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SAMPLE CHAPTER. CELL AND MOLECULAR BIOLOGY 3e: WHAT WE KNOW AND HOW WE FOUND OUT

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3rd edition (CMB 3e),
Sample Chapter

Cell and Molecular Biology

What We Know & How We Found Out

Gerald Bergtrom

Image Adapted From: Microarray

Cell and Molecular Biology

What We Know & How We Found Out

Annotated 3rd edition (CMB 3e)

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By

Gerald Bergtrom, Ph.D.

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- ✓ **new scientific updates (in text and *Challenge* boxes)**
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Dedicated to:

Sydell, Aaron, Aviva, Edan and our extended family whose patience and encouragement made this work possible; my students from whose curiosity I received as much as I gave; the memory of my mentor Herbert Oberlander, who gave me the time, opportunity and tools to do science.

Preface to CMB 3e

A grasp of the logic and practice of science is essential to understand the rest of the world around us. To that end, the **CMB3e iText** (like earlier editions) remains focused on experimental support for what we know about cell and molecular biology, and on showing students the relationship of cell structure and function. Rather than trying to be a comprehensive reference book, **CMB3e** selectively details investigative questions, methods and experiments that lead to our understanding of cell biology. This focus is nowhere more obvious than in the chapter ***learning objectives*** and in external links to supplementary material. In the freely available ***Basic CMB3e,*** the latter include the author's short *just-in-time* YouTube VOPs, embedded near relevant text. Each video is identified with a descriptive title and video *play* and *QR bar codes*:


*Note: Web links to external resources were live at the time of publication of the iText, but may disappear without notice!

The *Learning objectives* align with content and ask students to use new knowledge to make connections and deepen their understanding of concept and experiment. All external links are intended to expand or explain textual content and concepts and to engage student curiosity. The full *VOP* lectures (now at the back of the book) include optional edited closed captions.

All images in the iText are by the author or are from public domain or Creative Commons (CC) licensed sources. For all externally sourced images, CC licenses are indicated with the image. Beyond the ***Basic CMB3e***, the freely available ***Annotated* CMB*3e*** contains interactive links and formative assessments in the form of ***Challenge*** boxes. A **CMB3e *Sample Chapter*** and **CMB3e *iText for Instructors*** model additional interactive features, including short **25 Words or Less** writing assignments that can be incorporated into almost any course management system, and all of which the author assigned as homework in his *flipped*, *blended* course. These assessments aim to reinforce writing as well as critical thinking skills. The **CMB3e *Sample Chapter*** is freely available for download; the **CMB3e *for Instructors*** version of the iText is available on request.

As with previous editions of this iText, my goal i is to make the content engaging, free and comparable in accuracy and currency to commercial textbooks. I encourage instructors to use the interactive features of the iText (critical thought questions, YouTube videos, etc.) to challenge their students.

With all of these enhancements, I encourage students to think about

- how good and great experiments were inspired and designed,
- how alternative experimental results were predicted,
- how data was interpreted, and finally,
- investigators (and we!) arrive at the most interesting "next questions".

The online *iText* is the most efficient way to access links and complete online assignments. Nevertheless, you can download, read, study, and access many links with a smart phone or tablet. And you can add your own annotations digitally, or write in the margins of a printout the old-fashioned way! Your instructor may provide additional instructions for using your ***iText***.

Special Note to Instructors from the Author

The ***Basic CMB3e*** and the ***Annotated*** versions of the **CMB3e** *iText* is freely available as pdf files to you and your students. To get the **CMB3e *iText for Instructors*** you will need to fill out a short form identifying you as an instructor. When you submit the form, you will get pdf as well as MS-Word files for both the *Annotated iText* as well as *Instructor's iText*. Once you download the **CMB3e** *iText*, you should find it an easy matter to add, subtract, modify or embellish any part of either version to suit your purposes in accordance with the Creative Commons CC-BY license under which it is published. Note also that, if students access the iText through a CMS (Course Management System, e.g., BlackBoard, D2L, Canvas, etc.), you can create links to Discussion fora, DropBox, Quiz assignments, etc. directly in the iText. Thus, you are free to provide your customized version of the text to your students (e.g., as a pdf file). Feel free also to ask your students participate in the improvement of the iText (for fun or for credit!) and to share the results with others! One final bit of advice: whereas I provide content updates, please remember that some of the new content has significant potential subject to confirmation but is not necessarily definitive. I hope that you (and perhaps your students!) will enjoy creating and customizing interactive elements in the *iText*. Above all, I hope that your students will achieve a better understanding of how scientists use skills of inductive and inferential logic to ask questions and formulate hypotheses…, and how they apply concept and method to testing those hypotheses

Acknowledgements

Many thanks go to my erstwhile LTC colleagues Matthew Russell, Megan Haak, Melissa Davey Castillo, Jessica Hutchings and Dylan Barth for inspiration in suggesting ways to model how open course content can be made interactive and engaging. Their support continues to inform **CMB3e**. I thank my colleagues in the Golda Meir Library for their help in publishing the various versions of ***CMB*** on the Digital Commons open access platform.

Thanks also to M. Terry Bott for reviewing and vetting the images used in this iText as either in the public domain or designated with a Creative Commons (CC) license as an open resource (see *Creative Commons License* page, above). Last but not least, I must acknowledge my opportunity to teach, study and do research in science and interactive pedagogy for more than 35 years. My research and collegial experience at the University of Wisconsin-Milwaukee have left their mark on the content, concept and purpose of this digital *Open Education Resource* (OER).

About the Author

Dr. Bergtrom is Professor (Emeritus) of Biological Sciences and a former Learning Technology Consultant in the UW-Milwaukee *Center for Excellence in Teaching and Learning*. Scientific interests include cell and molecular biology and evolution. Pedagogic interests are blended and online instruction and the use of technology to serve more active and engaged teaching and learning. He has taught face-to-face, fully online, *blended* and *flipped* classes at both undergraduate and graduate levels. He also developed and co-instructed *Teaching with Technology*, an interdisciplinary course aimed at graduate students that might someday find themselves teaching. In his 40+ years of teaching and research experience, he has tested and incorporated pedagogically proven teaching technologies into his courses. Many research papers have been more recently supplemented with publications on active blended, online and flipped classroom methods[1-3]. The first edition of his *Open Access/Creative Commons* electronic iText, ***Cell and Molecular Biology***–*What We Know & How We Found Out*, appeared in 2015[4]. ***CMB 2e*** followed in 2016[5]. ***CMB3e*** (2017)[6] is now available at CMB3e Description and Available Versions. Access to older editions/versions may be available by request to the author.

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4. Bergtrom, G. (2015) *Cell and Molecular Biology: What We Know & How We Found Out* [*CMB1e*] (Please contact the author)
5. Bergtrom, G. (2016) *Cell and Molecular Biology: What We Know & How We Found Out* [*CMB2e*] (Please contact the author)
6. Bergtrom, G. (2018) *Cell and Molecular Biology: What We Know & How We Found Out* [*CMB3e*] (http://dc.uwm.edu/biosci_facbooks_bergtrom/)

Table of Contents

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Chapter 1: Cell Tour, Life's Properties and Evolution, Studying Cells

Scientific Method; Cell structure, methods for studying cells (microscopy, cell fractionation, functional analyses); Common ancestry, genetic variation, evolution, species diversity; cell types & the domains of life

I. Introduction

You will read in this book about experiments that revealed secrets of cell and molecular biology, many of which earned their researchers Nobel and other prizes. But let's begin here with a *Tale of Roberts*, two among many giants of science in the renaissance and age of enlightenment whose seminal studies came too early to win a Nobel Prize.

One of these, **Robert Boyle**, was born in 1627 to wealthy, aristocrat parents. In his teens, after the customary *Grand Tour* of renaissance Europe (Greece, Italy…) and the death of his father, he returned to England in 1644, heir to great wealth. In the mid-1650s he moved from his estates to Oxford where he set about studying physics and chemistry. He built a laboratory with his own money in order to do experiments on the behavior of gasses under pressure, and with a little help, discovered *Boyle's Law*, confirming that the gasses obey mathematical rules. He is also credited with showing that light and sound could travel through a vacuum, that something in air enables combustion, that sound travels through air in waves, that heat and particulate motion were related, and that the practice of alchemy was bogus! In fact, Boyle pretty much converted alchemy to chemistry by doing *chemical analysis*, a term he coined. As a chemist, he also rejected the old Greek concept of earth, air, fire and water elements. Instead, he defined elements as we still do today: the element is the smallest component of a substance that cannot be further chemically subdivided. He did this a century before Antoine Lavoisier listed and define the first elements! Based on his physical studies and chemical analyses, Boyle even believed that the indivisible unit of elements were atoms, and that the behavior of elements could be explained by the motion of atoms. Boyle later codified in print the scientific method that made him a successful experimental scientist.

The second of our renaissance Roberts was **Robert Hooke**, born in 1635. In contrast to Boyle parents, Hooke's were of modest means. They managed nonetheless to nurture their son's interest in things mechanical. While he never took the Grand Tour, he learned well and began studies of chemistry and astronomy at Christ Church College, Oxford in 1653. To earn a living, he took a position as Robert Boyle's assistant. It was

with Hooke's assistance that Boyle did the experiments leading to the formulation of Boyle's Law. While at Oxford, he made friends and useful connections. One friend was the architect Christopher Wren. In 1662, Boyle, a founding member of the Royal Society of London, supported Hooke to become the society's *curator of experiments*. However, to support himself, Hooke hired on as professor of geometry at Gresham College (London). After "the great fire" of London in 1666, Hooke, as city surveyor and builder, participated with Christopher Wren in the design and reconstruction of the city. Always interested in things mechanical, he also studied the elastic property of springs. This led him to *Hooke's Law*, which said that the force required to compress a spring was proportional to the length the spring was compressed. In later years these studies led Hooke to imagine how a coil spring might be used (instead of a pendulum) to regulate a clock. While he never invented such a clock, he was appointed to a Royal Commission to find the first reliable method to determine longitude at sea. He must have been gratified to know that the solution to accurate determination of longitude at sea turned out to involve a coil-spring clock! Along the way in his 'practical' studies, he also looked at little things, publishing his observations in *Micrographia* in 1665. Therein, he described microscopic structures of animal parts and even snowflakes. He also described fossils as having once been alive and compared structures in thin slices of cork that he saw in his microscope to monk's cells (rooms, chambers) in a monastery. Hooke is best remembered for his law of elasticity, and of course, for coining the word *cell*, which we now understand as the smallest unit of living things.

