1-2020

Sample Chapter. Cell and Molecular Biology 4e: What We Know and How Found Out

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4th edition (CMB 4e),
Sample Chapter

Cell and Molecular Biology

What We Know & How We Found Out

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Image Adapted From: Microarray

Cell and Molecular Biology

What We Know & How We Found Out

CMB 4e Sample Chapter

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By

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Revised January, 2020

ISBN# 978-0-9961502-5-5

New in CMB4e:

- ✓ *Reformatting, including*
  - *An expanded 'active' Table of Contents (the original Table of Contents remains as the Table of Chapters)*
  - *numbered figures with figure legends*
  - *New List of Figures and sources (Appendix I; other appendices renumbered)*
- ✓ *Many content updates (new illustrations, figures, links)*
- ✓ *More than 50 pages of new and reformatted content, with new sections on: Viruses, Proprioreception, Schrödinger's cat (!), history of CRISPR/cas, 'next gen' DNA sequencing, directed evolution and more*
- ✓ *New **Challenge** boxes (Annotated & Instructors' editions)*

Dedicated to:

Sydell, Aaron, Aviva, Edan, Oren 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.

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4th Edition, Published 2020

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M.T. Bott, Senior Lecturer, Biological Sciences, University of Wisconsin-Milwaukee

Preface to CMB4e

A grasp of the logic and practice of science is essential to understand the rest of the world around us. To that end, the **CMB4e** *iText* remains focused on experimental support for what we know about cell and molecular biology, and on showing students the relationship of cell structure and function.

Rather than trying to be a comprehensive reference book, the ***Basic* CMB4e** itext 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 *web-links. In addition to external online resources, links to the author's short YouTube voice-over PowerPoint (VOP) videos with optional closed captions are embedded near relevant text. Each video is identified by a *play-video* symbol and can be opened by clicking a descriptive title or using *QR bar codes*, such as the example below:

 **102 Golgi Vesicles & the Endomembrane System** 

The *Learning objectives* align with content and ask students to use new knowledge to make connections and deepen their understanding of concept and experiment. All external links are intended to expand or explain textual content and concepts and to engage student curiosity. All images in the iText and *just-in-time* VOPs are by the author or are from public domain or Creative Commons (CC) licensed sources.

Beyond the ***Basic CMB4e*,** a freely available ***Annotated*** version of the iText contains interactive links and formative assessments in the form of ***Challenge*** boxes. The ***Instructors* CMB4e** version models additional interactive features, including short ***25 Words or Less*** writing assignments that can be incorporated into almost any course management system, many of which the author has assigned as homework in his *flipped*, *blended* course. These assessments aim to reinforce writing as well as critical thinking skills. As a Sample Chapter, Chapter 1 of the ***Instructors*** version of CMB4e is freely available for download; the complete ***Instructors*** version is available on request.

My goal in writing and updating this *iText* is to make the content engaging, free and comparable in accuracy and currency to commercial textbooks. I encourage instructors to use the interactive features of the iText (critical thought questions, YouTube videos, etc.) to challenge their students.

***Note:** Web links to the author's own resources may occasionally be updated, but should remain active. Links to resources selected (but not created) by the author were live at the time of publication of the iText, but may disappear without notice!

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

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

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

Special to Instructors from the Author

All versions of the ***Basic*** and ***Annotated*** versions of **CMB4e** are freely available as pdf files to you and your students. To get the ***Instructors*** version you will need to fill out a short form identifying yourself as an instructor. When you submit the form, you will get pdf as well as MS-Word files for the *Basic*, *Annotated* and *Instructor’s* ***CMB4e.*** Once you download the ***CMB4e*** *iText(s)* of your choice, you should find it an easy matter to use the MS-Word file to add, subtract, modify or embellish any parts of it to suit your purposes (in accordance with the Creative Commons CC-BY license under which it is published). Common modifications are adding content of your own, or even that students uncover as part of their studies (or as an assignment!). A useful enhancement of the iText is to add links to your own assessments (quizzes, writing assignments) that take students directly to a Quiz, Discussion Forum, DropBox, etc. in your Learning Management System, e.g., D2L, BlackBoard, Canvas, etc.) course site. This is seamless if students open the iText from within your course site, but also works as long as both the iText and course site are open at the same time. You can provide a customized version of the iText to your students as a smaller pdf file (recommended) or as an MS-Word document.

As implied above, you ask your students participate in the improvement of the iText (for fun or for credit!) and to share the results with others! One final caveat: whereas I provide content updates, that have significant potential subject to confirmation, very current research is not necessarily definitive. I hope that you (and perhaps your students!) will enjoy creating and customizing interactive elements and digging in to some of the most recent research included in the *iText*. Above all, I hope that your students will achieve a better understanding of how scientists use skills of inductive and inferential logic to ask questions and formulate hypotheses…, and how they apply concept and method to testing those hypotheses.

Acknowledgements

Many thanks go to my erstwhile LTC (now CETL) colleagues Matthew Russell, Megan Haak, Melissa Davey Castillo, Jessica Hutchings and Dylan Barth for inspiration in suggesting ways to model how open course content can be made interactive and engaging. I also thank my colleagues Kristin Woodward and Ann Hanlon in the Golda Meir Library for their help in publishing the various online editions and versions of ***CMB*** on the University of Wisconsin-Milwaukee Digital Commons open access platform (http://dc.uwm.edu). I am most grateful to Ms. M. Terry Bott for reviewing and vetting the images used in this iText as either in the public domain or designated with a Creative Commons (CC) license as an open resource (see *Creative Commons License* page, above). Most recently, I owe a debt of gratitude to our departmental lab manager (and just down the hall neighbor) Jordan Gonnering for lots of hardware and software assistance during the preparation of CMB4e. Last but not least I must acknowledge the opportunity I was given at the University of Wisconsin-Milwaukee to teach, study and do research in science and interactive pedagogy for more than 35 years. My research and collegial experience at UW-M have left their mark on the content, concept and purpose of this digital *Open Education Resource* (OER).

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About the Author

Dr. Bergtrom is Professor (Emeritus) of Biological Sciences and a former Learning Technology Consultant in the UW-Milwaukee *Center for Excellence in Teaching and Learning*. Scientific interests include cell and molecular biology and evolution. Pedagogic interests are blended and online instruction and the use of technology to serve more active and engaged teaching and learning. He has taught face-to-face, fully online, *blended* and *flipped* classes at both undergraduate and graduate levels. He also developed and co-instructed *Teaching with Technology*, an interdisciplinary course aimed at graduate students that they might someday find themselves teaching. In his 40+ years of teaching and research experience, he has tested and incorporated pedagogically proven teaching technologies into his courses. His research papers have been supplemented with publications on active blended, online and flipped classroom methods[1-3].

The first edition of his *Open Access/Creative Commons* electronic iText, ***Cell and Molecular Biology***–*What We Know & How We Found Out* first appeared in 2015[4]. Subsequent editions and versions followed in 2016[5], 2018[6] and 2019[7]. The latest editions are available at http://dc.uwm.edu/biosci_facbooks_bergtrom/. Older editions (and versions) will remain available by request to the author.

1. Bergtrom, G. (2006) *Clicker Sets as Learning Objects*. Int. J. Knowl. & Learn. Obj. 2:105-110. (http://www.ijello.org/Volume2/v2p105-110Bergtrom.pdf)
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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 edition)
5. Bergtrom, G. (2016) *Cell and Molecular Biology: What We Know & How We Found Out* [**CMB2e**] (If necessary, please contact the author to access this edition)
6. Bergtrom, G. (2018) *Cell and Molecular Biology: What We Know & How We Found Out* [***CMB3e***] (If necessary, please contact the author to access this edition)
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Table of Chapters

(Click title to see first page of chapter)

Table of Contents

(Click Chapter title or numbered sections to navigate this e-text)

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

Comment [GKB1]: Use this space for your notes, questions and comments. Notes.

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

CELLS: *LEFT: Robert Hooke's drawing of cork slices seen through a microscope froom his 1665) Micrographia;* MIDDLE: Row of windows to monks' chambers (cells) as Hooke may have seen them. a monk is drawn in the window at the left; 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 a Nobel Prize.

One of these, **Robert Boyle**, was born in 1627 to wealthy, aristocrat parents. In his teens, after the customary *Grand Tour* of renaissance Europe (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 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 a little help, he discovered *Boyle's Law*, confirming that the gasses obey mathematical rules. He is also credited with showing that light and sound could travel through a vacuum, that something in air enables combustion, that sound travels through air in waves, that heat and particulate motion were related, and that the practice of alchemy was bogus! In fact, Boyle pretty much converted alchemy to chemistry by doing *chemical analysis*, a term he coined. As a chemist, he also rejected the old Greek concept of earth, air, fire and water elements.

Instead, he defined elements as we still do today: the element is the smallest component of a substance that cannot be further chemically subdivided. He did this a century before Antoine Lavoisier listed and define the first elements! Based on his physical studies and chemical analyses, Boyle even believed that the indivisible units of elements were atoms, and that the behavior of elements could be explained by the motion of atoms. Boyle later codified in print the scientific method that made him a successful experimental scientist.

