Third domain of life

Discovered in 1977 by Carl Woese
Common characteristics

- Transcription/translation similar to eukaryotes, not bacteria (TFIID, TFIIB, no sigma factors)
- Ether-linked branched membrane lipids
- Lipid backbone is glycerol-1P, not glycerol-3P
- No peptidoglycan (some have pseudomurein)
- Many are extremophiles – salt, temperature, pH

From Koga and Morii, 2007

Phylogenetic tree of Archaea
from Brochier et al., 2005

Archaeal “Tree of Life” project to fill in gaps – 11 archaea
Methanogens

- Catalyze final steps in anaerobic degradation of organic matter
- Produce 400,000,000 metric tons of methane each year
- Methane sources include wetlands, rice paddies, cows/sheep, biomass burning, automobiles

Methane importance: biofuel and greenhouse gas (21X more potent than CO₂)

Four methanogens in Archaea tree of life project

*Methanocorpusculum labreanum, Methanoculleus marisnigri*
- involved in biomethanation processes (anaerobic wastewater reactors, landfills, etc.)
- Methanomicrobiales – no genomes yet

*Methanobacterium formicicum*
- mesophile, important for comparison with thermophilic relatives to determine protein adaptations to high temperature

*Methanohalobium evestigatum*
- extreme halophilic methanogen, important for comparison with other methanogens to determine protein adaptations to high salt
Hyperthermophiles

Importance: production of heat-stable enzymes for industry (glycosidases, proteases)
- *Thermofilum pendens* – cellulase, amylase
- *Staphylothermus marinus*
- *Desulfurococcus fermentans* – cellulase, glycosidases

Thermoacidophiles

Importance: bioleaching agents for sulfide ores
- *Acidianus* sp. JP7 – grows at pH as low as 0.3

Halophiles

Importance: production of salt-stable enzymes for Industry, bioremediation of saline environments
- *Halorubrum lacusprofundi* – Antarctic lake, first psychrotolerant halophile to be sequenced
- “*Halococcoides aestuarii*” – low-salt-adapted halophile, important for comparative studies

Archaeoglobales

- *Ferroglobus placidus* – anaerobically oxidizes aromatic compounds (first archaeon found to do this)
Crenarchaeota physiology

Aerobes, facultative, anaerobes
Heterotrophs – grow on peptides, complex media
   many reduce sulfur producing H₂S
   (similar to Pyrococcus sp. from Euryarchaeota)
Autotrophs – hydrogen/sulfur, hydrogen/nitrate
Carbon fixation - reverse TCA cycle, hydroxypropionate cycle, other?
   (same phenotype, different genotype)

Much is unknown, because Crenarchaeota have not been studied as much as other archaea.

---

Hydrogen-sulfur autotrophy

\[ \text{H}_2 + \text{S} \rightarrow \text{H}_2\text{S} \]

hydrogenase

\[ \text{H}_2 \rightarrow 2 \text{H}^+ \]

sulfur reductase

\[ \text{S} \rightarrow \text{H}_2\text{S} \]

Cytoplasm

Acidianus ambivalens, Pyrodictium abyssi, Ignicoccus hospitalis
Wolinella succinogenes
Heterotrophy

Characterized in *Pyrococcus furiosus* (Euryarchaeota)

Similar pathways for degradation of branched-chain and aromatic amino acids and glutamate

Published Crenarchaeal genomes

- **Thermoproteales**
  - *Pyrobaculum aerophilum*
  - *Thermofilum pendens*

- **Sulfolobales**
  - *Sulfolobus acidocaldarius*
  - *Sulfolobus solfataricus*
  - *Sulfolobus tokodaii*
  - *Acidianus sp. JP7*

- **Desulfurococcales**
  - *Aeropyrum pernix*
  - *Hyperthermus butylicus*
  - *Staphylothermus marinus*