Now fast-forward almost 200 years to observations of plant and animal cells early in the 19th century. Many of these studies revealed common structural features including a nucleus, a boundary wall and a common organization of cells into groups to form multicellular structures of plants and animals and even lower life forms. These studies led to the first two precepts of ***Cell Theory***: (1) *Cells are the basic unit of living things*; (2) *Cells can have an independent existence*. Later in the century when Louis Pasteur disproved notions of *spontaneous generation*, and German histologists observed *mitosis* and *meiosis* (the underlying events of cell division in eukaryotes) a third precept rounded out Cell Theory: (3) *Cells come from pre-existing cells*. That is, they reproduce.

Comment [GKB1]: **Explain the statement that *Cells can have an independent existence* in 30 words or less. Put your word count in parenthesis after your response and submit it to the *Life is Good* DropBox by [insert date and time].**

We begin this chapter with a reminder of the ***scientific method***, that way of thinking about our world that emerged formally in the 17th century. Then we take a tour of the cell, reminding ourselves of basic structures and organelles. After the 'tour', we consider the ***origin of life*** from a common ancestral cell and the subsequent ***evolution*** of cellular complexity and the incredible diversity of life forms. Finally, we consider some of the *methods* we use to study cells. Since cells are small, several techniques of microscopy, cell dissection and functional/biochemical analysis are described to illustrate how we come to understand cell function.

Learning Objectives

When you have mastered the information in this chapter, you should be able to:

1. compare and contrast *hypotheses* and *theories* and place them and other elements of the scientific enterprise into their place in the cycle of the *scientific method.*
2. compare and contrast structures common to and that distinguish *prokaryotes*, *eukaryotes* and *archaea*, and groups within these *domains.*
3. articulate the function of different cellular substructures.
4. explain how *prokaryotes* and *eukaryotes* accomplish the same functions, i.e. have the same *properties of life*, even though prokaryotes lack most of the structures.
5. outline a procedure to study a specific cell *organelle* or other substructure.
6. describe how the different structures (particularly in eukaryotic cells) relate/interact with each other to accomplish specific functions.
7. describe some structural and functional features that distinguish prokaryotes (eubacteria), eukaryotes and archaea.
8. place cellular organelles and other substructures in their evolutionary context, i.e., describe their origins and the selective pressures that led to their *evolution.*
9. distinguish between the random nature of *mutation* and *natural selection* in evolution
10. relate archaea to other life forms and speculate on their origins in evolution.
11. suggest why evolution leads to more complex ways of sustaining life,
12. explain how *fungi* are more like animals than plants.

II. Scientific Method – The Practice of Science

For an amusing look at how scientists think, check out *The Pleasure of Finding Things Out: The Best Short Works of Richard Feynman (*1999, New York, Harper Collins). Here we focus on the essentials of the scientific method originally inspired by Robert Boyle, and then look at how science is practiced today. Scientific method refers to a standardized protocol for observing, asking questions about, and investigating natural phenomena. Simply put, it says look/listen, infer a cause and test your inference. As captured by the Oxford English Dictionary, the essential inviolable commonality of all scientific practice is that it relies on “systematic observation, measurement, and experiment, and the formulation, testing and modification of hypotheses."

A. The Method

Adherence to the method is not strict, and may sometimes breach adherence to protocol! In the end, scientific method in actual practice recognizes human biases and prejudices and allows deviations from the protocol. Nevertheless, an understanding of scientific method will guides the prudent investigator to balance personal bias against the leaps of intuition that successful science requires. The practice of scientific method by most scientists would indeed be considered a

success by almost any measure. Science "as a way of knowing" the world around us constantly tests, confirms, rejects and ultimately reveals new knowledge, integrating that knowledge into our worldview. Here in the usual order are the key elements of the scientific method:

- *Observe* natural phenomena (includes reading the science of others).
- Infer and propose an ***hypothesis*** (explanation) based on objectivity and reason. Hypotheses are declarative sentences that sound like a fact, but aren't! Good hypotheses are testable, easily turned into *if/then (predictive) yes-or-no* questions.
- Design an experiment to test the hypothesis: results must be measurable evidence for or against the hypothesis.
- Perform the experiment and then observe, measure, collect data and test for statistical validity (where applicable). Then, repeat the experiment.
- Consider how your data supports or does not support your hypothesis and then integrate your experimental results with earlier hypotheses and prior knowledge.

But, how do *theories* and *laws* fit into the scientific method?

A scientific ***theory***, contrary to what many people think, is not a guess. Rather, a theory is a statement well supported by experimental evidence and widely accepted by the scientific community. One of the most enduring, tested theories is of course the theory of evolution. Among scientists, theories might be thought of as 'fact' in common parlance, but we recognize that they are still subject to testing and, modification, and may even be overturned. While some of Darwin's notions have been modified over time, in this case, those modifications have only strengthened our understanding that species diversity is the result of natural selection. You can check out some of Darwin's own work (1859, 1860; *The Origin of Species*] at Origin of Species. For more recent commentary on the evolutionary underpinnings of science, check out Dobzhansky T (1973, *Nothing in biology makes sense except in the light of evolution*. Am. Biol. Teach. 35:125-129) and Gould, S.J. (2002, *The Structure of Evolutionary Theory*. Boston, Harvard University Press).

A **scientific *Law*** is thought of as universal and even closer to 'fact' than a theory! Scientific laws are most common in math and physics. In life sciences, we recognize Mendel's *Law of Segregation* and *Law of Independent Assortment* as much in his honor as for their universal and enduring explanation of genetic inheritance in living things. But Laws are not facts*!* Laws too, are always subject to experimental test. Astrophysicists are actively testing universally accepted laws of physics. Strictly speaking, even Mendel's *Law of Independent Assortment* should

not be called a law. Indeed, it is not true as he stated it! Check the Mendelian Genetics section of an introductory textbook to see how chromosomal crossing over violates this law.

In describing how we do science, the Wikipedia entry states: "the goal of a scientific inquiry is to obtain knowledge in the form of testable explanations (hypotheses) that can predict the results of future experiments. This allows scientists to gain an understanding of reality, and later use that understanding to intervene in its causal mechanisms (such as to cure disease)." The better an hypothesis is at making predictions, the more useful it is, and the more likely it is to be correct. In the last analysis, think of Hypotheses as *educated guesses* and think of Theories and/or Laws as one or more experimentally supported hypothesis that everyone agrees should serve as *guideposts* to help us evaluate new observations and hypotheses.

CHALLENGE: Since both theories and hypotheses are stated as declarative sentences and thus sound like facts, , articulate in your own words the difference between Hypothesis, Theory and Law.

A good hypothesis is a rational guess that explains scientific observations or experimental measurements. Therefore by definition, hypotheses are testable based on predictions based on logic. Additional observation can refine or change the original hypothesis, and/or lead to new hypothesis whose predictive value can also be tested. If you get the impression that scientific discovery is a cyclic process, that's the point! Exploring scientific questions reveals more questions than answers!

We now recognize that a key component of the scientific method is the requirement that the work of the scientist be disseminated by publication! In this way, shared data and experimental methods can be repeated and evaluated by other scientists.

B. Origins of the Scientific Method

Long before the word *scientist* began to define someone who investigated natural phenomena beyond simple observation (i.e., by doing experiments), philosophers developed formal rules of *deductive* and *inferential logic* to try to understand nature, humanity's relationship to nature, and the relationship of humans to each other. In fact, Boyle was not alone in doing experimental science. We therefore owe the logical underpinnings of science to *philosophers* who came up with systems of *deductive* and *inductive logic* so integral to the scientific method. The scientific method grew from those beginnings, along with increasing empirical observation and

experimentation. We recognize these origins when we award the Ph.D. (*Doctor of Philosophy),* our highest academic degree! We are about to learn about the life of cells, their structure and function, and their classification, or grouping based on those structures and functions. Everything we know about life comes from applying the principles of scientific method.

III. Domains of Life

We believe with good reason that all life on earth evolved from a common ancestral cell that existed soon after the origins of life on our planet. Too long ago, not all life was divided into two groups: the true bacteria and everything else! Now we group life into one of three ***domains***:

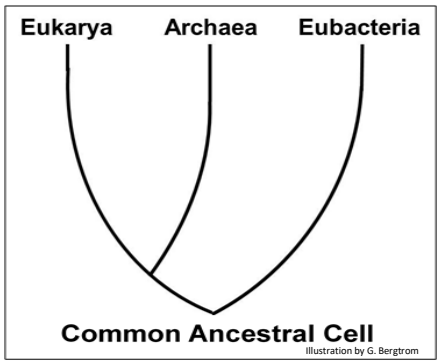
- ***Prokaryotes*** are among the first descendants of that common ancestral cell. They lack nuclei (*pro* meaning *before* and *karyon* meaning *kernel*, or *nucleus*). They include *bacteria* and *cyanobacteria* (blue-green algae).
- ***Eukaryotes*** include all higher life forms, characterized by cells with true nuclei (Eu, true; *karyon*, *nucleus*).
- ***Archaebacteria***, (meaning "old" bacteria) include many ***extremophile*** bacteria ('lovers' of life at extreme high temperatures, salinity, etc.). Originally classified as ancient prokaryotes, *Archaebacteria* were shown by 1990 to be separate from prokaryotes and eukaryotes, a third domain of life.

The archaea are found in such inhospitable environments as boiling hot springs or arctic ice, although some also live in conditions that are more temperate. Carl Woese compared the DNA sequences of genes for ribosomal RNAs in normal bacteria and extremophiles. Based on sequence similarities and differences, he concluded that the latter are in fact a domain separate from the rest of the bacteria as well as from eukaryotes. For a review, see (Woese, C. 2004; *A new biology for a new century*. Microbiol. Mol. Biol. Rev. 68:173-186)

The three domains of life (**Archaea**, **Eubacteria** and ***Eukarya***) quickly supplanted the older division of living things into Five Kingdoms, the *Monera (prokaryotes), Protista, Fungi, Plants, and Animals (all eukaryotes!)*. In a final surprise, the sequences of archaebacterial genes clearly indicate a common ancestry of archaea and eukarya.

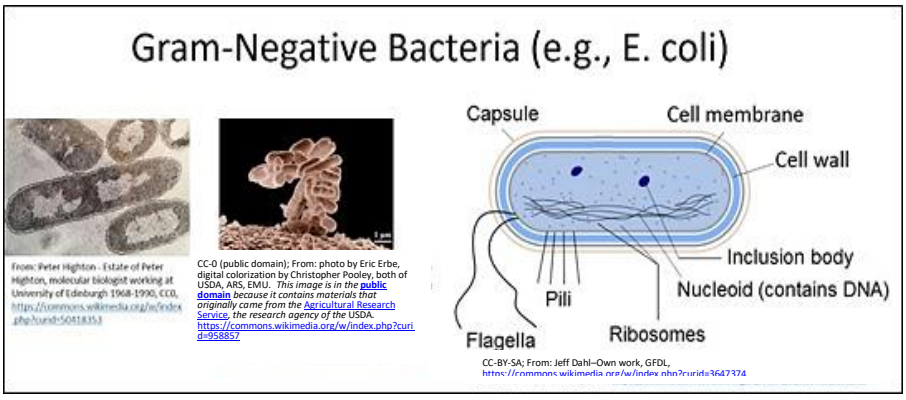
Thus, Archaea are *not* true bacteria! They share genes and proteins as well as metabolic pathways found in eukaryotes but *not* in bacteria, supporting their close evolutionary relationship to eukaryotes. That they also contain genes and proteins as well as metabolic pathways unique to the group is further testimony to their domain status. Understanding that all living organisms belong to one of three domains has dramatically changing our understanding of evolution.

