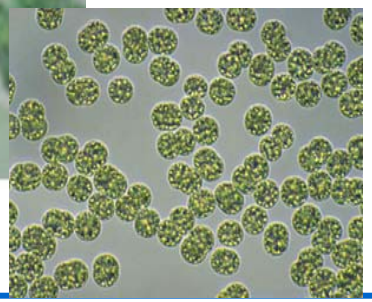
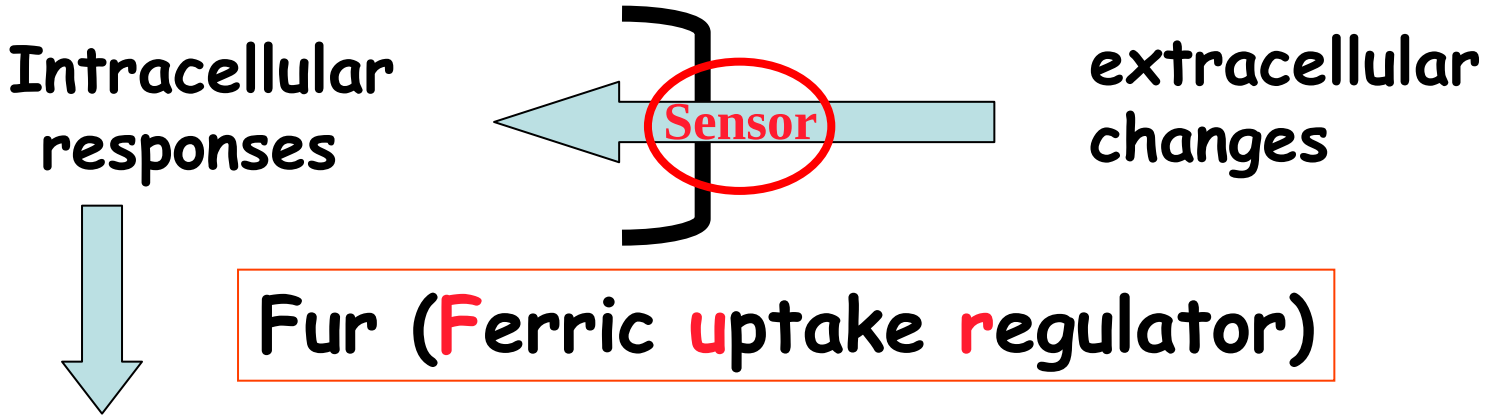


Fur (ferric uptake regulator) proteins in cyanobacteria: new roles for a master regulator

Dept. of Biochemistry and Mol. Cell Biol.
Complex Systems Physics Institute (BiFi),
University of Zaragoza, Zaragoza, Spain.

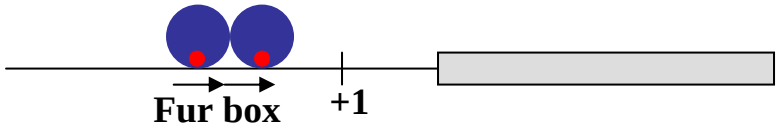
Iron deficiency < free [Fe^{2+}] < Toxic levels



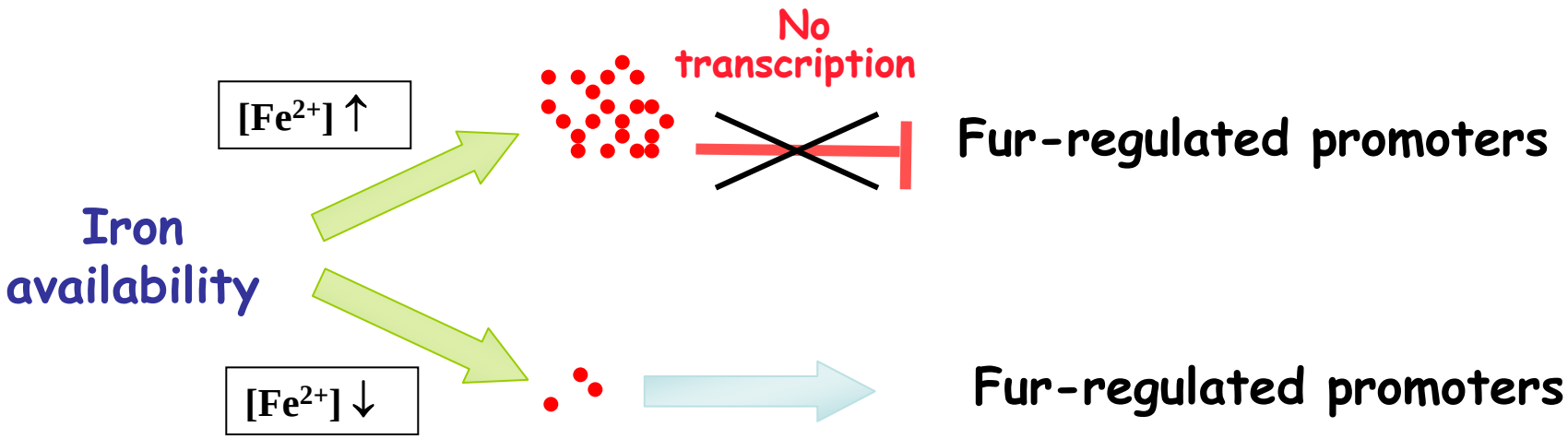
Fur (Ferric uptake regulator)

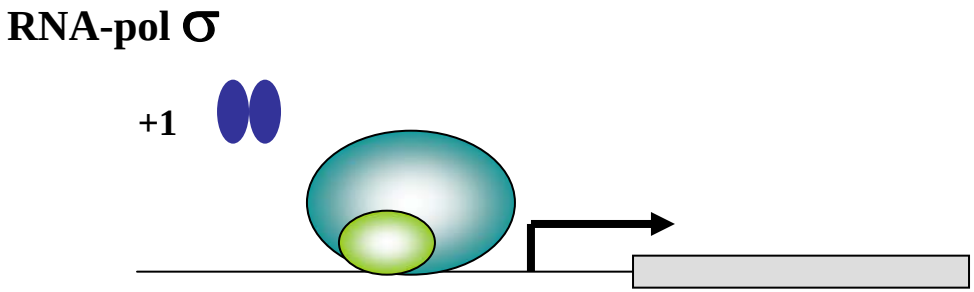
- Iron uptake and incorporation (siderophores)
- Trigger the production of virulence factors
- Acidic stress response
- Concerted response to oxidative stress
- Intermediate metabolism (aconitase, fumarase)

How does Fur work?



Active Fur repressor





Inactive Fur repressor

The Fur superfamily

Metal availability

Name	Co-repressor	Organism
Fur (*ferric uptake regulator*)	Fe^{2+}	*E. coli* *Anabaena 7120* *P. aeruginosa*
Zur (*zinc uptake regulator*)	Zn^{2+}	*M. tuberculosis* *B. subtilis*
Mur (*manganese uptake regulator*)	Mn^{2+}	*R. leguminosarum*
Nur (*nickel uptake regulator*)	Ni^{2+}	*S. coelicor*

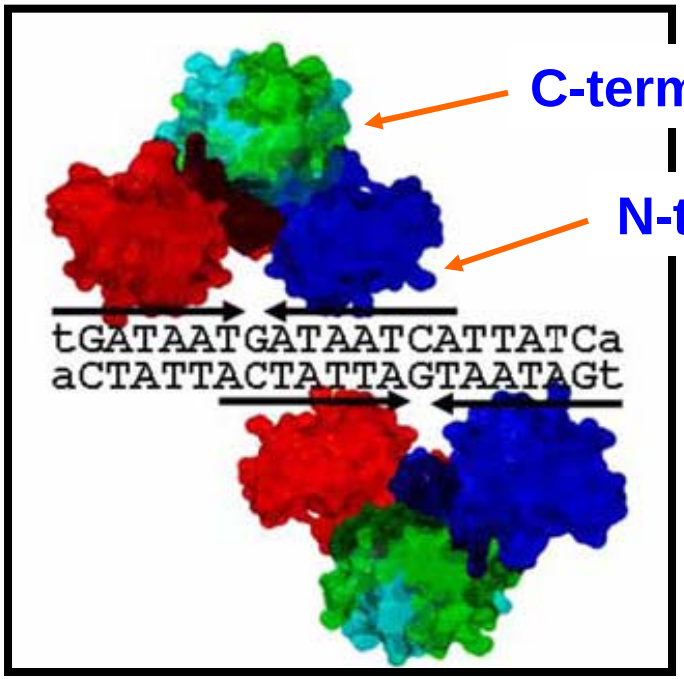
Oxidative stress

PerR (*peroxide stress response*)	Mn^{2+}/Fe^{2+}	*B. subtilis* *Synechocystis 6803*

Heme availability

Irr (*iron responsive regulator*)	Fe-hemo	*B. japonicum*

Common features of Fur proteins



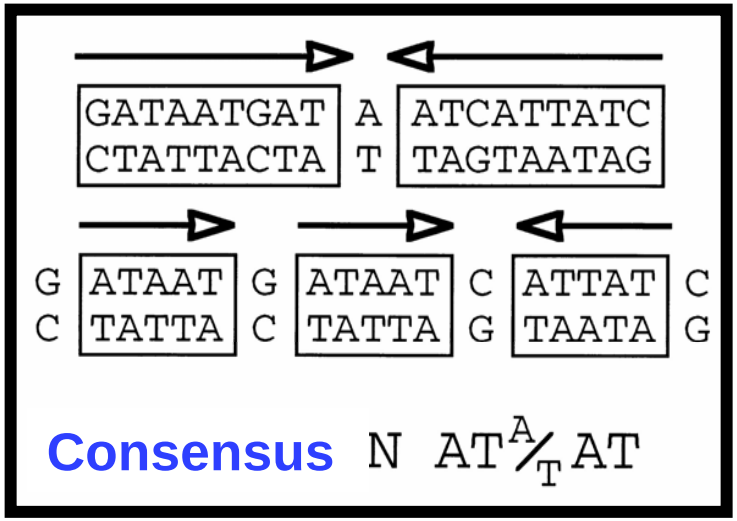
C-terminal domain: metal binding and dimerization

N-terminal domain: DNA binding

MW 13-19 kDa

pI 6-7

E. coli	HHHDHLICLDC
H. pylori	HHDHHIICLHC
S. aureus	HHHHHFICEKC
Consensus	$H_{3-5}X_2CX_2C$



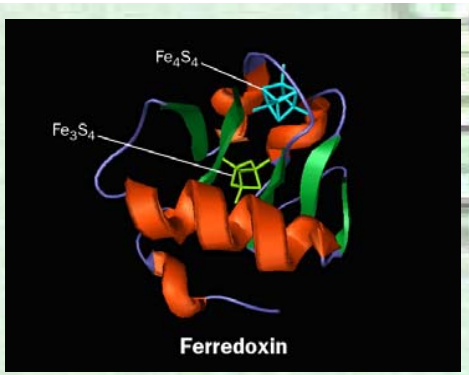
They recognize similar DNA sequences (iron boxes)

Pohl et al. (2003) Mol. Microbiol. 47:903-15

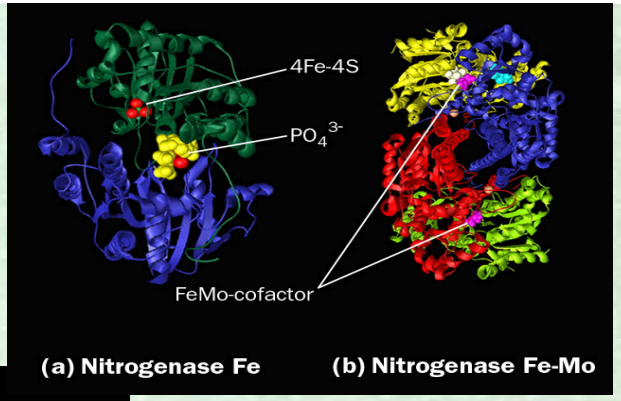
Lee *et al.* (2006) *Biometals* 20: 485-499

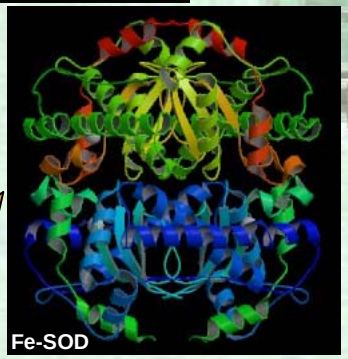
Why to study Fur Proteins in Cyanobacteria?