- **Cenarchaeales**
  - *Cenarchaeum symbiosum*
**Thermofilum pendens**

Optimal growth at 85-90°C, pH 5.0

Sulfur-dependent anaerobe (*P. aerophilum* is facultative, inhibited by sulfur)

Cells can be longer than 100 µm

No septum observed, “golf club” shaped cells

Requires lipid extract of *Thermoproteus tenax* for growth

Grows on peptide mixtures

---

**Genes found in *T. pendens* but not in other Crenarchaeota using IMG phylogenetic profiler**

<table>
<thead>
<tr>
<th>Taxon Name</th>
<th>With Lipid Extract</th>
<th>Without Lipid Extract</th>
<th>Spurring</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Archaea</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Crenarchaeota</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aeropyrum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hypothermus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Metalbosphaera</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sulfolobus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Deinococcus&lt;/br&gt; thermoacidophilus DSM 431</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Deinococcus&lt;/br&gt; radiothermus F1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sulfolobus&lt;/br&gt; solfataricus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Thermofilum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Thermofilum&lt;/br&gt; pendens&lt;/br&gt; lab</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pyrobaculum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pyrobaculum&lt;/br&gt; aerophilum&lt;/br&gt; IM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pyrobaculum&lt;/br&gt; aerophilum&lt;/br&gt; DSM 431</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Caldibacter</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Caldibacter&lt;/br&gt; thermophilus&lt;/br&gt; DSM 431</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pyrobaculum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pyrobaculum&lt;/br&gt; aerophilum&lt;/br&gt; DSM 431</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pyrobaculum&lt;/br&gt; aerophilum&lt;/br&gt; DSM 431</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Domains more abundant in *T. pendens* than in other Crenarchaeota using IMG abundance profile search
COGs not found in any other sequenced Archaea

<table>
<thead>
<tr>
<th>CDS</th>
<th>COG</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tpen_1241</td>
<td>0698</td>
<td>Ribose 5-phosphate isomerase rpiB</td>
</tr>
<tr>
<td>Tpen_1297</td>
<td>3525</td>
<td>Glycosyl hydrolase family 20</td>
</tr>
<tr>
<td>Tpen_1097</td>
<td>3444</td>
<td>Phosphotransferase system IIB subunit</td>
</tr>
<tr>
<td>Tpen_1100</td>
<td>3715</td>
<td>Phosphotransferase system IIC subunit</td>
</tr>
<tr>
<td>Tpen_1100</td>
<td>3716</td>
<td>Phosphotransferase system IID subunit</td>
</tr>
<tr>
<td>Tpen_1090</td>
<td>4821</td>
<td>Phosphosugar binding protein, SIS domain</td>
</tr>
</tbody>
</table>

COGs not found in any other sequenced Crenarchaeota

<table>
<thead>
<tr>
<th>CDS</th>
<th>COG</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tpen_1155</td>
<td>1554</td>
<td>Glycoside hydrolase family 65</td>
</tr>
<tr>
<td>Tpen_0948</td>
<td>0207</td>
<td>Thymidylate synthase</td>
</tr>
<tr>
<td>Tpen_1467</td>
<td>5598</td>
<td>Trimethylamine:corrinoid methyltransferase</td>
</tr>
<tr>
<td>Tpen_1211</td>
<td>1211</td>
<td>Monomethylamine:corrinoid methyltransferase</td>
</tr>
<tr>
<td>Tpen_1092</td>
<td>1080</td>
<td>Phosphoenolpyruvate-protein kinase (Enzyme I of PTS)</td>
</tr>
<tr>
<td>Tpen_1091</td>
<td>1925</td>
<td>Phosphotransferase system HPp protein</td>
</tr>
<tr>
<td>Tpen_1098</td>
<td>2893</td>
<td>Phosphotransferase system IIA component</td>
</tr>
<tr>
<td>Tpen_1491</td>
<td>1268</td>
<td>Biotin transporter bioY</td>
</tr>
<tr>
<td>Tpen_0929</td>
<td>3601</td>
<td>Riboflavin transporter</td>
</tr>
<tr>
<td>Tpen_0191</td>
<td>2116</td>
<td>Formate transporter</td>
</tr>
<tr>
<td>Tpen_1479</td>
<td>2060</td>
<td>Potassium-transporting ATPase, A chain</td>
</tr>
<tr>
<td>Tpen_1480</td>
<td>2216</td>
<td>Potassium-transporting ATPase, B chain</td>
</tr>
<tr>
<td>Tpen_1481</td>
<td>2156</td>
<td>Potassium-transporting ATPase, c chain</td>
</tr>
</tbody>
</table>
Genes present in all Crenarchaeota except \textit{T. pendens}:

<table>
<thead>
<tr>
<th>COG</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>0214</td>
<td>Pyridoxine biosynthesis enzyme (yaaD)</td>
</tr>
<tr>
<td>0311</td>
<td>Glutamine amidotransferase involved in pyridoxine synthesis (yaaE)</td>
</tr>
<tr>
<td>0413</td>
<td>Ketopantoate hydroxymethyltransferase</td>
</tr>
<tr>
<td>0452</td>
<td>Phosphopantothenoylcysteine synthetase/decarboxylase</td>
</tr>
<tr>
<td>0108</td>
<td>3,4-dihydroxy-2-butanone 4-phosphate synthase</td>
</tr>
<tr>
<td>1985</td>
<td>Pyrimidine reductase, riboflavin biosynthesis</td>
</tr>
<tr>
<td>0054</td>
<td>Riboflavin synthase beta chain</td>
</tr>
<tr>
<td>0163</td>
<td>3-polypropyl-4-hydroxybenzoate decarboxylase</td>
</tr>
<tr>
<td>1635</td>
<td>Flavoprotein involved in thiazole biosynthesis</td>
</tr>
<tr>
<td>0112</td>
<td>Glycine/serine hydroxymethyltransferase</td>
</tr>
<tr>
<td>0189</td>
<td>Glutathione synthase/Ribosomal protein S6P modification enzyme/L-2-aminoadipate N-acetyltransferase</td>
</tr>
<tr>
<td>0105</td>
<td>Nucleoside diphosphate kinase</td>
</tr>
<tr>
<td>2046</td>
<td>Sulfate adenylyltransferase</td>
</tr>
<tr>
<td>1650</td>
<td>Uncharacterized protein conserved in archaea</td>
</tr>
<tr>
<td>1701</td>
<td>Uncharacterized protein conserved in archaea</td>
</tr>
</tbody>
</table>

**Function Profile - 125 COGs involved in biosynthesis**

- \textit{T. pendens} – 11
- \textit{C. trachomatis} – 27
- \textit{R. prowazeckii} - 15

\textit{T. pendens} has fewer biosynthetic enzymes than obligate intracellular pathogens.
Does not make:
- Phenylalanine
- Glycine
- Histidine
- Isoleucine
- Lysine
- Leucine
- Arginine
- Threonine
- Valine
- Tryptophan
- Tyrosine

Biosynthesis

Partial pathway:
- Cysteine
- Glutamate
- Methionine
- Proline
- Serine

Can probably make:
- Alanine
- Aspartate
- Asparagine
- Glutamine
- Pyrimidines
- Deoxyribonucleotides

Obligate parasites have lower GC than relatives

What about *T. pendens*?

