Thioredoxin Dependent Changes in the Redox States of FurA from *Anabaena* sp. PCC 7120

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FurA (Ferric Uptake Regulator) from the cyanobacterium *Anabaena* sp. PCC 7120 is a transcriptional regulator that controls not only iron homeostasis but also other important cellular processes. In cyanobacteria, FurA contains five cysteines, four of them arranged into two CXXC motifs, and lack of a structural zinc ion allows the occurrence of intra and intermolecular thiol-disulfide exchanges. Consequently, FurA acts as a redox regulator able to integrate iron homeostasis with the redox status of its five cysteines. However, the precise mechanism underlying the reduction of FurA, as well as its functional electron donor, remain still unknown. As thioredoxins are essential players in thiol-based redox regulation and are involved, among many other processes, in the regulation of the activity of many redox-sensitive transcription factors, we sought to investigate the potential role of type *m* thioredoxin A (TrxA) in the redox modulation of FurA.

1. FurA interacts with Thioredoxin A

The **interaction** between **TrxA** and **FurA** was assessed both ***in vitro*** (Fig. 1) and ***in vitro*** (Fig. 2)

Cross-linking (*in vitro*)

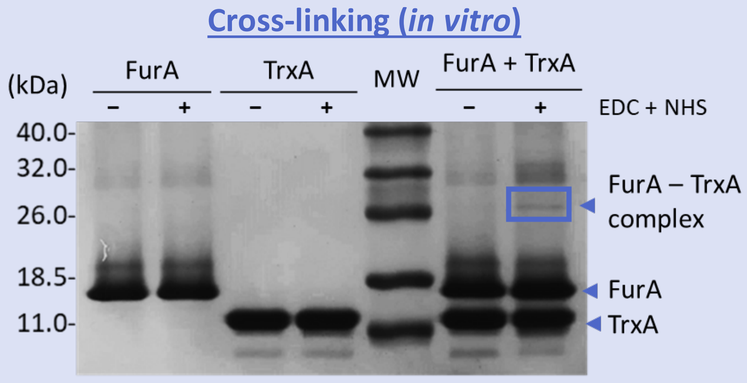

Figure 1. Cross-linking of FurA with TrxA. Equimolar concentrations (20 µM) of proteins incubated with 20 mM EDC and 20 mM NHS were analyzed by SDS-PAGE (15% gel). Samples without the cross-linker agent were used as control.

Bacterial two-hybrid assays (*in vivo*)

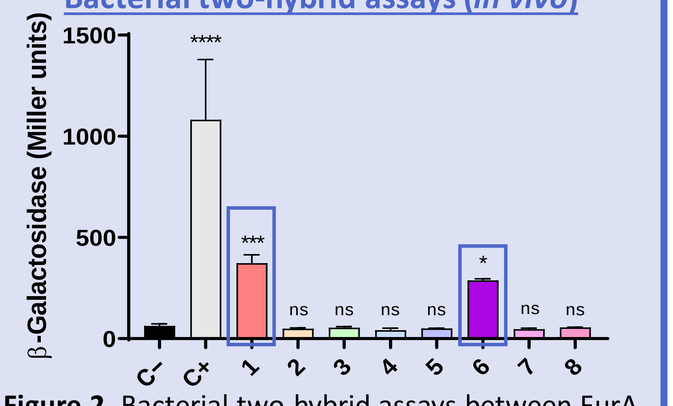

Figure 2. Bacterial two-hybrid assays between FurA and TrxA. *In vivo* interaction was quantified by measuring β-galactosidase activity in *E. coli* BTH101 harboring a pair of plasmids with *furA* and *trxA* fused with T25 or T18 adenylate cyclase domains.

