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SmtB is a metal-dependent repressor of the cyanobacterial metallothionein gene *smtA*: identification of a Zn inhibited DNA-protein complex

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ABSTRACT

The *smt* locus of *Synechococcus* PCC 7942 contains a metal-regulated gene (*smtA*), which encodes a class II metallothionein, and a divergently transcribed gene, *smtB*, which encodes a repressor of *smtA* transcription. Regions containing *cis*-acting elements required for efficient induction, and required for *smtB*-dependent repression, of the *smtA* operator-promoter were identified. Specific interactions between proteins extracted from *Synechococcus* PCC 7942 and defined regions surrounding the *smtA* operator-promoter were detected by electrophoretic mobility shift assays. Three metallothionein operator-promoter associated complexes were identified, one of which (MAC1) showed Zn-dependent dissociation and involved a region of DNA immediately upstream of *smtA*. Treatment with Zn-chelators facilitated re-association of MAC1 *in vitro*. MAC1 was not observed in extracts from *smt* deficient mutants but was restored in extracts from mutants complemented with a plasmid borne *smtB*. SmtB is thus required for the formation of a Zn-responsive complex with the *smt* operator-promoter and based upon the predicted structure of SmtB we propose direct SmtB-DNA interaction exerting metal-ion inducible negative control.

INTRODUCTION

Metal-induced expression of metallothionein (MT) genes in different animals involves the association of metal-activated factors, MTF-1, ZAP, MEP-1 with defined metal responsive elements (MRE's) (cited in 1). Cu-induced expression of *CUP1*, which encodes yeast Cu-thionein (MT), is mediated by ACE1 (2). Cu-ACE1 binds to *CUP1* upstream activator sequences (UAS) and stimulates MT expression.

We have recently isolated a prokaryotic MT locus, *smt*, from the cyanobacteria *Synechococcus* PCC 7942 and PCC 6301 (3, 4). Deletion of the *smt* locus reduces Zn/Cd tolerance (5). The *smt* locus includes *smtA*, which encodes a class II MT (6, 7) and a divergently transcribed gene *smtB* which encodes a repressor of *smtA* transcription (4). Transcription from the *smt* operator-promoter is stimulated by certain trace metal ions (notably Zn, Cu and Cd)(4). A 100 bp operator-promoter region lies between the *smtA* and *smtB* protein coding regions and contains divergent promoters that border a 7-2-7 hyphenated inverted repeat. An imperfect 6-2-6 hyphenated inverted-repeat and a 6-2-6 hyphenated direct repeat are located between the sites of transcriptional and translational initiation in *smtA*. Complementation studies show that *smtB* encodes a transcriptional regulator of *smtA* (4). The deduced SmtB polypeptide contains a region that scores highly (5.5) on a prediction matrix for the helix-turn-helix DNA-binding motif (4, 8). SmtB also has sequence similarity to a family of prokaryotic metal oxyanion-responsive factors, ArsR, (4, 9, 10) and MerR from *Streptomyces lividans* (11). These observations are suggestive of direct SmtB/DNA interaction. SmtB also shares sequence similarity to CadC, a protein of unknown function that is essential for high level Cd-resistance in *Staphylococcus aureus* (4, 12) and to CadC from *Bacillus firmus* OF4.

We report interactions between the *smt* operator-promoter region and proteins from *Synechococcus* PCC 7942.

MATERIALS AND METHODS

Bacterial strains

Derivatives of *Synechococcus* PCC 7942 referred to as R2-PIM8 (15), and R2-PIM8(*smt*) were used for protein extractions and phenotypic analyses. R2-PIM8(*smt*) is an *smt* mutant strain (4), in which the *smt* operator-promoter and 5′ regions of both genes are deleted and is therefore deficient in functional *smtA* and *smtB*. R2-PIM8 strains were cultured in Allens media (16, 3). *E.coli* SURE (Stratagene Ltd) was used for all genetic manipulations. *E.coli* was cultured in L-broth and plated on L-agar (17). Where appropriate, streptomycin (5 μg/ml), chloramphenicol (7.5/34 μg/ml) carbenicillin (10/50 μg/ml) and zinc chloride (2.5, 10 and 11 μM) were added to the growth medium (the concentrations given are for *Synechococcus*/*E.coli*).

