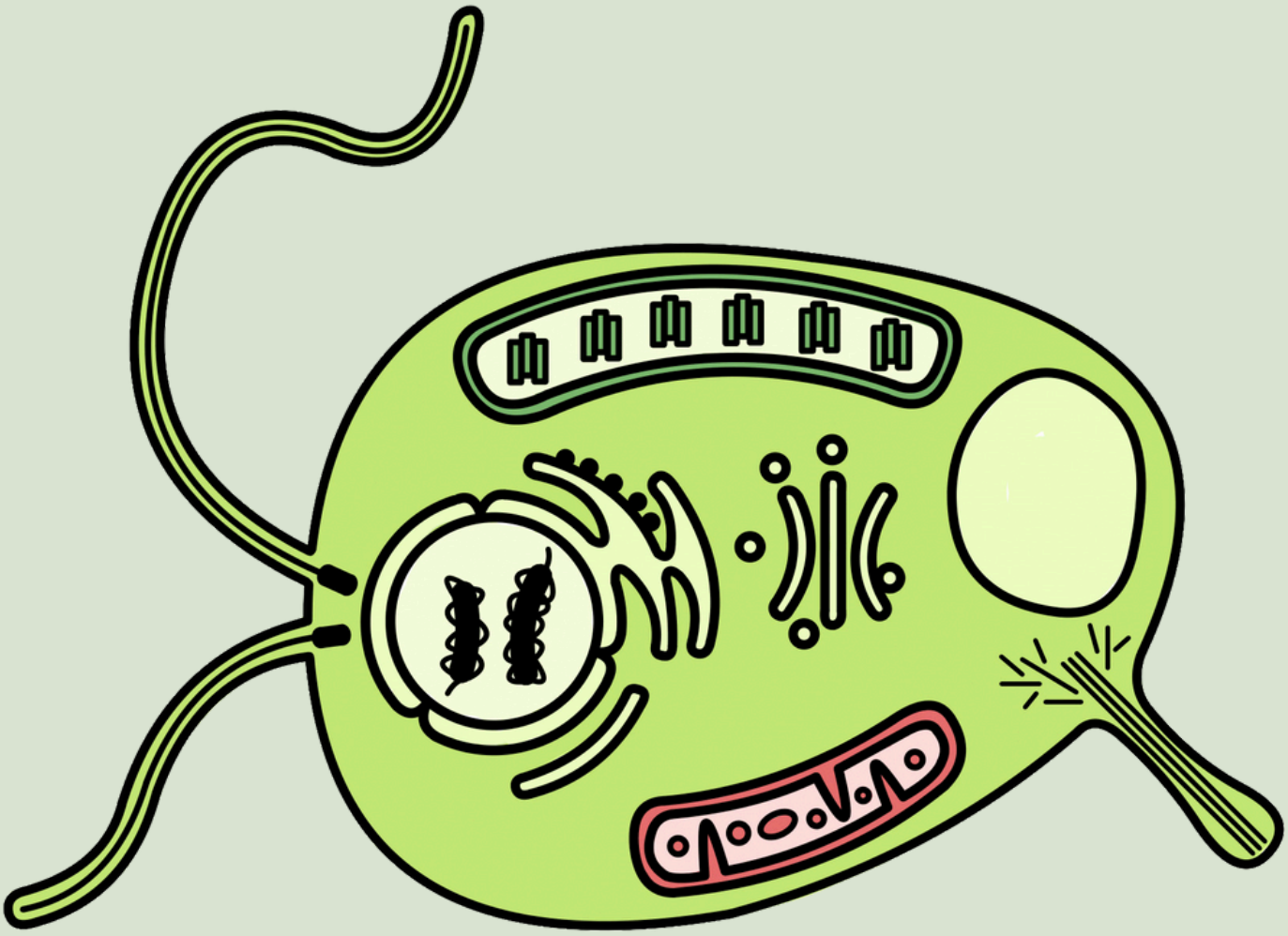

A Pocket-Sized Guide to
Protists

A BIOL203 Primer by Sirus Grames-Webb, Vittorio Boscaro, Patrick Keeling

Version 2.1; July 8th, 2025

Preface

This primer was ultimately inspired by Sirus Grames-Webb's experience taking notes while a student in Biol203. The idea to develop course notes into a formal primer for the subject and a core resource for the course eventually grew to become a multi-year challenge spread over many drafts and hundreds of hours of work. The primer has been checked and edited by a variety of experts, and it is fully compatible with the contents of Biol203. However, we want to stress that **it is NOT a study guide and is especially NOT a replacement for lectures**. Rather, it is a supplement to your experience in the class and to reinforce concepts and contents covered by lecture material.

It is important to **attend all the lectures and take your own notes**. This primer does not cover all the material in the class, and does not attempt to join different concepts from different sections. It is organized differently from the class and reading the primer cover to cover might help you understand lectures, but it will not substitute for them. **There are many topics and details covered in class, that are not in the manual**, and it is possible that some things included here might not be mentioned in the course. As always, **everything** done in class – and **only** things done in class – will be included in midterms, quizzes, and the final exam, regardless of the content of this manual.

The primer has been designed instead to help you fill in any gaps and clarify concepts or facts that remain fuzzy after lectures. You can search for terms or topics, to hone in on sections of the primer that you can then read thoroughly to correct misconceptions or fill in missing pieces. If used that way, as an aid to listening to lectures and taking your own notes, this resource should help you demystify concepts and clarify facts, and ultimately do well in the class, so we hope you use it thoroughly but do not over-rely on it.

Acknowledgments

We would like to thank members of the Keeling lab who provided feedback on this primer, in particular Anna Cho, Elizabeth Cooney, Daniele Giannotti, Gordon Lax, Mahara Mtawali, Ina Na, Noriko Okamoto, Lilith South, and Vasily Zlatogursky, who all provided comments on chapters closest to their research expertise. This project was partially supported by a Skylight Development Grant to PJK from the UBC Faculty of Science.

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Introductory Chapters

1 An Introduction to Protists

In many high school biology classes, students are taught that eukaryotes can be divided into three major groups: plants, animals, and fungi. Sometimes, a teacher may mention a fourth more obscure group, protists, but they are rarely discussed more than as a brief cameo in an introductory lesson. Also, protists are usually implied to be a "fourth lineage", of equal (or lower) standing than the three "major" groups.

In fact, the term "protists" refers to many, many lineages of eukaryotes (whereas plants, fungi, and animals each represent a single "twig" in the tree of eukaryotes) with immense biodiversity and ecological importance; many cause diseases in humans and other animals, or otherwise directly impact human society and economy, and a great number of them play essential roles in global ecosystems, in a way that make the Earth habitable for humans and other animals. Because the word "protist" is unfamiliar to most people, even extremely important or famous protists are more commonly referred to by other terms, such as "microbes", or "parasites" (when they are parasitic), or "algae" (when they are photosynthetic).

Protist (definition): A eukaryote that is not a plant, animal or fungus.

Pinning down an exact definition of protists is surprisingly difficult. This is because they are so diverse, and unlike animals, fungi, and plants they are not a single lineage in the tree of life. Of the few existing definitions, we will use the one above, which is still an odd one as it defines protists partly by what they are (eukaryotes), but partly by what they are not (plants, animals, or fungi).

Many plants, animals, and fungi are large, easily observed, and intuitively familiar to us, but most protists are microscopic and fly under the radar of our senses. Yet protists are found almost everywhere; lakes, streams, oceans, and soils are filled with these (usually) tiny creatures, and many live symbiotically inside other organisms. Autotrophic (photosynthetic) protists, commonly called algae, fix billions of tons of CO_2 each year, providing much of the primary production on which the majority of aquatic life depends – not to mention, much of the atmospheric oxygen we breath. Heterotrophic protists feast on bacteria, detritus, or other protists, regulating bacterial and algal populations and making nutrients and biomass available for other trophic levels. Many protists can be parasitic, causing diseases like malaria, beaver fever, sleeping sickness, and toxoplasmosis, or live mutualistically with other organisms, notably in the guts of many animals, or as photosymbionts, as in corals.

Some unicellular protists can be relatively simple, but others are amazingly complex, with cells ranging in size over orders of magnitude and exhibiting a wide variety of anatomical blueprints. Protist cells can harbour complex gun-like structures that fire toxic projectiles to capture prey or defend against predators, can have "mouths" and digestive tracts to eat other cells, armour or spines for protection, and complex behaviours for moving, eating, or sometimes for more mysterious purposes. In most protists, all of this takes place within a single cell! There are however also also colonial and multicellular protists – most of the seaweeds you know from the shores of BC, including giant kelps, are not actually plants, but large multicellular protists.

Collectively, protists make up most of the diversity of the eukaryotic tree of life – if animals, fungi, and plants are each a "kingdom", then there are dozens of protist kingdoms that are as old or older, and just as diverse. To understand the sheer breadth of this diversity, a tree of eukaryotes is shown in the next page, that outlines the major groups and subgroups. As an exercise, try to find plants, animals, and fungi on the tree; they don't stand out as much as you might think.

