# Identification of three new antisense RNAs in the *fur* locus from unicellular cyanobacteria.

**Emma Sevilla[(1)], Beatriz Martín-Luna[(1)], Andrés González[(1)], Jesús A. Gonzalo-Asensio[(2)], María Luisa Peleato[(1)] and María F. Fillat[(1)].**

[(1)]Department of Biochemistry and Molecular and Cell Biology, and Biocomputation and Complex Systems Physics Institute (BiFi), University of Zaragoza, Zaragoza, Spain.

[(2)] Laboratorio de Investigación Molecular, Hospital Universitario Miguel Servet. Instituto Aragonés de Ciencias de la Salud. Zaragoza

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Address correspondence to: María F. Fillat, University of Zaragoza, Pedro Cerbuna, 12, 50009 Zaragoza. Spain. Tel: +34 976761282; Fax: +34 976762123;

E-mail: fillat@unizar.es

## Summary

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 new 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. Syα-*fur* RNA covers only part of the coding sequence of the *fur* orthologue *sll0567*, whose flanking genes encode two hypotetical proteins. Transcriptional analysis of *fur* and their adjacent genes in *Microcystis* unravels a highly compact organization of this locus involving overlapping transcripts. Maα-*fur* RNA spans the whole Ma*fur* CDS and part of the flanking *dnaJ* and *sufE* sequences. In addition, Ma*fur* seems to be part of a dicistronic operon encoding this regulator and an *α–sufE* RNA. Those results contribute to gain new insights into the transcriptomes of two unicellular cyanobacteria and suggest that in *Microcystis aeruginosa* PCC7806 the α-*fur and* α-*sufE* RNAs might participate in a regulatory connection between the genes of the *dnaJ-fur-sufE* locus.

Keywords: antisense RNA; cyanobacteria; *Microcystis*; *Synechocystis*, Fur; *sufE*.

Abbreviations: CDS, coding sequence; Fur, ferric uptake regulator; α-*fur*, *fur* antisense RNA; Ma, *Microcystis aeruginosa*; Sy, *Synechocystis*; ncRNAs, non-coding RNAs; rbs, ribosome binding site; tsp, transcription start point.

# INTRODUCTION

In eubacteria, most regulatory RNAs identified to date are noncoding RNAs (ncRNAs) smaller than 300 nucleotides that are located far away from their target genes. The roles of ncRNAs are diverse, being 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 (Vecerek *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 modulate Fur activity. In addition, reciprocal regulation between Fur and several ncRNAs, as well as co-regulation of iron-responsive genes by Fur and ncRNAs have been described (Waldbeser *et al*., 1995) Identification of RhyB in *E. coli* cells provided the answer to the mechanism of activation of the *sodB* gene under iron-rich conditions (Masse & Gottesman, 2002) and linked the regulation of a set of iron-storage and iron-containing proteins to this small ncRNA, involving the own Fur as part of a backup mechanism.

In cyanobacteria, two cis-encoded regulatory RNAs linked to iron metabolism have been characterised. In the unicellular cyanobacterium *Synechocystis*, the regulatory RNA IsiR is expressed specifically during iron rich conditions and co-represses with Fur the synthesis of IsiA under iron-replete conditions (Duhring *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 6803 genome, the *furA* orthologue *sll0567* is flanked by genes encoding hypotetical 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), being its occurrence in *Microcystis* sp. much higher than in other unicellular cyanobacteria (Voss *et al*., 2009). More recently, the use of differential RNA sequencing of the model organism *Synechocystis* sp. PCC6803, together with complementary microarray-based RNA profiling show that a quarter of all chromosomal genes are subject to antisense transcription (Mitschke et al., 2011). Interestingly, those data also reveal that the majority of small RNAs are located at the complementary strand of mRNAs

In this work we provide experimental evidence for the transcription of three new antisense RNAs in unicellular cyanobacteria. Two of them have been identified in the *fur-sufE* locus of *Microcystis aeruginosa*. One 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.** *Microcystis aeruginosa* PCC 7806 was grown at 25ºC and 10 µmol of photons $m^{-2}$ $s^{-1}$ in modified BG11 medium with 2 mM $NaNO_3$ and 10 mM $NaHCO_3$ (Ripka *et al*., 1979) *Synechocystis* PCC6803 was grown in the same conditions using standard BG11.