Construction of *smt-lacZ* fusions and promoter deletions

PCR was used to generate two *smt* operator-promoter deletions flanked by *Sal*I and *Bam*HI sites, these sites were used to clone the truncated fragments into pGEM4Z and, after nucleotide sequence analysis, into the *lacZ* fusion vector, pLACPB2 (17),

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as described in (4). Standard cloning techniques were employed (18). Both truncated fragments (δ1, δ2) included the region downstream of the hyphenated inverted repeat, one (δ1) also contained the hyphenated inverted repeat (Figure 1E, F). The primers used to generate the PCR products were 5'-GGCGTCGACCTGAATCAAGATTCAGATGTTAGG-3' for δ1 and 5'-GGCGTCGACATGTTAGGCTTAAACACAT-3' for δ2, in conjunction with primers detailed in (4).

Protein extraction

Synechococcus cultures (1L, $O.D._{540}$ = 0.3) were harvested by centrifugation and resuspended in 1 ml of extraction buffer (10 mM Tris, 1 mM EDTA (or 10 μM $ZnCl_2$), 250 mM KCl, 0.5 mM DTT, 10% glycerol (v/v) and 1 mM PMSF). The cell suspension was frozen in liquid nitrogen and ground to a fine powder, suspended in 10 ml of extraction buffer, and sonicated. Cellular debris was pelleted (15,000×g, 20 mins) and protein was precipitated from the supernatant by the addition of $(NH_4)_2SO_4$ (0.4 g/ml). The protein precipitate was pelleted (15,000×g, 20 mins) and resolvated in 0.5 ml of extraction buffer without KCl. The protein extract was then dialysed for 12 h against 2L of extraction buffer without KCl. All manipulations were performed at 4°C.

