Iron stress and genetic response in cyanobacteria : *fur* genes from *Synechococcus* PCC 7942 and *Anabaena* PCC 7120

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Fur (Ferric uptake regulator) is a DNA-binding protein which represses the transcription of iron-regulated promoters using Fe²⁺ as a cofactor. In Gram negative bacteria, *fur* controls the expression of toxins and virulence factors when facing iron-stress. Iron is a key element limiting the growth and proliferation of phytoplankton. We have cloned the *fur*-gene from the cyanobacterium *Synechococcus* PCC 7942, applying the polymerase chain reaction (PCR) technique. The DNA fragments amplified encodes a Fur-type protein, which conserves the main motifs found in the family of Fur proteins. Recombinant Fur protein was successfully overproduced, reaching about 15 % of the total protein content. Also, a Fur protein was identified in the filamentous, nitrogen-fixing cyanobacterium *Anabaena* PCC 7120.

Réponse génétique au stress ferrique chez les Cyanobactéries : les gènes *fur* de *Synechococcus* PC 7942 et *Anabaena* PCC 7120

Mots-clés : ferric uptake regulator (*fur*), fer, cyanobactérie.

Fur (*Ferric uptake regulator*) est une protéine d'union à l'ADN qui réprime la transcription des promoteurs régulés par le fer, utilisé sous forme Fe²⁺ comme cofacteur. Chez les bactéries Gram négatif, *fur* contrôle l'expression des toxines et des facteurs de virulence lors de stress ferrique. Le fer est un élément fondamental qui limite la croissance et la prolifération du phytoplancton. Nous avons cloné le gène *fur* de la cyanobactérie *Synechococcus* PCC 7942, en appliquant la technique de la réaction en chaîne de la polymérase (PCR). Le fragment d'ADN exprime une protéine de la famille de Fur qui conserve les motifs principaux trouvés dans cette famille. La protéine Fur de *Synechococcus* a été produite avec succès, avec un rendement important de l'ordre de 15 % du contenu total des protéines. De plus, une autre protéine Fur a été identifiée dans une cyanobactérie filamenteuse fixative d'azote : *Anabaena* PCC 7120.

1. Introduction

Iron is the most important micronutrient used by microorganisms. Living beings have selected iron to act as a cofactor in a large number of enzymes and achieve several important biological processes (Crichton 1991), such as respiration, photosynthesis, dinitrogen

fixation or ribonucleotide synthesis. The unique suitability of iron comes from the extreme variability of the Fe³⁺ / Fe²⁺ redox potential which can be finely tuned by well-chosen ligands.

Although iron is one of the most abundant elements in Earth, it is not readily accessible to bacteria (Lewin 1984). Poor solubility in aqueous media and easy oxidation in aerobic atmospheres lead to the formation of insoluble hydroxides, which bacteria are unable to acquire. Its availability for microbial uptake is an important variable in determining the stability and composition of aquatic ecosystems.

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However, an excess of iron is toxic because of its ability to catalyse formation of reactive species of oxygen (Halliwell & Gutteridge 1984). Iron uptake has to be, therefore, exquisitely regulated to maintain the intracellular concentration of the metal between desirable limits. Considering that excretion mechanisms for iron are not known in bacteria, microorganisms appear to control iron homeostasis by regulating its transport through the membrane (Bagg & Neilands 1987).

Iron limitation has profound consequences for all microbial organisms so far identified on our planet. The limitation of soluble, biologically useful iron in aerobic environments, where the bulk of life resides on Earth, is as much a dilemma to planktonic microorganisms trying to sustain themselves in vast areas of the South Pacific (Behrenfeld & Kolber 1999). Species with stronger iron uptake systems would be able to preferentially use the available iron, as happens when microbial pathogens attempt to initiate and sustain commensal or pathological relationships with other organisms (Crosa 1997). The lack of iron is a major environmental signal that triggers expression of virulence determinants (Litwin & Calderwood 1993).

Iron availability and biolimitation by iron have been an important focus of discussion for oceanographers and limnologists. After the IronEx II (Coale et al. 1996, Behrenfeld et al. 1996; Kirchman 1996) it was established that deprivation of iron unequivocally limits cell division rates, abundance and production rate in phytoplankton (Frost 1996). Moreover, iron nutritional status of phytoplankton is of great interest, while toxin production (Lyck et al. 1996) or undesirable blooms of cyanobacteria may be related to iron availability (Behrenfeld et al. 1996).