**Sampling and RNA isolation.** 25 ml of cells were centrifuged and pellets re-suspended in 600 µl of 50 mM Tris-HCl (pH 8), 100 mM EDTA and 130 µl of chloroform and incubated in ice for 3 minutes. After centrifugation cell pellets were frozen and kept at -70ºC 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 µl of DEPC-$H_2O$ and treated with 40 units of 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 µg of RNA and 20 pmol of the primer in the annealing buffer (10 mM Tris-HCl (pH 8), 1 mM EDTA, 150 mM KCl) in a final volume of 10 µl. Samples were heated for 10 min at 85ºC and incubated for 60 min at 65ºC. Afterwards, 5 pmol of each dNTP, 0.2 mmol of DTT and 200 U of Superscript Reverse Transcriptase were added to the buffer (50 mM Tris-HCl pH

8.3, 75 mM KCl, 3 mM $MgCl_2$ and 20 mM DTT) to a final volume of 20 µl. Samples were incubated at 47ºC for 1 h and finally heated at 75ºC for 15 min. The products were amplified by PCR using 2 µl of each cDNA as template and analyzed in a 1% (w/v) agarose gel with a Gel Doc 2000 (BioRad).

**Transcription start point determination.** Potential Maα-*fur* and Ma*fur* tsps were determined by primer extension. Ma*α-fur* promoter region was amplified using oligonucleotides 10 (ATS5) and 11 (AsufE). To identify the tsp of Ma*fur*, oligonucleotides 6 (pFurN) and 13 were used for amplification of the promoter. The resulting fragments were cloned in pGEMT and introduced in *E.coli* JM 109 (Promega). The recombinant plasmids were purified and the correct integration of the inserts was ensured by restriction analyses with *EcoR*I. Those plasmids were used as templates in the sequencing reaction using the Termo Sequenase Fluorescent Primer Cycle Sequencing Kit (Amersham), where the labelled nucleotides primed the sequencing reaction used as molecular size marker. Reverse transcription was carried out using Expand Reverse Transcriptase (Roche) with 1 µg of total RNA as template and the Cy5-labelled primer 12 (ATS5-Cy5) for α-*fur* RNA and 14 (x-CY5) for *fur* RNA (Table 1). Samples were analyzed in an ALF sequencer (Pharmacia Biotech) as previously described (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 *Microcystis 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 primers 3 and 4 amplify an internal region of *fur* (195 bp). Fig. 1b shows a retrotranscription experiment with primer 1 followed by a PCR using primers 1 and 2 yields a 588-bp band, likely corresponding to an antisense RNA of the Ma*fur* gene. The sample treated with RNase did not show amplification, discarding the possibility that the amplification was due to chromosomal DNA contamination (Fig. 1b). The presence of Maα-*fur* RNA was confirmed by carrying out retrotranscription using primer 3 followed by a PCR with primers 3 and 4, which yielded a 195 bp amplification product (Fig. 1b). Again, the sample treated with RNAse did not show amplification.

The expression of the Syα-*fur* RNA was evidenced by RT-PCR (Fig. 2). Retrotranscription with oligo 7 followed by a PCR using oligos 7 and 8 yields a band of 145 bp corresponding to an antisense RNA of the Sy*furA* ortologue *sll0567*. The sample treated with RNase did not show amplification. This result is in good concordance with the recent data arose from the *Synechocystis* global tsp mapping (Mitschke et al., 2011) that reveals the presence of a tsp within *furA* (sll0567). The fact that a new retrotranscription experiment with oligo 7 followed by a PCR using oligos 7 and 9 did not show any amplification product, indicates that the length of Syα-*fur* RNA is smaller than in the cases of *Anabaena* or *Microcystis* and only covers part of the *fur* coding sequence.

**Characterization of Maα-*fur* RNA**

With the aim to know the extent of base-pairing of the Maα-*fur* RNA with the *fur* locus we intended to determine their boundaries. Primer extension analysis of Ma*fur* shows that

this gene is transcribed from a single tsp that is localized at -102 bp (Figs. 3 and S1), only at 3 bp distance of the translation start of *dnaJ*, indicating that the RNA-polymerase-binding site to promote *fur* transcription has to be imbricated in *dnaJ* CDS.