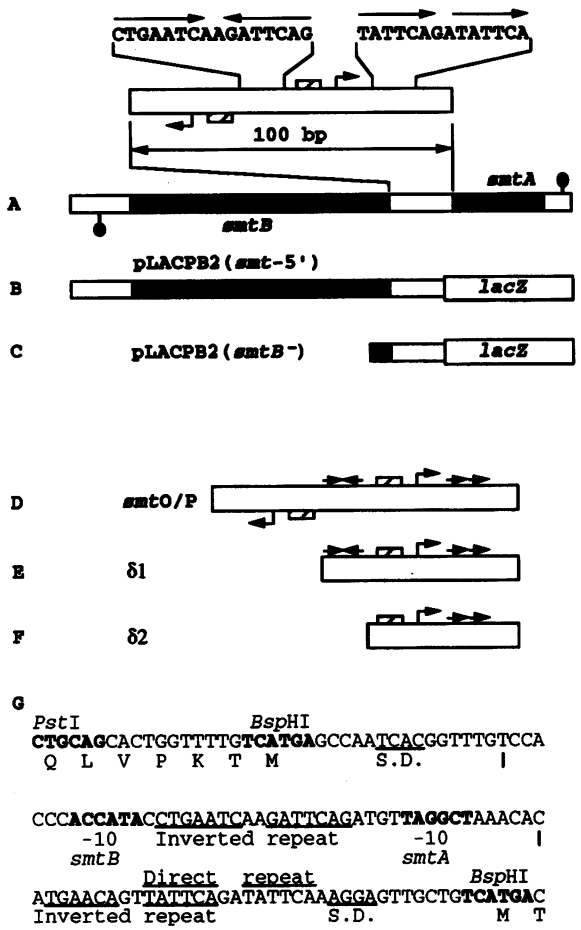

Figure 1. Organisation of the *smt* locus, reporter gene fusions and EMSA probes/competitors. A: *smt* locus. The divergent genes *smtA* and *smtB* are shown as black rectangles. The 100 bp operator-promoter region is expanded to show the sequence of the hyphenated inverted repeat (converging arrows) and hyphenated direct repeat (unidirectional arrows). Other features include determined transcript start sites (bent arrow), −10 motif (hatched box) and putative terminators (circle). B, C: Reporter gene fusions. *smtB* is again shown as a black rectangle within regions fused to a promoterless *lacZ* gene in the vector pLACPB2. D: *smt*O/P region used as a probe in EMSA. E, F: Deletion derivatives of the *smt*O/P region used as specific competitor DNA (δ1 and δ2), EMSA probe (δ2) and in reporter gene constructs. G: Sequence of the *smt* operator-promoter region showing partial amino acid sequence for SmtA and SmtB, *Bsp*HI and *Pst*I restriction enzyme sites (bold), −10 sequences (bold), Shine-Dalgarno sequences (underlined) and inverted/direct repeats (under/over-lined).

Electrophoretic mobility shift assays

This technique was performed as previously described (19) except the for the omission of EDTA from the binding buffer when using extracts enriched for Zn. Electrophoresis was for 150 mins at 140 V and the DNA-protein complexes were visualised by direct autoradiography. The probes used were *smt*O/P (100bp *Bsp*HI fragment, figure 1D, G), δ1 and δ2 (*Sal*I-*Bam*HI fragments, figure 1E, F).

β-galactosidase assays

These assays were performed using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) (Sigma, Dorset, UK) as the substrate (20). Optical density (O.D.) readings at 420 and 600 nm were normalised against water and Allens media respectively. β-galactosidase activity was calculated using a modified equation Activity(nmoles *o*-nitrophenol/min/mg protein) = $(O.D._{420(t)} - O.D._{420(0)})300/(t \times v \times O.D._{600})1.83$ Cells were lysed with chloroform/SDS.

Computer analysis

All computer analyses used the GCG package on the SERC Daresbury facilities DLVH and SEQNET.(21)

RESULTS

Analysis of metal-dependent β-galactosidase activity in an *smt* mutant

Metal dependent β-galactosidase activity was detected in R2-PIM8 containing pLACPB2(*smt*-5') (Figure 2). Cells containing a 5' deletion derivative of this construct,

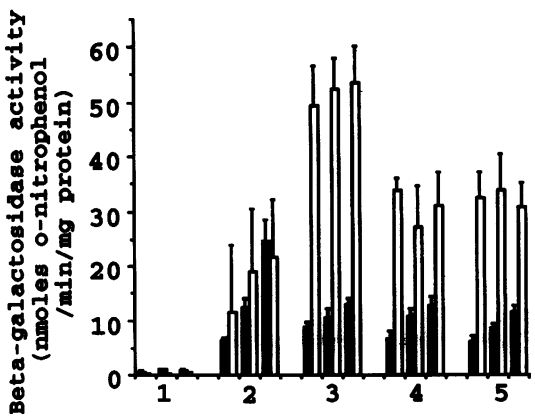

Figure 2. β-galactosidase activity measured in *Synechococcus* strains containing *smtA-lacZ* reporter gene fusions and deletions. Closed columns represent an R2-PIM8 background and open columns represent an R2-PIM8(*smt*) background. Each block of three values corresponds to 0, 2.5 and 11 μM Zn exposure from right to left. The constructs are; pLACPB2 (1), pLACPB2(*smt*-5') (2), pLACPB2(*smtB*⁻) (3), pLACPB2(δ1) (4) and pLACPB2(δ2).(5)

pLACPB2(*smtB*$^-$), showed elevated basal expression (in media containing no metal supplements) and significantly diminished maximal expression (in 11 μM Zn) (Figure 2). Highly elevated basal β-galactosidase activity and loss of metal-dependency was seen in R2-PIM8(*smt*) containing pLACPB2(*smtB*$^-$), devoid of a functional plasmid or genomic *smtB* (Figure 2)(4). All three sequential deletion derivatives (Figure 1C,E and F) showed similar activity in R2-PIM8, however, R2-PIM8(*smt*) containing pLACPB2(δ1 and δ2) had diminished activity in comparison to the equivalent strain carrying pLACPB2(*smtB*$^-$). The larger errors seen in assays of the R2-PIM8(*smt*) strain are possibly due to the lack of a functional *smtA* gene and consequent aberrant Zn homeostasis.