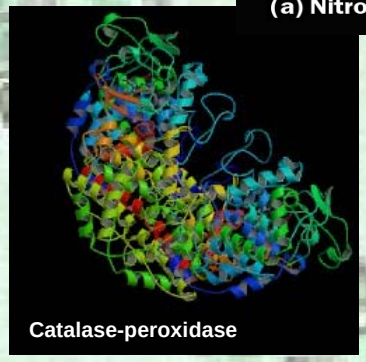
Photosynthesis



Nitrogen Metabolism



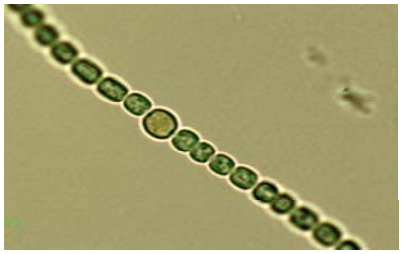




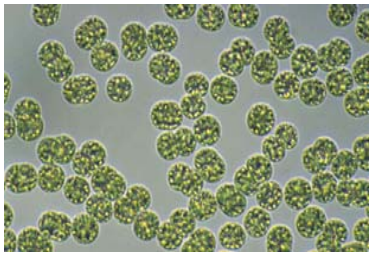
Oxidative stress defense

- **Fur is possibly involved in cyanotoxins production**

Fur Proteins in Cyanobacteria: Regulation and New Roles



Anabaena (Nostoc) **PCC 7120**



M. aeruginosa PCC 7806

- **Identification in the genome, purification, biochemical characterizacion…**

- **When and how they work?**

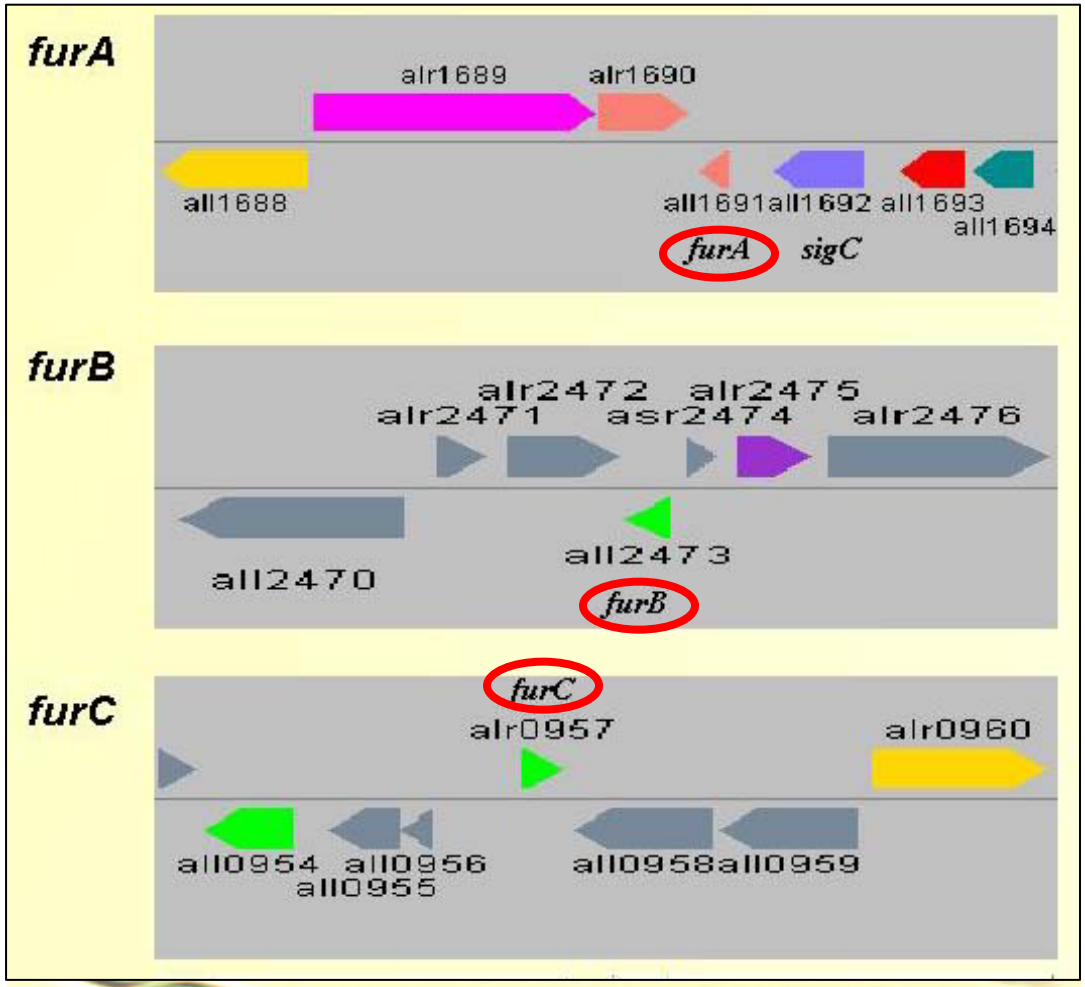
- Structure-function (mechanism)

+

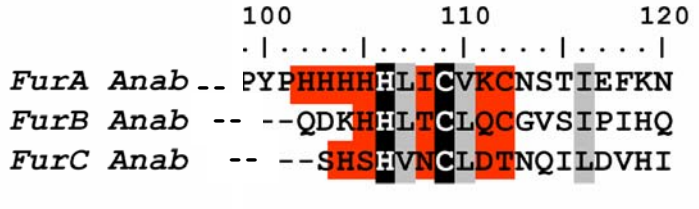
Regulation

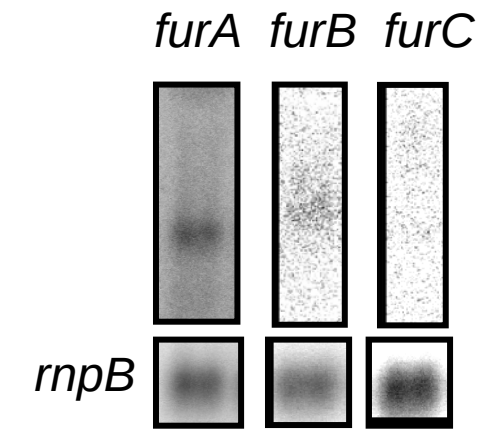
- **Which are their functions in cyanos?**

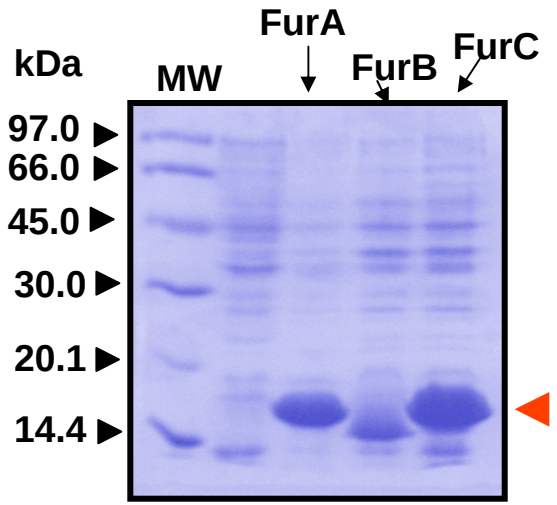
Fur proteins in *Anabaena sp.* PCC 7120



Hernández, JA et al. FEMS Letters, 2004







Characterization of FurA (*Anabaena*)

MW (IES)=17259 ± 7 Da

pI = 6.4

- Lack of structural metal { **ICP-mass** / **ESI-mass** } ⇒

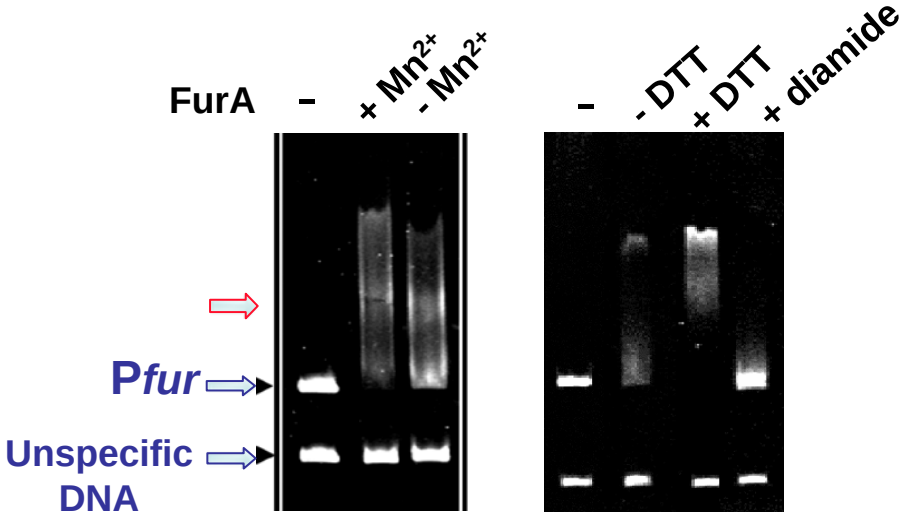
FurA *Anabaena* = 0 Zn^{2+}

Fur *E. coli* = 2 Zn^{2+} (positive control)

-Activity assays:

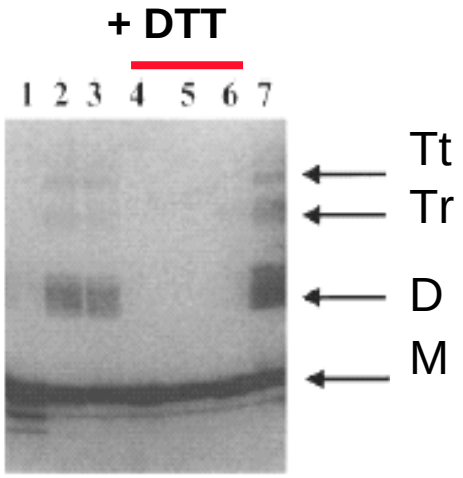
-FurA binds to specific DNA sequences

- Mn^{2+} and DTT are positive effectors



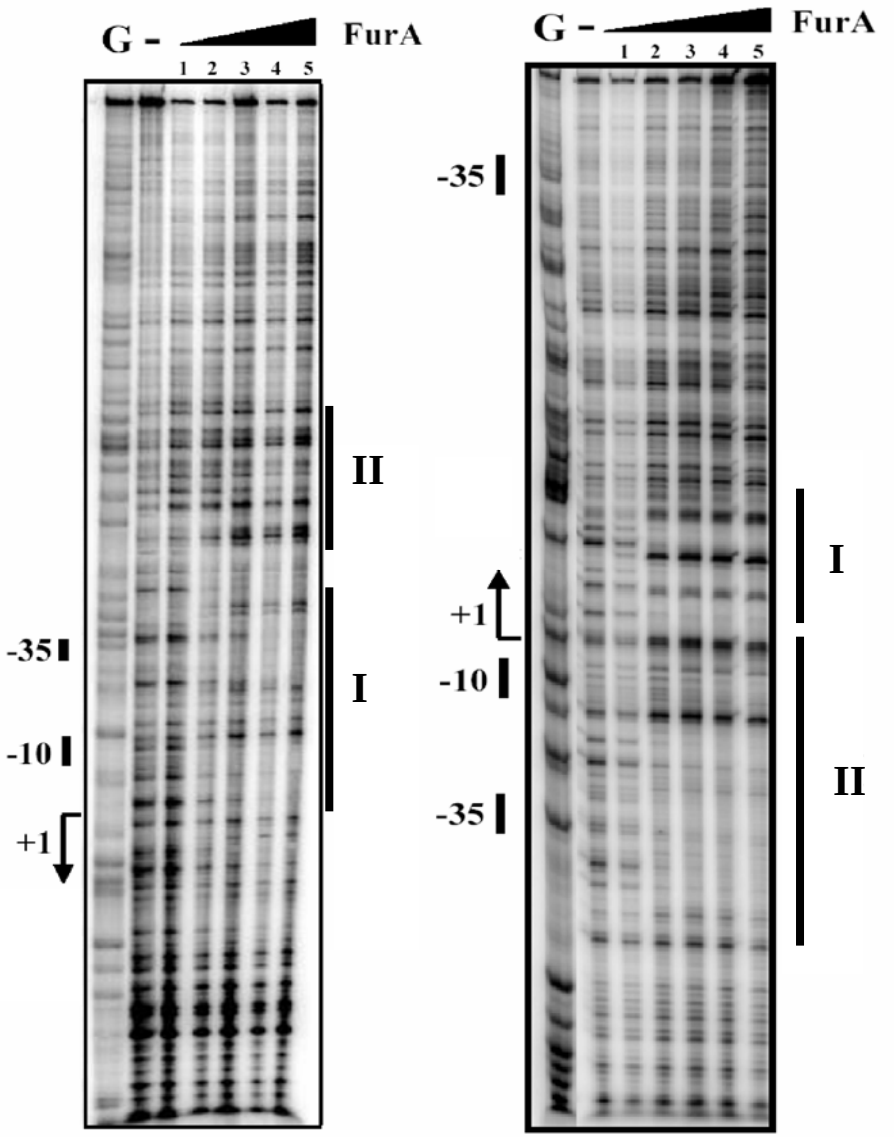
- Oligomerization ability:

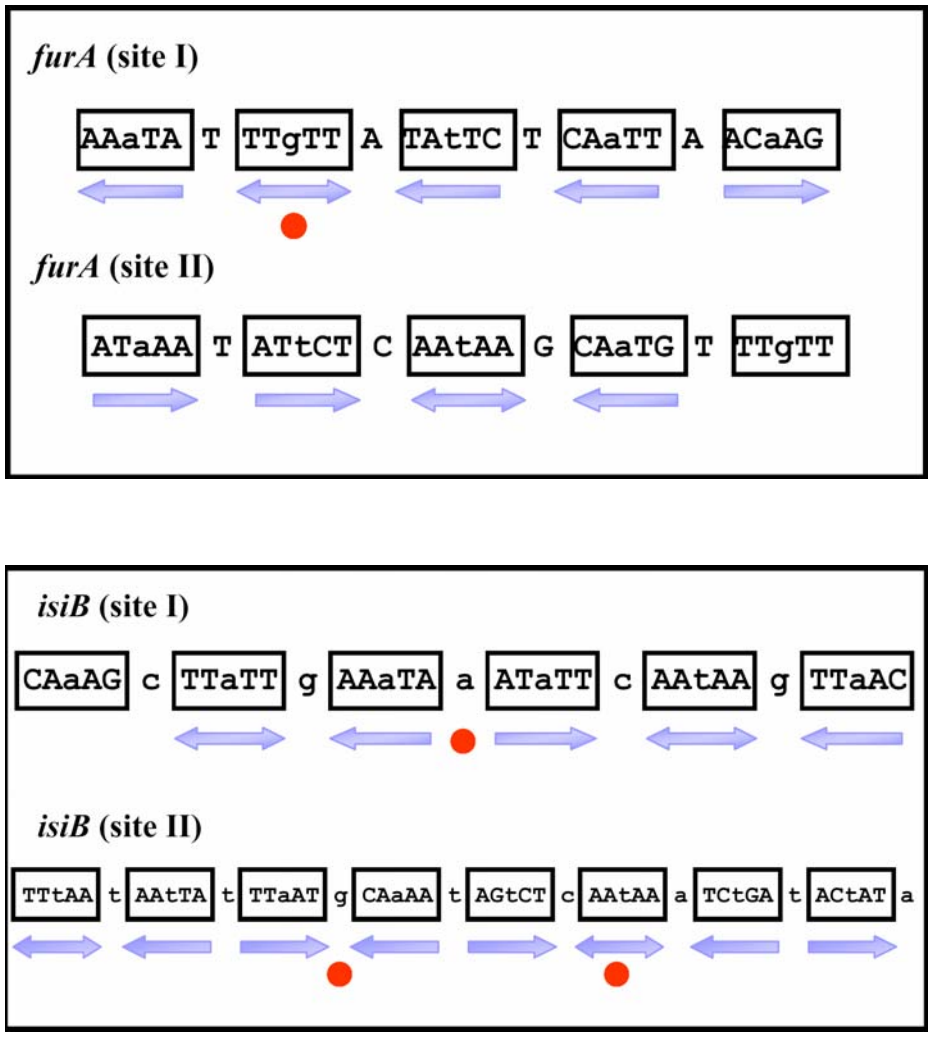
Involves S-S bridges



Hernández et al. Biochem.J., 2002

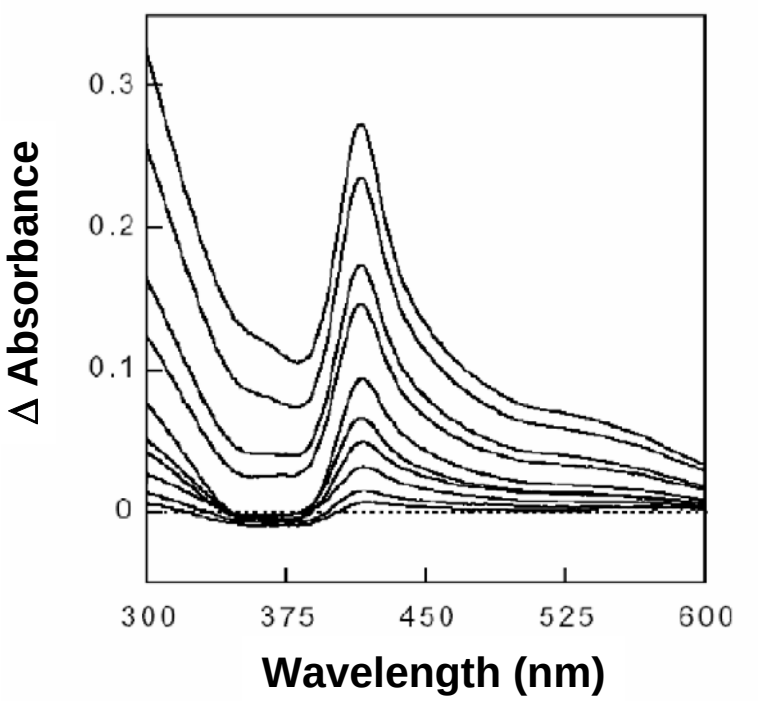
Identification of FurA-protected sites

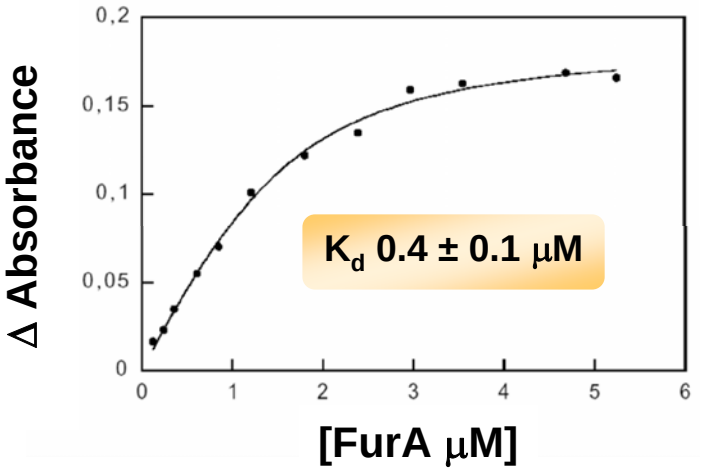


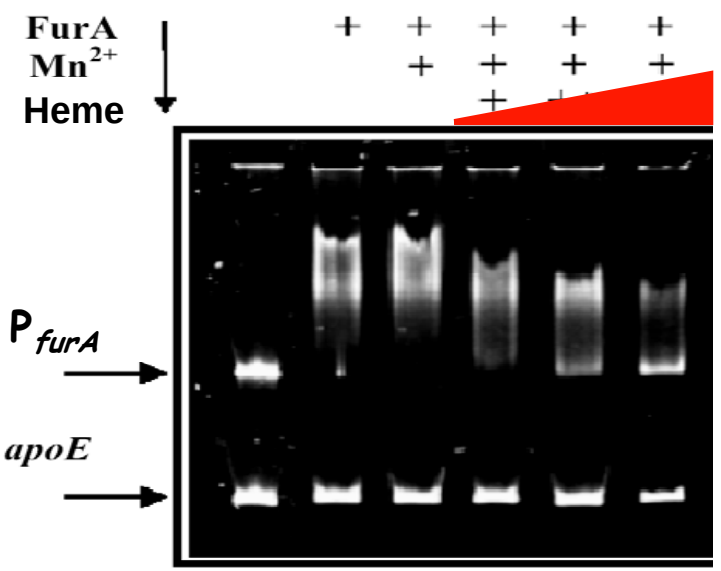


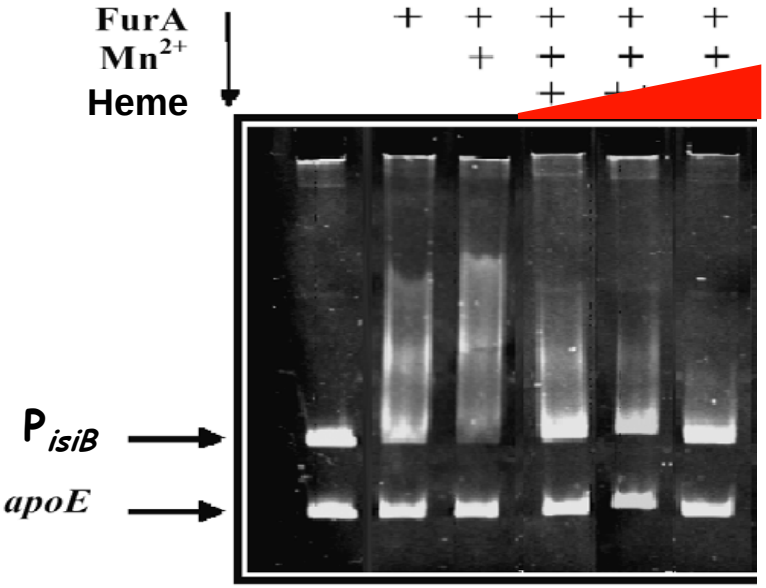
Hernández et al. BioMetals, 2006

FurA binds heme *in vitro and* impares FurA-DNA interaction





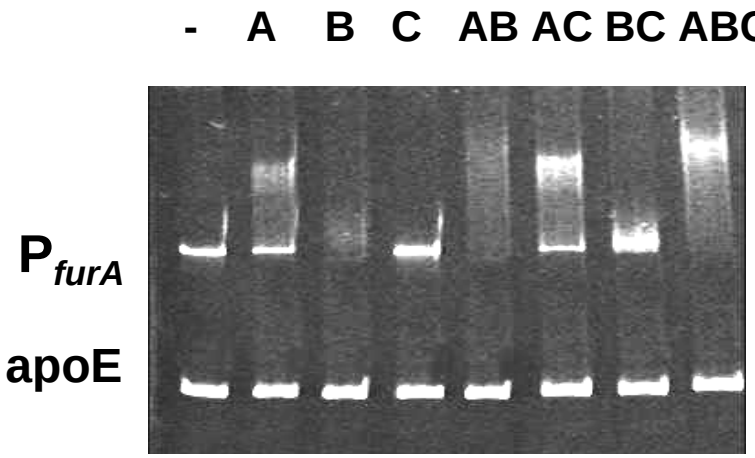


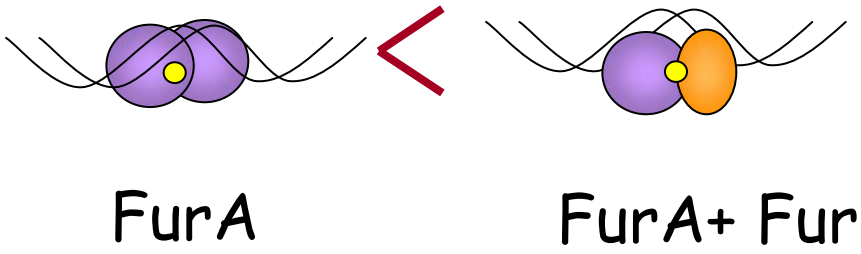


Hernández et al. FEBS Lett, 2004

Are Fur proteins interregulated?

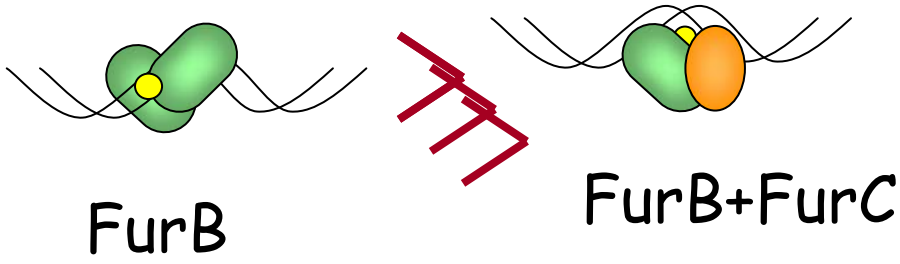
FurB binds to P_{furA}





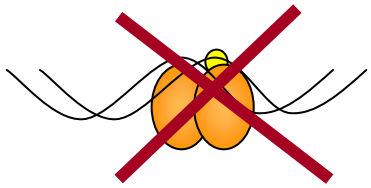
FurA

FurA+ FurC



FurB

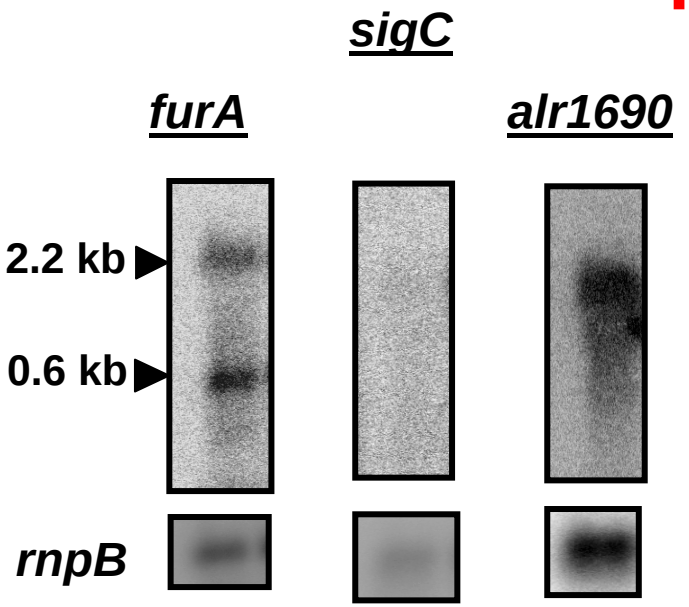
FurB+FurC

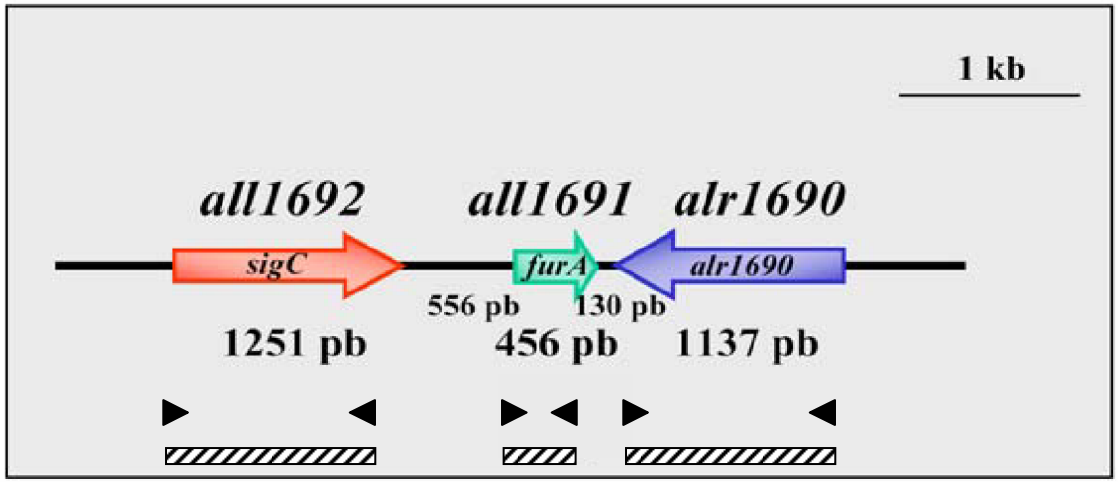


FurC

But FurC influences in the ability of FurA and FurB to bind DNA

Transcriptional analysis of *furA*



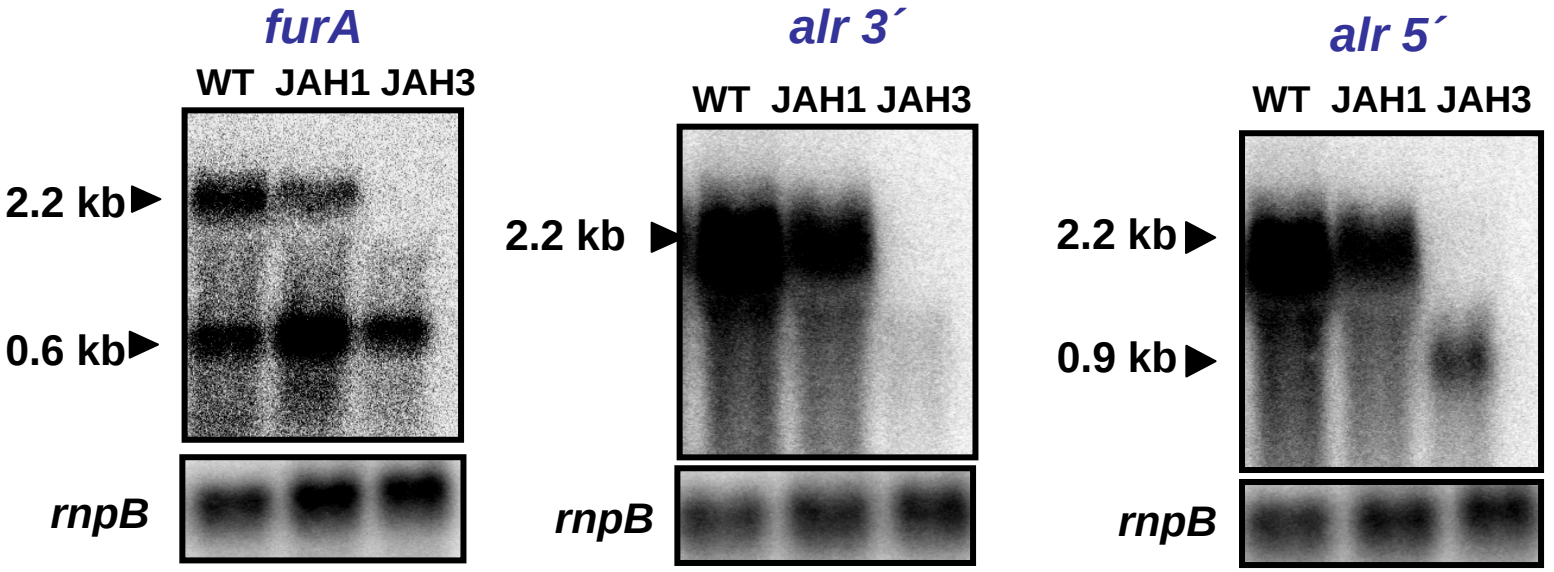


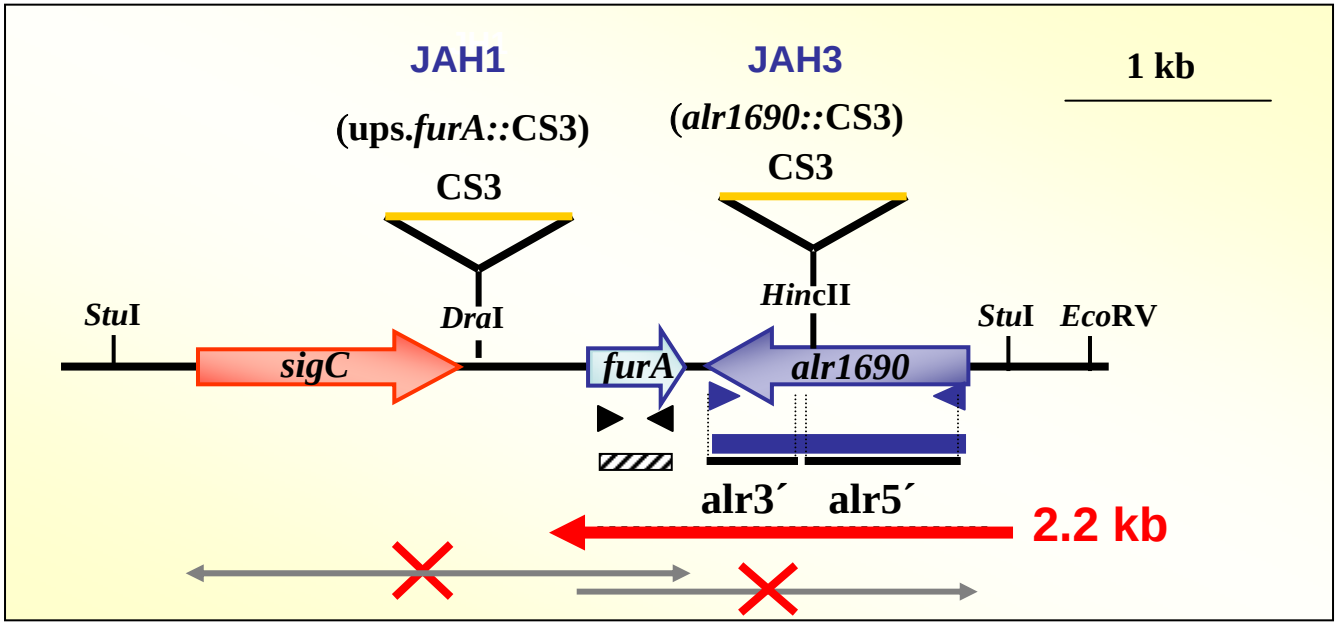
Assays performed several times using new probe and/or new RNA