Identification of the 5´ ends of the Maα-*fur* RNA shows three potential transcriptional start sites, whose presence was verified in RNA samples from different *Microcystis* cultures (Fig. S2). Localization of the potential Maα-*fur* tsps in the sequence of the Ma*fur* locus (Figs. 3 and 6) shows a partial overlapping between the Maα-*fur* transcript starting from tsp1 and the *sufE* gene that lies tail-to-tail with Ma*fur* and keep a distance of only 4 bp between both coding sequences, while the RNAs starting from tsp2 and/or tsp3 cover most of the Ma*fur* coding sequence.

Several attempts to identify the 3´end of Maα-*fur* by 3´RACE were unsuccessful. In order to estimate whether the Maα-*fur* RNA could work occluding Ma*fur* ribosome binding site, RT-PCR analysis were performed using primers at different positions to approximately define the end of the Maα-*fur* RNA. Fig. 4 shows the presence of amplification of the Maα-*fur* RNA when oligonucleotides 2 and 5 were used, while no amplification was detected when oligos 2 and 6 were used as primers. Those data allowed us to locate the 3´end of the Maα-*fur* RNA transcript between oligonucleotides 5 and 6 (see Figs. 3 and 6), indicating that the antisense extends beyond the putative Ma*fur* rbs and covers part of the *dnaJ* gene, that lies divergently to Ma*fur* and is located 105 bp upstream 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 Ma*fur* transcript, attempts to identify its 3´ were performed using RACE without concluding results. Therefore, the ability of several oligonucleotides

193 located at different distances to the Ma*fur* stop codon to allow amplification in RT-PCR
194 assays was tested. Retrotranscription using oligonucleotide 2 (MFurC) followed by PCR
195 with oligonucleotides 1 (MFurN) and 2 (MFurC) yielded one band of 588 bp (Fig. 5). A
196 second retrotranscription experiment performed with oligonucleotide 11 (ASufE) followed
197 by PCR using oligonucleotides 1 (MFurN) and 11 (ASufE) produced a band of 997 bp,
198 suggesting that Ma*fur* is transcribed in a dicistronic message together with a second
199 putative antisense RNA complementary to *sufE*. The identity of this transcript was
200 confirmed by sequencing the correspondent cDNA cloned in pGEMT (data not shown),
201 and performing the correspondent controls treating the samples with RNase (Fig. 5).
202
203 **DISCUSSION**
204
205 In this paper we report the expression of α-*fur* RNAs in three cyanobacterial strains with
206 important differences in their metabolism and also exhibiting very different *fur*
207 neighbourhoods (Fig. 6). In the nitrogen-fixing, filamentous *Anabaena* PCC 7120, *furA* is
208 surrounded by the sigma factor *sigC* and *all1690*. α-*furA* is transcribed in the same
209 message than Alr1690 and extends into the 5´UTR region of *furA* mRNA, being longer
210 than most ncRNAs described to date in eubacteria. In *Synechocystis* PCC 6803, the *furA*
211 orthologue *sll0567* is flanked by two hypothetical proteins, while the gene from
212 *Microcystis aeruginosa* PCC 7806, as its orthologue from *M. aeruginosa* NIES843 lies
213 between *dnaJ* and *sufE* (http://genome.kazusa.or.jp/cyanobase/; Martín-Luna *et al*., 2006).
214 Those results indicate that the expression of a cis α-*fur* RNA is not restricted to *Anabaena*
215 PCC 7120, but it is spread among different cyanobacterial genera and its occurrence is not
216 linked to a defined *furA* context.
217

Analysis of the Ma*fur* locus shows that it presents a rather compressed genetic arrangement. A region of the *dnaJ* promoter overlaps to Ma*fur* 5´-UTR and the 3´end of Maα-*fur* extends into *dnaJ* CDS. In addition the Maα-*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 iron-sulphur 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 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 iron-sulphur cluster biogenesis providing the possibility of differential regulation of those genes, increasing the versatility of this ubiquitous, potentially toxic cyanobacterium.

