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Determining Gene Specific Chromatin Differences in *Sulfolobus solfataricus*: Expression of MerR Protein for Targeted-ChIP Antibody Production

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Determining Gene Specific Chromatin Differences in Sulfolobus solfataricus: Expression of MerR Protein for Targeted-ChIP Antibody Production

Erica North, Sophie Payne, Sam McCarthy, Tyler Johnson, Paul Blum

Abstract

In this project the repressor protein MerR from the *Sulfolobus solfataricus* mercury resistance operon was cloned into pET28b and transformed into Rosetta 2 *E. coli* strains for overexpression and purification. Large quantities of recombinant MerR will be used for subsequent injection into a mammalian host for antibody production. These antibodies will be used in targeted-ChIP studies in which gene specific chromatin modification states will be analyzed. The overproduction of MerR is part of a larger project where future research could produce data on whether gene expression levels and chromatin modification states could be correlated at an individual gene level, possibly suggesting a novel epigenetic mechanism in Archaea.

Background

Sulfolobus solfataricus is a thermoacidophile crenarchaeon with an optimum temperature of 80C and pH of 3.0. Three separate lineages of super acid resistant strains of the crenarchaeaon (SARC) *Sulfolobus solfataricus* have been previously derived through adaptive laboratory evolution by serial passaging in increasing acid. This resulted in mutant strains which are capable of growth at pH 1, which greatly exceeds the capacity of the parental. These SARC strains have inheritable phenotypes and transcriptomes, however genomic analysis of 3 independently evolved SARC strains showed no conserved mutations. This suggests that the novel phenotype isn't due to changes in the genomic sequence.

Many abundant chromatin proteins are known to exist in *Sulfolobus solfataricus*. While there are no conserved genetic mutations in the SARC genome that accounts for the change in phenotype observed, it has been found that the abundant chromatin proteins Cren7 and Ssso7d are hypomethylated in the SARC strains as compared to the parental. The conservation of phenotype, transcriptomics and chromatin modification state and lack of conservation in mutations, suggest a potential relationship between the modification states of chromatin and the heritable phenotype of SARC. The mercury resistance operon in *Sulfolobus solfataricus* transcribes genes that are responsible for detoxifying high levels of mercury in the cell. The operon is induced as a stress response in the SARC strains, however it is not part of the conserved transcriptome of the SARC strains as it reverts back to normal levels of expression when the SARC strains are grown at pH 3. The operon has a repressor (MerR) which has been shown to very tightly bind its operator (*merO),* a characteristic which will be utilized in future experiments.

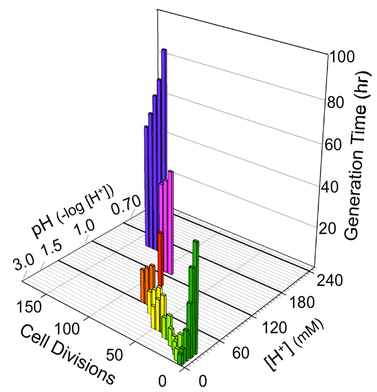


Fig. 1 Evolution of SARC strains to extreme thermoacidophily through adaptive laboratory evolution by serial passaging in acid. The strains saw a 178 fold increase in acid resistance.

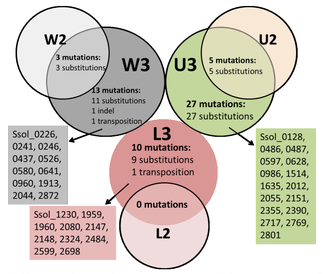


Fig. 2 Conservation of the overlapping mutations are presented as a venn diagram. Overlapping regions indicate common mutations. No common mutations were seen between lineages.

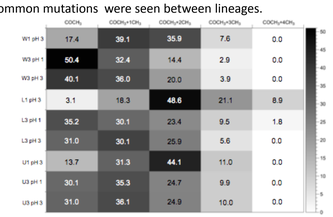
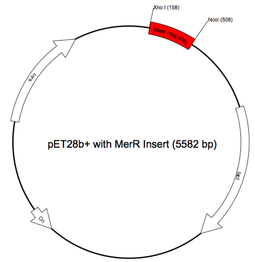


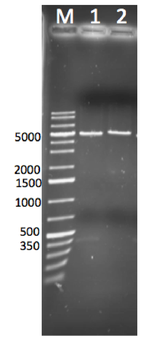
Fig. 3 Hypomethylation of Cren7 occurred independently in all evolved cell lines.

Results



Lanes
pET28b+:MerR(new start codon) in Rosetta 2
1- Crude
2- Heat fractionated

Expected Masses KDa
MerR 12 KDa



Lanes
M- Generuler 1 kb Plus ladder
1- pET28b+: MerR isolate double digested with NcoI/XhoI
2- pET28b+: MerR isolate single cut with NcoI

Expected Masses kb
MerR 350 bp
pET28b+ ~5,400 bp

Fig. 4 MerR was successfully cloned into pet28b+ as proven by the dropout on the DNA gel seen at 350 bp. It was successfully recovered in both the crude and heat fractionated samples, and purified in the heat fractionated sample.

Discussion and Future Goals

Recombinant MerR protein was successfully overproduced and purified. Scaled up cultures will produce enough MerR for use as an antigen for antibody production in a mammalian host. These antibodies will be used in a method called targeted ChIP, which will allow us to isolate specific gene regions and their associated chromatin. This chromatin can then be analyzed to determine how chromatin identity and modification state correlate with heritable transcriptomic patterns. Genes in the SARC transcriptome that were highly upregulated, downregulated and unaltered will be tested. We will then use targeted ChIP on these genes. This method is based on the idea that we can clone in *merO* next to genes of interest, and using the high binding affinity between *merO* and MerR we will be able to immunoprecipitate out regions of DNA that contain *merO*. Once these regions are successfully isolated, the chromatin proteins will be removed and run through both intact mass and fragmented peptide mass spectrometry to determine the quantity of the proteins, the identities of the proteins and their modification states .

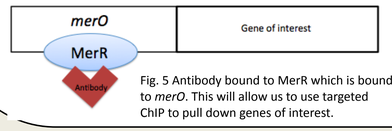


Fig. 5 Antibody bound to MerR which is bound to *merO*. This will allow us to use targeted ChIP to pull down genes of interest.

We hope to use this data to establish a correlation between the expression level and chromatin modification states of individual genes. This could provide solid evidence for a novel mechanism of epigenetics in Archaea.

Methods

MerR was cloned into the pet28b+ vector and transformed into Rosetta 2 E. coli strains. The E. Coli was then grown in liquid LB media with 100 µg/mL kanamycin and 25 µg/mL chloramphenicol and induced with 1mM IPTG to produce MerR. 50 mL of the induced media was then pelleted and resuspended in lysis buffer (20 mM $NaPO_4$, 0.5 M NaCl, 20 mM Imizadole and 1 mM Mercaptoethanol at pH 7.4). The solution was centrifuged at 8000 G for 30 minutes at 4°C to precipitate insoluble materials and the supernatant transferred to a new tube. Since MerR is a thermophilic protein expressed in the mesophile E.coli, the difference in temperature stability can be used as a purification step. The supernatant was subjected to heat fractionation by incubation in tubes in 90°C H_2O for one hour, which denatured the majority of E.coli proteins. The samples were then centrifuged one final time at 8000 G for 30 minutes at 4°C and the supernatant was transferred to a new tube. Purified extract was run on a 16% SDS-PAGE gel and bands were visualized using Coomassie Blue.

References

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