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Genomic Structure, Metagenomics, Horizontal Gene Transfer, and Natural Diversity of *Prochlorococcus* and *Vibrio*

PIs
Sallie Chisholm, Eric Alm and Martin Polz
MIT

Our overarching goal with this proposal was to develop a deep understanding of the design of *Prochlorococcus* and *Vibrio* cells, the variations in their designs, and the constraints that have shaped this variation at the cell-environment interface. That is, we wanted to develop our understanding of the biology of these microbes at all scales of biological organization, from individual cell design to the dynamics of large populations.

The complete list of publications that were supported in whole or in part from this grant are listed at the end of this document, and copies of the papers are supplied separately. Below we summarize some of the more salient findings.

Aim I: Identify the patterns of genome diversity within and among natural *Prochlorococcus* and *Vibrio* populations, and relate these patterns to the cellular metabolism, population genetics, and the ecology of these groups;

Prochlorococcus

We have assembled the genomes of 12 *Prochlorococcus* single-cells from the south Pacific ocean, which are part of two new high-light adapted phylogenic groups (Malmstrom, Rodrigue et al 2012). We have also flow-sorted and amplified the genomes of hundred of individual cells from different depths at the same location and at 3 different times during the same year (Kashtan et al in prep). Another important study was to compare the *Prochlorococcus* metagenomes from the Pacific and Atlantic oceans. Our results revealed that a major difference resides in phosphate acquisition genes, demonstrating that DNA sequence data can help inform selective pressure in the environment (Coleman and Chisholm 2010). We have also isolated new *Prochlorococcus* strains based on specific nutritional requirements. For example, we now have obtained nitrate-assimilating strains, which were long thought not to exist in *Prochlorococcus* and could play important ecological roles. We have recently sequenced the genomes of these new strains and these results will be published soon (Berube et al, in prep).

Vibrio

Population structure of Vibrios

Our overall approach has been to define population structure by sampling isolates from different microenvironments, modeling the genetic and ecological structure, and ultimately, sequencing genomes to identify ecologically associated genomic features as well as pathways of gene exchange within and among populations (e.g., see below software development). This approach was successfully carried out in an initial study (Hunt, David et al. 2008) where we explored population-level resource portioning in the coastal water column. From this initial collection, we were able to obtain 80 Illumina-sequenced genomes, which represent all major populations. We have also continued to explore the population structure of *Vibrionaceae* among marine animals as potential drivers for population-level differentiation and specialization (Preheim et al. 2011a; Preheim et al. 2011b; Szabo et al. 2012). This showed that in contrast to organic particles in the water column, larger animals are surprisingly non-selective in terms of the populations associated with them. Although zooplankton (e.g., copeopods) in the water column are colonized by more specific populations, larger animals (mussels and crabs) are primarily colonized by generalist populations. This can be explained by rapid colonization via food items and rapid turnover of populations within the animals, demonstrating that metapopulation dynamics may be highly important in determining local population structure.

In addition to the 80 *Vibrio* genomes, we have completed the sequencing of ~200 plasmids plasmids from our isolate collection in order to explore the diversity of backbone genes responsible for transfer and maintenance of the plasmids, and their accessory genes within and among the populations (Xue et al in preparation). We expect this work to be published within the year and to yield important insights into the ecology and evolution of this understudied group of extrachromosmal elements.

Development of the STARRInIGHTS software package

As part of our efforts in whole-genome analysis, we have developed the STARRInIGHTS software package. STARRInIGHTS can be used to perform 'population genomic' analysis in which multiple individuals from populations are targeted for genome sequencing. This powerful method promises to allow us to better understand the selective pressures on environmental bacteria, and the evolutionary forces shaping their genomes; however, analysis of this kind of genome-wide data is confounded by rampant recombination and horizontal gene transfer among isolates.

STARRInIGHTS account for recombination explicitly by parsing multiple genome alignments into blocks that are well-described by a single phylogenetic tree -- even when no single phylogenetic tree describes the genome-wide history. Numerous research groups are using

STARRInIGHTS, and we hosted a workshop in 2011 at the Human Microbiome Congress in Vancourver. We used STARRInIGHTS to analyze the 21 genomes from (Shapiro et al., 2012). This work is described below.

Genomic analysis of recently diverged populations
We fortuitously discovered two populations whose genetic structure and environmental distribution suggested that they had diverged very recently. This offered the unique opportunity to explore the genomic processes accompanying ecological specialization. We sequenced 20 genomes of *Vibrio cyclotrophicus*, 13 from a population found to be predominantly zooplankton associated and 7 from a population whose environmental occurrence suggested predominantly free-living lifestyle. Through careful analysis of genomic diversity and recombination, a surprising result was obtained shedding light on how adaptation spreads within nascent populations (Shapiro et al. 2012).