Cyanobacteria species have developed several mechanisms to avoid iron stress. They are able to produce powerful iron-binding compounds (siderophores) (Crosa 1989, Neilands 1989). Siderophore chelators can suppress growth of competing species in lake waters (Murphy et al. 1976). Cyanobacteria can also synthesize new proteins which substitute those proteins requiring iron to develop their function (flavodoxin-ferredoxin system, which has been used as a molecular marker of iron-stress) (Sandmann et al. 1990).

At a molecular level, cells must sense intracellular concentration of free iron and couple it to gene expression. The key element in this response, for cyanobacteria and Gram-negative bacteria, is a DNA-binding metaloprotein called Fur (Ferric uptake regulator). This protein, encoded by the *fur* gene, is able to repress the transcription of iron-regulated promoters in response to an increasing intracellular Fe^{2+} concentration. It has

been found that Fur binds to control sequences (*fur* boxes) (Escobar et al. 2000) overlapping Fur-regulated promoters when the intracellular concentration is high enough to allow the formation of a complex consisting of a Fur dimer and Fe^{2+} .

In cyanobacteria, Fur protein is the key element controlling expression of a number of Fur-regulated genes, such as siderophore biosynthesis enzymes or flavodoxin protein. However, the interest in Fur is not only the participation in most iron-dependent cellular systems. Also crucial functions have been found to be related with oxidative stress (Nunoshiba et al. 1999), acidic stress (Hall & Foster 1996) or toxin production in heterotrophic bacteria (Somerville et al. 1999).

Fur protein was first identified in *Salmonella typhimurium* (Ernst et al. 1978) and has been most extensively characterized in *Escherichia coli*. Fur homologues have been found in several other Gram negative bacteria, including some important human pathogens like *Pseudomonas* (Prince et al. 1993), *Vibrio* (Litwin et al. 1992), *Neisseria* (Thomas & Sparling 1994), *Yersinia* (Staggs & Perry 1991), *Helicobacter pylori* (Bereswill et al. 1998), *Bordetella* (Brickman & Armstrong 1995), *Campylobacter* (Wooldridge et al. 1994), *Acinetobacter baumannii* (Daniel et al. 1999), *Legionella* (Hinkey & Cianciotto 1994) or *Haemophilus* (Carson et al. 1996). However, Fur has not been deeply investigated in cyanobacteria, having only been described in the unicellular cyanobacteria *Synechocystis* PCC 6803 (Kaneko et al. 1996) and *Synechococcus* PCC 7942 (Ghassemian & Straus 1996), which are unable to fix nitrogen.

Our purpose is to characterise the product of the *fur* gene in cyanobacteria, since the Fur protein might be the key element controlling the expression of genes involved in cyanotoxin production, secondary metabolites produced by cyanobacteria which have hepatotoxic or neurotoxic effects on different species. Fur might be involved as well in the control of the occurrence of cyanobacterial blooms depending of the environmental iron level. With this purpose, we have amplified and overexpressed the Fur protein from the unicellular cyanobacterium *Synechococcus* PCC 7942. Moreover, a *fur* gene has also been identified in the filamentous nitrogen fixing cyanobacterium *Anabaena* sp. The high level of the expression system, about 20 % of the total protein content of the recombinant *E. coli* strain was Fur from *Synechococcus*, makes it very suitable for functional and structural studies of this protein whose three dimensional structure remains still unsolved.

2. Material and methods

2.1. Bacterial strains, plasmids and culture media

Genotypes and sources of the bacterial strains and plasmids used in this study are described in Table 1.

Synechococcus PCC 7942 was grown in BG11 medium liquid culture (Rippka et al. 1979).

2.2. PCR amplification and cloning of *Synechococcus fur* gene

The *fur* gene from *Synechococcus* was amplified by means of the polymerase chain reaction (PCR) using a Perkin Elmer Cetus DNA Thermal Cycler. Chromosomal DNA from *Synechococcus* PCC 7942 was isolated according to Cai & Wolk (1990), and used as a template for PCR reaction.

