

Microbial Evolution and Systematics

I. Early Earth and the Origin and Diversification of Life

14.1 Formation and Early History of Earth

- Origin and Diversification of Life
 - The theme that unifies all of biology is evolution
- Origin of Earth
 - Earth is thought to have formed about 4.5 billion years ago, based on data from slowly decaying radioactive isotopes
- Evidence for Microbial Life on Early Earth
 - Some ancient rocks contain what appear to be bacteria-like microfossils, typically as simple rods or cocci
 - In rocks of 3.5 billion years old or younger, **stromatolites** are abundant. Stromatolites are fossilized microbial mats consisting of layers of filamentous prokaryotes and trapped sediment

14.2 Origin of Cellular Life

- Surface Origin Hypothesis
 - One hypothesis for the origin of life is that the first membrane-enclosed, self-replicating cells arose out of a primordial soup that was rich in organic and inorganic compounds in a “warm little pond”
 - This hypothesis is unlikely as the surface conditions of early Earth would have been too hostile for life
- Subsurface Origin Hypothesis
 - An alternative hypothesis is that life originated at hydrothermal springs on the ocean floor, well below Earth’s surface. Conditions here would have been much less hostile and more stable
- An RNA World and Protein Synthesis
 - Another possibility is that there was an early *RNA world*, in which the first molecules were RNA. RNA might have catalyzed its own synthesis from the available sugars, base, and phosphate
 - RNA can also bind other molecules, such as amino acids, catalyzing the synthesis of primitive proteins. Eventually, DNA became a repository of genetic (coding) information and assumed the template role for RNA synthesis
- Lipid Membranes and Cellular Life
 - Other important factors were the buildup of lipids and the synthesis of phospholipid membrane vesicles that enclosed the cell’s biochemical and replication machinery. By entrapping RNA and DNA, these lipoprotein vesicles may have formed the first self-replicating cells
- Early Metabolism
 - From the time of its formation, the early ocean and all Earth was anoxic
 - The early energy-generating metabolism of primitive cells would have been anaerobic
 - The first cells may have been anaerobic autotrophs, obtaining their carbon from CO₂ and their energy from electrons used to reduce carbon dioxide to cellular material from H₂
 - Reactions with the substrate ferrous iron, which was abundant on early Earth, have also been proposed as energy-yielding reactions to primitive organisms

- These early forms of chemolithotrophic metabolism would have supported the production of large amounts of organic compounds from autotrophic CO₂ fixation

14.3 Microbial Diversification: Consequences for Earth's Biosphere

- Metabolic Diversification
 - Using information obtained from DNA to estimate a timescale, LUCA, the last universal common ancestor, may have existed as early as 4.25 billion years ago
 - Phototrophy arose somewhat later, about 3.2 billion years ago, and only in *Bacteria*
- The Rise of Oxygen: Banded Iron Formations
 - Molecular and chemical evidence indicates that oxygen-generating cyanobacteria first appeared on Earth about 2.7 billion years ago
 - The O₂ that cyanobacteria produced did not begin to accumulate in the atmosphere until it reacted with reduced materials, especially iron in the oceans
 - When O₂ began to accumulate in the atmosphere, major evolutionary events started
- New Metabolisms and the Ozone Shield
 - The evolution of oxygenic photosynthesis caused the atmosphere to gradually change from anoxic to oxic
 - During this time, organelle-containing eukaryotic microorganisms evolved
 - The formation of ozone from O₂ provided a barrier preventing much of the ultraviolet radiation of the sun from reaching the Earth

14.4 Endosymbiotic Origins of Eukaryotes

- Origin of Eukaryotes
 - Geological evidence from microfossils indicates unicellular eukaryotes arose on Earth about 2 billion years ago
- Endosymbiosis
 - A well-supported hypothesis for the origin of the eukaryotic cells is that of **endosymbiosis**, which states that the mitochondria and chloroplasts of modern-day eukaryotes arose from the stable incorporation into another type of cell of a chemooorganotrophic bacterium, which carried out facultative aerobic metabolism, and a cyanobacterium, which carried out oxygenic photosynthesis
 - The overall physiology and metabolism of mitochondria chloroplasts and the sequence and structures of their genomes support the endosymbiosis hypothesis
- Formation of the Eukaryotic Cell
 - One hypothesis is that the first eukaryotes began as a nucleus-bearing cell that later acquired mitochondria and chloroplasts by endosymbiosis
 - On the other hand, the *hydrogen hypothesis* proposes that the eukaryotic cell arose from an intracellular association between an oxygen-consuming, hydrogen-producing member of *Bacteria* (the symbiont, which gave rise to mitochondrion) and a member of *Archaea*, the host
- Chimeric Nature of the Eukaryotic Cell
 - Both hypotheses to explain the origin of eukaryotes suggest that the eukaryotic cell is a chimera, a cell made up of attributes of both *Bacteria* and *Archaea*
 - The development of the eukaryotic cell was a major step in evolution, creating complex, genetically chimeric cells with new capabilities, powered by mitochondria and containing oxygen-generating chloroplasts