The second of our renaissance Roberts was **Robert Hooke**, born in 1635. In contrast to Boyle parents, Hooke's were of modest means. They managed nonetheless to nurture their son's interest in things mechanical. While he never took the Grand Tour, he learned well and began studies of chemistry and astronomy at Christ Church College, Oxford in 1653. To earn a living, he took a position as Robert Boyle's assistant. It was with Hooke's assistance that Boyle did the experiments leading to the formulation of *Boyle's Law*. While at Oxford, he made friends and useful connections. One friend was the architect Christopher Wren. In 1662, Boyle, a founding member of the Royal Society of London, supported Hooke to become the society's *curator of experiments*. However, to support himself, Hooke hired on as professor of geometry at Gresham College (London). After "the great fire" of London in 1666, Hooke, as city surveyor and builder, participated with Christopher Wren in the design and reconstruction of the city. 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. In later years these studies led Hooke to imagine how a coil spring might be used instead of a pendulum to regulate a clock. While he never invented such a clock, he was appointed to a Royal Commission to find the first reliable method to determine longitude at sea. He must have been gratified to know that the solution to accurate determination of longitude at sea turned out to involve a coil-spring clock! Along the way in his 'practical' studies, he also looked at little things; he published 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 19th century. Many of these studies revealed common structural features including a nucleus, a boundary wall and a common organization of cells into groups to form multicellular structures of plants and animals and even lower life forms. These studies led to the first two precepts of ***Cell Theory***: (1) *Cells are the basic unit of living things*; (2) *Cells can have an independent existence*. Later in the century when Louis Pasteur disproved notions of *spontaneous generation*, and German histologists observed *mitosis* and *meiosis* (the underlying events of cell division in eukaryotes) a third precept rounded out Cell Theory: (3) *Cells come from pre-existing cells*. That is, they reproduce. We

Comment [GKB2]: Explain the statement that ***Cells can have an independent existence*** in 30 words or less. Put your word count in parenthesis after your response and submit it to the ***Life is Good*** DropBox by [insert date and time].

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

Learning Objectives

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

1. compare and contrast *hypotheses* and *theories* and place them and other elements of the scientific enterprise into their place in the cycle of the *scientific method.*
2. compare and contrast structures common to and that distinguish *prokaryotes*, *eukaryotes* and *archaea*, and groups within these *domains.*
3. articulate the function of different cellular substructures.
4. explain how *prokaryotes* and *eukaryotes* accomplish the same functions, i.e. have the same *properties of life*, even though prokaryotes lack most of the structures.
5. outline a procedure to study a specific cell *organelle* or other substructure.
6. describe how the different structures (particularly in eukaryotic cells) relate/interact with each other to accomplish specific functions.
7. describe some structural and functional features that distinguish prokaryotes (eubacteria), eukaryotes and archaea.
8. place cellular organelles and other substructures in their evolutionary context, i.e., describe their origins and the selective pressures that led to their *evolution.*
9. distinguish between the 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. Simply put, it says 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 an ***hypothesis*** (explanation) based on logic and reason.

- Hypotheses are declarative sentences that sound like fact but aren't! Good hypotheses are testable, easily turned into *if/then (predictive) statements*, or just as readily into *yes-or-no* questions.
- ***Design an experiment*** 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 repeated and evaluated by other scientists.

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

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

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

Astrophysicists are actively testing universally accepted laws of physics. Strictly speaking, Mendel's *Law of Independent Assortment* should not even be called a law. Indeed, it is not factual as he stated it! Check the Mendelian Genetics section of an introductory textbook to see how chromosomal crossing over violates this law.

To sum up, in describing how we do science, the 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 an *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.

In other words, hypotheses 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 the **Nobel Prize** in physics in 1933) once proposed a *thought experiment*. Schrödinger wanted his audience to understand the requirements of scientific investigation, but gained greater 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 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 door! 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 https://www.youtube.com/watch?v=IOYyCHGWJq4.

CHALLENGE: Assume that *Schrödinger's cat* is found dead when the box was opened. Can you suggest some controls for the experiment that could 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 guides 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.

A 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. In this day and age, most publications have two or more coauthors who contribute to a study. But the inherent collaborative nature of science extends beyond just the investigators in a study. In fact, when a paper (or a research grant for that matter) is submitted for consideration, other scientists are recruited to evaluate the quality of hypotheses, lines of experimentation, experimental design and soundness of its conclusions a submitted paper reports. This is *peer review* of fellow scientists is part and parcel of good scientific investigation.

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

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 underpinnings of science. They came up with systems of *deductive* and *inductive logic* so integral to the scientific method. The scientific method grew from those beginnings, along with increasing empirical observation and experimentation. We recognize these origins when we award the Ph.D. (*Doctor of Philosophy),* our highest academic degree! We are about to learn about the life of cells, their structure and function, and their classification, or grouping based on those structures and functions. Everything we know about life comes from applying the principles of scientific method to our intuition. For an 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 (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.

1.1 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, salinity, etc.). Originally classified as ancient prokaryotes, *Archaebacteria* were shown by 1990 to be separate from prokaryotes and eukaryotes, a third domain of life.

The archaea are found in such inhospitable environments as boiling hot springs or arctic ice, although some also live in conditions that are more temperate. Carl Woese compared the DNA sequences of genes for ribosomal RNAs in normal bacteria and extremophiles. Based on sequence similarities and differences, he concluded that the latter are in fact a domain separate from the rest of the bacteria as well as from eukaryotes. For a review, see (Woese, C. 2004; *A new biology for a new century*. Microbiol. Mol. Biol. Rev. 68:173-186) The three domains of life (**Archaea**, **Eubacteria** and ***Eukarya***) quickly supplanted the older division of living things into Five Kingdoms, the *Monera (prokaryotes), Protista, Fungi, Plants, and Animals (all eukaryotes!)*. In a final surprise, the sequences of archaebacterial genes clearly indicate a common ancestry of archaea and eukarya. The evolution of the three domains is illustrated below (Fig.1.1).

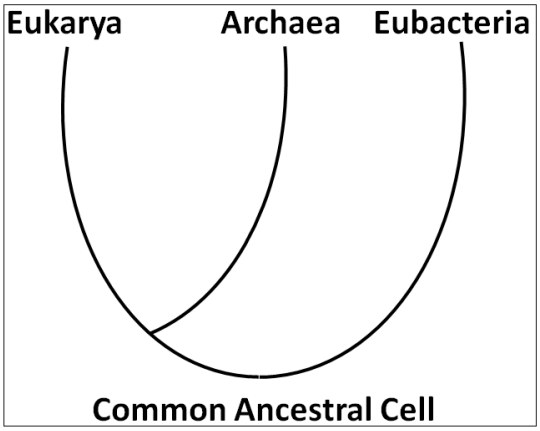

Fig. 1.1: Evolution of three domains showing a closer relationship between archaebacteria and Eukaryotes.

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

At this point you may be asking, “What about viruses?” Where do they belong in a 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 many that cause diseases in plants and animals. In humans, the corona viruses that cause influenza, the common cold, *SARS* and *COVID-19* are ***retroviruses***, with an RNA genome. Familiar retroviral diseases also include *HIV* (AIDS), Ebola, Zika, yellow fever and some cancers. On the other hand, Small pox, Hepatitis B, Herpes, chicken pox/shingles, adenovirus and more are caused by **DNA viruses**.

Viruses were not identified as agents of disease until late in the 19th 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 thus of agricultural significance) was the ***mosaic disease*** (Fig.1.2, below).

Fig. 1.2: Tobacco mosaic virus symptoms 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, one with a pore size so small that bacteria would not pass into the filtrate. Thus the infectious agent(s) were not 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*. 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. Because 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. Here we'll take a look at one of the more recent surprises from *virology*, 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 also shown by electron microscopy to be a virus…, albeit a ***giant virus***! Since this discovery, 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 Fig. 1.3 below.

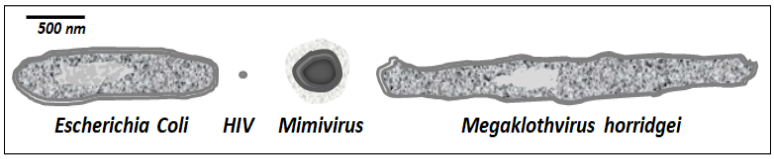

Fig. 1.3: Transmission electron micrographs of giant viruses, the AIDS (HIV) virus and the bacterium *E. coli*. *K. casanovai* is at least the same size and *M. horridgei* is twice the size of the bacterium. All the giant viruses, even the mimivirus, dwarf HIV, a typical eukaryotic virus.

Consider that a typical virus contains a relatively small genome, encoding an average of 10 genes. In contrast, the *M. chilensis* genome contains 2.5×10^6 base pairs that encode up to 1,100 proteins. Nevertheless, it still requires host cell proteins to infect and replicate. What's more surprising is that 75% of the putative coding genes in the recently sequenced 1.2×10^6 base-pair *mimivirus* genome ***had no counterparts*** in other viruses or cellular organisms! Equally surprising, some of the remaining 25%, of mimiviruses genes encode proteins homologous to those used for translation in prokaryotes and eukaryotes. If all, including the giant viruses, 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 of large size and large genomes?
- Were giant viruses once large free-living cells that invaded other cells, eventually becoming parasites and eventually losing most but not all of their genes? Or were they originally small viruses that incorporated host cell genes, resulting in increased genome size and coding capacity?

Challenge: What kinds of 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 are doing for the virus?

Clearly, viruses cause disease. Most were identified precisely because they are harmful to life. 2020 began with a novel viral epidemic in Wuhan, China. The disease, *COVID-19*, is caused by the ***SARS-CoV-2*** retrovirus. In a few short months *COVID-19* became a pandemic, one we are confronting as this is being written. It is interesting that so far, only a few viruses resident in human have been shown to be beneficial. This is in marked contrast to bacteria, some clearly harmful to humans. But many are beneficial, even necessary symbionts in our gut *microbiome*. The same is undoubtedly true of other living things, especially animals.

