

Peer-Review Record:

Metals in Cyanobacteria: Analysis of the Copper, Nickel, Cobalt and Arsenic Homeostasis Mechanisms

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Reviewer 1: Kevin Waldron

Reviewer 2: Anonymous

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First Round of Evaluation

Round 1: Reviewer 1 Report and Author Response

This manuscript is a review of recent developments in the study of metal homeostasis mechanisms (specifically Cu, Ni, Co, As) in cyanobacteria. It is generally good, covering all of the most important background and recent developments on the subject, with no obvious omissions.

One major issue needs to be addressed. In Section 2, Lines 111–130, the authors cover the potential role of CtaA as a copper importer. However, this model now seems unlikely, as the orientation of this P-type ATPase (with the N-terminal CopZ-like domain facing the cytosol) is inconsistent with a role in inward transport. See Raimunda *et al.* 2011, *Biometals* vol 24, pp. 467–475 (ref. 49 in the manuscript). Though such an import model for CtaA function was originally proposed, it now looks like both CtaA and PacS must transport copper out of the cytosol, yet also work in a linear, consecutive pathway in supplying copper to thylakoid-located plastocyanin, the periplasmic CucA, and membrane-located respiratory oxidase. This must presumably be related to the unusual subcellular organization of cyanobacteria. Clearly, the precise role of CtaA in cellular copper transport is unclear at this stage, but this section needs to reflect this ambiguity. Figure 1 should also be amended to incorporate this confusion in the current model.

Minor issues:

- The Irving-Williams series is mis-spelled throughout.
- On Line 141, it would make more sense to say “the soluble electron carrier cytochrome c6 is replaced by plastocyanin when copper is available”, as it is plastocyanin that contains copper, not c6.

Response: Thanks for your comments and criticism. All minor changes have been incorporated. The controversy about CtaA and PacS has now been included in the text and Figure 1 has been changed accordantly.

Round 1: Reviewer 2 Report and Author Response

The authors have done a good job producing a comprehensive review of elements of copper, nickel, cobalt, and arsenic homeostasis in cyanobacteria, particularly in the model organism, *Synechocystis*.

The following is a list of minor corrections, mainly spelling or grammatically errors, although there are some points that require clarification/ expansion, which I feel will be of benefit to the reader.

- Line 32: Impact should be plural
- Line 34: Perhaps homeostasis would be a better word than metabolism
- Line 41: Replace “much of the” with “many”
- Line 45: Replace “will be able to bind a metal unespecialy” with “bind metals with an order of affinity”, consider putting ‘for divalent cations’ in parenthesis
- Lines 54–55: “mine process” should be “mining processes”
- Line 62: Replace “is” with “are”
- Line 76: Metallothionein is misspelt
- Line 87: Oligoelement is not a commonly used word in English, perhaps replace with micro-nutrient (if I understand the context correctly)
- Line 106: Because of the cyt c6 switch, I don’t think plastocyanin can be described as essential
- Line 118: The sentence that starts “These phenotypes” seems to contradict the earlier assertions, as PacS would be localized to the plasma membrane and involved in the extrusion of copper from the cell
- Line 122: Replace “outside the cell” with “from the cell”
- Line 122: The fact that CtaA is required for copper to reach plastocyanin does not necessarily implicate CtaA as a copper importer
- Line 129: It was proposed that Fe(III) binding to FutA2 prevents aberrant binding of Fe(III) to sites for other metals including copper not “limits copper uptake”, the opposite
- Line 142: Replace “this regulation is known since a long time” with “this regulation has been known for a long time”
- Line 159: Replace “This allows CopS” with “This could allow CopS”
- Line 166: Thioredoxin is misspelt
- Figures 1 and 2: In these figures NrsC and CopC are represented as the outer membrane channels (OMC) of the HME-RND pumps, however neither of these two proteins display significant homology to OMCs (nrsC is predicted to encode a protein with similarity to lysozyme). Ideally

this should be noted in the figure legend or the authors should state on what basis they assign a role of these two genes as OMCs

