Identification of three novel antisense RNAs in the *fur* locus from unicellular cyanobacteria

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The interplay between Fur (ferric uptake regulator) proteins and small, non-coding RNAs has been described as a key regulatory loop in several bacteria. In the filamentous cyanobacterium *Anabaena* sp. PCC 7120, a large dicistronic transcript encoding the putative membrane protein Alr1690 and an α -*furA* RNA is involved in the modulation of the global regulator FurA. In this work we report the existence of three novel antisense RNAs in cyanobacteria and show that a *cis* α -*furA* RNA is conserved in very different genomic contexts, namely in the unicellular cyanobacteria *Microcystis aeruginosa* PCC 7806 and *Synechocystis* sp. PCC 6803. *Sy* α -*fur* RNA covers only part of the coding sequence of the *fur* orthologue *slr0567*, whose flanking genes encode two hypothetical proteins. Transcriptional analysis of *fur* and its adjacent genes in *Microcystis* unravels a highly compact organization of this locus involving overlapping transcripts. *Max*-*fur* RNA spans the whole *Mafur* CDS and part of the flanking *dnaJ* and *sufE* sequences. In addition, *Mafur* seems to be part of a dicistronic operon encoding this regulator and an α -*sufE* RNA. These results allow new insights into the transcriptomes of two unicellular cyanobacteria and suggest that in *M. aeruginosa* PCC 7806, the α -*fur* and α -*sufE* RNAs might participate in a regulatory connection between the genes of the *dnaJ*–*fur*–*sufE* locus.

Received 13 January 2011
Revised 20 July 2011
Accepted 8 September 2011

INTRODUCTION

In eubacteria, most regulatory RNAs identified to date are non-coding RNAs (ncRNAs) smaller than 300 nt that are located far away from their target genes. The roles of ncRNAs are diverse; they can be involved in the control of several stress responses, virulence and motility, among other functions (Waters & Storz, 2009). In particular, the interplay between Fur (ferric uptake regulator) proteins from different organisms and regulatory RNAs seems to play a major role in the control of iron homeostasis (Večerek *et al.*, 2007). It is assumed that, *in vivo*, Fur works as a classical repressor using Fe(II) as a cofactor to negatively regulate expression of their target genes through binding to Fur recognition sites (Bagg & Neilands, 1987). Cross-talk with other transcriptional regulators involved in carbon and nitrogen metabolism (Zhang *et al.*, 2005; López-Gomollón *et al.*, 2007) and with other members from the Fur superfamily (Fuangthong *et al.*, 2002; Hernández *et al.*, 2004) also contribute to the modulation of Fur activity. In addition, reciprocal regulation between Fur and several

ncRNAs, as well as co-regulation of iron-responsive genes by Fur and ncRNAs, has been described (Waldbeser *et al.*, 1995). Identification of RhyB in *Escherichia coli* cells provided the answer to the mechanism of activation of the *sodB* gene under iron-rich conditions (Massé & Gottesman, 2002) and linked the regulation of a set of iron storage and iron-containing proteins to this small ncRNA, involving its own Fur as part of a back-up mechanism.

In cyanobacteria, two *cis*-encoded regulatory RNAs linked to iron metabolism have been characterized. In the unicellular cyanobacterium *Synechocystis*, the regulatory RNA IsiR is expressed specifically during iron-rich conditions and, with Fur, co-represses the synthesis of IsiA under iron-replete conditions (Dühning *et al.*, 2006). In the filamentous, nitrogen-fixing cyanobacterium *Anabaena* (*Nostoc*) sp. PCC 7120 an α -*furA* is transcribed in the same message as the membrane protein Alr1690 and covers the complete coding sequence of *furA* (Hernández *et al.*, 2006). Disruption of the α -*furA*–*alr1690* message leads to an increased expression of FurA and the resulting mutant exhibits an iron-deficient phenotype (Hernández *et al.*, 2010). An *alr1690* homologue has also been identified in the context of the *fur* gene from other species, though this is not a fully conserved arrangement in cyanobacteria. In the *Synechocystis* sp. PCC

Abbreviations: CDS, coding sequence; ncRNA, non-coding RNA; tsp, transcription start point.

