A source of biological parts for Synthetic Biology. Xenologs?

Andrew Kuznetsov

ATG:Biosynthetics,
Merzhausen, Germany
Content

• (1) Design principles in SB
  – Dissatisfaction with bio-bricks
• (2) Dialectical cycle: analysis <> synthesis
• (3) Environmental scanning. Sulfur metabolism in bacteria (an example)
  – reference microorganism
  – min set of genes for sulfur metabolism
  – mapping to environments
  – xenologs vs. orthologs
  – design and simulation
Design principles – MIT & Co

• An idea of SB is to **build organisms that perform desired tasks**, rather than to modify existing ones
• A major challenge for SB is to **tackle complexity**
  – top-down
  – bottom-up
• “Cell as an agent” may be a very suitable concept
  – design of agents with adaptive strategies
  – swarm algorithms
• Molecular logic and DNA embedded programming on the different levels
  – recombination
  – transcription
  – RNA interference
  – GTPases
  – Phosphorylation
• Separated genetic programs within different types of cells that work together can be used to **avoid a cross talk**
  – partition of genomes (incompatible plasmids)
  – compartments (membranes)
  – scaffold (DNA, RNA, proteins)

• Karman-Knight’s strategy: **information is turned off → abstraction → design**
Dissatisfaction with the current bio-brick strategy

• new biological parts will lead to exponential growth of unspecific interactions in a target system – a cross talk

• standardization will lead to repeats in synthetic DNA and finally to recombinations – a genetic instability
Dialectical cycle in Freiburg

• “The central research idea of bioSS is to initiate and promote a dialectic process between scientists using analytical (dissecting) and synthetical (rebuilding) approaches in signalling research.”
Possible impact of bioinformatics on synthetic biology

- X-omics
- High-throughput sequencing technologies
  - 2d generation
    - Roche/454 FLX
    - Illumina/Solexa Genome Analyzer
    - Applied Biosystems SOLiD™ System
  - 3d generation
    - Helicos Heliscope™
    - Pacific Biosciences SMRT
- Understanding of biological complexity
The Project was initiated by the Gordon and Betty Moore Foundation, beginning in Jan 2006
Sampling (‘Sorcerer II’, and other)
Databases that were used

- Collection from the Sorcerer II expedition
- Hawaii Ocean Time Series station which presents microbial genomes down to 4000 m
- Ionian abyssal plain, a deep flat basin between Sicily and Greece in the Eastern Mediterranean that is reached by H₂S
- Microbial community from deep-sea hydrothermal vent polychaete worm *Alvinella pompejana*
- Symbionts from another worm, Mediterranean gutless oligochaete *Olavius algarvensis*
- Microbes from Acid Mine Drainage biofilm at Iron Mountain, California
- Soil bacteria from Waseca County, Minnesota
- And other
Fragment requirement plots of reference microorganisms

Thiomicrospira crunogena XCL-2
free-living bacterium
genome 2 427 674 nt
63 134 hits

Thiobacillus denitrificans ATCC 25259
free-living bacterium
genome 2 909 749 nt
42 275 hits

Vesicomyosocius okutanii HA
host Calyptogena okutanii
genome 1 022 154 nt
11 443 hits

Ruthia magnifica Cm
host Calyptogena magnifica
genome 1 160 782 nt
11 160 hits
V. okutanii is a symbiont in a deep-sea clam, Calyptogena okutanii [Kuwahara et al, 2007]
Sulfur metabolism in bacteria [lit. overview, KEGG, ERGO]

dissimilatory sulfite reductase (DsrAB), adenosine-5'-phosphosulfate reductase (APS), ATP sulfurylase (Sat) in cytoplasm, and Sox proteins in periplasm
The gene set from *V. okutanii* for a minimal sulfur metabolism