Identification of complexes which bind to the *smt* operator-promoter region

Three *smt* operator-promoter associated complexes (MAC1, MAC2 and MAC3) were detected using standard protein extracts from R2-PIM8 by electrophoretic mobility shift assays (EMSA) (Figure 3A) with *smt*O-P as probe (shown in Figure 1D). At high concentrations (0.3 μg μl^{-1}) of non-specific competitor DNA, MAC3 is more stable, while MAC1 is less stable, in reactions using extracts from Zn exposed cells (compare tracks 6 and 11, Figure 3A). To localise the DNA binding sites the experiment was repeated using different concentrations (0.01 to 0.5 μg μl^{-1}) of *smt* operator-promoter deletions, δ1 and δ2, in pGEM4Z,

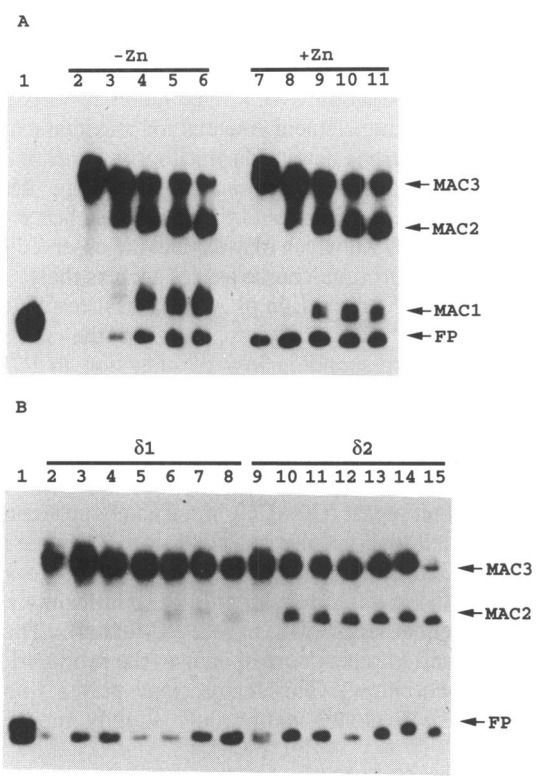

Figure 3. EMSA with *smt*O/P probe. A: Track 1 contains probe alone, tracks 2−6 and 7−11 correspond to reactions containing increasing amounts of poly-dIdC (0, .05, 0.1, 0.2, 0.3 μg/μl). Tracks 2−6 show free probe (FP) and three complexes (MAC1, MAC2 and MAC3) forming with protein extracts from non-Zn exposed cells. Tracks 7−11 show equivalent complexes with protein extracts from cells exposed to Zn *in vivo*. B: Track 1 contains probe alone. Tracks 2−8 and 9−15 correspond to reactions containing increasing concentrations (.01, .05, 0.1, 0.2, 0.3, 0.4 and 0.5 μg/μl) of pGEM4Z containing δ1 and δ2 respectively.

(Figure 1E and 1F) as specific competitors (Figure 3B). MAC1 was lost in reactions containing either δ1 or δ2. MAC2 was diminished in reactions containing δ1 compared to reactions containing δ2. MAC3 was retained in reactions containing either competitor.

Loss of MAC1 in extracts from an *smt* mutant

MAC2 and MAC3 form with the *smt*O-P probe using standard protein extracts from R2-PIM8(*smt*) (Figure 4A). MAC1 was not detected using extracts from these mutants.