Two supplementary figures are available with the online version of this paper.

6803 genome, the *furA* orthologue *sll0567* is flanked by genes encoding hypothetical proteins, while in *Microcystis aeruginosa* PCC 7806 and NIES-843 strains, the corresponding *furA* genes are flanked by *dnaJ* and cysteine desulfurase activator *sufE* genes (Martín-Luna *et al.*, 2006, <http://genome.kazusa.or.jp/cyanobase/>).

Comparative genome analysis of several cyanobacterial strains has allowed a reliable biocomputational prediction of a plethora of ncRNAs (Axmann *et al.*, 2005), and these are found to occur at a much higher rate in *Microcystis* species than in other unicellular cyanobacteria (Voß *et al.*, 2009). More recently, the use of differential RNA sequencing of the model organism *Synechocystis* sp. PCC 6803, together with complementary microarray-based RNA profiling, showed that a quarter of all chromosomal genes are subject to antisense transcription (Mitschke *et al.*, 2011). Interestingly, these data also reveal that the majority of small RNAs are located on the complementary strand of mRNAs.

In this work, we provide experimental evidence for the transcription of three novel antisense RNAs in unicellular cyanobacteria. Two of them have been identified in the *fur-sufE* locus of *M. aeruginosa*. The first is transcribed in the same message as *fur* and covers the complete CDS of the *sufE* gene. The second antisense RNA is an α -*fur* RNA that spans beyond *fur* and covers part of the flanking *sufE* and *dnaJ* genes. The occurrence of a smaller α -*fur* RNA has also been corroborated in *Synechocystis* PCC 6803, indicating that the presence of α -*fur* RNAs is not restricted to *Anabaena* PCC 7120 and seems to be independent of the *fur* gene context.

METHODS

Growth conditions. *M. aeruginosa* PCC 7806 was grown at 25 °C and 10 μ mol photons m⁻² s⁻¹ in modified BG11 medium with

2 mM NaNO₃ and 10 mM NaHCO₃ (Rippka *et al.*, 1979). *Synechocystis* PCC 6803 was grown in the same conditions using standard BG11 medium.

Sampling and RNA isolation. Cells (25 ml) were centrifuged and pellets were resuspended in 600 μ l 50 mM Tris/HCl (pH 8), 100 mM EDTA and 130 μ l chloroform and incubated on ice for 3 min. After centrifugation, cell pellets were frozen and kept at -70 °C until RNA isolation. Cells were disrupted in the 'Fastprep Instrument' using four cycles of 20 s at a setting of 6 m s⁻¹. Total RNA was extracted using the FastRNA Pro Blue kit (Qbiogene), resuspended in 50 μ l DEPC-H₂O and treated with 40 units DNase (Pharmacia).

RT-PCR analysis. RNA was reverse-transcribed using Superscript II Reverse Transcriptase (Invitrogen) and the specific oligonucleotide (Table 1). Residual DNA in RNA preparations was eliminated by digestion with RNase-free DNase I (Roche). The absence of DNA was checked by PCR. The RT-PCR mixture contained 1 μ g RNA and 20 pmol of the oligonucleotide in the annealing buffer [10 mM Tris/HCl (pH 8), 1 mM EDTA, 150 mM KCl] in a final volume of 10 μ l. Samples were heated for 10 min at 85 °C and incubated for 60 min at 65 °C. Afterwards, 5 pmol each dNTP, 0.2 mmol DTT and 200 U Superscript Reverse Transcriptase were added to the buffer [50 mM Tris/HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂ and 20 mM DTT] to a final volume of 20 μ l. Samples were incubated at 47 °C for 1 h and finally heated at 75 °C for 15 min. The products were amplified by PCR using 2 μ l of each cDNA as template and analysed in a 1 % (w/v) agarose gel with a Gel Doc 2000 (Bio-Rad).