## ACKNOWLEDGEMENTS

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## Figure legends

**Figure 1. Identification of Maα-*fur* RNA.**

(a) Schematic representation of two regions of the Ma*fur* gene amplified. Oligonucleotides are represented in black triangles. (b) RT-PCR analyses of the Maα-*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 left. Lane 1, molecular mass markers. Lane 2, PCR with primers 1 and 2 and chromosomic DNA of *Microcystis* (positive control), lane 3, RT step with primer 1 and PCR step with primer 1 and 2, lane 4, same as lane 3 in the presence of DNase free Rnase, lane 5, PCR with primers 3 and 4 and chromosomic DNA of *Microcystis* (positive control), lane 6, RT step with primer 3 and PCR step with primer 3 and 4, lane 7, same as lane 6 in the presence of DNase-free Rnase.

**Figure 2. Identification of Syα-*fur* RNA**. (a) Scheme of the *fur* locus in *Synechocystis* PCC 6803 and the primers used for identification of Syα-*fur* RNA. (b) RT-PCR analyses of the Syα-*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 left. Lane 1, molecular mass markers. Lane 2, RT step with primer 7 and PCR with primers 7 and 8, Lane 3 same as lane 2 in the presence of DNase free Rnase, Lane 4, RT step with primer 7 and PCR step with primer 7 and 9.

**Figure 3**. **Sequence of the Ma*fur* locus**. CDS of the *fur* gene is indicated in bold characters. Start and stop codons corresponding to FurA, the divergent DnaJ start codon, as well as start and stop codons corresponding to SufE are bold underlined. Transcriptional

start (+1) points of Ma*fur* and Ma*α-fur* are indicated. A putative iron-box overlapping tsp1Ma*α-fur*RNA is shadowed. 3´-end of Maα-*fur* RNA is located between deoxyoligonucleotides 5 (continuous underline) and 6 (broken underline). Primers 11 and 2 were used to localize Ma*fur* 3´-end.

**Figure 4. Screening of Maα-*fur* RNA 3´end through 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 left. Lane 1, molecular mass markers. Lane 2, PCR positive control, lane 3, RT step with primer 1 and PCR step with primer 1 and 2, lane 4, RT step with primer 5 and PCR step with primers 2 and 5, lane 5, RT step with primer 6 and PCR step with primers 2 and 6.

**Figure 5. Screening of Ma*fur* RNA 3´end through RT-PCR unravels that *fur* is encoded in the same message than an *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 left. Lane 1, molecular mass markers. Lane 2, PCR with primers 3 and 4 and chromosomic DNA of *Microcystis* (positive control), lane 3, RT step with primer 2 (MFurC) followed by PCR with primers 1 (MFurN) and 2 (MFurC), lane 4, RT step with primer 11 (ASufE) followed by PCR with primer 1 (MFurN) and 11 (ASufE), lane 5 same as lane 3 in the presence of DNase free RNase and lane 6, same as lane 4 in the presence of DNAse free Rnase.

**Figure 6. Genomic context of the Ma*fur* locus compared to those of *Synechocystis* PCC 6803 and *Anabaena* PCC 7120. (a)** Schematic representation of the results obtained after mapping the 3´and 5´ends of Ma*fur*-α-*sufE* and Maα-*fur* RNAs. Position of

transcription start points in Ma*fur* and Maα-*fur* RNA are represented as grey arrows. Deoxyoligonucleotides used are represented by black triangles. (b) Gene context of the Sy*fur* locus and (c) *Anabaena* PCC 7120.

## Supplementary files legends

**Figure S1. Primer extension analysis of Ma*fur* RNA.** The fluorogram shows a single transcription start point for the *fur* gene.

**Figure S2. Primer extension analysis of Maα-*fur* RNA.** Fluorogram of a representative primer-extension assay of Maα-*fur* RNA showing three potential transcription start sites. (a) tsp1. (b) tsp2 and tsp3. The corresponding DNA sequences are shown in the upper part of the fluorogram.

Figure 1(a)