<table>
<thead>
<tr>
<th>Organism Name</th>
<th>Genome Size (Mb)</th>
<th>GC Percent</th>
<th>Base Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thermofilum pendens</em> HK 15</td>
<td>1923</td>
<td>0.58</td>
<td>1811393</td>
</tr>
<tr>
<td><em>Pyrobaculum caldivolense</em> CCM 11544</td>
<td>2310</td>
<td>0.57</td>
<td>2009135</td>
</tr>
<tr>
<td><em>Aeropyrum pernix</em> K1</td>
<td>2015</td>
<td>0.56</td>
<td>1661695</td>
</tr>
<tr>
<td><em>Pyrobaculum aerophilum</em> DSM 13514</td>
<td>3400</td>
<td>0.55</td>
<td>2121076</td>
</tr>
<tr>
<td><em>Haloterraceae halb</em> DSM 5656</td>
<td>1654</td>
<td>0.54</td>
<td>1687163</td>
</tr>
<tr>
<td><em>Pyrobaculum aerophilum</em> AD4</td>
<td>2617</td>
<td>0.51</td>
<td>2221340</td>
</tr>
<tr>
<td><em>Pyrobaculum islandicum</em> DSM 4184</td>
<td>2062</td>
<td>0.50</td>
<td>1824602</td>
</tr>
<tr>
<td><em>Metallosphaera medusa</em> DSM 5541</td>
<td>3445</td>
<td>0.46</td>
<td>2191517</td>
</tr>
<tr>
<td><em>Caldivolera marina</em> JC-167</td>
<td>1986</td>
<td>0.43</td>
<td>2075755</td>
</tr>
<tr>
<td><em>Thalassobios scotia</em> DSM 658</td>
<td>3336</td>
<td>0.37</td>
<td>2237959</td>
</tr>
<tr>
<td><em>Thalassobios sulfureus</em> P2</td>
<td>3141</td>
<td>0.36</td>
<td>2032245</td>
</tr>
<tr>
<td><em>Phylophaga nissim</em> Ft</td>
<td>1675</td>
<td>0.36</td>
<td>1578435</td>
</tr>
<tr>
<td><em>Thalassobios icterica</em> 7</td>
<td>2964</td>
<td>0.35</td>
<td>2647556</td>
</tr>
</tbody>
</table>

*T. pendens* has highest GC of sequenced Crenarchaeota
Obligate parasites have fewer fusions than relatives

What about *T. pendens*?

<table>
<thead>
<tr>
<th>Rank</th>
<th>Species Name</th>
<th>Genes</th>
<th>GC Perc</th>
<th>Bases</th>
<th>Fused Genes</th>
<th>Fused Genes Perc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Prochlorothrix huskitecensis H16 5</em></td>
<td>1923</td>
<td>50</td>
<td>1813393</td>
<td>78</td>
<td>4.00%</td>
</tr>
<tr>
<td>2</td>
<td><em>Prochlorothrix acidophila DSM 439</em></td>
<td>2145</td>
<td>62</td>
<td>2203919</td>
<td>83</td>
<td>3.74%</td>
</tr>
<tr>
<td>3</td>
<td><em>Prochlorothrix aerophila DM2</em></td>
<td>2617</td>
<td>51</td>
<td>2221410</td>
<td>88</td>
<td>3.96%</td>
</tr>
<tr>
<td>4</td>
<td><em>Prochlorothrix ellipsoidea DSM 104</em></td>
<td>2062</td>
<td>50</td>
<td>1826482</td>
<td>65</td>
<td>3.13%</td>
</tr>
<tr>
<td>5</td>
<td><em>Caldolimnia rubra</em></td>
<td>2982</td>
<td>33</td>
<td>1694756</td>
<td>93</td>
<td>3.12%</td>
</tr>
<tr>
<td>6</td>
<td><em>Caldolimnia rubra</em></td>
<td>3141</td>
<td>36</td>
<td>1902245</td>
<td>50</td>
<td>2.75%</td>
</tr>
<tr>
<td>7</td>
<td><em>Stefanothermus maris F1</em></td>
<td>1655</td>
<td>36</td>
<td>1570415</td>
<td>46</td>
<td>2.78%</td>
</tr>
<tr>
<td>8</td>
<td><em>Methanothermobacter omelianski DSM 5381</em></td>
<td>2345</td>
<td>46</td>
<td>2191517</td>
<td>63</td>
<td>2.89%</td>
</tr>
<tr>
<td>9</td>
<td><em>Heliobacter kandleri</em></td>
<td>2339</td>
<td>46</td>
<td>2109315</td>
<td>63</td>
<td>2.69%</td>
</tr>
<tr>
<td>10</td>
<td><em>Caldolimnia rubra</em></td>
<td>1986</td>
<td>43</td>
<td>1079757</td>
<td>49</td>
<td>2.49%</td>
</tr>
<tr>
<td>11</td>
<td><em>Prochlorothrix aerophila</em></td>
<td>2408</td>
<td>55</td>
<td>1123070</td>
<td>58</td>
<td>2.41%</td>
</tr>
<tr>
<td>12</td>
<td><em>Heliobacillus bouquetii</em></td>
<td>1680</td>
<td>54</td>
<td>1667143</td>
<td>59</td>
<td>2.34%</td>
</tr>
<tr>
<td>13</td>
<td><em>Caldolimnia rubra</em></td>
<td>1901</td>
<td>56</td>
<td>1669685</td>
<td>61</td>
<td>3.17%</td>
</tr>
<tr>
<td>14</td>
<td><em>Stefanothermus maris F1</em></td>
<td>1494</td>
<td>57</td>
<td>1297318</td>
<td>20</td>
<td>1.87%</td>
</tr>
</tbody>
</table>