FurA and TrxA form a complex when cross-linking assays are performed (Fig. 1). Besides, **TrxA** is also able to **interact with FurA *in vivo***, as it can be seen in two hybrid assays (Fig. 2)

2. Thioredoxin A reduces FurA

To assess whether **TrxA was able to reduce FurA** the electron transport chain NADPH → NTR → TrxA → FurA was reconstituted *in vitro* and the **status of FurA cysteines** was evaluated using the alkylating agent AMS (Fig. 3)

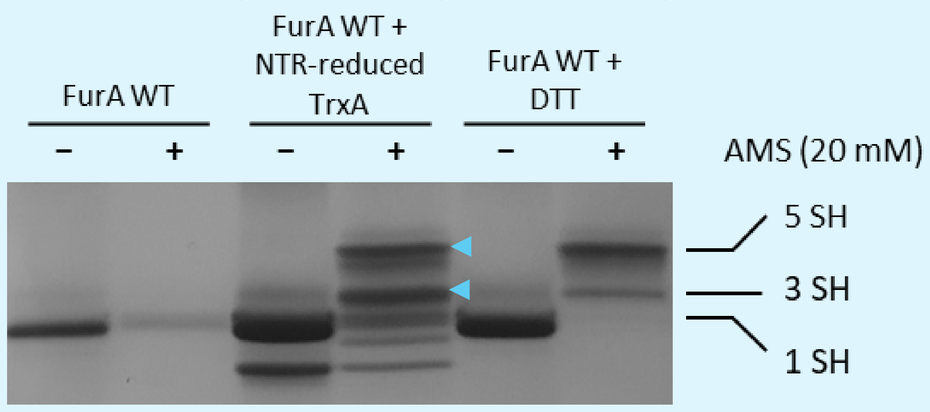

Figure 3. Reduction of FurA by TrxA**.** FurA WT (1 µM) was incubated with 0.5 µM TrxA in the presence of 0.2 mM NADPH, 0.5 mM EDTA, and 5 nM NTR to assess its reduction by TrxA.

As it can be seen in Fig. 3 **TrxA is able to reduce FurA**, generating **two redox isoforms**, one with 3 SH and other with 5 SH

3. TrxA reduces C_{141}-C_{144} disulfide bridge

As **FurA presents two disulfide bridges**, the ability of TrxA to reduce them was analysed using **FurA triple cysteine mutants** containing only cysteines involved in the formation these disulfide bridges (Fig. 4)

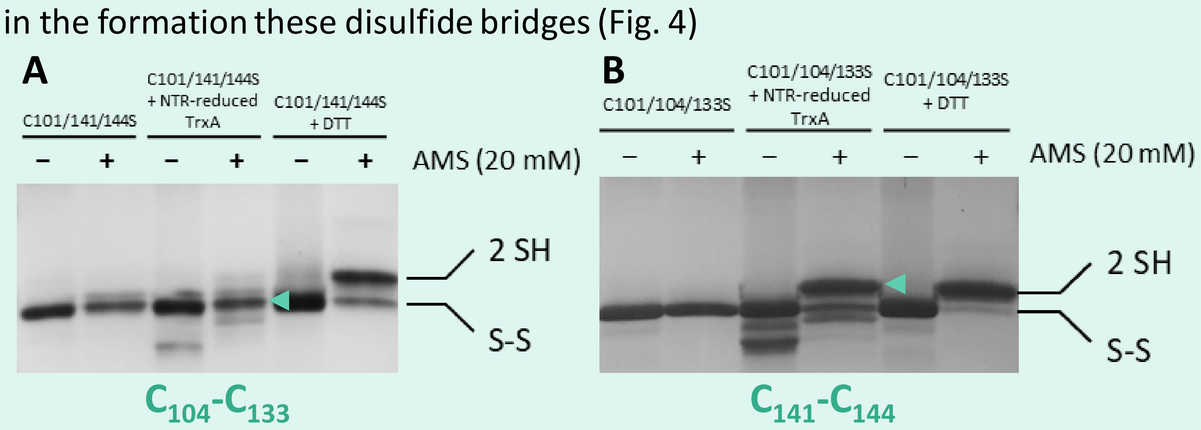

Figure 4. Reduction of disulfide bridges C_{104}-C_{133} and C_{141}-C_{144} of FurA by TrxA**.** 1 µM of FurA triple cysteine mutants C101/104/133S (**A**) and C101/104/133S (**B**) was incubated with 0.5 µM TrxA in the presence of 0.2 mM NADPH, 0.5 mM EDTA and 5 nM NTR to assess their reduction by TrxA.