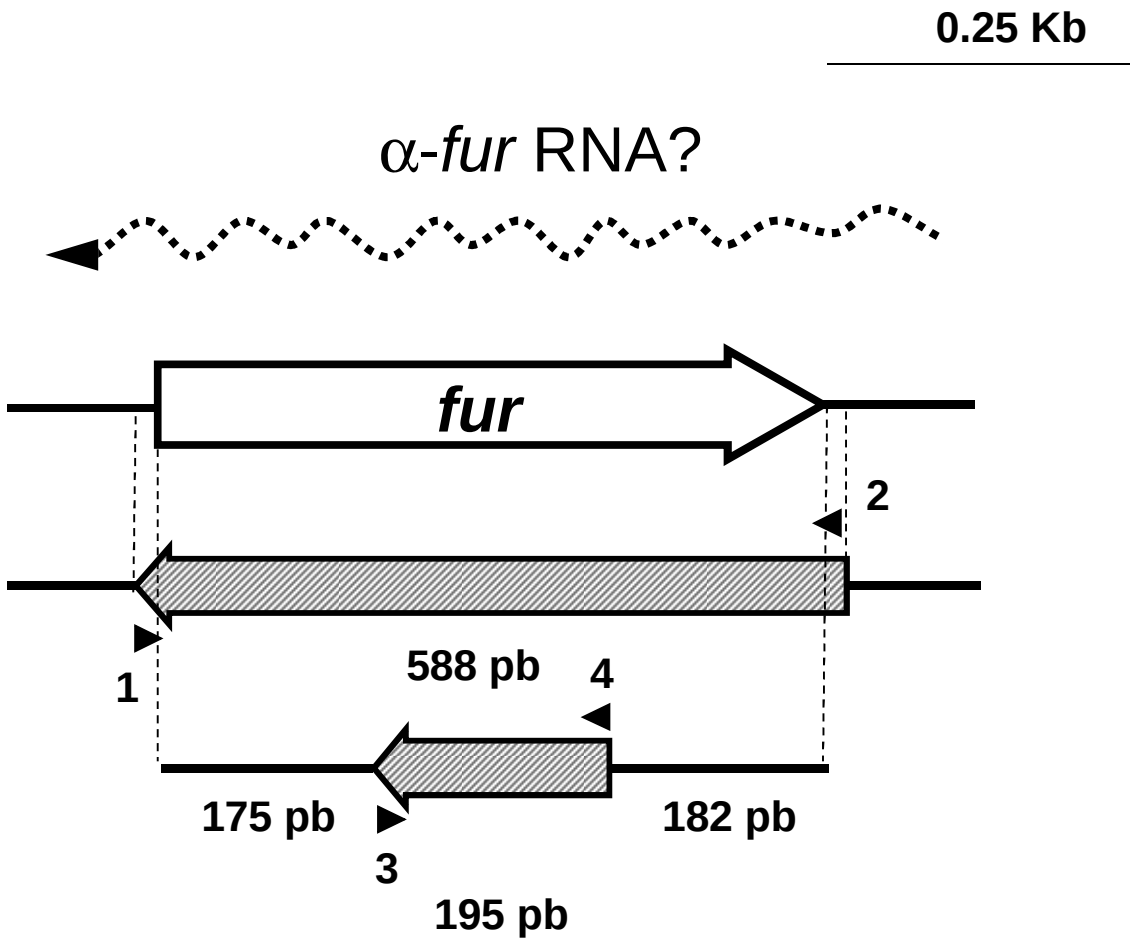


Figure 1(b)

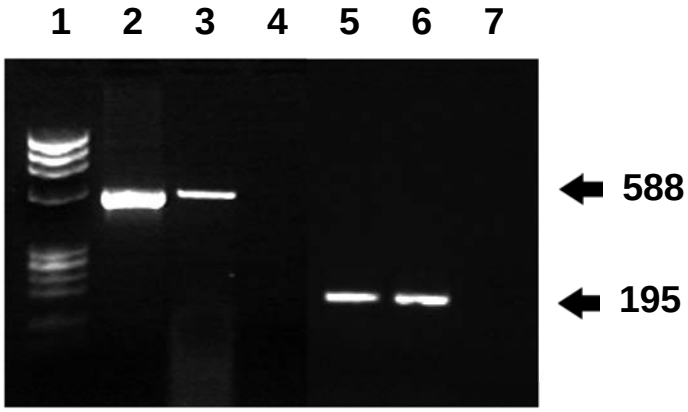


Figure 2(a)

