1

The Evolution of Microorganisms and Microbiology

1.1 Members of the Microbial World

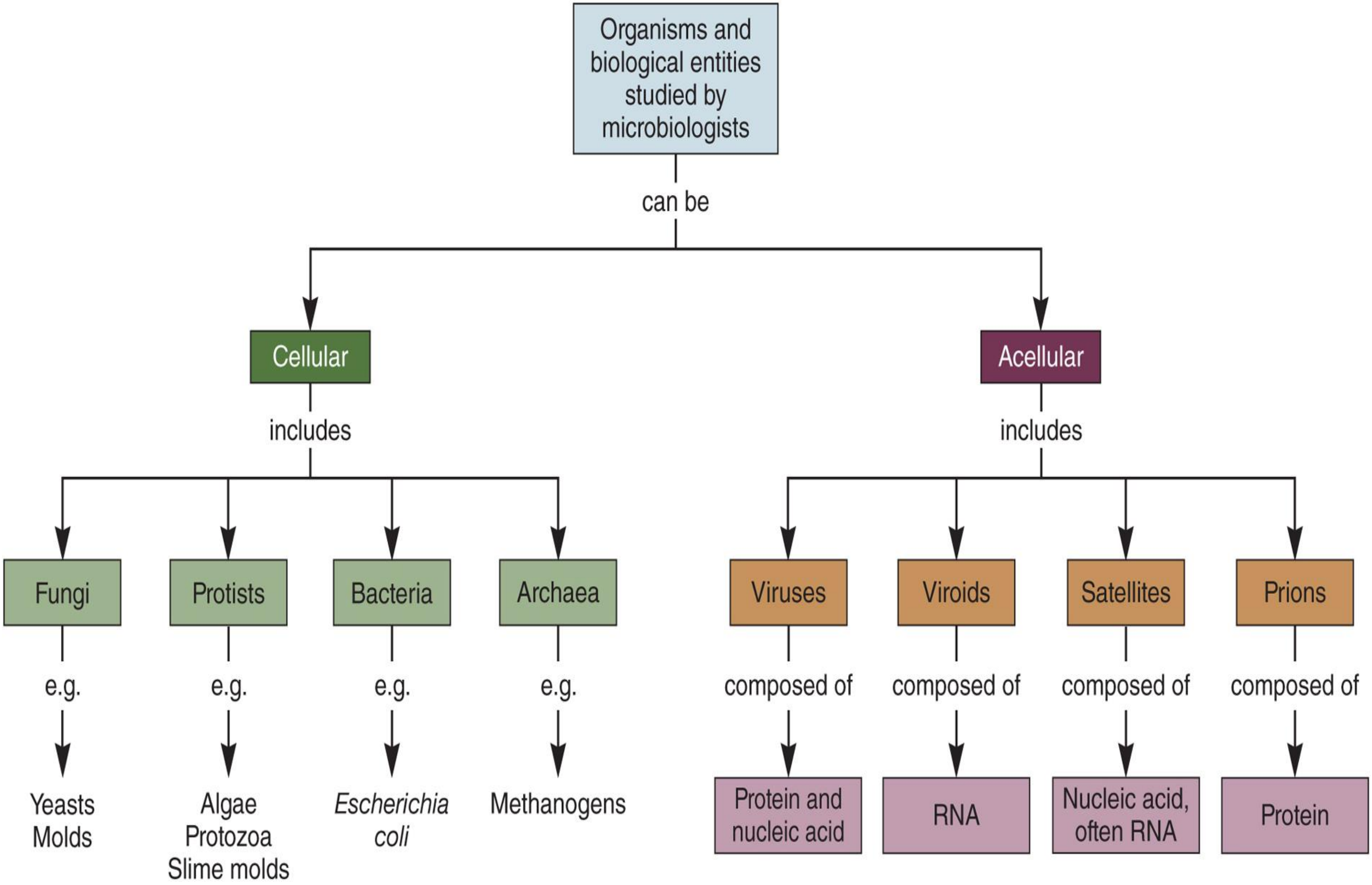
1. Differentiate the biological entities studied by microbiologists from those studied by other biologists
2. Explain Carl Woese’s contributions in establishing the three domain system for classifying cellular life
3. Provide an example of the importance to humans of each of the major types of microbes
4. Determine the type of microbe (e.g., bacterium, fungus, etc.) when given a description of a newly discovered microbe

The Importance of Microorganisms

- Most populous and diverse group of organisms
- Found everywhere on the planet
- Play a major role in recycling essential elements
- Source of nutrients and some carry out photosynthesis
- Benefit society by their production of food, beverages, antibiotics, and vitamins
- Some cause disease in plants and animals

Members of the Microbial World

- Organisms and acellular entities too small to be clearly seen by the unaided eye
  - some < 1 mm, some macroscopic
- These organisms are relatively simple in their construction and lack highly differentiated cells and distinct tissues



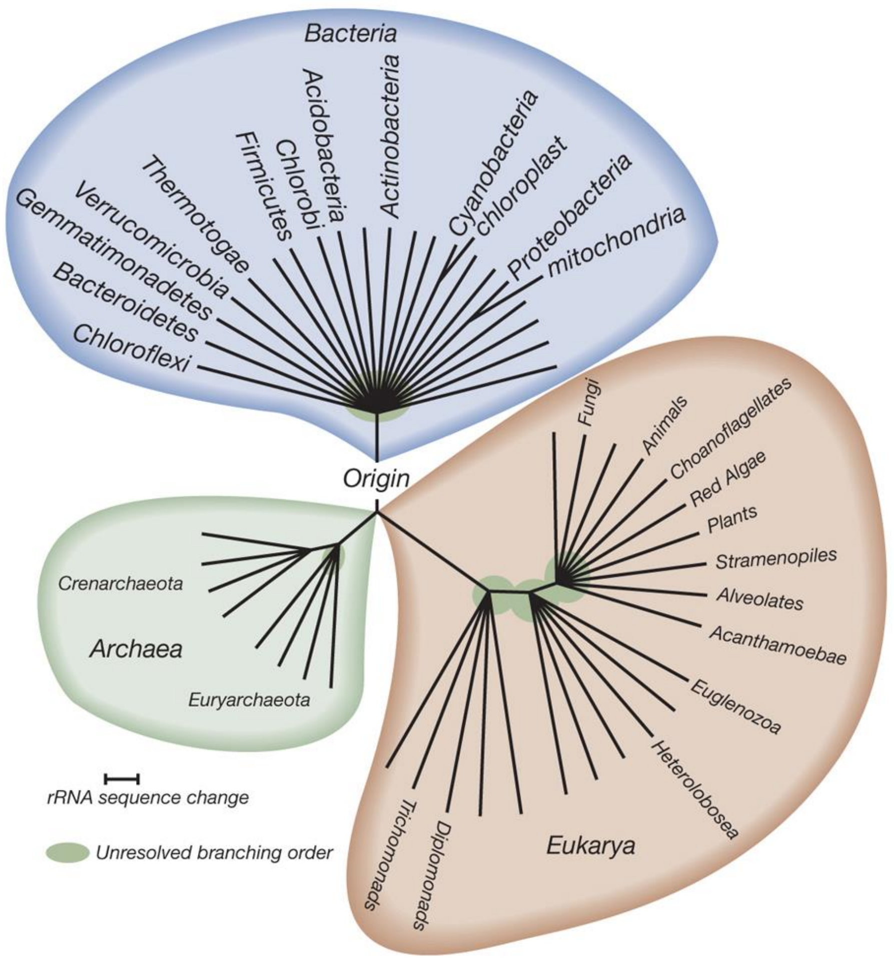
Organisms and biological entities studied by microbiologists
can be
Cellular
Acellular
includes
includes
Fungi
Protists
Bacteria
Archaea
Viruses
Viroids
Satellites
Prions
e.g.
e.g.
e.g.
e.g.
composed of
composed of
composed of
composed of
Yeasts Molds
Algae Protozoa Slime molds
Escherichia coli
Methanogens
Protein and nucleic acid
RNA
Nucleic acid, often RNA
Protein