As it can be seen in Fig. 4 **TrxA is able to reduce C_{141}-C_{144} disulfide bridge** but **not C_{101}-C_{104} disulfide bridge.**

4. Light-Dark Modulation of FurA Thiol Oxidation

Since **TrxA activity** is connected to photosynthetically-reduced ferredoxin, we sought to investigate the **thiol-redox dynamics of FurA under light to dark transitions**. With that purpose the redox status of FurA *in vivo* was analysed by using **Western Blot.**

In the **presence of light**, **FurA displays three redox isoforms** and the reduced isoforms (3 SH and 5 SH) are the predominant ones. In **darkness** the **proportion of more oxidized isoforms** (3 SH and 1 SH) **is incremented** and this oxidation is **reverted** when the culture is **reiluminated**.

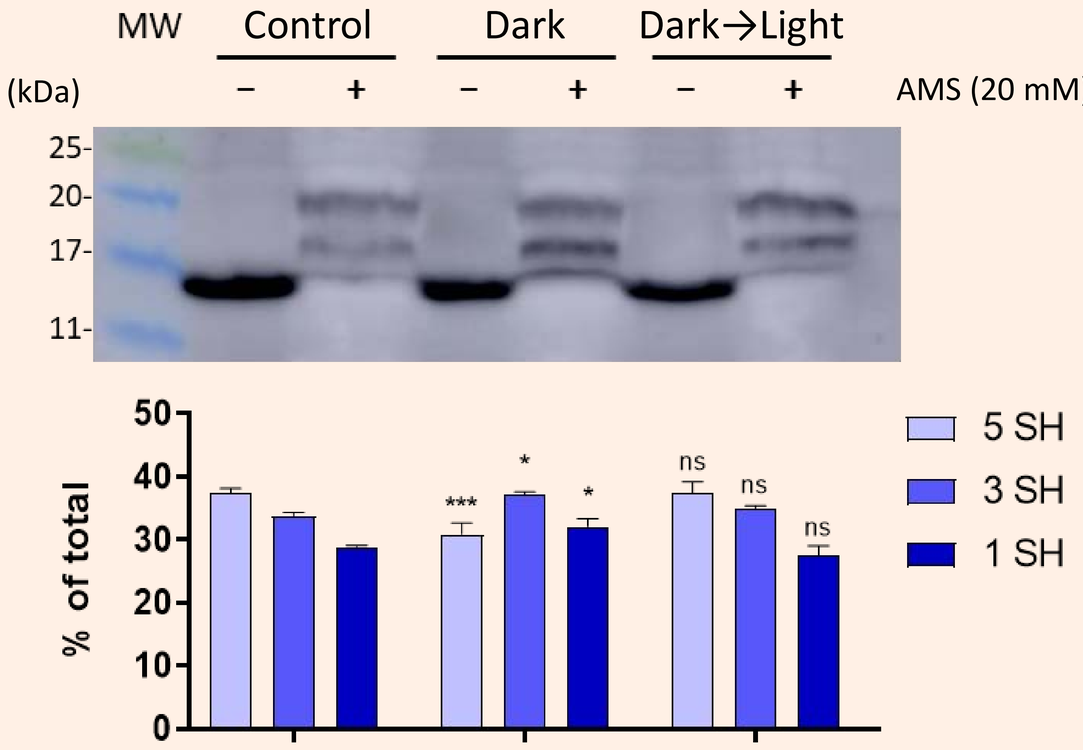

Figure 5. Light-dark modulation of FurA thiol oxidation. The *in vivo* redox status of FurA was assessed by Western blot under standard culture conditions of 30 µmol photons m^{-2} s^{-1} of white light (Control), after 15 min of darkness (Dark), after 15 min of darkness followed by 3 h of exposure to 30 µmol photons m^{-2} s^{-1} of white light (Dark→Light).

