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Sample Chapter Cell and Molecular Biology 5e: What We Know and How We Found Out

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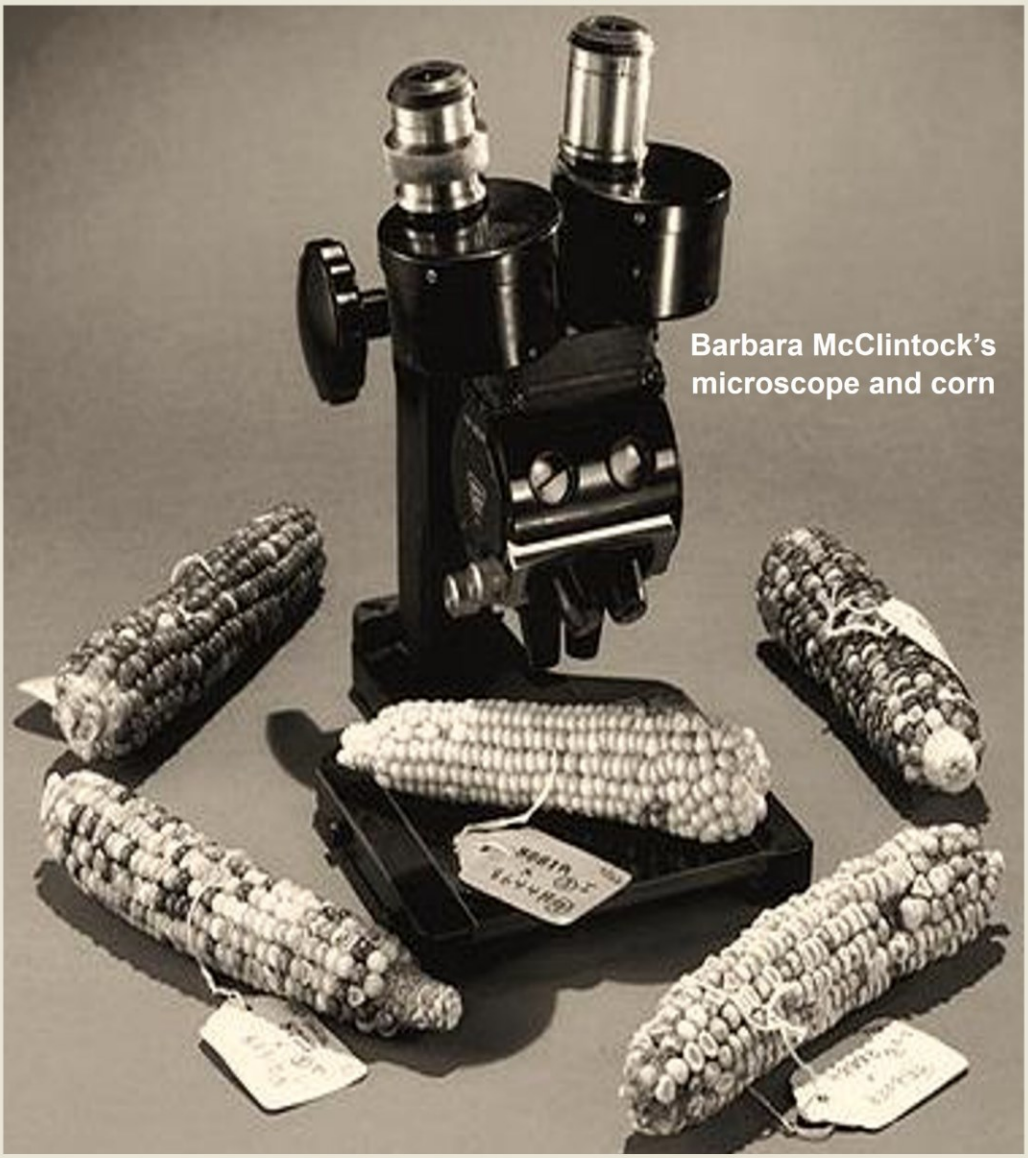
Cell and Molecular Biology

What We Know & How We Found Out

Sample Chapter



Barbara McClintock's microscope and corn

by

Gerald Bergtrom

Cell and Molecular Biology

What We Know & How We Found Out

Instructors' CMB5e iText (Digital Edition)

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Cell and Molecular Biology
What We Know & How We Found Out

Instrructors' CMB5e iText (Digital Edition)

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By
Gerald Bergtrom

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New in CMB5:

- ✓ *Reformatted to include*
  - ➢ *New typeface*
  - ➢ *QR codes to enable access to external websites from printed pages*
  - ➢ *A comprehensive index*
  - ➢ *Larger figures, now accompanied by Alternative Text to increase accessibility*
- ✓ *Many content updates*
- ✓ *New Challenge boxes*

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CMB5e Published 2022

Cover: Public Domain image from Microscope and Corn- Barbara McClintock

Dedicated to:

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

Preface to CMB5

Details, goals, and hopes for teaching and learning

A grasp of the logic and practice of science is essential to understanding the world around us. So, all editions/versions of **CMB** focus 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 comprehensive reference, the book 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 links to the author's short YouTube voice-over PowerPoint (VOP) videos. Numbered links to each of these VOPs are embedded near relevant text and includes edited, optional closed captions. These are easily launched by clicking a *play-video* symbol or descriptive title on a computer or tablet app or by using *QR codes*, as in the example below:

102 Golgi Vesicles & the Endomembrane System

Other ⋆***external online resource*** links are all numbered. The numbers refer to the QR codes for the links at the end of each chapter for use by students using a print edition or printed pages from the digital text.

All digital (the *Basic, Annotated,* and *Instructors* ***CMB5e***) as well as the hard-copy version (the *Annotated* ***CMB5p***) include these interactive features. In addition, the ***Annotated CMB5e*** and ***CMB5p*** have ***Challenge boxes*** that typically include questions about significant new science that is not necessarily definitive and still subject to confirmation. Finally, the **Instructors' CMB5e** adds interactive short ***25 Words or Less*** short writing assignments. The ***Instructors'*** CMB5e is available on request. All interactive elements are intended to expand on concepts discussed in the text. My hope is that that you will engage and experience some of them. In writing and updating ***CMB***, I tried to make it *user-friendly*, current, and accurate. I invite you to use the interactive features of the iText to think about:

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

Along the way, I hope active learning about cell and molecular biology will be an exciting way to reinforce your critical thinking and writing skills across the board.

⋆***External online resources*** links are active at the time of publication but may not remain so in the future.

A mission Statement

Ever since I wrote my first published research paper my goal was to make my science clear enough to be read by anyone interested in biology. Finding jargon-free ways to explain research is not always easy. Sometimes it involves metaphor or analogy and sometimes it requires linguistic precision. I've heard that writers of fiction and truth (novelists, storytellers, playwrights, poets…) spend a lot of time revising to make themselves understood. So, I agree with Eve. L. Ewing, a poet, Professor of Sociology of Education and *Marvel* comic writer, who said in an interview that a*cademic writing is a creative act* (Toor, R. 2020, *Scholars Talk Writing*, The Chronical of Higher Education). I have used interactive components of the early editions of ***CMB*** to create assessable homework assignments in my *flipped*, *blended* Cell Biology course. ***CMB5*** is the latest edition, the product of many revisions, updates, additions, and corrections. In aggregate, all editions and versions of ***CMB*** have been downloaded more than 60,000 times and have been adopted or made recommended reading in college and even high school and medical school courses in the U.S. and around the world. I would like to think that this universal appeal is due to some measure of imagination, clarity, and creative effort.

Some Notes on Using CMB5e and CMB5p

If you are reading the hardcopy *Annotated* ***CMB5p*** iText, you can also download the digital ***CMB5e*** version at ***https://dc.uwm.edu/*** into your browser's URL line. If your instructor has uploaded a digital version of CMB5 to your course site, expect further instructions on how you to access and use the book. ***CMB5p*** is an open access hardcopy version of the *Annotated* ***CMB5e***, for students that would like to study from a printed book. It is virtually identical in content and format to its digital cousin. All numbered text and QR code links should be active in the latest digital (***CMB5e***) versions. Hardcopy readers, please use QR codes to access web links. Links to the author's POVs should be stable (please notify the author or your instructor at of any exceptions). On the other hand, remember that links to ***external online resources*** that are active at the time of publication may not remain so in the future.

Special to Instructors from the Author

The complete digital ***Instructors CMB5e*** *iText* is available at *https://dc.uwm.edu/*. To get the complete ***Instructors CMB5e*** (with additional interactive features), you will need to fill out a short questionnaire identifying yourself as an instructor, your institutional and departmental affiliation, and course information (title, number). When you submit the form, you will get pdf as well as MS-Word files for all digital versions of the iText. When you have download the digital *iText(s)* of your choice, you should find it an easy matter to add, subtract, modify or enhance any parts of it to suit your purposes (in accordance with the ***Creative Commons CC-BY license*** under which it is published (*Creative Commons — Attribution 4.0 International — CC BY 4.0*). You are free to provide the original or a customized version to your students as a small pdf file (recommended) or the larger MS-Word file. If students access a digital iText through your CMS (Course Management System (e.g., Blackboard, D2L, Canvas), you can link assignments to Discussion Fora, a DropBox, or Quiz directly in the iText. Of

course, remember to provide instructions to students on how they should use the book in your class! Here are a few notes on the versioning of ***CMB5***.

1. If you modify the digital *Annotated* or *Instructors'* versions, the index (which is not *dynamic*) may no longer function as intended.
2. The *Basic* version of the digital iText has no index but can (like all versions) be searched online or after downloading.
3. An interactive OER, low-cost print version of the *Annotated CMB5 iText* (***CMB5p***) is now available for anyone that prefers a hardcopy textbook. ***CMB5p*** is still open access, with the a ***Creative Commons CC-BY-NC-SA license*** (*Creative Commons — Attribution-NonCommercial-ShareAlike 4.0 International — CC BY-NC-SA 4.0*).
4. And once again, please remember that links to ***external online resources*** that are active at the time of publication may not remain so in the future.

Finally, to assist instructors, *Open Access*, high resolution copies of the images in this book are provided with the *Instructors' CMB5e* or at https://dc.uwm.edu/ (search Bergtrom).

I hope that you (and maybe even your students!) will enjoy customizing interactive elements and digging into some of the more current research included in the latest edition of the ***CMB***. 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

I thank my erstwhile UW-M LTC (now CETL) colleagues Matthew Russell, Megan Haak, Melissa Davey Castillo, Jessica Hutchings and Dylan Barth for ideas on how to make OER content more interactive and engaging. Thanks to Ann Hanlon and all my Golda Meir Library colleagues for enabling publication of all digital ***CMB*** versions on UW-M Digital Commons. I am most grateful to Ms. M. Terry Bott for reviewing and vetting the images in the book, confirming their public domain or Creative Commons license status. I also owe a debt of gratitude to our departmental lab manager Jordan Gonnering for much hardware and software assistance during the preparation of CMB5, including the print edition. I also thank my copy editor at Elite Authors for her thorough and knowledgeable copy-editing and the team at Index Busters for their patient effort that eased the path to completion of the latest edition of my CMB textbook, especially the print edition. Last among these, but not least, thank you to all the artists, educators and scientists who generously gave Creative Commons license to others to use their work, without which this book would have been impossible. They are individually listed in Appendix I herein. Finally, I must acknowledge the University of Wisconsin-Milwaukee for the opportunity to research and learn both science and pedagogy for more than 35 years. My UW-M experience has left its mark on the content, concept and purpose all CMB editions and versions.

An OER hardcopy (print) version of the *Annotated CMB5e iText* (CMB5p) is available; search *Bergtrom* at https://www.lulu.com/shop/.

About the Author

Dr. Bergtrom is Professor (Emeritus) of Biological Sciences at the UW-Milwaukee and a former *Teaching and Learning* consultant in the UW-M *Center for Excellence in Teaching and Learning*. Scientific interests include cell, molecular and evolutionary biology. Pedagogic interests include the use of technology to serve active and engaged teaching and learning. He has taught face-to-face, fully online, *blended* and *flipped* classes. He also developed and co-instructed *Teaching with Technology*, an interdisciplinary graduate course for graduate students that might someday be teaching. In 40+ years of teaching and research, he has tested and adopted pedagogically proven teaching technologies in his courses. His scientific publications are supplemented by publications on active learning in all teaching modes[1-3]. The first edition of his ***Cell and Molecular Biology***– What We Know & How We Found Out appeared in 2015[4]. Further editions came out in 2016[5], 2018[6], 2019[7] and 2022[8, 9].