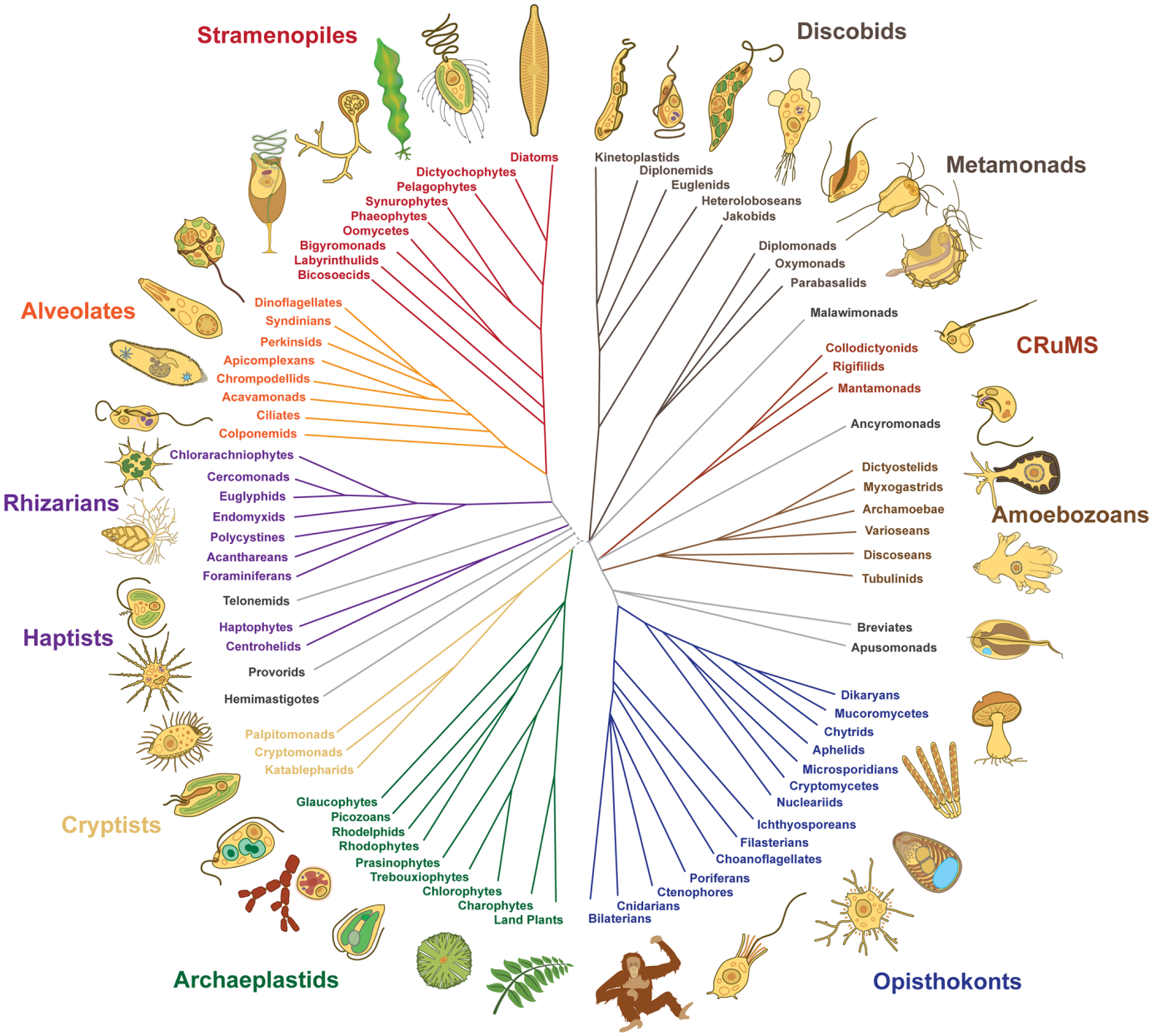


Figure 1: The Tree of Eukaryotes Showing Major Subgroups
Keeling & Eglit, PLoS Biol., 2022. (https://doi.org/10.1371/journal. pbio.3002395)

2 The Domains of Cellular Life

2.1 Overview

Cellular organisms can be divided into three major groups, or "domains": bacteria, archaea, and eukaryotes. Almost all bacteria and archaea are single-celled; bacteria are the most common life forms on the planet and their metabolic diversity is unparalleled, while archaea are well-known for their ability to thrive in extreme environments (although by no means all archaea are extremophiles). Eukaryotes can be unicellular or multicellular and are most commonly defined by the presence of a nucleus, but are better characterized by two larger and more comprehensive cellular systems: the cytoskeleton and the endomembrane system, the latter of which actually includes the nucleus in addition to many other organelles. Eukaryotes also possess organelles that originated by endosymbiosis, like mitochondria and (in some lineages) plastids. This chapter illustrates the unique characteristics of each domain, their similarities and differences, and how they express genetic information (bear in mind that, due to the huge variability in each domain, most of what follows is a simplification and there are exceptions to almost every statement).

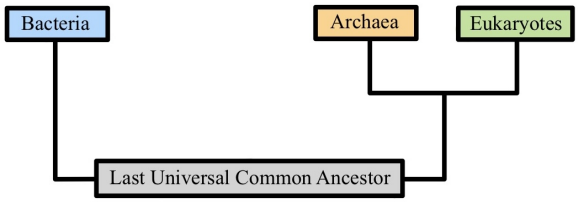


Figure 2: The Universal Tree of Life

2.2 Introduction to the Information Flow in the Cell

All cellular life uses proteins as building blocks, for chemical signalling, enzymatic activity, and many other functions. Proteins are polymers of amino acids arranged in a chain in a specific order, which are then folded into a precise three-dimensional structure. The information for these amino acid sequences is stored in DNA regions called genes, which are read, or "transcribed", by enzymes called RNA polymerases to make messenger RNAs (mRNAs), which are in turn read, or "translated", by large complexes called ribosomes that produce the corresponding proteins. A ribosome is composed of a few strands of ribosomal RNA (rRNA) complexed with dozens of proteins, and has two parts, called the small subunit (SSU) and large subunit (LSU).

Both transcription and translation rely on particularly complex processes for initiation, since initiation is often a point at which gene expression can be controlled by the cell. Initiation of transcription requires getting the RNA polymerase (or RNA pol) to bind to the right place on the DNA, typically a region upstream of a gene called the promoter. In the case of translation, initiation requires the ribosome to attach to an mRNA, find the three nucleotides where the transcribed gene begins coding the protein (the start codon), and then read the message by adding one specific amino acid to a growing polypeptide for every three-nucleotide codon in the correct frame. All cellular life requires these processes to survive, but the three domains have evolved somewhat different

strategies for each. These are interesting examples of just how diverse life is – indeed, the deeper you dig into the biology of the three domains of life, the more differences you find.

2.3 An Overview of Bacteria

Bacteria are by far the most abundant organisms on Earth, with roughly 5×10^{30} individuals alive today. Bacteria are highly studied because they cause many microbe-related diseases, have huge economic impacts on the food industry, and are deeply important ecologically in terrestrial and aquatic ecosystems, especially as consumers of organic compounds, primary producers, and sources of food for larger organisms. Most average bacteria are smaller than most eukaryotic organisms, usually measuring 1-3 μm in length, and are structurally relatively simple; however, bacteria commonly rely on metabolic processes that cannot be found in other domains. They feed mostly osmotrophically by secreting digestive enzymes near their food source and absorbing the broken-down nutrients via transporters in their membranes.

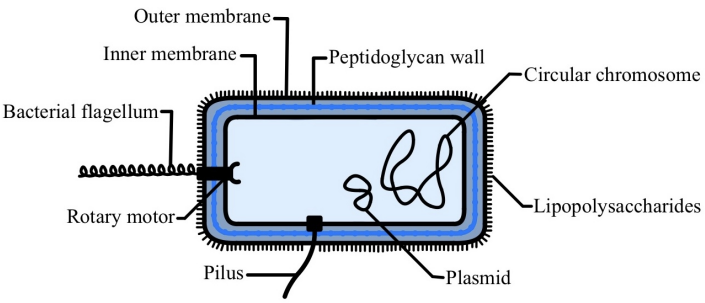


Figure 3: A Bacterial Cell

Most bacterial cells are surrounded by two membranes with a cell wall between them, which is made from a complex compound called peptidoglycan (or murein). Bacteria with two membranes are sometimes called Gram-negative for historical reasons, as opposed to the Gram-positive bacteria, which have a single membrane (the inner one) and a thicker peptidoglycan wall surrounding it. Gram-positive bacteria are less common and derived, meaning that they do not represent the ancestral state for bacteria. Many bacteria swim using a bacterial flagellum, which has a completely different structure, composition, origin, and motility mechanism from the eukaryotic flagellum, despite the two sharing the same name. A bacterial flagellum is proteinaceous, rigid, and coiled, and it twirls using a rotary motor embedded in the inner membrane.

Bacteria do not have an endomembrane system – for example, they don't have a nucleus. Their chromosomes are typically circular and sit directly in the cytoplasm. Bacteria also do not have a cytoskeleton like that of eukaryotes, and rely mostly on their wall for structural integrity.

2.4 Transcription in Bacteria

Because bacterial chromosomes are in the cytoplasm, transcription and translation happen simultaneously. Bacteria initiate transcription when a protein called the sigma (σ) factor binds to the RNA pol, allowing the complex to recognize and bind to promoter sequences approximately 35 and 10 base pairs upstream of where transcription begins (unimaginatively called the "–35" and "–10" regions). RNA pol then catalyzes the formation of an mRNA transcript complementary to one of

the DNA strands until it reaches a terminator sequence. Bacterial mRNA is often polycistronic, meaning a single transcript contains multiple protein-coding regions. The term "operon" is often used to describe a polycistronic transcript coding for proteins with related functions, which is quite common in bacteria and allows the cell to coordinate control over the expression of functionally related proteins.

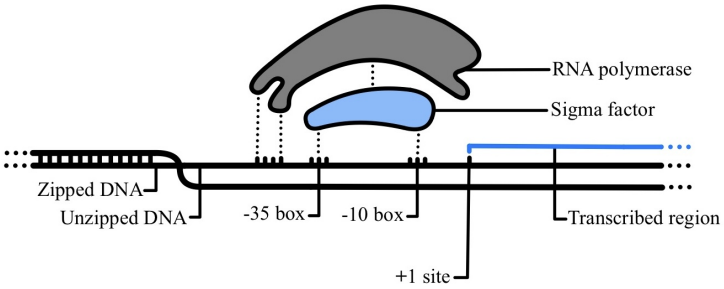


Figure 4: Transcription in Bacteria

2.5 Translation in Bacteria

Translation is initiated in bacteria by base-pairing between the rRNA in the ribosome small subunit and a region of the mRNA called the Shine-Dalgarno (SD) sequence, upstream of the start codon. In polycistronic mRNAs, there will be an SD sequence upstream of each transcribed gene, so ribosomes can bind to and initiate translation at multiple sites along one transcript. Because bacteria do not have a nucleus, mRNA is transcribed and translated simultaneously: as soon as a transcript begins to form, ribosomes will bind to it and begin translation.

