reason why so many *dps* homologues are present in *Anabaena*.

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**REFERENCES**


mutagenesis of the specific N-terminus of pea seed ferritin. *Biochem J* 305, 959–965.


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