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2. Bergtrom, G. (2009) *On Offering a Blended Cell Biology Course*. J. Res. Center Ed. Tech. 5(1) (***http://www.rcetj.org/?type=art&id=91609&***).
3. Bergtrom, G. (2011) *Content vs Learning: An Old Dichotomy in Science Courses*. J. Asynchr. Learning Networks 15:33-44 (***http://jaln_v15n1_bergtrom.pdf***)
4. Bergtrom, G. (2015) *Cell and Molecular Biology: What We Know & How We Found Out* (***CMB***) (If necessary, please contact the author to access this "first" edition)
5. Bergtrom, G. (2016) *Cell and Molecular Biology: What We Know & How We Found Out* (**CMB2e**).
6. Bergtrom, G. (2018) *Cell and Molecular Biology: What We Know & How We Found Out* (***CMB3e***)
7. Bergtrom, G. (2020) *Cell and Molecular Biology: What We Know & How We Found Out* (***CMB4e***)
8. Bergtrom, G. (2022) *Cell and Molecular Biology: What We Know & How We Found Out* (*Basic*, *Annotated* and *Instructors* OER digital ***CMB5e*** versions, available at ***https://dc.uwm.edu/biosci_facbooks_bergtrom/***).
9. Bergtrom, G. (2022) *Cell and Molecular Biology: What We Know & How We Found Out;* the hardcover Annotated ***CMB5p***; Search *Bergtrom* at ***https:Lulushop.com/***

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Use these links to navigate CMB5e (an online version of CMB5p)

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Use these links to navigate CMB5e (an online version of CMB5p)

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

Life's domains, scientific method, cell structures, Study methods (microscopy, cell fractionation, functional analyses); Common ancestry, Genetic variation, Evolution, Species diversity

Reminder: For inactive *links,* google key words/terms for alternative resources.

CELLS: *Left,* Robert Hooke's drawing of cork slices seen through a microscope from his 1665 *Micrographia;* Right, a monk's cell.

1.1 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 such coveted prizes.

One of these, **Robert Boyle**, was born in 1627 to wealthy, aristocrat parents. In his teens, after the customary *Grand Tour* of renaissance Europe (France, 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 where he had already set about studying physics and chemistry, to Oxford. There he built a laboratory with his own money to do experiments on the behavior of gasses under pressure. With some help, he discovered *Boyle's Law*, confirming that the gasses obey mathematical rules. He is also credited with showing that light 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 performing *chemical analysis*, a term he coined.

As a chemist, he also rejected the old Greek concept of the elements: earth, air, fire, and water. 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 defined the first elements! Based on his physical studies

and chemical analyses, Boyle even believed that the indivisible units of elements were atoms, and that the behavior of elements could be explained by the motion of atoms. Finally, Boyle 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 parents 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 other 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 also 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. Ever interested in things mechanical, he also studied the elastic property of springs, leading him to *Hooke's Law*, namely that the force required to compress a spring was proportional to the length that the spring was compressed. Later, these studies led Hooke to imagine how a coil spring might substitute for 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 microscopic structures he saw in thin slices of cork 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 know as the smallest unit of living things.

Now fast-forward almost 200 years to observations of plant and animal cells early in the nineteenth century. These observations revealed structural features common to all cells including a nucleus and a boundary (membrane or wall) and the common organization of cells in groups to form multicellular structures in lower life forms as well as in plants and animals. By the 1830s an enriched understanding of cell structure and the role of cells in the structure of animals and plants led botanist **Matthias Schleiden** and zoologist **Theodor Schwann** to propose the first two precepts of a unified ***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 finally disproved *spontaneous generation* and German histologists observed *mitosis* and *meiosis* (the underlying events of eukaryotic cell division), **Rudolf Virchow** added a third precept to round out ***Cell Theory***: (3) *Cells come from pre-existing cells*. That is, they reproduce. We begin this chapter with a reminder of the ***scientific method***, that way of thinking about our world that emerged formally in the seventeenth century. Then we'll 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.

Commented [GB1]: Explain the statement that ***Cells can have an independent existence.*** Put your word count in parenthesis after your response and submit it to the ***Life is Good*** DropBox by [insert date and time].

Finally, we consider some of the *methods* we use to study cells. Since cells are small, several techniques of microscopy, cell fractionation (in essence a biochemical dissection of the cell) 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 do the following:

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 of life.*
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 structures found in eukaryotes.
5. Outline a procedure to study a specific cell *organelle* or another substructure.
6. Describe or speculate on 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 could have led to their *evolution.*
9. Distinguish between the roles of random *mutations* 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.

1.2 Scientific Method – The Formal Practice of Science

Let's focus here on the essentials of the scientific method originally inspired by Robert Boyle, and then on how science is practiced today. *Scientific method* is one or another standardized protocol for observing, asking questions about, and investigating natural phenomena. Its simplest expressions are look/listen, infer, and test your inference. According to the Oxford English Dictionary, all scientific practice relies on the *systematic observation, measurement, and experiment, and the formulation, testing and modification of hypotheses*. Here is the scientific method as you might read it a typical science textbook:

- ***Read*** the science of others and ***observe*** natural phenomena on your own.
- ***Infer*** and state a ***hypothesis*** (explanation) based on logic and reason.
- Hypotheses are declarative sentences that sound like fact but aren't! Good hypotheses are testable ***predictions***, easily turned into *if/then statements* or *yes-or-no questions*.
- ***Design experiments*** to test the hypothesis: results must be ***measurable evidence*** for or against the hypothesis.
- ***Perform that 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.
- Finally, ***publish*** (i.e., make public) your experiments, results and conclusions. In this way, shared data and experimental methods can be evaluated (and repeated) by other scientists.

We'll return to the scientific method and how it is practiced shortly.

So, what are scientific *hypotheses*, *theories* and *laws* and how do they fit into the scientific method? A scientific *hypothesis*, as suggested above, is an inference, and *educated guess* about what might be going on based on evidence and logic. A hypothesis is a declarative sentence, for example "The Sun revolves around the Earth". This hypothesis was stated by **Aristotle** (among others)! Remember, a good hypothesis can be easily turned into a yes-or-no question, in this case "Does the sun revolve around the Earth?" By its nature, such yes-or-no questions can be answered (i.e., a good hypothesis can be tested) by gathering more evidence by observation and experiment. When Aristotle's hypothesis was finally tested by the observations and measurements of **Nicolaus Copernicus**, **Galileo Galilei** and others, it proved to be false! But you knew that, didn't you?

Contrary to what many people think, a ***scientific theory is not a guess***, neither an *educated* nor an *uneducated* one. Rather, a theory is a statement well supported by experimental evidence and widely accepted by the scientific community. Nevertheless, theories are not 'facts'. Scientists know that theories are subject to further test and modification and may even be overturned. Even scientific ***laws*** can be questioned. Astrophysicists actively test otherwise universally accepted physical ***laws***, occasionally threatening to modify them. In biology, Mendel's *Law of Independent Assortment* shouldn't even be called a law. Indeed, it was not factual as he stated it, or for that matter when he stated it. Check the Mendelian Genetics section of an introductory textbook to see how chromosomal crossing over violates this law, and a history of science book to see what happens when observations or experimental results are inexplicable or as we might say today, 'too far out'.

Even Darwin's ***Theory of Evolution***, one of the most enduring and tested biological theories, has been modified over time. But in this case, the modifications have only strengthened our understanding that biological diversity is the result of natural selection. For commentary on the evolutionary underpinnings of biology, 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). Or, check out some of Darwin's own work at [1.1]Origin of Species.

Commented [GB2]: ***Darwin's "Theory of Evolution" by natural selection should be promoted to a law.*** **Consider arguments for and against this statement as a way of deciding whether you agree or disagree with it. Write your answer in 75 words or less and submit it to the *Breakable Laws* DropBox by [insert date and time].**

Do you think that Darwin's *Theory of Evolution* by natural selection should be promoted to a law? To sum up, a Wikipedia entry states that *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 a hypothesis is at making predictions, the more useful it is. In the last analysis, think of hypotheses as *educated guesses* and think of theories and/or laws not as proofs of anything, but as one or more experimentally supported hypothesis that everyone agrees should serve as *guideposts* to help us evaluate new observations and hypotheses.

But do not making the mistake of placing hypotheses at the low end of a hierarchy of ideas. They are in fact are the bread and butter of the scientific enterprise. Good ones are testable and should predict either/or results of well-designed experiments. Those results (observations, experimental data) should support or nullify the hypotheses being tested. In either case, scientific data generates conclusions that inevitably lead to new hypotheses whose predictive value will 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!

A word about well-designed experiments. **Erwin Schrödinger (**winner of a **Nobel Prize** in physics in 1933) once proposed a *thought experiment*. He wanted his audience to understand the requirements of scientific investigation, but gained a fame (and notoriety) far beyond the world of theoretical physics. Perhaps you have heard of his cat! Considered a founding father of quantum physics, he recognized that adherence to scientific method is not strict and that we can (and should) occasionally violate adherence to the dictates of scientific method. In the now popular story of ***Schrödinger's Cat***, Schrödinger stated that if you sealed a cat in a box with a toxic substance, how could you know if the cat was alive or dead unless you open the box. Wearing his philosopher's hat (yes, he had one!), he postulated that until you open the box, the cat is both "dead and alive". That is, until the box was opened, the cat was in a sense, neither dead nor alive, but both! Often presented as little more than an amusing puzzle, Schrödinger was in fact illustrating that there were two alternate hypotheses: (1) *the cat exposed to toxin survived*, ***or*** (2) *the cat exposed to toxin died*. Note that either hypothesis is a declarative sentence, and that either one could be tested. Just open the box!

In a twist however, Schrödinger added that by opening the box, the investigator would become a factor in the experiment. For example, let's say (for the sake of argument) that you find a dead cat in the box. Is it possible that instead of dying from a poison, the cat was scared to death by your act of opening the box? Or that the toxin made the cat more likely to die of fright but was not lethal by itself? How then to determine whether it was the toxin or your action that killed the cat? This made the puzzle even more beguiling, and to the many laypersons, his greatest scientific contribution! But to a scientist, the solution to the puzzle just means that a scientist must take all possible outcomes of the experiment into account, including the actions of the experimenter, ensuring sound experimental design with all necessary controls. The bottom line, and often the reason that scientific manuscripts suffer negative peer review, is the absence or inadequacy of control experiments. See more about ***Schrödinger's cat*** at [1,2]A Cat Video.

CHALLENGE: Assume that *Schrödinger's cat* is found dead when the box was opened. Suggest some controls for the experiment to eliminate an alternative to the hypothesis that it was the toxin that caused the death?

1.2.1 The Method as It Is Really Practiced!

If you become a scientist, you may find that adherence to the 'rules' of scientific method are honored as much in the breach as in their rigorous observance. An understanding of those rules, or more appropriately principles of scientific method guide prudent investigators to

balance personal bias against the leaps of intuition that successful science requires. Deviations from protocol are allowed!

I think that we would all acknowledge that the actual practice of science by would be considered a success by almost any measure. *Science is a way of knowing* the world around us through constant test, confirmation, and rejection that ultimately reveals new knowledge, integrating that knowledge into our worldview.

An element often missing but integral to any scientific method is that *doing science is collaborative*. Less than a century ago, many scientists worked alone. Again, Gregor Mendel is an example, and his work was not appreciated until decades after he published it. Today, most publications have two or more coauthors. And the inherent collaborative nature of science doesn't end with the investigators in a study. When a paper (or a research grant for that matter) is submitted for consideration, other scientists are recruited to evaluate the quality of hypotheses, experimental design, and soundness of conclusions reported in a manuscript. This *peer review* of fellow scientists is part and parcel of good scientific investigation.

CHALLENGE: Since laws, theories, and hypotheses are each stated as declarative sentences and thus sound like facts, articulate the difference between them in your own words.

1.2.2 Logic and the Origins of the Scientific Method

The scientist, defined as a both observer and investigator of natural phenomena, is only a few centuries old. Long before that, philosophers developed formal rules of *deductive* and *inferential logic* to try and understand nature, humanity's relationship to nature, and the relationship of humans to each other. We owe to those *philosophers* the logical basis of the scientific enterprise. They came up with the rules and systems of *deductive* and *inductive logic* now integral to the practice of science. 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 now going 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 to our intuition. For a bemused take on how scientists think, check out *The Pleasure of Finding Things Out: The Best Short Works of Richard Feynman (*1999, New York, Harper Collins).