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

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

Prokaryotic cells lack a nucleus and other eukaryotic organelles such as mitochondria, chloroplasts, endoplasmic reticulum, and assorted eukaryotic vesicles and internal membranes. Bacteria do contain *bacterial microcompartments* (BMCs), but these are made up entirely of protein and are ***not*** surrounded by a phospholipid membrane. These function for example in CO_2 fixation to sequester metabolites toxic to the cells. Click Bacterial Organelles for more information. Bacteria are typically unicellular, although a few (like some cyanobacteria) live colonial lives at least some of the time. See electron micrographs and a drawing of rod-shaped bacteria in Fig. 1.4, below.

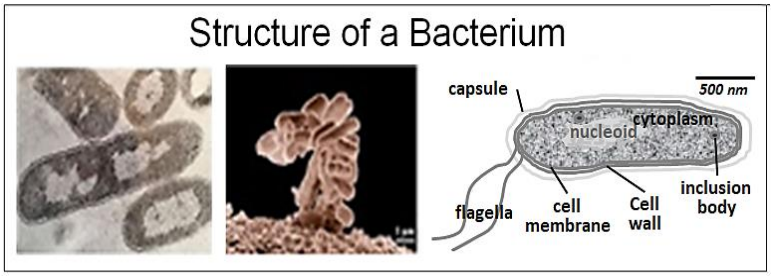

Fig. 1.4: Transmission and scanning electron micrographs of the gram-negative *E. coli* bacterium (left and middle), with its basic structure illustrated at the right).

1.3.2.a Bacterial Reproduction

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

1.3.2.b Cell Motility and the Possibility of a Cytoskeleton

Movement of bacteria is typically by *chemotaxis*, a response to environmental chemicals. Some may respond to other stimuli such as light (*phototaxy*). They can move to or away from nutrients, noxious/toxic substances, light, etc., and achieve motility in several ways. For example, many move using flagella made up largely of the protein ***flagellin***. Flagellin is absent from eukaryotic cell. On the other hand, the cytoplasm of eukaryotic cells is organized within a complex cytoskeleton of rods and tubes made of *actin* and *tubulin* proteins. Prokaryotes were long thought to lack these or similar cytoskeletal components. However, two bacterial genes that encode proteins homologous to eukaryotic actin and tubulin were recently discovered. The *MreB* protein forms a *cortical ring* in bacteria undergoing *binary fission*, similar to the actin cortical ring that pinches dividing eukaryotic cells during *cytokinesis* (the actual division of a single cell into two smaller daughter cells). This is modeled below (Fig.1.5) in the cross-section (right) near the middle of a dividing bacterium (left).

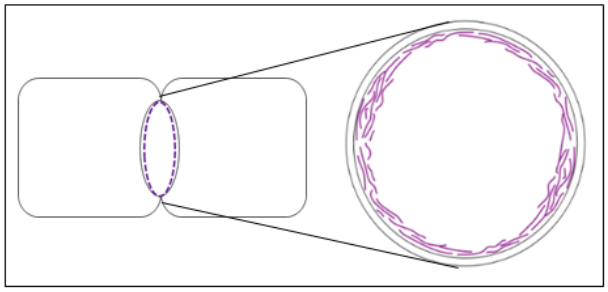

Fig. 1.5: Illustrated cross section of a dividing bacterium showing location of MreB cortical ring protein (purple).

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

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*). *Carboxysomes* (Fig. 1.6) are membrane bound photosynthetic vesicles in which CO_2 is fixed (reduced) in cyanobacteria. Photosynthetic bacteria have less elaborate internal membrane systems.

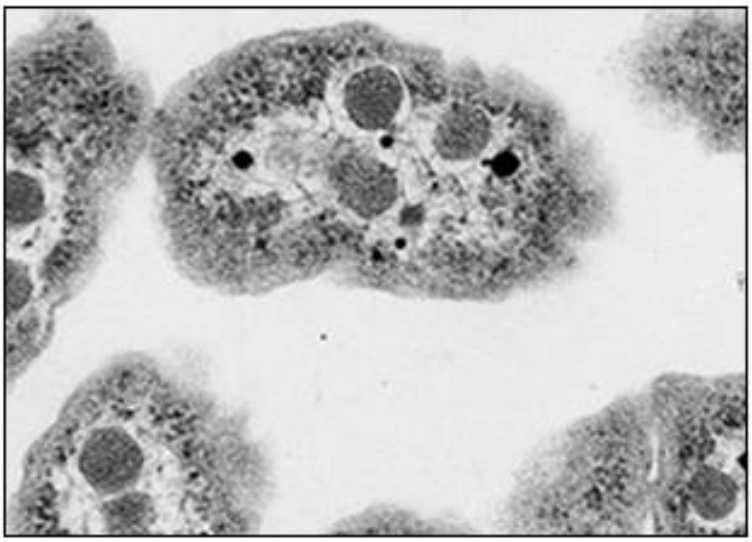

Fig. 1.6: Carboxysomes in a cyanobacterium, as seen by transmission electron microscopy.

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

Ribosomes are the protein-synthesizing machines of life. *Ribosomes* of prokaryotes are smaller than those of eukaryotes but are able to translate eukaryotic messenger RNA (mRNA) *in vitro*. Underlying this common basic function is the fact that the ribosomal RNAs of all species share base sequence

and structural similarities indicating a long and conserved evolutionary relationship. Recall the similarities between RNA sequences that revealed the closer relationship of archaea to eukarya than prokarya.

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

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 also generate methane gas in the process. It was not until the 1960s that Thomas Brock (from the University of Wisconsin-Madison) discovered *thermophilic* bacteria living at temperatures approaching 100^{o}C in Yellowstone National Park in Wyoming. Organisms living in any extreme environment were soon nicknamed ***extremophiles***. One of the thermophilic bacteria, now called *Thermus aquaticus*, became the source of *Taq* polymerase, the heat-stable DNA polymerase that made the *polymerase chain reaction* (PCR) a household name in labs around the world!

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

- *Acidophiles*: grow at acidic (low) pH.
- *Alkaliphiles*: grow at high pH.
- *Halophiles*: require high [salt], e.g., *Halobacterium salinarium*.
- *Methanogens*: produce methane.
- *Barophiles*: grow best at high hydrostatic pressure.
- *Psychrophiles*: grow best at temperature 15 °C or lower.

- *Xerophiles*: growth at very low water activity (drought or near drought conditions).
- *Thermophiles* and *hyperthermophiles*: organisms that live at high temperatures. *Pyrolobus fumarii*, a hyperthermophile, lives at 113°C! *Thermus aquaticus* (noted for its role in developing the polymerase chain reaction)normally lives at 70^{o}C.
- *Toxicolerants*: grow in the presence of high levels of damaging elements (e.g., pools of benzene, nuclear waste).

Salt-loving and *heat-loving* bacteria are shown in the micrographs in Fig. 1.7 and 1.78 below.

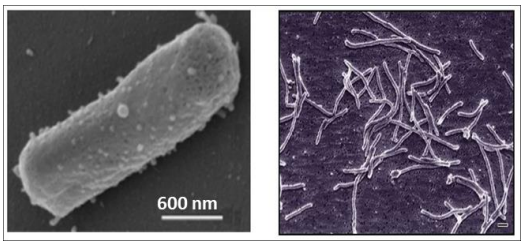

Fig. 1.7: Left: Scanning electron micrograph of *Halobacterium salinarium*, a salt-loving bacterium.
Fig. 1.8: Right: Scanning electron micrograph of 'heat-loving' *Thermus aquaticus* bacteria.

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

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 would expect many smaller rooms inside such a large space, each with its own door(s). The eukaryotic cell is a lot like that large space, with lots of interior "rooms" (i.e., organelles) with their own entryways

and exits. 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, enabling required environmental chemicals to enter and quickly diffuse throughout the cytoplasm of the bacterial cell. Chemical communication between parts of a small cell is therefore rapid. In contrast, the communication over a larger expanse of cytoplasm inside a eukaryotic cell requires the coordinated activities of subcellular components and compartments. Such communication can be relatively slow. In fact, eukaryotic cells have lower rates of metabolism, growth and reproduction than do prokaryotic cells. The existence of large cells required an evolution of divided labors supported by *compartmentalization*.

Fungi, more closely related to animal than plant cells, are a curious beast for a number of reasons! For one thing, the organization of fungi and fungal cells is somewhat less defined than animal cells. Structures between cells called *septa* separate fungal *hyphae*, allow passage of cytoplasm and even organelles between cells. Some primitive fungi have few or no septa, in effect creating *coenocytes*, which are single giant 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 (Fig.1.9, Fig.1.10).

