Evolution and Functional Characterization of the *RH50* Gene from the Ammonia-Oxidizing Bacterium *Nitrosomonas europeae*

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Why study *Nitrosomonas europaeae*?
Nitrosomonas europeae

- Identified by Winogradsky (1892)
- Gram (-) Beta proteobacter
- Obligate chemolithoautotroph
- Uses ammonia and carbon dioxide and mineral salts for growth,
- Is an important part of the global biogeochemical nitrogen cycle

The Nitrogen Cycle

http://www.life.umd.edu/classroom/bsci424/BSCI223WebSiteFiles/NitrogenCycle.gif
Bioremediation and *N. europeae*

Found in industrial and sewage waste treatment in the first step of oxidizing ammonia to nitrate.

*N. Europeae* s also capable of degrading halogenated organic compounds:

- trichloroethylene (Arciero et al 1989)
- benzene (Hyman et al 1985)
- vinyl chloride

Bioremediation of MTBE
Evolution of Genes
Evolution of Genes

- **Homologous Genes:**
  - derived from a common ancestor by vertical decent

- **Orthologous genes:**
  - related by vertical descent from a common ancestor and encode proteins of the same function in different species

- **Paralogous genes:**
  - are homologous genes that have evolved by duplication and code for proteins of similar but not identical function
Horizontal Gene Transfer

• Horizontal (lateral) gene transfer is defined as the transfer of genes between different species

  – Subject of debate in eukaryotes—defies the biological definition of "species: (reproductive isolation)

  – HGT recognized in the transfer of genes from
    • mitochondria and chloroplast (endosymbiotic organelles) to nuclear genome

  – With the advent of complete genome sequencing it is becoming apparent that HGT is not a minor player in evolution of prokaryotes
Mechanisms of HGT in Bacteria

Naturally Competent cells
(1% of described species)

Transposable elements
(plasmid, phage)
Natural Transformation

• Release of extracellular DNA to environment
  – From dead cells
  – Actively excreted DNA from living cells (reported for Actinobacter, Alcaligens, Azotobacter, Bacillus, Flavobacterium, Micrococcus, Pseudomonas (780 ug per ml), and Streptococcus)

• Persistence of DNA
  • One study showed persistence of small plasmid DNA pBR322 and RSF1030 in blood serum
  • Uptake of DNA by competent bacteria rapid in vitro -(100 bp/sec in S. pneumoniae)

Recombination can occur through:

– homologous recombination
– possible illegitimate recombination
  • by double stranded break -end joining (E. coli),
  • or additive integration (twp flaking regions of High DNA similarity initiate recombination)
Fates of Horizontally Transferred Genes

1) Deleterious genes are eliminated from population

2) Some genes are neutral- their survival depends on chance

3) If horizontally acquired chromosomal DNA or transposable elements that encode their own transfer and maintenance confer an advantage they have the potential to spread rapidly through the population (e.g. antibiotic resistance)
1) Multifactorial Analysis of codon frequencies in *E. coli* showed 15% of genes deviated from normal frequencies-related to bacteriophage genes.

2) Major difference in genome between bacteria of same lineage, *E. coli* and *Haemophilus influenza*, cannot be explained by evolution by vertical descent.

3) Genome sequence of hyperthermophilic bacteria *Aquifex aeolicus* and *Thermotoga maritima* reveal a larger fraction of archaeal genes than any other bacterial genomes.

4) *Synechocystis* sp. genome encodes signaling proteins that are believed to be of eukaryotic origin.
Methods of Detecting HGT

1) Protein database search reveals a gene sequence of an organism in question with highest similarity to a homolog from a distant taxon

2) Phylogenetic tree analysis:

For example—in a well supported tree a protein groups with homologs from eukaryotes and excludes homologs from other bacteria

3) Unusual phyletic patterns:

Clusters of Orthologous Groups (COG’s) of proteins show presence of bacterial-archaeal protein in a single bacterial lineage. (for example DNA pol II in gamma-proteobacteria)

4) Conservation of Gene order between distantly related groups-
1) three or more genes in the same order in distantly related groups is unlikely unless genes are an operon-
2) probability is each operon evolved once and has been maintained-
3) therefore the same operon in distantly related groups is evidence of HGT
<table>
<thead>
<tr>
<th>Operon</th>
<th>Genes</th>
<th>Phyletic distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate reductase</td>
<td>NarGHJI</td>
<td>Ec, Mtu, Bs, Pa, Ap</td>
</tr>
<tr>
<td>Urease</td>
<td>UreABCFG</td>
<td>Mtu, Bs, Bh, Hi, Pa, Hp, Dr, Uu</td>
</tr>
<tr>
<td>Formate hydrogen lyase</td>
<td>HyfBCDEFG</td>
<td>Ec, Mtu</td>
</tr>
<tr>
<td>Na(^+)-transporting NADH:ubiquinone oxidoreductase</td>
<td>NqrABCEF</td>
<td>Nm, Vc, Pa, Tm, Cp, Ct</td>
</tr>
<tr>
<td>Archael/vacuolar-type H(^+)-ATPase</td>
<td>NtpABCDEFGIK</td>
<td>All archaea, Dr, Tp, Bb, Ct, Cp</td>
</tr>
<tr>
<td>(\text{Na}^+/\text{H}^+)-antiporter</td>
<td>MnhABCDEFG</td>
<td>Dr, Bs, Bh, Vc, Tm</td>
</tr>
</tbody>
</table>

And now the rest of the story!