Lack of signal using *furB* y *furC* as probes

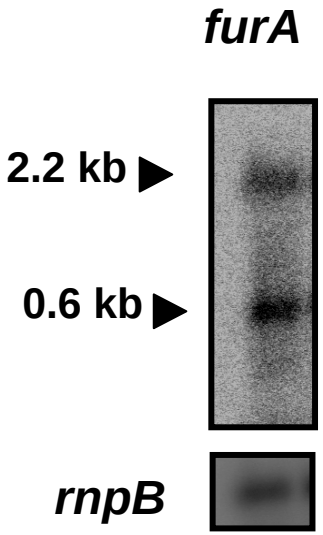
The 2.2 kb signal appeared probing with *alr1690*, but not with *sigC*

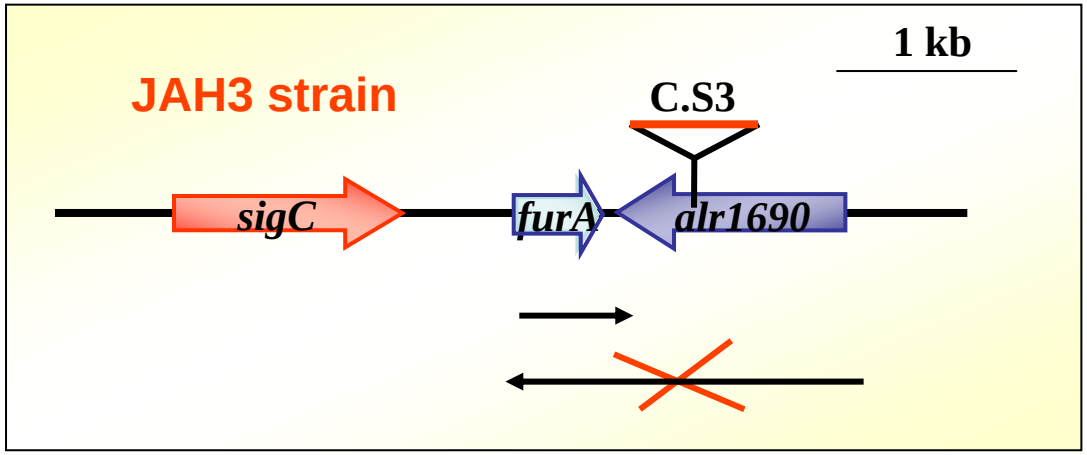
Northern blot analysis of mutants in regions flanking *furA*





The 2.2 kb signal is an antisense RNA (α-*furA* RNA)



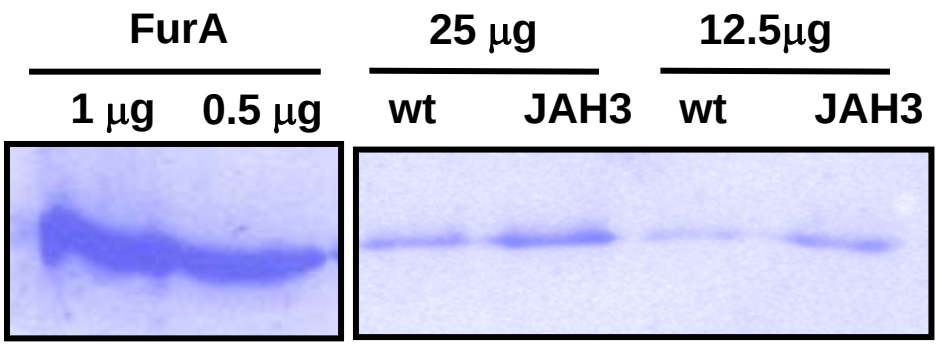


JAH3 cells show:

- ✓ Higher FurA expression
- ✓ Lower iron content in cells

wt = 1326.9 μg Fe/ g células

H3 = 830.65 μg Fe/ g células



Hernández JA et al. J.Mol.Biol., 2006

Nitrogenase reductase

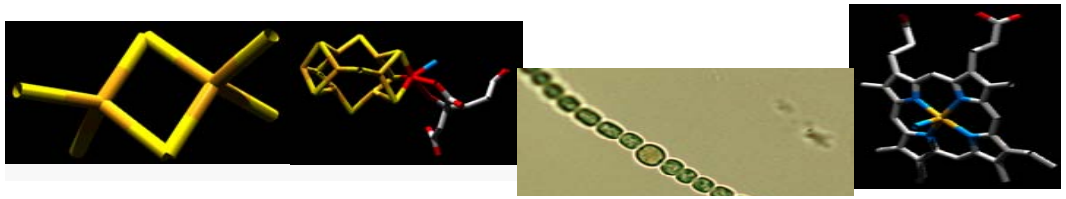
Dinitrogenase

Nitrate reductase

Nitrite reductase

GOGAT

Ferredoxin



Hypothesis:

+Iron and N metabolism are interregulated

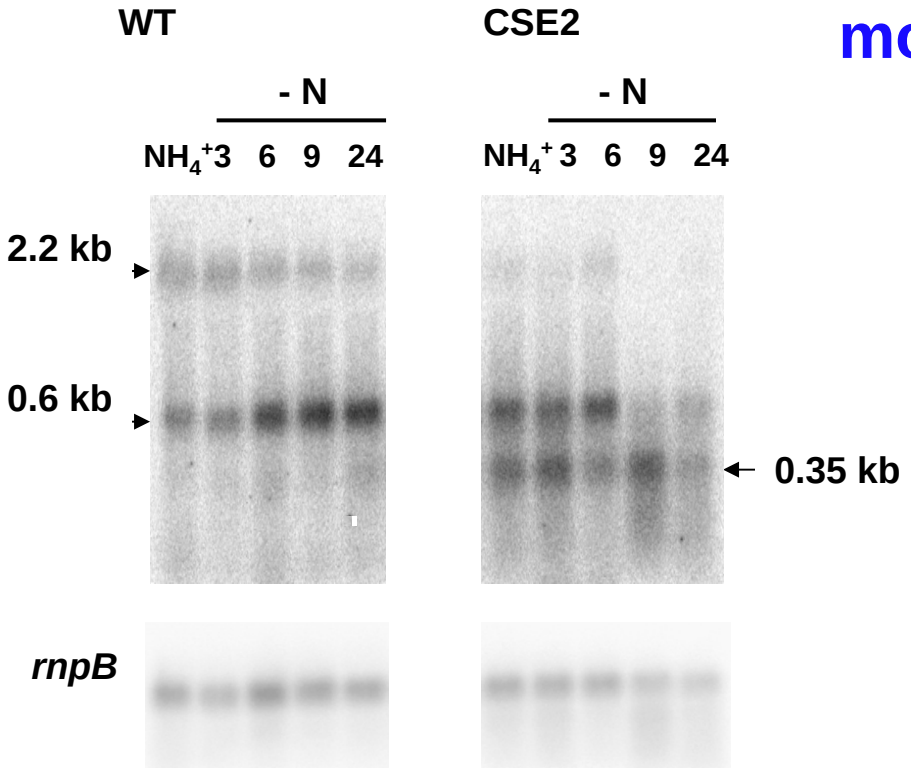
Is FurA involved in the network that controls nitrogen metabolism ?

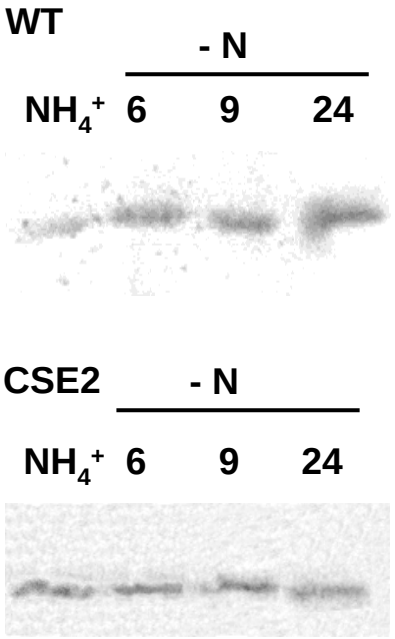
-Is FurA expression dependent of N status?

-Identification of Fur-regulated genes involved in N metabolism

Influence of nitrogen deprivation in the transcription and translation of *furA*

Is NtcA involved in the modulation of *furA*?

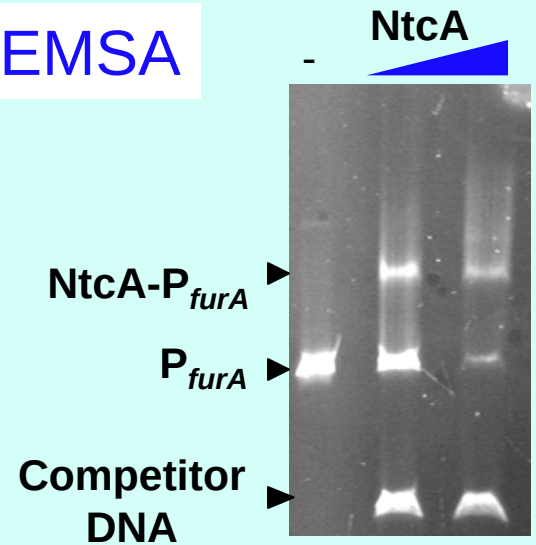




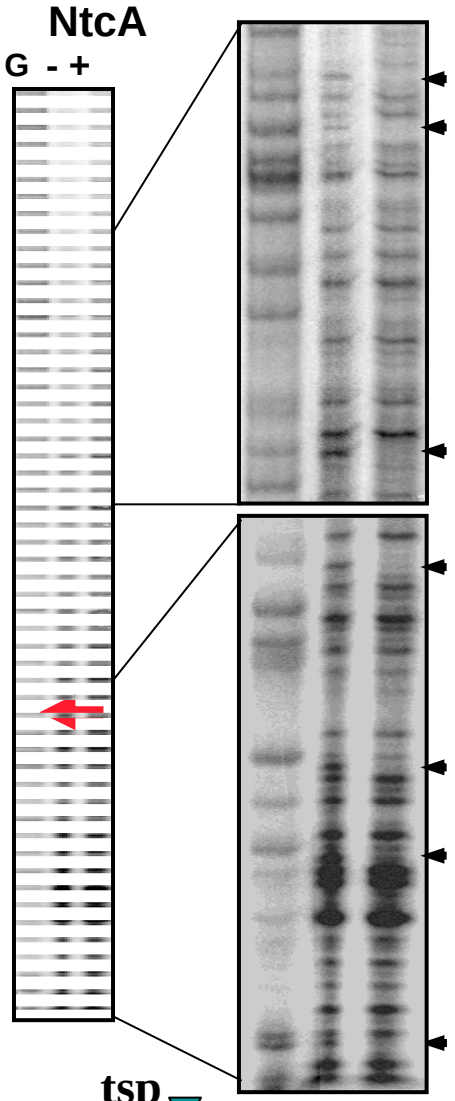
López-Gomollón et al., Microbiology (2007)

Does NtcA bind to P_{furA} ?

