2-oxoglutarate modulates the affinity of FurA for the *ntcA* promoter in *Anabaena* sp. PCC 7120

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In cyanobacteria, 2-oxoglutarate (2-OG) is a metabolite whose function is to provide carbon skeletons that allow the incorporation of ammonium through the GS-GOGAT cycle, connecting both carbon and nitrogen metabolism. It has also been described as a signal molecule that reflects the cellular carbon/nitrogen balance, modulating the DNA-binding activity of the key regulator of nitrogen metabolism NtcA. Since FurA (Ferric uptake regulator) from the cyanobacterium *Anabaena sp.* PCC7120 is a global regulator that controls several cellular processes, including nitrogen metabolism, we sought to investigate whether this transcriptional regulator could also be sensing carbon/nitrogen balance via 2-OG.

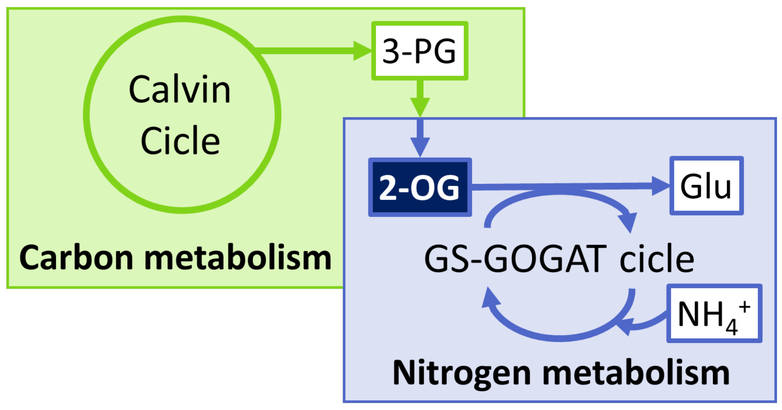

Figure 1. Schematic representation of the role of 2-OG as a metabolite that connects carbon and nitrogen metabolisms in cyanobacteria

1. FurA contains two hypothetical 2-OG binding sites

Firstly, in order to **predict** if **2-OG** was able to **bind to FurA**, a model of **FurA three-dimensional structure** was built and **docked with 2-OG**.

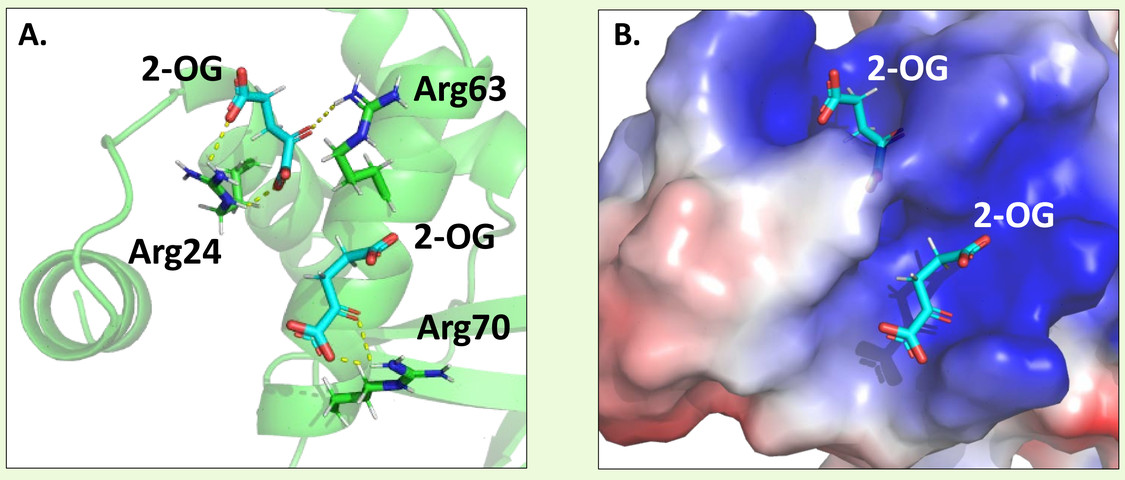

Figure 1. Prediction of the 2-OG binding sites in the modelled structure of FurA from *Anabaena sp.* PCC7120. **A.** Cartoon representation of FurA (green) in complex with 2-OG (cyan). Residues involved in 2-OG binding are represented as sticks and by atom type (C, green; N, blue). **B.** Surface representation of FurA in complex with 2-OG (cyan).

These simulations **predicted** that **2-OG could bind to FurA** and revealed the existence of two potential 2-OG binding sites (Fig. 1). One of this sites involved **Arg24** and **Arg63** and the other involved **Arg70**, residues that were predicted to interact with 2-OG by **hydrogen bonds**.

2. FurA binds to 2-OG *in vitro*

ITC assays were performed to confirm if **FurA was able to bind 2-OG**.