Type of Microbial Cells

- Prokaryotic cells lack a true membrane-delimited nucleus
  - this is not absolute!
- Eukaryotic cells have a membrane-enclosed nucleus, are more complex morphologically, and are usually larger than prokaryotic cells

Classification Schemes

- Three domain system, based on a comparison of ribosomal RNA genes, divides microorganisms into
  - *Bacteria* (true bacteria),
  - *Archaea*
  - *Eukarya (eukaryotes)*



Domain *Bacteria*

- Usually single-celled
- Majority have cell wall with peptidoglycan
- Most lack a membrane-bound nucleus
- Ubiquitous and some live in extreme environments
- Cyanobacteria produce significant amounts of oxygen

Domain *Archaea*

- Distinguished from *Bacteria* by unique rRNA gene sequences
- Lack peptidoglycan in cell walls
- Have unique membrane lipids
- Some have unusual metabolic characteristics
- Many live in extreme environments

Domain *Eukarya* - *E*ukaryotic

- Protists – generally larger than *Bacteria* and *Archaea*
  - algae – photosynthetic
  - protozoa – may be motile, “hunters, grazers”
  - slime molds – two life cycle stages
  - water molds – devastating disease in plants
- Fungi
  - yeast - unicellular
  - mold - multicellular

Acellular Infectious Agents

- Viruses
  - smallest of all microbes
  - requires host cell to replicate
  - cause range of diseases, some cancers
- Viroids and virusoids
  - infectious agents composed of RNA
- Prions – infectious proteins

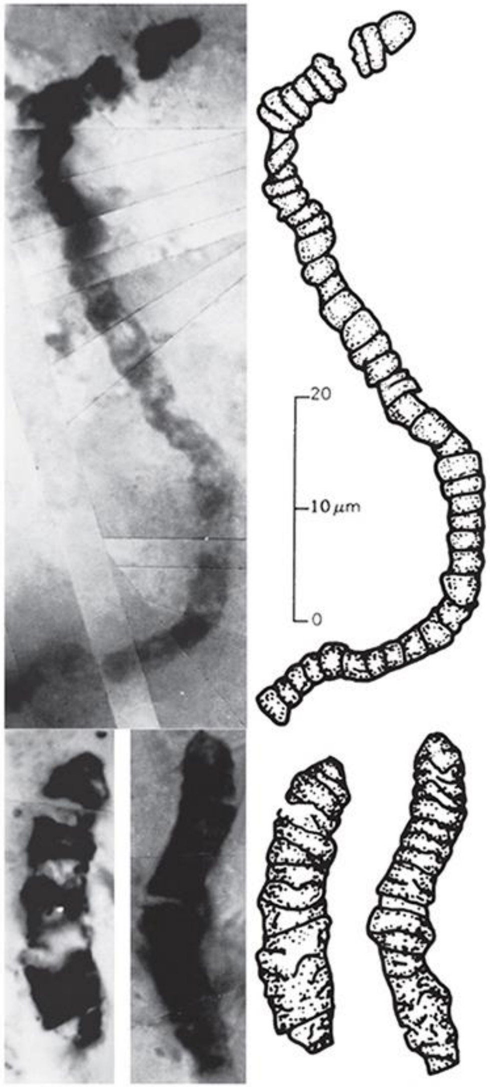
1.2 Microbial Evolution

1. Propose a time line of the origin and history of microbial life and integrate supporting evidence into it
2. Design a set of experiments that could be used to place a newly discovered cellular microbe on a phylogenetic tree based on small subunit (SSU) rRNA sequences
3. Compare and contrast the defi nitions of plant and animal species, microbial species, and microbial strains

Microbial Evolution

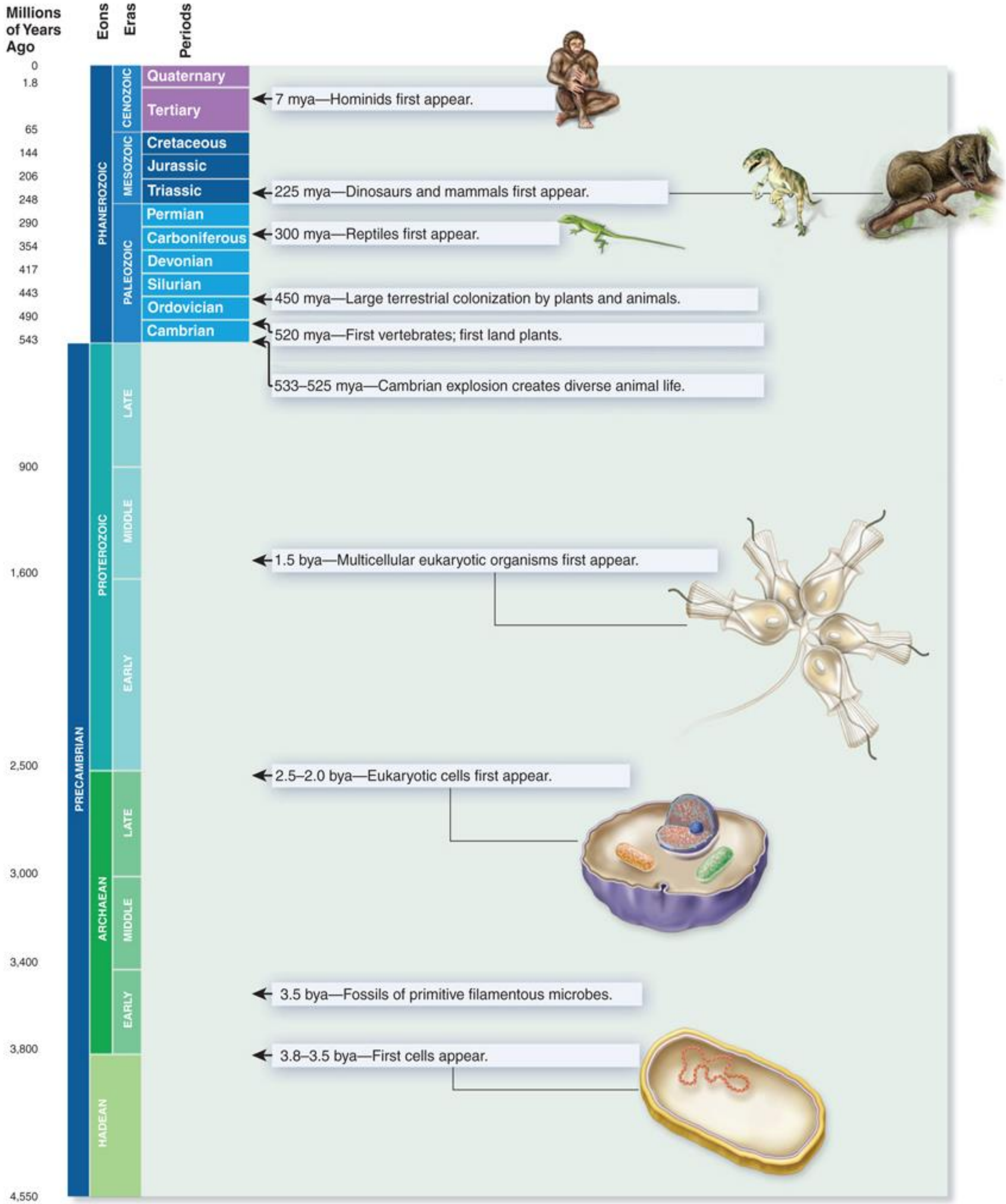
- Definition of life
  - cells and organization
  - response to environmental changes
  - growth and development
  - biological evolution
  - energy use and metabolism
  - regulation and homeostasis
  - reproduction