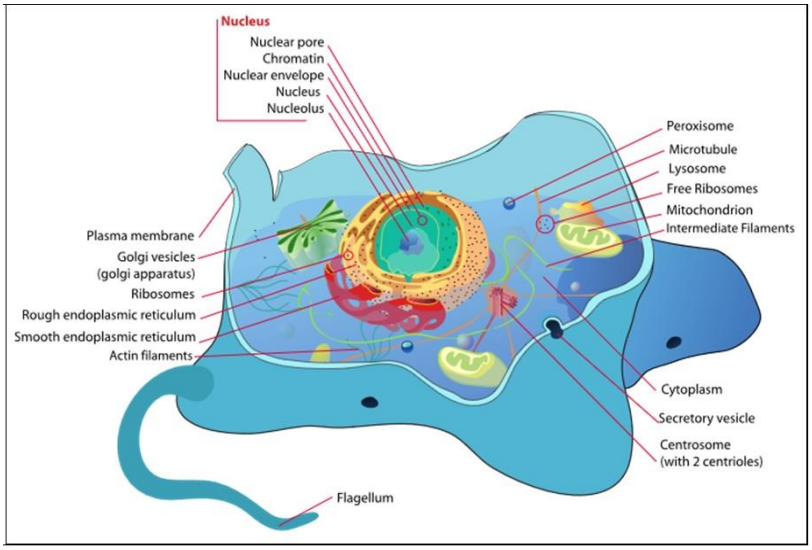

Fig. 1.9: Illustration of the structural components of a typical animal cell.

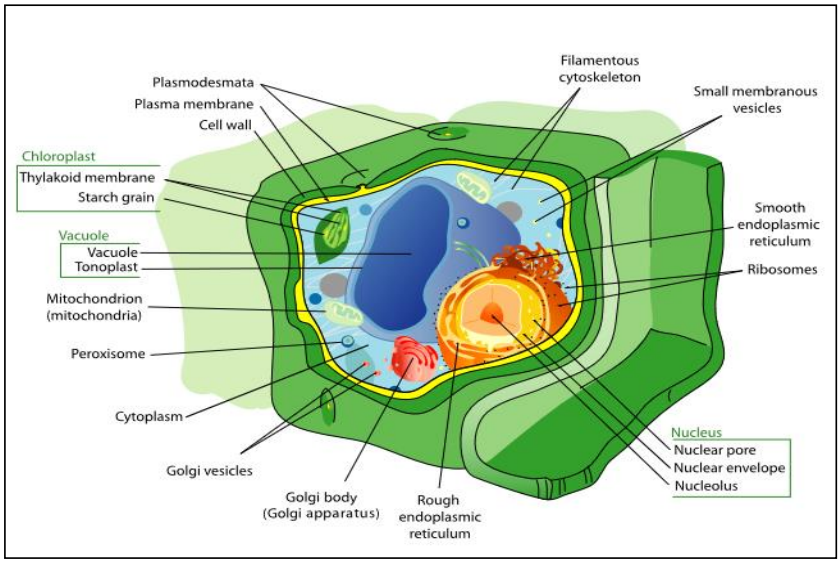

Fig. 1.10: Illustration of the structural components of a typical plant cell.

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 as much of its existence to its microbiome as it does to its 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 separates 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

Comment [GKB3]: Check out the VOP clip at this link: http://youtu.be/Bw23E7e0YNk. (You may need to right-click on the link and select "open" to access it). Then answer the question in the last slide in the clip by completing the sentence "If these structures are nuclei, then..." in 30 words or less. Put your word count in parenthesis after your response. Submit your answers to the *ID Nuclei* DropBox in D2L no later than [insert date and time].

time between cell divisions. This is where the status of genes and therefore of the proteins produced in the cell, are regulated. *rRNA*, *tRNA* and *mRNA* are transcribed from genes, processed in the nucleus, and exported to the cytoplasm through ***nuclear pores***. Other RNAs remain in the nucleus, typically participating in the regulation of gene activity. In all organisms, dividing cells must produce and partition copies of their duplicated genetic material equally between new daughter cells. Let's look first at the structural organization of the nucleus, and then at its role in the genetics of the cell and of the whole organism.

1.4.1.a Structure of the Interphase Nucleus

A typical electron microscope and cartoon of a nucleus, the largest eukaryotic organelle in a cell, are shown in Fig. 1.11 (below). This cross-section of an interphase nucleus reveals its double membrane, or ***nuclear envelope***. The outer membrane of the nuclear envelope is continuous with the *RER* (rough endoplasmic reticulum). Thus, the lumen of the RER is continuous with the space separating the nuclear envelope membranes. The electron micrograph also shows a prominent ***nucleolus*** (labeled **n**) and a darkly granular RER surrounding the nucleus.

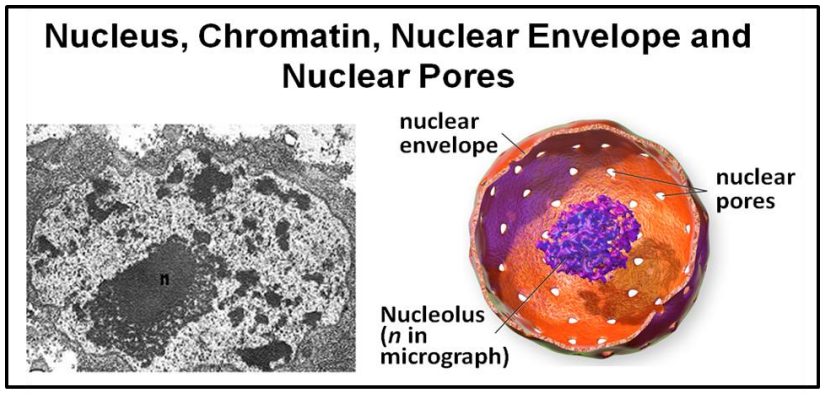

Fig. 1.11: LEFT - Transmission electron micrograph of an insect cell nucleus indicates the nucleolus(**n**)); RIGHT – Illustration of a nucleus showing chromatin (purple) and pores in the nuclear envelope (orange).

Zoom in on the micrograph; you may see the double membrane of the nuclear envelope. You can also make out ribosomes (small granules) bound to both the RER and the outer nuclear membrane. Nuclear envelope ***pores*** (illustrated in the

cartoon at the right) allow large molecules and even particles to move in and out of the nucleus across both membranes. We learn what some of this nuclear pore traffic is all about in later chapters.

104-2 The nucleus

The nucleus is *not* an unorganized space surrounded by the nuclear envelope, as might appear in transmission electron micrographs. The nucleolus is 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 long before the advent of photomicrography) can be seen at Cajal's Nuclear Bodies and 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! Different nuclear bodies are associated with specific proteins. Fig. 1.12 illustrates the results of immunofluorescence localization of nuclear body proteins.

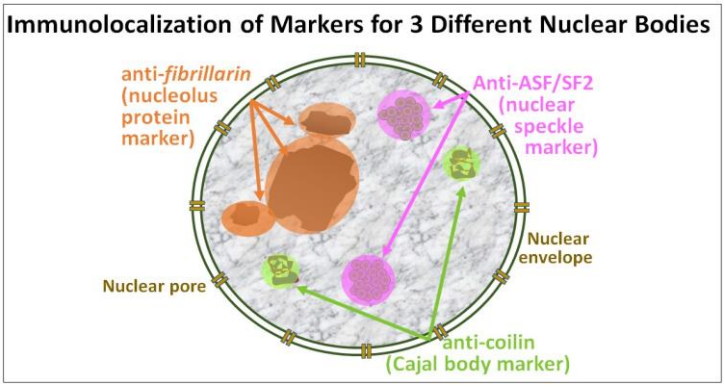

Fig. 1.12: Simulated fluorescence micrograph showing the immunolocalization of antibodies against fibrillarin, coilin and ASF/SF2 protein to *nuclear bodies* (nucleolus, *Cajal Bodies* and nuclear speckles, respectively).

Nucleoli contain ***fibrillarin*** proteins and stain red because they have been treated with red-fluorescence-tagged ***antifibrillarin*** antibodies. CBs contain the protein ***coilin***. They fluoresce pink because the nuclei were treated with fluorescence-

tagged ***anticoilin*** antibodies. Green-fluorescent antibodies to the **ASF/SF2** protein localize to nuclear speckles. As part of, or included in a nuclear matrix, nuclear bodies organize and regulate different aspects of nuclear activity and molecular function. The different nuclear bodies perform specific functions and interact with each other and with proteins DNA and RNA to do so. We will revisit some nuclear bodies in their working context in later chapters.

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

We read earlier that bacteria are busy doubling and partitioning their naked DNA chromosomes at the same time as they grow and divide by binary fission. In eukaryotic cells, a ***cell cycle*** divides life into discrete consecutive events. During most of the cell cycle, cells are in interphase and DNA is wrapped up in proteins in a structure called ***chromatin***. It is not merely the DNA, but chromatin that must be duplicated when cells reproduce. Duplication of DNA also involves rearranging, or disturbing the chromatin proteins resting on the DNA. This occurs before cell division (***mitosis*** and ***cytokinesis***). As the time of cell division nears, chromatin associates with even more proteins, condensing to form *chromosomes*, while the nuclear envelope dissolves. You may recall that every somatic cell of a eukaryotic organism contains paired *homologous chromosomes*, and therefore two copies of every gene an organism owns. On the other hand, sperm and eggs contain one of each pair of chromosomes, and thus one copy of each gene. Whether by mitosis or meiosis, cytokinesis separates duplicated chromosomes to *daughter* cells. In the fluorescence micrograph of a cell in the *metaphase* stage of *mitosis* (Fig.1.13), the chromosomes (blue) are just about to be pulled apart by microtubules of the spindle apparatus (green).