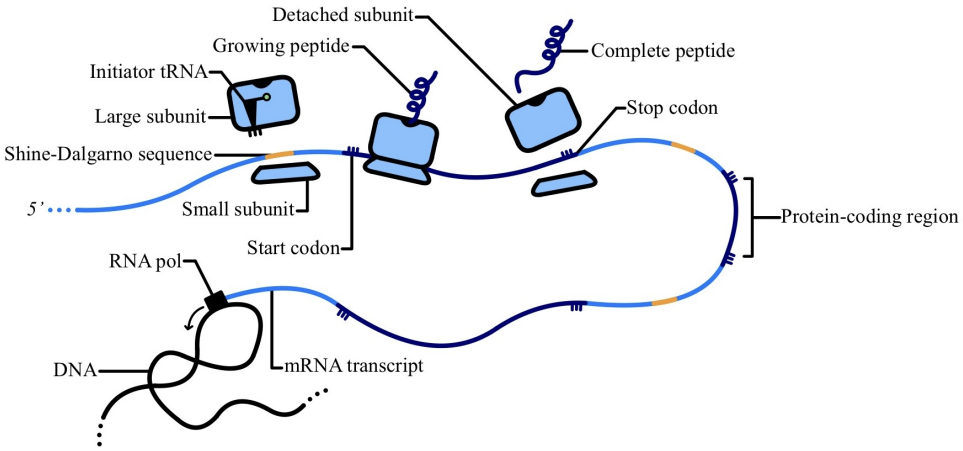


Figure 5: The Flow of Information in Bacteria

2.6 An Overview of Eukaryotes: Cellular Complexity Based on the Cytoskeleton and Endomembrane Systems

While bacteria exhibit an astounding range of metabolic pathways and archaea can occupy the most extreme environments, eukaryotes' specialty lies in structural complexity and the behaviours this allows. One of the key features that distinguishes eukaryotes is phagocytosis, the ability of a cell to ingest large particles. While some eukaryotes feed osmotrophically, most heterotrophic eukaryotes actively engulf their food, for example prey cells, enveloping it with their plasma membrane and sequestering it into specialized compartments for digestion. This process has allowed eukaryotic cells to bypass the diffusion barrier problem and grow much larger than most bacteria and archaea. Because bacteria and archaea rely on the diffusion and/or transport of nutrients across their membrane(s), and since volumes increase faster than surface areas for most common cell shapes, the larger they are, the less effective diffusion and membrane transport become. Phagocytosis overcomes this limitation by bringing packages of concentrated nutrients (e.g., prey) into the cell.

This is all possible because eukaryotes have evolved two major systems in their cell that impact almost every cellular function: the endomembrane and cytoskeleton systems. Eukaryotic cells are distinguished by possessing separate subcellular compartments called organelles, which are structures within the cell with specialized functions. A few membrane-bound organelles, like mitochondria and plastids, are derived from endosymbiotic events, as we will see in a later chapter, but most (nucleus, endoplasmic reticulum, Golgi apparatus, lysosomes, ...) are part of the endomembrane system. The endomembrane system is a collection of internal membranes within the cell, which are able to fuse and exchange material with each other and the plasma membrane in a highly dynamic and regulated fashion. The endomembrane system allows eukaryotes to efficiently sort and compartmentalize almost everything inside a cell: storing food in food vacuoles, sequestering digestive enzymes into lysosomes, allowing controlled entry of materials in and out of the cell using vesicles, and separating proteins destined for modification or secretion in the endoplasmic reticulum (ER) and Golgi.

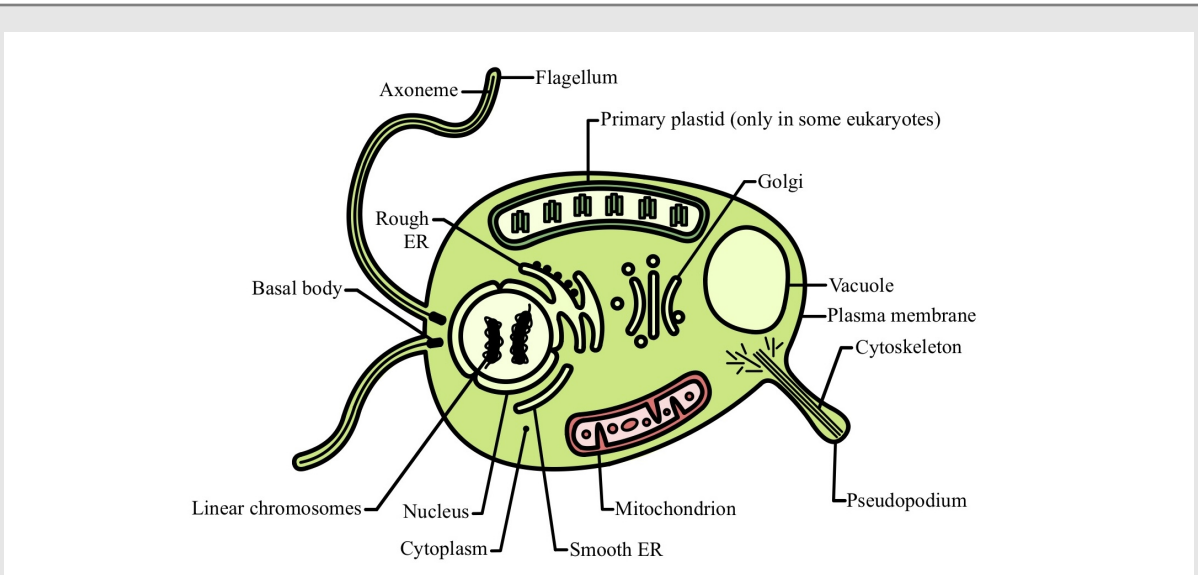


Figure 6: A Eukaryotic Cell

The nucleus is the part of the endomembrane system enveloping the cell's DNA: eukaryotic DNA is packed tightly by proteins (chiefly histones) and wrapped in a sheet of endomembrane that folds back on itself, forming two stacked lipid bilayers. These bilayers constitute the nuclear envelope, which is supported by the cytoskeleton and is continuous with the ER. The nuclear envelope has pores, corresponding to the regions where it folds in on itself, that allow regulated transport of materials in and out of the nucleus, including mRNA transcripts and ribosomal subunits (which operate in the cytoplasm but are assembled in a specialized region of the nucleus, the nucleolus).

This endomembrane system works closely with the other major system that distinguishes eukaryotic cells, the cytoskeleton. The cytoskeleton is a network of filamentous protein polymers that exist in a constant and regulated polymerization/depolymerization dynamic, and that associate with specific molecular motor proteins that allow the cell to either move cytoskeletal filaments around, or move other material along them. There are two main kinds of cytoskeletal polymers, present (in different relative amounts) in all eukaryotes: microtubules and microfilaments.

Microfilaments are built from monomers of actin, which bind together forming two strands that twist into a tight double helix. Microfilaments are described as having a + end and a - end, preferentially polymerizing at the + end and depolymerizing at similar rates at both ends. Microfilaments are associated with the motor protein myosin, which consumes ATP to "walk" along microfilaments. Microfilaments have various functions, including the formation of contractile rings during cytokinesis and certain forms of cellular motility like amoeboid locomotion and some variants of gliding.

Microtubules are larger and less flexible than microfilaments. Each microtubule is composed of 13 parallel protofilaments forming a cylindrical, hollow tube, with each protofilament built from repeating units of tubulin heterodimers (each dimer is formed by one alpha-tubulin and one beta-tubulin molecule). Microtubules also have a + and a – end, and preferentially polymerize and depolymerize at the + end. On their – end, microtubules are in contact with large protein complexes called microtubule organizing centers (MTOCs) that regulate their polymerization and order their direction. Two common types of MTOCs are basal bodies, which support flagella, and centrosomes, which organize the spindle fibres during mitosis and meiosis (the "centrioles" often mentioned in cell biology are conspicuous components of one type of centrosome found in animals and a few other groups, and are in fact homologous to basal bodies).

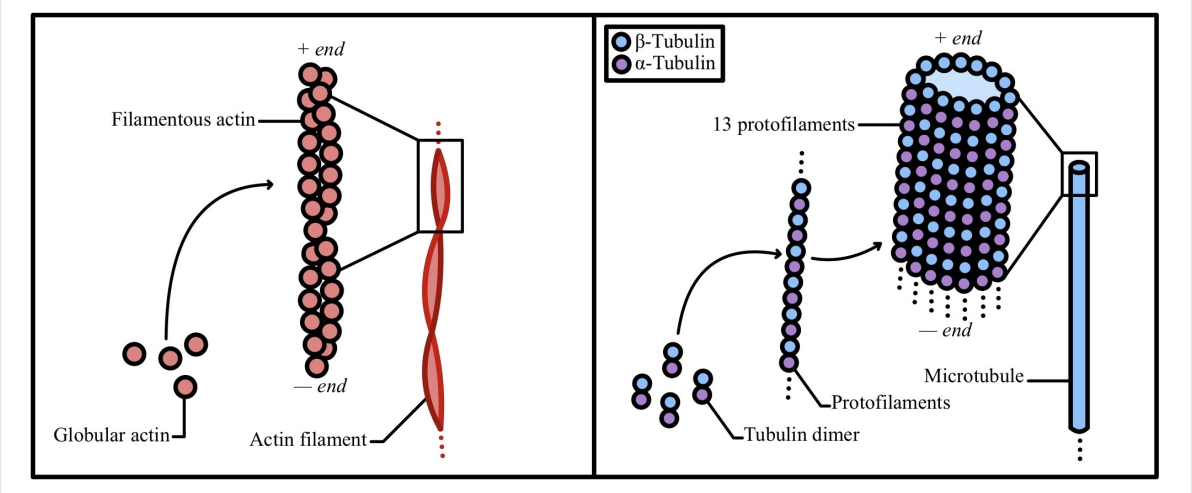


Figure 7: Microfilaments and Microtubules

Eukaryotic flagella are cellular projections often used to swim, feed, and sense the environment. They are actually intracellular structures, entirely bounded by the plasma membrane, unlike bacterial flagella and archaella, which are rigid structures made entirely of proteins, situated outside of the plasma membrane. The main component of a eukaryotic flagellum is the axoneme, a microtubule-based structure which grows from a basal body. Microtubules in the axoneme are organized in a so-called "9+2" arrangement: nine microtubule pairs – each with one complete and one incomplete microtubule – surrounding two unpaired, central microtubules. Basal bodies are made of microtubules organized in a "9+0" arrangement: nine microtubule triplets – each with one complete and two incomplete microtubules – with no central microtubules. The axoneme and basal body are separated by a more variable region called the transition zone, where the two central microtubules of the axoneme originate; the peripheral microtubules of the axoneme are continuous with two of the three microtubules in each basal body triplet.