The evolution of the three domains is illustrated below.



A. The Prokaryotes (Eubacteria = *Bacteria* and C*yanobacteria*)

Prokaryotic cells lack a nucleus and other organelles such as mitochondria, chloroplasts, endoplasmic reticulum, and assorted eukaryotic vesicles and internal membranes. Bacteria do contain *bacterial microcompartments* (BMCs), but these are made up entirely of protein and are ***not*** surrounded by a phospholipid membrane. These function for example in CO_2 fixation to sequester metabolites toxic to the cells. Click Bacterial Organelles for more information. Bacteria are typically unicellular, although a few (like some cyanobacteria) live colonial lives at least some of the time. Transmission and scanning electron micrographs of rod-shaped bacteria are shown in the example below at the left. A diagram of bacterial structure is also shown (right).

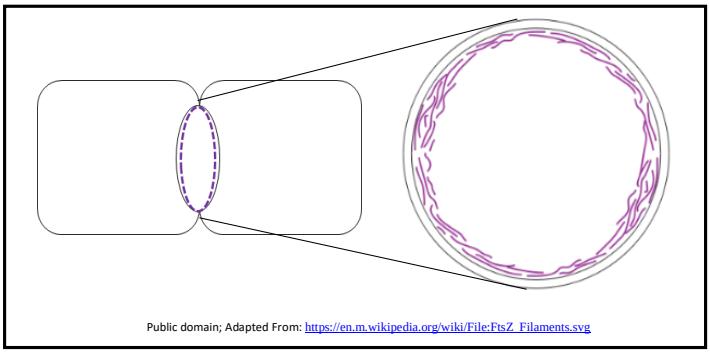


1. Bacterial Reproduction

Without the compartments afforded by the internal membrane systems common to eukaryotic cells, intracellular chemistries, from DNA replication, transcription, translation, and all the metabolic biochemistry of life, happen in the cytoplasm of the cell. DNA is a circular double helix that duplicates as the cell grows. While not enclosed in a nucleus, bacterial DNA is concentrated in a region of the cell called the ***nucleoid***. When not crowded at high density, bacteria replicate their DNA throughout the life of the cell, dividing by ***binary fission***. The result is the equal partition of duplicated bacterial *"chromosomes"* into new cells. The bacterial chromosome is essentially naked DNA, unassociated with proteins.

2. Cell Motility and the Possibility of a Cytoskeleton

Movement of bacteria is typically by *chemotaxis*, a response to environmental chemicals. Some may respond to other stimuli such as light (*phototaxy*). They can move to or away from nutrients, noxious/toxic substances, light, etc., and achieve motility in several ways. For example, many move using flagella made up largely of the protein ***flagellin***. Flagellin is absent from eukaryotic cells. On the other hand, the cytoplasm of eukaryotic cells is organized by a complex cytoskeleton of rods and tubes made of *actin* and *tubulin* proteins. Prokaryotes were long thought to lack these or similar cytoskeletal components. However, two bacterial genes that encode proteins homologous to eukaryotic actin and tubulin were recently discovered. The *MreB* protein forms a *cortical ring* in bacteria undergoing *binary fission*, similar to the actin cortical ring that pinches dividing eukaryotic cells during *cytokinesis* (the actual division of a single cell into two smaller daughter cells). This is modeled below in the cross-section (right) near the middle of a dividing bacterium (left).

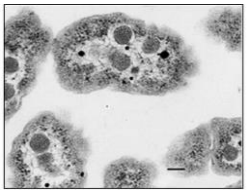


Public domain; Adapted From: https://en.m.wikipedia.org/wiki/File:FtsZ_Filaments.svg

The *FtsZ* gene encodes a homolog of tubulin proteins. It seems that together with flagellin, the MreB and FtsZ proteins may be part of a primitive prokaryotic *cytoskeleton* involved in cell structure and motility.

3. Some Bacteria Have Internal Membranes

While bacteria lack organelles (the membrane-bound structures of eukaryotic cells), internal membranes in some bacteria form as inward extensions (*invaginations*) of plasma membrane. Some of these capture energy from sunlight (photosynthesis) or from inorganic molecules (*chemolithotrophy*). *Carboxysomes* are membrane bound photosynthetic vesicles in which CO_2 is fixed (reduced) in cyanobacteria (shown below).



Photosynthetic bacteria have less elaborate internal membrane systems.

4. Bacterial Ribosomes Do the Same Thing as Eukaryotic Ribosomes… and Look Like Them!

Ribosomes are the protein synthesizing machines of life. *Ribosomes* of prokaryotes are smaller than those of eukaryotes, but are able to translate eukaryotic messenger RNA (mRNA) *in vitro*. Underlying this common basic function is the fact that the ribosomal RNAs of all species share base sequence and structural similarities indicating a long evolutionary relationship. Recall similarities revealed the closer relationship of archaea to eukarya than prokarya.

Clearly, the prokarya (Eubacteria) are a diverse group of organisms, occupying almost every wet, dry, hot or cold nook and cranny of our planet. Despite this diversity, all prokaryotic cells share many structural and functional metabolic properties with each other… and with the archaea and eukaryotes! As we have seen with ribosomes, shared structural and functional properties support the common ancestry of all life. Finally, we not only share common ancestry with prokaryotes, we even share living arrangements with them. Our gut bacteria represent up to 10X more cells than our own! Read more at The NIH Human Microbiome Project. Also check out the following link for A Relationship Between Microbiomes, Diet and Disease.

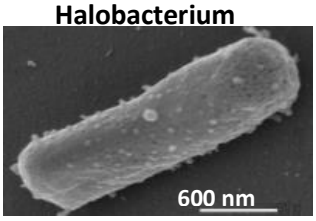
B. The Archaebacteria (Archaea)

Allessandro Volta, a physicist who gave his name to the Volt, discovered methane producing bacteria (*methanogens*) way back in 1776! He found them living in the extreme environment at the bottom of Lago Maggiore, a lake shared by Italy and Switzerland. These unusual bacteria are *cheomoautotrophs* that get energy from H_2 and CO_2 and also generate methane gas in the process. It was not until the 1960s that Thomas Brock (from the University of Wisconsin-Madison) discovered *thermophilic* bacteria living at temperatures approaching 100^{o}C in Yellowstone National Park in Wyoming. Organisms living in any extreme environment were soon nicknamed *extremophiles*. One of the thermophilic bacteria, now called *Thermus aquaticus*, became the source of *Taq* polymerase, the heat-stable DNA polymerase that made the *polymerase chain reaction* (PCR) a household name in labs around the world!

Extremophile and "normal" bacteria are similar in size and shape(s) and lack nuclei. This initially suggested that most extremophiles were prokaryotes. But as Carl Woese demonstrated, it is the archaea and eukarya that share a more recent common ancestry! While some bacteria and eukaryotes can live in extreme environments, the archaea include the most diverse extremophiles. Here are some examples of extremophiles:

- *Acidophiles*: grow at acidic (low) pH.
- *Alkaliphiles*: grow at high pH.
- *Halophiles*: require high salt concentrations; see *Halobacterium salinarium* below.

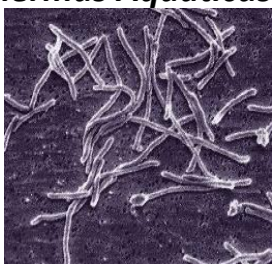
Halobacterium



- *Methanogens*: produce methane; a cross section of *Methanosarcina acetivorans* is shown above (right). Note the absence of significant internal structure.
- *Barophiles*: grow best at high hydrostatic pressure.
- *Psychrophiles*: grow best at temperature 15 °C or lower.
- *Xerophiles*: growth at very low water activity (drought or near drought conditions).

- *Thermophiles* and *hyperthermophiles*: organisms that grow best at 40°C or higher, or 80°C or higher, respectively. *Pyrolobus fumarii*, a hyperthermophile, can live at a temperature 113°C. Another thermophile *Thermus aquaticus,* noted for its role in developing the polymerase chain reaction, is shown below.

Thermus Aquaticus



CC-0 (public domain) Adapted From: https://upload.wikimedia.org/wikipedia/commons/4/48/Thermus_aquaticus.JPG

- *Toxicolerants*: grow in the presence of high levels of damaging elements (e.g., pools of benzene, nuclear waste).

Archaea were originally seen as oddities of life, thriving in unfriendly environments. They also include organisms living in less extreme environments, including soils, marshes and even in the human colon. They are also abundant in the oceans where they are a major part of plankton, participating in the carbon and nitrogen cycles. In the guts of cows, humans and other mammals, methanogens facilitate digestion, generating methane gas in the process. In fact, cows have even been cited as a major cause of global warming because of their prodigious methane emissions! On the plus side, methanogenic Archaea are being exploited to create biogas and to treat sewage. Other extremophiles are the source of enzymes that function at high temperatures or in organic solvents. As already noted, some of these have become part of the biotechnology toolbox.