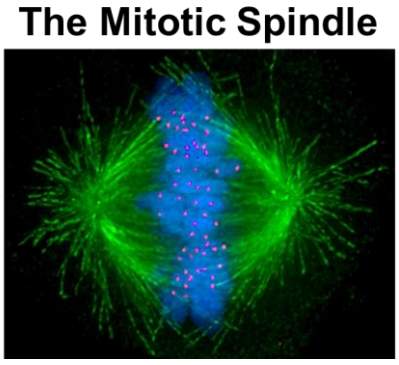

Fig. 1.13: Fluorescence micrograph of a mitotic spindle treated with antibodies to chromosomal proteins (blue) and spindle fiber proteins (green).

As the chromosomes separate and daughter cells form, nuclei reappear and chromosomes de-condense. These events mark the major visible difference between cell division in bacteria and eukaryotes. Cytokinesis begins near the end of mitosis. *Sexual reproduction*, a key characteristic of eukaryotes, involves ***meiosis*** rather than mitosis. The mechanism of *meiosis*, the division of *germ cells* leading to production of sperm and eggs, is similar to mitosis except that the ultimate daughter cells have just one each of the parental chromosomes, eventually to become the gametes (eggs or sperm). 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 understood since mitosis and meiosis were first described in the late 19th century. However, it was finally demonstrated in 1962, when John Gurdon and Shinya Yamanaka transplanted nuclei from the intestinal cells the frog *Xenopus laevis* into enucleated eggs (eggs from which its own nucleus had been removed). These 'eggs' grew and developed into normal tadpoles, proving that no genes are lost during development, but just expressed differentially. We will revisit animal cloning later in this book. But for now, it's sufficient to know that Molly the cloned frog was followed in 1996 by Dolly, the first cloned sheep, and then other animals, all cloned from enucleated eggs transplanted with differentiated cell nuclei. Click Cloning Cuarteterra for the *60 Minutes* story of the cloning of *Cuarteterra*, a champion polo mare whose clones are also champions! For their first animal cloning experiments, Gurdon and Yamanaka shared the 2012 Nobel Prize form Physiology or medicine.

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

1.4.2 Ribosomes

On the other end of the size spectrum, ribosomes are evolutionarily conserved protein synthesizing machines in all cells. They consist of a large and a small subunit, each made up of multiple proteins and one or more molecules of ribosomal RNA (rRNA). Ribosomes bind to messenger RNA (mRNA) molecules and then move along the mRNA as they translate 3-base code words (codons) to link amino acids into polypeptides. Multiple ribosomes can move along the same mRNA, becoming a

polyribosome, simultaneously translating the same polypeptide encoded by the mRNA. The granular appearance of cytoplasm in electron micrographs is largely due to the ubiquitous distribution of ribosomal subunits and polysomes in cells. Fig. 1.14 shows a ***polyribosome*** 'string' of ribosomes, or ***polysome*** for short.

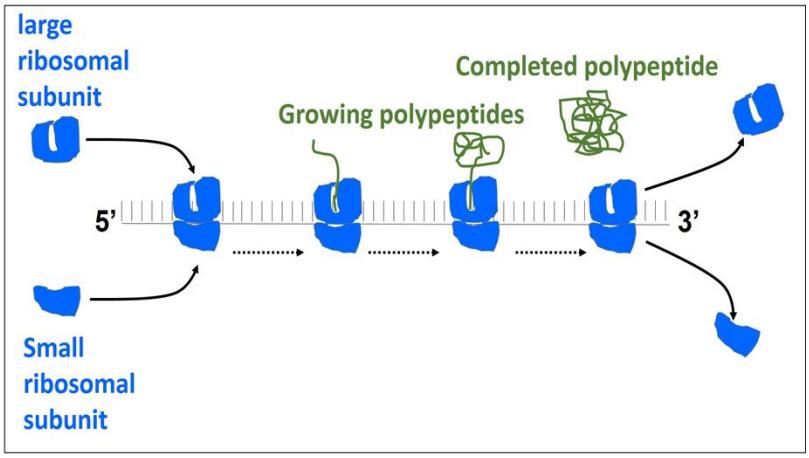

Fig. 1.14: To form a polysome, ribosomes (blue) assemble at the left on an mRNA molecule. As they move along the mRNA from left to right, they translate the message into a polypeptide (green), shown growing and emerging from ribosomes in the polysome.

In the illustration, ribosomes assemble at the left of the messenger RNA (mRNA) to form the polysome. When they reach the end of the message, the ribosomes disassemble from the RNA and release the finished polypeptide. In electron micrographs of leaf cells from the dry, quiescent desiccation-tolerant dessert plant, *Selaginella lepidophylla* (Fig.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).

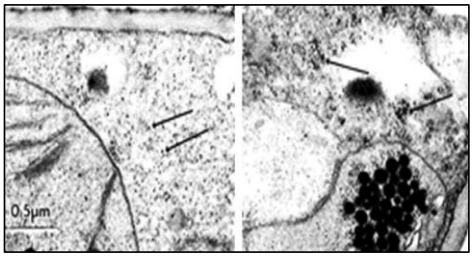

Fig. 1.15: Transmission electron micrographs of cells from desiccated and fully hydrated *Selaginella lepidophylla* plants. Free ribosomes or ribosomal subunits in the desiccated cells (LEFT) appear to have organized to form polysomes in the hydrated plant cells (RIGHT).

Eukaryotic and prokaryotic ribosomes differ in the number of RNAs and proteins in their large and small subunits, and thus in their overall size. Isolated ribosomes and their subunits can be separated based on differences in mass. Fig.1.16 shows the difference in ribosomal subunit 'size', protein and ribosomal RNA (rRNA) composition.

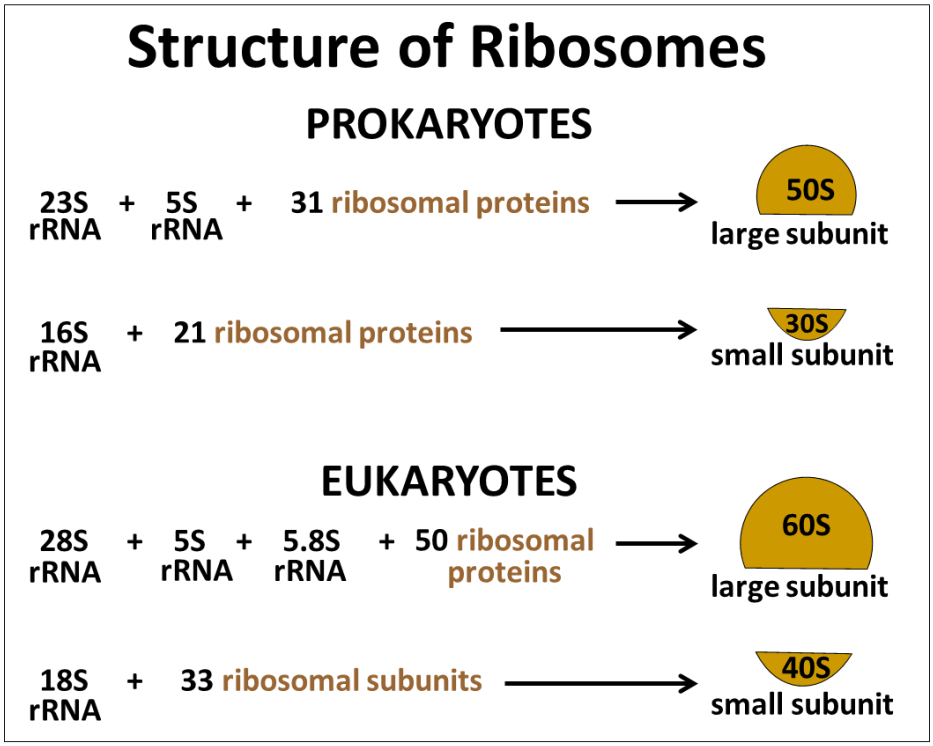

Fig. 1.16: Key differences between prokaryotic and eukaryotic ribosomes: Eukaryotic ribosomes and their subunits are larger and contain more proteins and larger ribosomal RNAs (rRNAs) than those of bacteria. The components were separated by sucrose density gradient centrifugation in which particles and macromolecules (like RNA) move through a sugar gradient at rates dependent on their mass (in effect, their size).

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

101 Ribosomes & Polysomes

1.4.3 Internal membranes and the Endomembrane System

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

Many ***vesicles*** and ***vacuoles*** in cells, including Golgi vesicles, are part of the ***endomembrane system*****.** Proteins synthesized on the ribosomes of the **RER (*rough endoplasmic reticulum*)** can enter the interior space (*lumen*) or can become part of the RER membrane itself. The synthesis of ***RER***, ***SER*** *(**smooth endoplasmic reticulum**)*, ***Golgi bodies***, ***lysosomes***, ***microbodies*** and other vesicular membranes (as well as their protein content) all begin in the RER. The RER and protein contents bud into *transport vesicles* that fuse with *Golgi Vesicles* (**G** in the electron micrograph in Fig. 1.17).

Comment [GKB4]: 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/SIM6U0Y6BxQ*. (You may need to right-click on the link and select "open" to access it). You can spend some time looking up details of endomembrane system function (OK), or answer more simply, using logic to suggest what is going on (Preferred!). Submit your answers to the *Endomembrane Traffic* DropBox no later than [insert date and time].