Synthetic oligonucleotides, designed according to the *fur* sequence reported by Ghassemian & Straus (1996), were used to generate double-stranded DNA in addition to restriction enzyme sites for cloning. The forward primer 5'- GACTCGC-CATGGCCTACACAGCG-3' carried a *NcoI* restriction site. The reverse primer 5'-GCGGAAGCTTCG-CTAGCTGCACC-3' carried a *HindIII* restriction site. The reaction mix contained 0.5 µM of each sense and antisense primer, 200 µM of each dNTP, 1.5 mM MgCl₂ and 2.5 U of Taq DNA. Amplification was carried out as follows : denaturation at 95°C for 3 min., then 30 cycles at 93°C (0.5 min.) for denaturation, annealing at 65°C (1 min.) and extension at 72°C (1 min.). A final extension step at 72°C was performed for 10 min.

Table 1. Bacterial strains and plasmids.
Tableau 1. Souches bactériennes et plasmides.

Bacterial strains and plasmids	Relevant characteristics	Source or reference
Strains		
<u>E.coli</u>		
JM109	<u>recA1</u> , <u>endA1</u> , <u>gyrA96</u> , <u>thi</u> , <u>hsdR17</u> (r _k ⁻ , m _k ⁺), <u>reiA1</u> , <u>supE44</u> , Δ(<u>lac-proAB</u>), [F', <u>traD36</u> , <u>proAB</u> , <u>lacI9ZAM15</u>]	Messing et al. 1981
BL21-DE3 strain	F' <u>dcm omp</u> T <u>hsdS</u> (r _B ⁻ m _B ⁻) <u>gal</u> , λ(DE3)	Stratagene
<u>Synechococcus</u>		
7942	<u>Synechococcus</u> sp. strain PCC7942	
Plasmids		
pGEM-T Easy	Cloning vector with single 3'-T overhanging at the insertion site. It is flanked by recognition sites for the restriction enzymes EcoRI, BsfZI and NotI	Promega
pET-28a(+)	Expression vector carrying an N- terminal His•Tag/thrombin/T7•Taq configuration plus an optional C-terminal His•Taq sequence	Novagen

2.3. Sequencing of the DNA fragment

The PCR product (a single band in agarose gels) was cloned, without further purification, into the pGEM®-T Easy Vector System (Promega, Madison, WI). *E. coli* JM109 cells were transformed with these constructs. Sequencing in both directions was performed, using the Thermo Sequenase fluorescent labelled primer cycle sequencing kit (Amersham Pharmacia Biotech, UK) and an automated ALFexpress™ DNA sequencer (Amersham Pharmacia Biotech, UK). Synthetic oligonucleotides used as primers for PCR and DNA sequencing were purchased from Pharmacia (Sweden).

2.4. Construction of the *Synechococcus fur* gene expression vector

The pGEM®-T Easy/insert construct was digested with the restriction enzymes *NcoI* and *HindIII* to generate cohesive ends, and the digestion products separated in a 2 % agarose gel. The band containing the *fur*-gene was purified (see Bes et al. 2000) and this DNA was cloned into the *NcoI* and *HindIII* cloning sites of the expression vector pET-28a(+) using T4-DNA ligase. *E. coli* BL21-DE3 cells were transformed with the construct pET-28a(+)/*fur*, and colonies were selected in Luria Bertani plates containing kanamycin (30 µg/ml). Automatic DNA sequencing was performed to confirm the sequence.

2.5. Analysis of expressed Fur protein

Several colonies containing the pET-28a(+)/*fur* plasmid were grown overnight in Luria Bertani medium supplemented with 30 mg/ml of kanamycin. After 14 hours of incubation with shaking at 37°C, cultures were diluted 70-fold in Luria Bertani medium containing 30 mg/ml of kanamycin. After 3 hours growth at 37°C, when the absorbance at 600 nm. had reached a value of 0.7-0.9, isopropyl β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM. In control cultures, IPTG was omitted. Cells were allowed to grow at 37°C, harvesting tubes 2 hours later. The level of expression was monitored by evaluating total protein content of different samples by electrophoresis on denaturing SDS-PAGE gels (Laemmli 1970) and further analysis with a Gel Doc 2000 image analyser from Bio-Rad.