C. The Eukaryotes

1. Large Compartmentalized Cells

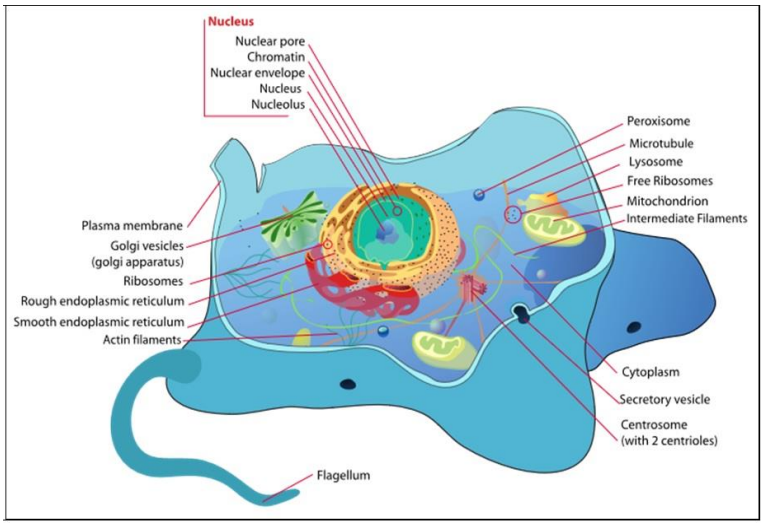
The volume of a typical eukaryotic cell is some 1000 times that of a typical bacterial cell. Eukaryotic life would not even have been possible if not for a division of labor of eukaryotic cells among different *organelles* (membrane-bound structures). Imagine a bacterium as a 100 square foot room (the size of a small bedroom, or a large walk-in closet!) with one door. Now imagine a room 1000 times as big. That is, imagine a 100,000 square foot 'room'. You would expect many smaller rooms inside such a large space, each with its own door(s). The

eukaryotic cell is a lot like that large space, with lots of interior “rooms” (i.e., organelles) with their own entryways and exits. The smaller prokaryotic “room” has a much larger plasma membrane *surface area/volume ratio* than a typical eukaryotic cell, enabling required environmental chemicals to enter and quickly diffuse throughout the cytoplasm of the bacterial cell. Chemical communication between parts of a small cell is therefore rapid. In contrast, the communication over a larger expanse of cytoplasm inside a eukaryotic cell requires the coordinated activities of subcellular compartments. Such communication might be relatively slow. In fact, eukaryotic cells have lower rates of metabolism, growth and reproduction than do prokaryotic cells. The existence of large cells required the evolution of a *division of labor* supported by *compartmentalization*. .

CHALLENGE: Early prokaryotes must have had membrane-bound structures, later inherited as organelles by early eukaryotes. Google a bit to see what such precursor organelles might have been and how they may have formed.

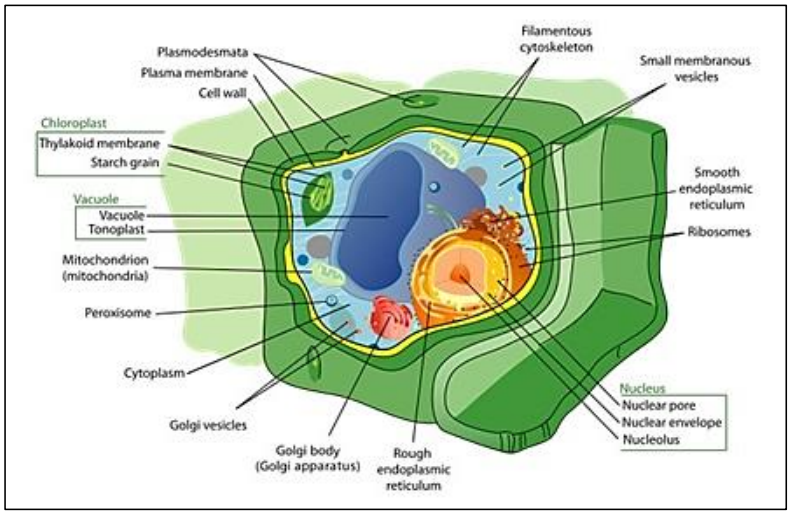
2. Animal and Plant Cell Structure Overview

A typical animal cell with its organelles and other structures is illustrated below.



Public Domain; From Mariana Ruiz, Image:Animal cell structure.svg, https://commons.wikimedia.org/w/index.php?curid=4266142

A typical plant cell showing its organelles and other structures is illustrated below.



Public Domain; From Mariana Ruiz, Image:Animal cell structure.svg, https://commons.wikimedia.org/w/index.php?curid=4266142

A plasma (cell) membrane surrounds all cells. A cell wall further surrounds prokaryotic, algal, fungal and plant cells, creating rigid structure around the cell membrane and supporting cell shape. Bacterial cell walls are composed of ***peptidoglycan***, long polysaccharide chains attached to polypeptide (amino acid) chains. ***Cellulose***, ***hemicellulose***, and ***pectin*** are major polysaccharides of the plant cell wall. Fungal cells contain a wall, whose principal component is ***chitin***. Chitin is the same material that makes up the exoskeleton or arthropods (including insects and lobsters!). Fungi, more closely related to animal than plant cells, are a curious beast for a several reasons! For one thing, the organization of fungi and fungal cells is somewhat less defined than animal cells. Structures between cells called *septa* separate fungal *hyphae*, allow passage of cytoplasm and even organelles between cells. Some primitive fungi have few or no septa, in effect creating *coenocytes*, which are single giant cells, with multiple nuclei.

We end this look at the domains of life by noting that, while eukaryotes are a tiny minority of all living species, “their collective worldwide biomass is estimated at about equal to that of prokaryotes” (Wikipedia). On the other hand, our bodies contain 10 times as many microbial cells as human cells! In fact, it is becoming increasingly clear that a human owes as much of its existence to its microbiota (see above) as it does to its human cells. Keeping in mind that plants and animal

Cells share many of internal structures and organelles that perform the same or similar functions, we'll now look at cell structures and organelles with a brief description of their functions.

IV. Tour of the Eukaryotic Cell

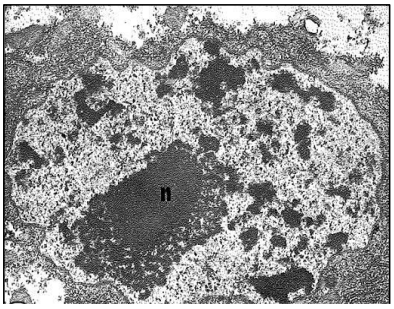
A. The Nucleus

The nucleus separates the genetic blueprint, i.e., DNA from the cell cytoplasm. Although the eukaryotic nucleus breaks down during mitosis and meiosis as chromosomes form and cells divide, it spends most of its time in ***interphase***, the time between cell divisions. This is where the status of genes (and therefore of the proteins produced in the cell) is regulated. *rRNA*, *tRNA* and *mRNA* are transcribed from genes, processed in the nucleus, and exported to the cytoplasm through *nuclear pores*. Some other RNAs remain in the nucleus, typically participating in the regulation of gene activity. In all organisms, dividing cells must produce and partition copies of their duplicated genetic material equally between new daughter cells. Let's look first at the structural orgabno9zation of the nucleus, and then at its role in the genetics of the cell and of the whole organism.

Comment [GKB2]: Check out the VOP clip at this link: http://youtu.be/Bw23E7e0YNk. (You may need to right-click on the link and select "open" to access it). Then answer the question in the last slide in the clip by completing the sentence "If these structures are nuclei, then..." in 30 words or less. Put your word count in parenthesis after your response. Submit your answers to the *ID Nuclei* DropBox in D2L no later than [insert date and time].

1. Structure of the Interphase Nucleus

The nucleus is the largest organelle in the cell. A typical electron microscope image of a nucleus, the largest eukaryotic organelle in a cell, is shown below.



From Bergtrom et al. (1977) J. Ultrastr. Res. 60:395-405; Research by G. Bergtrom

This cross-section of an interphase nucleus reveals its double membrane, or ***nuclear envelope***. Nuclear envelope ***pores*** (not visible in this micrograph) allow large molecules and even particles to move in and out of the nucleus across both membranes. The outer membrane of the nuclear envelope is continuous with the *RER* (rough endoplasmic reticulum). Thus, the lumen of the RER is continuous

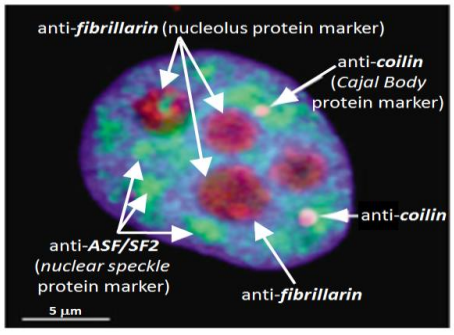
with the space separating the nuclear envelope membranes. The electron micrograph also shows a prominent ***nucleolus*** (labeled **n**) and a darkly granular RER surrounding the nucleus. Zoom in on the micrograph; you may see the double membrane of the nuclear envelope. You might also make out ribosomes (small granules) bound to both the RER and the outer nuclear membrane.

104 The Nucleus

The nucleus is *not* an unorganized space surrounded by the nuclear envelope, as seems to appear in the transmission electron micrographs. The nucleolus is just the largest of several nuclear inclusions that seem to segregate nuclear functions. ***Santiago Ramón y Cajal*** reported more structures in the nuclei of neurons more than 100 years ago, drawing his observations before modern photomicrographic technology became widely available. See what he saw at Cajal's Nuclear Bodies, including the nucleolus and what came to be known as ***Cajal bodies*** (*CBs*). As we saw earlier, *Ramón y Cajal* shared the *Nobel Prize* in Physiology or Medicine 1906 with *Camillo Golgi* for their studies of nerve cell structure. Check out a gallery of Cajal's hand-drawn micrographs of brain nerve cells in Cajal's Beautiful Brain Cells.

Later seen in an electron microscope, *CBs* look like coils of tangled thread, and were thus called ***coiled bodies*** (conveniently, also CBs). Other nuclear bodies since identified include ***Gems***, ***PML bodies***, nuclear speckles (or ***splicing speckles***), ***histone locus bodies*** (*HLBs*) **…,** and more! Different nuclear bodies turn out to be associated with specific proteins. The localization of specific proteins to different nuclear bodies can be seen in the immunofluorescence micrograph below.

Overlay of Immunofluorescence Localizations of Four *Nuclear Body* Markers

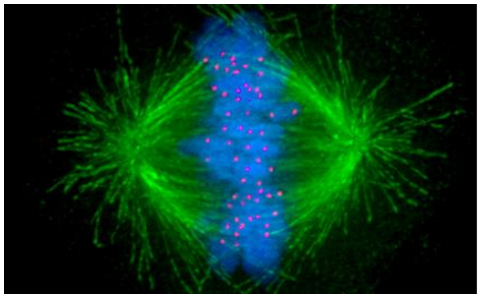


Nucleoli contain ***fibrillarin*** proteins and stain red because they have been treated with red-fluorescence-tagged ***antifibrillarin*** antibodies. CBs contain the protein ***coilin***. They fluoresce pink because the nuclei were treated with fluorescence-tagged ***anticoilin*** antibodies. Green-fluorescent antibodies to the **ASF/SF2** protein localize to nuclear speckles. As part of, or included in a nuclear matrix, nuclear bodies organize and regulate different aspects of nuclear activity and molecular function. The different nuclear bodies perform specific functions and interact with each other and with proteins DNA and RNA to do so. We will revisit some nuclear bodies in their working context in later chapters.