Transcription start point (tsp) determination. Potential Max-*fur* and Mafur tsps were determined by primer extension. The Max-*fur* promoter region was amplified using oligonucleotides 10 (ATS5) and 11 (AsufE). To identify the tsp of Mafur, oligonucleotides 6 (pFurN) and 13 were used for amplification of the promoter. The resulting fragments were cloned in pGEMT and introduced into *E. coli* JM109 (Promega). The recombinant plasmids were purified and the correct integration of the inserts was confirmed by restriction analysis with *Eco*RI. These plasmids were used as templates in the sequencing reaction using the Thermo Sequenase Fluorescent Primer Cycle Sequencing kit (Amersham), where the labelled nucleotides primed

Table 1. Oligonucleotides used in this work

Oligonucleotide (number)	Oligonucleotide sequence (5'-3')	Description	5' Position relative to the <i>fur</i> ORF
MFurN (1)	GTCGATCGCCCATGGCTGCCTAC	Forward primer for <i>fur</i> gene in <i>Microcystis</i>	-11
MFurC (2)	CAGTTGGGAATCCCGCTTAGATG	Reverse primer for <i>fur</i> gene in <i>Microcystis</i>	553
Mintfw (3)	CGACGATTACCGCAGTG	Forward primer for internal region of <i>fur</i> gene in <i>Microcystis</i>	176
Mintrev (4)	CACACTGTTTGAGACTGTG	Reverse primer for internal region of <i>fur</i> gene in <i>Microcystis</i>	351
NdnaJ (5)	CGGGGAATTTGGAGGATTTTC	Reverse primer N-terminal region of <i>dnaJ</i> gene	-140
pFurN (6)	CTCTTGAGCGTAGTGCCATC	Internal <i>dnaJ</i> gene and forward primers for cloning <i>fur</i> promoter	-381
FurSyneRev (7)	CATGTCCTACACCGCCGATTC	Forward primer for <i>fur</i> gene in <i>Synechocystis</i>	-1
FurSyneInt (8)	CAGACGATGGTGTAACCTCCTC	Reverse primer for <i>fur</i> gene in <i>Synechocystis</i>	-144
FurSyneFor (9)	CCTAGGCCAAGGAAATACTGC	Internal primer <i>fur</i> gene <i>Synechocystis</i>	503
ATS5 (10)	CGGGAATACCGAGTAATTGG	Forward primer for cloning α - <i>fur</i> promoter region	451
ASufE (11)	CTCGCAATATGCTCCCGTCTAATCTC	Reverse primer for cloning α - <i>fur</i> promoter region	961
ATS5-Cy5 (12)	CGGGAATACCGAGTAATTGG-Cy5	Forward primer for α - <i>fur</i> primer extension	451
Tsp fur (13)	CTGAGATGATTGCCTTTTCGG	Reverse primer for cloning <i>fur</i> promoter region	102
Tsp fur-Cy5 (14)	CTGAGATGATTGCCTTTTCGG-Cy5	Reverse primer for <i>fur</i> primer extension	102

Fig. 1. Identification of *Max-fur* RNA. (a) Schematic representation of two regions of the *Mafur* gene that were amplified. Oligonucleotides are indicated by black arrowheads. (b) RT-PCR analyses of the *Max-fur* RNA. The 1 % agarose gel shows the amplified fragments after the reverse transcription and PCR steps. The sizes (in bp) of the molecular mass markers are indicated on the right. Lanes: 1, molecular mass markers; 2, PCR with oligonucleotides 1 and 2 and chromosomal DNA of *Microcystis* (positive control); 3, reverse transcriptase step with oligonucleotide 1 and PCR step with oligonucleotides 1 and 2; 4, sample as in lane 3, in the presence of DNase-free RNase; 5, PCR with oligonucleotides 3 and 4 and chromosomal DNA of *Microcystis* (positive control); 6, RT step with oligonucleotide 3 and PCR step with oligonucleotides 3 and 4; 7, sample as in lane 6 in the presence of DNase-free RNase.

the sequencing reaction which was used as molecular size marker. Reverse transcription was carried out using Expand Reverse Transcriptase (Roche) with 1 µg total RNA as template and the Cy5-labelled oligonucleotide 12 (ATS5-Cy5) for α -*fur* RNA and 14 (x-CY5) for *fur* RNA (Table 1). Samples were analysed in an ALF sequencer (Pharmacia Biotech) as described previously (Sola-Landa *et al.*, 2005).