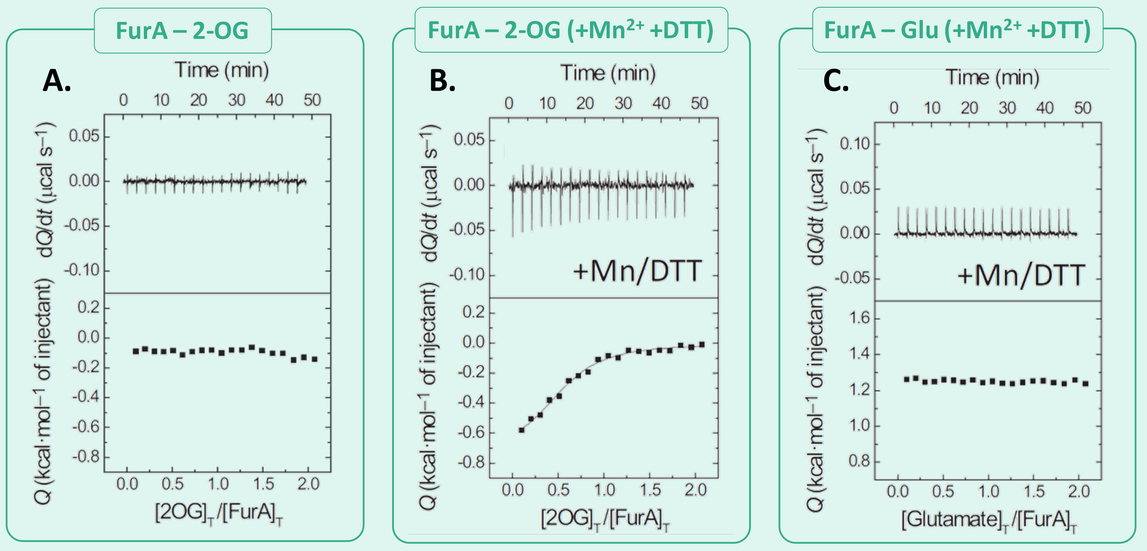

Figure 2. Analysis of the interaction between FurA from *Anabaena sp.* PCC7120 and 2-OG by ITC. **A.** Titration of FurA with 2-OG. **B.** Titration of FurA with 2-OG in the presence of 100 µM Mn^{2+} and 1 mM DTT. **C.** Titration of FurA with glutamate in the presence of 100 µM Mn^{2+} and 1 mM DTT.

Results showed that **FurA** was able to **interact *in vitro*** with **2-OG** but only in the **presence** of both **Mn^{2+}** and **DTT**, the same conditions that FurA requires to bind to DNA (Fig 2A and 2B). Besides, this interaction was proven to be **specific**, since **FurA was not able to bind glutamate**, a molecule which a similar structure to 2-OG (Fig 2C).

3. 2-OG enhances DNA binding activity of FurA to *ntcA* promoter

The effects of **2-OG** on **DNA binding activity of FurA** were studied by **Electrophoretic Mobility Shift Assays (EMSA).**

***ntcA* promoter**

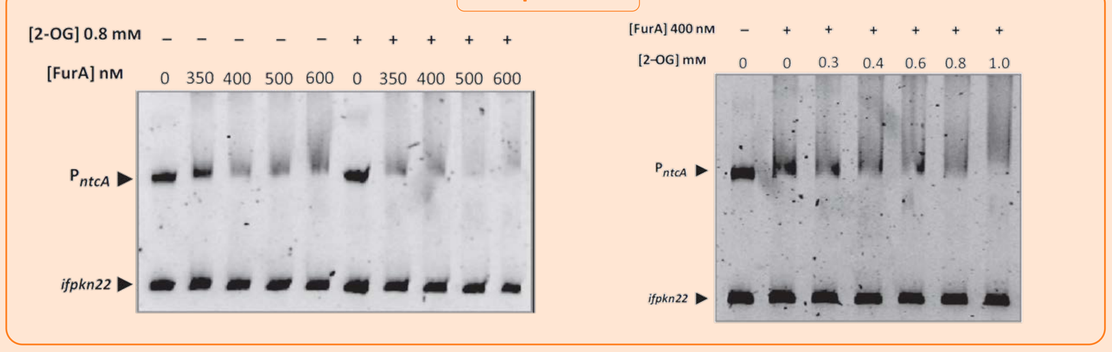

Figure 4. EMSA assays showing the effect of 2-OG on FurA binding to the *ntcA* promoter. The *pkn22* internal fragment was used as control for unspecific binding.