- Line 173: There is an extra space before PSI
- Line 206: Cobalamin is misspelt
- Line 211: Replace “related with” to “related to”
- Line 214: Replace “based in” with “based on”
- Line 219: Maturation is misspelt
- Line 221: Remove domains
- Line 221: Earlier, it was stated that UreH and HupE/UreJ are related to NiCoT transporters. Here, it suggests HupE/UreJ represent another type of secondary transporter, please clarify
- Line 222: Secondary transporter, singular
- Line 232: Replace “consist in” with “consist of”
- Line 237: Replace “enumerated” with “described”
- Line 238: Replace “metals needs” with “metal needs” or “metals need”
- Line 241: The meaning of the sentence starting “In a bioinformatic screening” was very unclear, please restructure this sentence
- Line 243: Cobalamin is misspelt
- Line 244: Replace “change its expression” with “change expression”
- Line 254: The discussion of HspA and GroES seems a little out of sequence here, as you mention efflux pumps in the previous sentence and then resume the discussion of these pumps in the following paragraph. Consider restructuring this section slightly
- Line 262: Replace “The genes involved in these two metals resistance” with “The genes involved in resistance to these two metals”
- Line 266 and 267: Why are CorT and ZiaA described as putative cobalt and zinc ATPases?
- Line 267: ATPase, singular
- Line 269: Replace “by CorR (or CoaR), that is a MerR homolog” with “of CorR (or CoaR), a MerR homolog”
- Line 271: Transcription factor
- Line 285: Isn’t the nrsBACD operon also induced by cobalt?
- Line 286: The homology to methyl Co-M reductase is very interesting, but doesn’t this suggest that NrsS would be unlikely to bind nickel directly, but rather factor-430?
- Line 288: I think this should be F430
- Line 295: The nickel/cobalt sensing RcnR was discovered at a very similar time to copper sensing CsoR
- Line 296: Replace “residues to bind copper” with “copper binding residues”
- Line 307: Replace “where arsenic contents in drinking water are well over” with “where the arsenic content of drinking water is well over”
- Line 318: Replace “as electron acceptor in an anaerobic respiratory chain, electron donor” with “as an electron acceptor in anaerobic respiration, an electron donor”
- Line 321: Molecular oxygen
- Line 323: Replace “consist in” with “consist of”

- Line 334: Isn't this due to the redundancy of ArsI and ArsC rather than/ in addition to low expression?
- Line 336: Oxidative stress response
- Line 352: Replace “contain also” with “also contain”
- Line 359: This is a bit confusing, is arsB an ACR3 family member? It seemed in the earlier sentence that ArsB and ACR3 were different protein families
- Line 368: Replace “expression have been” with “expression has been”
- Line 370: Remove “it” from “what it seems”
- Line 376: Glyoxalase misspelt
- Line 382: Glyoxalase misspelt
- Line 383: Replace “influence in” with “influence on”
- Line 406: (s) following ion
- Line 407: Interaction between
- Line 410: Replace “use them as” with “use as”
- Line 414: Replace man with human or anthropogenic
- Line 418: Replace “accumulated” with “accumulate”

Like I said, there are mainly spelling or grammatical errors, which the authors can use or discard as they see fit, but the points of clarity should be addressed. Great review.

Response: Thanks for the exhaustive revision of our manuscript. Your comments and suggestions have been very fruitful and have much improved the review.

Minor changes have been incorporated.

- *Line 122: This has now been changed to clarify the direction of copper transport and an alternative explanation for the phenotypes observed is also suggested.*
- *Line 129: This has been now clarified.*
- *Figs. 1 and 2: These assignments are based on the mutant data, but not on homology. It is true that especially nrsC present a strong homology to lysozyme and that further experiments are needed to address this question. However, we think that for simplification, this is better to left as stated.*
- *Line 221: Earlier, it was stated that UreH and HupE/UreJ are related to NiCoT transporters. Here, it suggests that HupE/UreJ represent another type of secondary transporter. This has been changed according to the reviewer's suggestion.*
- *Line 254: This section has been changed as indicated.*
- *Line 285: Yes, but it has no effect on cobalt resistance and therefore, it could lead to the assignment of a wrong gene function.*
- *Line 286: That has been changed to clarify this.*