Analysis of whole genomes provided evidence that specific genome regions but not whole genomes have swept each of the two populations (Shapiro et al. 2012). Moreover, several of these regions are consistent with differentiation into attached vs. free-living lifestyles providing evidence for differential acquisition of adaptive genes/alleles. Finally, analysis of homologous recombination within and across populations demonstrated that both populations had been actively recombining in the past but that the most recent events had already become population specific, suggesting an independent evolutionary trajectory of these nascent populations (Shapiro et al. 2012).

These observations suggest a new model of genotypic cluster formation in which an ancestral, ecologically uniform and recombining population differentiates into novel, ecologically distinct populations, which gradually develop into genotypic clusters. The first step in this process is evolution of an adaptive allele or gene, either via mutation or recombination (both homologous and non-homologous) in at least one member of the ancestral population. Such adaptive genes may subsequently spread by recombination to other genomes within the ancestral populations. Importantly, if this process triggers differential environmental association, new subpopulations are formed with decreased gene flow between them due to spatial separation. Finally, if the new population structure remains stable over time, accumulation of population-specific mutations will start to genetically differentiate genomes. In bacteria, this may strongly and quickly enhance cluster formation by inhibiting homologous recombination whose rate decreases exponentially with sequence divergence.

Aim II: Characterize the design of the cellular machineries of *Prochlorococcus* and Vibrio

Prochlorococcus

We have characterized and quantified the complete transcriptome and proteome (Rodrigue et al 2012) of *Prochlorococcus* cells synchronized to a light dark cycle. Directional RNA sequencing was performed to identify and quantify all RNA species and proteins species from the same samples were also extracted and identified by mass spectrometry. A procedure involving labeling with radio-isotopes was also developed to obtain a relative quantification of the proteins during the whole diel cycle. We observed that most proteins reach their maximal level 4 to 6 hours after the corresponding transcript, suggesting that post-transcriptional regulatory mechanisms play a very important role in gene expression diel cycle adaptation in *Prochlorococcus*.

On a different note, we have also studied adaptation to light shock in many *Prochlorococcus* strains. We have exposed *Prochlorococcus* strains thought to be adapted to different light levels in the environment to brief light shocks and monitored their recovery. We have observed that some strains tolerate important variations in light intensity, which could explain their temporal distribution in the oceans.

We have also studied the response of the *Prochlorococcus* transcriptome to iron-starvation (Thompson et al 2011a), light shock (Thompson et al in prep), and phage infection (Thompson et al 2011b, Zeng and Chisholm 2012)

Vibrio

The functional significance of the flexible genome and its role in ecological adaptation are poorly understood. The strain collection we assembled as part of this project provided a unique opportunity to examine the roles of these genome regions in the context of ecological populations.

Drawing from our strain collection of the ubiquitous marine bacteria Vibrionaceae with characterized microhabitat preferences, we sequenced, assembled, and analyzed 82 new genomes of Vibrionaceae. Complete draft genome sequences were constructed *de novo* from Illumina short reads using a cost-effective assembly approach we developed, leveraging synteny information from closely related strains to improve assembly contiguity by 2-3 fold. We identified gene coding regions, built orthologous groups, and chronicled the history of gene gain, loss, and transfer in 22 ecologically-defined populations spanning >8% 16S divergence.

We found very rapid gene flux occurring in short divergence times between nearly identical isolates (>100kb new DNA per 100 new point mutations), in addition to gene content divergence of ~25% within traditional OTU boundaries (3% 16S). However, most of these apparently flexible genes are ubiquitous to smaller, ecologically defined populations within traditional OTUs, and are also distinct between populations, even when those populations are not distinguishable by marker genes or even core-genome trees. This suggests much of the genome content that is "flexible" within arbitrarily defined phylogenetic groups, such as OTUs, is

involved in the ecological differentiation of their subgroups. Thus, traditionally defined OTUs merge populations that are ecologically and genomically distinct, as well as merging flexible genes with those that are core to a particular niche.

The vibrios have a particularly large flexible genome and exhibit very high rates of recent horizontal transfer compared to a recent bacteria-wide survey of HGT (Smillie & Smith et al, 2011). Despite the established propensity for recent horizontal transfers to occur between same-ecology bacteria, overall, horizontally acquired DNA accumulating in a vibrio lineage was not more similar to that of Vibrio lineages found in similar microhabitats in space in time, and neither was metabolic potential (Biolog). Instead, both were largely explained by phylogenetic distance. Thus, while flexible genes are ecologically adaptive, lineages adapted in parallel to the same microhabitat did so via largely non-convergent genomic and metabolic adaptive strategies. We are currently preparing a manuscript to describe these findings.