Origins of Life

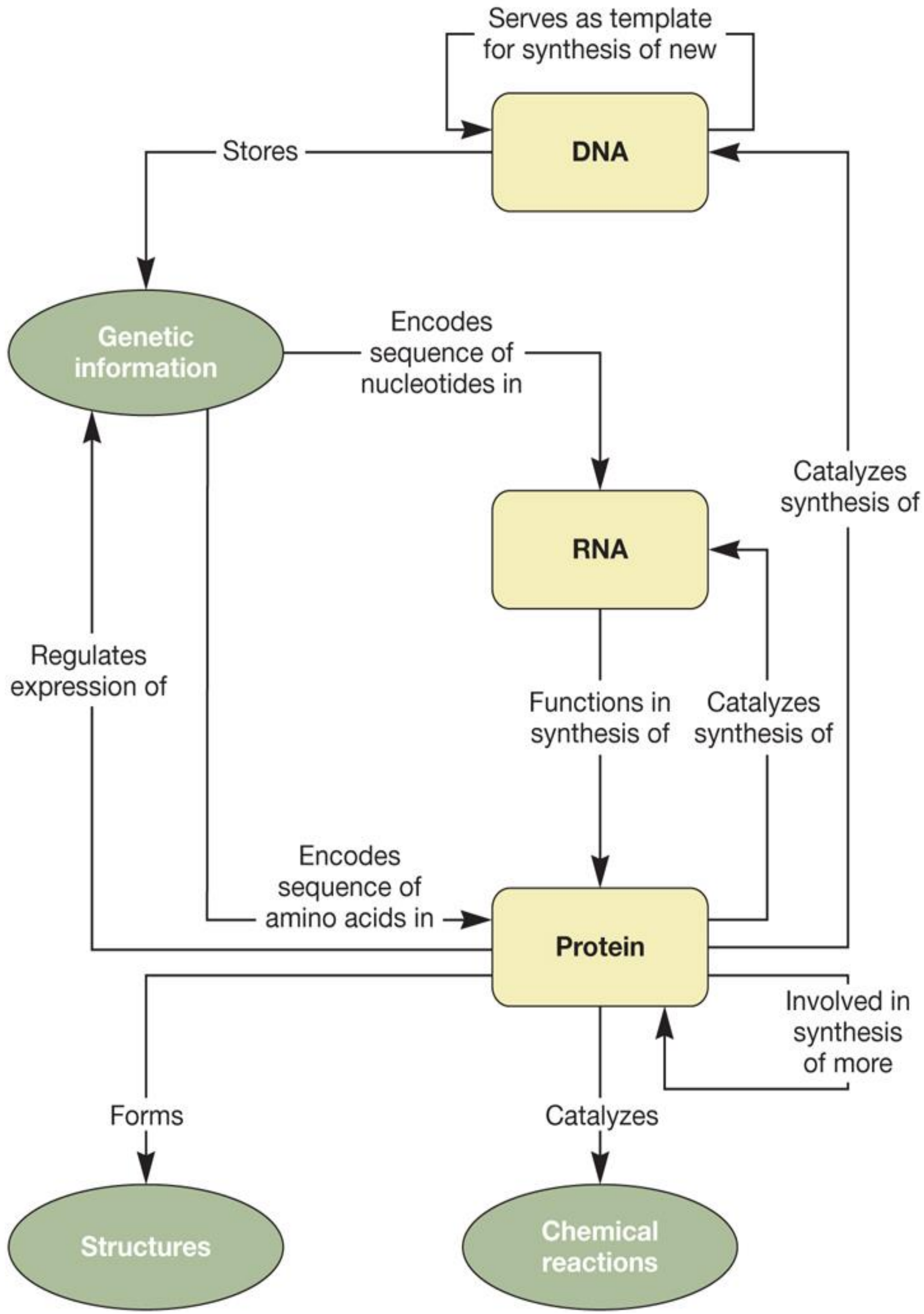


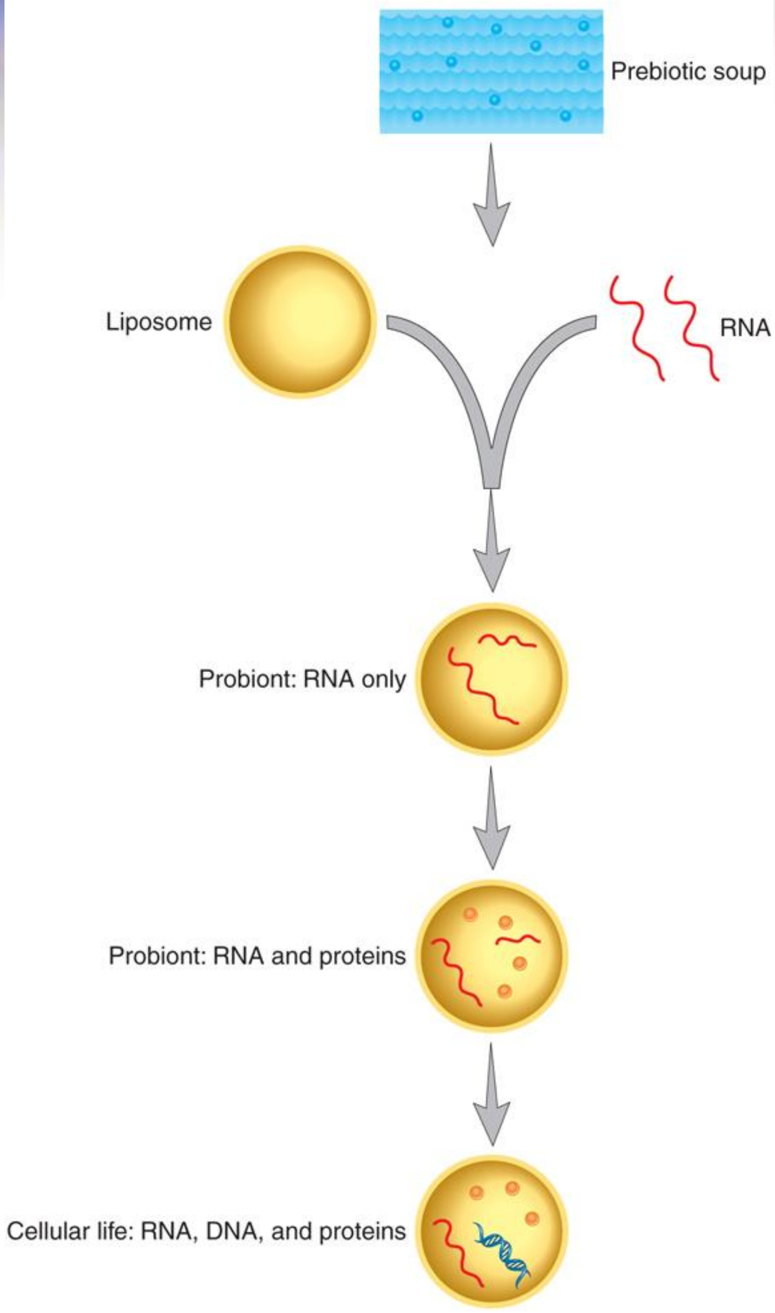
J. William Schopf. Microfossils of the Early Archean Apex Chert: New Evidence of the Antiquity of Life. Reprinted with permission from *Science* 260:640 © 1993 Sept. 30, fi g 4 a, f, g. AAAS