A single complex (C1) was formed with the *smt* operator-promoter deletion fragment δ2 using a protein extract from R2-PIM8 (Figure 4B). This complex was absent when extracts from R2-PIM8(*smt*) were used (Figure 4B, compare tracks 1−3 with 4−6). The complex was again detected using extracts from R2-PIM8(*smt*) containing pLACPB2(*smt*-5′), which reintroduces a plasmid borne *smtB*, but not using extracts from R2-PIM8(*smt*) containing pLACPB2 alone (Figure 4B, compare tracks 7−9 with 10−12).

Effects of Zn on complexes formed with *smt* operator-promoter deletion δ2

To further examine the effects of Zn on complexes forming with the *smt* operator-promoter region extracts were prepared with EDTA free buffers supplemented with $ZnCl_2$ (5 μM), and binding reactions performed in the absence of EDTA. A single major complex (C1) was again formed with promoter deletion δ2 (Figure 5, track 2) using extracts from cells which had not been exposed *in vivo* to elevated Zn. Using extracts from cells exposed (1 h) *in vivo* to $ZnCl_2$ (10 μM) the abundance of C1 declined (Figure 5, compare tracks 2 and 6), and was absent in extracts from R2-PIM8(*smt*) (Figure 5, track 10). Treatment of identical reactions with increasing concentrations (0.1 mM and 1 mM) of a metal chelator, 1,10-phenanthroline resulted in increased abundance of C1 in extracts from R2-PIM8 (Figure 5, compare track 2 with 3 and 4; track 6 with 7 and 8), again no complex was seen using extracts from R2-PIM8(*smt*) (Figure 5, tracks 11 and 12). It is also noted that in the presence of the highest concentration of 1,10-phenanthroline, a second prominent complex (C2) was detected in reactions containing extracts from R2-PIM8 cells which had not been exposed to elevated Zn (Figure 5, track 4). Furthermore, a minor higher M_r complex was also seen in all extracts.

Multiple alignment of SmtB with similar proteins

The OWL 18.0 database was searched for proteins whose primary amino-acid sequence was similar to SmtB. Sequences were aligned and a consensus sequence generated with a plurality of 6 (Figure 6). The accession numbers of the included proteins are OWL: SmtB-S19927; 773ArsR-P15905; 2258ArsR-M86824; CadC-P20047; MerR-S23610; NolR-S19675. GenBank: 267ArsR-M80565; OF4CadC-M90750.

DISCUSSION

Abnormal over expression from the *smt* operator-promoter is (partially) complemented in R2-PIM8(*smt*) by plasmid borne *smtB*, demonstrating that SmtB is a *trans*-acting repressor of *smtA* (figure 2)(4). A direct interaction between SmtB and DNA may be mediated by residues 61 to 81, inclusive, which score highly on a prediction matrix for the helix-turn-helix DNA-binding motif (4). Such inducible negative control would be similar to that