RESULTS

Occurrence of a *cis* α -*fur* RNA is conserved in two unicellular cyanobacteria with different gene contexts

Previous identification of an α -*furA* RNA as part of a dicistronic operon in the nitrogen-fixing *Anabaena* PCC 7120 led us to seek the existence of this kind of RNA in unicellular cyanobacteria exhibiting different *furA* gene contexts. The occurrence of α -*fur* RNA in the toxigenic cyanobacterium *M. aeruginosa* PCC 7806 was demonstrated by performing RT-PCR analysis using two pairs of deoxyoligonucleotides (Fig. 1a). Oligonucleotides 1 and 2 cover the whole *fur* gene (588 bp) and oligonucleotides 3 and 4 amplify an internal region of *fur* (195 bp). Fig. 1(b) shows a retrotranscription experiment with oligonucleotide 1 followed by a PCR using oligonucleotides 1 and 2, which yields a 588 bp band, which likely corresponds with an antisense RNA of the *Mafur* gene. The sample treated with RNase did not show amplification, removing the possibility that the amplification was due to chromosomal DNA contamination (Fig. 1b). The presence of *Max-fur* RNA

Fig. 2. Identification of *Syx-fur* RNA. (a) Scheme of the *fur* locus in *Synechocystis* PCC 6803 and the oligonucleotides used for identification of *Syx-fur* RNA. (b) RT-PCR analysis of the *Syx-fur* RNA. The 1 % agarose gel shows the amplified fragments after the reverse transcription and PCR steps. The size (in bp) of the molecular mass marker are indicated on the right. Lanes: 1, molecular mass markers; 2, reverse transcriptase step with oligonucleotide 7 and PCR with oligonucleotides 7 and 8; 3, sample as in lane 2 in the presence of DNase-free RNase; 4, reverse transcriptase step with oligonucleotide 7 and PCR step with oligonucleotides 7 and 9.

was confirmed by carrying out retrotranscription using oligonucleotide 3 followed by a PCR with oligonucleotides 3 and 4, which yielded a 195 bp amplification product (Fig. 1b). Again, there was no amplification product from the sample treated with RNase.

The expression of the *Sy α -fur* RNA was shown by using RT-PCR (Fig. 2). Retrotranscription with oligonucleotide 7 followed by a PCR using oligonucleotides 7 and 8 yielded a band of 145 bp corresponding to an antisense RNA of the *SyfurA* orthologue *sll0567*. There was no amplification product from the sample treated with RNase. This result is in concordance with the recent data taken from the *Synechocystis* global *tsp* mapping (Mitschke *et al.*, 2011) which reveals the presence of a *tsp* within *furA* (*sll0567*). The fact that a new retrotranscription experiment with oligonucleotide 7 followed by a PCR using oligonucleotides 7 and 9 did not show any amplification product, indicates

that the length of *Sy α -fur* RNA is smaller than those of *Anabaena* or *Microcystis* and only covers part of the *fur* coding sequence.

Characterization of *Max-fur* RNA

With the aim of determining the extent of base-pairing of the *Max-fur* RNA with the *fur* locus, we intended to determine their boundaries. Primer extension analysis of *Max-fur* shows that this gene is transcribed from a single *tsp* that is localized at -102 bp (Fig. 3 and Supplementary Fig. S1, available with the online version of this paper), only 3 bp from the translation start site of *dnaJ*, indicating that the RNA polymerase-binding site to promote *fur* transcription has to overlap the *dnaJ* CDS.