- Microbial fossils
  - Swartkoppie chert – granular silica
  - 3.5 billion years old
- Fossil record sparse
- Indirect evidence and scientific method are used to study origins of life



Millions of Years Ago
Eons
Eras
Periods
0
1.8
65
144
206
248
290
354
417
443
490
543
900
1,600
2,500
3,000
3,400
3,800
4,550
PHANEROZOIC
PRECAMBRIAN
CENOZOIC
MESOZOIC
PALEOZOIC
PROTEROZOIC
ARCHAEAN
HADEAN
LATE
MIDDLE
EARLY
Quaternary
Tertiary
Cretaceous
Jurassic
Triassic
Permian
Carboniferous
Devonian
Silurian
Ordovician
Cambrian
7 mya—Hominids first appear.
225 mya—Dinosaurs and mammals first appear.
300 mya—Reptiles first appear.
450 mya—Large terrestrial colonization by plants and animals.
520 mya—First vertebrates; first land plants.
533–525 mya—Cambrian explosion creates diverse animal life.
1.5 bya—Multicellular eukaryotic organisms first appear.
2.5–2.0 bya—Eukaryotic cells first appear.
3.5 bya—Fossils of primitive filamentous microbes.
3.8–3.5 bya—First cells appear.


Serves as template for synthesis of new
DNA
Stores
Genetic information
Encodes sequence of nucleotides in
RNA
Catalyzes synthesis of
Regulates expression of
Functions in synthesis of
Catalyzes synthesis of
Encodes sequence of amino acids in
Protein
Involved in synthesis of more
Forms
Catalyzes
Structures
Chemical reactions



Earliest Molecules - RNA

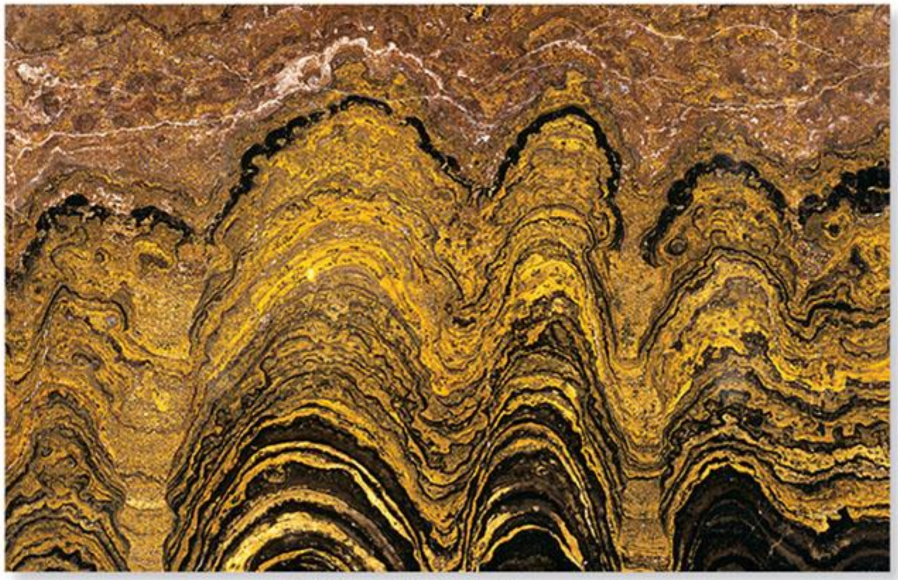
- Original molecule must have fulfilled protein and hereditary function
- Ribozymes
  - RNA molecules that form peptide bonds
  - perform cellular work and replication
- Earliest cells may have been RNA surrounded by liposomes

Earliest Molecules – RNA - 2

- Cellular pool of RNA in modern day cells exists in and is associated with the ribosome (rRNA, tRNA, mRNA)
  - RNA catalytic in protein synthesis
  - RNA may be precursor to double stranded DNA
- Adenosine 5’ triphosphate (ATP) is the energy currency and is a ribonucleotide
- RNA can regulate gene expression

Earliest Metabolism

- Early energy sources under harsh conditions
  - inorganics, e.g., FeS
- Photosynthesis
  - cyanobacteria evolved 2.5 billion years ago
  - stromatolites – mineralized layers of microorganisms



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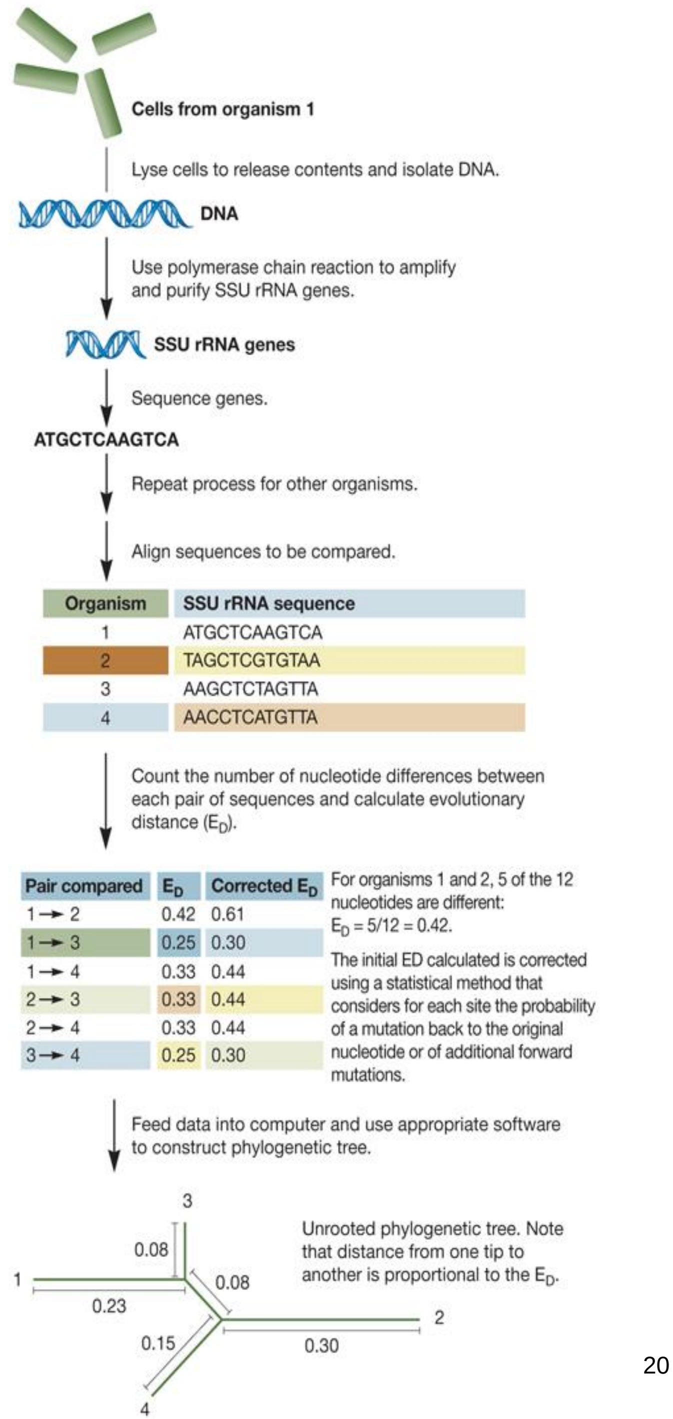
Evolution of 3 Domains of Life