Paul Harvey
Morning News
7:30 & 9:30 am
Noon News
The Rest of the Story
4:15 pm

1918-2009
Rh (Rhesus factor) Proteins

• Landsteiner and Weiner- Rh antigen 1940

• Human Rh antigens are two erythrocyte transmembrane proteins- RhD and RhCE- aka Rh30

• Rh50A erythrythroid specific- forms multi-protein complex
• Required for Rh blood group antigen expression

• When human Rh50A and Rh50C are expressed in yeast mutant growth on ammonium is restored ([Cherif-Zahar et al., 2007](http://example.com); [Lupo et al., 2007](http://example.com)).
Rh50 protein in *Nitrosomonas europeae*

Rh50 has long evolutionary history—present in basal deuterostomes-sea urchins, tunicates, lancets

NeRh50 is a homo-trimer

Ne RH50 structurally very similar to AmtB of *E.coli*

20-25% sequence identity to Amt proteini family- homologous to Amt genes

Are assigned to same protein family: Pfam PF00909)

Function of Rh50 genes has been subject of debate: $CO_2$ transport or ammonium

http://www.jic.ac.uk/staff/mike-merrick/
The evolutionary scenarios

• Until Rh50 was identified in the *Nitrosomonas europeae* genome RH genes were thought of as strictly a eukaryotic gene:
  – It was thought bacterial and archaeal genomes only encoded *Amt* genes
  – Some protist genomes (unicellular protists, cellular slime molds), and some animals (cnidarians, nematodes, insects) had both *Amt* and RH50
  – Vertebrates possessed only RH
Construction of Phylogenetic Trees

- Joint Genome Institute (JGI) sequences Ne0441 to Ne0457 were used as Blast query (Rh50 is Ne0448) against NCBI and TIGR databases.

- Orthologous genes retrieved from the databases were added.

- Sequences were aligned, and ProtTest1.3 was used to determine tree (WAG, RTREv, and Blossom 62 substitution matrices were used).

- Maximum-likelihood trees were computed by using PHYML 2.4.4 from datasets comprising 38-taxa (238 amino acid positions), 34-taxa (297 amino acid positions), and 38-taxa (316 nucleotide sites, first and second codon positions) in Ne0448 (A), Ne0445 (B), and Ne0446 (C), respectively.
Unable to Prove HGT by Phylogenetic Analysis

• The basal position of the RH50 gene for *N. europeae* on the Phylogenetic tree make it impossible to show HGT

• RH50 could have been present in the common ancestor and the differences shown on the tree due to divergence of the gene in these lineages.
"If at first you don’t succeed, shift the blame, change the rules, redirect the focus of your critics, spin the media, redefine success, and there won’t be any need to try, try again!"
Evidence of HGT

• Rh50 Genes were identified in four species out of 700 Blast Searches:
  - two β-proteobacter AOB- N. europeae and Nitrospira multiformis
    Acidobacteria bacterium, and Kuenenia stuttgartiensis
• AMT genes and Rh are missing in the two AOB-N. eutropha and Nitrosococcus oceani
• RH genes missing in other members of Planctomycetes and Acidobacter
• Possible though unlikely explanation is loss of RH genes in these bacteria
• Most parsimonious explanation is acquisition of RH genes by the bacteria through HGT
Phylogenetic Analysis of Adjacent genes

- Decided to perform the same analysis on the 16 genes neighboring RH50
- Ne0445 (uroporphyrinogen decarboxylase) showed no HGT
- Three orthologous genes from *N. europeae* all clustered together
- Located in β-proteobacteria (99% bootstrap value)
Phylogenetic Analysis of Ne0446

- Ne0446 codes for 3-demethylubiquinone-9 3 methyltransferase
- Clusters with Geobacter (δ-proteobacter)
- Ortholog in Nitrospira clusters with the other β-proteobacteria
- Results suggest this gene entered Nitrosomonas via HGT
Further Evidence of HGT

• Three genes adjacent and upstream of NeRH50 on the same strand code integron sequences:
  – integron-integrase *intI* (Ne0450)
  – A *catalytic core integrase* (Ne0451)
  – Another integrase (Ne0454) on opposite strand (clusters with α-proteobacteria)
  – And a transposase, IS911, (Ne0452)
Probable HGT Events

- One region- Ne0455 and possibly Ne0456 (NE0454 code for integrase)
- Second region- Ne0446, Ne0447 and Ne0449
- By Inference Ne448 likely due to HGT
Possible Evolutionary Scenarios

• Assuming you accept that the Rh50 Gene was acquired by HGT
  – A single HGT event occurred in the common ancestor of the AOB
    • This leads to the conclusion that gene loss occurred in *N. eutropha* lineage
    Or a single HGT occurred in the *N. europeae* lineage
Phylogeny of RH50

Three independent HGT events may have occurred leading to the AOB, Ca. Kuenenia, and Acidobacteria- Short branches may indicate recent events or possibly slow evolution rates

Separation from eukaryotic homologs may be explained by a eukaryote-intermediate prokaryote-prokaryote HGT event!

Evidence of DNA transfer from Eukaryote to prokaryotes is extremely rare

May be a case of xenologous gene displacement