Review of: "TrmB family transcription factor as a thiol-based regulator of oxidative stress response"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

Review comments

TrmB family transcription factor as a thiol-based regulator of oxidative stress response
Paula Mondragon et al.

This paper identifies the function of TrmB family transcription regulator with about 123 amino acid lengths that have not yet been known. The site of action controlled by candidate transcription regulator without the C-terminal in Halophilic archaea was discovered through ChIP-Seq, and interestingly, it was found to have a dual function of an activator and an inhibitor. In particular, it is a transcription regulator sensitive to thiols in vivo, which has not been known in this field study, and has a characteristic selectively induced by hypochlorite.

The manuscript is judged to have high completeness overall in terms of experimental processes and results derivation, and the discovery of new functions is a challenge to expand our knowledge.
Detailed comments are described below.
OxsR exhibits an activator and an inhibitor regulation. OxsR is the smallest size TrmB-like regulator which first time characterized one.

OxsR binding sites on different locations in promoter regions in targets from ChIP-Seq for specific binding and non-specific binding because some candidate's sites revealed GC-rich sequence but others did not. Maybe there is several possible technology to show direct interaction ratio between specific or non-specific binding sites.

Targets have participated in thiol relay and small thiol compound biosynthesis. What are real LMW thiols in OxsR sensing?

OxsR revealed a homodimeric form using dimerization helix under hypochlorite stress conditions. N-terminal helix near DNA recognition included a conserved Cys residue which made a covalent bond. Does this disulfide bond help dimerization of conformation or DNA-binding? Do you detect disulfide binding between C residues?

Why OxsR specifically sensed by hypochlorite but not any other oxidative resources such as H2O2 and others? Can OxsR has directly sensed hypochlorite? Or corroborate other interaction partner for hypochlorite sense?

Please choose the single term qRT-PCR or RT-qPCR.

<In Discussion>
What is the evidence for OxsR binding partners for GC-rich motif recognition?

What are the dimerization inducers for OxsR? Peroxide, NO, and oxidized glutathione are enough? Why not H2O2?

What is the meaning of the nitrosylation of the Fe-S cluster?

It is correct to enter Lrs14 (Lsr14 is written in the text).

Phosphosites? Phosphorylated sites of AbfR1?

<In Materials and Methods>
Usually used NaOCl. But HOCl was also treated?

For the ChIP-Seq, 5 ml precultured cell (OD600 ~1,0) inoculated to 100 ml GMM contained 2.5 mM NaOCl and incubated for 20 min, then OD reached log phase (0.3~0.5). I guess this protocol is just diluted to OD600 of 0.5. How to decide the log phase because preculture OD600 of 1.0 is too enough stationary phase samples, isn't it?

Please use the single term H. volcanii or Hfx. volcanii.

At western blotting analysis, what is 18? 18 hours maybe or other units?

Where is came from BSA, Sigma Life Science or Sigma Aldrich, or Life Science?

In Acknowledgements, AS or AKS for Fund receiver.

Fig. 2. Why oxsR-disrupted mutants revealed two growth curves, curve 1 and curve 2. Do you find any clues this phenomenon? Need to explanation.
In Fig. 2, why curve 2 was grown up at 4 replicates in deleted oxsR mutant and OxsR C24A at 1 replicate, too? Gene deletion method used markerless deletion. Thus, maybe there are several unexpected mutations inclusive?
In text: "By contrast, the ΔoxsR mutant displayed no growth (curve 1, 8 replicates) or recovered after an extended lag (117 ± 23 h) (curve 2, 4 replicates) under these same conditions (Figure 2B). All replicates of the mutant retained the

markerless deletion of the oxsR gene, consistent with whole genome resequencing analysis which revealed oxsR to be deleted from all genomic copies (Figure S2A-C, Table S1). One distinction was identified among the ΔoxsR mutant replicates: those that that did not recover from hypochlorite stress initially (curve 1) were no longer viable, whereas the replicates that displayed an extended lag (curve 2) were viable, **but no longer able to recover after exposure to a second dose of hypochlorite and were not viable once this stress agent was removed (Figure S2D).** When performing complementation assays, the ΔoxsR mutant was found to be uniformly hypersensitive to hypochlorite in the presence of the empty vector, suggesting that the plasmid posed an extra burden to the cells under these conditions (Figure 2B). Expression of the oxsR gene from this multicopy plasmid restored the ΔoxsR mutant to recover from the hypochlorite stress at parental levels (Figure 2B). **This finding suggests that the second site point mutation observed in the ΔoxsR genome sequence (non-coding intergenic G>A mutation between hvo_RS01570 and hvo_RS01575, Table S1) was not the source of the observed hypochlorite recovery defect of ΔoxsR.** Based on these results, OxsR is important for the recovery of H. volcanii from hypochlorite stress. Furthermore, the minimal effect of the C-terminal HA-tag on OxsR function during hypochlorite stress (Figure 2B) indicated this construct could be used for downstream immunoprecipitation analysis."

- Thus, need for a concrete conclusion for the recovery growth of OxsR deficient mutants. Is it the same mutation between curve 2 and recovery of C>A mutation with HA tag.
- It would have been more helpful to understand if I could see the supplementary data.

Fig. 7B

In text: "The anti-HA antibodies were found to be specific to the strains expressing OxsR-HA variants, as no signal was detected in the parent strain devoid of the HA tag. Furthermore, on visual inspection of the immunoblots, the OxsR-HA C24A and OxsR-HA were comparable in protein abundance, suggesting the C24A modification did not impact OxsR expression or stability. While amino acid exchange at C24 did not alter OxsR protein abundance, it did eliminate the ability of OxsR to facilitate the recovery of cells and upregulate the level of hvo_1043 transcript during hypochlorite stress. Thus, C24 appears to be important for OxsR function as a transcriptional activator when strong oxidants of cellular thiols are introduced into the environment."

- It would have been clearer if the positive control experiment (an induced band in total lysates) had been included.

In text: "Arrangement of the 3D-model into a homodimer was by comparison to the X-ray crystal structure of the biofilm regulator Sulfolobus acidocaldarius AbfR2 (Saci_1223; PDB: 6CMV). DNA interactions were predicted by comparison to the Xray crystal structure of the Streptococcus pneumoniae FabT:DNA complex (PDB: 6JBX)."

- To understand the binding properties, I am not sure how certain the structural prediction is by comparing it with other known protein structures. Additional information on comparative proteins is required.

- Minor comments:

In Important at Page 2, OxxR changed to OxsR

In reference number 9, please fill volume and paper

- It is difficult to indicate the exact location because there is no side line mark.