CHALLENGE: The article at ([1.3]How to Defend Against Science Deniers) has a clear point of view, i.e., it takes sides!). The author feels that defending valid science by offering up *the scientific method* (i.e., how science is done) is flawed because it invites rebuttal. Summarize his argument, list some take-home messages you feel are important, and why… either because you agree or because you disagree with them.

1.3 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. At one time, 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 temperatures, high salinity, and the like). Originally classified as ancient prokaryotes, *Archaebacteria* were shown by 1990 to be separate from prokaryotes and eukaryotes, in fact a third domain of life.

The archaea are found in such inhospitable environments as boiling hot springs or arctic ice, though some also live in conditions that are more temperate. Based on comparison of the DNA sequences of genes for ribosomal RNAs in eukaryotes, normal bacteria (*eubacteria*) and extremophiles, Carl Woese proposed the three-domain phylogeny illustrated in Figure 1.1.

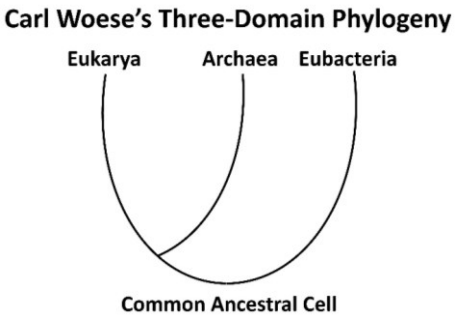

Fig. 1.1: A three-domain phylogeny showing a closer relationship between archaea and eukaryotes.

Based on sequence similarities and differences, Woese concluded that the archaebacteria (Archaea) are not just a domain separate from the rest of the bacteria and from eukaryotes but are more closely related to eukarya than eubacteria! In fact, the Achaea share genes, proteins, and metabolic pathways found in eukaryotes but *not* in bacteria, supporting their close evolutionary relationship to eukaryotes. This unique sharing is further testimony to their domain status. Understanding that all living organisms belong to one of three domains has dramatically changed our understanding of evolution. Archea may be prokaryotes, but their immediate ancestors are more closely related to us than to *E. coli*! 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 replaced the older division of living things into Five Kingdoms, the *Monera (prokaryotes), Protista, Fungi, Plants, and Animals (all eukaryotes!)*.

At this point you may be asking, "What about viruses?" Where are they on, or do they even belong in the tree of life? You may already know that viruses require live cellular hosts to reproduce, but that they are not themselves alive. In fact, much about the place of viruses in evolution is an open question that we will consider in a later chapter. For now, let's look at how we come to know about viruses and some of their peculiarities.

1.3.1. Viruses: Dead or Alive; Big and Small - A History of Surprises

Viruses that infect bacteria are called *bacteriophage* (phage meaning eaters, hence *bacteria eaters*). Eukaryotic viruses include DNA and RNA viruses, with DNA and RNA genomes. Smallpox, hepatitis B, herpes, chicken pox/shingles, and adenovirus are caused by DNA viruses. Common colds, influenza, SARS, and COVID-19 are caused by *positive strand RNA viruses* that upon infecting a cell, replicate their RNA genome to make RNA *negative strand RNA*s encoding all necessary information to make new viruses. HIV AIDS, Ebola, Zika, yellow fever, and some cancers are caused by *retroviruses*, RNA viruses whose genome is reverse-transcribed into a cDNA intermediate that replicates and is transcribed to generate new viruses. Viruses were not identified as agents of disease until late in the nineteenth century, and we have learned much in the ensuing century. In 1892, **Dmitri Ivanofsky**, a Russian botanist, was studying plant diseases. One that damaged tobacco (and was therefore of agricultural significance) was the *mosaic disease* (Figure 1.2, below).

Fig. 1.2: Tobacco mosaic virus symptoms (white patches) on a tobacco leaf.

Ivanofsky showed that extracts of infected tobacco leaves were themselves infectious. The assumption was that the extracts would contain infectious bacteria. But his extracts remained infectious even after passing them through a *Chamberland-Pasteur* filter with a pore size so small that bacteria would not pass into the filtrate. Thus, the infectious agent(s) couldn't be bacterial. Since the infectious material was not cellular and depended on a host for reproduction with no independent life of its own, they were soon given the name *virus*, a term that originally just meant *toxin*, or *poison*. This marked the start of ***virology***, the study of viruses. The virus that Ivanofsky studied is now called *Tobacco Mosaic Virus*, or TMV.

Invisible by light microscopy, viruses are sub-microscopic non-cellular bits of life-chemistry that only become reproductive (come alive) when they parasitize a host cell. Since many viruses cause disease in humans, we have learned much about how they are similar and how they differ. In other chapters, we'll learn how viruses have even become tools for the study of cell and molecular biology. Let's start with a recent surprise from the study of viruses.

As eventually seen in the electron microscope, viruses (called virions or viral particles) are typically 150 nm or less in diameter. And that is how we have thought of viruses for over a century! But in 2002, a *particle* inside an amoeba, originally believed to be a bacterium, was shown by electron microscopy to be a *giant virus*! Since then, several more *giant*, or *Megavirales* were discovered.

Megavirales fall into two groups, *pandoraviruses* and *mimiviruses*. At 1000nm (1 μm) *Megavirus chilensis* (a *pandoravirus*) may be the largest. Compare a few giant viruses to a bacterium (*E. coli*) and the AIDS virus in Figure 1.3 below.

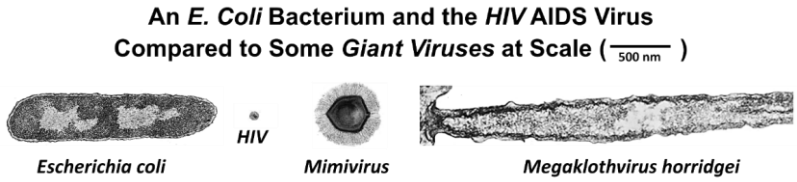

Fig. 1.3: Transmission electron micrographs of 2 giant viruses, the AIDS (HIV) virus and an *E. coli* bacterium. *M. horridgei* is twice the size of *E. coli*. All giant viruses dwarf HIV, a typical eukaryotic virus.

Consider that a typical virus contains a small genome, encoding an average of 10 genes. In contrast, the *Megaviurus chilensis* genome contains 2.5 x 10^6 base pairs (bp) encoding up to 1,100 proteins. Still, it requires host cell proteins to infect and replicate. More surprising is that 75% of the sequenced 1.2 x 10^6 bp *mimivirus* genome code putative proteins *with no counterparts* in other viruses or cells! Equally surprising, some mimiviruses genes encode proteins homologous to those used for translation in prokaryotes and eukaryotes. If all viruses, including the *Megavirales*, only use host cell enzymes and ribosomal machinery to synthesize proteins, what are these genes doing in a mimivirus genome? Think of the surprises here as questions. The big ones concern where and when *Megavirales* (giant viruses) evolved:

- What are those genes with no cellular counterparts all about?
- What were the selective advantages to a virus of large size and large genomes?
- Were *Megavirales* once large cells that invaded other cells, eventually becoming viral parasites and losing most but not all of their genes? Or were they once small viruses that incorporated host cell genes, increasing their genome size and coding capacity?

CHALLENGE: What information would you need (or what questions would you ask and/or what experiments could you do) to find out what the unique proteins encoded by those uniquely viral genes in e.g., mimivirus are doing for the virus?

Viruses are typically identified because they are harmful. The discovery of HIV earned Luc Montagnier, Françoise Barré-Sinoussi, and Harald Z. Hausen the 2008 Nobel Prize in Physiology or Medicine. More recently, as this is written, we are (we hope) near the end of the *COVID-19* viral pandemic caused by the *SARS-CoV-2* retrovirus. In fact, few viruses resident in

humans are known to be beneficial. In contrast, some bacteria are harmful to humans and animals but many are beneficial (even necessary) as symbionts in our many ***microbiomes***.

CHALLENGE: Robert Koch discovered bacterial causes of many diseases and won the 1905 Nobel prize in Physiology or Medicine for showing that [1.4]*Mycobacterium tuberculosis* caused tuberculosis. What other bacterial diseases did he discover and why are his *four postulates* still relevant?

Let's now turn our attention to cells, entities that we define as living, with *all* the *properties of life…,* starting with *eubacteria*.

1.3.2 The Prokaryotes (Eubacteria = *Bacteria* and C*yanobacteria*)

Prokaryotic cells lack nuclei and other eukaryotic organelles, such as mitochondria, chloroplasts, endoplasmic reticulum, and assorted eukaryotic vesicles and internal membranes. Transmission and scanning electron micrographs and an illustration of rod-shaped bacteria are shown below (Figure 1.4).

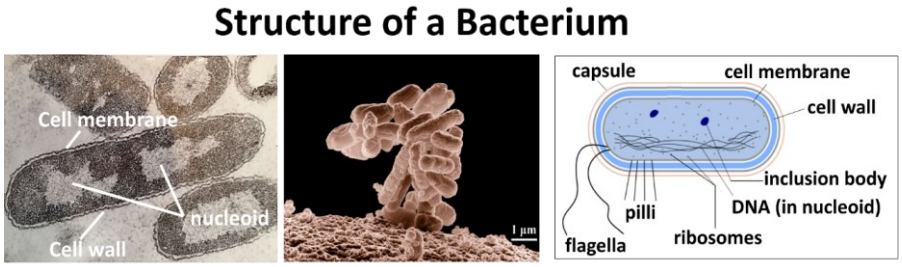

Fig. 1.4: Transmission electron micrograph (left) and drawing of the gram-negative *E. coli* bacterium (right) labeling its structural components, and a scanning electron micrograph of a cell cluster (middle).

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. Check out [1.5]Bacterial Organelles for more information. Bacteria are typically unicellular, although a few (like some cyanobacteria) live colonial lives at least some of the time.

1.3.2.a Bacterial Reproduction

Without the compartments afforded by the internal membrane systems of eukaryotic cells, all intracellular chemistries, (reproduction and gene expression (DNA replication, transcription, translation, and all the metabolic biochemistry of life) happen in the cytoplasm. Bacterial 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 basically naked DNA, unassociated with proteins.

1.3.2.b Cell Motility and the Possibility of a Cytoskeleton

Movement of cells is a response to environment. Some respond to chemicals (chemotaxis), some to light (*phototaxis*) or even gravity (*geotaxis*). Bacteria move to or from nutrients, noxious chemicals, light, dark, gravitational force, etc., by one of several mechanisms. Some use a flagellum made up largely of the bacterial protein ***flagellin***. The main proteins of eukaryotic cell flagella and cilia are the ***tubulins***. Together with *actin* and other proteins, tubulins are also part of the eukaryotic cell cytoskeleton of rods and tubes. Prokaryotes were long thought to lack similar cytoskeletal components. But two bacterial homologues of eukaryotic actin and tubulin genes were recently discovered. ***MreB*** is the actin homologue. Like actin, *MreB* monomers polymerize to form filaments that lie under the cell membrane of bacteria (e.g., *E. coli*), helping to maintain their rod-like shape. In fact, *E. coli* with a mutant MreB gene is spherical…, and normally spherical bacteria lack an *MreB* gene! *MreB* was also thought to form an actin-like ***cortical ring*** that in dividing eukaryotic cells constricts to pinch off two new cells. But this function seems to be served by the ***FtsZ*** protein that encodes a eukaryotic *tubulin homologue*. *FtsZ* polymers form filaments that are seen in a ***Z ring*** at the center of a bacterial cell during *binary fission*. FtsZ mutants divide, but abnormally; thus the role of *FtsZ* in separating bacterial cells during *binary fission* is not yet clear. Figure 1.5 shows a micrograph and an illustration of FtsZ in the Z rings of dividing *E. coli* cells.