*T. pendens* has the highest percentage of fused genes in Crenarchaeota

**Obligate intracellular pathogens**

- Reduced genome size: *normal genome size*
- Less signal transduction: *more signal transduction*
- Fewer DNA repair genes: *normal DNA repair genes*
- High %AT: *low %AT*
- Increased noncoding DNA: *less noncoding DNA*
- Lower fusions: *higher fusions*

Conclusion: *T. pendens* does not show signs of becoming a parasite.

It has adapted to a nutrient-rich environment.
Transport capabilities

- More sugar ABC transporters than other archaea
- PTS, GPH, thiamin ABC rare in archaea
- RFT, BioY not in other Crenarchaeota

Lack of biosynthesis → Increased transport

<table>
<thead>
<tr>
<th>Sugar transport</th>
<th>Peptides</th>
<th>Amino acids</th>
<th>Cofactors</th>
<th>Polyamines</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABC</td>
<td>PTS</td>
<td>GPH</td>
<td>ABC</td>
<td>ABC</td>
</tr>
<tr>
<td>(10)</td>
<td>(2)</td>
<td>(3)</td>
<td>(3)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

Function profile – all published archaeal genomes

T. pendens

Haloarcula marismortui
Other sources of energy?

Hydrogen and formate may be prevalent

Formate without sulfur

T. pendens is the only Crenarchaeote to have a formate transporter and formate hydrogen-lyase.

In E. coli, FHL is used when no electron acceptors other than protons are present.
Monomethylamine:corrinoid methyltransferase
● only found before in Methanosarcinales

Function in Methanosarcinales

\[
\begin{align*}
\text{CH}_3\text{NH}_2 \quad \text{NH}_3 \\
\text{CH}_3\text{-corrinoid protein} \\
\text{CH}_3\text{-Coenzyme M} \\
\text{Coenzyme B} \\
\text{CoM-CoB heterodisulfide} \\
\text{CoM + CoB}
\end{align*}
\]

Proposed function in \textit{T. pendens}

\[
\begin{align*}
\text{CH}_3\text{NH}_2 \quad \text{NH}_3 \\
\text{CH}_3\text{-corrinoid protein} \\
\text{homocysteine} \\
\text{S-adenosylhomocysteine} \\
\text{methionine} \\
\text{S-adenosylmethionine} \\
\text{T. pendens also contains trimethylamine:corrinoid methyltransferase}
\end{align*}
\]

Presenilin

● part of gamma-secretase protease complex in animals
● implicated in Alzheimer's disease
● involved in Notch signaling
● structure is still being debated

images from Dewji, 2005
T. pendens and Pyrobaculum aerophilum have presenilin-related proteins
Predicted highly expressed (PHX) genes in *T. pendens*