While the basal body is the essential organizing center (the MTOC) of the flagellum, it is absolutely not sufficient to provide anchoring for such a large, often fast-beating structure by itself. All basal bodies are held in place by complex, extensive cytoskeletal elements called flagellar roots, which stretch around the margin of the cell, often right below the plasma membrane, and vary massively in arrangement and composition in different groups of eukaryotes.

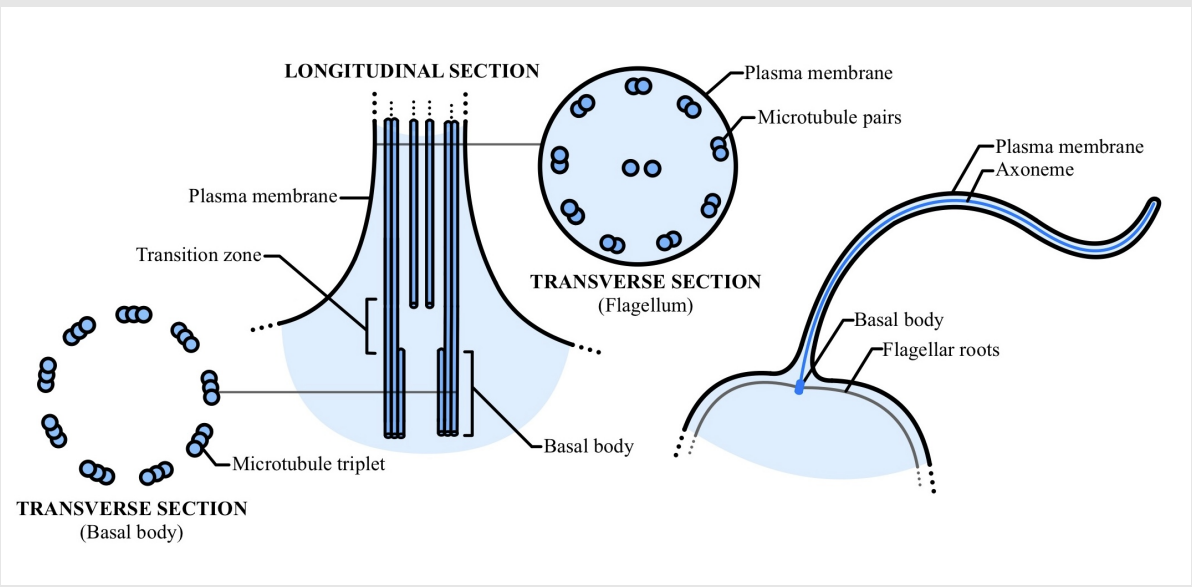


Figure 8: Eukaryotic Flagella

2.7 Transcription and mRNA Processing in Eukaryotes

Eukaryotic transcription happens in the nucleus, isolated from ribosomes, meaning transcription and translation can never happen simultaneously. Eukaryotes use a promoter region called the TATA box, which is recognized and bound to by a transcription factor – specifically, the TATA-binding protein (TBP) subunit of the larger TFIID complex. This first transcription factor is recognized by a second, then a third, a fourth, and so on until a very large aggregate of different transcription factors has clumped around the TATA box. This complex (and not the TATA-box itself) is recognized by RNA pol II (one of several eukaryotic RNA polymerases) which binds to the DNA and slides to the +1 site where it initiates transcription.

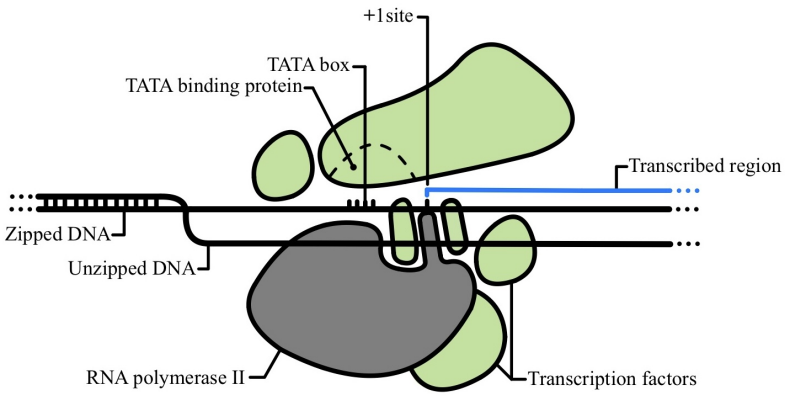


Figure 9: Transcription in Eukaryotes

Once transcribed, eukaryotic mRNAs must undergo further modifications before they leave the nucleus. Nuclear enzymes trim the 3' end and add a chain of adenines called the poly-A tail. Different enzymes also add a modified guanosine nucleotide to the 5' end, called the 5' cap. These modifications, which are not present in bacteria and archaea, preserve the transcript from degradation and help initiate translation (see below).

Many eukaryotic genes also have sequences that are not part of the information used to build a protein. These sequences are called introns, while the surrounding coding regions of the gene are called exons. Transcripts containing introns must undergo additional processing to precisely remove these introns before they are translated. Intron removal is conducted by intranuclear protein-RNA complexes called spliceosomes. Here is a step-wise walkthrough of a typical spliceosomal mRNA splicing:

1. Introns have a guanine-uracil (GU) sequence at their 5' end, an adenine-guanine (AG) sequence at their 3' end, and a key adenine (A) somewhere between the two, called the "branching point". In a reaction catalyzed by the spliceosome, the branching point adenine nucleotide attacks the guanine nucleotide in the GU at the 5' splice site in a reaction called transesterification, cleaving the 5' end of the intron from the 3' end of the preceding exon. The branching point nucleotide is now covalently bound to the splice site guanine nucleotide in the process, while still being bound to the preceding and following nucleotides in the intron, in a very unusual configuration.

2. In a second transesterification reaction, the exposed last nucleotide of the upstream exon then attacks the nucleotide after the guanine in the AG at the 3' splice site (the first nucleotide of the downstream exon), which results in the two exons being covalently bound and the intron being "spliced out" of the mRNA.

3. Once this process is complete for all the introns in a gene, the exons are arranged consecutively and each intron is left in a lasso-like structure called a lariat, which is degraded. The mRNA transcript is now readable.

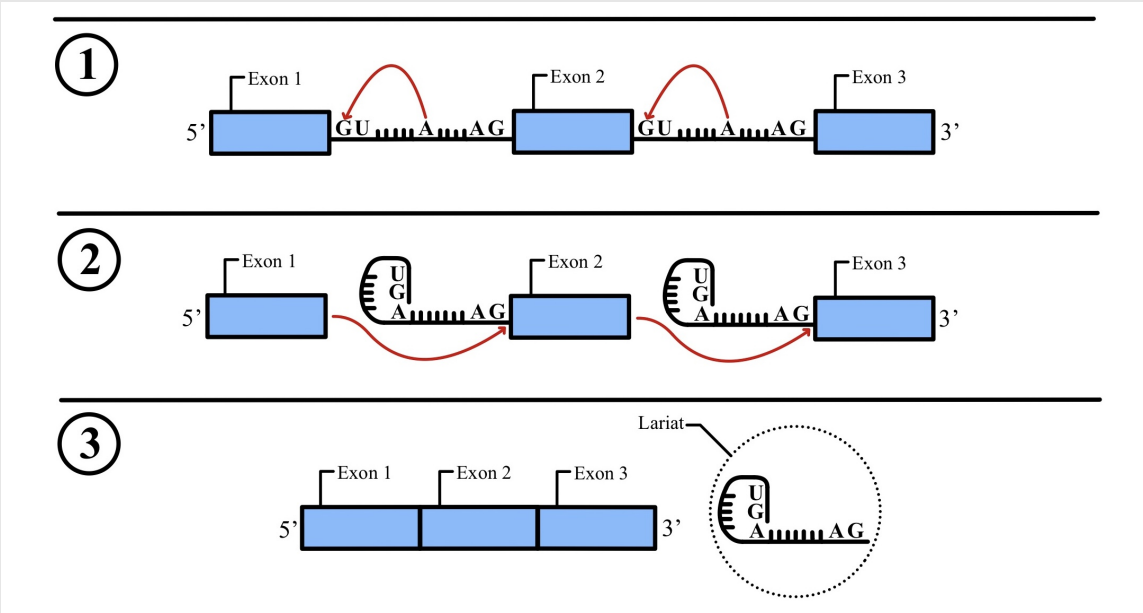


Figure 10: Mechanism of Spliceosomal mRNA Splicing

2.8 Translation in Eukaryotes

Processed, mature mRNA is exported from the nucleus via a nuclear pore. While bacteria rely on Shine-Dalgarno sequences to recruit and position ribosomes on mRNAs, eukaryotic ribosomes use the 5' cap to bind to mRNA, and then "scan" (sliding over the mRNA molecule) for the first start codon they can find. This is a significant difference because it means that there can only be one initiation site per transcript: eukaryotic ribosomes cannot translate polycistronic mRNAs. Translation then proceeds somewhat similarly to that in bacteria, although many more translation factors are involved.