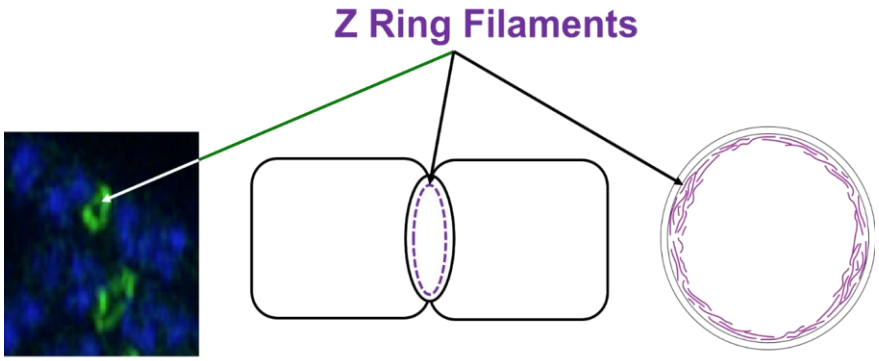

Fig. 1.5: Immunofluorescence localization of FtsZ proteins in Z rings in cross-sections in the middle of dividing E. coli cells (left). Drawings represent the location of Ftz protein in Z rings between dividing cells (middle) and in cross section (right).

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, from which our own evolved!

1.3.2.c 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, or *invaginations* of plasma membrane. Some of these capture energy from sunlight (photosynthesis) or from inorganic molecules (*chemolithotrophy*). Photosynthetic vesicles called *Carboxysomes* (Figure 1.6) are membrane bound structures in which CO_2 is fixed (reduced) in cyanobacteria. Photosynthetic bacteria have less elaborate internal membrane systems.

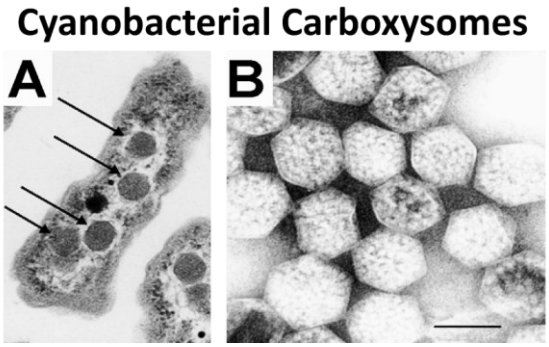

Fig. 1.6: Transmission electron micrograph of carboxysomes in a cyanobacterium (arrows, (left) and isolated from a cyanobacterium (right).

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

Ribosomes are protein-synthesizing machines. Those of prokaryotes are smaller than those of eukaryotes but can translate eukaryotic messenger RNA (mRNA) in vitro. This is because the sequences and structures of ribosomal RNAs are shared by all species, indicating long conserved evolutionary relationships. Recall that it was ribosomal sequence similarities that revealed our closer relationship to archaea than bacteria.

The prokarya (*eubacteria*) are a diverse group, occupying almost every wet, dry, or hot and cold nook-and-cranny of our planet. Yet, all prokaryotic cells share structural and functional metabolic properties with each other and with archaea and eukaryotes! As we've seen with ribosomes, this sharing supports the common ancestry of all life.

Finally, we share not only common ancestry, but living arrangements with bacteria. There are microbiomes in our gut, on our lips, in belly buttons, and in fact all over our skin (see [1.6]Our Skin Microbiome for more about *that*!). Gut microbiome bacteria alone number ~10X more than our own cells! And microbiomes are invisible but not quiet ([1.7]The Human Microbiome). Interest in our microbiomes even earned them their own [1.8]The NIH Human Microbiome Project.

Challenge: Your microbiome is unique and could be another 'fingerprint' ([1.9]Microbiomes are Fingerprints). Why is this so? Suggest circumstances in which microbiome fingerprinting might be unreliable.

1.3.3 The Archaebacteria (Archaea)

Allessandro Volta, a physicist who gave his name to the 'volt' (electrical potential energy), 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 *chemoautotrophs* that get energy from H_2 and CO_2 and generate methane gas in the process. It was not until the 1960s that Thomas Brock (at the University of Wisconsin-Madison) discovered *thermophilic* bacteria living at temperatures approaching 100ºC 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), now 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 extremophilic archaebacteria:

- *Acidophiles* grow at acidic (low) pH.
- *Alkaliphiles* grow at high pH.
- *Halophiles* require high [salt] (e.g., *Halobacterium salinarium* (Figure 1.7).
- Heat-loving *Thermophiles* and *hyperthermophiles* live at high temperatures. *Thermus aquaticus* (Figure 1.8) is athermophile that lives at 70ºC, and is noted for its role in developing the polymerase chain reaction. *Pyrolobus fumarii* is a hyperthermophile, lives at 113°C!

Thermophilic Bacteria

Fig. 1.7: *Pyrolobus fumarii* **Fig. 1.8: *Thermus Aquaticus***

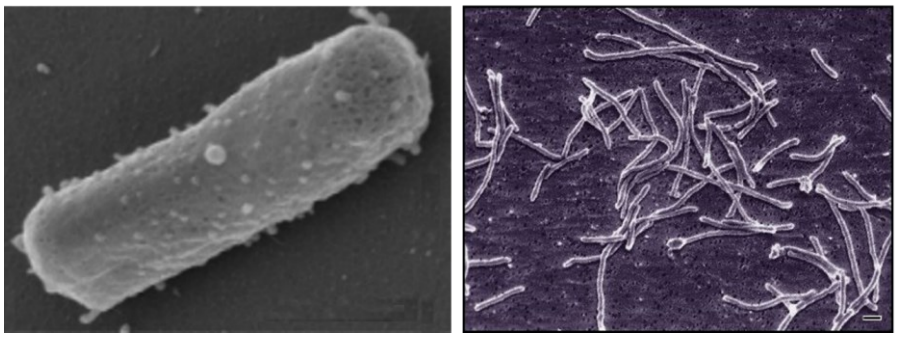

Fig. 1.7 (left): Scanning electron micrograph of *Halobacterium salinarium*, a 'salt-loving' bacterium (**Fig. 1.7**). Right: Scanning electron micrograph of 'heat-loving' *Thermus aquaticus* bacteria (**Fig. 1.8**).

- *Methanogens* produce methane.
- *Barophiles* grow best at high hydrostatic pressure.
- *Psychrophiles* grow best at temperature 15 °C or lower.

- *Xerophiles* grow at very low *water activity* (i.e., drought or near drought conditions).
- *Toxicolerants* grow in the presence of high levels of damaging chemicals, for example, pools of benzene, nuclear waste.

Archaea were originally seen as oddities of life, thriving in unfriendly environments. But they 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.

1.3.4 The Eukaryotes

The volume of a typical eukaryotic cell is some 1000 times that of a typical bacterial cell. 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 might expect many smaller rooms inside this room for such a large space to be functional. The eukaryotic cell is a lot like that large space, with lots of interior *rooms* (i.e., organelles) with their own entryways and exits. In fact, eukaryotic life would not even be possible without a division of labor of eukaryotic cells among different *organelles* (the equivalence to the small rooms in our metaphor).

The smaller prokaryotic "room" has a much larger plasma membrane *surface area-to-volume ratio* than a typical eukaryotic cell. This enables required environmental chemicals to enter and quickly diffuse throughout the cytoplasm of e.g., an *E. coli* cell. The communication between chemicals and structures in a small cell is therefore rapid. In contrast, the communication over a larger expanse of cytoplasm inside a eukaryotic cell requires the coordinated (not to mention regulated!) activities of subcellular components and compartments. Such communication can be relatively slow in a large space. In fact, eukaryotic cells have lower rates of metabolism, growth, and reproduction than prokaryotic cells. Thus, the existence of large cells required the evolution of divided labors supported by *compartmentalization*.

Fungi are more closely related to animal than plant cells. They are a curious beast for several reasons! For one thing, the organization of fungi and fungal cells is somewhat less defined than animal cells. *Septa* are structures between cells that separate fungal *hyphae*. They allow passage of cytoplasm and even organelles between cells. On the other hand, some primitive fungi have few or no septa, in effect creating ***coenocytes***, which are single giant cell with multiple nuclei. Fungal cells are surrounded by a wall, whose principal component is ***chitin***. Chitin is the same material that makes up the exoskeleton of *arthropods* (which includes insects and lobsters!). Typical animal and plant cells with organelles and other structures are illustrated below, in Figure 1.9 and in Figure 1.10).

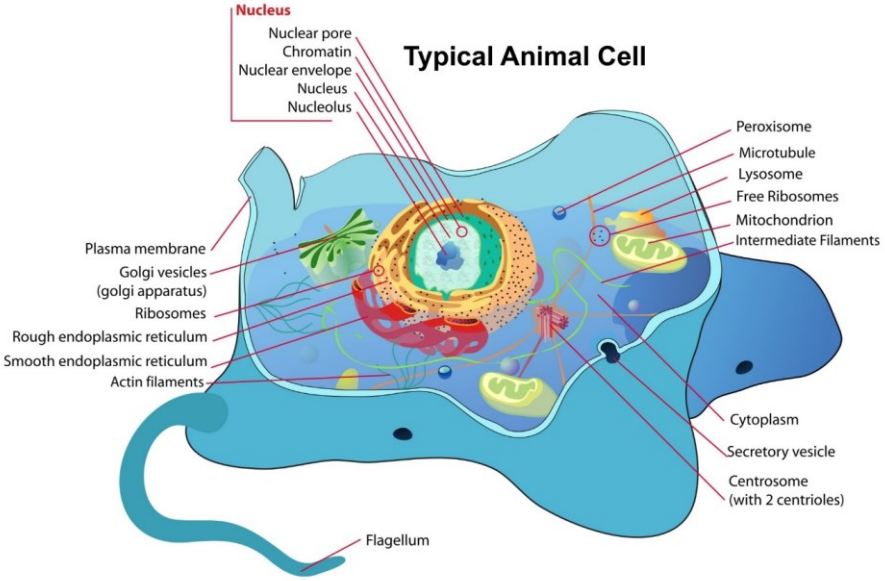

Fig. 1.9: Labeled drawing of the structural components of a typical animal cell.

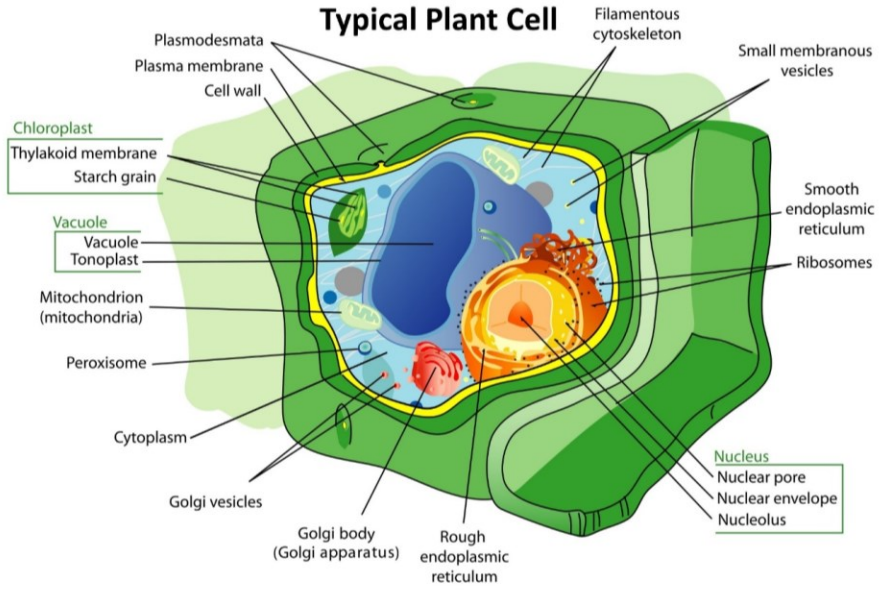

Fig. 1.10: Labeled drawing of the structural components of a typical plant cell.

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 to be equal to that of prokaryotes" (Wikipedia). And we already noted that the bacteria living commensally with us humans represent 10 times as many cells as our own human cells! Clearly, each of us (and probably most animals and even plants) owes our existence to its microbiome as much we do to our own human cells. For now, keeping in mind that plants and animal cells share many internal structures and organelles that perform the same or similar functions, let's look at them and briefly describe their functions.

1.4 Tour of the Eukaryotic Cell

Here we take a closer look at the division of labors among the organelles and structures within eukaryotic cells. We'll look at cells and their compartments in a microscope and see how the organelles and other structures were isolated from cells and identified not only by microscopy, but by biochemical and molecular analysis of their isolates.

1.4.1 The Nucleus

The nucleus is the largest organelle in the cell, separating the genetic blueprint (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 its familiar form during ***interphase***, the time between cell divisions. The structural organization of an interphase nucleus is shown in Figure 1.11 below.