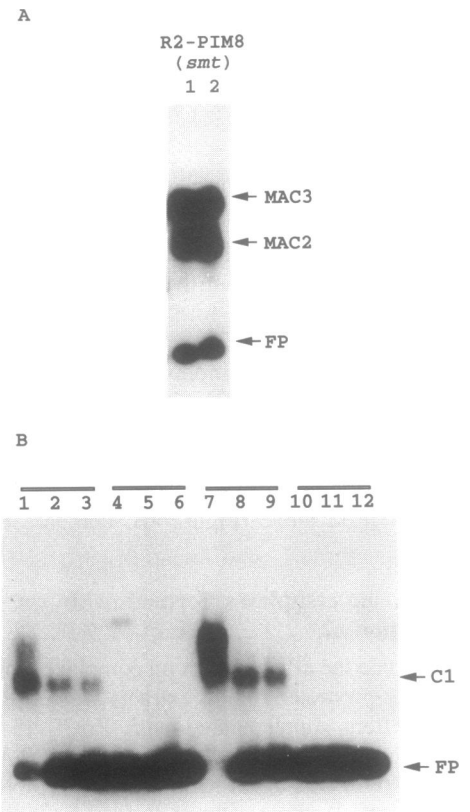

Figure 4. EMSA with protein extracts from R2-PIM8(*smt*) and derivative *smtB* complemented strains. A: Tracks 1 and 2 correspond to reactions containing the *smt*O/P probe with 0.2 and 0.3 μg/μl of poly-dIdC respectively. The protein extract was from non-Zn exposed R2-PIM8(*smt*). B: The δ2 probe was used with protein extracts from non-Zn exposed R2-PIM8 (1−3), R2-PIM8(*smt*) (4−6), R2-PIM8(*smt*) containing pLACPB2(*smt*-5′) (7−9) and R2-PIM8(*smt*) containing pLACPB2 (10−12). Each set of three reactions contained increasing concentrations (0, 0.1 and 0.2 μg/μl) of poly-dIdC. Free probe(FP), MAC2, MAC3 and C1(MAC1) are labelled.

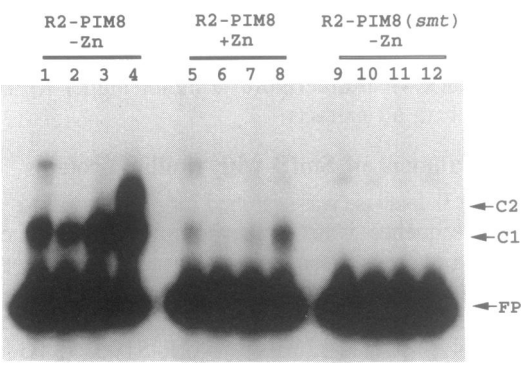

Figure 5. EMSA with protein extracts from R2-PIM8 and R2-PIM8(*smt*) in conjunction with the δ2 probe. Tracks 1,5 and 9 correspond to reactions devoid of non-specific competitor DNA, all other reactions contained 0.2 μg/μl of poly-dIdC. Each set of 4 tracks correspond to reactions containing 0,0,0.1 and 1mM 1,10-phenanthroline. Free probe(FP), C1(MAC1) and C2 are labelled.

demonstrated for the arsenic resistance operon (*ars*) from *E.coli* in which ArsR mediated transcriptional repression is alleviated by exposure to the metal-oxyanions arsenate, arsenite, antimonite and bismuthite (9).

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                1                                                  50
     SmtB  MTKPVLQDGE TVVCQGTHAA IASELQAIAP EVAQSLAEFF AVLADPNRLR
 773 ArsR  .......... .......... .......... MLQLTPLQLF KNLSDETRLG
 267 ArsR  .......... .......... .......... MSYKELSTIL KVLSDPSRLE
 258 ArsR  .......... .......... .......... MSYKELSTIL KILSDSSRLE
 OF4 CadC  ....VNKKDT CEIFCYDEEK VNRIQGDLKT IDIVSVAQML KAIADENRAK
 258 CadC  ....MKKKDT CEIFCYDEEK VNRIQGDLQT VDISGVSQIL KAIADENRAK
     MerR  .......... ...MKSPALA GSLATAEVPC THPDTTARFF RALADPTRLK
     NolR  .......... ...MNFRMEH TMQPLPPEKH EDAEIAAGFL SAMANPKRLL
Consensus  ---------- ---------- ---------- ---------L --L-D--RL-

                51                                                 100
     SmtB  LLSLL.ARSE LCVGDLAQAI GVSESAVSHQ LRSLRNLRLV SYRKQGRHVY
 773 ArsR  IVLLLREMGE LCVCDLCMAL DQSQPKISRH LAMLRESGIL LDRKQGKWVH
 267 ArsR  ILDLL.SCGE LCACDLLEHF QFSQPTLSHH MKSLVDNELV TTRKNGNKHM
 258 ArsR  ILDLL.SCGE LCACDLLEHF QFSQPTLSHH MKSLVDNELV TTRKDGNKHW
 OF4 CadC  ITYALCQDEE SCVCDIANII GITAANASHH LRTLHKQGIV RYRKEGKLAF
 258 CadC  ITYALCQDEE LCVCDIANIL GVTIANASHH LRTLYKQGVV NFRKEGKLAL
     MerR  LLQFI.LRGE RTSAECVEHA GISQPRVSVH LSCLVDCGYV SARRDGKKLR
     NolR  ILDSL.VKEE MAVGALAHKV GLSQSALSQH LSKLRAQNLV STRRDAQTIY
Consensus  I---L----E LC--D----- --S----S-H L--L-----V --RK-G----