2. Every Cell (i.e., Every Nucleus) of an Organism Contains the Same Genes

We read earlier that bacteria are busy doubling and partitioning their naked DNA chromosomes at the same time as they grow and divide by binary fission. In eukaryotic cells, a ***cell cycle*** divides life into discrete consecutive events. During most of the cell cycle, cells are in interphase and DNA is wrapped up in proteins in a structure called ***chromatin***. It is not merely the DNA, but chromatin that must be duplicated when cells reproduce. Duplication of DNA involves rearranging chromatin proteins. This occurs before cell division (***mitosis*** and ***cytokinesis***). As the time of cell division nears, chromatin associates with even more proteins, condensing to form *chromosomes*, while the nuclear envelope dissolves. You may recall that every somatic cell of an organism contains paired *homologous chromosomes*, and therefore two copies of every gene an organism owns. On the other hand, sperm and eggs contain one of each pair of chromosomes, and thus one copy of each gene. Whether by mitosis or meiosis, cytokinesis separates duplicated chromosomes to *daughter* cells. In the fluorescence micrograph of a cell in the *metaphase* stage of *mitosis* (below), the chromosomes (blue) are just about to be pulled apart by microtubules of the spindle apparatus (green).

The Mitotic Spindle



As the chromosomes separate and daughter cells form, nuclei reappear and chromosomes de-condense. These events mark the major visible difference between cell division in bacteria and eukaryotes. Cytokinesis begins near the end of mitosis. *Sexual reproduction*, a key characteristic of eukaryotes, involves ***meiosis*** rather than mitosis. The mechanism of *meiosis*, the division of *germ cells* leading to production of sperm and eggs, is similar to mitosis except that the ultimate daughter cells have just one each of the parental chromosomes, eventually to become the gametes (eggs or sperm).

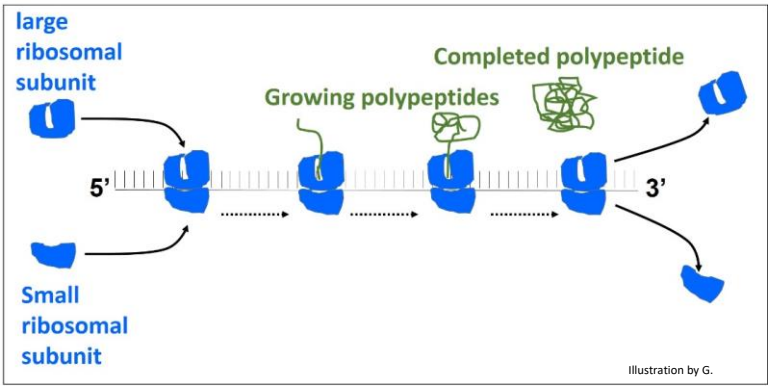
A key take-home message here is that every cell in a multicellular organism, whether egg, sperm or somatic, contains the same genome (genes) in its nucleus. This was understood since mitosis and meiosis were first described in the late 19th century. However, it was finally demonstrated in 1962, when John Gurdon and Shinya Yamanaka transplanted nuclei from the intestinal cells the frog *Xenopus laevis* into enucleated eggs (eggs from which its own nucleus had been removed). These 'eggs' grew and developed into normal tadpoles, proving that no genes are lost during development, but just expressed differentially. We will revisit animal cloning later in this book. But for now, it's sufficient to know that Molly the cloned frog was followed in 1996 by Dolly, the first cloned sheep, and then other animals, all cloned from enucleated eggs transplanted with differentiated cell nuclei. Click Cloning Cuarteterra for the *60 Minutes* story of the cloning of *Cuarteterra*, a champion polo mare whose clones are also champions! For their first animal cloning experiments, Gurdon and Yamanaka shared the 2012 Nobel Prize form Physiology or medicine

CHALLENGE: One group of bacteria (*Planctomycetes*) do surround their nucleoid DNA with a membrane! How do you think these cells divide their DNA equally between daughter cells during cell division?

A. Ribosomes

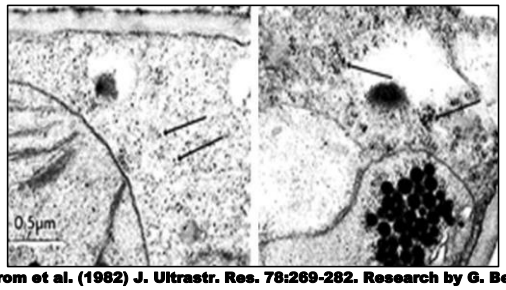
On the other end of the size spectrum, ribosomes are evolutionarily conserved protein synthesizing machines in all cells. They consist of a large and a small subunit, each made up of multiple proteins and one or more molecules of ribosomal RNA (rRNA). Ribosomes bind to messenger RNA (mRNA) molecules, moving along the mRNA as they translate 3-base code words (codons) to link amino acids into polypeptides. Multiple ribosomes can move along the same mRNA, becoming a polyribosome, simultaneously translating the same polypeptide encoded by the mRNA. The granular appearance of cytoplasm in electron micrographs is largely due

to the ubiquitous distribution of ribosomal subunits and polysomes in cells. The illustration below shows a 'string' of ribosomes, the ***polyribosome*** or ***polysome*** for short.



In the illustration, ribosomes assemble at the left of the messenger RNA (mRNA) to form the polysome. When they reach the end of the message, the ribosomes disassemble from the RNA and release the finished polypeptide.

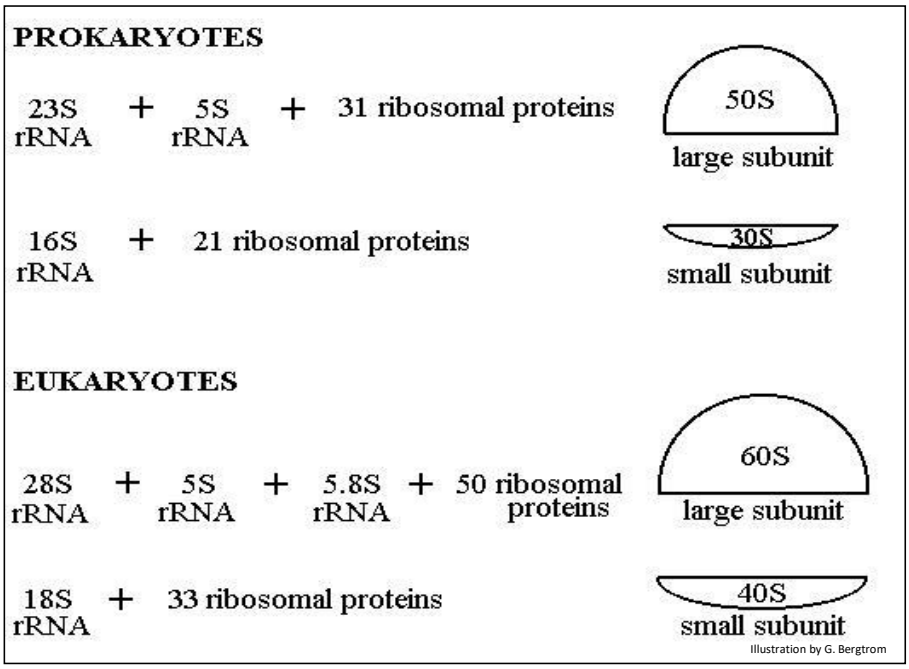
In an electron micrograph of leaf cells from a quiescent desiccated dessert plant, *Selaginella lepidophylla*, you can make out randomly distributed ribosomes and ribosomal subunits (arrows, below left). In cells from a fully hydrated plant, you can see ***polysomes*** as more organized strings of ribosomes (arrows, below right).



From Bergtrom et al. (1982) J. Ultrastr. Res. 78:269-282. Research by G. Bergtrom

Eukaryotic and prokaryotic ribosomes differ in the number of RNA and proteins in their large and small subunits, and thus in their overall size. Isolated ribosomes centrifuged in a sucrose density gradient move at a rate based on their size (or more specifically, their *mass*).

The illustration below shows the difference in ribosomal 'size', their protein composition and the number and sizes of their ribosomal RNAs.



The position of ribosomal subunits in the gradient is represented by an ***S value,*** after *Svedborg*, who first used sucrose density gradients to separate macromolecules and particles by mass. Note that the ribosomal RNAs themselves also separate on sucrose density gradients by size, hence their different S values.

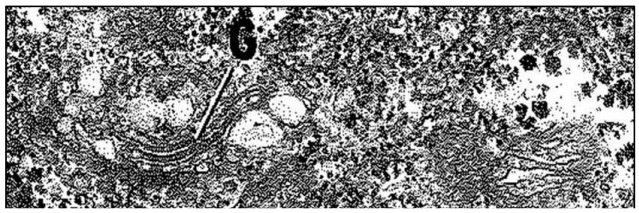
101 Ribosomes & Polysomes

B. Internal membranes and the Endomembrane System

Microscopists of the 19th century saw many of these structures using the art of histology, staining cells to increase the visual contrast between cell parts. One of these, ***Camillo Golgi***, an early neurobiologist, developed a silver (black) stain that first detected a network of vesicles we now call Golgi bodies (***Golgi vesicles***) in nerve cells. For their discoveries in cellular neuroscience, Golgi and ***Santiago Ramón y Cajal*** shared the 1906 Nobel prize for Medicine or Physiology.

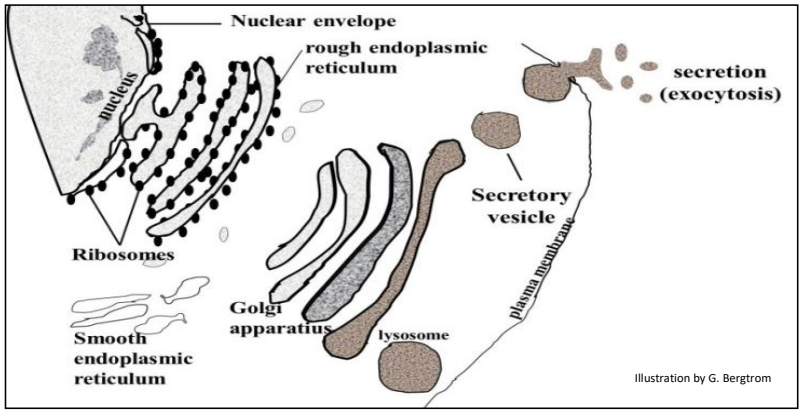
Many ***vesicles*** and ***vacuoles*** in cells, including Golgi vesicles, are part of the ***endomembrane system***. Proteins synthesized on the ribosomes of the **RER (*rough endoplasmic reticulum*)** can enter the interior space (*lumen*) or can become part of the RER membrane itself. Production of ***RER***, ***SER*** *(**smooth endoplasmic reticulum**)*, ***Golgi bodies***, ***lysosomes***, ***microbodies*** and other vesicular membranes, as well as their protein content all begin in the RER. The RER and protein contents bud into *transport vesicles* that fuse with *Golgi Vesicles* **(**G in the electron micrograph below).