II. Microbial Evolution

14.5 The Evolutionary Process

- Evolution

- Evolution is descent with modification, a change in the sequence of an organism's genomic DNA and the inheritance of that change by the next generation
- Mutations and Selection
 - Mutations occur due to errors in replication. They are essential in order for life to be able to change and adapt through natural selection. Adaptive mutations are those that improve the **fitness** of an organism, increasing its survival or reproduction compared to other organisms
 - Gene duplication can lead to evolutionary developments of new functions as the sequence of the gene changes over time
 - **Horizontal gene transfer** can bring genes together from distantly related lineages
 - Gene loss can lead to the genome reduction often seen in obligate symbionts and parasites
 - As environments change, cells must either survive and successfully compete for nutrients or become extinct

14.6 Evolutionary Analyses: Theoretical Aspects

- Phylogeny
 - The evolutionary history of a group of organisms is called its **phylogeny**
- Genes Employed in Phylogenetic Analysis
 - Most widely used and useful for defining relationships are the genes encoding 16S rRNA and its counterpart in eukaryotes, 18S rRNA. These **small subunit ribosomal RNA (SSU rRNA)** genes have been used extensively for sequence-based evolutionary analysis
 - Carl Woese established the presence of three domains of life, *Bacteria*, *Archaea*, and *Eukarya*, and established for the first time a unified phylogenetic framework for bacteria
 - The 23S large subunit rRNA (LSU-rRNA) gene is also phylogenetically highly informative, with its longer sequence providing additional information
 - The highly conserved SSU and LSU genes have changed slowly and provide a view of evolution that is deep enough to encompass all living organisms
- Molecular Clocks
 - If DNA (and protein) sequences change at a constant rate, then the amount of change between two homologous sequences would serve as an approximate **molecular clock** of evolution, allowing the time in the past when the two sequences diverged from a common ancestral sequence to be estimated
 - The main problem with the molecular clock approach is that DNA sequences change at different rates

14.7 Evolutionary Analyses: Analytical Methods

- Obtaining DNA Sequences
 - Phylogenetic analysis using DNA sequences relies on the polymerase chain reaction (PCR) to obtain sufficient copies of a gene for sequencing
- Sequence Alignment
 - Phylogenetic analysis is based on homology, analysis of DNA sequences that are related by common ancestry
 - Homologous genes in different organisms may be either *orthologs*, which differ because of sequence divergence as the organisms followed different evolutionary paths, or *paralogs*, which arise through gene duplication
- Phylogenetic Trees
 - Reconstructing evolutionary history from observed nucleotide sequence differences involves construction of a *phylogenetic tree*

- A *phylogenetic tree* is a graphic illustration of the relationships among the sequences of the organisms under study, much like a family tree
- The relationship between two organisms should be read in terms of common ancestry; the more recently two species share a common ancestor, the more closely related they are
- Tree Reconstruction
 - Cladistics is the classification of species based on ancestry
 - A **monophyletic group** describes how phylogenetically informative characters are recognized
 - Computer-based analysis of these changes generates a phylogenetic tree, or *cladogram*
 - A widely used cladistic method is *parsimony*, which is based on the assumption that evolution proceeded along the path requiring the fewest changes
 - Other cladistic methods, *maximum likelihood* and *Bayesian analysis*, proceed like parsimony, but differ by assuming a model of evolution wherein certain kinds of nucleotide changes are more common than others

14.8 Microbial Phylogeny

- Microbial Phylogeny
 - Biologists previously grouped living organisms into the five kingdoms: plants, animals, fungi, protists, and bacteria
 - Cellular life on Earth has evolved along three primary lineages called **domains**, the *Bacteria*, the *Archaea*, and the *Eukarya*. The first two domains are composed only of cells that lack a membrane-enclosed nucleus (prokaryotic cells). The third is primarily microbial and includes plants, animals, fungi, and protists
- A SSU rRNA Gene-Based Phylogeny of Life
 - The **universal phylogenetic tree** based on SSU rDNA genes is a genealogy of all life on Earth
- *Bacteria*
 - At least 80 major evolutionary groups (phyla or divisions) of *Bacteria* have been discovered so far
 - Many groups have been defined from environmental sequences only
 - Most major groups consist of species that are related from a phylogenetic standpoint, but lack strong phenotypic cohesiveness
 - Eukaryotic organelles clearly originated from within the domain *Bacteria*. Mitochondria arose from the *Proteobacteria*
- *Archaea*
 - The domain *Archaea* consists of two groups, the *Crenarchaeota* and *Euryarchaeota*. Branching close to the root of the universal tree are hyperthermophilic *Crenarchaeota*
 - The *Euryarchaeota* include the methane-producing *Archaea* and the extreme halophiles
 - There are some branches in the *Crenarchaeota* lineage that are known only from environmental sampling of ribosomal genes from the environment
- *Eukarya*
 - Phylogenetic trees of species in this domain are generated from comparative sequencing analysis of the 18S rRNA gene, the functional equivalent of the 16S rRNA gene
 - Include a wide diversity of organisms
- Distinguishing Characteristics of the Domains of Life
 - Each domain can be characterized by various phenotypic properties