- Universal phylogenetic tree
  - based on comparisons of small subunit rRNA (SSU rRNA)
  - aligned rRNA sequences from diverse organisms are compared and differences counted to derive a value of evolutionary distance
  - relatedness, but not time of divergence, is determined this way



Last Universal Common Ancestor (LUCA)

- The root or origin of modern life is on bacterial branch but nature still controversial
- *Archaea* and *Eukarya* evolved independently of *Bacteria*
- *Archaea* and *Eukarya* diverged from common ancestry

Endosymbiotic Hypothesis

- Origin of mitochondria, chloroplasts, and hydrogenosomes from endosymbiont
- Mitochondria and chloroplasts
  - SSU rRNA genes show bacterial lineage
  - genome sequences closely related to *Rickettsia* and *Prochloron,* respectively
- Hydrogenosomes
  - anaerobic endosymbiont

Evolution of Cellular Microbes

- Mutation of genetic material led to selected traits
- New genes and genotypes evolved
- *Bacteria* and *Archaea* increase genetic pool by horizontal gene transfer within the same generation

Microbial Species

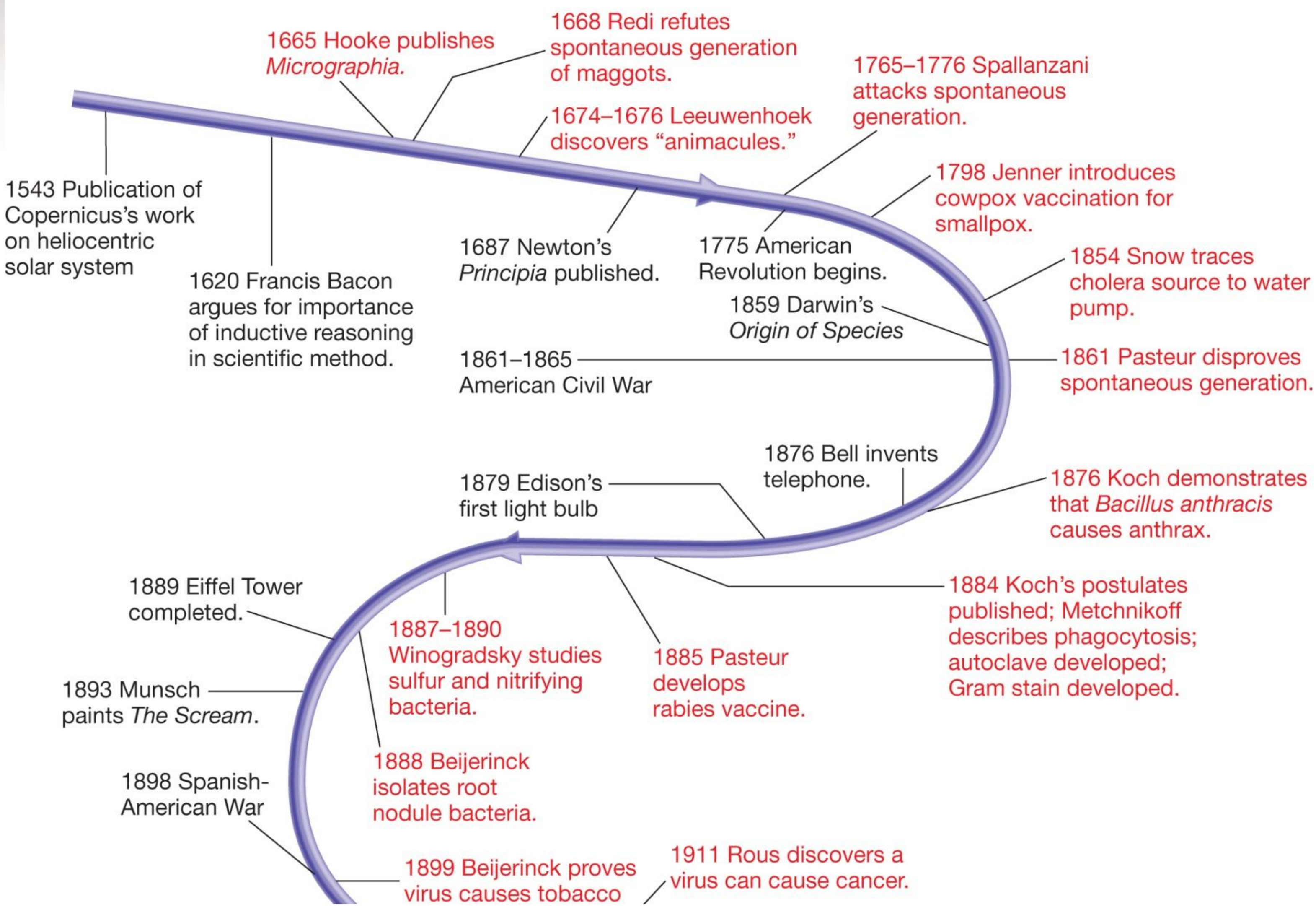
- Eukaryotic microbes fit definition of reproducing isolated populations
- *Bacteria* and *Archaea* do not reproduce sexually and are referred to as strains
  - a strain consists of descendents of a single, pure microbial culture
  - may be biovars, serovars, morphovars, pathovars
- binomial nomenclature
  - genus and species epithet

