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Summary of Research Interests

1. Gene identification and genome annotation
2. The evolution of genome-sequence compositional properties
3. Models of protein evolution
4. The phylogeny of Bacteria
5. Measures of differentiation from genomic and metagenomic data
6. The evolution of chaperone proteins

1. Gene identification and genome annotation

- From gene prediction to prokaryotic genome annotation
  - Missing genes are recognized in bacterial annotations
  - New methods and bioinformatics tools are needed to translate information from computational gene prediction and from conservation into accurate genome annotations

- Frame analysis
  - Frame analysis provides an effective way of visualizing frame-specific contrasts in sequence composition that reveal genes missed in genome annotations.
  - Tools are needed to efficiently translate the qualitative information provided by frame analysis into genome-wide annotations.

- We are developing methods for:
  - Quantitative characterization of sequence regions with compositional periodicity.
  - Statistical characterization of the intensity of compositional contrasts
  - Graphical representation of results in relation to gene-prediction information for sequence annotation

Quantitative frame analysis

- To verify expression of predicted genes we use:
  - RNAseq: genome-wide sequencing of the trancriptome, measuring mRNA expression levels.
  - Ribosome Profiling (RIBO-seq): genome-wide analysis of translation of coding regions.

2. Sequence compositional properties

- What are the determinants of the composition of bacterial genomes?
  - Bacterial genomes encompass a wide variety of compositional properties.
  - In bacterial GC content ranges from about 16% to 75% GC. Does GC content reflect different selective pressures in different organisms?
  - Codon usage is highly variable across bacterial species. What are the determinants of codon usage?

- How does GC content vary in different regions of the genomes?
  - We are investigating the relation of GC content with coding capacity and how GC content .
  - How does GC content relate to site-specific variation in selective pressures for optimal codon usage and for amino acid composition across species and across protein families?

2. Sequence compositional properties
What are the determinants of GC content?

- How does codon usage vary across species?
  - How does codon usage relate to GC content?
  - What are the optimal codons for each organism?
  - How does optimal codon usage relate to patterns of gene conservation?
- 1000 prokaryotic genomes analysis
  - Definition of amino-acid-specific optimal codon types in 1000 genomes
  - We are interested in investigating how optimal codon usage relates to patterns of gene conservation across 1000 genomes.

2. Sequence compositional properties

What are the determinants of optimal codon usage?

3. Models of protein evolution

Protein functional differentiation has been most commonly associated with substitution events between amino acid types belonging to different classes of physico-chemical properties (Hanada et al. 2007) implying that substitutions between similar types prevalently involve preservation of the protein functional/structural properties.

Classes	**Amino acid type**
Hydrophilic and small (MW: 75–146)	A N C G P S T
Hydrophobic and small (MW: 117-149)	I L M V
Negatively charged (MW: 133–147)	D E
Positively charged, aromatic and relatively large (MW: 146-204)	R Q H K F W Y

Hanada et al. 2007

3. Models of protein evolution

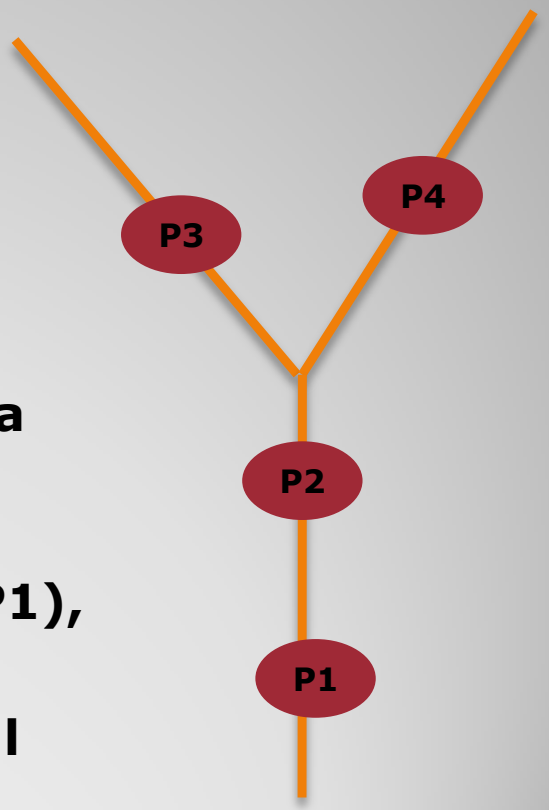
Long-term protein evolution as a mixture of neutral substitution and functional differentiation

A protein can be modeled as a sequence of profiles of site-specific amino acid frequencies.

