Redox control of copper homeostasis in cyanobacteria

Luis López-Maury, Joaquín Giner-Lamia, Francisco J. Florencio*.

Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Américo Vespucio 49, E-41092 Sevilla, Spain.

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* Correspondence to:
Francisco J. Florencio, Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla-CSIC, Av Américo Vespucio 49, E 41092 Sevilla, Spain;

Telephone: +34 954489509; FAX: +34 954460065; E-Mail: floren@us.es
Abstract

Copper is essential for all living organisms but is toxic when present in excess. Therefore organisms have developed homeostatic mechanisms to tightly regulate its cellular concentration. In a recent study we have shown that CopRS two-component system is essential for copper resistance in the cyanobacterium *Synechocystis* sp. PCC 6803. This two-component regulates expression of a heavy-metal RND type copper efflux system (encoded by *copBAC*) as well as its own expression (in the *copMRS* operon) in response to an excess of copper in the media. We have also observed that both operons are induced under conditions that reduce the photosynthetic electron flow and this induction depends on the presence of the copper-protein, plastocyanin. These findings, together with CopS localization to the thylakoid membrane and its periplasmic domain being able to bind copper directly, suggest that CopS could be involved in copper detection in both the periplasm and the thylakoid lumen.

TEXT

Copper is an essential micronutrient that acts as a cofactor in fundamental processes like respiration and photosynthesis. The same redox properties that makes it an excellent metal cofactor also makes it extremely toxic when it is in excess, generating reactive oxygen species through Fenton-like reactions, destabilizing Fe-S clusters and competing for the binding sites of other metalloproteins.\(^1\,^2\) Furthermore, most metal-containing proteins will prefer to bind copper over other divalent metals in vitro, following the Irving-Williams series.\(^3\) These have forced living organisms to dedicate specific machineries to handle copper, ensuring that copper gets delivered to every copper containing protein and preventing spurious copper binding to other metalloproteins. In bacteria most copper
proteins are mostly located either in the plasma membrane or the periplasm, to avoid copper entering the cytosol. In these regard cyanobacteria are unusual among bacteria as they have an extra internal copper requirement, like most photosynthetic organisms (from cyanobacteria to higher plants), in the form of the blue-copper protein plastocyanin, the electron transport protein between the cytochrome $b_6f$ and photosystem I.

The pathway for copper incorporation in cyanobacteria has been analyzed mainly in *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*). Copper import is mediated by two P$_1$-type ATPases, CtaA and PacS, and a small soluble copper metallochaperone Atx1. These three proteins, together with glutathione, collaborate to deliver copper to the thylakoid lumen, where it is incorporated into plastocyanin and cytochrome c oxidase, preventing copper binding to undesired proteins. This proposed pathway is conserved in plant chloroplasts where two P$_1$-type ATPases are present in the inner chloroplast and the thylakoid membranes. Another protein that have been implicated in copper transport is FutA2, which electrophoretic mobility changes in the presence of the Cu$^+$-chelator, and a mutant in the corresponding gene is impaired in copper import and has reduced levels of intracellular copper proteins. CtaA and PacS are thought to transport reduced copper, although copper is present in the growth medium in its oxidized state, pointing to the existence of an unidentified copper reductase in cyanobacteria.

Until now nothing was known about copper resistance mechanism in cyanobacteria, but we have recently shown that CopRS (previously also known as Hik31/Rre34) two-component system is involved in copper resistance in *Synechocystis*. CopRS directly regulates a HME-RND export system (CopBAC; encoded by ORFs *slr6042, slr6043* and *slr6044*), its own
expression and a protein of unknown function CopM (encoded by ORFs sll0788 and slr6039) in response to an excess of copper in the media. CopS belongs to the membrane attached histidine kinases and we have shown that its periplasmic domain is able to bind copper with high affinity. In addition CopS is partially localized to the thylakoid membrane where it could be able to bind Cu^{2+} in the thylakoid lumen. This two-component has been previously suggested to play a role in redox regulation mediated by plastoquinone pool in *Synechocystis* based on its differential induction after DCMU (which allows cyclic electron flow) and DBMIB (which completely blocks electron flow) treatments.\(^9\) Later it was also shown to be induced under other conditions that alter the electron transport rate around PSI, such as sulfur and nitrogen starvation or low oxygen.\(^{10-12}\) We have shown that copper is strictly required for these inductions, suggesting that these are indirect effects of reduction of photosynthetic electron transport. Plastocyanin, which is the main copper containing protein in *Synechocystis*, it is also the major difference between photosynthetic electron transport chains copper replete and copper free medium. Plastocyanin alternates between it reduced and oxidized states during electron transfer (Figure 1), but accumulates in the oxidized state after DBMIB treatment (Figure 1B). Under this condition plastocyanin levels decrease and copper (that will be in its oxidized state) will be released in the thylakoid lumen where it could be detected by CopS, activating the CopMRS system. We have also shown that induction of *copMRS* partially depends on the presence of copper loaded plastocyanin, as mutants in the gene coding for plastocyanin (*petE*) or lacking both PacS and CtaA (which lacks copper loaded plastocyanin) showed reduced induction of the system. Why does CopS need to detect thylakoid copper levels? Plastocyanin have been estimated to be in millimolar concentration in the thylakoid lumen\(^{13}\) and therefore even degradation of a small amount of plastocyanin, after a reduction in the photosynthetic
electron flux (due to DBMIB treatment or nitrogen starvation), will release high amounts of free copper in the thylakoid lumen. Oxidized copper is very hazardous in this compartment due to presence of essential metal containing proteins in photosynthesis. CopS sensing domain will most probably face the thylakoid lumen where it could directly detect copper released from plastocyanin (which will be in its oxidized state). This mechanism ensure that the copper resistance system will be activated (through CopRS) when copper requirements are lower due to reduced plastocyanin contents. Hence, induction of the copper resistance system will prevent intracellular copper overload, even if no additional copper is added, and will protect the photosynthetic machinery in the thylakoid. Finally, CopRS could control other genes involved in copper homeostasis which have not been characterized yet, but that are expected to exist such as a copper reductase, additional copper transporters (besides CtaA and PacS), or a chaperone for cytochrome c oxidase assembly.

References


**Figure Legend**

**Figure 1**

Redox control of plastocyanin stability.

Schematic representation of the photosynthetic electron flow from H$_2$O to NADPH in *Synechocystis* sp. PCC 6803 in copper containing media: **A**, under normal growth conditions (with an active photosynthetic electron flow) the pool of reduced plastocyanin (PC-Cu$^{+}$) is higher than oxidized pool (PC-Cu$^{2+}$) or **B**, after addition of DBMIB (or other conditions that reduces the photosynthetic electron flow) the pool of reduced plastocyanin (PC-Cu$^{+}$) is lower than the oxidized pool (PC-Cu$^{2+}$). Under these conditions, oxidized plastocyanin is degraded and free oxidized copper is released to the thylakoid lumen. PSII, photosystem II; PQ, plastoquinone pool; b$_{6}$f, cytochrome b$_{6}$f complex; PC, plastocyanin; PSI, photosystem I; Fd, ferredoxin; FNR, ferredoxin-NADP$^{+}$ reductase; FQR, ferredoxin-quinone oxidoreductase.
A CYTOSOL

THYLAKOID LUMEN

B CYTOSOL

THYLAKOID LUMEN

2H₂O → O₂ + 4H⁺