Identification of the 5' ends of the *Max-fur* RNA shows three potential transcriptional start sites, whose presence

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GCCTGCCAAAATTGCGGATTAATTGCGATCGCTTGCTGGTAAAATGCGATCGCTAGTTGATAATTTTCTCTTGAGCGTAGTGCC
ATCCCTGTTGATAAAAATCCTCGGCACTGCGCTGAAAAGTGGTATTTCTGGGGATAAACTGCGATCATACTCTTTTCTTTGTCT
TTATCGCTCAAAACCGGATAAGCTTGTTCAATTTGCGGAAATTTGTCACTGCCTCTGGATCATTGGGATTATAATCGGGGTGAT
ATTGCCGGGCCAGACGACGAAAAGCGGCTTTTATCTGATTATTACTGGCATTACGGGGAATTTGGAGGATTTTCATAGTAATTTT
CATACCTTGAATGGGGGGAGAATTTCAACTACTGAAAAGTGTGCAATGGCTCGCCGAAACGACTATCATTGTCAATTAATAG
CTTTTATTGAGAGTCGATCGCTTATGTCTGCTACACTGCCTCCTCCCTGAAAGCGGAAGTCAATGCGCGGGGGTGGCGCTTA
ACTCCCAGCGAGAGAAAATTCTGCACGTTTTCCAGAATTTACCGAAAGGCAATCATCTCAGTGCCGAAGAATTGCAGGAAT
TACTGGACAAACGGGGGGAAGGAATTAGTTTATCGACGATTTACCGCAGTGTTAACTAATGTCGCGGATGGGAATTTGCG
GGAGTTGGAATTGGCCGAGGGTCATAAACACTACGAACTCAATCAACCCATCCCCATCACCATCATCATTTGGTCTGTATTC
AGTGAATAAAACCATCGAATTTAACAATGATTCCATTCTCAAACACAGTCTCAAACAGTGTGAGAAAGAGGGTTTTTCAGTTA
ATTGACTGTCAGCTAACGGTGATGGCCATCTGTCCGGAAGCTTTGCGGATGGGTGGCCGTCGGAATACCGAGTAATTGGG
GTTGCACGCGTTGCTGGTGGATACTCGTTTCCA+1AACTGCGAGATTCCTGAATCAAAGGAAC+1CGGAACAGAAAAGTAC+1
ATCTAAGCGGACATTCCCAACTGAAAACCGAGAGCTTTTTCTGCATTAAATTGGAAAA+1ATTATAAAATCCGTTGSCGCGGGAAG
GGGTAAGACTGACTTTTAAACCGGTTTCTTCGATAAAATCCGGGGTGACTTGCAGGATTTCCGAGGGAGTCAGTCCATTTAACC
CCTCGATTAACAAAGCCACTAAACCTTGACTAATTGCGCGTCGGAATCCCTTTGTACCAAACCTGGCCATTTTCGAGGTCCG
CAGTGATATAAACTTGGGATACACAGCCATGGACTTTATTAGCGGGTATTTGGCAGATTCTGGTATTGGTTCTAGTTTTTTAGC
AAAAGCTAGTAATTGTTCTGATTTTTGCTTAGGATCGGTACGACGTTTGAGGCGTTTCGACGATCGCATCGAGATTAGACGGGAG
CATATTGCGAGTTAAATAGGAAAAAATTAGCC...

