Expanding the FurC (PerR) regulon in *Anabaena* sp. PCC 7120: involvement of FurC in the regulation of processes dependent of C/N ratio

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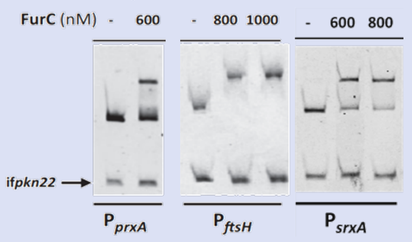
FurC (PerR) from *Anabaena* sp. PCC7120 is a global regulator that controls several important processes in cyanobacteria such as nitrogen metabolism, photosynthesis and oxidative stress defenses. Previous studies from our research group identified a putative FurC DNA-binding consensus sequence. In the present work, this putative FurC DNA binding-box was searched in the promoter regions of the whole *Anabaena* sp. PCC 7120 genome in order to expand the regulon of FurC. All the newly predicted FurC targets were evaluated by electrophoretic mobility shift assays (EMSA), allowing the identification of 19 novel FurC targets. Some of these newly identified targets display relevant roles in nitrogen fixation (*hetR* and *hgdC*) and carbon assimilation processes (*cmpR*, *glgP1* and *opcA*) suggesting a key regulatory role of FurC in the control of C and N metabolisms. Besides, 2-oxoglutarate (2-OG) a molecule that acts as a signal of C/N balance was found to be able to bind to FurC and to have an effect on its DNA binding activity. Taken together, these data suggest that apart from redox signals and metal availability, FurC could also respond to C/N ratio. Thus, FurC would be an additional player for the harmonization of carbon and nitrogen metabolisms.

1. Prediction of novel FurC-binding sites in *Anabaena* sp. PCC 7120 genome

Bioinformatics tools were use to build a **FurC DNA binding-box weight matrix** and look for putative **FurC-binding sites** in ***Anabaena* sp. PCC 7120 genome**

Identification of FurC binding regions in previous studies

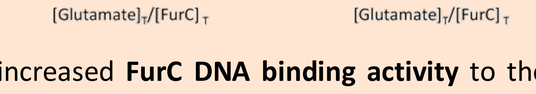


MEME — Multiple Em for Motif Elicitation

Prediction of putative FurC boxes

Gene	*p*-value	FurC box
prxA_alr4641	2.40x10^{-9}	CATAGTCATAACGATTTTG
ftsH_all4776	1.05x10^{-8}	CGAAGTCATTACGAATTTG
furC_alr0957	1.25x10^{-8}	CAAACTCATTACAACTTTA
srxA_asl4146	1.98x10^{-8}	CGAAGTCATAATGACTATG
nifH2_alr0874	2.78x10^{-8}	AAAAATCATAACGATATTG
ahpC_all1541	3.65x10^{-7}	TATAATCATAATGACTACG

Construction of a FurC weight matrix



FIMO — Find Individual Motif Occurences

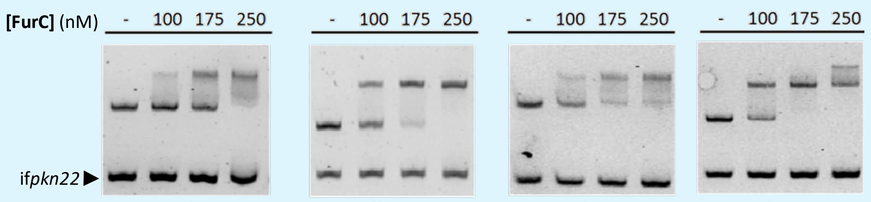
Prediction of novel FurC-binding sites

Gene	p-value	Predicted FurC-box
psbZ	6.42x10^{-6}	AGAAGTCAAAATGACCATA
hetR	6.85x10^{-6}	AGTAGTCATAATGGCTTAA
fas1	7.05x10^{-6}	AAATCTCATAACTAATTTG

2. Identification of novel FurC direct targets

Electrophoretic Mobility Shift Assays (EMSA) were used to determine whether **FurC** was able to **bind** to the **predicted binding sites** and allowed the identification of **19 new FurC direct targets**