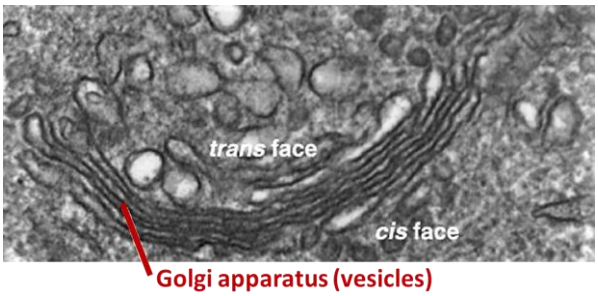

Fig. 1.17: Transmission electron micrograph of an insect cell showing Golgi bodies (G).

In moving through the endomembrane system, *packaged proteins* undergo stepwise modifications (*maturation*) before becoming biologically active (Fig.1.18, below). 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.

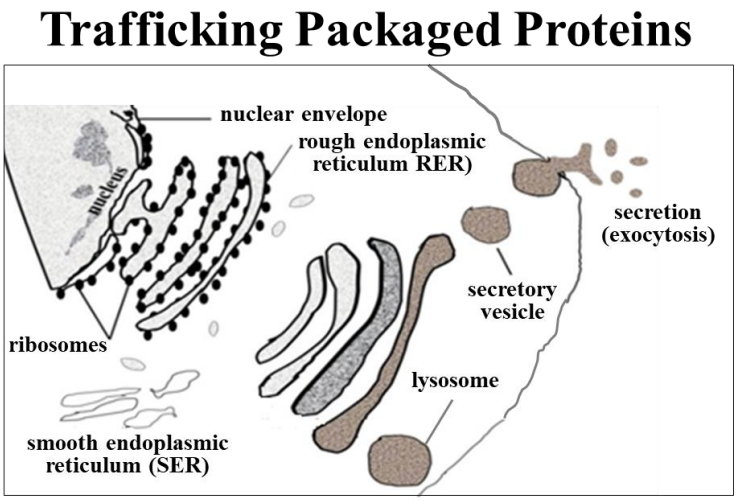

Fig. 1.18: Illustration of 'packaged' protein traffic through a cell, from the RER to organelles (e.g., lysosomes) or to the plasma membrane for exocytosis (i.e., secretion). RER and Golgi vesicles are major sites for the modification (i.e., maturation) of packaged proteins.

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 stems upright.

1.4.4 Mitochondria and Plastids

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

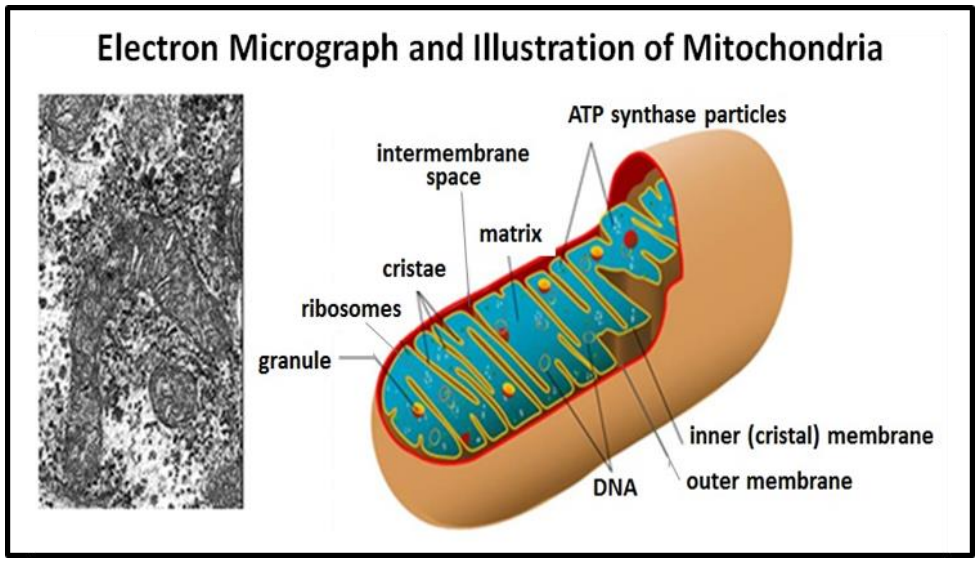

Fig. 1.19: Transmission electron micrograph (LEFT) and drawing of a mitochondrion (RIGHT).

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

Earlier, we speculated that some eukaryotic organelles could have originated within bacteria. But mitochondria most likely evolved from a complete aerobic bacterium (or proto-bacterium) that was engulfed by a primitive eukaryotic cell. The bacterium escaped destruction, becoming an ***endosymbiont*** in the host cell cytoplasm. Lynn Margulis proposed the ***Endosymbiotic Theory*** in 1967 (Margulis, L. [*Sagan, L*], *1967. On the origin of mitosing cells.* Journal of Theoretical Biology **14** (3): 225–274). Read her paper at Margulis-Endosymbiosis. She proposed that chloroplasts (one among several different ***plastids***) also started as *endosymbionts*. Both mitochondria

and the plastids of plants and some algae have their own DNA, supporting their origins as bacteria and cyanobacteria engulfed by primitive eukaryotic cells. Living at first in symbiosis with the rest of the cell, they would eventually evolve into the organelles that we are familiar with.

A handful of protozoa were discovered that lack mitochondria and other organelles. This had suggested they might share ancestry with those primitive eukaryotes that acquired mitochondria by endosymbiosis. However, since such cells contain other organelles such as *hydrogenosomes* and *mitosomes*, it is thought more likely that these species *once had, but then lost mitochondria*. Therefore, descendants of ancient eukaryotic cells missing mitochondria probably no longer exist…, if they ever existed at all! More evidence for the *Endosymbiotic Theory* is discussed elsewhere.

CHALLENGE: To see why Nick Lane favors an early endosymbiotic event where one prokaryote engulfed another to form a eukaryotic progenitor…, and to read some eye-opening challenges to orthodox thinking about life origins in general, check out http://nick-lane.net/2018/01/Lane-Mol-Frontiers.pdf. We'll look at some of Lane's ideas more closely in a later chapter.

Chloroplasts and *cyanobacteria* contain chlorophyll and use a similar photosynthetic mechanism to make glucose. Typical chloroplasts are shown (Fig. 1.20, below). The one on the right is shows a few starch granules.

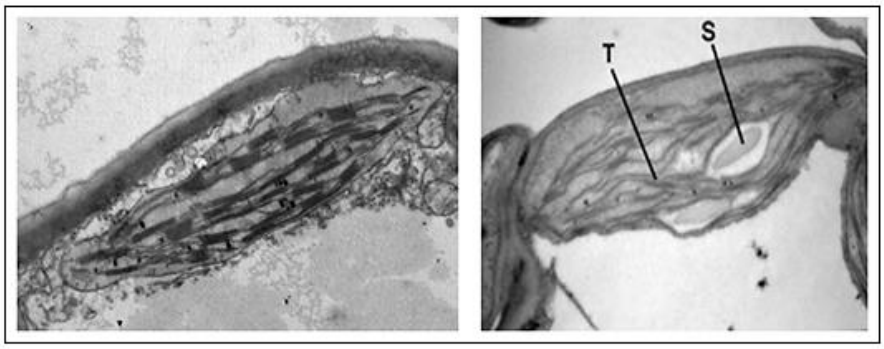

Fig. 1.20: Transmission electron micrograph of chloroplast that could have begun photosynthesizing (LEFT), and one that has photosynthesized long enough to accumulated starch granules (RIGHT). S, starch granule; T, thylakoids.

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

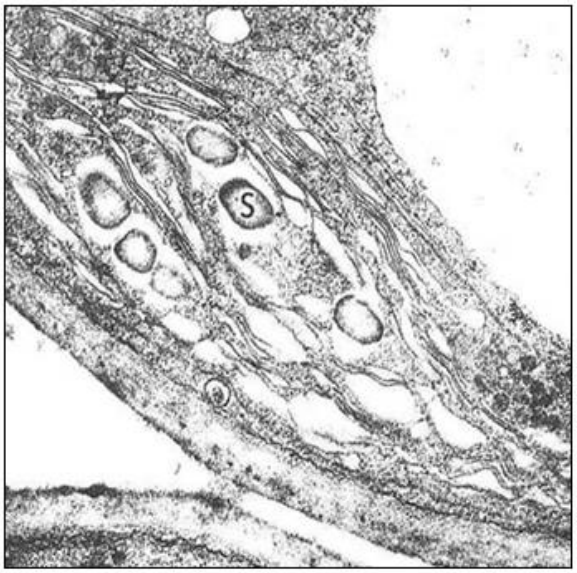

Fig. 1.21: Transmission electron micrograph of a leucoplast, a chloroplast that has become filled with starch granules (S).

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 components of this ***cytoskeleton*** are ***microfilaments***, ***intermediate filaments*** and ***microtubules.***

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

We have already noted that a prokaryotic cytoskeleton is composed in part of proteins homologous to actins and tubulins. As in a eukaryotic cytoskeleton, these bacterial proteins may play a role in maintaining or changing cell shape. On the other hand, flagellum-powered movement in bacteria relies on flagellin, a protein not found in eukaryotic cells.

A bacterial flagellum is actually a rigid hook-like structure attached to a molecular motor in the cell membrane that spins to propel the bacterium through a liquid medium. In contrast, eukaryotic *microtubules* slide past one another causing a more flexible flagellum to undulate in wave-like motions. The motion of a eukaryotic cilium is also based on sliding microtubules, but in this case causing the cilia to beat rather than undulate. Cilia are involved not only in motility, but also in feeding and sensation. The main cytoskeletal components are shown in Fig.1.22.