3. Results

3.1. Amplification and cloning of the *fur* gene from *Synechococcus* PCC 7942

The *Synechococcus fur*-gene has already been cloned, by screening in a DNA library (Ghassemian &

Straus 1996). In our work, we applied a different procedure, using PCR. Chosen oligonucleotides correspond to a sequence complementary to the beginning and to the end of *Synechococcus fur*-gene, modified to include *NcoI* and *HindIII* restriction sites in 5' and 3', respectively (Material and Methods).

PCR reactions were performed with this oligonucleotides, using total DNA from *Synechococcus* PCC 7942 as template. In this way, several PCR products were obtained, one of them showing similar weight than that of a putative *fur*-gene, approximately 450 pb. This PCR product was purified, cloned in the pGEM-T Easy plasmid and sequenced. The sequence was compared with *fur*-gene from *Synechococcus* PCC 7942, deposited in the GenBank database, and showed complete identity, except in one nucleotide. However, this did not imply any change in the protein sequence (Fig. 1).

3.2. Identification of a *fur* homologue from *Anabaena* PCC 7120

In order to detect the presence of a Fur-like protein in *Anabaena* PCC 7120, we took into account the fact that for all microorganisms the described Fur proteins share regions fully conserved. This is the case of the HHXHXXCXXC motif (Hennecke 1990), a consensus sequence indispensable for the binding of the metal ion which activates Fur in order to interact with the DNA operator regions. This is an infrequent amino acid sequence which is not found in other protein families, probably because of the presence of several bulky amino acids consecutively placed. Using the computer sequence searching tools present in the CyanoBase, we searched for any sequence encoding the histidine motif characteristic of Fur proteins. We localized only one region in the complete genomic sequence of *Anabaena* PCC 7120 where this motif was contained in an open reading frame that presented all the characteristics of a *fur* gene. It is shown in the figure 2, together with the deduced protein sequence.

3.3. Sequence analysis of the identified *fur* genes

The isolated *fur* gene from *Synechococcus* PCC 7942 was an open reading frame with only one nucleotide differing with the already reported sequence, but coding for the same 147 amino acid protein.

The sequence selected in the CyanoBase coding a *fur*-like gene from *Anabaena* PCC 7119 was an open reading frame coding for a sequence of 151 amino acids that was homologous to several Fur proteins and contained the putative iron-binding domain typical of Fur repressors family reported up to date, indicating

1	ATG	ACC	TAC	ACA	GCG	GCC	TCC	CTC	AAA	GCG	GAA	TTG	AAC	GAG	CGA	45
1	M	T	Y	T	A	A	S	L	K	A	E	L	N	E	R	15
46	GGC	TGG	CGA	TTG	ACG	CCT	CAG	CGC	GAA	GAA	ATT	CTG	CGA	GTC	TTT	90
16	G	W	R	L	T	P	Q	R	E	E	I	L	R	V	F	30
91	CAA	AAT	CTC	CCA	GCA	GGT	GAA	CAC	CTC	AGT	GCT	GAG	GAC	CTT	TAC	135
31	Q	N	L	P	A	G	E	H	L	S	A	E	D	L	Y	45
136	AAT	CAT	TTG	CTG	AGT	CGC	AAT	TCA	CCC	ATC	AGC	CTC	TCG	ACT	ATC	180
46	N	H	L	L	S	R	N	S	P	I	S	L	S	T	I	60
181	TAT	CGC	ACC	CTG	AAG	CTA	ATG	GCT	CGC	ATG	GGT	CTC	CTG	CGA	GAG	225
61	Y	R	T	L	K	L	M	A	R	M	G	L	L	R	E	75
226	CTA	GAT	CTG	GCC	GAA	GAT	CAC	AAG	CAC	TAC	GAG	CTC	AAT	CAA	CCC	270
76	L	D	L	A	E	D	H	K	H	Y	E	L	N	Q	P	90
271	CTG	AAG	CAT	CAC	CAT	CAC	TTG	ATC	TGC	GTC	AGT	TGC	AGC	AAG	ACG	315
91	L	K	H	H	H	H	L	I	C	V	S	C	S	K	T	105
316	ATT	GAG	TTC	AAG	AGC	GAT	TCC	GTG	CTC	AAA	ATT	GGC	GCC	AAG	ACC	360
106	I	E	F	K	S	D	S	V	L	K	I	G	A	K	T	120
361	TCC	GAG	AAG	GAG	GGC	TAC	CAC	CTC	TTG	GAT	TGT	CAG	CTC	ACC	ATT	405
121	S	E	K	E	G	Y	H	L	L	D	C	Q	L	T	I	135
406	CAT	GGC	GTC	TGC	CCA	ACC	TGT	CAG	CGA	TCG	CTG	GTT	TAG			444
136	H	G	V	C	P	T	C	Q	R	S	L	V	STOP			