1. Training set (Karlin et al., 2005)
2. Run EMBOSS program cusp to get codon usage table
3. Run EMBOSS program cai to get codon adaptation index
4. Top 5% of genes taken as PHX

- Tpen_1511 – cytosolic alpha-glucosidase
- Tpen_1269 – secreted glycosidase
- Tpen_1458 – cyclomaltodextrinase
- Tpen_1055 – ABC maltose-binding protein
- Tpen_1208 – ABC sugar-binding protein
- Tpen_1257 – ABC sugar-binding protein
- Tpen_1649 - phosphofructokinase

Carbohydrate metabolism – 7 PHX genes
Peptide metabolism – 10 PHX genes

- Tpen_1350 – peptidase
- Tpen_1225 – peptidase
- Tpen_1635/1636/1638 – peptide ABC transporter subunits
- Tpen_1245/1247/1248/1249 – peptide ABC transporter subunits
- Tpen_1579 – branched chain AA ABC transporter ATPase

CRISPR-associated – 6+ PHX genes

- Tpen_1263 – APE2256 family
- Tpen_1287 – SSO1426 family (RAMP)
- Tpen_1288 – SSO1426 family (RAMP)
- Tpen_1316 – Crm2 family
- Tpen_1342 – APE2256 family
- Tpen_1356 – Csa2 family

Tpen_1287-1293 all PHX (7 genes)
Tpen_1289/1291/1293 RAMP family
Other stress-associated – 6 PHX genes

- Tpen_1301 – nucleotidyltransferase
- Tpen_0991 – nucleotidyltransferase
- Tpen_1459 – nucleotidyltransferase
- Tpen_1430 – peroxiredoxin
- Tpen_1223 – drug resistance ABC membrane protein
- Tpen_1136 – drug resistance ABC ATPase

PHX Conclusions

- Nutrient acquisition (both peptides and carbohydrates) is a high priority for *T. pendens* because of the lack of biosynthetic capabilities
- Stress responses (to viruses, toxins, and oxygen) are another high priority
- Transcription, translation, and replication-associated proteins are not PHX, suggesting fast growth is not a priority
**Staphylothermus marinus**

Isolated from shallow heated sediment and from black smoker

Grows up to 98°C

Sulfur-dependent anaerobe

Forms clusters of up to 100 cells

Forms giant cells (15 μm diameter) at high nutrient concentrations

Grows on complex media, heterotroph

---

**Domains unique among published Crenarchaeal genomes**

<table>
<thead>
<tr>
<th>Domain ID</th>
<th>Gene ID</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>COG1883</td>
<td>Smar_1504</td>
<td>Na⁺-transporting decarboxylase, beta subunit</td>
</tr>
<tr>
<td>pfam04277</td>
<td>Smar_1503</td>
<td>Na⁺-transporting decarboxylase, gamma chain</td>
</tr>
<tr>
<td>COG1006</td>
<td>Smar_0026, Smar_0654, Smar_1069</td>
<td>Multisubunit Na⁺/H⁺ antiporter, MnhC subunit</td>
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<tr>
<td>COG1320</td>
<td>Smar_0023, Smar_0651, Smar_1066</td>
<td>Multisubunit Na⁺/H⁺ antiporter, MnhG subunit</td>
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<tr>
<td>COG1863</td>
<td>Smar_0027, Smar_0655, Smar_1070</td>
<td>Multisubunit Na⁺/H⁺ antiporter, MnhE subunit</td>
</tr>
<tr>
<td>COG2111</td>
<td>Smar_0025, Smar_0653, Smar_1068</td>
<td>Multisubunit Na⁺/H⁺ antiporter, MnhB subunit</td>
</tr>
<tr>
<td>COG0286</td>
<td>Smar_0761</td>
<td>Type I restriction-modification system methyltransferase</td>
</tr>
<tr>
<td>COG0610</td>
<td>Smar_0763</td>
<td>Type I restriction-modification system, R subunit</td>
</tr>
<tr>
<td>COG0732</td>
<td>Smar_0762</td>
<td>Restriction endonuclease S subunits</td>
</tr>
<tr>
<td>COG0424</td>
<td>Smar_0734</td>
<td>Protein implicated in inhibition of septum formation</td>
</tr>
<tr>
<td>pfam03169</td>
<td>Smar_1400</td>
<td>OPT oligopeptide transporter</td>
</tr>
</tbody>
</table>
Sodium-transporting decarboxylases – 2 possibilities
both involved in amino acid degradation