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Primer 6

Primer 5

Primer 2

Primer 11

Fig. 3. Tsps and approximated localization of the 3' ends of *Max-fur* and *Mafur* RNAs in the *Mafur* locus. The CDS of the *fur* gene is indicated by bold type. Start and stop codons corresponding to *FurA*, the divergent *DnaJ* start codon, and the start and stop codons corresponding to *SufE* are underlined and in bold type. Tsps of *Mafur* and *Max-fur* are indicated as +1. A putative iron box overlapping *tsp1* *Max-fur* RNA is indicated by grey shading. The 3' end of *Max-fur* RNA is located between oligonucleotides 5 (continuous underline) and 6 (dotted underline). Oligonucleotides 11 and 2 were used to localize the 3' end of *Mafur*.

was verified in RNA samples from different *Microcystis* cultures (Supplementary Fig. S2, available with the online version of this paper). Localization of the potential *Max-fur* tsps in the sequence of the *Mafur* locus (Figs 3 and 4) shows a partial overlap between the *Max-fur* transcript starting from tsp1 and the *sufE* gene, which lies tail-to-tail with *Mafur* and keeps a distance of only 4 bp between both coding sequences, while the RNAs starting from tsp2 and/or tsp3 cover most of the *Mafur* coding sequence.

Several attempts to identify the 3' end of *Max-fur* by 3' RACE were unsuccessful. In order to estimate whether the *Max-fur* RNA could work by occluding the *Mafur* ribosome-binding site, RT-PCR analysis was performed using oligonucleotides at different positions to approximately define the end of the *Max-fur* RNA. Fig. 5 shows that there was an amplification product from *Max-fur* RNA when oligonucleotides 2 and 5 were used, while there was no amplification detected when oligonucleotides 2 and 6 were used. These data allowed us to locate the 3' end of the *Max-fur* RNA transcript between oligonucleotides 5 and 6 (see Figs 3 and 4), indicating that the antisense extends beyond the putative *Mafur* ribosome-binding site and covers part of the *dnaJ* gene that lies divergently to *Mafur* and is located 105 bp upstream of the coding sequence of the regulator.

RT-PCR analysis suggests the presence of a second antisense RNA complementary to *sufE*

In order to define the *Mafur* transcript, attempts to identify its 3' end were performed using RACE without concluding results. Therefore, the ability of several oligonucleotides located at different distances from the *Mafur* stop codon to allow amplification in RT-PCR assays was tested. Retrotranscription using oligonucleotide 2 (MFurC) followed by PCR with oligonucleotides 1 (MFurN) and 2 (MFurC) yielded one band of 588 bp (Fig. 6). A second retrotranscription experiment performed with oligonucleotide 11 (ASufE) followed by PCR using oligonucleotides 1 (MFurN) and 11 (ASufE) produced a band of 997 bp, suggesting that *Mafur* is transcribed in a dicistronic message together with a second putative antisense RNA complementary to *sufE*. The identity of this transcript was confirmed by sequencing the corresponding cDNA cloned in pGEMT (data not shown), and performing the corresponding controls using RNase-treated samples (Fig. 6).

DISCUSSION

In this paper we report the expression of α -*fur* RNAs in three cyanobacterial strains with important differences in their metabolism and also exhibiting very different *fur* regions (Fig. 4). In the nitrogen-fixing, filamentous

Fig. 4. Genomic context of the *Mafur* locus compared with that of *Synechocystis* PCC 6803 and *Anabaena* PCC 7120. (a) Schematic representation of the results obtained after mapping the 3' and 5' ends of *Mafur-α-sufE* and *Max-fur* RNAs. Position of the tsps in *Mafur* and *Max-fur* RNA are represented as grey arrows. The oligonucleotides used are represented by black arrowheads. (b, c) Gene context of the *Syfur* locus (b) and context of the *fur* locus in *Anabaena* PCC 7120 (c).

Fig. 5. Screening of *Max-fur* RNA 3' end by RT-PCR. (a) The 1 % agarose gel shows the amplified fragments after the reverse transcription and PCR steps. The sizes (in bp) of the molecular mass markers are indicated on the right. Lanes: 1, molecular mass markers; 2, PCR positive control; 3, reverse transcriptase step with oligonucleotide 1 and PCR step with oligonucleotides 1 and 2; 4, reverse transcriptase step with oligonucleotide 5 and PCR step with oligonucleotides 2 and 5; 5, reverse transcriptase step with oligonucleotide 6 and PCR step with oligonucleotides 2 and 6.