Comment [GKB3]: Golgi vesicles are part of an intracellular network of membranes called the 'endomembrane system'. Run the animated PowerPoint slide at the following link and answer the question on the slide in 30 words or less: *http://youtu.be/SlM6U0Y6BxQ*. (You may need to right-click on the link and select "open" to access it). You can spend some time looking up details of endomembrane system function (OK), or answer more simply, using logic to suggest what is going on (Preferred!). Submit your answers to the *Endomembrane Traffic* DropBox no later than [insert date and time].



Adapted from Bergtrom and Robinson (1977) J. Ultrastr. Res. 60:395-405. Research by G. Bergtrom

In their journey through the endomembrane system, *packaged proteins* undergo stepwise modifications (maturation) before becoming biologically active (below).



102 Golgi Vesicles & the Endomembrane System

Some proteins made in the endomembrane system are secreted by ***exocytosis***. Others end up in organelles like ***lysosomes*** that contain hydrolytic enzymes. These enzymes are activated when the lysosomes fuse with other organelles destined for degradation. ***Food vacuoles*** form when a plasma membrane *invaginates,* engulfing food particles. They then fuse with lysosomes to digest the engulfed nutrients.

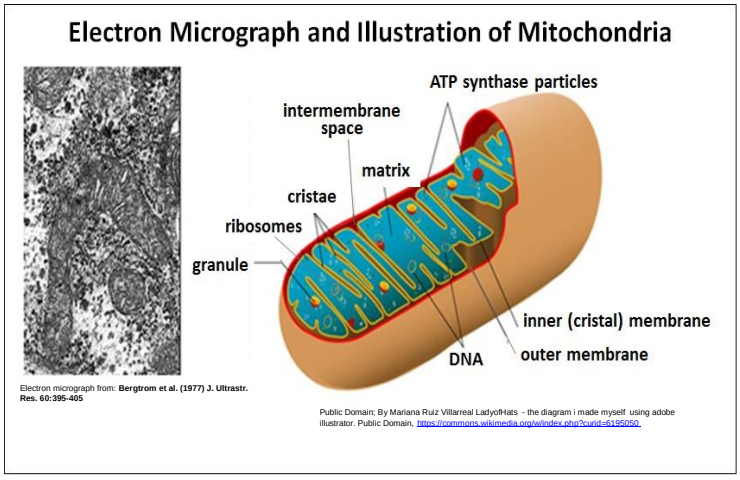
Autophagosomes are small vesicles that surround and eventually encapsulate tired organelles (for example, worn out mitochondria), eventually merging with lysosomes whose enzymes degrade their contents. In 2016, Yoshinori Ohsumi earned the Nobel Prize in Physiology and Medicine for nearly 30 years of research unraveling the cell and molecular biology of autophagy. ***Microbodies*** are a class of vesicles smaller than lysosomes, but formed by a similar process. Among them are peroxisomes that break down toxic peroxides formed as a by-product of cellular biochemistry. Some vesicles emerging from the RER will become part of the SER, which has several different functions (e.g., alcohol detoxification in liver cells).

103 Smooth Endoplasmic Reticulum

Other organelles include the ***contractile vacuoles*** of freshwater protozoa that expel excess water that enters cells by osmosis. Some protozoa have *extrusomes*, vacuoles that release chemicals or structures that deter predators or enable prey capture. A large aqueous central vacuole dominates the volume of many higher plant cells. When filled with water, they will push all other structures against the plasma membrane. In a properly watered plant, this water-filled vacuole exerts osmotic pressure that among other things, keeps plant leaves from wilting and stems upright.

C. Mitochondria and Plastids

Nearly all eukaryotic cells contain *mitochondria,* shown below.

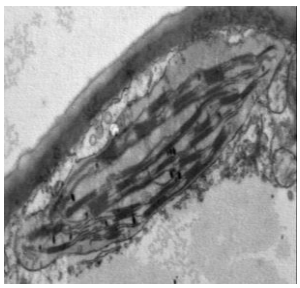


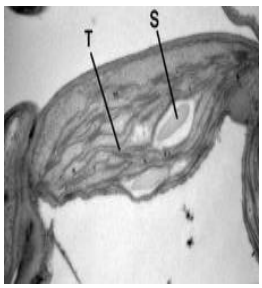
A double membrane surrounds the mitochondrion. Each contains and replicates its own DNA containing genes encoding some mitochondrial proteins. Note that the surface area of the inner mitochondrial membrane is increased by being folded into ***cristae***, which are sites of ***cellular respiration*** (aerobic nutrient oxidation).

Earlier, we speculated eukaryotic organelles that could have originated within bacteria. Mitochondria most likely evolved from a complete aerobic bacterium (or proto-bacterium) that was engulfed by a primitive eukaryotic cell. The bacterium escaped destruction, becoming an *endosymbiont* in the host cell cytoplasm. Lynn Margulis first proposed the ***Endosymbiotic Theory*** (Margulis, L. [*Sagan, L*], *1967. On the origin of mitosing cells.* Journal of Theoretical Biology **14** (3): 225–274; available at: Margulis L. Endosymbiotic theory). Margulis proposed that chloroplasts also started as ***endosymbionts***. Like mitochondria, the plastids of plants and some algae have their own DNA, most likely originating as cyanobacteria engulfed by primitive eukaryotic cells. Living in symbiosis with the rest of the cell, they would eventually evolve into plastids, including chloroplasts. Detailed evidence for the *Endosymbiotic Theory* is discussed elsewhere.

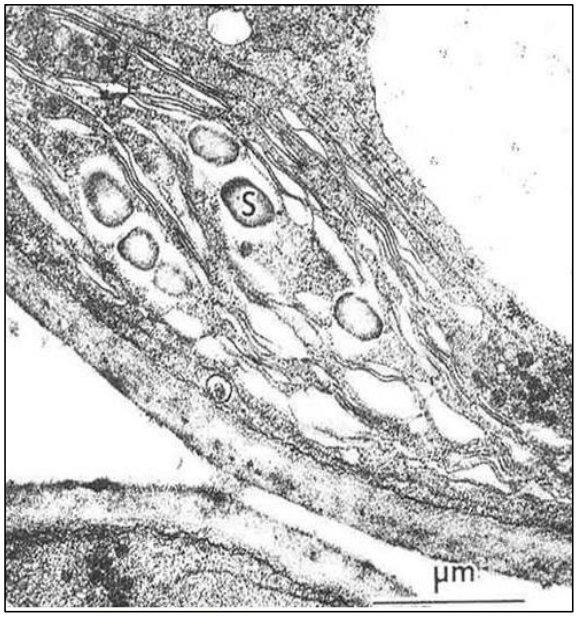
A handful of protozoa were found lacking mitochondria and other organelles. This had suggested they might share ancestry with those primitive eukaryotes that acquired mitochondria by endosymbiosis. However, since such cells contain other organelles such as *hydrogenosomes* and *mitosomes*, it is thought more likely that these species *once had, but then lost mitochondria*. Therefore, descendants of ancient eukaryotic cells missing mitochondria probably no longer exist.

Chloroplasts and *cyanobacteria* contain chlorophyll and use a similar photosynthetic mechanism to make glucose. A typical chloroplast is shown in the micrograph below (left). A chloroplast beginning to store nutrient sugar as starch is at the right.





A *leucoplast* is a plastid a chloroplast that has become filled with starch granules. In the micrograph below, you can see that, because of starch accumulation, the grana have become dispersed and indistinct, forming a leucoplast.



From Bergtrom et al., J. Ultrastr. Res. 78:269-282 (S: starch granule). Research by G. Bergtrom

105 Endosymbiosis-Mitochondria & Chloroplasts

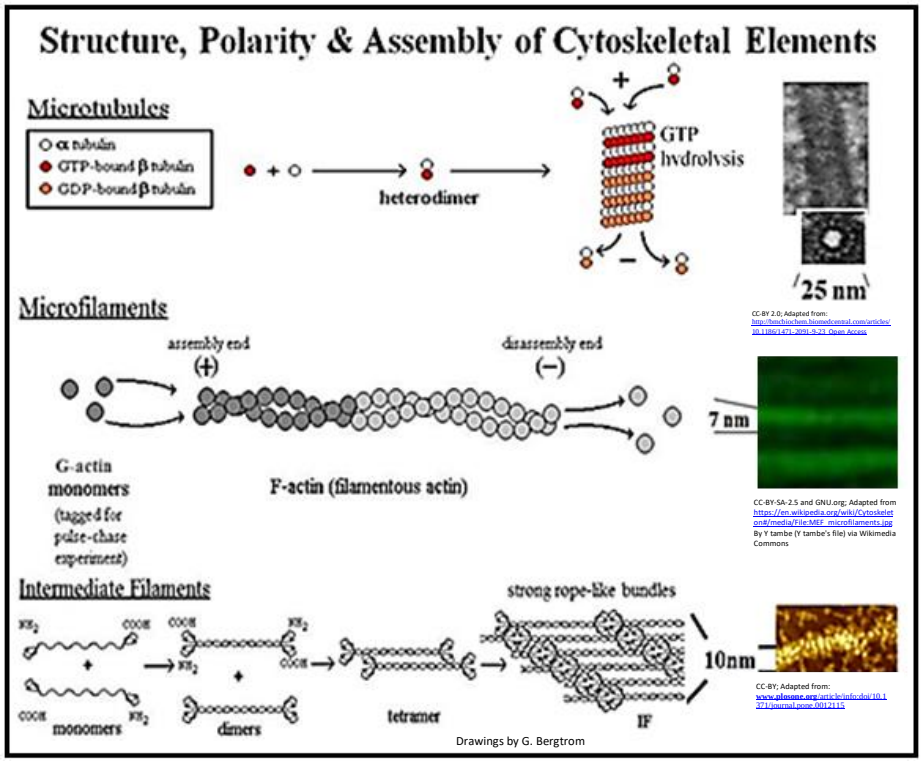
D. Cytoskeletal structures

We have come to understand that the cytoplasm of a eukaryotic cell is highly structured, permeated by rods and tubules. The three main components of this cytoskeleton are ***microfilaments***, ***intermediate filaments*** and ***microtubules.***

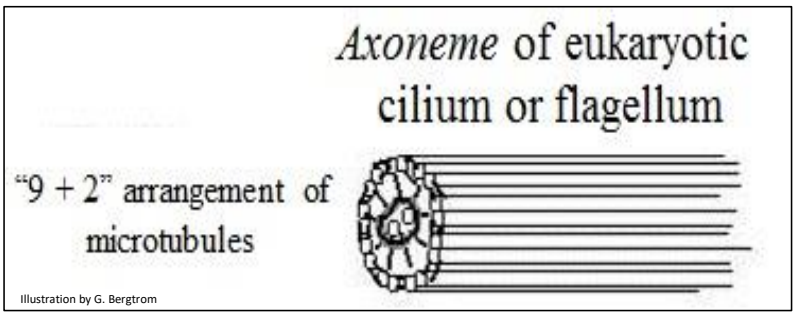
Microtubules are composed of α– and β-*tubulin* protein monomers. Monomeric *actin* proteins make up microfilaments. Intermediate filament proteins are related to *keratin*, a protein found in hair, fingernails, bird feathers, etc. Cytoskeletal rods and tubules not only determine *cell shape*, but also play a role in *cell motility*. This includes the movement of cells from place to place and the movement of structures within cells.