Photosynthesis and photoprotection



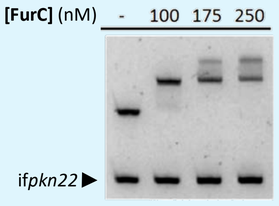
psbZ PSII structural subunit

all1123 Orange Carotenoid Protein NTD-paralog

fas1 Protein involved in the response to UV-B radiation stress

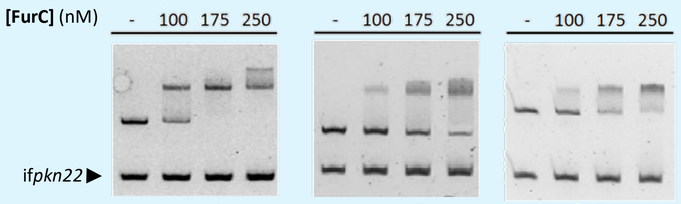
degQ Protease involved in the degradation of PsbO subunit

Zinc homeostasis



zupT Zinc transporter

Carbohydrate metabolism and carbon fixation

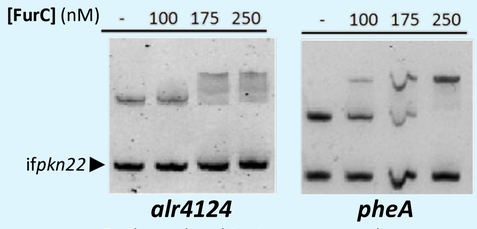


opcA Glucose-6-phosphate DH assembly protein

cmpR low CO_2-responsive LysR transcriptional regulator

glgP1 glycogen phosphorylase

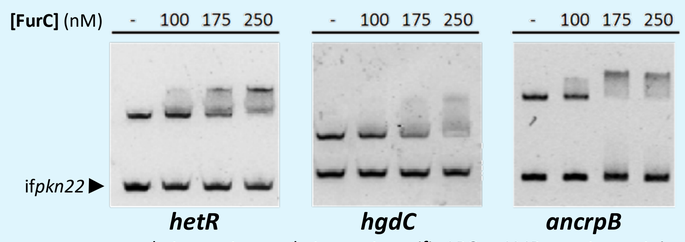
Aminoacid metabolism



alr4124 S-adenosylmethionine synthetase

pheA prephenate dehydratase

Nitrogen metabolism and heterocyst differentiation

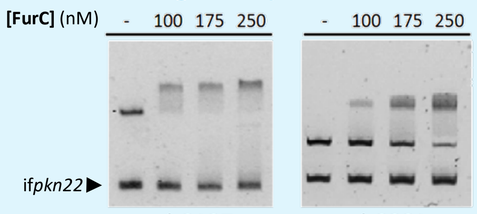


hetR heterocyst development regulator

hgdC heterocyst specific ABC-transporter

ancrpB cAMP receptor protein transcriptional regulator

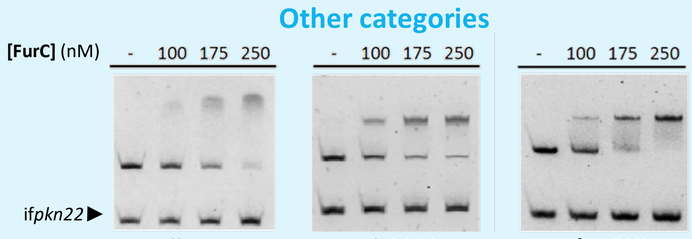
Regulatory functions



alr2137 two-component system, NarL family, sensor kinase

alr3281 putative transcriptional regulator