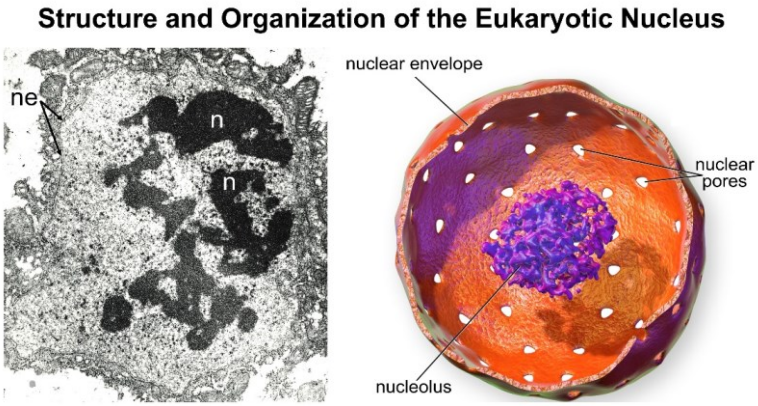

Fig. 1.11: LEFT: Transmission electron micrograph of an insect cell nucleus showing the nuclear envelope (ne) and nucleolus (**n**); RIGHT: Drawing of a nucleus with chromatin (purple) and nuclear pores.

The cross-section of the interphase nucleus in the electron micrograph shows a prominent ***nucleolus*** (labeled **n**). The nucleus is enclosed in a ***nuclear envelope*** and surrounded by a darkly granular ***RER*** (rough endoplasmic reticulum). You can make out ribosomes (small granules) bound to the RER and to the outer nuclear membrane. The space enclosed by the RER (the ***lumen***) is in fact continuous with the space separating the inner and outer membranes of the nuclear envelope, as illustrated in the drawing (above right). ***Nuclear pores*** in the nuclear envelope (look at the drawing) let large molecules and even particles move in

Commented [GB3]: Check out the VOP clip at this link: http://youtu.be/Bw23E7e0YNk.

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].

and out of the nucleus across both membranes. The eukaryotic nucleus is where genes and RNA transcription are regulated and thus one place where cellular protein levels are controlled. *RNAs*, once transcribed from genes and processed, are exported to the cytoplasm through the nuclear pores. Even completely assembled ribosomal subunits are exported from the nucleus. Other RNAs remain in the nucleus, often participating in the regulation of gene activity. We learn some details of nuclear pore traffic, DNA replication, and the dynamics of cell division in later chapters.

104-2 The nucleus

Beyond its nucleolus and nuclear envelope, the nucleus is more organized than it appears in conventional transmission electron micrographs. The nucleolus is just the largest of several inclusions that seem to segregate nuclear functions. Over 100 years ago ***Santiago Ramón y Cajal*** reported other structures in the nuclei of neurons, including what came to be known as ***Cajal bodies*** *(CBs)*. His elegant hand-drawn illustrations of nuclear bodies (made before the advent of photomicrography) can be seen at [1.10]Cajal's Nuclear Bodies and [1.11]Cajal's Beautiful Brain Cells. ***Cajal*** and ***Camillo Golgi*** shared the Nobel Prize in Physiology or Medicine 1906 for their studies of nerve cell structure. In the electron microscope, ***Cajal bodies*** *(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! The results of immunofluorescence localization studies show that different nuclear bodies are associated with specific proteins (Figure 1.12, below).

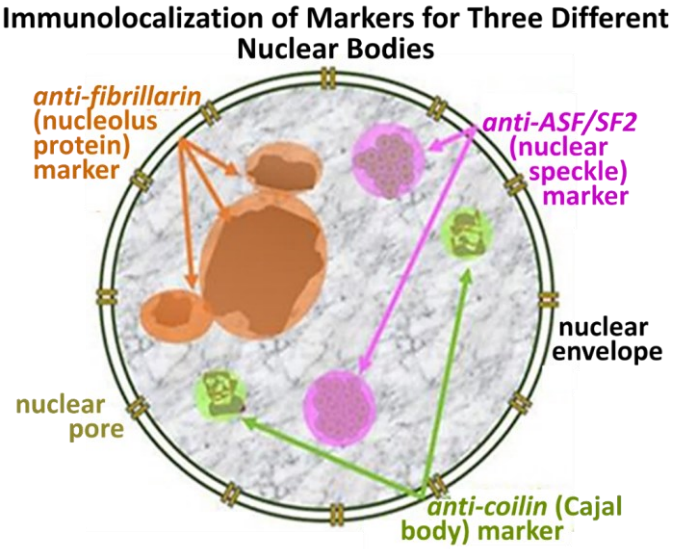

Fig. 1.12: Simulated immunolocalization of different proteins (fibrillarin, coilin and ASF/SF2) to nuclear bodies (nucleolus, Cajal Bodies and nuclear speckles, respectively), using fluorescent antibodies.

Nucleoli contain ***fibrillarin*** proteins, stained red by treating cells with red-fluorescence-tagged antibodies to ***fibrillarin***. Pink-fluorescence-tagged ***anticoilin*** antibodies light up the coilin proteins of CBs. Green-fluorescing **ASF/SF2** antibodies 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 nuclear bodies in their working context later.

1.4.1.a 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 ***chromatin*** inside a nucleus. It is not merely the DNA, but chromatin that must be duplicated when cells reproduce. Duplication of DNA also involves disturbing and rearranging the chromatin proteins resting on the DNA. This occurs before cell division. As the time of cell division nears, chromatin associates with even more proteins, condensing to form *chromosomes*, while the nuclear envelope dissolves, marking the start of ***mitosis*** (***meiosis*** in germline cells) and ***cytokinesis***. You may recall that each somatic cell of a eukaryotic organism has paired *homologous chromosomes* and thus two copies of every gene the organism owns. But sperm and eggs emerge from meiosis with one of each pair of chromosomes and only one copy of each gene. Whether by mitosis or meiosis, duplicated chromosomes (chromatids) lined up at metaphase attach to spindle fibers (as seen in Figure 1.13) to be separated and drawn into new daughter cells formed during cytokinesis.

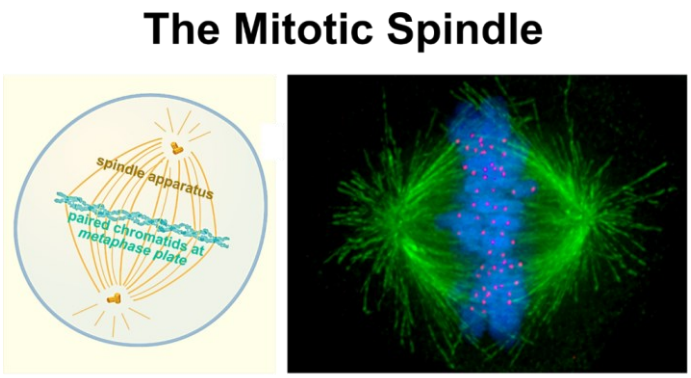

Fig. 1.13: Drawing (left) and fluorescence micrograph (right) of a cell in metaphase of mitosis: aligned chromosomes (chromatids) at the center of the cell (blue in the micrograph) are just about to be pulled apart by microtubules of the spindle apparatus (green) extending from the poles to the center of the cell.

As 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 like mitosis except that the ultimate daughter cells have just one each of the parental chromosomes, eventually to

become the gametes (eggs or sperm). Google *meiosis* and/or *mitosis* to remind yourself about the differences between the two processes, meiosis and mitosis. 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 already understood from the time that mitosis and meiosis were first described in the late nineteenth century.

That every cell of an organism really does contain copies of all of its genes was finally demonstrated by John Gurdon and Shinya Yamanaka in 1962. They transplanted nuclei from the intestinal cells the frog *Xenopus laevis* into *enucleated eggs* (eggs from which their own nuclei had been removed). These 'eggs' grew and developed into normal tadpoles, proving that no genes are lost during development, but are just expressed differentially. For these cloning experiments, Gurdon and Yamanaka shared the 2012 Nobel Prize for Physiology or Medicine. We'll revisit animal cloning later. For now, it's enough to know that Molly the cloned frog was followed by Dolly, the first cloned sheep (1966) and then other animals, all cloned from enucleated eggs transplanted with differentiated cell nuclei. See [1.12]Cuarteterra to read about the cloning a champion polo mare whose clones are also champions!

CHALLENGE: One group of bacteria ([1.13]Planctomycetes) does in fact surround their nucleoid DNA with a membrane! How do you think these cells divide their DNA equally between daughter cells during cell division?

1.4.2 Ribosomes

On the tiny end of the size spectrum, ribosomes are protein-making machines found in all cells. They consist of large and small subunits, each made up of proteins and ribosomal RNAs (rRNAs). Ribosomes bind to messenger RNAs (mRNAs), moving along the mRNA to translate 3-base code words (codons) into polypeptides. Multiple ribosomes can move along the same mRNA, forming ***polyribosomes*** (or ***polysomes***) that simultaneously translate the same polypeptide encoded by the mRNA as shown in Figure 1.14.

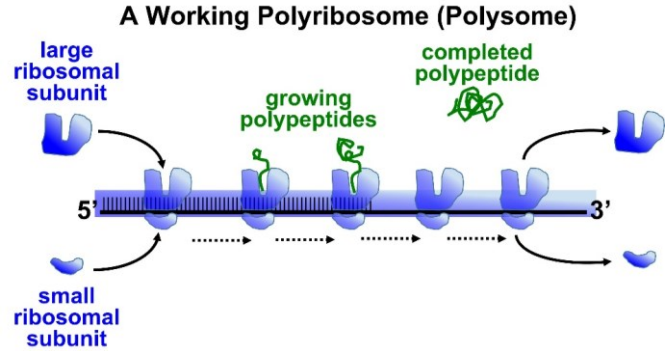

Fig. 1.14: Polysomes form when ribosomes (blue) assembled at the left on an mRNA molecule move. As they move from left to right more ribosomes can assemble at the left, each translating the message; a polypeptide (green) grows, emerging from the ribosomes. To accommodate size the allosteric changes during polypeptide synthesis, there are at least 35 nucleotides separating ribosomes on polysomes.

In the illustration, ribosomes assemble on the left (5′) end of the messenger RNA to form the polysome. When they reach the other (3′) end of the mRNA, the ribosomes disassemble from the RNA and release the finished polypeptide. The granular appearance of cytoplasm in electron micrographs is largely due to the ubiquitous distribution of ribosomal subunits and polysomes in cells. In electron micrographs of leaf cells from a dry, desiccation-tolerant dessert plant, *Selaginella lepidophylla* (Figure 1.15), 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).

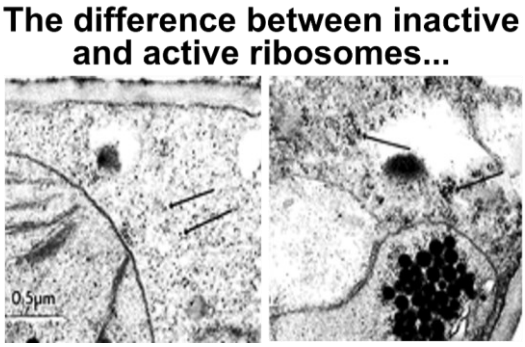

Fig. 1.15: Transmission electron micrographs of Selaginella lepidophylla plant cells. Small, free ribosomal subunits in desiccated cells (left) seem to have formed larger structures (ribosomes) aligned on polysomes in the hydrated plant cells (right). Zoom in to see these details.

Isolated ribosomes and subunits can be separated by sucrose-density-gradient centrifugation based on differences in mass. Figure 1.16 compares ribosomal subunit 'size', protein, and ribosomal RNA (rRNA) composition in eukaryotes and prokaryotes.

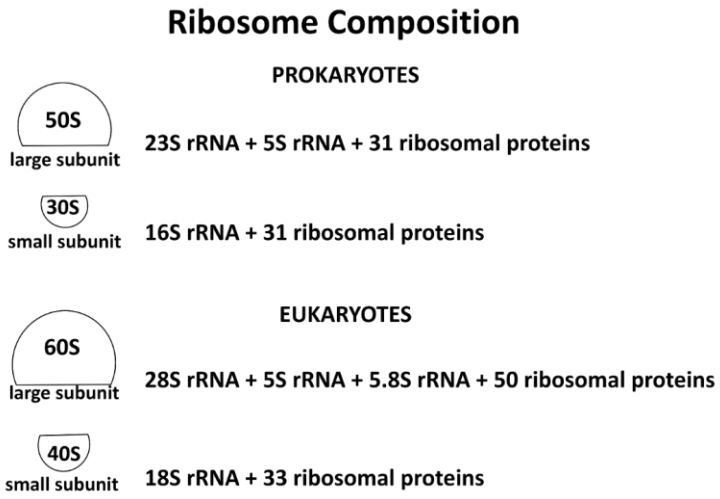

Fig. 1.16: Comparison of prokaryotic (upper) and eukaryotic (lower) ribosomal subunit, rRNA and protein composition. The subunits and their component rRNAs were separated by mass (i.e., size) by sucrose density gradient ***ultracentrifugation***. Proteins were isolated from separated subunits for analysis.

