RosR's Substitutes in the *H. salinarum* Regulatory Network Under ROS Conditions

Abstract

Regulatory networks made up of genes regulated by transcription factors are essential to an organism's survival response to abiotic stress. In past research, RosR has been identified as the primary transcription factor that drives *H. salinarum*'s response to oxidative stress. This poster explores other parts of the response network structure, specifically analyzing the other transcription factors that have a significant role in the oxidative stress reactionary mechanism. This includes understanding secondary or parallel reactionary mechanisms in the wild type that are not directly regulated by RosR, and exploring the regulatory network of cellular response to oxidative stress in a RosR-deleted mutant type. Further data and analysis will be needed to properly construct the full regulatory network in *H. salinarum.*

Methods

- Separated genes into bound and unbound by RosR
  - Filtered ChIP-chip data with a p-value of 0.05
- Performed histogram analysis to determine statistically significant gene expression to build sets of relevant genes
  - Used difference in expression levels between wild and mutant type as well as wild and mutant type gene expression individually
- Compared gene sets to identify critical genes, gene functions, and transcription factors in secondary ROS response network

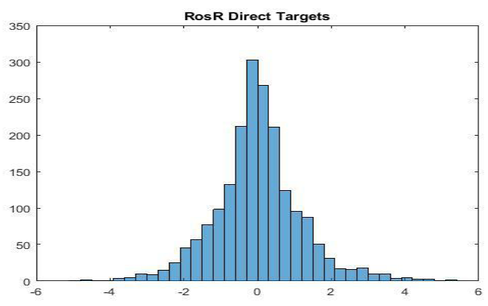


Figure 1. Example histogram found during our histogram analysis. This shows an example of difference analysis between the wild and mutant type (p-value = 0.05).

Results

Initial ROS Response Analysis

- Explored activity of RosR-bound genes in mutant types
- Figure 2 shows the results of hierarchical, complete-linkage clustering of the gene expression data.

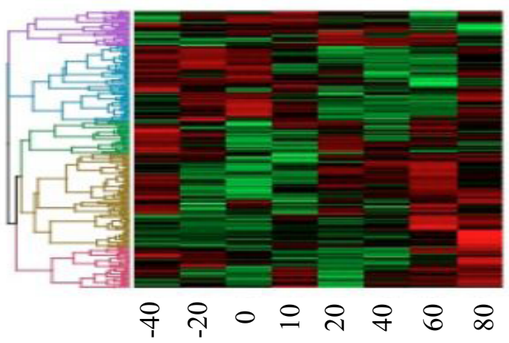


Figure 2. Dendrogram of RosR-bound genes in mutant type, clustered by gene expression in response to oxidative stress, and heatmap of gene expression at different time points of ROS exposure response

- Identified five clusters of gene expression timelines
- Contradicts expectation of a large number of clusters with very few genes.
- Despite RosR-deletion in the mutant type, RosR bound genes were still involved in coherent regulatory network in response to oxidative stress.
- This conclusion served as justification to continue with our analysis.

Results (cont.)

Oxidative Stress Response Secondary Network Structure

Figure 3. Venn Diagram of RosR indirect targets and genes active in response to ROS in the **wild** type.

Figure 4. Venn Diagram of RosR indirect targets and genes active in response to ROS in the **mutant** type.

Our analysis yielded three critical datasets:

- **Group A:** Non-RosR targets that are active in the wild type. These genes and their common transcription factors make up the secondary and parallel regulatory networks in the wild type (Green Region of Figure 1).
- **Group B:** Non-RosR targets that are active in the mutant type. These genes and their common transcription factors make up the definitive substitutional oxidative stress response when RosR is not present. (Green Region of Figure 2).
- **Group C:** RosR targets that are active in response to ROS in the mutant type. The most common transcription factors amongst those genes may be direct substitutes for RosR in the mutant type - mimicking RosR's role when it is not present. (Yellow Region of Figure 2).

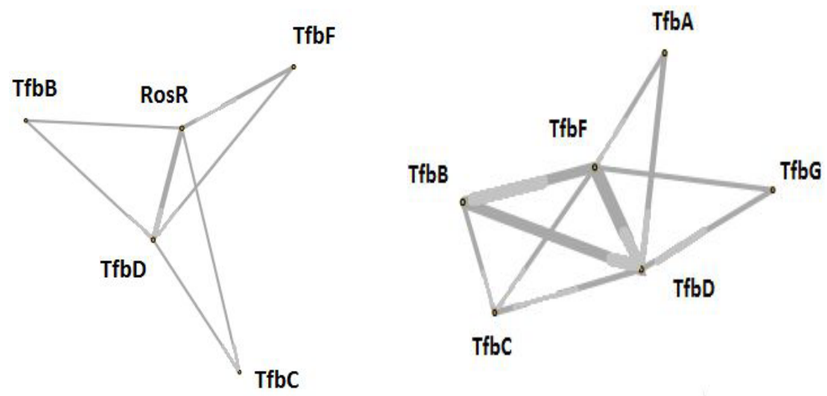
arCOG Analysis of Secondary Network Structure

Table 1. Functions of identified gene groups

Genes	Function	Probability	Expect	Count
Group A	Amino acid transport and metabolism	0.086291	21.82081	27
	Function unknown	0.008289	34.83673	47
	General function prediction only	0.000408	45.36431	65
	RNA processing and modification	0	0.191411	1
Group B	Defense mechanisms	0.036221	1.279526	3
	Nucleotide transport and metabolism	0.085284	2.369493	4
	RNA processing and modification	0	0.04739	1
	Amino acid transport and metabolism	0.088912	19.16179	24
Group C	Function unknown	0.001712	30.59163	45
	General function prediction only	0.02876	39.83636	50

Results (cont.)

Transcription Factor Network Analysis



a) **b)**

Figure 5. a) Transcription factor network for RosR direct targets (Weight= 10). b) Transcription factor network for Group C (Weight = 5). Line thickness indicates relative number of common gene targets

- TfbD, TfbF, and TfbB have the highest number of common targets with RosR
  - Have potential to replicate RosR transcription patterns
- TfbD, TfbF, and TfbB are the most common transcription factors amongst genes in group C
  - Heavy involvement in regulating RosR target genes in ROS response when RosR is not present

Conclusions

- Discovered 104 RosR direct targets - similar to Tonner's discovery "of over 100 target genes" (Tonner et al., 2015)
- Identified gene sets and associated common transcription factors that make up:
  - secondary and parallel regulatory network structure in wild type in response to oxidative stress
  - substitutional regulatory network in response to oxidative stress when RosR is not present
  - direct substitutes for RosR when RosR is not present
- TfbD, TfbF, and TfbB may act as potential substitutes for RosR in response to oxidative stress in the mutant type

Further Analysis

- Need to identify transcription factors associated with identified gene sets relevant to ROS stress
- Limited by scope of ChIP-chip dataset
  - Only contains data for other transcription factors under non-ROS conditions
- Requires ChIP-chip data for many transcription factors under condition of oxidative stress

References

1. Sharma, K., Gillum, N., Boyd, J. L., & Schmid, A. (2012). The RosR transcription factor is required for gene expression dynamics in response to extreme oxidative stress in a hypersaline-adapted archaeon. *BMC genomics*,*13* (1), 1.
2. Tonner, P. D., Pittman, A. M., Gulli, J. G., Sharma, K., & Schmid, A. K. (2015). A Regulatory Hierarchy Controls the Dynamic Transcriptional Response to Extreme Oxidative Stress in Archaea. *PLOS Genet*, *11*(1), e1004912.