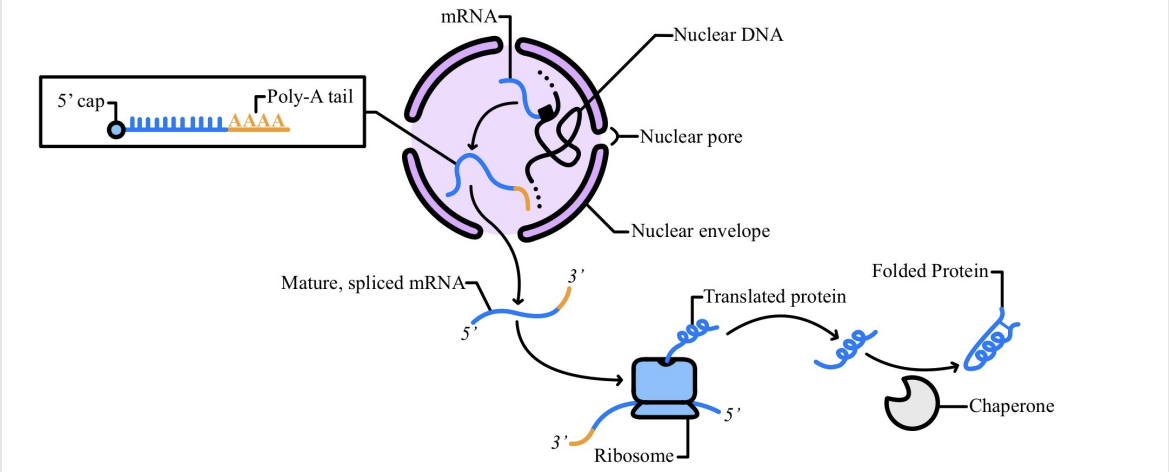


Figure 11: The Flow of Information in Eukaryotes

Another wrinkle in eukaryotic protein expression comes from the fact that eukaryotes have to ensure each protein is not only expressed correctly and at the right level, but also sent to the correct cellular compartment. Proteins destined for compartments other than the cytoplasm are described as being "targeted" to those specific compartments. This is a feature of the endomembrane system. Here is a stepwise description of one kind of protein targeting, sorting, and processing, typical of secreted proteins:

1. A gene coding for a secreted protein is transcribed into mRNA in the nucleus. The mRNA is processed like any other, but the 5' end of the gene's coding region encodes a short amino acid sequence called a signal peptide that will dictate where the protein ends up. The mRNA transcript is processed and released from the nucleus, like normal.

2. The mRNA binds a ribosome in the cytoplasm, and translation initiates in the usual way, but then quickly stops after the signal peptide is completed. That is because a cytoplasmic complex called the signal recognition particle (SRP) binds to the signal peptide, interrupting translation, and guides the ribosome to the rough endoplasmic reticulum.

3. The ribosome and SRP lock onto the membrane of endoplasmic reticulum, the signal peptide is inserted into a channel in the ER membrane, and the SRP is released, allowing translation to resume. But now the protein is simultaneously being polymerized while passing through the ER membrane into the ER lumen. Enzymes inside the ER lumen cleave the signal peptide and degrade it, before translation is even complete.

4. The translated protein is folded by chaperonins in the ER lumen, and it is then exported from the ER inside a vesicle.

5. The protein reaches the Golgi, where it is typically glycosylated, further sorted, and packaged in a vesicle that will eventually fuse with the plasma membrane, releasing the protein either onto the surface of the cell or into the surrounding medium.

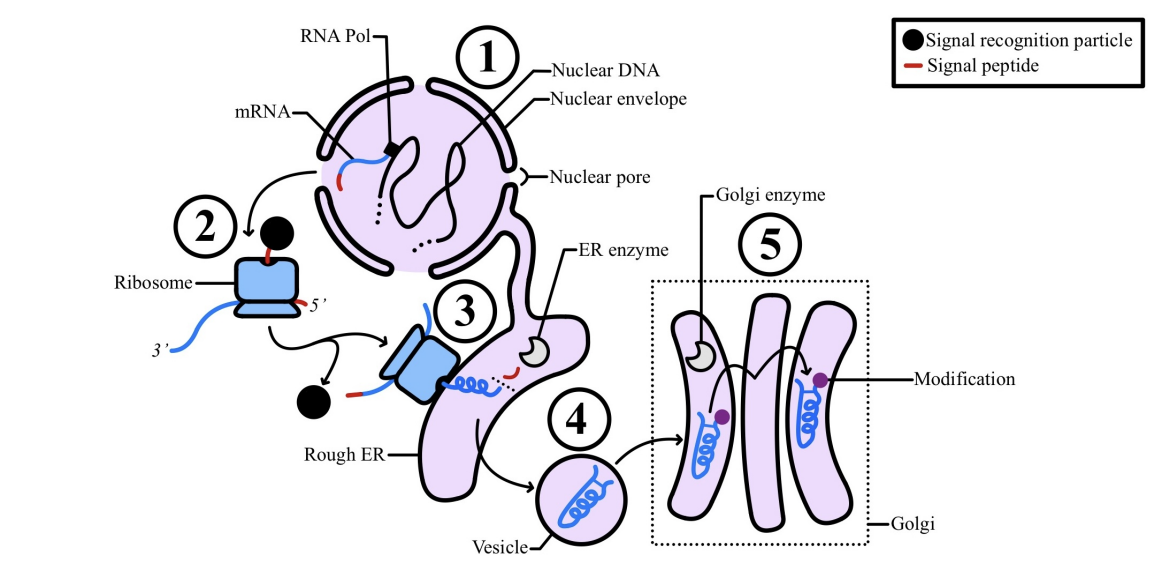


Figure 12: Protein Processing by the Endomembrane System

2.9 Archaea

Archaea are the least studied domain of life, but they are important microorganisms that play a role in global carbon and nitrogen cycles, and are common in a variety of ecosystems, although their exact ecological roles are not always well understood. Many archaea, especially among those that are best known, are extremophiles, living in thermal vents, hot springs, and environments with extreme acidity or salinity. Archaea have approximately the same size range as bacteria, and they also feed osmotrophically. They have circular chromosomes and lack both an endomembrane system and a true cytoskeleton, making them appear more similar to bacteria than eukaryotes (both are often described as "prokaryotes") – although, critically, they have a single plasma membrane and no peptidoglycan wall, so are structurally quite different from bacteria.

Archaea are actually more closely related to eukaryotes than they are to bacteria. One recently discovered group called the Lokiarcheaota appears to be especially closely related to eukaryotes – indeed, Loki archaea are closer to eukaryotes than to other archaea, so one could argue that they should then not be called archaea... but this is a classification issue. The similarities between archaea and eukaryotes emerge not from their structural features, but in their molecular biology, and specifically in the flow of information. Archaea package DNA with histones, use TATA box promoters, and have a eukaryote-like RNA polymerase that docks not on the promoter but rather on a cluster of transcription factors, although fewer than those found in eukaryotes. But they also have characteristics in common with bacteria, for instance polycistronic mRNAs with Shine-Dalgarno sequences, no 5' cap or poly-A tail, and no spliceosomes or spliceosomal introns. Since there is no nucleus, transcription and translation co-occur in archaea, just like they do in bacteria.

Of course, archaea have unique features too, shared with neither bacteria nor eukaryotes. For example, they often have a cell wall called the S-layer made from membrane proteins, and a unique locomotive apparatus called the archaellum, which is superficially similar to the bacterial flagellum, but is made of completely different proteins. But one of the most puzzling and important features that distinguish archaea is the nature of their membrane phospholipids. Archaea make their plasma membrane from isoprenoids ether-linked to glycerol-1-phosphate, whereas the membrane phospholipids in both bacteria and eukaryotes are made of fatty acids ester-linked to glycerol-3-phosphate. This is a major difference in how the cell is constructed, requiring different enzymes, exhibiting different chiralities, and conferring very different properties to their membranes. Furthermore, although in most archaea the phospholipids are organized in a typical bilayer, in some the isoprenoid tails of phospholipids on the two layers are covalently bound, so the plasma membrane remains polarized but it is not strictly speaking a lipid bilayer but rather a less fluid bi-polar monolayer.

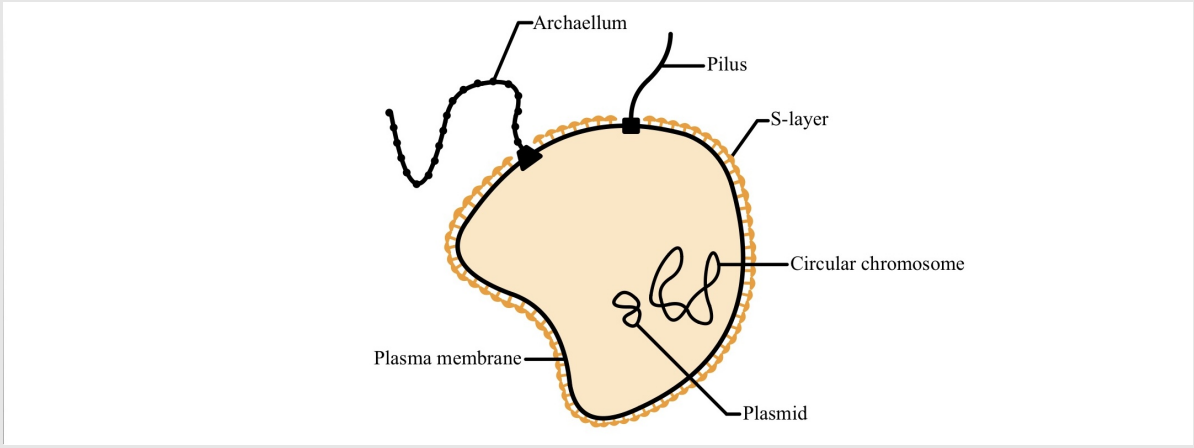


Figure 13: An Archaeal Cell

3 Mitochondria

3.1 Overview

Mitochondria are often described as the powerhouse of the cell, but they are a lot more than that. Mitochondria are the descendants of an ancient bacterium that lived endosymbiotically inside an ancestor of eukaryotes, and over millions of years lost many of its bacterial structures and transferred the majority of its genes to the host genome. Most mitochondria produce ATP through cellular respiration, but they also synthesize crucial cofactors like heme and iron-sulphur (Fe-S) clusters, oxidize fatty acids, and regulate calcium homeostasis, among other functions. They also perform a number of housekeeping processes, including maintaining, replicating, and expressing their own genome, which means they have their own RNA polymerase, tRNAs, ribosomes, etc. Modified mitochondria in certain lineages have lost the ability to produce ATP, or they produce ATP in different ways; some mitochondria are so functionally reduced they are almost unrecognizable, and are kept exclusively for Fe-S cluster biosynthesis.