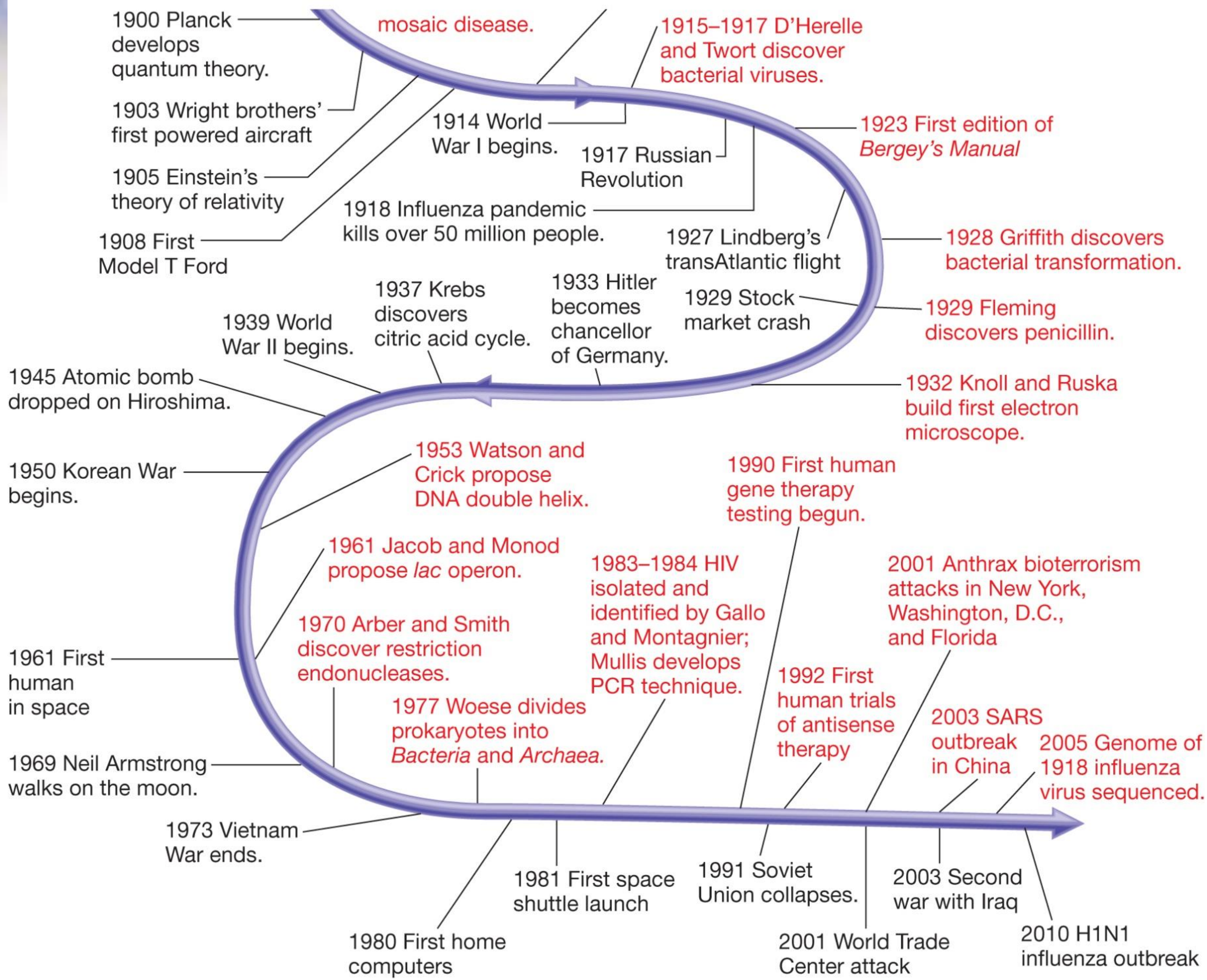
1.3 Microbiology and Its Origins

1. Evaluate the importance of the contributions to microbiology made by Hooke, Leeuwenhoek, Pasteur, Koch, Cohn, Beijerinck, von Behring, Kitasato, Metchnikoff , and Winogradsky
2. Outline a set of experiments that might be used to decide if a particular microbe is the causative agent of a disease
3. Predict the difficulties that might arise when using Koch’s postulates to determine if a microbe causes a disease unique to humans

Microbiology - Origins

- Study of microorganisms
- Tools used for the study
  - microscopes
  - culture techniques
  - molecular genetics
  - genomics

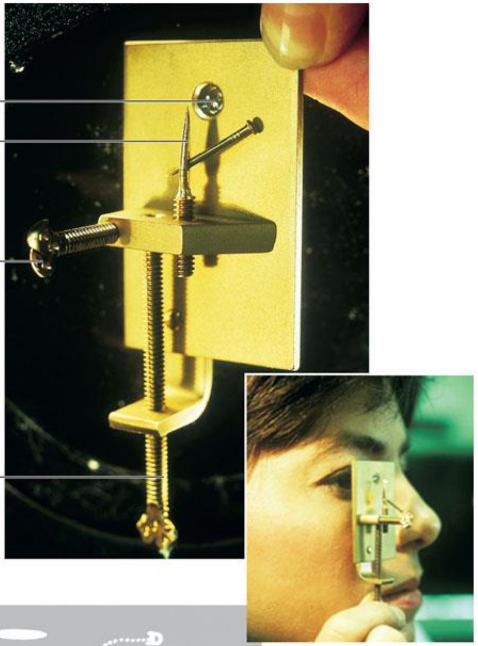

1543 Publication of Copernicus's work on heliocentric solar system
1620 Francis Bacon argues for importance of inductive reasoning in scientific method.
1665 Hooke publishes *Micrographia.*
1668 Redi refutes spontaneous generation of maggots.
1674–1676 Leeuwenhoek discovers "animacules."
1687 Newton's *Principia* published.
1765–1776 Spallanzani attacks spontaneous generation.
1775 American Revolution begins.
1798 Jenner introduces cowpox vaccination for smallpox.
1854 Snow traces cholera source to water pump.
1859 Darwin's *Origin of Species*
1861–1865 American Civil War
1861 Pasteur disproves spontaneous generation.
1876 Bell invents telephone.
1876 Koch demonstrates that *Bacillus anthracis* causes anthrax.
1879 Edison's first light bulb
1884 Koch's postulates published; Metchnikoff describes phagocytosis; autoclave developed; Gram stain developed.
1885 Pasteur develops rabies vaccine.
1887–1890 Winogradsky studies sulfur and nitrifying bacteria.
1888 Beijerinck isolates root nodule bacteria.
1889 Eiffel Tower completed.
1893 Munsch paints *The Scream*.
1898 Spanish-American War
1899 Beijerinck proves virus causes tobacco
1911 Rous discovers a virus can cause cancer.



mosaic disease.
1900 Planck develops quantum theory.
1903 Wright brothers' first powered aircraft
1905 Einstein's theory of relativity
1908 First Model T Ford
1914 World War I begins.
1915–1917 D'Herelle and Twort discover bacterial viruses.
1917 Russian Revolution
1918 Influenza pandemic kills over 50 million people.
1923 First edition of *Bergey's Manual*
1927 Lindberg's transAtlantic flight
1928 Griffith discovers bacterial transformation.
1929 Stock market crash
1929 Fleming discovers penicillin.
1932 Knoll and Ruska build first electron microscope.
1933 Hitler becomes chancellor of Germany.
1937 Krebs discovers citric acid cycle.
1939 World War II begins.
1945 Atomic bomb dropped on Hiroshima.
1950 Korean War begins.
1953 Watson and Crick propose DNA double helix.
1961 Jacob and Monod propose *lac* operon.
1961 First human in space
1969 Neil Armstrong walks on the moon.
1970 Arber and Smith discover restriction endonucleases.
1973 Vietnam War ends.
1977 Woese divides prokaryotes into *Bacteria* and *Archaea*.
1980 First home computers
1981 First space shuttle launch
1983–1984 HIV isolated and identified by Gallo and Montagnier; Mullis develops PCR technique.
1990 First human gene therapy testing begun.
1991 Soviet Union collapses.
1992 First human trials of antisense therapy
2001 Anthrax bioterrorism attacks in New York, Washington, D.C., and Florida
2001 World Trade Center attack
2003 SARS outbreak in China
2003 Second war with Iraq
2005 Genome of 1918 influenza virus sequenced.
2010 H1N1 influenza outbreak

Discovery of Microorganisms

- Antony van Leeuwenhoek (1632-1723)
  - first person to observe and describe microorganisms *accurately*



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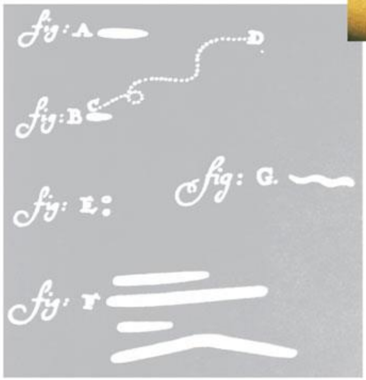

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The Conflict over Spontaneous Generation

- Spontaneous generation
  - Idea that living organisms can develop from nonliving or decomposing matter
- Francesco Redi (1626-1697)
  - discredited spontaneous generation
  - showed that maggots on decaying meat came from fly eggs

But Could Spontaneous Generation Be True for Microorganisms?