S (***Svedberg***) units are calculated from the position of particles and molecules in the gradient after separation. Theodor Svedberg earned the 1926 Nobel Prize in Chemistry for among other things, applying ***analytical ultracentrifugation*** to the separation and determination of particulate and molecular masses.

101 Ribosomes & Polysomes

1.4.3 Internal membranes and the Endomembrane System

Microscopists of the nineteenth century saw many subcellular structures using the art of histology, staining cells to increase the visual contrast between cell parts. One of these microscopists was the early neurobiologist, ***Camillo Golgi***. He developed a silver (black) stain that first detected a network of vesicles which we now call ***Golgi bodies*** (or ***Golgi vesicles***) in nerve cells. For his studies of the membranes now named after him, ***Camillo Golgi*** shared the 1906 Nobel prize for Medicine or Physiology with ***Santiago Ramón y Cajal***.

Golgi vesicles along with other ***vesicles*** and ***vacuoles*** in cells, including, comprise the ***endomembrane system***. Proteins made by ribosomes of the ***rough endoplasmic reticulum*** (**RER**) either enter the interior space (*lumen*) or become part of the RER membrane itself. The syntheses of ***RER***, ***smooth endoplasmic reticulum*** (***SER***), ***Golgi bodies***, ***microbodies***, ***lysosomes***, and other vesicular membranes (and their protein content) all start in the RER. ***transport vesicles*** that bud off from RER fuse with *Golgi Vesicles* at their *cis face* (Figure 1.17).

Commented [GB4]: 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 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].

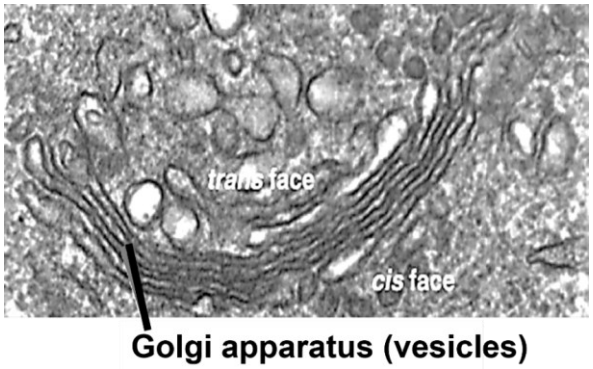

Fig. 1.17: Transmission electron micrograph of an insect cell Golgi body showing cis and trans faces (labeled in white). Vesicles from the RER at the cis face (below, right) will fuse with the Golgi vesicles. Vesicles emerging at the trans face (above, left) are progenitors of a variety of different organelles.

Some proteins made in the endomembrane system are secreted by ***exocytosis***. Others end up in organelles such as ***lysosomes*** that contain hydrolytic enzymes. These enzymes are activated when the lysosomes fuse with other organelles destined for degradation. For example, *food vacuoles* form when a plasma membrane *invaginates,* engulfing food particles. They then fuse with lysosomes to digest the engulfed nutrients. Still other proteins synthesized by ribosomes on the RER are incorporated into the RER membranes, destined to become part of lysosomes, peroxisomes, and even the plasma membrane itself. In moving through the

endomembrane system, *packaged proteins* undergo stepwise modifications (*maturation*) before becoming biologically active (Figure 1.18, below).

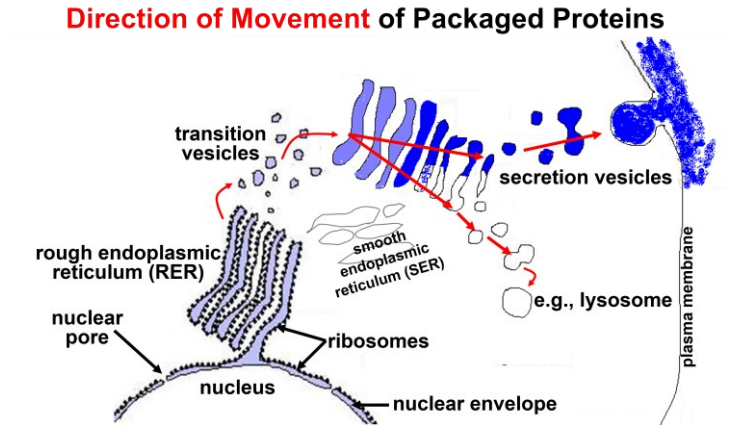

Fig. 1.18: Illustration of 'packaged' protein traffic through a cell from the RER (lower left) through Golgi vesicles (middle) to organelles (e.g., lysosomes) and the plasma membrane (upper right) for exocytosis (i.e., secretion). RER and Golgi vesicles are major sites for the modification (i.e., maturation) of packaged proteins.

100-2 The RER-Rough Endoplasmic Reticulum

102 Golgi Vesicles & the Endomembrane System

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 lose their ribosomes to become part of the SER, which has several different functions (e.g., alcohol detoxification in liver cells).

103-2 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 keeps stems upright.

1.4.4 Mitochondria and Plastids

Nearly all eukaryotic cells contain *mitochondria,* shown in Figure 1.19.

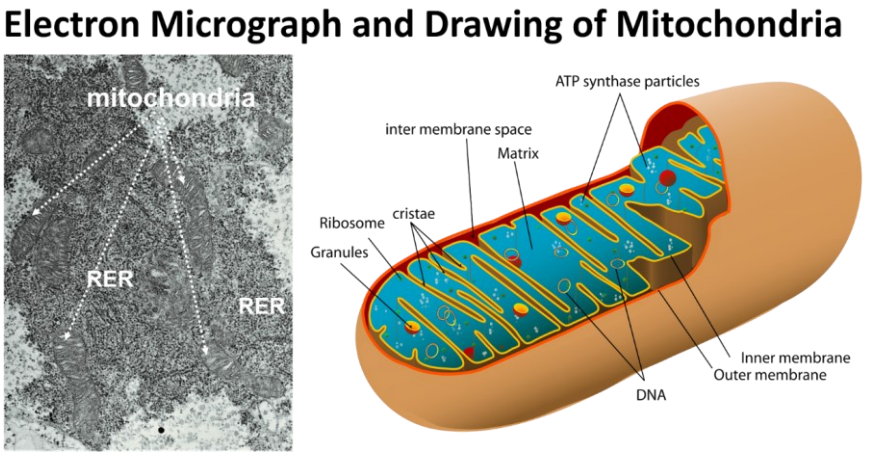

Fig. 1.19: Transmission electron micrograph of mitochondria embedded in RER (left) and a labeled drawing of a single mitochondrion (right).

The ***matrix*** of the mitochondrion is enclosed by a **cristal membrane** surrounded by an ***outer membrane***. Each contains and replicates its own DNA, which contains genes encoding some of the mitochondrial proteins. The surface area of the inner mitochondrial membrane is increased by being folded into ***cristae***, which are sites of ***cellular respiration*** (aerobic nutrient oxidation). Later, we'll consider the role of mitochondria in respiration in more detail.

Earlier, we speculated that some eukaryotic organelles could have originated within bacteria. But mitochondria probably evolved from an aerobic bacterium that was engulfed by another cell that escaped destruction to become an ***endosymbiont*** in the host cell. Lynn Margulis first proposed this in her ***Endosymbiotic Theory***, in which a primitive eukaryotic cell acquired a bacterial endosymbiont (Margulis, L. [*Sagan, L*], 1967, *On the origin of mitosing cells.* Journal of Theoretical Biology **14**: 225–274). She proposed that chloroplasts also started as *endosymbionts*. Both mitochondria and the plastids of plants contain their own DNA, transcribe it into RNA and use their own translational machinery (i.e., ribosomes) to synthesize proteins, further supporting their bacterial and cyanobacterial origins. Living at first in symbiosis with the rest of the cell, these endosymbionts would eventually evolve into the organelles that we are familiar with.

Several protozoa lacking mitochondria and other organelles were discovered and suggested to be "first ingestors" of an ancestral endosymbiont, but since these cells contain other organelles (e.g., *hydrogenosomes*, *mitosomes*) it is thought more likely that these species *once had, but then lost mitochondria*.

Therefore, the descendants of ancient eukaryotic cells missing mitochondria probably no longer exist, if they ever existed at all! More evidence for the *Endosymbiotic Theory* is discussed elsewhere.

CHALLENGE: Nick Lane favors an endosymbiotic event where one prokaryote engulfed another prokaryote (1.14Mitochondria evolve from Bacterium-in-Bacterium Endosymbiosis). What is the dramatic, unorthodox consequence to evolutionary thought if Lane is right about this?

Chloroplasts, photosynthetic protozoa, and *cyanobacteria* contain chlorophyll and use similar photosynthetic mechanisms to make glucose. Transmission electron micrographs of chloroplasts are shown in the Figure 1.20. The one on the right shows a few starch granules.

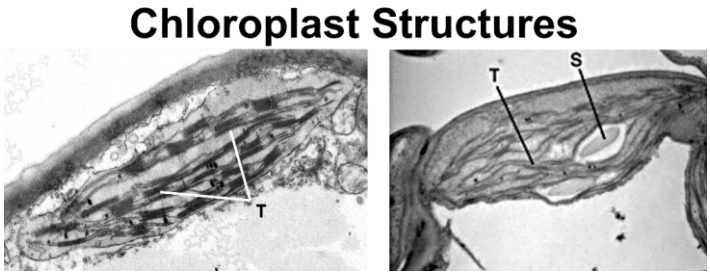

Fig. 1.20: Transmission electron micrographs of a typical photosynthetic chloroplast (LEFT), and one that has photosynthesized long enough to accumulated starch granules (RIGHT). S, starch granule; T, thylakoids.

A *leucoplast* is also a plastid, a chloroplast that has become filled with starch granules. In the electron micrograph of a leucoplast in Figure 1.21, you can see that, because of the accumulation of starch, the grana have become dispersed and indistinct.

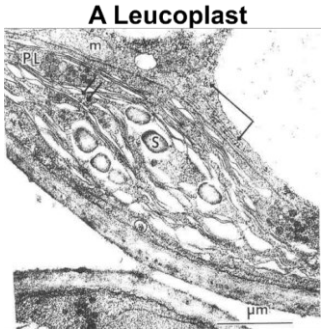

Fig. 1.21: Electron micrograph of a leucoplast, a chloroplast that has become filled with starch granules (S).

105-2 Endosymbiosis-Mitochondria & Chloroplasts

1.4.5 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 structural components of this ***cytoskeleton*** are ***microfilaments***, ***intermediate filaments***, and ***microtubules.*** The structure and ***polarity*** of these structures are shown in Figure 1.22.