5. Proposed reduction mechanism of FurA

Based on the fact that **TrxA** is able to **reduce only one of the two disulfide bridges present** in FurA but it is able to generate **completely reduced isoforms**, a mechanism for the reduction of FurA has been proposed. As it can be seen, we propose that **TrxA will reduce C_{141}-C_{144} disulfide bridge** and C_{104}-C_{133} disulfide bridge will be reduced by C_{141} C_{144} pair which, according to previous studies, displays disulfide reductase activity.

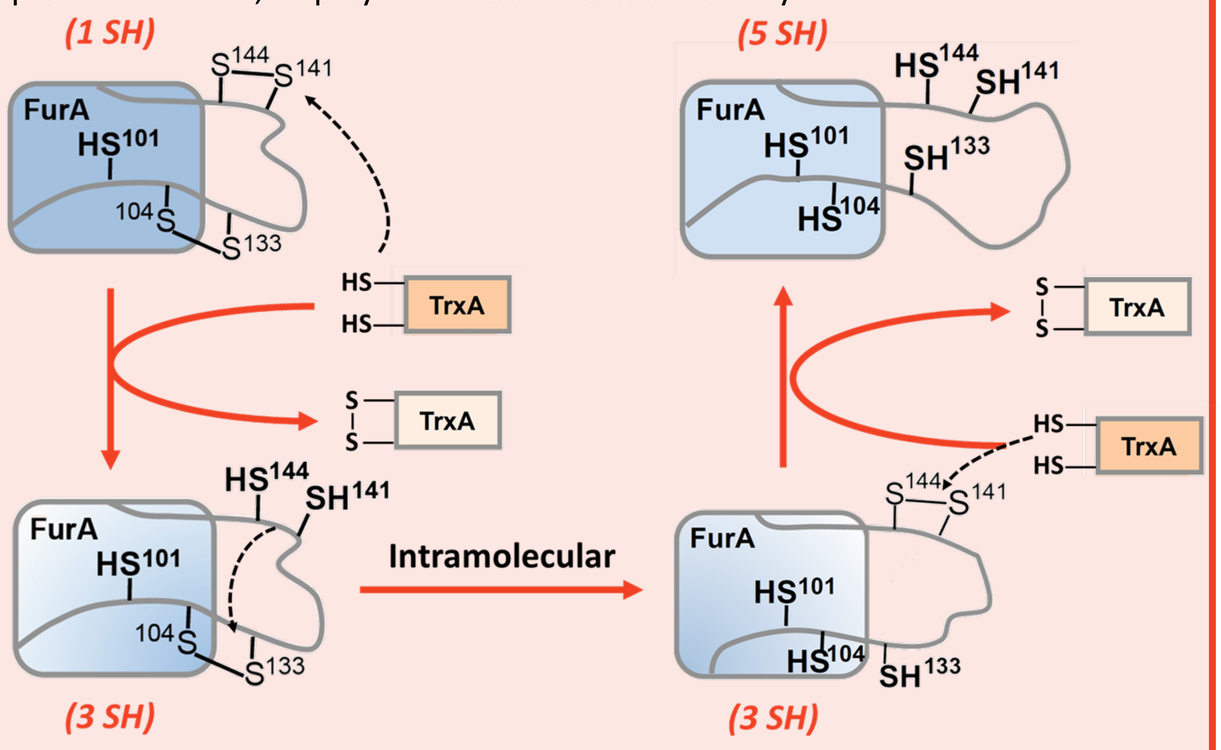

Figure 6. Proposed TrxA-mediated reduction mechanism of FurA from *Anabaena* sp. PCC7120. Arrowed dashed lines reflect the electron movement from dithiols to the disulfides.

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

CONCLUSIONS

- **TrxA** modulates the **different redox states** of FurA, which determine its ability to interact with DNA and different metabolites
- **Reversible changes** of **FurA redox states** in response to **light/dark transitions** link this regulator to **photosynthesis**

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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