- John Needham (1713-1781)
  - his experiment:

    mutton broth in flasks → boiled →sealed
  - results: broth became cloudy and contained microorganisms
- Lazzaro Spallanzani (1729-1799)
  - his experiment:

    broth in flasks →sealed → boiled
  - results: no growth of microorganisms

Louis Pasteur (1822-1895)

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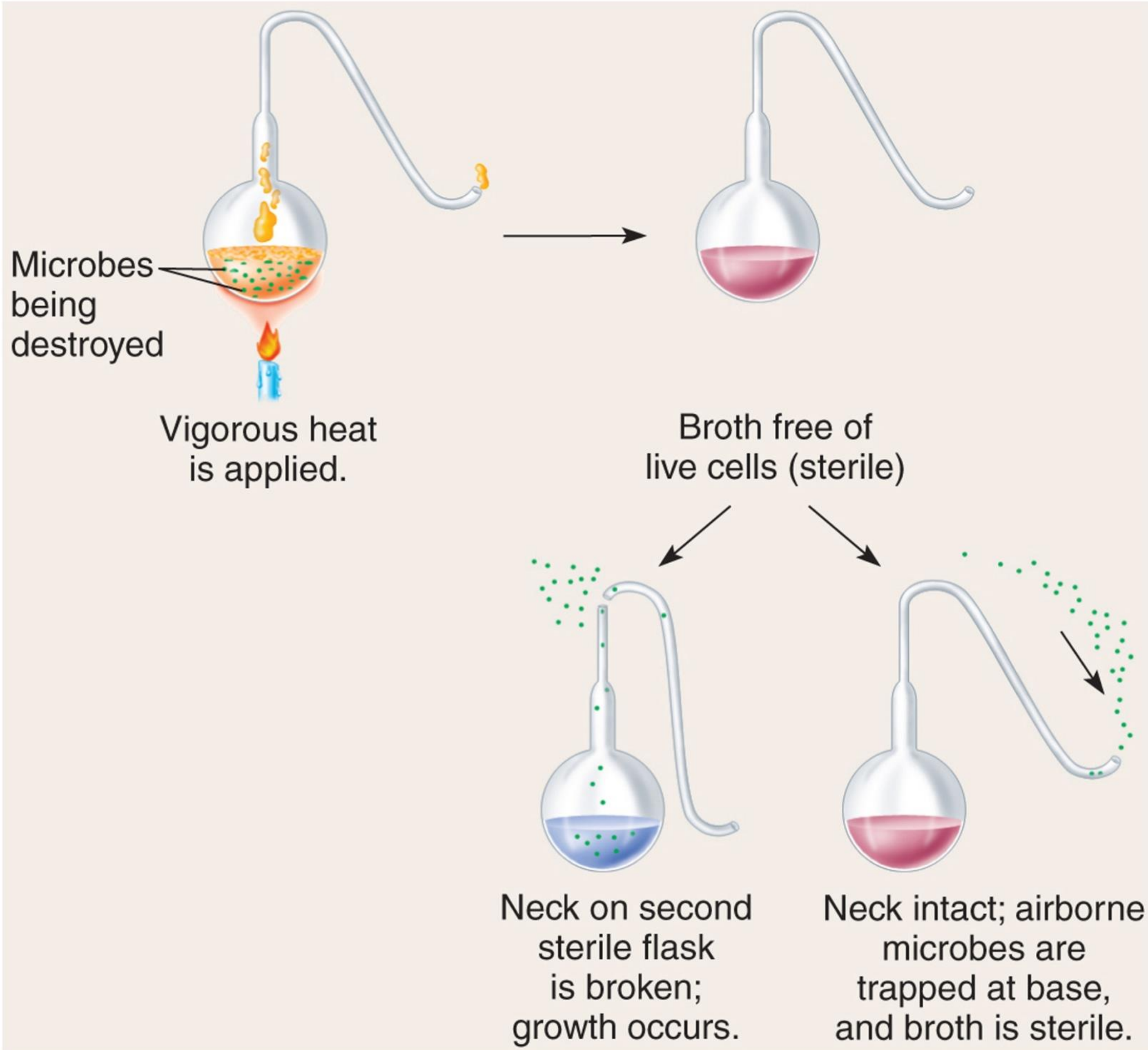
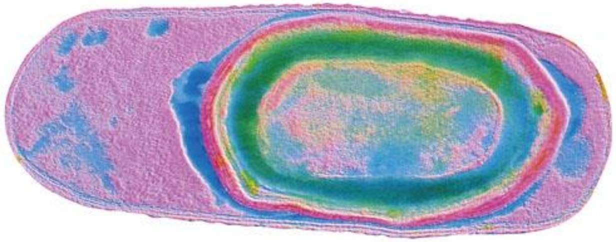
- 'Swan-neck flask' experiments
  - – placed nutrient solution in flasks
  - – created flasks with long, curved necks
  - – boiled the solutions
  - – left flasks exposed to air
- results: no growth of microorganisms


Microbes being destroyed
Vigorous heat is applied.
Broth free of live cells (sterile)
Neck on second sterile flask is broken; growth occurs.
Neck intact; airborne microbes are trapped at base, and broth is sterile.

Final Blow to Theory of Spontaneous Generation

- John Tyndall (1820-1893)
  - demonstrated that dust carries microorganisms
  - showed that if dust was absent, nutrient broths remained sterile, even if directly exposed to air
  - also provided evidence for the existence of exceptionally heat-resistant forms of bacteria
- Ferdinand Cohn (1828-1898)
  - heat-resistant bacteria could produce endospores



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The Role of Microorganisms in Disease

- Was *not* immediately obvious
- Infectious disease believed to be due to supernatural forces or imbalances of 4 bodily-fluid ‘humors’
- Establishing connection depended on development of techniques for studying microbes

Evidence for the Relationship between Microorganisms and Disease

- Agostini Bassi (1773-1856)
  - showed that a disease of silkworms was caused by a fungus
- M. J. Berkeley (ca. 1845)
  - demonstrated that the great Potato Blight of Ireland was caused by a water mold
- Heinrich de Bary (1853)
  - showed that smut and rust fungi caused cereal crop diseases

More Evidence…

- Louis Pasteur
  - demonstrated microorganisms carried out fermentations, helping French wine industry
  - developed pasteurization to avoid wine spoilage by microbes
  - showed that the pébrine disease of silkworms was caused by a protozoan

Other Evidence…

- Joseph Lister
  - provided indirect evidence that microorganisms were the causal agents of disease
  - developed a system of surgery designed to prevent microorganisms from entering wounds as well as methods for treating instruments and surgical dressings
  - his patients had fewer postoperative infections