Fig. 1. Nucleotide sequence of *Synechococcus* PCC 7942 *fur* gene. The deduced amino acid sequence is shown below the nucleotide sequence in one-letter code. The nucleotides corresponding to the translational start site and stop site are in bold letters and underlined. The only nucleotide differing from the reported sequence is underlined.

Fig. 1. Séquence des nucléotides du gène *fur* de *Synechococcus* PCC 7942. La séquence déduite des acides aminés figure en dessous de la séquence des nucléotides, avec un code d'une lettre. Les nucléotides correspondant au début et à la fin de la traduction apparaissent en gras et soulignés. Le seul nucléotide qui diffère de la séquence existante est souligné.

that it was likely to encode a Fur protein. Fig. 2 shows the translated amino acid sequence encoded by the open reading frame from *Anabaena*, which was analyzed for homologous proteins in the SwissProt data base. Optimal alignment (Fig. 3) with the sequences of the two cyanobacterial Fur proteins available in the database shows the highest homology, 73 %, with the protein from *Synechococcus*, and a 66 % homology with Fur from *Synechocystis*. Much lower homology was found with Fur from *E.coli* (30 %). This degree of dissimilarity can explain its lack of activity in Western blots treated with antiserum raised against Fur from *E.coli* (Figure 4B).

3.4. Expression of Fur from *Synechococcus* in *E.coli*

From the several expression systems that are available to overproduce foreign proteins in *E.coli*, the pET-28a(+) vector was the most efficient. This system is based on the strong promoter for RNA polymerase of the T7 phage (Novagen). When BL21-DE3 (Gold) *E.coli* cells transformed with the expression plasmid containing the *fur*-gene were induced to overexpress the gene, an increase in the level of synthesis of one of the proteins was observed at growing time intervals by SDS-PAGE.

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1   ATG GCT GTC TAC ACA AAT ACT TCG CTC AAG GCA GAA TTA AAC GAA 45
1   M  A  V  Y  T  N  T  S  L  K  A  E  L  N  E 15