3.2 Structure and Function

Mitochondria are membrane-bound organelles that typically perform both catabolic and synthetic functions in eukaryotes. They have two membranes: a porous outer membrane (OM) and a highly folded inner membrane (IM), which surrounds the innermost compartment, the matrix.

Mitochondria are typically involved in cellular respiration, fatty acid oxidation, and the synthesis of heme and iron-sulphur clusters. Cellular respiration begins with glucose, which is broken down into pyruvate in the cytoplasm by glycolysis. Pyruvate is moved into the mitochondrial matrix by specific transporters, where it enters the Krebs cycle (also called the Citric Acid Cycle, or Tricarboxylic Acid Cycle, or TCA cycle), a cyclic series of enzymatic steps that generates ATP, CO_2, and electrons to provide reducing potential. These high-energy electrons subsequently enter the electron transport chain (ETC), which is a series of large protein complexes on the inner membrane, where the energy is harnessed to create a proton gradient that in turn powers the synthesis of even more ATP.

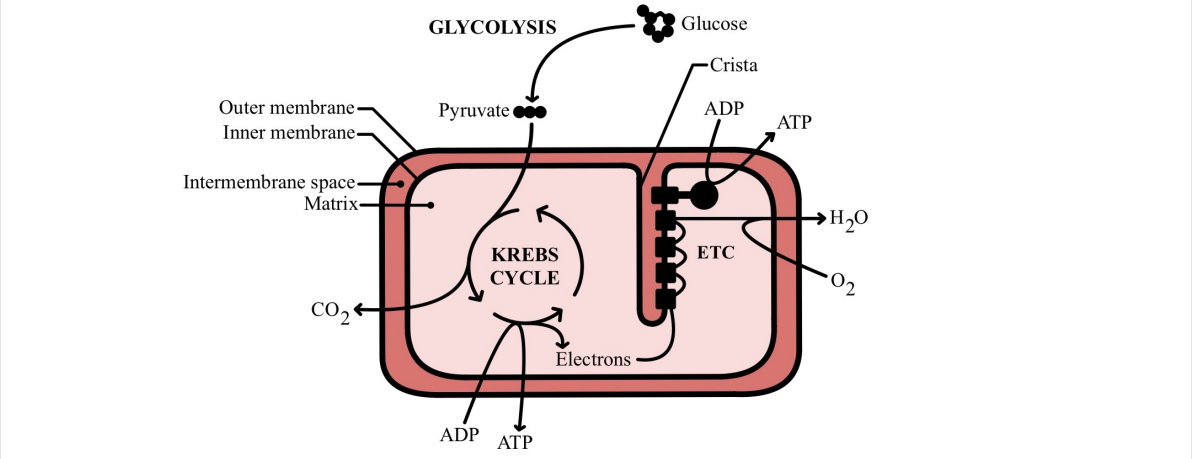


Figure 14: Metabolic Function of Mitochondria

In addition to producing ATP, mitochondria synthesize precursors for several amino acids, as well as important cofactors like heme and Fe-S clusters. Heme is an organic molecule that forms a ring around a central iron atom and is used as a catalyst in the redox reactions of many enzymes. Fe-S clusters are molecular ensembles of iron and sulphur, which bind together in tight pyramidal or square-shaped complexes. Fe-S clusters are essential in electron-transporting proteins such as ferredoxin, a protein participating in electron transport chains.

The inner membrane of canonical mitochondria has many folds called cristae, which increase its surface area and allow the mitochondria to be densely packed with ETCs. Cristae are typically classified by shape and can be tubular **(1)**, flat/lamellar **(2)**, or discoid **(3)** depending on the group of eukaryotes; for example, the mitochondria of animals, plants, and some protists have flat cristae, while most protists have tubular or, occasionally, discoid cristae.

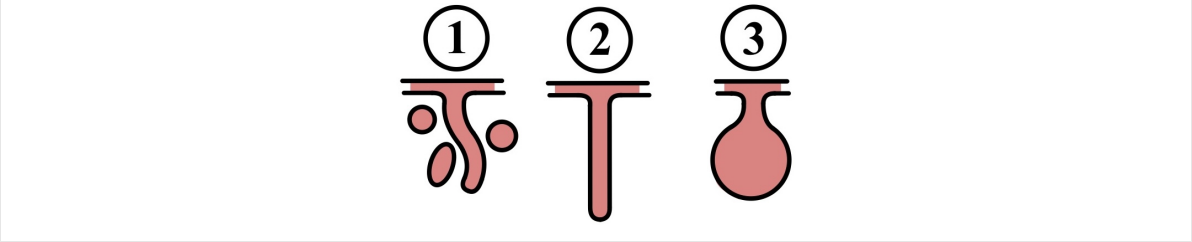


Figure 15: Tubular, Flat/Lamellar, and Discoid Cristae

3.3 Primary Endosymbiosis and the Origin of Mitochondria

Plastids and mitochondria both evolved from the uptake and integration of once free-living bacteria in a process called "primary endosymbiosis". While we know these two organelles originated from endosymbiotic bacteria, exactly how this process took place has remained a matter of some debate. One of the undisputed hallmarks of this process, however, is the genetic integration of the endosymbiont with the host, which is also often referred to as the point where an "endosymbiont" becomes an "organelle". By genetic integration we mean that genes from the endosymbiont move to the host nuclear genome, are then expressed by the host transcription and translation apparatus, and their protein products are then targeted back to the endosymbiont. This transfer of genes resulted in the endosymbiont genome getting smaller and smaller, as more and more essential genes were moved to the nucleus. Over the course of evolution, the symbiont also lost unnecessary structures like its peptidoglycan wall and bacterial flagellum.

Mitochondria originated ∼2.0 - 1.7 billion years ago when an alphaproteobacterium was taken up by an ancestor of all known extant eukaryotes. Alphaproteobacteria are Gram-negative bacteria, and so the endosymbiont would have had two membranes; the two membranes of mitochondria most likely correspond to the inner and outer membranes of the bacterium.

3.4 Protein Targeting

Most mitochondria retain their own small genome with a handful of protein-coding genes, but the mitochondrion requires hundreds of proteins to function. The many "missing" mitochondrial genes have been transferred to the nucleus, and are translated by eukaryotic, cytoplasmic ribosomes. In order to function, therefore, these proteins have to enter the mitochondrion. The cell has a specific targeting system to ensure this happens. Protein targeting to the mitochondrial matrix can be

summarized as follows (note that modifications to this general scheme also allow for targeting to other mitochondrial compartments, like the intermembrane space or one of the two membranes):

1. In the nucleus, a mitochondrial-specific gene is transcribed into an mRNA transcript.
2. The mRNA transcript is processed like any other, transported out of the nucleus, and translated in the cytoplasm by a ribosome. The resulting protein includes a short N-terminal extension called a transit peptide, which is recognized by receptors in the mitochondrial membranes.
3. The transit peptide is first recognized by a complex in the outer mitochondrial membrane called the Translocon of the Outer Mitochondrial membrane (TOM for short), which pulls the protein through the membrane to another complex, the Translocon of the Inner Mitochondrial membrane (TIM for short), required for the protein to cross the inner membrane.
4. The protein ends up in the mitochondrial matrix, where the transit peptide is cleaved from the protein by mitochondrial peptidases and the rest of the protein is re-folded by chaperonins.

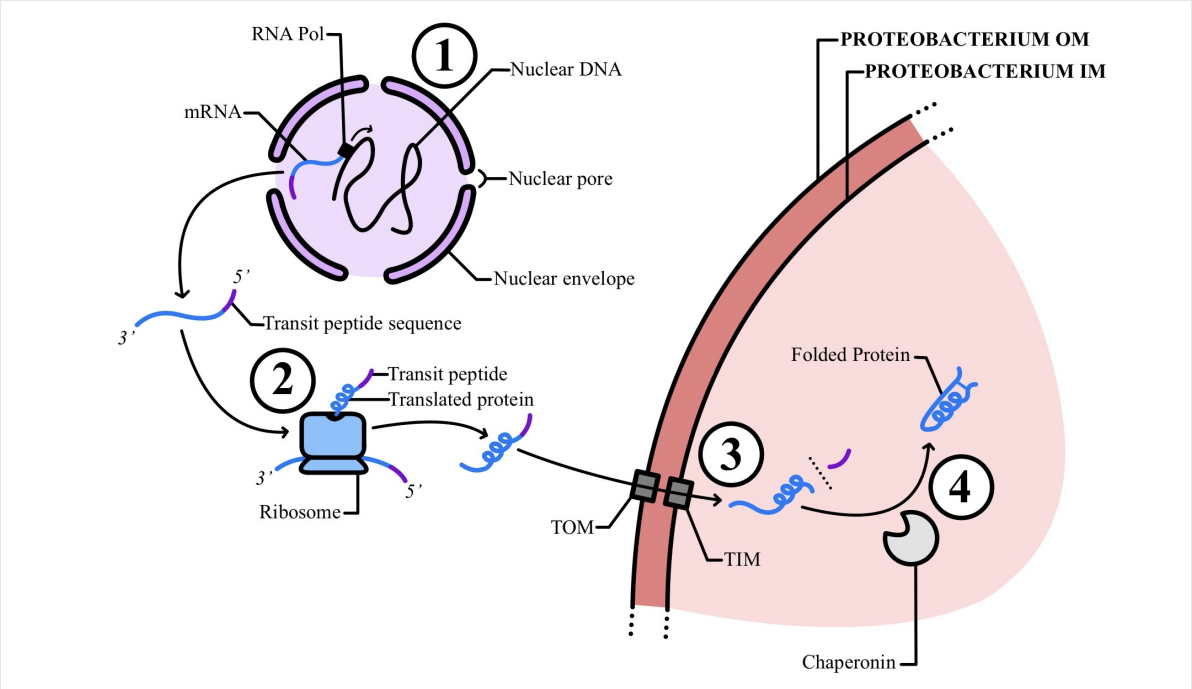


Figure 16: Protein Targeting in Mitochondria

3.5 Modified Mitochondria

When we think of mitochondria we generally have a picture of animal mitochondria (i.e. bean-shaped organelles with flat cristae carrying out oxidative phosphorylation), but protist mitochondria can be quite diverse: many have other functions or a reduced number of functions, they can be structurally modified, or completely lost. The best-known cases where mitochondria have significantly changed are found in anaerobic protists, some of the best studied examples of which are parabasalians, diplomonads, oxymonads, archamoebae, and microsporidians. These protists were