Final Proof…

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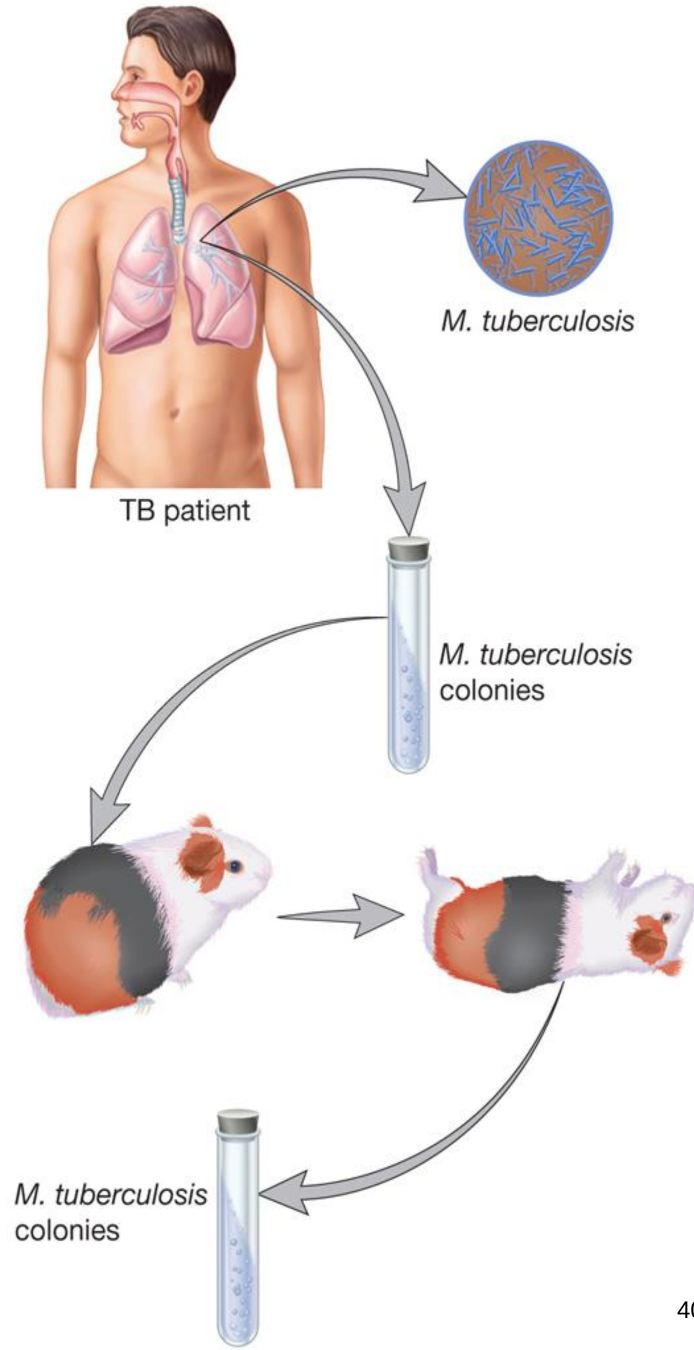
- Robert Koch (1843-1910)
  - established the relationship between *Bacillus anthracis* and anthrax
  - used criteria developed by his teacher Jacob Henle (1809-1895)
  - these criteria now known as Koch's postulates
    - still used today to establish the link between a particular microorganism and a particular disease

Postulate	Experimentation
1. The microorganism must be present in every case of the disease but absent from healthy organisms.	Koch developed a staining technique to examine human tissue. *Mycobacterium tuberculosis* could be identified in diseased tissue.
2. The suspected microorganisms must be isolated and grown in a pure culture.	Koch grew *M. tuberculosis* in pure culture on coagulated blood serum.
3. The same disease must result when the isolated microorganism is inoculated into a healthy host.	Koch injected cells from the pure culture of *M. tuberculosis* into guinea pigs. The guinea pigs subsequently died of tuberculosis.
4. The same microorganisms must be isolated again from the diseased host.	Koch isolated *M. tuberculosis* in pure culture on coagulated blood serum from the dead guinea pigs.



Limitations of Koch’s Postulates

- Some organisms cannot be grown in pure culture
- Using humans in completing the postulates is unethical
- Molecular and genetic evidence may replace and overcome these limits

The Development of Techniques for Studying Microbial Pathogens

- Koch's work led to discovery or development of:
  - agar
  - Petri dishes
  - nutrient broth and nutrient agar
  - methods for isolating microorganisms

Other Developments…

- Charles Chamberland (1851-1908)
  - developed porcelain bacterial filters used by Ivanoski and Beijerinck to study tobacco mosaic disease
    - determined that extracts from diseased plants had infectious agents present which were smaller than bacteria and passed through the filters
    - infectious agents were eventually shown to be viruses

Other Developments…

- Pasteur and Roux
  - discovered that incubation of cultures for long intervals between transfers caused pathogens to lose their ability to cause disease (termed ‘attenuation’)
- Pasteur and his coworkers
  - developed vaccines for chicken cholera, anthrax, and rabies

Immunological Studies

- once established, led to study of host defenses - immunology
- Edward Jenner (ca. 1798)
  – used a vaccination procedure to protect individuals from smallpox

    NOTE: this preceded the work establishing the role of microorganisms in disease!

More Developments…

- Emil von Behring (1854-1917) and Shibasaburo Kitasato (1852-1931)
  - developed antitoxins for diphtheria and tetanus
  - evidence for humoral (antibody-based) immunity
- Elie Metchnikoff (1845-1916)
  - discovered bacteria-engulfing, phagocytic cells in the blood
  - evidence for cellular immunity

The Development of Industrial Microbiology and Microbial Ecology

- Louis Pasteur
  - demonstrated that alcohol fermentations and other fermentations were the result of microbial activity
  - developed the process of pasteurization to preserve wine during storage

Developments in Microbial Ecology

- Sergei Winogradsky (1856-1953) and Martinus Beijerinck (1851-1931)
  - studied soil microorganisms and discovered numerous interesting metabolic processes (e.g., nitrogen fixation)
  - pioneered the use of enrichment cultures and selective media

1.4 Microbiology Today

1. Construct a concept map, table, or drawing that illustrates the diverse nature of microbiology and how it has improved human conditions
2. Support the belief held by many microbiologists that microbiology is experiencing its second golden age

Microbiology Has Basic and Applied Aspects

- Basic aspects are concerned with individual groups of microbes, microbial physiology, genetics, molecular biology and taxonomy
- Applied aspects are concerned with practical problems – disease, water, food and industrial microbiology

Molecular and Genomic Methods

- Led to a second golden age of microbiology (rapid expansion of knowledge)
- Discoveries
  - restriction endonucleases (Arber and Smith)
  - first novel recombinant molecule (Jackson, Symons, Berg)
  - DNA sequencing methods (Woese, Sanger)
  - bioinformatics and genomic sequencing and analysis

Major Fields in Microbiology

- Medical microbiology – diseases of humans and animals
- Public health microbiology – control and spread of communicable diseases
- Immunology – how the immune system protects a host from pathogens

More Fields…

- Microbial ecology is concerned with the relationship of organisms with their environment
  - less than 1% of earth's microbial population has been cultured
- Agricultural microbiology is concerned with the impact of microorganisms on agriculture
  - food safety microbiology
  - animal and plant pathogens

More Fields….

- Industrial microbiology began in the 1800s
  - fermentation
  - antibiotic production
  - production of cheese, bread, etc.
- Microbial physiology studies metabolic pathways of microorganisms

More Fields….

- Molecular biology, microbial genetics, and bioinformatics study the nature of genetic information and how it regulates the development and function of cells and organisms
- Microbes are a model system of genomics