***isiB* promoter**

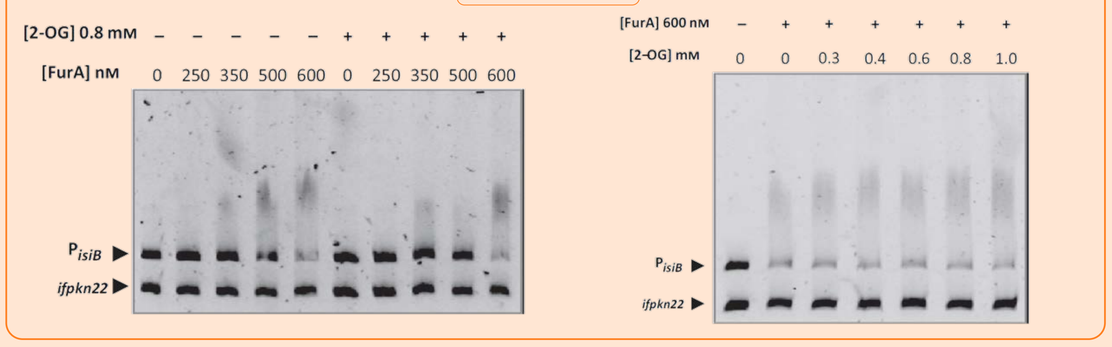

Figure 5. EMSA assays showing the effect of 2-OG on FurA binding to the *isiB* promoter. The *pkn22* internal fragment was used as control for unspecific binding.

As it is showed in Fig 4, 2-OG was able to **mildly enhance** the **binding of FurA to the *ntcA* promoter**. However no effects were observed when this experiment was carried out with the flavodoxin (*isiB*) promoter region (Fig 5).

4. Arg70 is involved in 2-OG binding

As docking simulations suggested two putative binding sites, we constructed **two variants of FurA** in which the **residues** that were **predicted to interact with 2-OG** were replaced by **alanines**.

R24R63 FurA

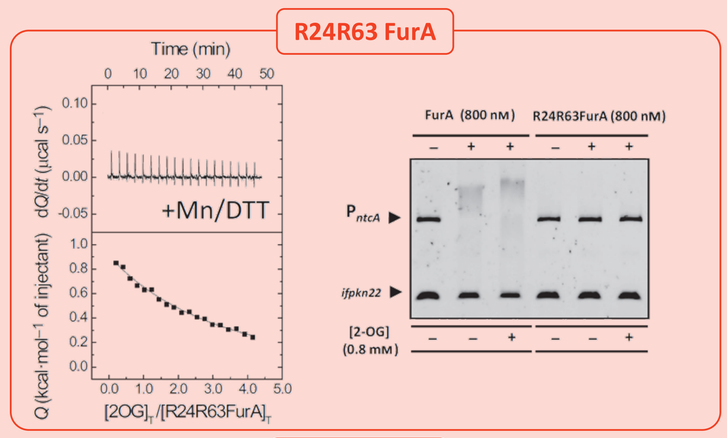

Figure 6. **A.** Analysis of the interaction between R24R63FurA and 2-OG by ITC. **B.** EMSA assays showing that R24R63FurA was unable to bind to the *ntcA* promoter. The *pkn22* internal fragment was used as control for unspecific binding.

R70 FurA

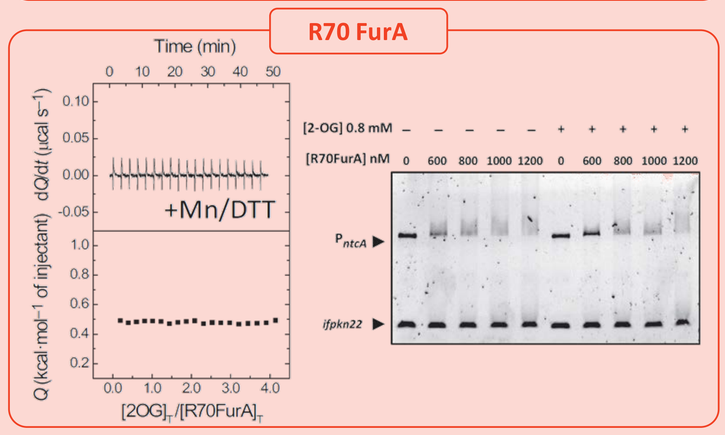

Figure 7. **A.** Analysis of the interaction between R70FurA and 2-OG by ITC. **B.** EMSA assays showing the effect of 2-OG on FurA binding to the *ntcA* promoter. The *pkn22* internal fragment was used as control for unspecific binding.

ITC and **EMSA** assays showed that **FurA variant lacking both Arg24 and Arg63** (R24R63) was **able to bind to 2-OG** but **unable to bind to DNA** (Fig 6). On the contrary, **FurA variant lacking Arg70** (R70) was **unable to interact with 2-OG** and this metabolite **had no effects on its DNA binding activity** (Fig 7), revealing that **Arg70 was involved in 2-OG binding.**

CONCLUSIONS

- A **2-OG binding site in FurA** from *Anabaena sp.* PCC7120 has been identified by combining **bioinformatic predictions** and **experimental procedures**.
- FurA from *Anabaena sp.* PCC 7120 probably acts as a **sensor of carbon/nitrogen balance**, since it **binds 2-OG** and this interaction influences its **DNA binding activity**

This study is published in FEBS Letters. If you are interested, you can find it here:

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

Funding for this study was from 'Ministerio de Ciencia, Innovación y Universidades' (PID2019-104889GB-I00 to M.F.F. and grant FPU2018/03619 to J.G.)