We have already noted that a prokaryotic cytoskeleton is composed in part of proteins homologous to actins and tubulins. As in a eukaryotic cytoskeleton, these bacterial proteins may play a role in maintaining or changing cell shape. On the other hand, flagellum-powered movement in bacteria relies on flagellin, a protein not found in eukaryotic cells. A bacterial flagellum is actually a rigid hook-like structure attached to a molecular motor in the cell membrane that spins to propel the bacterium through a liquid medium.

In contrast, eukaryotic *microtubules* slide past one another causing a more flexible flagellum to undulate in wave-like motions. Likewise, the motion of a eukaryotic cilium is based on sliding microtubules, in this case causing the cilia to beat rather than undulate. Cilia are involved not only in motility, but also in feeding and sensation. The structures and assembly of the main cytoskeletal components are shown below.



Microtubules in eukaryotic flagella and cilia arise from a *basal body* (similar to *kinetosomes* or *centrioles*). Aligned in a flagellum or cilium, microtubules form an ***axoneme*** surrounded by plasma membrane. In electron micrographs of cross sections, a ciliary or flagellar *axoneme* is typically organized as a ring of nine paired microtubules (called *doublets*) around two *singlet* microtubules (illustrated below).



Centrioles are themselves comprised of a ring of microtubules. In animal cells they participate in spindle fiber formation during mitosis and are the point from which microtubules radiate thorough the cell to help form and maintain its shape. These structures do not involve axonemes. The spindle apparatus in plant cells, which typically lack centrioles, form from an amorphous structure called the *MTOC*, or *MicroTubule Organizing Center*, which serves the same purpose in mitosis and meiosis as centrioles do in animal cells.

106 Filaments & Tubules of the Cytoskeleton

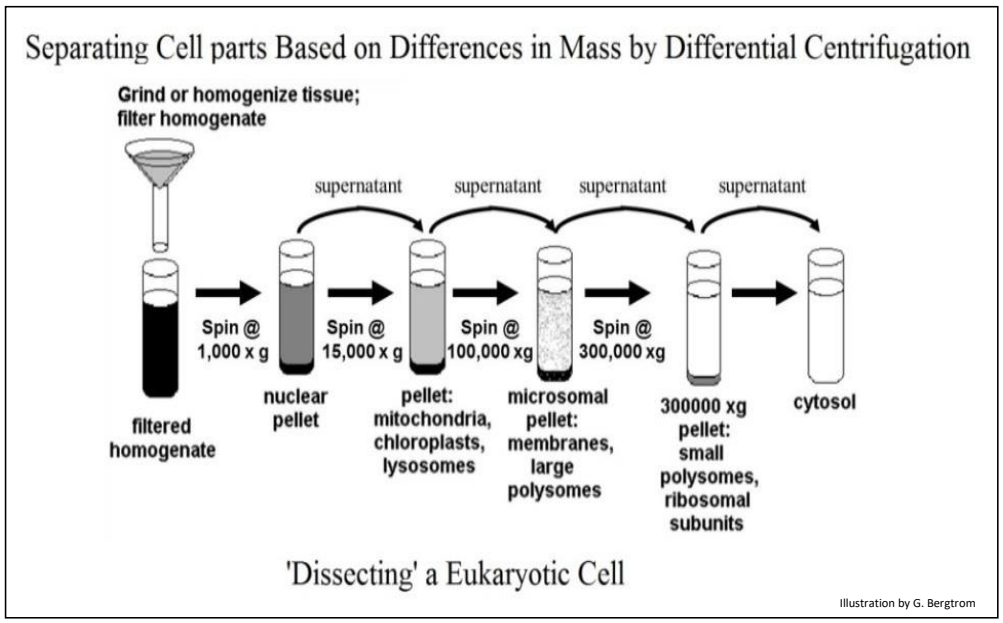
Elsewhere, we describe how microfilaments and microtubules interact with motor proteins (*dynein*, *kinesin*, *myosin, etc.*) to generate force that results in the sliding of filaments and tubules to allow cellular movement. You will see that motor proteins can also carry cargo molecules from one place to another in a cell.

V. How We Know the Functions of Cellular Organelles and Structures

A. Cell Fractionation

We can see and describe cell parts in the light or electron microscope, but we could not definitively know their function until it became possible to release them from cells and separate them from one another. This became possible with the advent of

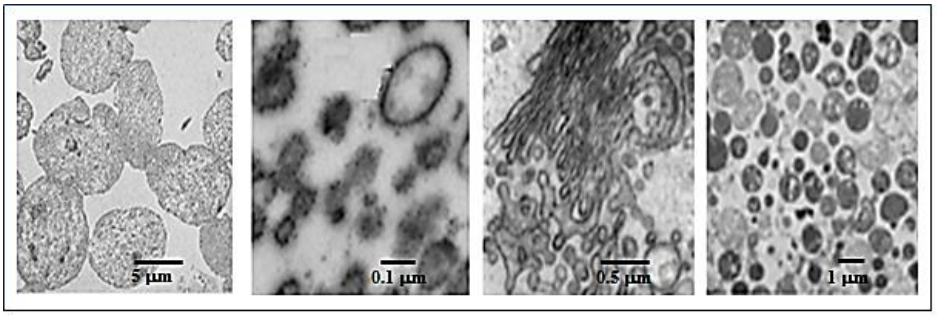
differential centrifugation. Under centrifugal force generated by a spinning centrifuge, subcellular structures separate by differences in mass. Structures that are more massive reach the bottom of the centrifuge tube before less massive ones. A cell fractionation scheme is illustrated below. Biochemical analysis of the isolated cell fractions can reveal what different organelles and cellular substructures do.



107 Dissecting the Cell; a Cell Fractionation Scheme

Cell fractionation separates cells into their constituent parts. The first step of a cell fractionation is to break open the cells and release their contents. This can be done by physical means such as grinding in a mortar and pestle, tissue grinder or similar device, exposure to ultrasound or high pressure, or exposure to enzymes or other chemicals that can selectively degrade the plasma membrane. The next step is to isolate the subcellular organelles and particles from the cytoplasm (i.e., cytosol) by differential centrifugation. As noted, centrifugation of broken cells at progressively higher centrifugal force separates particulate cell components based on their mass. At the end of this process, a researcher will have isolated mitochondria, chloroplasts, nuclei, ribosomes etc. After re-suspension, each pellet can be re-suspended and prepared for microscopy.

Below are electron micrographs of several isolated subcellular fractions.



These structures can be tentatively identified by microscopy based on their dimensions and appearance. Molecular analyses and biochemical tests on the cell fractions then help to confirm these identities.

108 Isolated Nuclei **109 Isolated RER**

110 Isolated Golgi vesicles **111 Lysosomes & Peroxisomes**

112 Isolated Mitochondria **113 Isolated Chloroplasts**

114 Isolated Plasma Membrane

Can you tell what organelles have been purified in each of these fractions based on the electron micrographs alone? Consider the structures on the left as an example. These were found in a low speed centrifugal pellet, implying that they are large structures. They look a bit like nuclei, which are also the largest structures in a eukaryotic cell. What biochemical or functional tests might you do to confirm that the four structures shown from left to right are isolated nuclei, rough endoplasmic reticulum, Golgi vesicles and mitochondria? Physical separation combined with biochemical-molecular analysis of subcellular structures has revealed their basic functions and continue to reveal previously un-noticed structures and functions in cells.

Comment [GKB4]: Look at the *phase contrast* micro-graph of isolated chloroplasts in this link: http://youtu.be/oZX1H0X7xQY. (You may need to right-click on the link and select "open" to access it). In 30 words or less, state a working, testable hypothesis consistent with your suspicion that these structures are isolated chloroplasts. Remember that an hypothesis is a declarative sentence, usually stated as an "if..., then..." statement. Put your word count in parenthesis after your response and submit it to the *Chloroplast* D2L DropBox by [insert date and time].

All of cell and molecular biology is devoted to understanding how prokaryotic and eukaryotic cells (and organisms) use their common structural and biochemical inheritance to meet very different survival strategies. As you progress in your studies, watch for experiments in which cell parts are separated and reassembled, or reconstituted. *Reconstitution* is one of the recurring experimental themes involving the functional analysis of cell components. Look for this theme as you continue your studies. Look also for another theme, namely how evolution can account for the common biochemistry and genetics of life…, *and* its structural diversity!

VI. The Origins, Evolution, Speciation, Diversity and Unity of Life

The question of how life began has been with us since the beginnings or recorded history. It is now accepted that there was a time, however brief or long, when the earth was a lifeless (prebiotic) planet. Life's ***origins*** on earth date to some 3.7-4.1 billion years ago under conditions that favored the formation of the first cell, the first entity with all of the properties of life. But couldn't those same conditions have spawned multiple cells independently, each with all of the properties of life? If so, from which of these did life, as we know it today, descend? Whether there were one or more different "first cells", evolution (a property of life) only began with those cells.

115 Properties of Life

The fact that there is no evidence of cells of independent origin may reflect that they never existed. Alternatively, the cell we call our ancestor was evolutionarily successful at the expense of other life forms, which thus became extinct. In any event, whatever this successful ancestor may have looked like, its descendants would have evolved quite different appearances, chemistries and physiologies. These descendant cells would have found different genetic and biochemical solutions to achieving and maintaining life's properties. One of these descendants evolved the solutions we see in force in all cells and organisms alive today, including a common (*universal*) genetic code to store life's information, as well as a common mechanism for retrieving the encoded information. Francis Crick called is commonality the "Central Dogma" of biology. That ancestral cell is called our ***Last Universal Common Ancestor***, or ***LUCA***.

116 The Universal Genetic Code

117 Origins of Life

118 Life Origins vs Evolution

Elsewhere we consider in more detail how we think about the origins of life. For now, our focus is on evolution, the property of life that is the basis of speciation and life's diversity.

Natural selection was Charles Darwin's theory for how evolution led to the *structural* diversity of species. New species arise when beneficial traits are naturally selected from genetically different individuals in a population, with the concomitant culling of less fit individuals from populations over time. If natural selection acts on individuals, evolution results from the persistence and spread of selected, heritable changes through successive generations in a population. Evolution is reflected as *an increase in diversity and complexity* at all levels of biological organization, from species to individual organisms to molecules. For an easy read about the evolution of eyes (whose very existence according to creationists could only have formed by intelligent design by a creator), see the article in National Geographic by E. Yong (Feb., 2016, with beautiful photography by D. Littschwager).