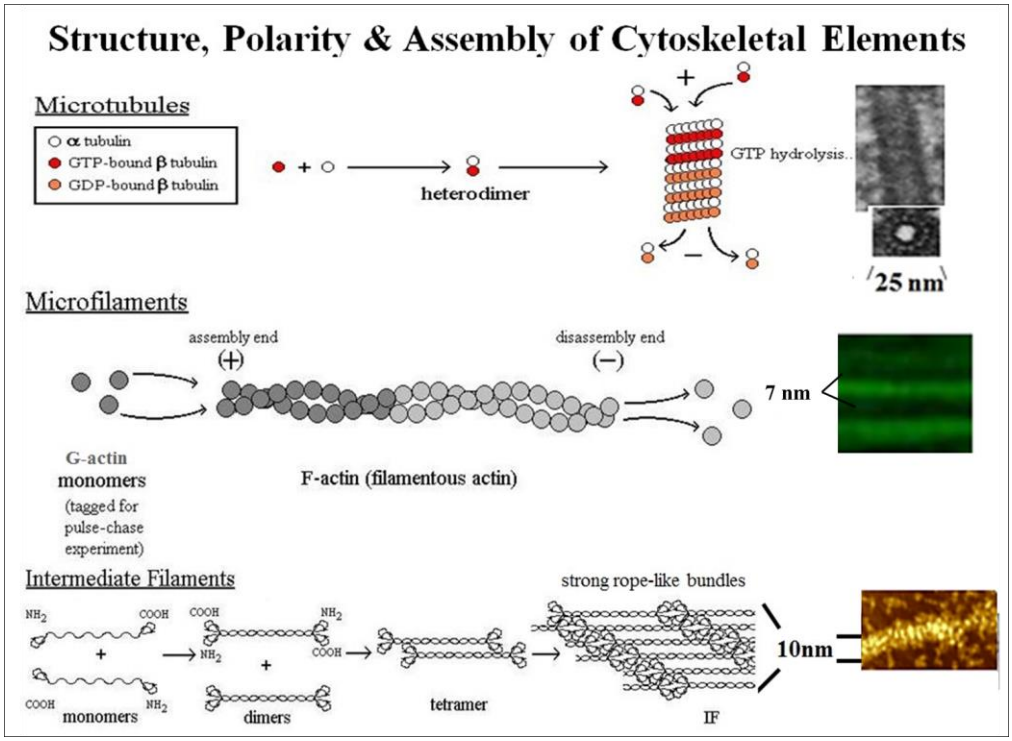

Fig. 1.22: Illustration of assembly, structure and polarity of microtubules, microfilaments and intermediate filaments alongside fluorescence micrographs made using fluorescent antibodies against isolated microtubule, microfilament and intermediate filament proteins (TOP, MIDDLE and BOTTOM, respectively).

Microtubules in eukaryotic flagella and cilia arise from a *basal body* (similar to *kinetosomes* or *centrioles*) such as the one in Fig. 1.23 below.

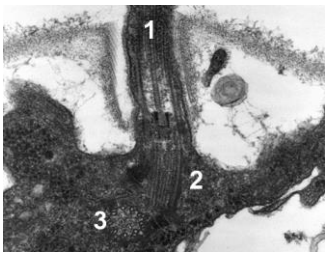

Fig. 1.23: Transmission electron micrograph showing a flagellum (#1) emerging from a basal body (2). Number 3 is another basal body, this time in cross section.

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. This ***9+2*** arrangement of microtubules is illustrated in Fig. 1.24.

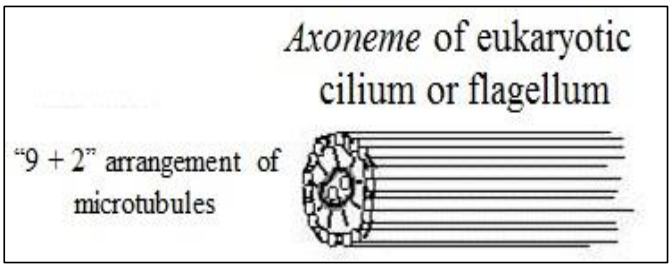

Fig. 1.24: The characteristic "9+2" arrangement of microtubules seen in cross-sections of eukaryotic cilia and flagella is maintained the *axoneme*, a structure remaining after removing the plasma membrane from isolated cilia or flagella.

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

106-2 Filaments & Tubules of the Cytoskeleton

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

1.5 How We Know the Functions of Cellular Organelles and Structures: Cell Fractionation

We can see and describe cell parts in the light or electron microscope, but we could not definitively know their function until it became possible to release them from cells and separate them from one another. This became possible with the advent of differential centrifugation (the example of the separation of ribosomes and ribosomal components was mentioned earlier). Under centrifugal force generated by a spinning centrifuge, subcellular structures separate by differences in mass. Structures that are more massive reach the bottom of the centrifuge tube before less massive ones. A cell fractionation scheme is illustrated in Fig 1.25. Biochemical analysis of the isolated cell fractions can reveal what different organelles and cellular substructures do.

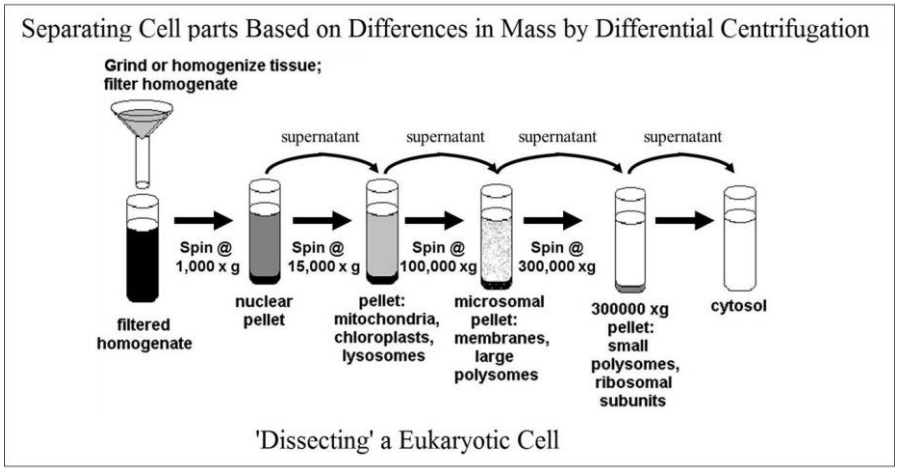

Fig. 1.25: A cell fractionation involving disruption of cells and the isolation of sub-cellular components, including organelles, ribosomes and a soluble fraction, the cytosol. Separation is achieved by successive centrifugations at different speeds (centrifugal forces) that sediment subcellular structures on the basis of mass; the lowest mass structures require the highest G-forces (fastest spin), requiring an ultracentrifuge.

107-2 Dissecting the Cell-a Cell Fractionation Scheme

Cell fractionation separates cells into their constituent parts. The first step of a cell fractionation is to break open the cells and release their contents. This can be done by physical means such as grinding in a mortar and pestle, tissue grinder or similar device, exposure to ultrasound or high pressure, or exposure to enzymes or other chemicals that can selectively degrade the plasma membrane. The next step is to isolate the subcellular organelles and particles from the cytoplasm (i.e., cytosol) by differential centrifugation. The centrifugation of broken cells at progressively higher centrifugal force separates particulate cell components based on their mass. At the end of this process, a researcher will have isolated mitochondria, chloroplasts, nuclei, ribosomes etc. After re-suspension, each pellet can be re-suspended and prepared for microscopy. Electron micrographs of several isolated subcellular fractions are shown in Fig. 1.25, below.

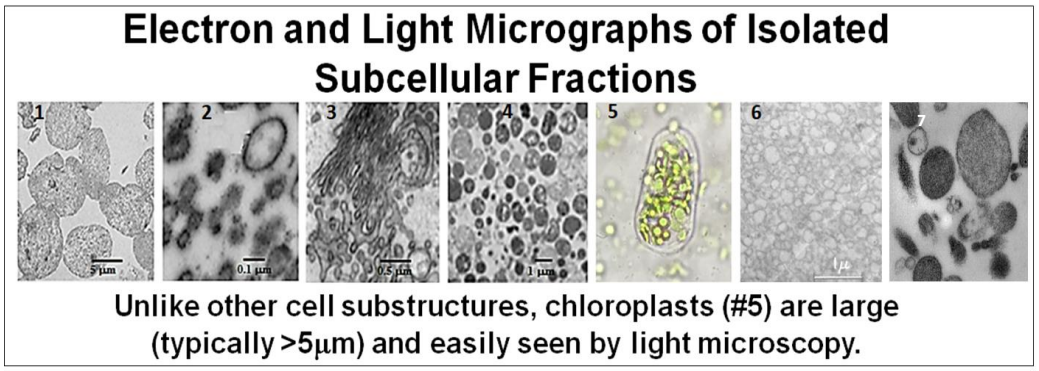

Fig. 1.26: Organelles isolated by cell fractionation from eukaryotic cells.

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 also the largest structures in a eukaryotic cell…, and indeed that's what they are! Physical separation combined with biochemical-molecular analysis of subcellular structures has revealed their basic functions and continue to reveal previously un-noticed structures and functions in cells. What biochemical tests might you do to confirm the identities of the structures shown?

Comment [GKB5]: Look at the *phase contrast* micro-graph of isolated chloroplasts in this link: http://youtu.be/oZX1H0X7xQY. (You may need to right-click on the link and select "open" to access it). In 30 words or less, state a working, testable hypothesis consistent with your suspicion that these structures are isolated chloroplasts. Remember that an hypothesis is a declarative sentence, usually stated as an "if..., then..." statement. Put your word count in parenthesis after your response and submit it to the *Chloroplast* D2L DropBox by [insert date and time].