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Start</th>
<th>End</th>
<th>Strand</th>
<th>Length</th>
<th>Locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP sulfurylase</td>
<td>98093</td>
<td>99301</td>
<td>+</td>
<td>402</td>
<td>sat</td>
</tr>
<tr>
<td>adenyllysulfate reductase membrane anchor</td>
<td>99516</td>
<td>100385</td>
<td>+</td>
<td>289</td>
<td>aprM</td>
</tr>
<tr>
<td>adenyllysulfate reductase β-subunit</td>
<td>100417</td>
<td>100896</td>
<td>+</td>
<td>159</td>
<td>aprB</td>
</tr>
<tr>
<td>adenyllysulfate reductase</td>
<td>100896</td>
<td>102779</td>
<td>+</td>
<td>627</td>
<td>aprA</td>
</tr>
<tr>
<td>sulfur oxidation protein SoxB</td>
<td>172596</td>
<td>174485</td>
<td>+</td>
<td>629</td>
<td>soxB</td>
</tr>
<tr>
<td>sulfur oxidation protein SoxA</td>
<td>770792</td>
<td>771607</td>
<td>-</td>
<td>271</td>
<td>soxA</td>
</tr>
<tr>
<td>sulfur oxidation protein SoxZ</td>
<td>771635</td>
<td>771937</td>
<td>-</td>
<td>100</td>
<td>soxZ</td>
</tr>
<tr>
<td>sulfur oxidation protein SoxY</td>
<td>771971</td>
<td>772414</td>
<td>-</td>
<td>147</td>
<td>soxY</td>
</tr>
<tr>
<td>sulfur oxidation protein SoxX</td>
<td>772425</td>
<td>772772</td>
<td>-</td>
<td>115</td>
<td>soxX</td>
</tr>
<tr>
<td>intracellular sulfur oxidation protein DsrR</td>
<td>817196</td>
<td>817537</td>
<td>-</td>
<td>113</td>
<td>dsrR</td>
</tr>
<tr>
<td>intracellular sulfur oxidation protein DsrP</td>
<td>818938</td>
<td>820140</td>
<td>-</td>
<td>400</td>
<td>dsrP</td>
</tr>
<tr>
<td>intracellular sulfur oxidation protein DsrO</td>
<td>820166</td>
<td>820897</td>
<td>-</td>
<td>243</td>
<td>dsrO</td>
</tr>
<tr>
<td>intracellular sulfur oxidation protein DsrJ</td>
<td>820894</td>
<td>821277</td>
<td>-</td>
<td>127</td>
<td>dsrJ</td>
</tr>
<tr>
<td>putative glutamate synthase (NADPH) small subunit</td>
<td>821307</td>
<td>823271</td>
<td>-</td>
<td>654</td>
<td>dsrL</td>
</tr>
<tr>
<td>intracellular sulfur oxidation protein DsrK</td>
<td>823327</td>
<td>824892</td>
<td>-</td>
<td>521</td>
<td>dsrK</td>
</tr>
<tr>
<td>intracellular sulfur oxidation protein DsrM</td>
<td>824894</td>
<td>825667</td>
<td>-</td>
<td>257</td>
<td>dsrM</td>
</tr>
<tr>
<td>intracellular sulfur oxidation protein DsrC</td>
<td>825744</td>
<td>826067</td>
<td>-</td>
<td>107</td>
<td>dsrC</td>
</tr>
<tr>
<td>intracellular sulfur oxidation protein DsrB</td>
<td>827224</td>
<td>828297</td>
<td>-</td>
<td>357</td>
<td>dsrB</td>
</tr>
<tr>
<td>intracellular sulfur oxidation protein DsrA</td>
<td>828373</td>
<td>829674</td>
<td>-</td>
<td>433</td>
<td>dsrA</td>
</tr>
<tr>
<td>rhodanese family protein</td>
<td>950273</td>
<td>950752</td>
<td>-</td>
<td>159</td>
<td></td>
</tr>
<tr>
<td>sulfide-quinone reductase</td>
<td>995954</td>
<td>997240</td>
<td>+</td>
<td>428</td>
<td>sqr</td>
</tr>
</tbody>
</table>
sox operon: SoxX, SoxY, SoxZ, SoxA, SoxB (CAMERA search)

1  Punta Cormorant, Hypersalin...
2  Sargasso Station 11; Sargasso...
3  Mangrove on Isabella Island
4  Yucatan Channel
5  Warm seep, Roca Redonda
6  Upwelling, Fernandina Island
7  Newport Harbor, RI
8  Gulf of Maine
9  Sargasso Stations 3
10  South of Charleston, SC
11  30 miles from Cocos Island
12  Hydrostation S
13  Cabo Marshall, Isabella Island
14  Off Key West, FL
15  Cape May, NJ
16  Wolf Island
17  Northern Gulf of Maine
18  134 miles NE of Galapagos
19  Outside Halifax, Nova Scotia
20  Rosario Bank
21  Sargasso Station 13
22  Gulf of Panama
23  Northeast of Colon
Stations with multiple Sox and Dsr hits, the normal values

- Punta Cormorant, Hypersaline Lagoon, Floreana Island
- Upwelling, Fernandina Island
- Sargasso Station 11,13
- Richmond Acid Mine
- Deep Mediterranean, Ionian 3Km station
- FarmSoil, Waseca County
- Alvinella Pompejana, East Pacific Rise
- Gutless Worm, Bay off Capo di Sant' Andrea, Elba

(normalized matching sequences)

- Sox
- Dsr
location of stations with a plenty of Dsr matching sequences
Xenologs vs. Orthologs

• *dsr* reads generated trees incompatible with the corresponding 16S rRNA phylogeny
• due to the low quality of databases (random and partial DNA reads)?
• due to BLAST local alignment removes the most divergent regions from the sequences?
• or due to a lateral gene transfer? That is in agreement with [Klein et al, 2001; Boucher et al, 2003]
Bio-bricks for sulfur metabolism

• Fragments of \textit{V. okutanii} HA genetic map with genes coding sulfur metabolism
Simulation (1) within JDesigner

$\text{k2} = 0.01$

$\text{k2} = 0.1$
(2) within the stochastic pi-Machine
Conclusion

• *sat-apr, sox* and *dsr* locuses from *V. okutanii* are very suitable for design of sulfur metabolism

• xenologs as bio-bricks is an attractive idea which needs more detailed analysis
Thanks

- Nelli Sergeeva and Maksim Gulin, Institute of Biology of the Southern Seas, Sevastopol, Ukraine
- Jan Kuever, Max-Planck-Institute for Marine Microbiology, Bremen, Germany
- Saul Kravitz, CAMERA Development, J. Craig Venter Institute

References