once thought to lack mitochondria entirely, and were even proposed to have diverged from other eukaryotes before the primary endosymbiosis event (they were considered "ancestrally amitochondriate", meaning that neither them nor any of their ancestors ever had a mitochondrion), and were referred to as the "Archezoa". This view turned out to be incorrect. Phylogenetic trees eventually showed that the Archezoa do not branch at the base of the eukaryotic tree, and in fact belong to many different groups of eukaryotes. Moreover, most archezoans were also shown to have mitochondria, albeit reduced or modified to the point of being unrecognizable. Organisms typically lose unnecessary features quickly in the course of evolution; in mitochondria, the most commonly lost function is actually aerobic respiration, which is useless in anoxic environments. For example, diplomonads, oxymonads, and parabasalians usually live in the guts of larger organisms and rely on fermentation to make ATP, and microsporidians are parasites that live inside other cells and can steal ATP directly from their host.

Parabasalians have mitochondria-derived organelles called hydrogenosomes, which have evolved a novel energy metabolism that produces hydrogen gas and CO_2 instead of water and CO_2. They do not possess cristae nor a genome. Other anaerobic protist mitochondria are more extremely reduced to organelles called mitosomes, which have further lost all energy-related functions. Mitosomes function exclusively to synthesize Fe-S clusters: you may have never heard of Fe-S clusters, but all organisms need these cofactors to survive, and their biosynthesis is the most conserved pathway of mitochondria. Oxymonads are the only known group of eukaryotes that have lost all traces of their mitochondria; so how do they survive without Fe-S clusters? The answer is they can't survive without them either, but they acquired the genes for a bacterial Fe-S cluster biosynthesis pathway via horizontal gene transfer; the enzymes in this alternative pathway are active in the cytoplasm, so when the ancestor of oxymonads became anaerobic they could completely lose their mitochondria.

4 Plastids

4.1 Overview

"Plastid" is a general term that refers to all the organelles ultimately deriving from an ancient primary endosymbiosis between a eukaryote and a cyanobacterium. We will use it for simplicity, but keep in mind that there are a variety of terms for specific types of plastids: chloroplasts are green plastids typical of plants and algae related to plants, but there are also rhodoplasts, amyloplasts, leucoplasts, apicoplasts, etc.

Plastids are generally, and for good reasons, associated with photosynthesis, however they also synthesize numerous essential cellular compounds like Fe-S clusters, some amino acids, fatty acids, heme, and isoprenoids. Plastids arose from a primary endosymbiosis similar to the one originating mitochondria, but they have engaged in further rounds of endosymbiosis called secondary, or tertiary, endosymbioses. Primary plastids have two membranes, whereas secondary plastids can have three to four membranes and result from an endosymbiosis with a primary alga. Tertiary plastids are only known in dinoflagellates, and are the result of an organism with a secondary plastid being taken in as an endosymbiont.

4.2 Structure and Function

Primary plastids have two membranes, which correspond to the two membranes of the ancestral Gram-negative cyanobacterial endosymbiont: an outer membrane (OM) and an inner membrane (IM), the latter of which is highly folded, forming many stacked compartments called thylakoids. The compartment surrounded by the IM is called the stroma. Some plastids have a dedicated region of the stroma called a pyrenoid where carbon fixation by the enzyme RuBiSCo is concentrated. Secondary and tertiary plastids share the same overall structure, but are surrounded by additional membranes.

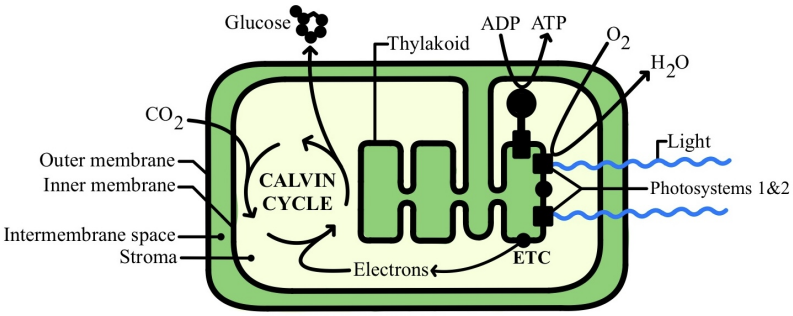


Figure 17: Metabolic Function of Plastids

The main function of most plastids is obviously photosynthesis, which is the harnessing of energy from light to drive the fixation of CO_2 into glucose. There is a lot of variation in this process between different kinds of plastids, but in general light strikes a protein complex embedded in the thylakoid membrane, providing the energy to split a water molecule into oxygen gas, protons, and electrons. The electrons travel down the plastid electron transport chain (ETC), driving the protons across the thylakoid membrane and creating an electrochemical gradient that powers an ATP synthase protein complex, which synthesizes ATP. At the end of the ETC, electrons are picked

up by molecular couriers that carry them to the Calvin Cycle, where a series of enzymes use these electrons plus the protons split from water to reduce carbon dioxide into glucose, consuming energy stored in ATP to do so. Like mitochondria, plastids also carry out a number of other metabolic pathways that we often forget: various plastids synthesize Fe-S clusters, amino acids, isoprenoids, heme, fatty acids, and also convert glucose to numerous complex carbohydrates for storage.

4.3 Origin of Primary Plastids

Primary plastids originated ∼1.5 billion years ago from a primary endosymbiosis between an ancestor of the archaeplastid supergroup of eukaryotes and a cyanobacterium. As with mitochondria, the exact process is not known, but there is little doubt that it involved phagocytosis. Over time, the cyanobacterium transferred the majority of its genes to the eukaryotic nucleus, and the corresponding proteins are now targeted back to the organelle. Plastids also became reduced by losing structures and functions; most plastids have lost their peptidoglycan wall, with some interesting exceptions, like the plastids of glaucophytes and some land plants. Most plastids have lost the ancestral phycobiliproteins, large protein complexes that help collect sunlight, which were however retained in the plastids of red algae and some secondary plastids. Plastids also gained some functions, the most conspicuous of which is the light harvesting complex (LHC) proteins, a family of proteins that form huge light-collecting antennae that more efficiently transfer energy from light to the reaction centres. Three major algal lineages have primary plastids: red algae, green algae, and glaucophytes, which collectively make up the supergroup Archaeplastida.

4.4 Secondary Plastids

Secondary endosymbiosis is the process in which an alga with a primary plastid is engulfed by another eukaryote, and it is itself retained and integrated as an organelle (a secondary plastid) in the host. Unlike primary plastids, secondary plastids remain surrounded by the endomembrane of the new host (originally, the membrane of the food vacuole). Most secondary plastids have four membranes, but in some protists (dinoflagellates and euglenids), the plasma membrane of the endosymbiotic alga is lost and the plastids only contain two cyanobacterial membranes surrounded by secondary host endomembrane. In two groups (cryptomonads and chlorarachniophytes) the nucleus of the endosymbiotic alga has also been retained in a reduced form, which appears as a vestigial structure called the nucleomorph, containing a small genome with a few hundred genes.

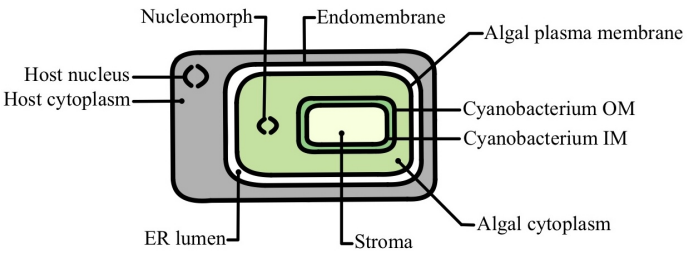


Figure 18: Secondary Plastid with a Remnant Algal Membrane and Nucleomorph

Secondary plastids originate either from red or green algae. There have been two events of secondary endosymbiosis with green algae, one resulting in the chlorarachniophyte plastid and another resulting in the euglenid plastid. The plastids in a wide variety of protist groups (cryptomonads,

haptophytes, photosynthetic stramenopiles, dinoflagellates, and apicomplexans) are derived from secondary endosymbioses with a red alga, however, the total number of endosymbiosis events that led to this distribution is still unknown.