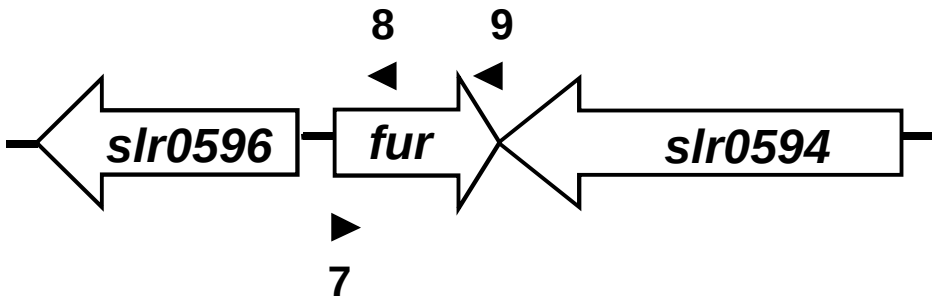
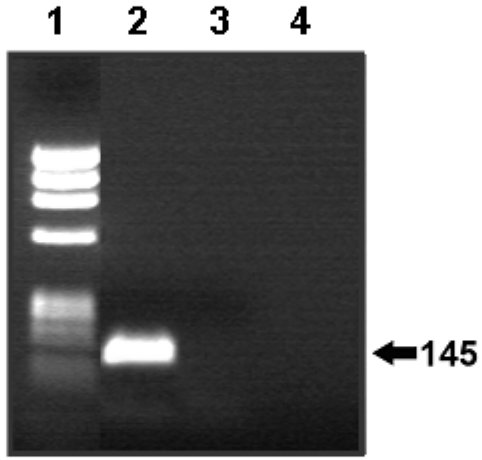


Figure 2(b)