EMSA



footprinting



…TGTTAATAATAAGCATTGTTACTAGGTTTTT

GTAGGTGATT TTCACTA… …TAATAAATAT TCTCAATAA

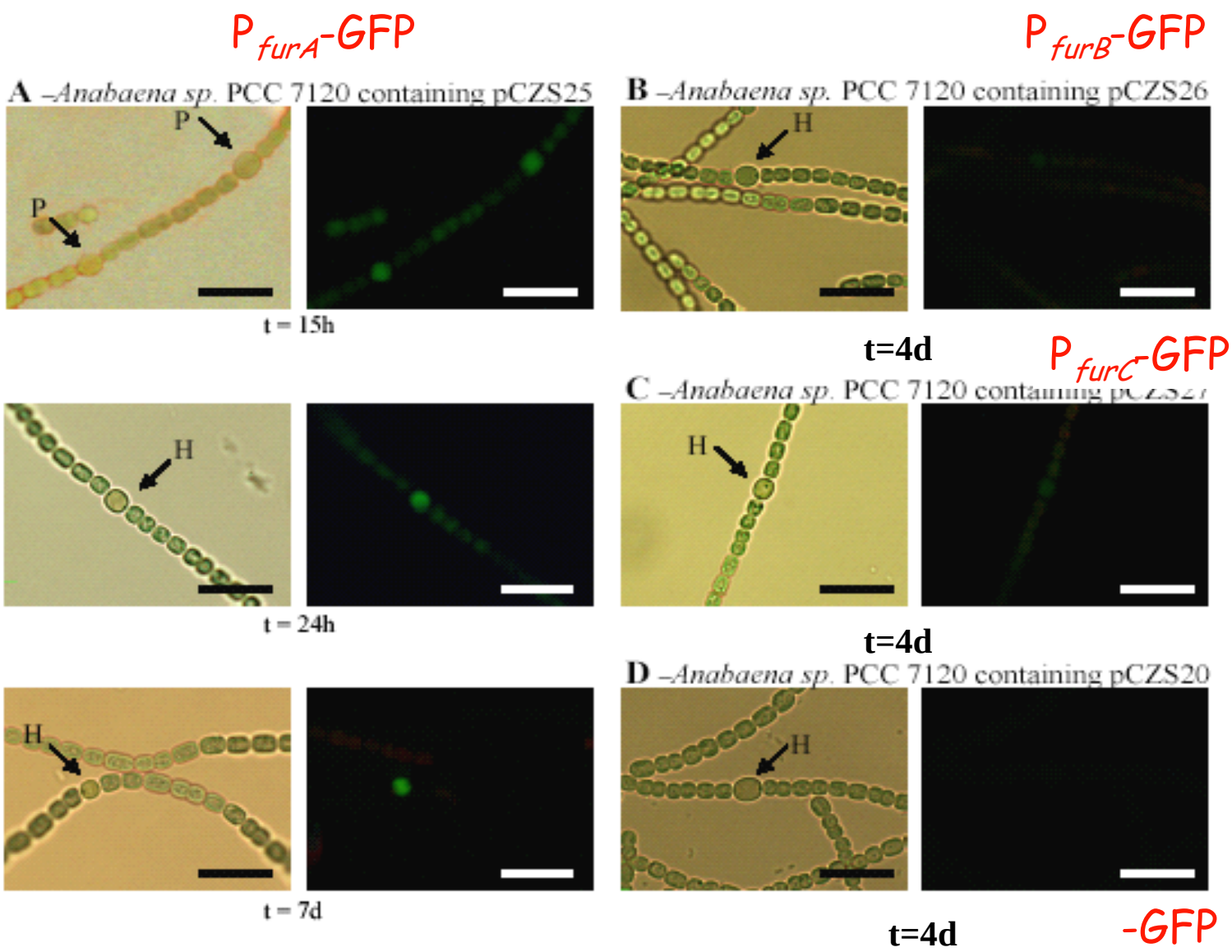
-10

GCAATGTTTG TTGCAAATCA CTCCAAATAT TTGTTATATT CTCAATTAAC

AAGCTTTGTT GAGAAAAATT AGTATGACTG TCTACACAAA TACTTCGCTC AAGGC

Met Thr Val Tyr Thr...

Expression of *furA* is strongly enhanced in heterocysts

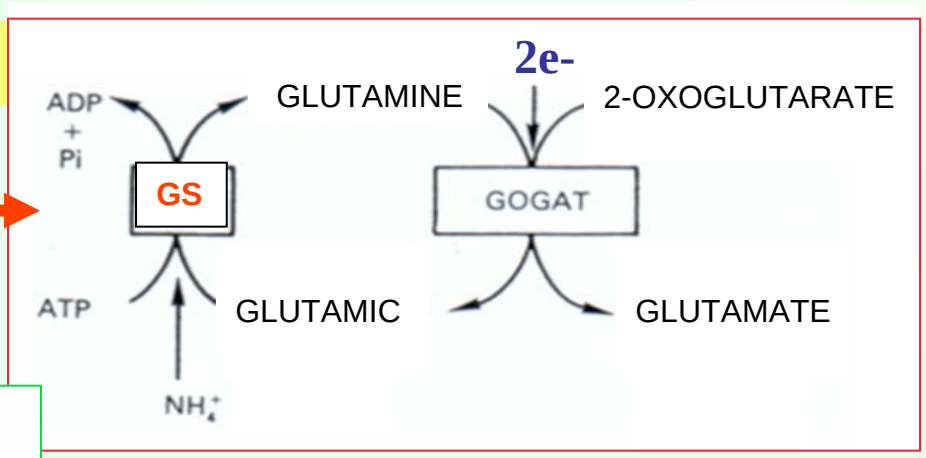


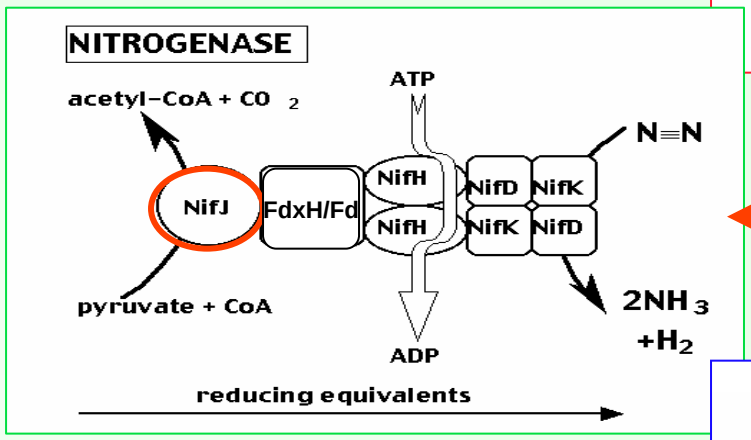
López-Gomollón et al. Microbiology, 2007

FurA binds to the promoters of key genes involved in nitrogen metabolism

glnA **(glutamine sinthetase)**

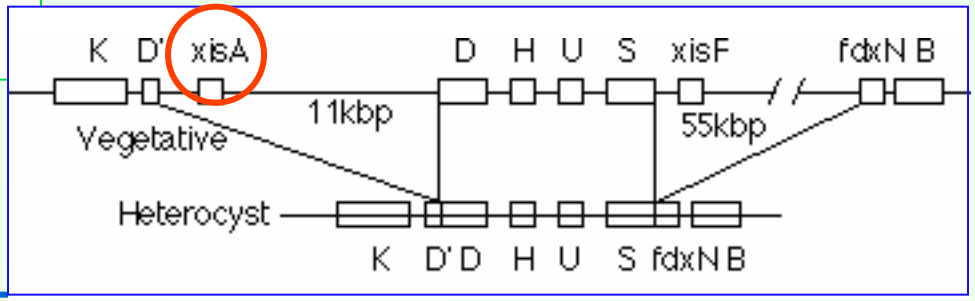
Gateway of ammonium to carbonated skeletons



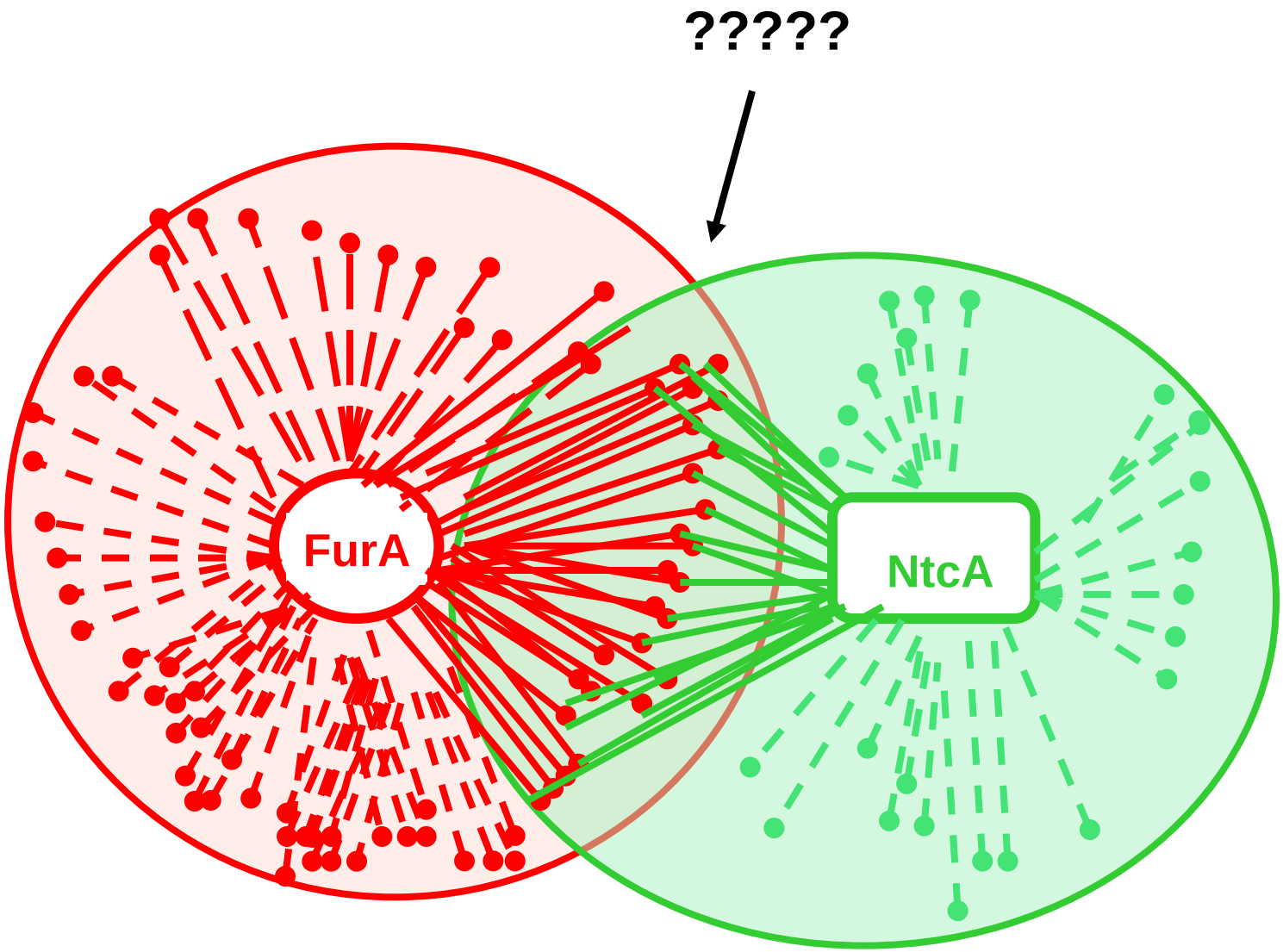


nifJ **(piruvate-flavodoxin oxido-reductase)**

xisA **(excisase)**



How important is the overlap between both regulons?