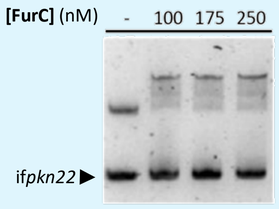
Other categories



all4441 glycosyltransferase family 2 protein

alr0252 Na^+/H^+ antiporter

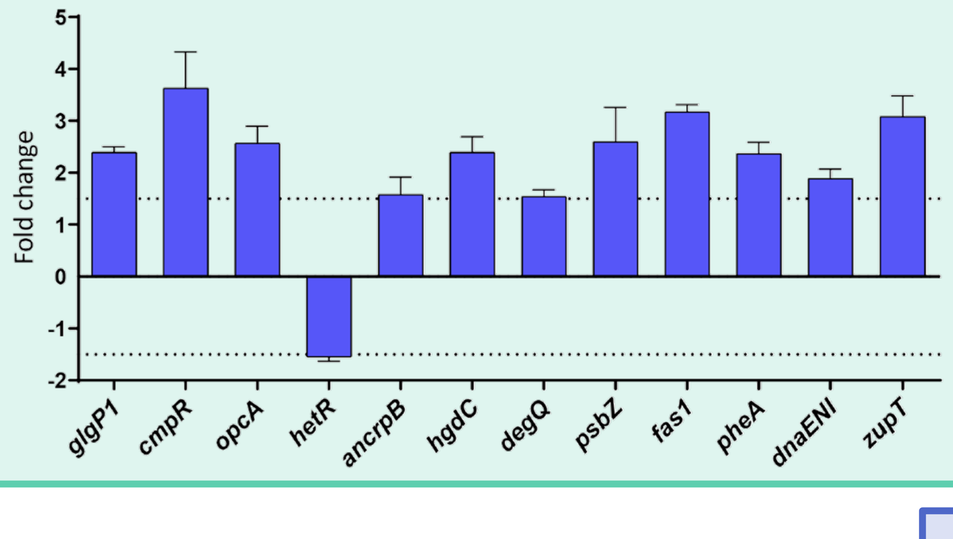
dnaENI DNA polymerase subunit III



asr2041 putative XRE family transcriptional regulator

3. Differential gene expression analysis of newly identified FurC targets

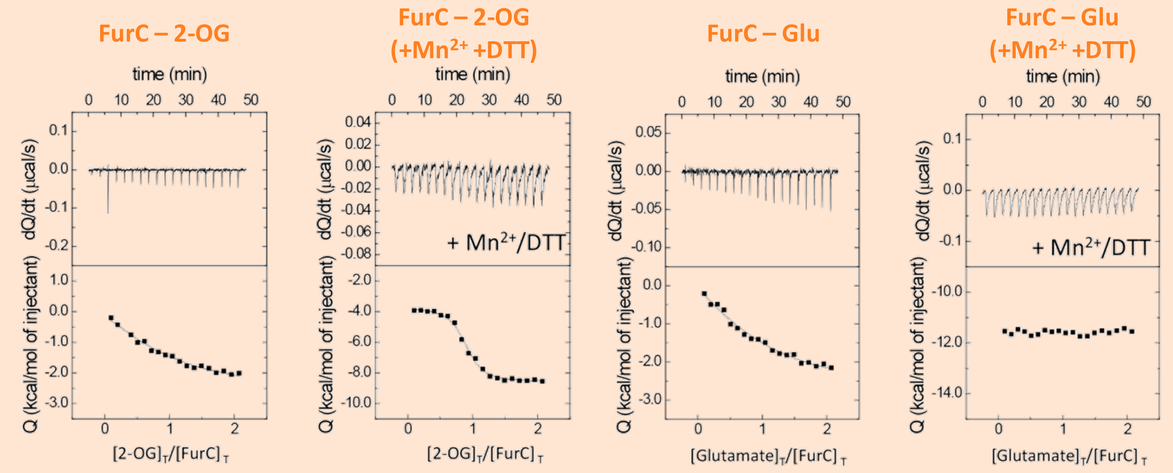
Transcriptional analysis were used to compare the **expression levels** of some **novel FurC direct targets** in a ***furC*-overexpressing strain** (EB2770FurC) versus the wild type strain *Anabaena* sp. PCC7120. **All the tested genes** showed **altered expression** in the ***furC*-overexpressing strain** thus confirming their **direct regulation by FurC**



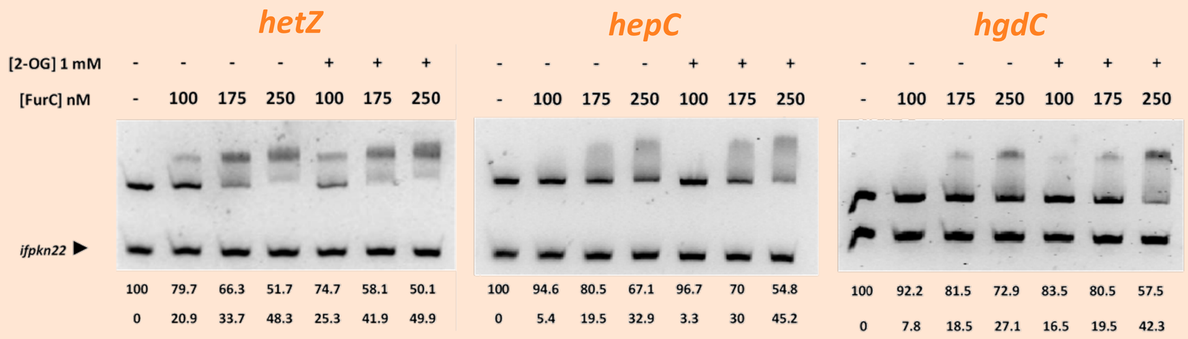
4. FurC binds to 2-OG

ITC assays showed that **FurC was able to bind to 2-OG**, a molecule that acts as a signal of **carbon/nitrogen balance**, in the presence of **Mn^{2+}** and **reducing conditions.** Besides, this interaction was proven to be **specific**, since **FurC was not able to bind glutamate**, a molecule which a similar structure to 2-OG