GCCTGCCAAAATTGCGGATTAATTGCGATCGCTTGCTGGTAAAATGCGATCGCTAGTTGATAATTTTTCTCTTGAGCGTAGTGCC

Primer 6

ATCCCTGTTGATAAAAATCCTCGGCACTGCGCTGAAAAGTGGTATTTCTGGGGATAAACTGCGATCATACTCTTTTCTTTTGTCT

TTATCGCTCAAAACCCGATAAGCTTGTTCAATTTCGCGAAATTTTGTCACTGCCTCTGGATCATTGGGATTATAATCGGGGTGAT

ATTGCCGGGCCAGACGACGAAAAGCGGCTTTTATCTGATTATTACTGGCATTACGGGGAATTTGGAGGATTTCATAGTAATTTTT

Primer 5

**+1**

**CAT**ACTTGAATGGGGGGGAGAATTTTCAACTACTGAAAACTGTTGCCAATGGCTCGCCGAAACGACTATCATTGTCAATTAAATAG

CTTTTATTGAGAGTCGATCGCTT**ATGTCTGCCTACACTGCCTCCTCCCTGAAAGCGGAACTCAATGCGCGGGGGTGGCGCTTA**

**ACTCCCCAGCGAGAGAAAATTCTGCACGTTTTCCAGAATTTACCGAAAGGCAATCATCTCAGTGCCGAAGAATTGCAGGAAT**

**TACTGGACAAACGGGGGGGAAGGAATTAGTTTATCGACGATTTACCGCAGTGTTAAACTAATGTCGCGGATGGGAATTTTGCG**

**GGAGTTGGAATTGGCCGAGGGTCATAAACACTACGAACTCAATCAACCCTATCCCCATCACCATCATCATTTGGTCTGTATTC**

**AGTGTAATAAAACCATCGAATTTAACAATGATTCCATTCTCAAACACAGTCTCAAACAGTGTGAGAAAGAGGGTTTTCAGTTA**

**ATTGACTGTCAGCTAACGGTGATGGCCATCTGTCCGGAAGCTTTGCGGATGGGTTGGCCGTCGGGAATACCGAGTAATTGGG**

**+1** **+1**

**GTTGCACGCGTTCGCTGGTGGATACTCGTTTCCAAAACTGCGAGATTCCCGAATCAAAGGAACCGGAACCAGAAAACTAG**AC

**+1**

AT**CTA**AGCGGACATTCCCAACTGAAAACCGAGAGCTTTTTTCTGCATTAATTGGAAAATATTATAAAATCCGTTGGCGCGGGAAG

Primer 2

GGGTAAGACTGACTTTTAAACCGGTTTCTTCGATAAAATCCGGGGTGACTTGCAGGATTTCCGAGGGAGTCAGTCCATTTAACC

CCTCGATTAACAAAGCCACTAAACCCTTGACTAATTGCGCGTCGGAATCCCCTTTGTACCAAACTTGGCCATTTTCGAGGTCGG

CAGTGATATAAACTTGGGATACACAGCCATGGACTTTATTAGCGGGTATTTTGGCAGATTCTGGTATTGGTTCTAGTTTTTTAGC

AAAAGCTAGTAATTGTTCGTATTTTTGCTTAGGATCGGTACGACGTTTGAGGCGTTCGACGATGCGATCGAGATTAGACGGGAG

Primer 11

**CAT**ATTGCGAGTTAAAATAGGAAAAAATTAGCC…

Figure 4

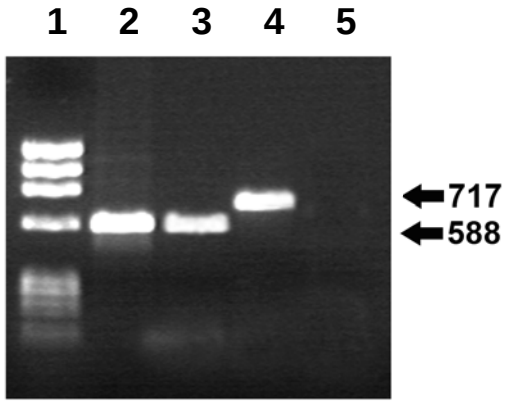


Figure 5

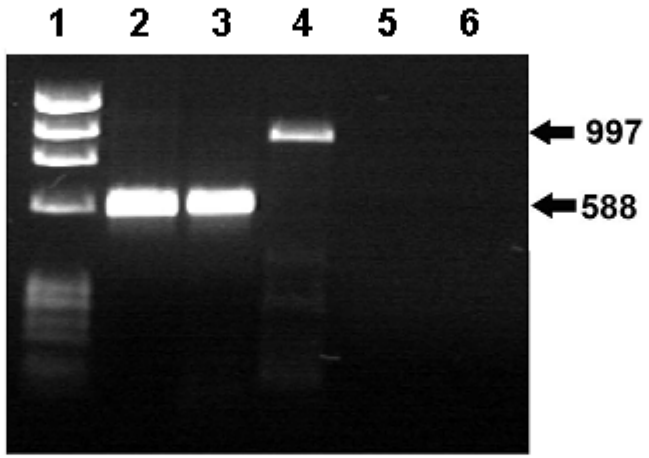


Figure 6