Identification of genes modulated by FurA & NtcA

Metodology:

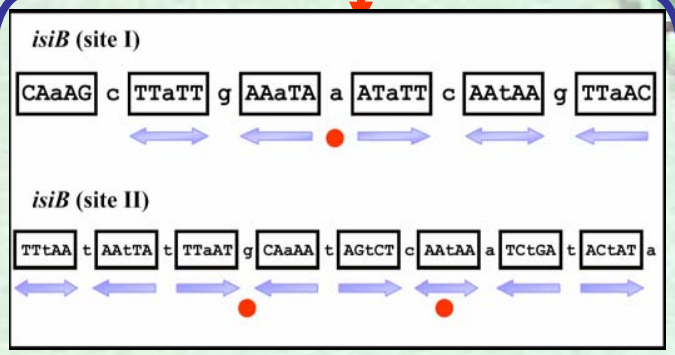
- Low cost

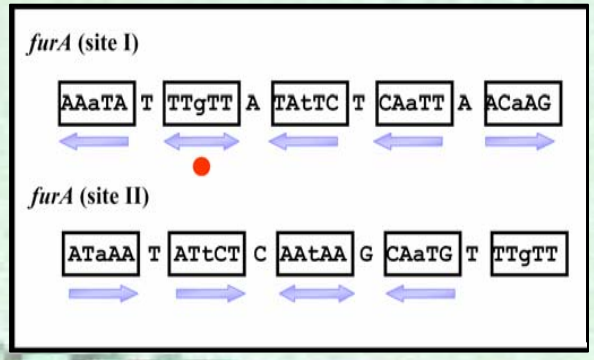
~~micro-arrays~~

- FurA is essential for the cell

~~- ΔfurA~~

1.- Experimental identification of target sequences of FurA in *Anabaena* (P_{furA} and P_{isiB})





Consensus

↓

PSSM-matrix +

PATSER

CUT-OFF: mean + SD

2.- Computational prediction of potential targets

3.- Experimental validation (PCR+EMSA)

Computational prediction of genes modulated by FurA & NtcA

Regulatory regions of 123 genes:

-108 bearing binding sites for NtcA (*)

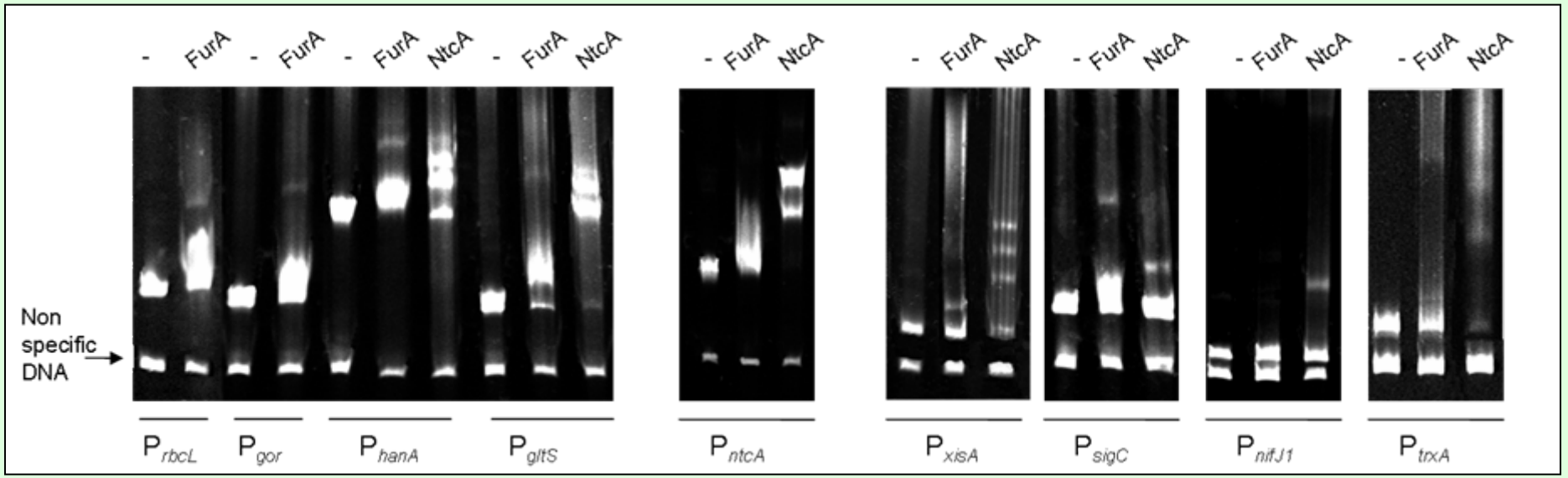
-The other 15 were likely to be under the control of both transcription factors.

PATSER

CUT-OFF: mean + SD

54 promoters potentially regulated by FurA & NtcA

30 Regulatory regions were amplified by PCR and tested by EMSA using FurA & NtcA



(*) Su et al. NAR, 2005

CATEGORY	GENE	INTERACTS WITH	
		FurA	NtcA
Fur family	*furA*	Yes	Yes
	furB	Yes	Yes
	furC	Yes	Yes
Nitrogen Metabolism	*ntcA*	Yes	Yes
	glnA	Yes	Yes
	gltS	Yes	Yes
Nitrogen Fixation	*nifH*	Yes	Yes
	nifJ1	No	Yes
	nifJ2	Yes	Yes
	xisA	Yes	Yes
Photosynt. & Respiration	*isiA*	Yes	Yes
	isiB	Yes	No
	rbcL	Yes	Yes
	prk	Yes	Yes
	psaL	Yes	Yes
	psbZ	Yes	Yes
	coxB2	Yes	Yes
	all1127	Yes	Yes
	ndhF	Yes	Yes
	petH	Yes	Yes

CATEGORY	GENE	INTERACTS WITH	
		FurA	NtcA
Biosynthesis of cofactors, prostetic groups and electron carriers	*trxA*	Yes	Yes
	ftrC	Yes	Yes
	trxQ	Yes	Yes
	all2367	Yes	Yes
	alr2205	Yes	Yes
	gor	Yes	Yes
Translation	*hanA*	Yes	Yes
Transcription	*sigC*	Yes	Yes
Others	*dpsA*	Yes	No
	alr1690-α-furA	Yes	Yes

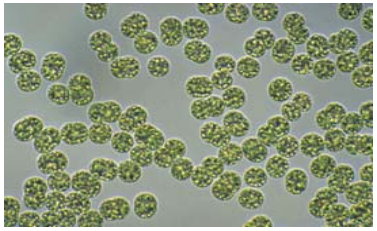
From the 30 regulatory regions tested, 27 bound to FurA and NtcA.

Blue boxes show FurA and/or NtcA targets reported for the first time

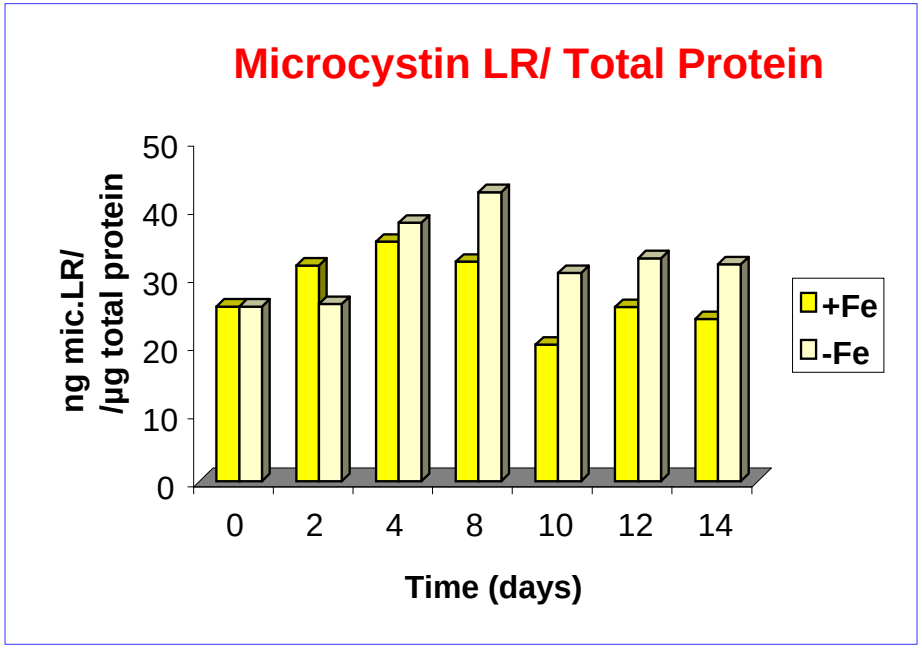
Is Fur involved in the regulation of microcystin ?