Aim III: Characterize the role of phage and horizontal gene transfer in shaping genome diversity, population structure, and metabolism in *Prochlorococcus*;

The most striking feature of our work with *Prochlorococcus* cyanophage over the years is the host-like genes (Anciallary Metabolic Genes, or AMGs) that they carry. These include genes involved in the light reactions of photosynthesis, the pentose phosphate cycle, ribonucleotide production, and phosphorus acquisition – to mention a few of the more interesting categories. These genes serve as signals to us regarding key bottlenecks the phage encounters in the phage-host interaction, as it strives to take over host metabolism and replicate its genome. We have recently shown, for example, that phage isolated from P-limited environments are more likely to carry high affinity phosphate transport genes (eg *pstS*) (Kelly et al in press.), and we have shown that these genes are upregulated in phage that are infecting P-limited cells relative to P-replete cells (Zeng and Chisholm 2012). Strikingly, some phage also carry a regulatory protein, cp12 (Sullivan et al 2009, Thompson 2010, PhD thesis), that in host cells shuts down the Calvin Cycle and shunts carbon metabolism toward the pentose phosphate pathway. We hypothesize that the phage employs this protein to redirect host metabolism during infection, so it can channel the ATP and reducing power produced by the light reactions of photosynthesis toward nucleotide synthesis rather than carbon dioxide reduction (Thompson et al, 2012b). This obviously has implications for channeling the products of photosynthesis toward selected ends.

Another very interesting feature of the AMGs carried by cyanophage is that they are on average, smaller than their host counterparts (Thompson et al 2012b). In one case (the transaldolase gene) we have shown that the phage version of the gene is a less efficient catalyst than the host version, but still functional. It appears that the constraints of an extremely small genome in the phage have systematically reduced the size of these genes due to the tradeoffs of function over genome compactness, and resource efficiency.

Aim IV: Harnessing natural genetic diversity and processes for strain engineering

Development of Illumina-based metagenomics

Metagenomics has become a standard approach for describing the functional potential for the genetic diversity carried within an environmental sample. Metagenomic sequencing, however, is still too expensive to use in many applications. We developed new experimental and computational techniques that lower the cost of a metagenomic survey of ~30 million reads to ~$3000 by taking advantage of the Illumina sequencing platform (Rodrigue et al 2010). The technique works by creating a highly efficient size selection of DNA fragments ~180 bp in length (using SPRI beads), sequencing these using overlapping forward and reverse reads, and combines the two overlapped fragments using a new software package called SHE-RA that we have released to the public, and is currently in use by several groups.

Additional Achievements of Note

Evolution of the Hutchinsonian niche

Our characterization of the temperature and salinity tolerance of wild *Vibrio* strains has enabled us to, for the first time, describe the evolution of the Hutchinsonian niche along these ecological dimensions (Materna et al., 2012). In this study, we combine theory and experiment to address three fundamental questions: (i) what basic niche shapes (*e.g.*, rectangular) are anticipated and observed, (ii) what organismal physiology cause different shapes, and (iii) how do niche shapes vary within and between populations? We used 17 environmental isolates of the genus *Vibrio*, comprising ecologically well-characterized and genetically diverse species, and measured their ability to grow across a range of temperatures and salinities using inexpensive two-dimensional salinity-temperature gradients on solid media.

Development of ProPortal: a resource for integrated systems biology of *Prochlorococcus* and its phage.

ProPortal (http://proportal.mit.edu/) is a public database containing genomic, metagenomic, transcriptomic and field data for the marine cyanobacterium *Prochlorococcus* (Kelly et al 2011). Our goal was to provide a source of cross-referenced data across multiple scales of biological organization—from the genome to the ecosystem—embracing the full diversity of ecotypic variation within this microbial taxon, its sister group, *Synechococcus* and phage that infect them. The site currently contains the genomes of 13 *Prochlorococcus* strains, 11 *Synechococcus* strains and 28 cyanophage strains that infect one or both groups. Cyanobacterial and cyanophage genes are clustered into orthologous groups that can be accessed by keyword search or through a genome browser. Users can also identify orthologous gene clusters shared by cyanobacterial and cyanophage genomes. Gene expression data for *Prochlorococcus* ecotypes MED4 and MIT9313 allow users to identify genes that are differentially expressed in response to environmental stressors. In addition, the transcriptome in synchronized cells grown on a 24-h

light–dark cycle reveals the choreography of gene expression in cells in a 'natural' state. Metagenomic sequences from the Global Ocean Survey from *Prochlorococcus*, *Synechococcus* and phage genomes are archived so users can examine the differences between populations from diverse habitats. (Abstract of Kelly et al 2011).

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