46  AGA GGC TGG CGT TTG ACC CCT CAA CGG GAA ACT ATC TTA CAT ATC 90
16  R  G  W  R  L  T  P  Q  R  E  T  I  L  H  I 30

91  TTT CAA GAA CTT CCT CAA GGC GAA CAC CTG AGT GCA GAA GAT TTA 135
31  F  Q  E  L  P  Q  G  E  H  L  S  A  E  D  L 45

136 TAT CAT CGC TTG GAA GCT GAC GGT GAA GGA ATC AGC CTT TCA ACG 180
46  Y  H  R  L  E  A  D  G  E  G  I  S  L  S  T 60

181 ATT TAC CGG ACG TTG AAG TTG ATG GCT CGG ATG GGG ATT TTA CGT 225
61  I  Y  R  T  L  K  L  M  A  R  M  G  I  L  R 75

226 GAA CTG GAA TTA GGC GAA GGA CAC AAG CAC TAC GAA ATT AAC CAG 270
76  E  L  E  L  G  E  G  H  K  H  Y  E  I  N  Q 90

271 CCT TAT CCC CAT CAT CAC CAT CAC CTA ATT TGT GTC AAA TGT AAC 315
91  P  Y  P  H  H  H  H  H  L  I  C  V  K  C  N 105

316 TCT ACT ATT GAA TTC AAA AAC GAT TCA ATC TTA AAA ATT GGG GCA 360
106 S  T  I  E  F  K  N  D  S  I  L  K  I  G  A 120

361 AAA ACT GCC CAG AAA GAA GGT TTT CAC CTA CTG GAC TGC CAA ATG 405
121 K  T  A  Q  K  E  G  F  H  L  L  D  C  Q  M 135

406 ACA ATT CAT GCA GTC TGC CCC AAG TGC CAA CGT GCG CTC ATG CCA 450
136 T  I  H  A  V  C  P  K  C  Q  R  A  L  M  P 150

451 CTT TAG 456
151 L  STOP

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Fig. 2. Nucleotide sequence of *Anabaena* PCC 7120 *fur* gene. The deduced amino acid sequence is shown below the nucleotide sequence in one-letter code. The nucleotides corresponding to the translational start site and stop site are in bold letters and underlined.

Fig. 2. Séquence des nucléotides du gène *fur* d'*Anabaena* PCC 7120. La séquence déduite des acides aminés figure en dessous de la séquence des nucléotides, avec un code d'une lettre. Les nucléotides correspondant au début et à la fin de la traduction apparaissent en gras et soulignés.

Alignment of several Fur proteins : CLUSTAL W (1.8) multiple sequence alignment

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Anabaena PCC7119      MTVYTNTSLKAELNERGWRLTPQRETILHIFQELPQGEHLSAEDLYHRLEADGEGISLST 60
Synechococcus PCC7942 -MTYTAASLKAELNERGWRLTPQREEILRVFQNLPAEHLAEDLYNHLLSRNSPISLST 59
Synechocystis PCC6803 -MSYTADSLKAELNARGWRLTPQREKILTIFQNLPEGEHLSAEELHHRLEEEEREKISLST 59
E.coli                -----MTDNNTALKKAGLKVTLPRLKILEVLQ--EPDNHHSVSAEDLYKRLIDMGEEIGLAT 54
                        .  ::  * :  *  ::*  *  **  ::*  *  ..*:****:***:  .  *.*:*

Anabaena PCC7119      IYRTLKLMARMGILRELELGEGHKHYEINQPYPHHHHLICVKCNSTIEFKNDSILKIGA 120
Synechococcus PCC7942 IYRTLKLMARMGLLRELDLAEDHKHYELNQP-LKHHHHLICVSCSKTIEFKSDSVLKIGA 118
Synechocystis PCC6803 VYRSVKLMSRMGILRELELAEGHKHYELQQASPHHHHHVVCVQCNRRTIEFKNDSILKQSL 119
E.coli                VYRVLNQFDDAGIVTRHNFEGGKSVFELTQQ--HHHDHLICLDGKVIIEFSDDSIEARQR 112
                        :**  ::  :  *::  .  ::  .  .  :*:  *  :*:***:***:..  ****.*:*

Anabaena PCC7119      KTAQKEGFHLLDCQMTIHAVCPKCQRALMPL----- 151
Synechococcus PCC7942 KTSEKEGYHLLDCQLTIHGVCPTCQRLV----- 147
Synechocystis PCC6803 KQCEKEGFQLIDCQLTVTTICPEAIRMGWPSTLPSNWACTRSISLA 165
E.coli                EIAAKHGIRLTNHSLYLYGHCAEGDCREDEHAHEGK----- 148
                        :  .  *.*  :*  :  .  :  :  *

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Fig. 3. Multiple sequence alignment. Sequence alignment was obtained by using the Clustal W (version 1.8) program.

Fig. 3. Alignement multiple des séquences. L'alignement a été effectué à l'aide du programme Clustal W (version 1.8).

In Figure 4A, the overexpression of the Fur protein from *Synechococcus* is presented. A new band with a mobility of 15.4 kDa was found after 2 hours from induction, even though some residual expression was present in absence of IPTG. Densitometric scans indicate that Fur represents at least 20 % of the total protein content in the host cells.

The mobility of the putative Fur band, about 17.4 kDa, is fairly coincident with the theoretical molecular weight of 16.8 kDa for the *Synechococcus* protein.