Hypothesis:

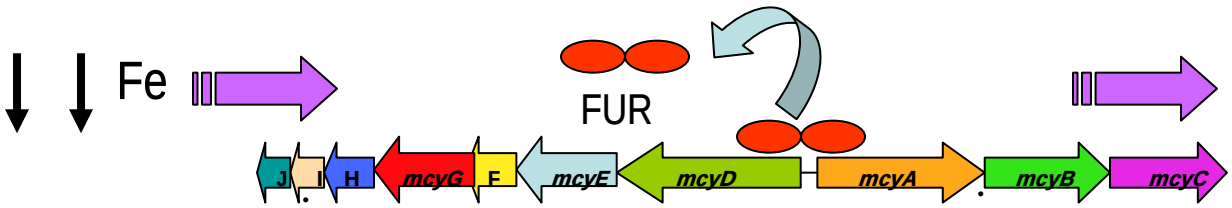
+Iron can be one of the main factors controlling microcystin synthesis

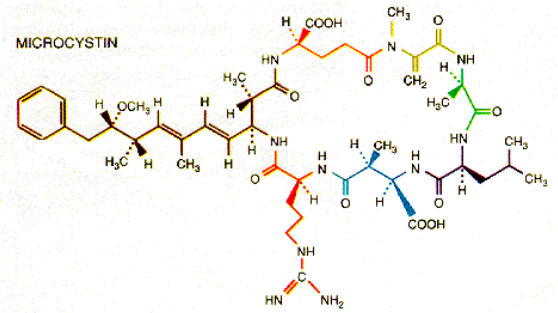


***Microcystis* PCC 7806**

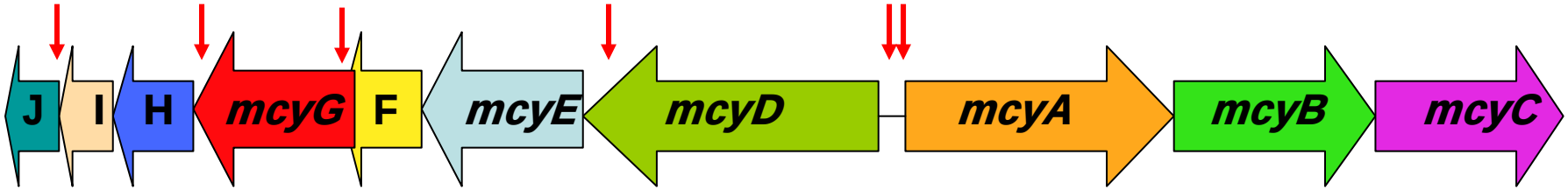


Synthesis of microcystin

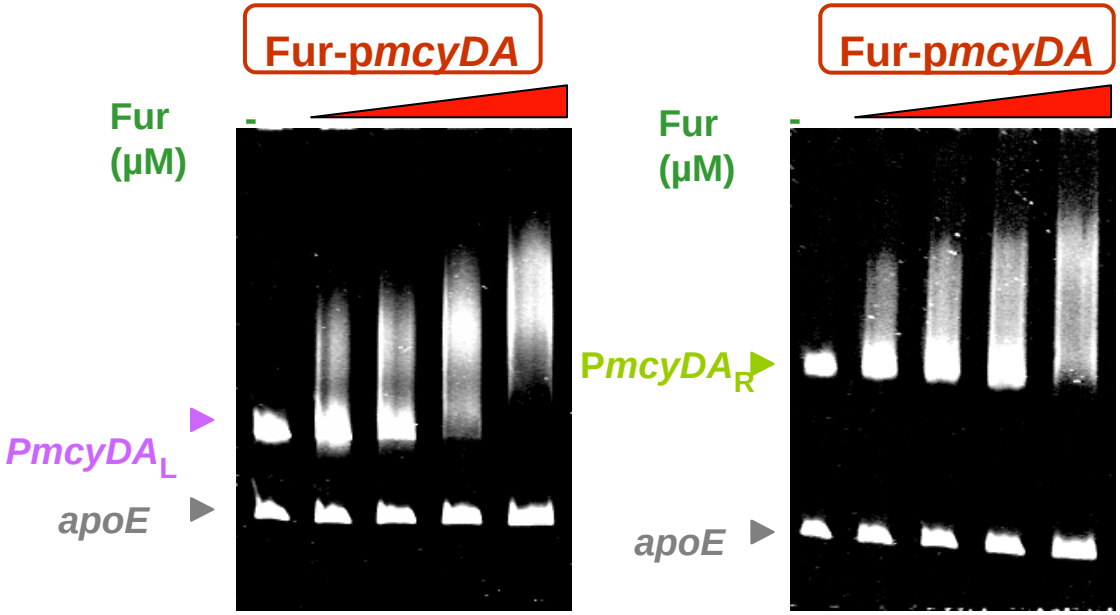




Fur recognises and binds to *mcy* promoters



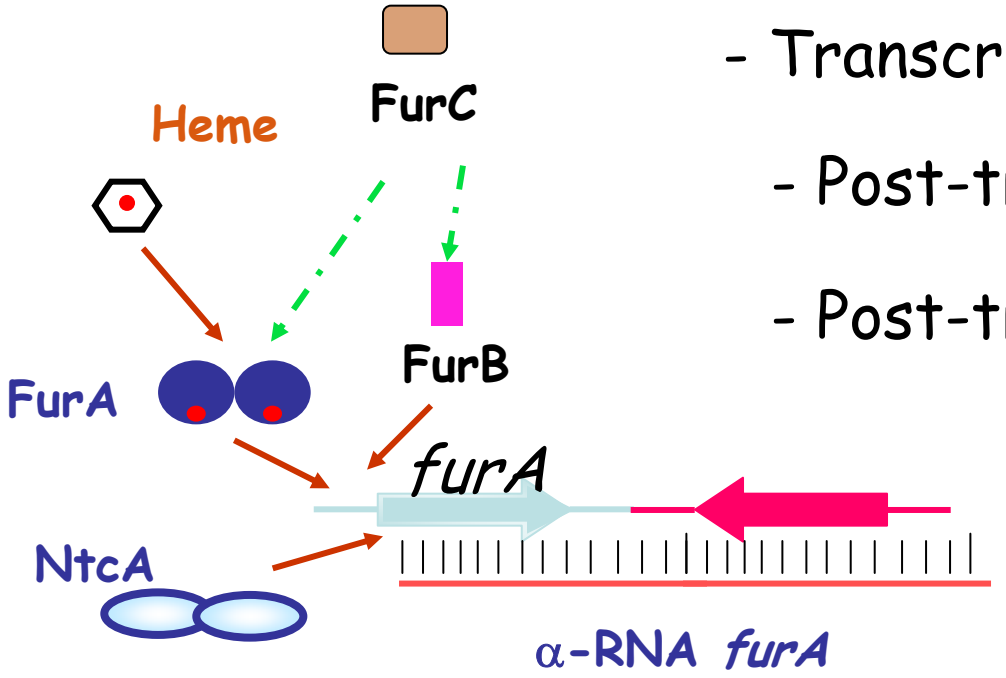
Promoter region	$K_{d(app)}$ (μM)
mcyDA$_{331}$	0.28± 0.03
mcyDA$_{438}$	0.36± 0.04
mcyH	0.48± 0.03
mcyG	0.60± 0.07
mcyE	0.77± 0.03
mcyJ	1.53± 0.1



Martín-Luna et al. Phytochemistry, 2006

Conclusions (I)

The regulation of FurA is complex

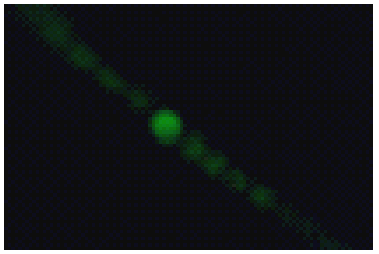


- Transcriptional level
- Post-transcriptional
- Post-translational

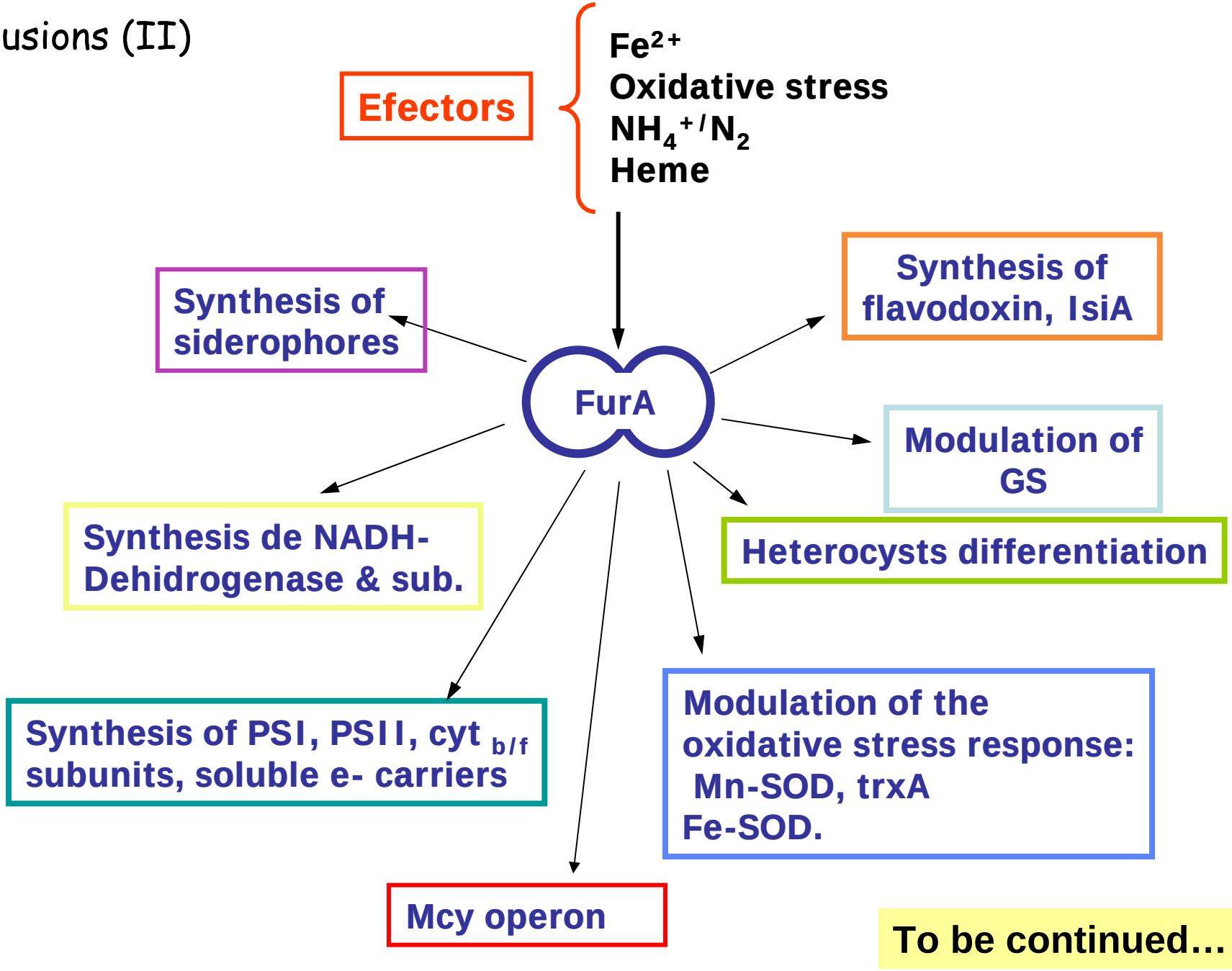
Mn^{2+}
DTT
Heme

FurA is involved in the regulatory network that controls nitrogen metabolism

At least 27 genes seems to be coordinately regulated by FurA and NtcA



Conclusions (II)



To be continued…

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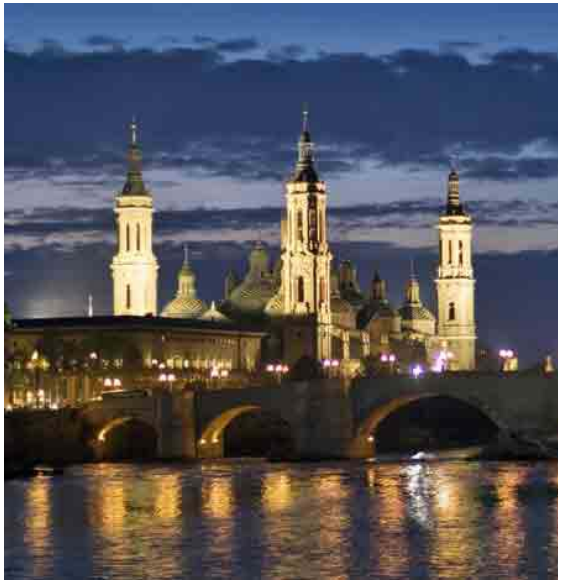

Thanks for your attention!!!