

Atypical Iron Sulfur Cluster Biosynthesis in Sulfate Reducing Bacteria

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Project Goals: One of the key aims of Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) is to obtain insight into the functionally important activities of key microbes and their interactions in the environment. ENIGMA investigators are specifically interested in how microbes respond to environmental changes and have developed techniques to enable evidence-based annotation of gene function using high throughput mutagenesis and phenotyping of mutants under a range of stress conditions. Here, using the model sulfate-reducing bacterium *Desulfovibrio vulgaris*, our goal was to utilize a combination of traditional biochemistry and molecular biology together with a high throughput functional genomics approach to screen for genetic interactions. Together these data are permitting us to gain detailed insight into the key essential process of iron sulfur cluster biosynthesis, which is known to be impacted by environmentally encountered stresses.

Iron sulfur (FeS) cluster containing proteins make essential contributions to many key cellular processes but can readily be damaged by environmentally encountered oxidative stress, exposure to toxic metals or reactive N-oxyanions. Damage of key FeS enzymes such as pyruvate-ferredoxin oxidoreductase (PFOR) has been proposed as the source of inviability of sulfate reducing bacteria (SRBs) and other anaerobes when encountering oxygen. Using *Desulfovibrio vulgaris* Hildenborough (DvH) as a model SRB, we have characterized DvH strains harboring mutations in known FeS cluster biosynthesis factors and assayed the relative contributions of these factors to FeS cluster biosynthesis by monitoring activity of FeS dependent enzymes including PFOR. To date, enzymatic assays suggests that mutations in DVU1021(*sufB*), DVU1382 (*sufA*) and DVU0664 (*nifS*) have a detrimental effect on multiple FeS enzymes, although functional redundancy between biosynthesis factors appears to be significant. Only DvH strains lacking DVU1021 or DVU1382 displayed severe growth defects in rich growth media, consistent with the hypothesis that the SufBC predicted FeS scaffold complex is the likely primary site of *de novo* FeS cluster assembly in DvH and may work with DVU1382 to mature multiple FeS enzymes, deficiencies in which can impact the growth rate of the cell. In order to fully uncover the relationships between DvH FeS cluster biosynthesis factors, we have utilized a TnSeq-based procedure (RB-TnSeq; [1]) to conduct high throughput genetic interaction screening. We have observed synthetic lethality between SufBC and NifSU systems (no *sufB/C* transposon insertions in *nifS/U* background or *v.v.*) which is consistent with partial

functional redundancy. Strikingly, the observed viability of a *nifS* mutant in DvH where it is predicted to be the sole cysteine desulfurase, contradicts current FeS cluster biosynthesis dogma regarding the essentiality of cysteine desulfurases as a sulfur source for *de novo* FeS cluster formation. Further work is ongoing to confirm *nifS* genetic interactions.

References

1. Wetmore, K.M., et al., *Rapid quantification of mutant fitness in diverse bacteria by sequencing randomly bar-coded transposons*. MBio, 2015. 6(3): p. e00306-15.

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