	K (M^{-1})	K_d (μM)	ΔH (kcal/mol)	n
2OG	1.3 · 10^5	7.6	4.4	0.9
2OG DTT Mn	4.9 · 10^6	0.2	4.9	0.86
Glu	1.0 · 10^5	10	5.7	0.9
Glu DTT Mn	--	--	--	--

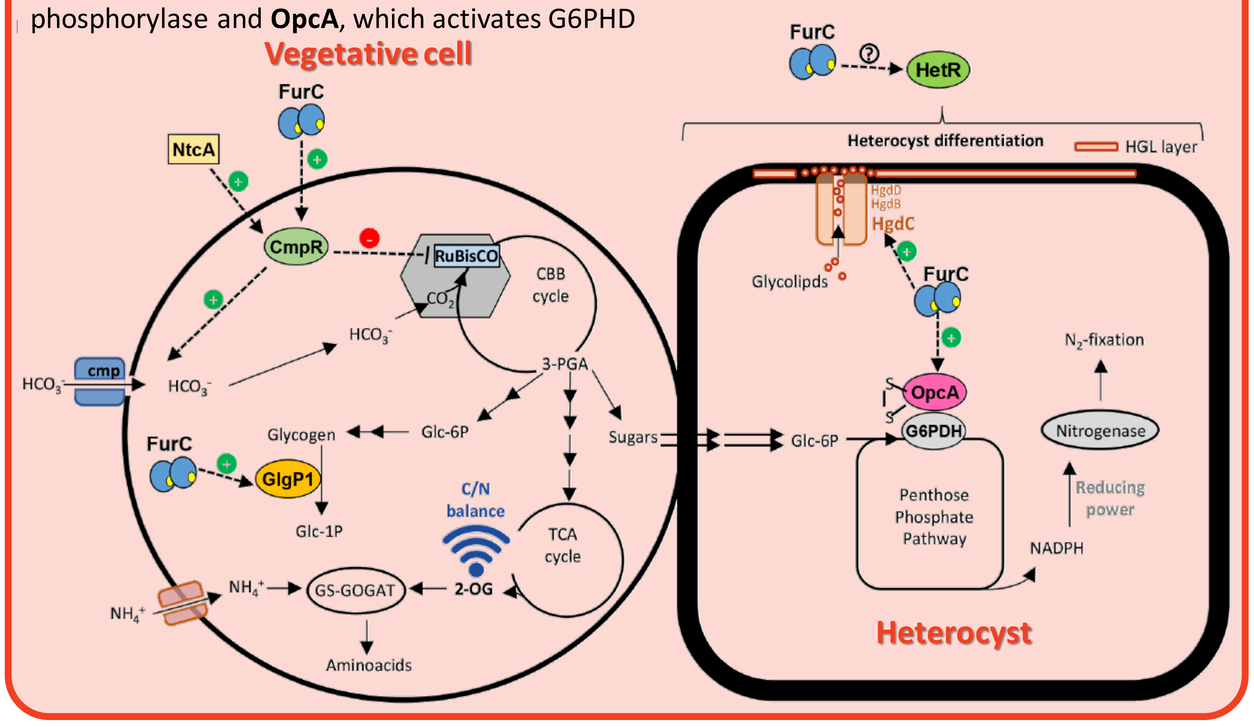


EMSA assays showed that **the presence of 2-OG** increased **FurC DNA binding activity** to the **promoter regions** of genes involved in **nitrogen metabolism** and **heterocyst differentiation**



5. FurC is involved in the control of processes dependent on C/N ratio

FurC seems to **control important processes** essential for the maintenance of **C and N homeostasis:** genes involved in **heterocyst formation** such as the **HgdC** transporter and the master regulator of heterocyst development **HetR**; genes involved in **carbon metabolism** such as **CmpR**, a transcriptional regulator that controls the carbon fixation, **GlpP1** glycogen phosphorylase and **OpcA**, which activates G6PHD



CONCLUSIONS

- **This work allowed the identification of several novel FurC targets** displaying key roles in **carbon and nitrogen metabolism** (*hgdc*, *hetR*, *glgp1*, *cmpR*, *opcA*) but also genes involved in **photosynthesis** (*psbZ*, *fas1*), **zinc homeostasis** (*zupT*) or **aminoacid metabolism** (*pheA*)
- **FurC** could regulate these processes by **integrating 2-OG levels,** a metabolite that informs about the **cellular C/N balance**

If you have any questions or are interested in our work please do not hesitate to contact me: jguio@unizar.es • Follow us on Twitter! @cyanofur

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