Repeated speciation occurs with the continual divergence of life forms from an ancestral cell through natural selection and evolution. Our shared cellular structures, nucleic acid, protein and metabolic chemistries (the 'unity' of life) supports our common ancestry with all life. These shared features date back to our LUCA! Most living things even share some early *behaviors*. Take our ***biological clock***, an adaptation to our planet's 24 hour daily cycles of light and dark that have been around since the origins of life; all organisms studied so far seem to have one!. The discovery of the genetic and molecular underpinnings of ***circadian rhythms*** (those daily cycles) earned Jeffrey C. Hall, Michael Rosbash and Michael W. Young the 2017 Nobel Prize in Medicine or Physiology (click Molecular Studies of Circadian Rhythms wins Nobel Prize to learn more)!

The molecular relationships common to all living things largely confirm what we have learned from the species represented in the fossil record. Morphological, biochemical and genetic traits that are shared across species are defined as ***homologous***, and can be used to reconstruct evolutionary histories. The biodiversity that scientists (in particular, environmentalists) try to protect is the result of millions of years of speciation and extinction. Biodiversity needs protection from the unwanted acceleration of evolution arising from human activity, including blatant extinctions (think passenger pigeon), and near extinctions (think American bison by the late 1800s). Think also of the consequences the introduction of invasive aquatic and terrestrial species and the effects of climate change.

Let's look at the biochemical and genetic unity among livings things. We've already considered what happens when cells get larger in evolution when we tried to explain how larger cells divided their labors among smaller intracellular structures and

organelles. When eukaryotic cells evolved into multicellular organisms, it became necessary for the different cells to communicate with each other and to respond to environmental cues.

Some cells evolved mechanisms to "talk" directly to adjacent cells and others evolved to transmit electrical (neural) signals to other cells and tissues. Still other cells produced hormones to communicate with cells to which they had no physical attachment. As species diversified to live in very different habitats, they also evolved very different nutritional requirements, along with more extensive and elaborate biochemical pathways to digest their nutrients and capture their chemical energy. Nevertheless, despite billions of years of obvious evolution and astonishing diversification, the underlying genetics and biochemistry of living things on this planet is remarkably unchanged. Early in the 20th century, Albert Kluyver first recognized that cells and organisms vary in form appearance in spite of the essential biochemical unity of all organisms (see Albert Kluyver in Wikipedia). This unity amidst the diversity of life is a paradox of life that we will probe further in this course.

A. Genetic Variation, the Basis of Natural Selection

DNA contains the genetic instructions for the structure and function of cells and organisms. When and where a cell or organism's genetic instructions are used (i.e., to make RNA and proteins) are regulated. Genetic variation results from random mutations. Genetic diversity arising from mutations is in turn, the basis of natural selection during evolution.

119 The Random Basis of Evolution

B. The Genome: An Organism's Complete Genetic Instructions

We've seen that every cell of an organism carries the DNA including gene sequences and other kinds of DNA. The genome of an organism is the *entirety* of its genetic material (DNA, or for some viruses, RNA). The genome of a common experimental strain of *E. coli* was sequenced by 1997 (Blattner FR et al. 1997*The complete genome sequence of Escherichia coli K-12.* Science 277:1452-1474). Sequencing of the human genome was completed by 2001, well ahead of the predicted schedule (Venter JC 2001*The sequence of the human genome*. Science 291:1304-1351). As we have seen in the re-classification of life from five kingdoms into three domains, nucleic acid sequence comparisons can tell us a great deal about evolution. We now know that evolution depends not only on gene sequences, but also, on a much grander scale, on the structure of genomes. Genome sequencing has confirmed not only genetic variation between species, but also

considerable variation between individuals of the same species. Genetic variation within species is in fact the raw material of evolution. It is clear from genomic studies that genomes have been shaped and modeled (or remodeled) in evolution. We'll consider genome remodeling in more detail elsewhere.

C. Genomic 'Fossils' Can Confirm Evolutionary relationships.

It had been known for some time that gene and protein sequencing could reveal evolutionary relationships and even familial relationships. Read about an early demonstration of such relationships based on amino acid sequence comparisons across evolutionary time in Zuckerkandl E and Pauling L. (1965) *Molecules as documents of evolutionary theory.* J. Theor. Biol. 8:357-366. It is now possible to extract DNA from fossil bones and teeth, allowing comparisons of extant and extinct species. DNA has been extracted from the fossil remains of humans, other hominids, and many animals. DNA sequencing reveals our relationship to each other, to our hominid ancestors and to animals from bugs to frogs to mice to chimps to Neanderthals to… Unfortunately, DNA from organisms much older than 10,000 years is typically so damaged or simply absent, that relationship building beyond that time is impossible. Now in a clever twist, using what we know from gene sequences of species alive today, investigators recently *'constructed'* a genetic phylogeny suggesting the sequences of genes of some of our long-gone progenitors, including bacteria (click here to learn more: Deciphering Genomic Fossils). The comparison of these *'reconstructed'* ancestral DNA sequences suggests when photosynthetic organisms diversified and when our oxygenic planet became a reality.

120 Genomic Fossils- Molecular Evolution

VII. Microscopy Reveals Life's Diversity of Structure and Form

Broadly speaking, there are two kinds of microscopy. In *Light Microscopy*, the specimen on the slide is viewed through optical glass lenses. In *Electron Microscopy*, the viewer is looking at an image on a screen created by electrons passing through or reflected from the specimen. For a sampling of light and electron micrographs, check out this Gallery of Micrographs. Here we compare and contrast different microscopic techniques.

A. Light Microscopy

Historically one form or other of light microscopy has revealed much of what we know of cellular diversity. Check out the Drawings of Mitosis for a reminder of how eukaryotic cells divide and then check out The Optical Microscope for descriptions of

different variations of light microscopy (e.g., *bright-field, dark field*, *phase-contrast*, *fluorescence*, etc.). Limits of *magnification* and *resolution* of 1200X and 2 μm, (respectively) are common to all forms of light microscopy. The main variations of light microscopy are briefly described below.

1. *Bright-Field microscopy* is the most common kind of light microscopy, in which the specimen is illuminated from below. Contrast between regions of the specimen comes from the difference between light absorbed by the sample and light passing through it. Live specimens lack contrast in conventional bright-field microscopy because differences in refractive index between components of the specimen (e.g., organelles and cytoplasm in cells) diffuse the resolution of the magnified image. This is why *Bright-Field microscopy* is best suited to fixed and stained specimens.
2. In *Dark-field* illumination, light passing through the center of the specimen is blocked and the light passing through the periphery of the beam is diffracted ("scattered") by the sample. The result is enhanced contrast for certain kinds of specimens, including live, unfixed and unstained ones.
3. In *Polarized light microscopy*, light is polarized before passing through the specimen, allowing the investigator to achieve the highest contrast by rotating the plane of polarized light passing through the sample. Samples can be unfixed, unstained or even live.
4. *Phase-Contrast* or *Interference microscopy* enhances contrast between parts of a specimen with higher refractive indices (e.g., cell organelles) and lower refractive indices (e.g., cytoplasm). *Phase–Contrast* microscopy optics shift the phase of the light entering the specimen from below by a half a wavelength to capture small differences in refractive index and thereby increase contrast. Phase–Contrast microscopy is a most cost-effective tool for examining live, unfixed and unstained specimens.
5. In a *fluorescence microscope*, short wavelength, high-energy (usually UV) light is passed through a specimen that has been treated with a fluorescing chemical covalently attached to other molecules (e.g., antibodies) that fluoresces when struck by the light source. This fluorescent *tag* was chosen to recognize and bind specific molecules or structures in a cell. Thus, in *fluorescence microscopy*, the visible color of fluorescence marks the location of the target molecules and structures in the cell.
6. *Confocal microscopy* is a variant of fluorescence microscopy that enables imaging through thick samples and sections. The result is often 3D-like, with much greater depth of focus than other light microscope methods. Click at Gallery of Confocal Microscopy Images to see a variety of confocal micrographs and related images; look mainly at the specimens.

7. *Lattice Light-Sheet Microscopy* is a 100 year old variant of light microscopy that allows us to follow subcellular structures and macromolecules moving about in living cells. It was recently applied to follow the movement and sub-cellular cellular location of RNA molecules associated with proteins in structures called *RNA granules* (check it out at RNA Organization in a New Light).

B. Electron Microscopy

Unlike light (optical) microscopy, electron microscopy generates an image by passing electrons through, or reflecting electrons from a specimen, and capturing the electron image on a screen. Transmission Electron Microscopy (TEM) can achieve much higher magnification (up to 10^6X) and resolution (2.0 nm) than any form of optical microscopy! *Scanning Electron Microscopy* (SEM) can magnify up to 10^5X with a resolution of 3.0-20.0 nm. TEM, together with biochemical and molecular biological studies, continues to reveal how different cell components work with each other. The higher voltage in *High Voltage Electron microscopy* is an adaptation that allows TEM through thicker sections than regular (low voltage) TEM. The result is micrographs with greater resolution, depth and contrast. SEM allows us to examine the surfaces of tissues, small organisms like insects, and even of cells and organelles. Check this link to Scanning Electron Microscopy for a description of scanning EM, and look at the gallery of SEM images at the end of the entry.

121 Electron Microscopy

Some iText & VOP Key words and Terms

Actin	**Eukaryotes**	**Nuclear envelope**
Archaea	**Eukaryotic flagella**	**Nuclear pores**
Bacterial cell walls	**Evolution**	**Nucleoid**
Bacterial Flagella	**Exocytosis**	**nucleolus**
Binary fission	**Extinction**	**Nucleus**
Cell fractionation	**Hypothesis**	**Optical microscopy**
Cell theory	**Inference**	**Plant cell walls**
Chloroplasts	**Intermediate filaments**	**Plasmid**
chromatin	**keratin**	**Progenote**
Chromosomes	**Kingdoms**	**Prokaryotes**
Cilia	**LUCA**	**Properties of life**

Confocal microscopy	**Lysosomes**	**Rough endoplasmic reticulum**
Cytoplasm	**Meiosis**	**Scanning electron microscopy**
Cytoskeleton	**Microbodies**	**Scientific method**
Cytosol	**Microfilaments**	**Secretion vesicles**
Deduction	**Microtubules**	**Smooth endoplasmic reticulum**
Differential centrifugation	**Mitochondria**	**Speciation**
Diversity	**Mitosis**	**Theory**
Domains of life	**Motor proteins**	**Tonoplast**
Dynein	**Mutation**	**Transmission electron microscopy**
Endomembrane system	**Natural selection**	**Tubulins**