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

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 some 3.7-4.1 billion years ago under conditions that favored the formation of the first cell, the first entity with all of the properties of life.

But couldn't those same conditions have spawned multiple cells independently, each with all of the properties of life? If so, from which of these did life, as we know it today, descend? Whether there were one or more different "first cells", evolution (a property of life) could only begin with 'that or those' cells.

115 Properties of Life

The fact that there is no evidence of cells of independent origin may reflect that they never existed. Alternatively, we can propose that the cell we call our ancestor was evolutionarily successful at the expense of other early life forms, which thus became extinct. In any event, whatever this successful ancestor may have looked like, its descendants would have evolved quite different biochemical and physiological solutions to achieving and maintaining life's properties. One of these descendants evolved the solutions we see in force in all cells and organisms alive today, including a common (*universal*) genetic code to store life's information, as well as a common mechanism for

retrieving the encoded information. Francis Crick called is commonality the "Central Dogma" of biology. That ancestral cell is called our ***Last Universal Common Ancestor***, or ***LUCA***.

116 The Universal Genetic Code **117 Origins of Life**

118 Life Origins vs Evolution

Elsewhere we consider in more detail how we think about the origins of life. For 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***, 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, while less fit individuals are culled from the population. If natural selection acting 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 diversity and complexity* at all levels of biological organization, from species to individual organisms and all the way down to biomolecules. Darwin recognized the discord his theory would generate 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."*

According to creationists, the exquisite eyes could only have formed by the intelligent design of a creator. But 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 20th century , it became increasingly clear that it is an organism's genes that are inherited, are passed down the generations, and are the basis of an organism's traits. It also became clear that Mendel had found the a genetic basis for Darwin's theory and the evolution of eyes can be explained. Over time, science and religion have found ways to co-exist but as we know, 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) supports our common ancestry with all life. These shared features date back to our LUCA! Take as an example the fact that most living things even share some early *behaviors*. Our ***biological clock*** is an adaptation to our planet's 24-hour daily cycles of light and dark that have been around since the origins of life; all organisms studied so far seem to have one!. The discovery of the genetic and molecular underpinnings of ***circadian rhythms*** (those daily cycles) earned Jeffrey C. Hall, Michael Rosbash and Michael W. Young the 2017 Nobel Prize in Medicine or Physiology (click Molecular Studies of Circadian Rhythms wins Nobel Prize to learn more)!

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

Let's look at the biochemical and genetic unity among livings things. We've already considered what happens when cells get larger 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 20th century, Albert Kluyver first recognized that cells and organisms vary in form appearance in spite of an essential biochemical unity of all organisms (see Albert Kluyver in Wikipedia). 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 or organism's genetic instructions are used (i.e., to make RNA and proteins) are regulated. Genetic variation results from random mutations. Genetic diversity arising from mutations is in turn, the basis of natural selection during evolution.


119 The Random Basis of Evolution

1.6.2 The Genome: An Organism's Complete Genetic Instructions

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

It had been known for some time that gene and protein sequencing could reveal evolutionary relationships and even familial relationships. Read about an early demonstration of such relationships based on amino acid sequence comparisons across evolutionary time in Zuckerkandl E and Pauling L. (1965) *Molecules as documents of evolutionary theory.* J. Theor. Biol. 8:357-366. It is now possible to extract DNA from fossil bones and teeth, allowing comparisons of extant and extinct species. DNA has been extracted from the fossil remains of humans, other hominids, and many animals. DNA sequencing reveals our relationship to animals (from bugs to frogs to mice to chimps…) and to Neanderthals 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.

Now in a clever twist, using what we know from gene sequences of species alive today, investigators recently *'constructed'* a genetic phylogeny suggesting the sequences of genes of some of our long-gone progenitors, including bacteria (click here to learn more: Deciphering Genomic Fossils). The comparison of these *'reconstructed'* ancestral DNA sequences suggests when photosynthetic organisms diversified and when our oxygenic planet became a reality.

Closer to home, many remains of ancestral humans have been discovered in the Americas. These promise to unlock mysteries of human settlement of the continents, but 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 discovered in the Nevada desert in the 1940s. Tests of hair and clothing fragments revealed that this *Spirit Cave mummy* was over 10,000 years old. The *Fallon Paiute-Shoshone* tribe that lives near the burial site asserted a cultural relationship to the body and requested the right to its return to the tribe in compliance with the *Native American Graves Protection and Repatriation Act*. Anthropologists 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. By the time DNA tests were allowed, the results established that the remains *was* indeed that of an ancestor to the tribe, and the *Spirit Cave mummy* was reburied with tribal rites at the beginning of 2018. To read more, see Resolving American Indian Ancestry - a 60-Year Old Controversy or The-worlds-oldest-natural-mummy-unlocks-secrets-ice-age-tribes-americas

120-2 Genomic Fossils-Molecular Evolution

CHALLENGE: Tracing ancient remains to tribal descendants continues to cause culture/science tension. See *Mungo Man, a* 42,000 year-old Australian Aboriginal, (Perrotet, T. & Smith, D.M. 2019 *The Homecoming*; Smithsonian 50: 38-49). 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 kinds of microscopy. In *Light Microscopy*, the specimen on the slide is viewed through optical glass lenses. In *Electron Microscopy*, the viewer is looking at an image on a screen created by electrons passing through or reflected from

the specimen. For a sampling of light and electron micrographs, check out this Gallery of Micrographs. Here we compare and contrast different microscopic techniques.

1.7.1 Light Microscopy

Historically one form or other of light microscopy has revealed much of what we know of cellular diversity. Check out the Drawings of Mitosis for a reminder of how eukaryotic cells divide and then check out The Optical Microscope for descriptions of different variations of light microscopy (e.g., *bright-field, dark field*, *phase-contrast*, *fluorescence*, etc.). Limits of *magnification* and *resolution* of 1200X and 2 μm, (respectively) are common to all forms of light microscopy. The main variations of light microscopy are briefly described below.

- *Bright-Field microscopy* is the most common kind of light microscopy, in which the specimen is illuminated from below. Contrast between regions of the specimen comes from the difference between light absorbed by the sample and light passing through it. Live specimens lack contrast in conventional bright-field microscopy because differences in refractive index between components of the specimen (e.g., organelles and cytoplasm in cells) diffuse the resolution of the magnified image. This is why *Bright-Field microscopy* is best suited to fixed and stained specimens.

- 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 polarized before passing through the specimen, allowing the investigator to achieve the highest contrast by rotating the plane of polarized light passing through the sample. Samples can be unfixed, unstained or even live.

- *Phase-Contrast* or *Interference microscopy* enhances contrast between parts of a specimen with higher refractive indices (e.g., cell organelles) and lower refractive indices (e.g., cytoplasm). *Phase–Contrast* microscopy optics shift the phase of the light entering the specimen from below by a half a wavelength to capture small

- differences in refractive index and thereby increase contrast. Phase–Contrast microscopy is a most cost-effective tool for examining live, unfixed and unstained specimens.

- In a *fluorescence microscope*, short wavelength, high-energy (usually UV) light is passed through a specimen that has been treated with a fluorescing chemical covalently attached to other molecules (e.g., antibodies) that fluoresces when struck by the light source. This fluorescent *tag* was chosen to recognize and bind specific molecules or structures in a cell. Thus, in *fluorescence microscopy*, the visible color of fluorescence marks the location of the target molecule/structure in the cell.

- *Confocal microscopy* is a variant of fluorescence microscopy that enables imaging through thick samples and sections. The result is often 3D-like, with much greater depth of focus than other light microscope methods. Click at Gallery of Confocal Microscopy Images to see a variety of confocal micrographs and related images; look mainly at the specimens.

- *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. There has been renewed interest in this form of light microscopy. Read more about this technique at Lattice Light Sheet Microscopy**.**

1.7.2 Electron Microscopy

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

121-2 Electron Microscopy

Some iText & VOP Key words and Terms

Actin	Eukaryotes	Nuclear envelope
Archaea	Eukaryotic flagella	Nuclear pores
Bacterial cell walls	Evolution	Nucleoid
Bacterial Flagella	Exocytosis	nucleolus
Binary fission	Extinction	Nucleus
Cell fractionation	Hypothesis	Optical microscopy
Cell theory	Inference	Plant cell walls
Chloroplasts	Intermediate filaments	Plasmid
chromatin	keratin	Progenote
Chromosomes	Kingdoms	Prokaryotes
Cilia	LUCA	Properties of life
Confocal microscopy	Lysosomes	Rough endoplasmic reticulum
Cytoplasm	Meiosis	Scanning electron microscopy
Cytoskeleton	Microbodies	Scientific method
Cytosol	Microfilaments	Secretion vesicles
Deduction	Microtubules	Smooth endoplasmic reticulum
Differential centrifugation	Mitochondria	Speciation
Diversity	Mitosis	Theory
Domains of life	Motor proteins	Tonoplast
Dynein	Mutation	Transmission electron microscopy
Endomembrane system	Natural selection	Tubulins

CHAPTER ONE QUIZ

Comment [B6]: When you feel you are ready, open your LMS and click on on the link below to open the CHAPER ONE QUIZ LINK. You may take this quiz twice; your highest score will be recorded in the gradebook.