4. Discussion

Iron availability has been thought as the limitant element for microorganisms growth. After the IronEx II (Coale et al. 1996, Behrenfeld et al. 1996, Kircham 1996), this hypothesis was definitively established : iron unequivocally limits cell division rates, abundance and production rate in phytoplankton (Frost 1996). This fact has lead to the use of remarkably sophisticated mechanisms to scavenge iron from the sources in the environment where microorganisms reside.

Cyanobacteria are one of the most ancient groups of bacteria living in Earth. Throughout their evolution, they have developed a large number of systems to take advantage in competitive media, such as dinitrogen fixation, high ability to retain phosphorus or CO₂, gas vesicle to regulate their flotation, formation of high resistance forms (akinetes) or the existence of exceptionally developed iron-uptake systems. Altogether, these factors make cyanobacteria successful in the use of en-

vironmental nutrients, made clear by the appearance of cyanobacterial blooms. In fact, sudden dominance of cyanobacteria in eutrophic lakes coincided with the production of strong iron chelators and the rapid uptake of iron (Murphy et al. 1976).

Cyanotoxin production can also be related to iron availability. It has been shown (Utkilen & Gjølme 1995) that toxin synthesis increases together with iron concentration, suggesting that toxins could act as an intracellular chelator which keeps the cellular level of free iron low. However, an opposite relationship between toxin production and iron concentration has also been reported (Lukac & Aegerter 1993). Whether cyanotoxin production can also be considered as another cyanobacterial response to ferric-stress, in order to eliminate competitors and enrich iron content in the medium, remains to be investigated. In this way, Fur might play a key role in sensing intracellular iron contents and regulating expression of peptide and polyketide synthetases involved in cyanotoxin production. A deep study of their promoters and regulation of gene expression should be performed to a complete understanding of toxin function in cyanobacteria.

In this work, we report the cloning and overexpression of the Fur protein from unicellular cyanobacterium *Synechococcus* PCC 7942. Moreover, a sequence highly homologous to the *fur*-gene has also been found in the genome of the nitrogen-fixing *Anabaena* PCC 7119 (filaments former). Their sequences show significant homology with all the Fur sequences known in

Fig. 4. SDS-PAGE and Western-blot analysis of the expression and cross-reactivity of Fur from *Synechococcus* PCC 7942.

A : SDS-PAGE in a 20 % polyacrilamide gel, showing overexpression of the protein collected at different times. Lanes 1, 4 and 6 : cells with plasmid carrying the *fur* gene collected at 0 min. Lanes 2, 5 and 7 : cells with plasmid carrying the *fur* gene collected 120 min. after induction. Lane 3, molecular weight markers (in Kda.) 94, 67, 43, 30, 20.1 and 14.4.

B: Western-blot of the same gel as in figure 4A, transferred to a PVDF membrane and developed with antibodies raised against Fur from *E.coli*. A band from *E.coli* Fur appears in all lanes, but no band corresponding to the Fur protein from *Synechococcus* was seen, showing that there is no cross-reaction of anti-Fur from *E.coli* with Fur from *Synechococcus*.

Fig. 4. Résultats des analyses du SDS-PAGE et du Western Blot, quant à l'expression et la réactivité croisée de la protéine Fur de *Synechococcus*.

A : Le test SDS-PAGE a été réalisé dans un gel de 20 % de polyacrilamide, montrant la protéine produite collectée à différents intervalles de temps. Pistes 1, 4 et 6 : les cellules avec le plasmide et le gène *fur* collectés au temps 0. Pistes 2, 5 et 7 : les cellules avec le plasmide et le gène *fur* collectés 120 min. après l'induction. Piste 3, marqueur du poids moléculaire (en Kda.) 94, 67, 43, 30, 20,1 et 14,4.

B : Western-blot du gel de la figure 4A, transféré à une membrane PVDF et révélé avec des anticorps préparés contre Fur de *E.coli*. La bande de Fur de *E.coli* apparaît dans toutes les pistes, mais il n'y a aucune bande de la protéine Fur de *Synechococcus*, ce qui montre qu'il n'y a pas eu de réaction croisée entre les anti-Fur du *E.coli* et la protéine Fur de *Synechococcus*.

cyanobacteria, and low homology with *E. coli* Fur sequence.

Future work will focus on characterization of Fur protein and further work about Fur binding with suitable gene promoters, including those involved in cyanotoxin production.

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