**Known Bacterial Phylogenetic Divisions**

1987 (5 divisions; 4 cultured; 1 uncultured)

2004 (10 divisions; 3 cultured; 7 uncultured)

---

**“Metagenomics” approaches**

- **Environmental Sample**
- **Clone (30 directly sequenced)**
- **Shotgun library**
- **Fosmid (large-insert) 40 kb**
- **BAC (large-insert) BIG STUFF!**

**Library Types:**
- Shotgun (small-insert) 3kb
- Fosmid (large-insert) 40 kb
- BAC (large-insert) BIG STUFF!

**Processes:**
- Assemble reads
- Call genes
- Bin fragments
- High throughput sequencing

**Monterey Bay National Marine Sanctuary**

**Genotypes**
- Genotyping
- Allelic diversity
- Metabolic pathways
- Functional guilds
- Regulatory elements

**Phenotypes**
- Genomics
- Metagenomics
- Ecology
- Communities & environment

- Environmental variability
- Community composition
- Population genetics
- Community dynamics
- Ecosystem response

---

**CULTIVATION INDEPENDENT MICROBIAL SURVEYS**
Norm Pace and colleagues, ~ 1985

Stahl et al., ASM News 51: 4-11 (1985)
Haloarchaeal bacteriorhodopsin is light driven proton pump.

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Fast photcycle kinetics

Expression of proteorhodopsin in E. coli

UBIQUITOUS, ABUNDANT, & MANY VARIATIONS ON A THEME

Depth-specific differences in proteorhodopsin variants

PROTEORHODOPSIN NATURAL VARIATION = FUNCTIONAL VARIATION

“Green” rhodopsin  “Blue” rhodopsin

Leu105  ->  Gln105

DIFERENT ‘FLAVORS’ OF RHODOPSIN

Beja et al, 2003

Sensory rhodopsins lack the cytoplasmic proton donor - 22 of Sargasso Sea PR variants have either Thr (18), Ile (3), or Lys (1). Each appears linked in an operon to a putative sensory rhodopsin.

UBIQUITOUS, ABUNDANT, & MANY VARIATIONS ON A THEME
Proteorhodopsin photosystem gene organization


A single genetic event can confer photoheterotrophy


Possible uses of pmf in cellular physiology: Ion transport, motility, ATP synthesis, reverse electron transport, etc, etc !

Station ALOHA - North Pacific Subtropical Gyre

Hawaii Ocean Time-series (HOT) program begun in October 1988

- monthly cruises to measure hydrography, chemistry and biology of the water column

Light-stimulates growth of proteorhodopsin-containing marine Flavobacteria

Marine Proteorhodopsin Genomic Distributions

http://kela.soest.hawaii.edu/ALOHA/hawaiianislands.html

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Light-powering Escherichia coli with proteorhodopsin

1) Prochlorococcus phylotypes evident
2) Viral recovery maxima - 70 m
3) Viral recovery decrease below PZ
Cluster based expression ratio = number of cDNA reads mapped to each GOS protein cluster / number of DNA reads mapped to each GOS protein cluster

Prochlorococcus in the wild (75 m): DNA coverage and gene expression

What do non rRNAs and mRNAs represent??

RNAse P RNA

Tryptophanyl-tRNA synthetase 5'UTR

Prochlorococcus sRNA

15.48 15.56
There are many unknown but functional ncRNAs. Many ncRNAs may play important regulatory roles.

Regulation of Bacterial Gene Expression by Riboswitches


The synthesis and functions (mechanisms) of small RNAs

sRNA evolution is constrained by its secondary structure

- Drastic sequence changes can be tolerated.
- Compensatory mutations are very common.
  - One basepair mutates into another basepair.
  - Doesn't change its secondary structure.

http://www.sanger.ac.uk/Software/Rfam/

How many known sRNA families in our data sets?

- tRNA
- tmRNA
- RNaseP
- SRP

Known sRNA families in our data sets

<table>
<thead>
<tr>
<th>sRNA</th>
<th>Known sRNA families</th>
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<tbody>
<tr>
<td>DMP</td>
<td>tRNA, tmRNA, RNaseP, SRP</td>
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<tr>
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<td>tRNA, RNaseP, SRP</td>
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<tr>
<td>tRNA</td>
<td>Pre-tRNA splicing in tRNA</td>
</tr>
<tr>
<td>RNaseP</td>
<td>Pre-RNaseP splicing in RNaseP</td>
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<th>Pre-sRNA splicing in sRNA</th>
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</table>
Read identity breakdown in metatranscriptomic datasets

Compensatory base-pairing

Categories of clustered small RNA sequence groups identified in depth profile

Lack of coding potential
sRNA candidate Group 4 (Proteobacteria)

Blocks translation initiation in pyruvate kinase ???

Group 5 sRNAs: a novel riboswitch??

Found on Pelagibacter metagenomic contigs, but not cultured genomes

Metatranscriptomics and metagenomics data summary (HOT 179)

<table>
<thead>
<tr>
<th>Depth</th>
<th>cDNA</th>
<th>rRNA</th>
<th>non-rRNA</th>
<th>HIT to mRNAs</th>
<th>HIT to Bios</th>
<th>HIT to tRNA</th>
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<td>30.5</td>
</tr>
</tbody>
</table>

25m: 10 PM 75m: 3:30 AM 125m: 6 AM 500m: 6 AM

Metatranscriptomic and metagenomic depth profile (HOT 179)

HOT179 16S rRNA profile
Similarity of reads to genomes in the DNA and cDNA

Genome-specific ORF expression profile (I)

Genome-specific ORF expression profile (II)

Protein family (GOS cluster) expression profile

Gene and transcript occurrence in an oceanic depth profile
Comparison of two depth profiles at station ALOHA

- HOT 179
  - March 2006
    - 25m: 22:00
    - 75m: 3:30
    - 125m (DCM): 6:00
    - 500m: 6:00
- HOT 186
  - October 2006
    - 25m: 22:00; 13:00
    - 75m: 17:00
    - 110m (DCM): 21:00
    - 500m: 15:30

Genome-wide expression correlation in samples taken 6 months apart

The Central Dogma

Biotic niche space

DNA → RNA → Protein

HOT179
HOT186
Monitoring microbial Community response to environmental change...

Use combined metagenomic, transcriptomic & environmental approaches in field studies.

- Monitor natural variation or perturbations
- Track changes in community gene expression and population structure
- Microcosms, mesocosms, or in situ

Experimental community transcriptomics

- 2000 L surface seawater
- ~2 L HMWDOM concentrate
- Microcosms, mesocosms, or in situ

Students shape science futures

DeLoos Lab
- Justin Buck
- Rachel Barry
- John Eppley
- Sara Lincoln
- Jay McCarron
- Julie Marteza
- Elsabeth Oleson
- Turlon Palden
- Vinh Pham
- Yikeei Shi
- Frank Stewart
- Gene Tyson
- Laura-Anna Veitkouras

Collaborators
- MIT - Chisholm Lab
- Penn State - Stephan Schuster
- Dave Karl, CMORE & HOT Teams