Anabaena PCC 7120, *furA* is surrounded by the sigma factor *sigC* and *all1690*. α -*furA* is transcribed in the same message as *Alr1690* and extends into the 5'-untranslated region of *furA* mRNA; this is longer than most ncRNAs described to date in eubacteria. In *Synechocystis* PCC 6803, the *furA* orthologue *sll0567* is flanked by two hypothetical proteins, while the gene from *M. aeruginosa* PCC 7806 lies between *dnaJ* and *sufE*, as does its orthologue from *M. aeruginosa* NIES843 (<http://genome.kazusa.or.jp/cyanobase/>; Martín-Luna *et al.*, 2006). These results indicate that the expression of a *cis* α -*fur* RNA is not restricted to *Anabaena* PCC 7120, but it is spread among different cyanobacterial genera and its occurrence is not linked to a defined *furA* context.

Analysis of the *Mafur* locus shows that it presents a rather compressed genetic arrangement. A region of the *dnaJ* promoter overlaps the *Mafur* 5'-untranslated region and the 3' end of *Max-fur* extends into the *dnaJ* CDS. In addition, the *Max-fur* *tsp1* is located within *sufE* CDS. The prevalence of overlapping genomic signals, such as functional promoters inside CDSs, as well as the overlap of 3' ends of 137 transcript pairs has been reported in the archaea *Halobacterium salinarum* (Koide *et al.*, 2009). This phenomenon has also been observed in yeast (Nagalakshmi *et al.*, 2008) and has important consequences on the transcriptional regulation of these organisms. In the case of *Microcystis*, the presence of α -*fur* and α -*sufE* RNAs might add new levels of information to the *fur*-*sufE* locus, whose physiological implications should be investigated. In *E. coli*, the *sufBCD* operon is regulated by Fur and specifically adapted to synthesize Fe-S clusters when iron or sulphur metabolism is disrupted by iron starvation or oxidative stress (Outten *et al.*, 2004). *SufE* is involved in the assembly of Fe-S clusters and works coordinately with the *SufBCD* complex to increase the activity of the cysteine desulfurase *SufS* (Outten *et al.*, 2003). These proteins are particularly important in cyanobacteria, since they are indirectly

Fig. 6. Screening of *Mafur* RNA 3' end by RT-PCR reveals that *fur* is encoded in the same message as α -*sufE* RNA. The 1 % agarose gel shows the amplified fragments after the reverse transcription and PCR steps. The sizes (in bp) of the molecular mass markers are indicated on the right. Lanes: 1, molecular mass markers; 2, PCR with oligonucleotides 3 and 4 and chromosomal DNA from *Microcystis* (positive control); 3, reverse transcriptase step with oligonucleotide 2 (MFurC) followed by PCR with oligonucleotides 1 (MFurN) and 2 (MFurC); 4, reverse transcriptase step with oligonucleotide 11 (ASufE) followed by PCR with oligonucleotides 1 (MFurN) and 11 (ASufE); 5, sample as in lane 3 in the presence of DNase-free RNase; 6, sample as in lane 4 in the presence of DNase-free RNase.

involved in the regulation of PSI and its activation by a shift to high light conditions links their synthesis to the photosynthetic activity (Seki *et al.*, 2006). The existence of α -*fur* and α -*sufE* in *Microcystis* might relate Fur expression to Fe-S cluster biogenesis providing the possibility of differential regulation of those genes, increasing the versatility of this ubiquitous, potentially toxic, cyanobacterium.

ACKNOWLEDGEMENTS

This work was funded by the Spanish Ministerio de Ciencia y Tecnología (BFU2009-07424) and Fundación La Caixa-Diputación General de Aragón (grant 2009/00372). A. G. was supported by Fondos Feder 2007-2013. The authors thank Dr Maribel Muro-Pastor for the kind gift of *Synechocystis* PCC 6803 RNA.

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Edited by: C.-C. Zhang