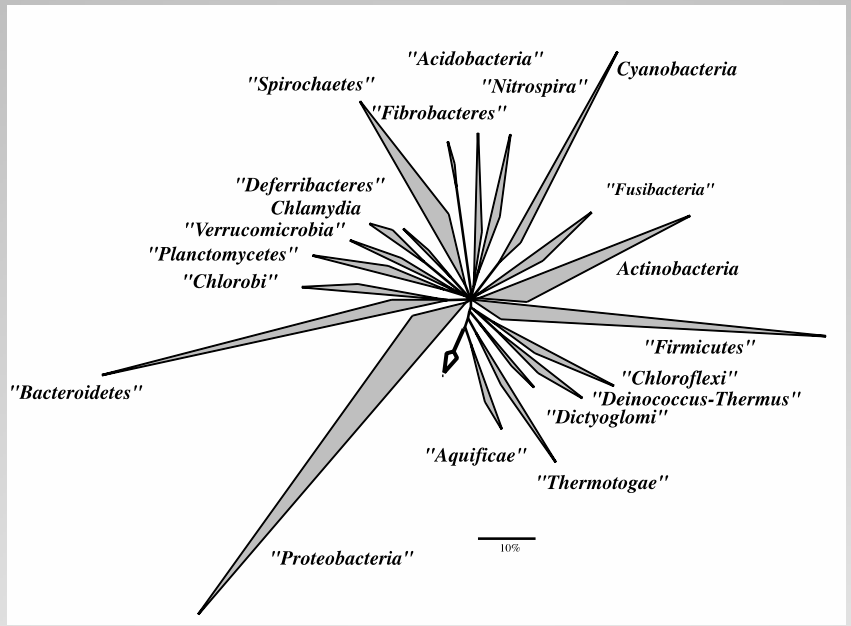
We are modeling protein evolution as a mixture of two processes:

1) frequent neutral amino acid substitutions within profiles (e.g., P1), punctuated by

2) profile changes related to functional differentiation (P1 -> P2 -> P3 ….).



4. The phylogeny of Bacteria



Determining the evolutionary relationships among bacterial phyla based on phylogenetic trees of protein evolution

