Regulons, Uptake and Salvage Pathways that Mediate Metabolite Exchange in Hot Spring Microbial Mats.

Dmitry A. Rodionov[1]* (rodionov@burnham.org), Frank Collart,[2] Aaron T. Wright,[3] Margie Romine,[3] Jim Fredrickson,[3] **Andrei L. Osterman**[1]

[1]Sanford-Burnham Medical Research Institute, La Jolla, CA; [2]Argonne National Laboratory, Argonne, IL; [3]Pacific Northwest National Laboratory, Richland, WA

Project Goals: The objective of the Pacific Northwest National Laboratory Foundational Scientific Focus Area (FSFA) is to develop a predictive, genome-enabled understanding of how microbial interactions impart stability, robustness, and functional efficiency to microbial communities. As part of this multi-institutional effort, we are utilizing genomic sequence to identify candidate systems that mediate and control the synthesis and secretion of chemical commodities by the autotroph and the corresponding systems that enable their uptake and salvage by interacting heterotrophs. By compiling this knowledge and employing various experimental methods to validate novel functional predictions, we intend to improve our ability to predict the nature of beneficial partnerships that occur between microorganisms that occupy the same niche.

Research is being conducted on three separate microbial communities; phototrophic mats that occur in alkaline siliceous hot springs in Yellowstone National Park (YNP) or a meromictic, hypersaline lake in northern Washington state and chemotrophic mats that occur in acidic hyperthermophilic springs in YNP. As sequence becomes available from organisms (or their near relatives) that occupy these communities we use integrative genomics-based reconstruction and experimental assessment of metabolic and regulatory networks to understand metabolic interactions and associated regulatory interactions in the target microbial communities. Our general workflow includes: (i) the subsystems-based genomic reconstruction of selected pathways and regulons; (ii) identification of currently unknown components of these pathways, primarily transporters, sensory and regulatory proteins using comparative genomics and high-throughput -*omics* data; (iii) experimental testing of selected functional predictions combining biochemical and genetic methods; and (iv) use model co-cultures to assess metabolic exchange in microbial communities.

Our current research focuses on members of the Cyanobacteria and Chloroflexi phyla that were isolated from or related to isolates detected in YNP phototrophic mats. In these mats, anoxygenic phototrophic bacteria (e.g., *Roseiflexus*, *Chloroflexus*) are thought to function predominantly as photoheterotrophs using metabolites produced by cyanobacteria (e.g., *Synechococcus*) as carbon sources. Whereas typical metabolic byproducts may provide a major flux of carbon and energy, vitamins (precursors of key cofactors) are required in relatively small amounts. We hypothesize that vitamin exchange may be rather widespread phenomena contributing to "opportunistic" relationships between species in mat communities. In a previous genomic survey, we observed a mosaic distribution of *de novo* and salvage pathways for biogenesis of major vitamins (such as niacin, pantothenate, biotin, thiamin, riboflavin) that leads to the presence in communities of the strict auxotrophs and strict prototrophs with respect to one or another vitamin. In this work, we focused on reconstruction of metabolic pathways and regulons involved in metabolite salvage and exchange in two groups of environmental bacteria, Chloroflexi and Cyanobacteria. Several co-cultures (e.g., *Synechococcus sp.* PCC 7002 and *Roseiflexus castenholzii*) have been established by combining isolates available in pure culture and thus provide tractable models for studying naturally occurring interactions.

In Cyanobacteria, we used the comparative genomic approach to reconstruct regulons for 18 transcription factors (including 14 previously known in the literature) and 10 riboswitches. This approach led to the discovery of a putative B_{12} transport uptake system (BtuFCD) in *Synechococcus sp.* PCC 7002. This strain lacks the ability to synthesize B_{12} and thus is dependent on acquiring it from other microbes that are able to produce it. The transporter prediction will be validated through knock-out construction (in collaboration with Don Bryant) and *in vitro* assay. In addition to using regulon/subsystem analysis to discover additional novel B_{12} transporters we will use chemical probe profiling to discover them experimentally.

In Chloroflexi, a repertoire of all transcription factors and environmental sensors encoded in 5 sequenced genomes from this phylum was identified and analyzed. Several bioinformatics workflows are used for identification of conserved DNA and RNA motifs and *ab initio* reconstruction of associated regulons. Currently this approach resulted in reconstruction of 20 regulons operated by either transcription factors or riboswitches, and the number of reconstructed regulons is growing.

The reconstructed regulons lead to the discovery of multiple novel uptake transport systems for essential vitamins (thiamin/B_1, riboflavin/B_2, B_{12}), metal ions (manganese, zinc, ferrous iron, molybdenum, cobalt), and carbohydrates (e.g., rhamnose). These novel transport systems fill the gaps in the respective metabolic pathways. Several examples of novel vitamin uptake systems were selected for further experimental validation. First, a putative B_2 transporter (RibXY) complements the absence of the *de novo* riboflavin biosynthesis pathway in *Roseiflexus* spp. The riboflavin transfer to *Roseiflexus* could be important in co-culture with B_2-prototrophic strains *Synechococcus* and/or *Thermochlorobacter*. Second, we identified putative B_1 precursor transporters (ThiXYZ, ThiW, CytX) that are required for B_1 salvage pathways in the auxotrophic bacteria from the Chloroflexi phylum. The ABC transporter predictions (ThiY, RibY) will be validated through *in vitro* assay. Third, we found a novel ECF-type transport system of yet unknown specificity that is involved in B_{12} metabolism in Chloroflexi. Finally, we found multiple predicted transport systems that are involved in the uptake of biometals (Fe, Zn, Mn, Co, Mo). They are regulated by committed transcription factors to maintain the cell homeostasis.

L-rhamnose is an essential component of the external polysaccharide layer in the cyanobacterial cells. Analysis of *Chloroflexus/Roseiflexus* genomes revealed a conserved gene cluster encoding enzymes from the rhamnose catabolic pathway. We hypothesize that transfer of carbon from YNP *Synechococcus* spp. to YNP *Chloroflexus* via L-rhamnose is an example of metabolite exchange that occurs in native YNP mats. We have identified a novel ABC-type transport system (RhaGHJF) and a DeoR-type transcription factor (RhaR) encoded in the rhamnose gene cluster and propose that they are involved in uptake and sensing of L-rhamnose to induce the catabolic pathway. The RhaR DNA binding sites were predicted and the respective rhamnose regulon was reconstructed by comparative genomics. Experimental validation of predicted functional roles of the novel rhamnose transporter and regulator in a selected model strain of *Roseiflexus castenholzii* is ongoing. In addition, we intend to test the hypothesis of L-rhamnose exchange between Cyanobacteria and Chloroflexi in a model co-culture combining gene expression, proteomics and metabolomics analyses.

This research is supported by the Genomic Science Program (GSP), Office of Biological and Environmental Research (OBER), U.S. Department of Energy (DOE), and is a contribution of the Pacific Northwest National Laboratory (PNNL) Foundational Scientific Focus Area.