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.

Addendum to:

Giner-Lamia J, López-Maury L, Reyes JC, Florencio FJ. The CopRS two-component system is responsible for resistance to copper in the cyanobacterium *Synechocystis* sp. PCC 6803. Plant Physiol 2012; 159:1806-18.

Submitted: 20 September 2012

Accepted:

Key words: copper, histidine kinase, two-component system, thylakoid, *Synechocystis*.

*** 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 mechanism 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 condition that reduces the photosynthetic electron flow and this induction depends of 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 Irwing-Williams series.[3] These have forced living organism 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_I-type ATPases, CtaA and PacS, and a small soluble copper metallochaperone Atx1.[4,5] 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.[6] This proposed pathway is conserved in plant chloroplasts where two P_I-type ATPases are present in the inner chloroplast and the thylakoid membranes.[7] 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.[8] CtaA and PacS are thought to transport reduced copper, although copper is present in the growth medium in it 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

1. Macomber L, Imlay JA. The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. Proc Natl Acad Sci USA 2009; 106:8344-9.
2. Robinson NJ, Winge DR. Copper metallochaperones. Annu Rev Biochem 2010; 79:537-62.
3. Waldron KJ, Robinson NJ. How do bacterial cells ensure that metalloproteins get the correct metal? Nat Rev Microbiol 2009; 7:25-35.
4. Tottey S, Rich PR, Rondet SA, Robinson NJ. Two Menkes-type atpases supply copper for photosynthesis in *Synechocystis* PCC 6803. J Biol Chem 2001; 276:19999-20004.

5. Tottey S, Rondet SA, Borrelly GP, Robinson PJ, Rich PR, Robinson NJ. A copper metallochaperone for photosynthesis and respiration reveals metal-specific targets, interaction with an importer, and alternative sites for copper acquisition. J Biol Chem 2002; 277:5490-7.
6. Tottey S, Patterson CJ, Banci L, Bertini I, Felli IC, Pavelkova A, Dainty SJ, Pernil R, Waldron KJ, Foster AW, Robinson NJ. Cyanobacterial metallochaperone inhibits deleterious side reactions of copper. Proc Natl Acad Sci USA 2012; 109:95-100.
7. Puig S, Penarrubia L. Placing metal micronutrients in context: transport and distribution in plants. Curr Opin Plant Biol 2009; 12:299-306.
8. Waldron KJ, Tottey S, Yanagisawa S, Dennison C, Robinson NJ. A periplasmic iron-binding protein contributes toward inward copper supply. J Biol Chem 2007; 282:3837-46.
9. Hihara Y, Sonoike K, Kanehisa M, Ikeuchi M. DNA microarray analysis of redox-responsive genes in the genome of the cyanobacterium *Synechocystis* sp. strain PCC 6803. J Bacteriol 2003; 185:1719-25.
10. Osanai T, Imamura S, Asayama M, Shirai M, Suzuki I, Murata N, Tanaka K. Nitrogen induction of sugar catabolic gene expression in *Synechocystis* sp. PCC 6803. DNA Res 2006; 13:185-95.
11. Summerfield TC, Nagarajan S, Sherman LA. Gene expression under low-oxygen conditions in the cyanobacterium *Synechocystis* sp. PCC 6803 demonstrates Hik31-dependent and -independent responses. Microbiology 2011; 157:301-12.
12. Zhang Z, Pendse ND, Phillips KN, Cotner JB, Khodursky A. Gene expression patterns of sulfur starvation in *Synechocystis* sp. PCC 6803. BMC Genomics 2008; 9:344.