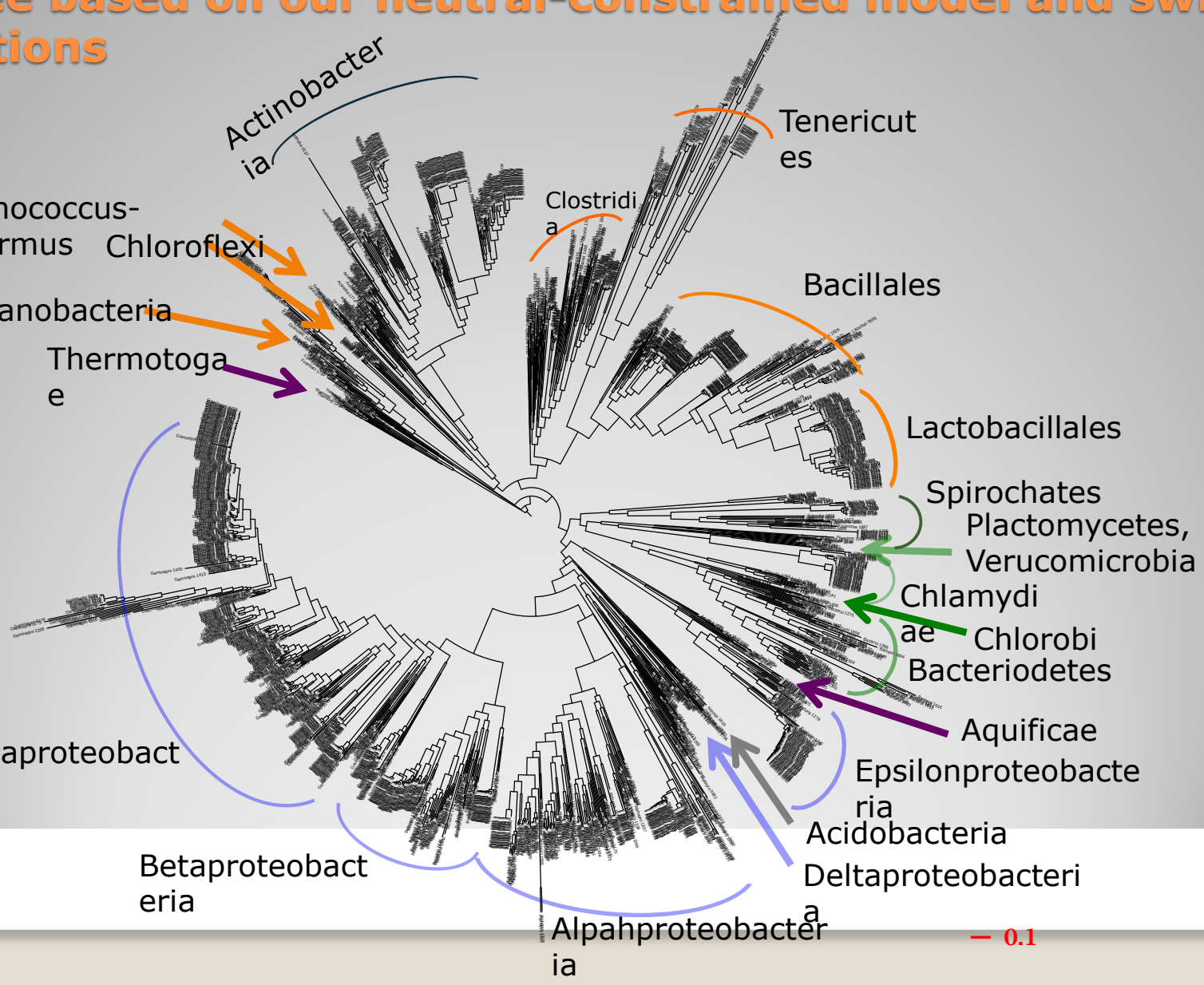
Reconstruction of phylogenetic trees

Development of amino acid substitution models

Identification of characters preserving the phylogenetic signal

4. The phylogeny of bacteria

A tree based on our neutral-constrained model and switch positions



5. Measures of differentiation from genomic and metagenomic data

- We are developing measures of diversity within samples of data (e.g., from genome projects). to help direct future sequencing projects in an effort to maximize diversity.
- We are also interested in developing measures of diversity that will allow for meaningful comparisons between sets of data. For example, comparisons between microbiomes that take into consideration the evolutionary relations of the different sequences.
- We are working on measures of diversity based on the concept of genetic relatedness (Wright, 1922), calculated over given evolutionary trees.

5. Measures of differentiation
Expected Genetic Diversity

- A measure of genetic diversity (as opposed to phenotypic diversity)
- It estimates how many states not identical by descent are expected to be represented at the leaves of a phylogenetic tree (or of a cluster).
- In our implementation states correspond to amino acid types.
- Estimates of Expected Genetic Diversity depend on the substitution model as well as on branch lengths and tree topology.
- Associated with this measure are measures of sampling density, group relatedness, and leaf-weights.

6. Evolution of chaperone proteins

- Chaperone proteins are well known for the critical role they play in assisting cellular activities, such as protein folding, and their role in in disease.
- Recent analyses indicated that the number and functional differentiation of eukaryotic chaperone genes is larger than previously thought.
- The availability of complete genome sequences makes it possible to pursue characterization of the complete set of chaperone genes encoded by human and other species.

6. The evolution of chaperone proteins

Human chaperonin-genes

- Chaperonins are involved in folding nascent peptides and in rescuing mis-folded proteins. We identified fifty-four chaperonin-like sequences in the human genome including a newly-defined class of chaperonin genes named CCT8L, represented in human by the two sequences CCT8L1 and CCT8L2 (Mukherjee *et al*, *BMC Evol Biol* 2010).
- The characterization of many newly-discovered chaperonin pseudogenes uncovered the intense duplication activity of eukaryotic chaperonin genes (Mukherjee *et al*, *BMC Evol Biol* 2010).
- By extensive searches of chaperonin-like genes, we uncovered the ancient origin of chaperonin-like BBS genes, associated with the human developmental disorder Bardet-Biedl Syndrome (BBS), and previously believed to be metazoan-specific (Mukherjee and Brocchieri, *J Phylogen Evolution Biol* 2013).

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Editor Signature

Luciano Brocchieri

Thank you.