Oxaloacetate decarboxylase
Smar_0341 - alpha
Smar_1504 - beta
Smar_1503 - gamma

aspartate

\[
\begin{align*}
\text{oxaloacetate} & \rightarrow \text{CO}_2 \\
\text{pyruvate} & \rightarrow \text{Fd}_{\text{ox}} \\
\text{CO}_2 & \rightarrow \text{Fd}_{\text{red}} \\
\text{acetyl-CoA} & \rightarrow \text{ADP} \\
\text{acetate} & \rightarrow \text{ATP}
\end{align*}
\]

Methylmalonyl-CoA decarboxylase
Smar_1426 - alpha
Smar_1504 – beta
Smar_1427 - gamma
Smar_1503 - delta

glutamate

\[
\begin{align*}
\text{glutamate} & \rightarrow 2\text{-oxoglutarate} \\
\text{CoA} & \rightarrow \text{Fd}_{\text{ox}} \\
\text{CO}_2 & \rightarrow \text{Fd}_{\text{red}} \\
\text{methylmalonyl-CoA} & \rightarrow \text{CO}_2 \\
\text{propionyl-CoA} & \rightarrow \text{ADP} \\
\text{propionate} & \rightarrow \text{ATP}
\end{align*}
\]

Multisubunit membrane protein complexes

- **S. marinus** has three operons with antiporter subunits and NADH dehydrogenase-related genes
- They are related to **P. furiosus** mbh and mbx operons
- Mbh = membrane bound hydrogenase
- Mbx = unknown function in electron transport/sulfur reduction
**S. marinus** antiporter subunits are closely related, but NADH dehydrogenase subunits are not → cassette
### Do all sulfur-dependent Crenarchaeota use the same sulfur reduction pathways?

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>S. marinus</th>
<th>T. pendens</th>
<th>H. butylicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfur/polysulfide reductase (molybdoenzyme)</td>
<td>-</td>
<td>Tpen_1121-1123</td>
<td>Hbut_0371-0373</td>
</tr>
<tr>
<td>Sulphhydrogenase</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sulfide dehydrogenase</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NADPH:sulfur oxidoreductase</td>
<td>-</td>
<td>Tpen_0143</td>
<td>Hbut_0802</td>
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<tr>
<td>mbh/mbx-related</td>
<td>Smar_0018-0030, Smar_0645-0657, Smar_1057-1071</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Same phenotype, different genotype

### Acknowledgments

**Genome Biology Program**
- Nikos Kyrpides
- Natalia Ivanova
- Thanos Lykidis
- Costas Mavrommatis
- Sean Hooper
- Edwin Kim
- Natalia Mikhailova

**Virginia Bioinformatics Institute**
- Biswarup Mukhopadhyay
- Jason Rodriguez
- Dwil Susanti
- Lakshmi Dharmarajan

**University of Georgia**
- Barby Whitman
- Iris Porat

**University of Illinois**
- Gary Olsen
- Claudia Reich

**JGI**
- Alla LAPIDUS
- Hui Sun
- Susan Lucas
- Alex Copeland
- Matt Nolan

**LANL**
- Chris Dettter
- Linda Thompson

**Oak Ridge**
- Miriam Land
- Igor Zhulin
- Luke Ulrich
- Jim Elkins

**University of Regensburg**
- Harald Huber