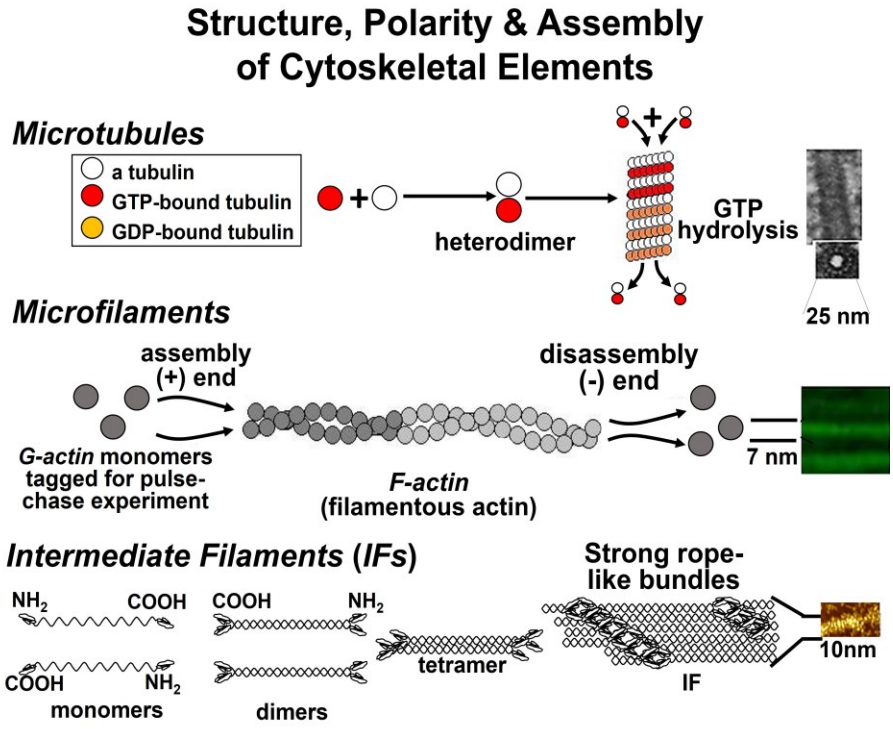

Fig. 1.22: Assembly, structure and polarity of microtubules (upper), microfilaments middle), and intermediate filaments (lower) with electron micrographs of isolated microtubules upper right) and immunofluorescence micrographs of using fluorescent antibodies to microfilament and intermediate filament proteins (middle and bottom right, respectively).

Microtubules are composed of α– and β–*tubulin* protein monomers. Monomeric *actin* proteins make up microfilaments. Intracellular intermediate filament proteins are related to the extracellular *keratin* of hair, fingernails, claws, and bird feathers. These 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 the 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, *flagellin* (a protein not found in eukaryotic cells) powers the movement of bacterial flagella.

A bacterial flagellum is 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 and a cilium to beat rather than undulate. Cilia are involved not only in motility, but also in feeding and sensation. Microtubules in eukaryotic flagella and cilia arise from a *basal body* (similar to *kinetosomes* or *centrioles*) such as the one in Figure 1.23.

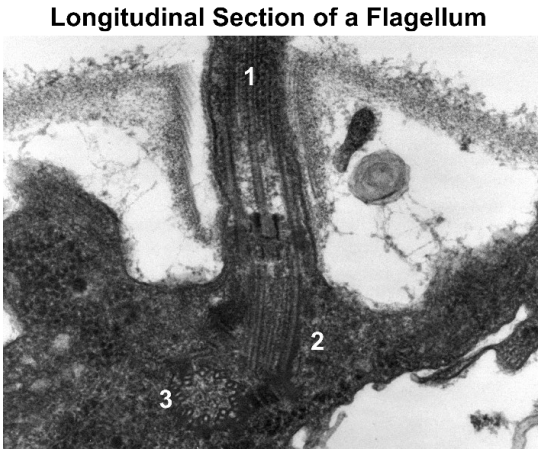

Fig. 1.23: Transmission electron micrograph of a longitudinal section of a flagellum (#1) emerging from a basal body (2). Number 3 is another basal body, this time in cross section.

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. Figure 1.24 shows the *9+2* microtubule arrangement of an isolated axoneme.

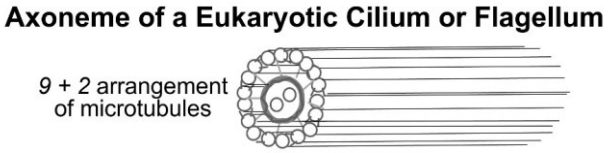

Fig. 1.24: Drawing of an axoneme isolated from a eukaryotic cilium or flagellum, by removing their plasma membrane, showing their characteristic 9+2 arrangement of microtubules in cross section (at the left) and longitudinal section projecting to the right.

Centrioles are themselves comprised of a ring of microtubules. In animal cells they participate in spindle fiber formation during mitosis and meiosis 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 typically lack centrioles but form from an amorphous *MTOC*, or *MicroTubule Organizing Center*. The MTOC serves the same purpose in mitosis and meiosis as centrioles serve in animal cells.

106-2 Filaments & Tubules of the Cytoskeleton

Elsewhere, we describe how microfilaments and microtubules interact with motor proteins (e.g., *dynein*, *kinesin*, and *myosin*) to generate force that results in the sliding of filaments and tubules to allow cellular movement. You'll see that motor proteins also transport molecular cargo from one place to another in a cell.

1.5 How we Know Functions of Organelles and Cell Structures: Cell Fractionation

We could 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 in a spinning centrifuge rotor, 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 in Fig 1.25. Biochemical analysis of the isolated cell fractions can reveal what different organelles and cellular substructures do.

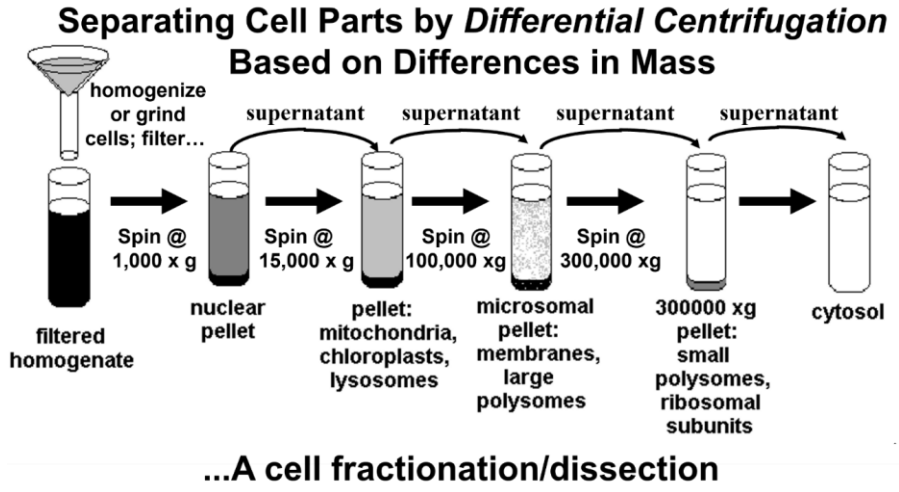

Fig. 1.25: Cells are broken open to release their contents and then filtered to remove unbroken cells (far left). Centrifugation at sequentially higher speed (G-force) sediments progressively smaller cellular parts (organelles, ribosomes, etc.) in *centrifugal pellets* (the 4 tubes in the middle), leaving behind a final supernatant, the soluble cell fraction or cytosol (tube at the far right). The smallest cell parts (membranes, ribosomes) require ultracentrifugation at the highest G-forces.

107-2 Dissecting the Cell-a Cell Fractionation Scheme

CHALLENGE: The cell fractionation scheme pictured in Fig. 1.25 does not include sucrose density gradient centrifugation. Offer an explanation.

Cell fractionation separates cells into their constituent parts. The first step 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.

Commented [GB5]: Consider how you would fractionate a prokaryotic cell type of your choice. Using the cell fractionation shcheme above as a guiside, draw a new scheme (protoocol) for such a fraction-ation. Submit your protocol with an brief explanation of its steps (no word limits here!) to the *Bacterial Bits* DropBox no later than [insert date and time].

The next step is to isolate the subcellular organelles and particles from the cytoplasm (i.e., cytosol) by differential centrifugation. The centrifugation of broken cells at progressively higher centrifugal force separates (fractionates) particulate cell components based on their mass. At the end of this process, a researcher will have isolated ribosomes, mitochondria, chloroplasts, nuclei, and other subcellular structures. After re-suspension, each pellet can be prepared for microscopy. Micrographs of some isolated subcellular fractions are shown in Figure 1.26.

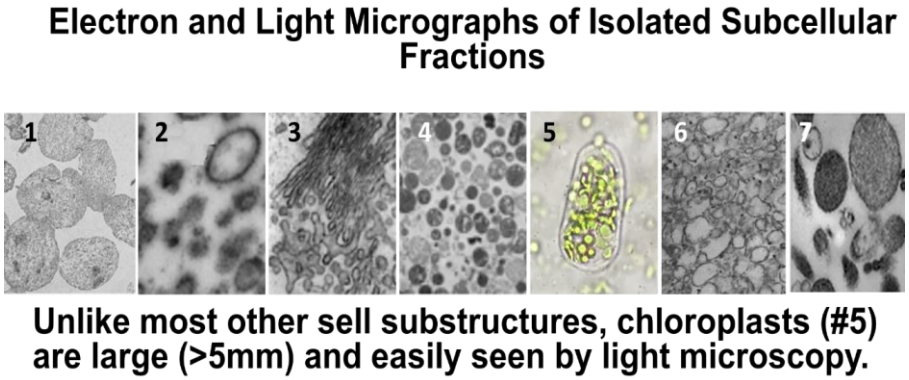

Fig. 1.26: Transmission electron micrographs of organelles isolated by eukaryotic cell fractionation: 1, nuclei; 2, RER; 3, Golgi vesicles; 4, mitochondria; 6, membrane vesicles; 7, lysosomes. The chloroplast (5) is a light micrograph.

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.

108-2 Isolated Nuclei

109-2 Isolated RER

110-2 Isolated Golgi Vesicles

111-2 Lysosomes & Peroxisomes

112-2 Isolated Mitochondria

113-2 Isolated Chloroplasts

114-2 Isolated Membranes Form Vesicles

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 in fact, the largest structures in a eukaryotic cell)—and indeed that's what they are!

Physical separation and the biochemical and molecular analysis of subcellular structures have revealed their basic functions and continue to reveal previously un-noticed structures and functions in cells. What biochemical tests might you do to confirm the identities of the structures shown? At this point you may realize that all 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 keep studying, watch for experiments in which cell parts are separated and reconstituted. *Reconstitution* is a recurring experimental theme in the functional analysis of cell parts. Also look for another, even bigger theme: how evolution accounts for the common biochemistry and genetics of life— *and* its structural diversity!

Commented [GB6]: Look at the ***phase contrast*** micro-graph of isolated chloroplasts at http://youtu.be/oZX1H0X7xQY.
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].

1.6 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 about 4 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) could only begin with 'that or those' cells.

115 Properties of Life

The ***progenote*** has been defined as the first cell from which all life then descended. This implies that the origin of a cell was a unique, one-time only event. The fact that there is no evidence of multiple, independent origins of cellular life might be evidence (albeit negative evidence) that life originated only once, to produce a progenote as defined, and that multiple first cells (or potential progenotes) never existed. Alternatively, we can propose that the cell we call our ancestral progenitor originally had company, but that this progenote was evolutionarily successful at the expense of other early life forms, which thus became extinct.

Whatever our progenote may have looked like, one of its descendants later evolved the solutions to living that 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, what Francis Crick called the *Central Dogma* of biology. That ancestral cell is called our ***Last Universal Common Ancestor***, or ***LUCA***. We will consider ideas about life's origins, progenotes, and universal ancestors in the last chapter of this book. For now, feel free to check out the links below for more information.

116 The Universal Genetic Code

117 Origins of Life

118 Life Origins vs Evolution

For the moment, our focus is on evolution, the property of life that is the basis of speciation and life's diversity. Charles Darwin's theory of evolution was an explanation of the *structural* diversity of species. A naturalist, Darwin lived at a time of ferment where scientific discovery was challenging religion. But by 1839, Charles Darwin had published his ***Narrative of the Surveying Voyages of His Majesty's Ships Adventure and Beagle***. This was the first of many reports of his careful observations of nature, with the seeds of what was to become his theory of ***natural selection***. He published his more fully formed theory of evolution by natural selection in 1859 in ***The Origin of Species***. There, he finally acknowledged his evidence-based belief that that new species arise when beneficial traits are selected from random genetic differences in individuals in a population. At the same time, less fit individuals would be culled from the population. If natural selection acts on individuals, the emergence of new species (evolution) results from the persistence and spread of selected, heritable changes through successive generations in a population. In this way, evolution results in *an increase in biological diversity and complexity* at all levels of biological organization, from species to individual organisms and all the way down to biomolecules.

Darwin recognized that his theory would generate discord between science and biblical accounts of purposeful creation. He addressed the issue with great tact in introducing *The Origin of Species*: *"Although much remains obscure, and will long remain obscure, I can entertain no doubt, after the most deliberate study and dispassionate judgement of which I am capable, that the view which most naturalists entertain, and which I formerly entertained–namely, that each species has been independently created–is erroneous."* Yet today, according to creationists, our exquisite eyes could only have formed by the intelligent design of a creator.

