nature portfolio

Peer Review File

A ‘through-DNA’ mechanism for co-regulation of metal uptake and efflux

Corresponding Author: Professor Peng Chen

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Chakraborty et al. present evidence for a "through-DNA" model in the fine-tuning of the Zn-deficiency response in E. coli. In this model, a Zn-responsive transcription factor involved in activating Zn-efflux promoters can function as a non-conventional activator-of-sorts at promoters for Zn influx. In the apo-state, ZntR appears to be able to, presumably through a physical interaction with Zur and DNA, increase the unbinding of the Zn-responsive repressor Zur at target promoters. The authors use a combination of in vivo imaging and in vitro DNA-binding experiments to support their model and quantify the kinetics of these interactions. They also provide some evidence that this Zur-ZntR-DNA interaction model changes the response time of gene expression upon Zn depletion (based on single-cell imaging of a ZinT-GFP fusion). This study provides significant new insight into the regulation of Zn and sets the stage for follow-on experimentation on the systems-level impact of such a mechanism as a fitness advantage for balancing the essentiality and toxicity of metal nutrients.

The authors have adequately responded to my previous comments, and I only have minor suggestions remaining:

Pg 4 Line 33: “because the apo-state of wild-type ZntR cannot be rigorously achieved inside cells”, I would suggest deleting this statement. Alternatively, the sentence could be re-worded, such as “Because quantifying the metalated state of ZntR in vivo is challenging, we used…”

“Regulon” is usually used to refer to multiple genes. In the legend of Fig. 6, “a zur regulon” could be edited to “a Zur-regulated gene” or something.

Perhaps for the discussion: the manuscript focuses on the impact of ZntR on Zur dissociation from target promoters, but can the authors simulate what impact this interaction would have on ZntR-binding to its cognate promoters? Or do the authors speculate that binding of ZntRapo to Zur-bound promoters (and thus reducing [ZntR] available to bind at Zn-efflux promoters), would have a negligible impact on ZntR binding Zn-efflux promoters when [Zn] increases in the cell?

Throughout, to help the reader, temporary compounds used as adjectives could be hyphenated as follows:
Zn-uptake regulation
Zn-uptake genes
Zn-efflux genes
Zn-excess conditions
Zn-deficient environment
And elsewhere

(Remarks on code availability)

Reviewer #3

(Remarks to the Author)
I have read the revised version of the manuscript, supplementary information and responses and believe that the authors have satisfactorily addressed all raised concerns.

(Remarks on code availability)

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

Chakraborty et al. present evidence for a "through-DNA" model in the fine-tuning of the Zn-deficiency response in E. coli. In this model, a Zn-responsive transcription factor involved in activating Zn-efflux promoters can function as a non-conventional activator-of-sorts at promoters for Zn influx. In the apo-state, ZntR appears to be able to, presumably through a physical interaction with Zur and DNA, increase the unbinding of the Zn-responsive repressor Zur at target promoters. The authors use a combination of in vivo imaging and in vitro DNA-binding experiments to support their model and quantify the kinetics of these interactions. They also provide some evidence that this Zur-ZntR-DNA interaction model changes the response time of gene expression upon Zn depletion (based on single-cell imaging of a ZinT-GFP fusion). This study provides significant new insight into the regulation of Zn and sets the stage for follow-on experimentation on the systems-level impact of such a mechanism as a fitness advantage for balancing the essentiality and toxicity of metal nutrients.

The authors have adequately responded to my previous comments, and I only have minor suggestions remaining:

Pg 4 Line 33: “because the apo-state of wild-type ZntR cannot be rigorously achieved inside cells”, I would suggest deleting this statement. Alternatively, the sentence could be re-worded, such as “Because quantifying the metalated state of ZntR in vivo is challenging, we used…”

[**Reply**] Thank you so much for your suggestion. We have re-worded this sentence accordingly.

“Regulon” is usually used to refer to multiple genes. In the legend of Fig. 6, “a zur regulon” could be edited to “a Zur-regulated gene” or something.

[**Reply**] Thank you for the comment. We have made the correction.

Perhaps for the discussion: the manuscript focuses on the impact of ZntR on Zur dissociation from target promoters, but can the authors simulate what impact this interaction would have on ZntR-binding to its cognate promoters? Or do the authors speculate that binding of ZntRapo to Zur-bound promoters (and thus reducing [ZntR] available to bind at Zn-efflux promoters), would have a negligible impact on ZntR binding Zn-efflux promoters when [Zn] increases in the cell?

[**Reply**] Thank you for the comment. There are limited number of genes regulated by Zur (e.g., four known ones), while the copy number of ZntR is in many tens to hundreds in the cell. Therefore, the impact of ZntRapo-binding to Zur promoters should have minimal effect on [ZntR] availability. This would be more true with increasing [Zn] in the cell, as holo-ZntR appears to not bind to Zur promoters (Fig 5c,d).

Throughout, to help the reader, temporary compounds used as adjectives could be hyphenated as

follows:
Zn-uptake regulation
Zn-uptake genes
Zn-efflux genes
Zn-excess conditions
Zn-deficient environment
And elsewhere

[**Reply**] Thank you for the comment. We have made the correction.

Reviewer #3 (Remarks to the Author):

I have read the revised version of the manuscript, supplementary information and responses and believe that the authors have satisfactorily addressed all raised concerns.

[**Reply**] Thank you.