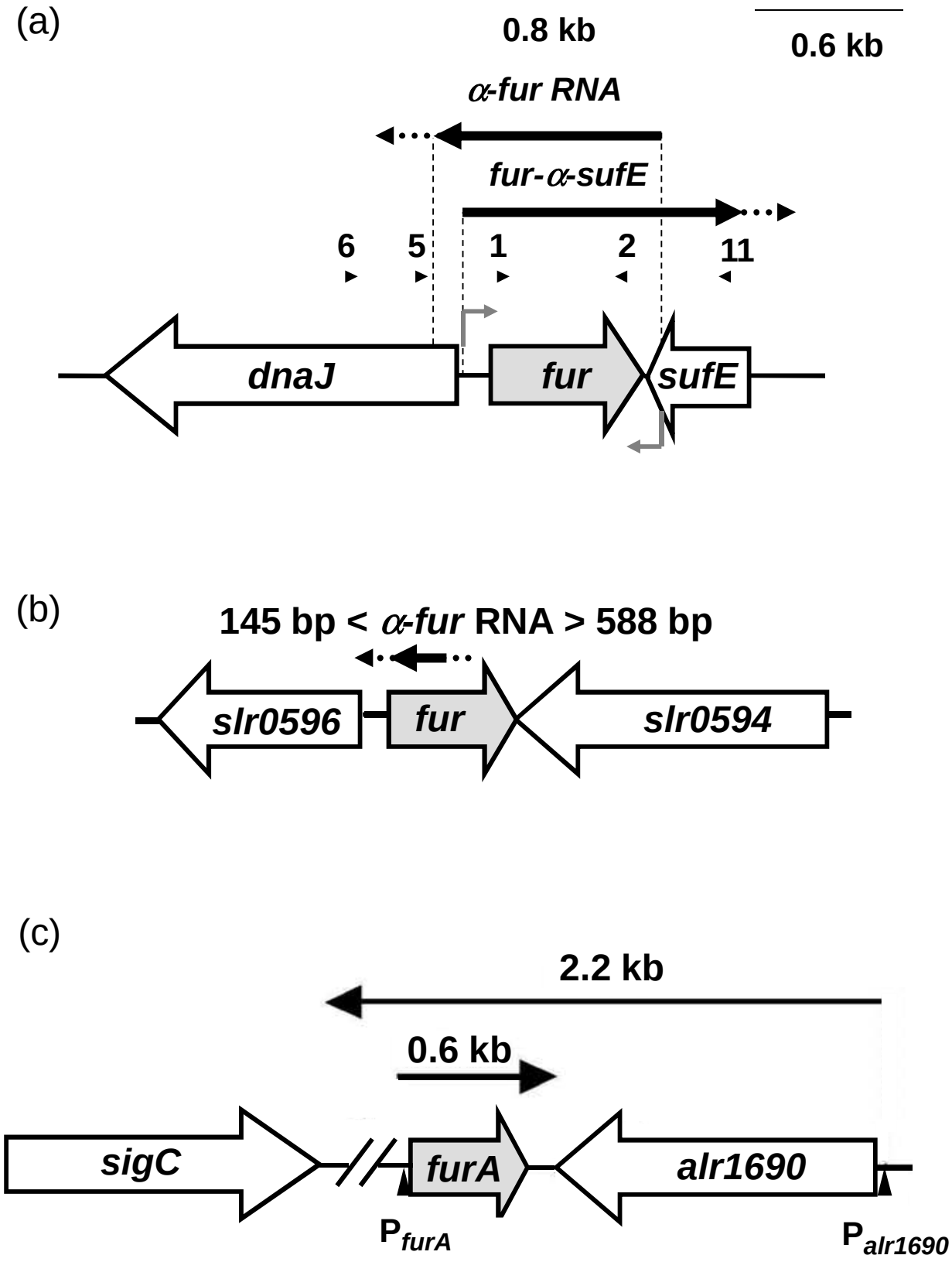

(a)
0.8 kb
0.6 kb
α-fur RNA
fur-α-sufE
6
5
1
2
11
dnaJ
fur
sufE
(b)
145 bp < α-fur RNA > 588 bp
slr0596
fur
slr0594
(c)
2.2 kb
0.6 kb
sigC
furA
alr1690
P furA
P alr1690

Table 1-Deoxyoligonucleotides used in this work

| Primer | Primer sequences (5´-3´) | Description | 5´ Position relative to Fur ORF |
|---|---|---|---|
| MFurN (1) | GTCGATCGCCC**ATG**GCTGCCTAC | Forward primer *fur* gene *Microcystis* | - 11 |
| MFurC (2) | CAGTTGGGAATTCCCGCTTAGATG | Reverse primer *fur* gene *Microcystis* | 553 |
| Mintfw (3) | CGACGATTTACCGCAGTG | Forward primer internal region *fur* gene *Microcystis* | 176 |
| Mintrev (4) | CACACTGTTTGAGACTGTG | Reverse primer internal region *fur* gene *Microcystis* | 351 |
| NdnaJ (5) | CGGGGAATTTGGAGGATTTC | Reverse primer N-terminal region of *dnaJ* gene | -140 |
| pFurN (6) | CTCTTGAGCGTAGTGCCATC | Internal *dnaJ* gene and forward primers for cloning *fur* promoter | -381 |
| FurSyneRev (7) | CATGTCCTACACCGCCGATTC | Forward primer *fur* gene *Syneccocystis* | -1 |
| FurSyneInt (8) | CAGACGATGGTGTAACTCCTC | Reverse primer *fur* gene *Syneccocystis* | -144 |
| FurSyneFor (9) | CCTAGGCCAAGGAAATACTGC | Internal primer *fur* gene *Syneccocystis* | 503 |
| ATS5 (10) | CGGGAATACCGAGTAATTGG | Forward primer for cloning α-*fur* promoter region | 451 |
| ASufE (11) | CTCGCAATATGCTCCCGTCTAATCTC | Reverse primer for cloning α-*fur* promoter region | 961 |
| ATS5-Cy5 (12) | CGGGAATACCGAGTAATTGG-Cy5 | Forward primer for *α-fur* primer extension | 451 |
| Tsp fur (13) | CTGAGATGATTGCCTTTCGG | Reverse primer for cloning *fur* promoter region | 102 |
| Tsp fur-Cy5 (14) | CTGAGATGATTGCCTTTCGG-Cy5 | Reverse primer for *fur* primer extension | 102 |