For the evolutionary perspective, see the article in National Geographic by E. Yong (Feb., 2016, with photography by D. Littschwager). Over time science favored Darwin. With the rediscovery of Mendel's genetic experiments at the turn of the twentieth century, it became increasingly clear that the genes of an organism are the basis of an organism's inherited physical and chemical traits, those traits that are passed down through the generations. It also became clear that Mendel had found the genetic basis for Darwin's theory and that the evolution even of *miraculous* eyes can be explained. Science and religion found ways to co-exist but, the controversy persists.

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) are testimony to our common ancestry with all life, dating back to our LUCA! Living things even share some early *behaviors,* governed at least in part by genes.

Take as an example the fact that our ***biological clock*** is an evolutionary adaptation to our planet's 24-hour daily cycles of light and dark. Day and night have been around since the origins of life, and all organisms studied so far seem to have a biological clock! 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 (check out [1.15]Circadian Rhythms Win 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 adaptation (natural selection), 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 of the introduction of invasive aquatic and terrestrial species and the looming 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 when we tried to explain how larger cells divide their labors among smaller intracellular structures and organelles. When eukaryotic cells evolved further 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 far away, 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, through billions of years of evolution and astonishing diversification, the underlying genetics and biochemistry of living things on this planet is remarkably unchanged. Early in the twentieth century, Albert Kluyver first recognized that cells and organisms vary in form appearance in spite of an essential biochemical unity of all organisms (see [1.16]Albert Kluyver). This unity amidst the diversity is a life paradox that we examine in this course.

1.6.1 Random Acts of 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's or organism's genetic instructions are used (i.e., to make RNA and proteins) are highly regulated. Genetic variation results from random mutation. Genetic diversity arising from random mutations is in turn, the basis of natural selection during evolution.

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1.6.2 The Genome: An Organism's Complete Genetic Instructions

Recall that every cell of an organism carries the same genome as every other cell. The genome of an organism is the entirety of its genetic material (DNA, or for some viruses, RNA), including genes and other kinds of DNA sequences. 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 (more or less!) by 2001, well ahead of schedule (Venter JC 2001, The sequence of the human genome. Science 291:1304-1351). Recall also that the analysis of rRNA gene sequences resulted in the dramatic re-classification of life from five kingdoms into three domains.

Thus, comparisons of specific gene or other DNA sequences can tell us a great deal about evolution. We now know that evolution depends not only on individual gene sequences, but on a much grander scale, on the structure of genomes. Genome sequencing has confirmed not only genetic variation between species, but also much variation between individuals of the same species. It is the genetic variation within species that is 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.

1.6.3 Genomic 'Fossils' Can Confirm Evolutionary Relationships.

We have been looking to gene and protein sequencing to find evolutionary relationships and even, familial relationships. You can 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. In addition, it has been possible for some time now, 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 animals (from bugs to frogs to mice to chimps…) and to Neanderthals (with whom we share some genes!) and our other hominid ancestors. 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.

Using what we know from gene sequences of species alive today, investigators have recently *'reconstructed'* a genetic phylogeny suggesting the sequences of genes of some of our long-gone progenitors, including bacteria (to learn more, check out: [1.17]Deciphering Genomic Fossils). The comparison of these *'reconstructed'* ancestral DNA sequences suggests when photosynthetic organisms diversified and when our oxygenic planet became a reality. Closer to home, many remains of ancestral humans have been discovered in the Americas. These promise to unlock the mysteries of human settlement of the continents, though not without controversy. Indian tribal cultures treat their ancestors as sacred and argue against sampling such remains for DNA Analysis. In one example, a well-preserved mummified body was discovered in the Nevada desert in the 1940s. Tests of clothing fragments and hair revealed that this *Spirit Cave mummy* was over 10,000 years old. DNA sequence analysis was proposed to confirm the origins of the mummy. But then the *Fallon Paiute-Shoshone* tribe, which lives near the burial site, asserted a cultural relationship to the body and requested the right of its

return in compliance with the *Native American Graves Protection and Repatriation Act*. Anthropologists then counter-asserted a need for further study of the body to learn more about its origins and about native American origins in general. The dispute ended only after 20 years, when the time the tribe consented DNA tests were allowed. When the DNA sequence analysis results established that the remains *were* indeed that of an ancestor to the tribe, the *Spirit Cave mummy* was returned to the *Fallon Paiute-Shoshone* to be reburied with full tribal rites in 2018. To read more, see [1.18]Resolving American Indian Ancestry or [1.19]Ice Age Mummy DNA Analysis Unlocks Tribal Secrets.

120-2 Genomic Fossils-Molecular Evolution

CHALLENGE: Tracing ancient remains to tribal descendants continues to cause culture/science tension. See the 42,000 year-old Australian aboriginal *Mungo Man,* (Perrotet, T. & Smith, D.M. 2019, *The Homecoming*; Smithsonian 50: 38-49), and then reflect on what the discovery can tell us and how the conflict was resolved.

1.7 Microscopy Reveals Life's Diversity of Structure and Form

Broadly speaking, there are two main categories of microscopy. In *Light Microscopy*, the slide is viewed through optical glass lenses that see visible light reflected from or passing through the specimens on the slide. In *Electron Microscopy*, the viewer is looking at an image on a screen created by electrons passing through or reflected from the specimen, usually mounted on a copper grid. For a sampling of light and electron micrographs, check out this [1.20]Micrograph Gallery. Here we compare and contrast different microscopic techniques.

1.7.1 Light Microscopy

Historically one or another version of light microscopy has revealed much of what we know of the structural diversity of cells, especially eukaryotic cells. Check out the [1.21]Mitosis Drawings for a reminder of how eukaryotic cells divide, and then check out [1.22]The Optical Microscope for descriptions of different variations of light microscopy (e.g., *bright-field, dark field*, *phase-contrast*, and *fluorescence*.). Limits of *magnification* and *resolution* of 1200X and 2 μm, (respectively) are common to all forms of light microscopy. Some variations of light microscopy are briefly described here:

- *Bright-Field microscopy* is the most common kind of light microscopy. 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 in refractive index between components of the specimen (e.g., organelles and cytoplasm in cells) diffuse the resolution of the magnified image. Therefore *Bright-Field microscopy* is best suited to fixed and stained specimens.

- 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.
- In *Polarized light microscopy*, light is passed through a polarizing filter before passing through the specimen. The resulting *incident light* (the light beamed at the object on the slide) to pass through the specimen where it may be bent (diffracted), thereby increasing the contrast of the specimen. The microscopist achieves the highest contrast by rotating the plane of polarized light passing through the sample. A valuable feature of polarized light microscopy is that samples can be unfixed, unstained or even live.
- *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 to increase contrast. Phase–Contrast microscopy is a most cost-effective tool for examining live, unfixed, and unstained specimens.
- In a *fluorescence microscope*, a specimen is, for example, treated with a molecule tagged with (covalently attached to) a *fluorophore* that fluoresces (emits visible light) when exposed to short wavelength, high-energy (usually UV) light. The tagged molecule are often fluorescent antibodies that will bind to specific molecules in a cell. In *fluorescence microscopy*, the visible fluorescent light localizes the target molecule/structure in the cell.
- *Confocal microscopy,* a variant of fluorescence microscopy, enables imaging through thick samples and sections. The result is often 3D-like, with much greater depth of focus than other light microscope methods. Look at [1.23]Microscope Image Gallery-Confocal Included to see a variety of confocal micrographs and related images; look mainly at the specimens.
- *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. Read more about the renewed interest in this technique at [1.23]Lattice Light Sheet Microscopy.

1.7.2 Electron Microscopy

Transmission Electron Microscopy (TEM) microscopy generates an image by passing electrons through a specimen treated with an electron-opaque stain (e.g., osmium). , or reflecting electrons from a specimen, and capturing the electron image on a screen. TEM can achieve much higher magnification (up to 10^6X) and resolution (2.0 nm) than any form of optical microscopy. The higher voltage of ***High Voltage Electron microscopy*** allows passage of electrons through thicker sections than regular (low voltage) TEM. The result is micrographs with much greater resolution, depth, and contrast. ***Scanning Electron Microscopy*** (SEM), with magnifying power up to 10^5X and a 3.0-20.0 nm resolution. SEM lets us to examine the surfaces of tissues, small organisms like insects, and even of cells and organelles. Objects of SEM must be conductive, so that biological samples are usually spray-coated with a thin layer of metal (e.g., palladium, platinum) (check the link to [1.25]Scanning Electron Microscopy for more on SEM and look at the gallery of SEM images at the end of the entry). ***Helium Ion Microscopy*** is a form of SEM that substitutes helium ions for the vacuum in which SEM samples are normally viewed, eliminating the need for metal spray coating. Thus, ***HIM*** enables investigators to examine e.g., cells and viruses in a more natural

state (you can google this on your own to see some examples of this). Electron microscopy, together with biochemical and molecular biological studies have revealed how interacting cellular and molecular components work with each other, and continue to do so, shedding light on all manner of biological processes and interactions.

121-2 Electron Microscopy

Some iText & VOP Key words and Terms

actin	eukaryotes	Natural Selection
Archaea	eukaryotic flagella	nuclear envelope
bacterial cell walls	evolution	nuclear pores
bacterial flagella	exocytosis	nucleoid
binary fission	extinction	nucleolus
cell fractionation	hypothesis	nucleus
cell theory	inference	optical microscopy
chloroplasts	intermediate filaments	plant cell walls
chromatin	keratin	progenote
chromosomes	kingdoms	prokaryotes
cilia	LUCA	Properties of life
confocal microscopy	lysosomes	rough endoplasmic reticulum (RER)
cytoplasm	meiosis	scanning electron microscopy
cytoskeleton	microbiome	Scientific Method
cytosol	microbodies	secretion vesicles
deductive logic	microfilaments	smooth endoplasmic reticulum (SER)
differential centrifugation	microtubules	speciation
diversity	mitochondria	theory
domains of life	mitosis	transmission electron microscopy
dynein	motor proteins	tubulins
endomembrane system	mutation	

CHAPTER 1 WEB LINKS

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Reviews/Comments for, earlier editions/versions of
Cell and Molecular Biology
What We Know & How We Found Out

Reviews (Open Textbook Library, University of Minnesota)

2022: "No other text offers a broader understanding of this exciting science." Zhiming Liu, Professor of Biology, New Mexico University: 

2021: "Gerald Bergtrom's Basic Cell and Molecular Biology… textbook is a tour de force showcasing his passion for teaching... Bergtrom's periodic updates makes sure that the text stays accurate and relevant." Adriana LaGier, Assoc.Professor, Grand View University: 

2021: "This as comprehensive and up to date as any upper-level Cell Biology text I have used. I think is suitable for both higher-level college and introductory graduate school cell biology classes… It is excellent…" Philip Rock, Professor of Biology, Virginia Wesleyan University: 

2020: "The text is easy to read. It almost feels as if the instructor is talking to the reader. Topics are presented in a clear manner with the learning objectives in mind. Meltem Arikan, Adj. Faculty, Massachusetts Maritime Academy: 

2017: "…great introductory text that includes the basics of cell and molecular biology. Each chapter includes… video presentations which add to comprehension. There is a theme based on evolution utilized throughout each chapter with appropriate examples. Main points are reinforced with guided exercises for students." Kate Kenyon, Assoc. Professor, Umpqua Community College: 

2016: "(The book) is written in a narrative form with a somewhat casual tone which makes it easy to read and easy to follow… Learning objectives are outlined at the beginning of each chapter. Throughout the entire text evolution is a constant theme, providing context, rationale, and examples of its importance in the biological sciences". Brendan Mattingly, Acad. Prog. Associate, University of Kansas: 

Comments

2022: "Thank you for providing such a valuable open access text…" (Ali Azghani, Fulbright Scholar & Professor of Biology, Univ. of Texas at Tyler)

2020: "I appreciate the approach your textbook takes…, providing students with a relatable narrative on how things were discovered, rather than simply stating the facts." (Dr. Maria Vassileva, MVSc, PhD; Assoc. Prof. of Biological Science, Nagoya University)

2020: "I have used Campbell and Raven, Hillis, and am now considering the Openstax textbook and find yours so much superior. And then the additional resources like your short, recorded PowerPoints (are) amazing in their conciseness and clarity" (Crima Pogge, Instructor, City College of San Francisco)

2019: "I simply LOVE your book, your historical approach to the study of biology, your challenges for the students that stimulate critical thinking... (Daniela Fadda, Istituto di Istruzione Superiore Desanctis Deledda [Linguistic H. S.], Sardinia Italy)

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