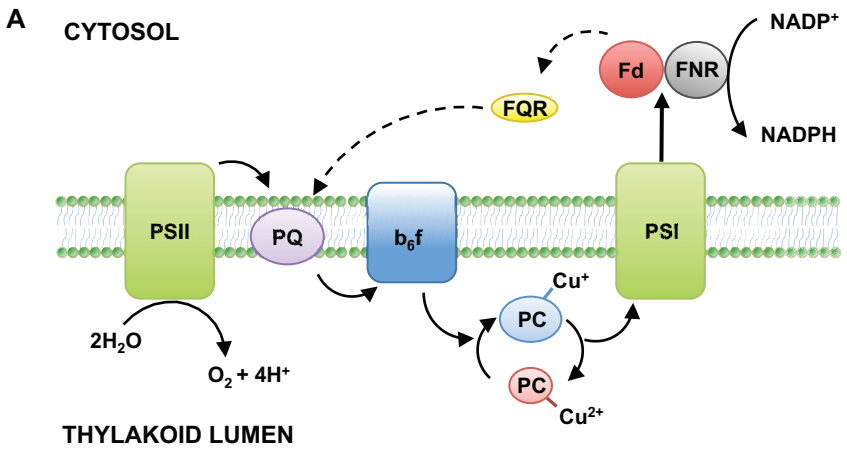
13. Finazzi G, Sommer F, Hippler M. Release of oxidized plastocyanin from photosystem I limits electron transfer between photosystem I and cytochrome b6f complex in vivo. Proc Natl Acad Sci USA 2005; 102:7031-6.

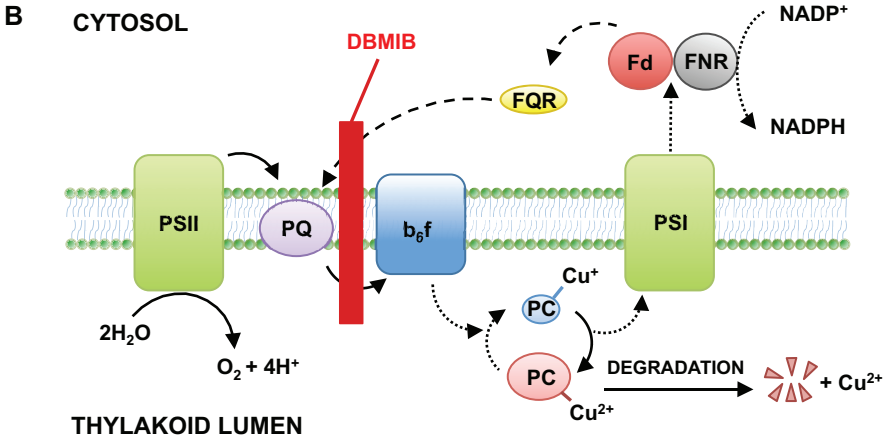
Figure Legend

Figure 1

Redox control of plastocyanin stability.

Schematic representation of the photosynthetic electron flow from H_2O 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 ($PC\text{-}Cu^{+}$) is higher than oxidized pool ($PC\text{-}Cu^{2+}$) or **B,** after addition of DBMIB (or other conditions that reduces the photosynthetic electron flow) the pool of reduced plastocyanin ($PC\text{-}Cu^{+}$) is lower than the oxidized pool ($PC\text{-}Cu^{2+}$). Under these conditions, oxidized plastocyanin is degraded and free oxidized copper is released to the thylakoid lumen. PSII, photosystem II; PQ, plastoquinone pool; b_6f, cytochrome b_6f complex; PC, plastocyanin; PSI, photosystem I; Fd, ferredoxin; FNR, ferredoxin-$NADP^{+}$ reductase; FQR, ferredoxin-quinone oxidoreductase.


A
CYTOSOL
NADP+
Fd
FNR
FQR
NADPH
PSII
PQ
b6f
PSI
Cu+
PC
2H2O
O2 + 4H+
PC
Cu2+
THYLAKOID LUMEN


B
CYTOSOL
DBMIB
NADP+
Fd
FNR
FQR
NADPH
PSII
PQ
b6f
PSI
Cu+
PC
2H2O
O2 + 4H+
PC
DEGRADATION
+ Cu2+
Cu2+
THYLAKOID LUMEN