                101                                                147
     SmtB  YQLQDH..HI VALYQNALDH LQECR..... .......... .......
 773 ArsR  YRLSPHIPSW AAQIIEQAWL SQQDDVQVIA RKLASVNCSG SSKAVCI
 267 ArsR  YQL.NH..EF LDYINQNLDI INTSDQGCAC KNMKSGEC.. .......
 258 ArsR  YQL.NH..AI LDDIIQNLNI INTSNQRCVC KNVKSGDC.. .......
 OF4 CadC  YSLDDEHIRQ IMMIVLEHKK EVNVNV.... .......... .......
 258 CadC  YSLGDEHIRQ IMMIALAHKK EVKVNV.... .......... .......
     MerR  YSVGDP..RV ADLVMLARCL AADNAAALDC CTRIPGEGEQ R......
     NolR  YSSSSD..AV LKILGALSDI YGDDTDAVEE KPLVRKSA.. .......
Consensus  Y-L------- ---I------ ---------- ---------- -------
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Figure 6. Multiple sequence alignment of SmtB from *Synechococcus* PCC 7942, ArsR from *E.coli* (plasmid R773), ArsR from *Staphylococcus xylosus* (plasmid pSX267), ArsR from *Staphylococcus aureus* (plasmid pI258), CadC from *Bacillus firmus* OF4, CadC from *Staphylococcus aureus* (plasmid pI258), MerR from *Streptomyces lividans* and NolR from *Rhizobium meliloti*. A concensus sequence is shown with a plurality of 6. The putative DNA-binding region of SmtB is underlined.

There is a significant loss of inducible reporter gene activity in R2-PIM8 containing pLACPB2(*smtB*$^-$), lacking ca. 500 bp of *smtA* 5′-sequence, (figure 1C). This either reflects the loss of a 'remote' *cis*-acting element essential for maximal expression and/or the loss of *trans*-acting plasmid borne *smtB* and hence inequity between copies of SmtB and its target site. The latter might result in increased basal *smtA* expression and hence reduced available endogenous Zn which may explain the observed patterns of expression in short time course assays such as these. Further deletion of the *smt* 5′-region, in pLACPB2(δ1), resulted in loss of induction which was only apparent in the *smt* mutant background. A corresponding loss of induction in R2-PIM8, where the major element of transcriptional control (SmtB) was still functional, was not seen. The region lost corresponds to the proposed MAC3 binding site and represents a *cis*-acting activatory region. Computer analysis has identified a 5 bp overlapping direct repeat (CCACC) immediately upstream of the 7-2-7 hyphenated inverted repeat, which is a candidate binding site for MAC3. Further deletion, pLACPB2(δ2), (loss of the 7-2-7 hyphenated inverted repeat) did not significantly alter the induction in either R2-PIM8 or R2-PIM8(*smt*). The 7-2-7 hyphenated inverted repeat corresponds to the proposed MAC2 binding site (see below). This feature may play a role in the regulation of *smtB* alone, and/or only slightly modify *smtA* expression.