14.9 Applications of SSU rRNA Phylogenetic Methods

- Signature Sequences
 - Computer analyses of ribosomal rRNA sequences have revealed **signature sequences**, short oligonucleotides unique to certain groups of organisms

III. Microbial Systematics

- Microbial Systematics
 - **Systematics** is the study of the diversity of organisms and their relationships
 - The science of characterizing, naming, and placing organisms into groups is called **taxonomy**
 - Bacterial taxonomy uses a *polyphasic* approach that uses three kinds of methods for the identification and description of bacteria: phenotypic, genotypic, and phylogenetic

14.10 Phenotypic Analysis

- Phenotypic Analysis
 - The observable characteristics of a bacterium provide many traits that can be used to differentiate between species

14.11 Genotypic Analysis

- DNA–DNA Hybridization
 - When two organisms share many highly similar genes, their DNAs would be expected to hybridize to one another in approximate proportion to their gene sequences. Measurement of **DNA-DNA hybridization** between the genomes of two organisms provides a rough index of their similarity to each other
- GC Ratios
 - **GC ratios** measure the percentage of guanine plus cytosine in an organism's genomic DNA
 - GC ratios vary over a wide range, with values from 20% to 80% among *Bacteria* and *Archaea*
 - Two organisms can have identical GC ratios and be unrelated

14.13 The Species Concept in Microbiology

- Species Concept in Microbiology
 - At present, there is no universally accepted concept of **species** for prokaryotes
- Current Definition of Prokaryotic Species
 - A prokaryotic species presently is defined as a collection of strains that share a high degree of similarity
 - The biological species concept—which states that a species is an interbreeding population that is reproductively isolated from other interbreeding populations—is not effective or meaningful for the *Bacteria* and *Archaea*, which are haploid and do not undergo sexual reproduction
 - The genealogical species concept—which is a group of strains that cluster closely with each other phylogenetically and are distinct from other groups of strains—is suitable for haploid organisms
- Speciation in Prokaryotes
 - Periodic selection occurs in a population of bacteria that originated from a single cell and that occupy a particular niche in a habitat. If cells in this population share a particular resource (a nutrient), this population of cells can be called an **ecotype**

- Repeated rounds of mutation and selection in this ecotype may lead it to become more genetically distinct from the other ecotypes
- How Many Prokaryotic Species Are There?
 - Nearly 7,000 species in *Bacteria* and *Archaea* are already known and perhaps as many as 100,000-1,000,000 in total are thought to exist
 - Microbial community analyses indicate that we have only scratched the surface in our ability to culture the diversity of *Bacteria* and *Archaea* in nature

14.14 Classification and Nomenclature

- Classification
 - Classification is the organization of organisms into progressively more inclusive groups on the basis of either phenotypic similarity or evolutionary relationship
- Nomenclature
 - Nomenclature is the application of formal rules for naming organisms
 - In the **binomial system**, prokaryotes are given genus names and species epithets
 - The terms used are Latin or Latinized Greek derivations, often of some descriptive property appropriate for the organism, and are set in print in *italics*
- *Bergey's Manual* and *The Prokaryotes*
 - There is no “official” classification of *Bacteria* and *Archaea*
 - The classification system most widely accepted by microbiologists is the “Taxonomic Outline of the Prokaryotes” arising out of the second edition of *Bergey's Manual of Systematic Bacteriology*
 - A second major reference in bacterial diversity is *The Prokaryotes*, which provides detailed information on the enrichment, isolation, and culture of the many groups of *Bacteria* and *Archaea*
- Culture Collections
 - National microbial culture collections are permanent collections that catalog and store microorganisms and provide them upon request, usually for a fee, to researchers in academia, medicine, and industry
 - A related and key role of culture collections is as repositories for *type strains*
- Describing New Species
 - To achieve formal validation of taxonomic standing as a new genus or species, viable cultures of the organism are deposited in at least two international culture collections and a detailed description of the organism's characteristics and traits, along with its proposed name, is published
 - The description and new name should be published in the *International Journal of Systematic and Evolutionary Microbiology (IJSEM)*, the official publication of record for the taxonomy and classification of *Bacteria* and *Archaea*
 - Publication in *IJSEM* paves the way for inclusion in *Bergey's Manual of Systematic Bacteriology*
 - The International Committee on Systematics of Prokaryotes (ICSP) is responsible for overseeing nomenclature and taxonomy of *Bacteria* and *Archaea*