Three complexes, MAC1, MAC2 and MAC3, form with the *smt* operator-promoter region using protein extracts from R2-PIM8. MAC1 was absent from reactions containing competitor δ2 (and δ1) demonstrating MAC1 formation with the region lying between the 7-2-7 hyphenated inverted repeat and Shine-Dalgarno sequence of *smtA* (Figure 3B). MAC2 is diminished in reactions containing competitor d1, but not δ2. These data suggest that MAC2 associates with the 7-2-7 hyphenated inverted repeat which is only present in δ1. MAC3

was not significantly diminished by either competitor and is proposed to bind upstream of the 7-2-7 hyphenated inverted repeat. As anticipated, only one major complex (C1), corresponding to MAC1, forms with δ2 (Figure 4B).

MAC1 (Figure 4A), and hence C1 (Figure 4B), do not form when using extracts from R2-PIM8(*smt*), which lacks functional *smtA* and *smtB* genes, however, MAC2 and MAC3 are retained (Figure 4A). C1 is restored upon reintroduction of a plasmid borne *smtB* (Figure 4B) demonstrating that SmtB is necessary for C1 (MAC1) formation.

The stability of MAC1 is slightly diminished by treatment with Zn *in vivo* (Figure 3A). A more pronounced diminution of C1 (MAC1), in response to *in vivo* Zn, was observed using extracts prepared with EDTA-free buffers (Figure 5). In these extracts, C1 increased following *in vitro* addition of 1,10-phenanthroline. These data suggest a direct interaction of this factor with metals. To date it has not been possible to affect binding via the addition of Zn *in vitro* (data not shown). This is also apparent in Figure 5 where a difference in binding between extracts from *in vivo* Zn exposed and non-exposed cells is observed when both extracts have been extensively dialysed against buffers containing 5 μM Zn. This may indicate a requirement for other factors or conformational change in the ligand *in vitro* (such as oxidation of a metal-binding site). Zn-dependent dissociation is consistent with the observations that C1 (MAC1) is *smtB* dependent and that SmtB is a repressor of *smtA* expression. The association of this complex with the δ2 fragment (figure 1), containing only the *smtA* promoter and downstream regions, is consistent with a role in transcriptional repression. A 6-2-6 direct repeat (TATTCA-GA-TATTCA) is present in the region retained in the δ2 fragment and represents a candidate for DNA-protein interaction, however, prokaryotic proteins employing helix-turn-helix structure generally bind to inverted repeats rather than to directly repeated sequences. Another candidate site is a degenerate 6-2-6 inverted repeat, which incorporates the left half of the 6-2-6 direct repeat (TGAACA-GT-TATTCA)(figure 1G).

SmtB shows significant similarity to a range of proteins within the OWL 18.0 database. This family of related proteins includes several transcriptional regulators and a number of proteins involved in metal metabolism. Within this family only NolR is not involved in metal-ion metabolism.

All of the known metal responsive transcription factors involved in the regulation of eukaryotic MT genes directly bind metals and, as a result, are activated to bind specific promoter-DNA sequences to activate transcription (1). Similarly other eukaryotic transcription factors, Zn-fingers, Zn-clusters and Zn-twists (22), require Zn for DNA-'association'. By contrast, although the SmtB-dependent complex (C1, MAC1), also interacts directly with metals, this interaction mediates dissociation from specific promoter-DNA sequences rather than binding. This component of prokaryotic MT gene regulation imparts inducible negative control compared with the inducible positive control seen in eukaryotic MT genes. The presence of other factors (MAC2, MAC3) suggests additional regulatory mechanisms.

In summary, *smtB* encodes a transcriptional repressor of *smtA* and is required for the formation of a complex, MAC1, with a region immediately upstream of the *smtA* coding sequence. MAC1 shows Zn-dependent dissociation from its target sequence. Circumstantial evidence supports the proposal that MAC1 is formed by the interaction of SmtB with DNA. Two other DNA-protein complexes have been identified, MAC2 and MAC3. MAC3 forms with an upstream region which plays a positive regulatory role in expression from the *smtA* operator-promoter. To our knowledge *Synechococcus* genes encoding *trans*-acting DNA-binding proteins have not previously been characterised and the observed Zn-dependent dissociation is unique among known metal-responsive transcription factors.

ACKNOWLEDGEMENTS

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