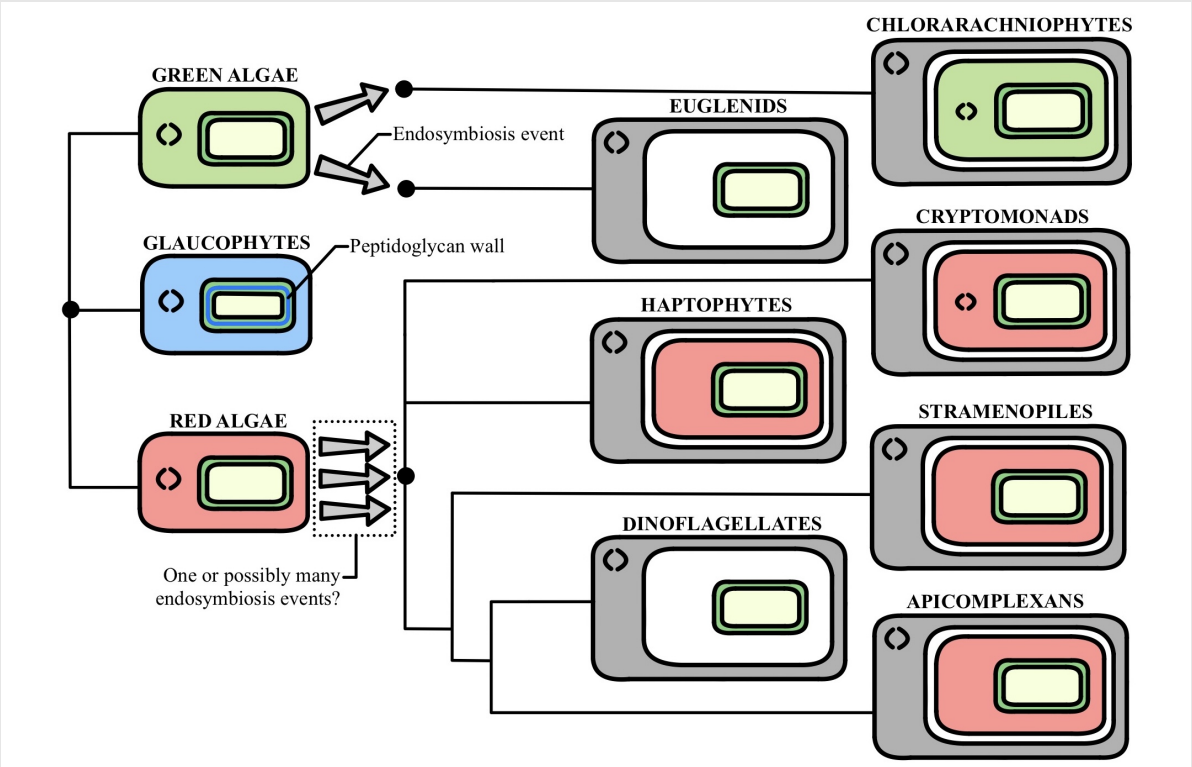


Figure 19: Secondary Plastids - Origins and Membranes

4.5 Protein Targeting

Protein targeting in primary plastids is highly similar to protein targeting in mitochondria: targeted proteins have an N-terminal transit peptide, and cross the two plastid membranes using protein complexes that are similar to those of the mitochondrion, called TOC and TIC (which stand for Translocon of the Outer Chloroplast membrane and Translocon of the Inner Chloroplast membrane). Protein targeting in secondary plastids is more complicated because there are more membranes to cross. Here is an example of how proteins are targeted to the stroma of secondary plastids with four membranes (there is some variation between different lineages, but this is a good generalization):

1. In the nucleus, the gene for a plastid-targeted protein is transcribed into an mRNA transcript, which is processed and transported to the cytoplasm for translation by cytoplasmic ribosomes. The 5' end of the protein-coding gene encodes a "bipartite" leader sequence, which first has a signal peptide followed by a transit peptide.

2. Signal peptides are used by all eukaryotes in the fundamental process of directing protein synthesis to the endomembrane system, for example to express secretory proteins (see chapter 2). When such proteins are translated, the ribosome will stop after synthesizing the signal

peptide because the peptide will bind to the signal recognition particle (SRP), a complex that binds to the signal peptide and guides the ribosome to the endomembrane. Normally this would mean guiding secretory proteins to the endoplasmic reticulum, but in secondary plastids this mechanism has been co-opted to guide ribosomes to the endomembrane surrounding the secondary plastid.

3. When the ribosome binds to the endomembrane, the SRP falls off and translation resumes, but now the protein is being co-translationally inserted through the membrane, i.e. pushed into the lumen of the endomembrane system, where a peptidase cleaves the signal peptide exposing the transit peptide.

4. To cross the next membrane the transit peptide of the fully-translated protein is first recognized by another protein complex called SELMA, which is a special duplicated version of a complex found in all eukaryotes: the ERAD complex (which stands for Endoplasmic-Reticulum-Associated protein Degradation). All eukaryotes use the ERAD complex to identify and remove misfolded or mutated proteins from the endoplasmic reticulum, transporting them back into the cytoplasm to be destroyed. In secondary plastid protein targeting, the algal ERAD system has been duplicated and one copy modified into the SELMA complex, which stands for Symbiont-derived ERAD-Like Machinery. SELMA still performs a function similar to ERAD, but it is inserted in what was once the endosymbiotic alga plasma membrane, so it transports proteins out of the endomembrane lumen (but by recognizing the transit peptide, rather than because they are misfolded), and moves them across a membrane (but in this case not into the cytoplasm, but further inside the plastids).

5. At this point, protein targeting can follow the same two steps seen in primary plastids (and mitochondria). The transit peptide is recognized by TOC and TIC and pulled through the membranes of cyanobacterial origin and into the plastid stroma, where an enzyme cleaves the transit peptide, and the protein is re-folded by chaperonins.

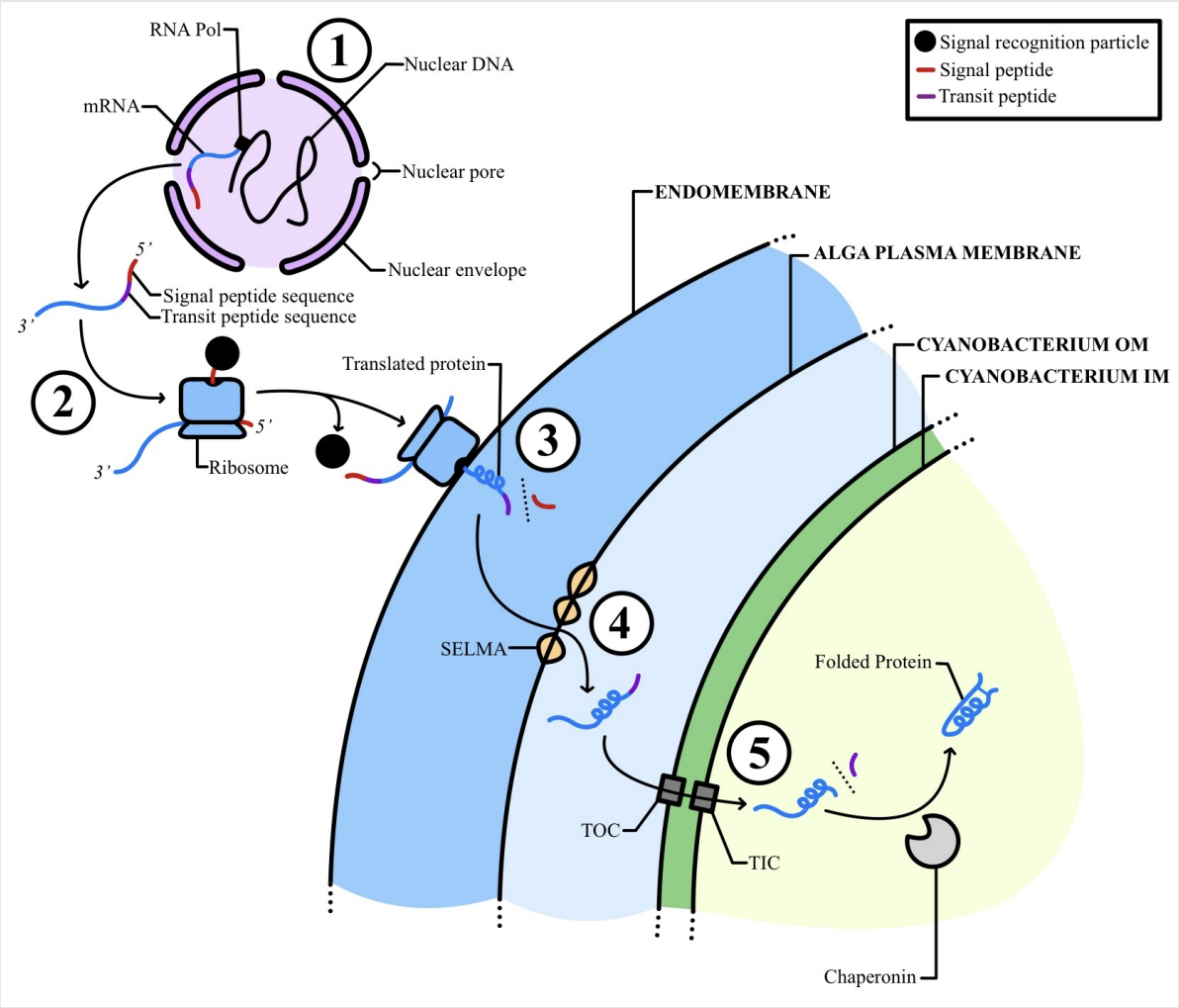


Figure 20: Protein Targeting in Secondary Plastids

5 Notes on Some Major Ways Protists Impact Ecosystems

Protists impact every major ecosystem on Earth in some way, and the importance of specific groups of protists on specific ecosystems is touched upon in their respective chapters. However, there are processes that involve a number of different protists that are of global significance, and here we summarize two major examples.

5.1 Nitrogen Cycling in the Rhizosphere

Protists are key players in the terrestrial nitrogen cycle. Nitrogen is one of the most important limiting factors for the growth of plants, which implement multiple strategies to overcome this limitation. Many rely heavily on nitrogen-fixing bacteria, who transform inaccessible atmospheric nitrogen into NH_4, a form that can be absorbed and metabolized by the plant. Even more plant species (the majority, in fact) increase the reach of their root system through symbioses with fungi (mycorrhizae). Usually, this is not enough – in repeated experiments, removing *protists* from the soil stunts plant growth. Why?

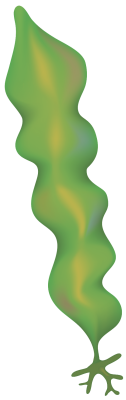
Plants actively secrete sugars into the soil, contributing to the total dissolved organic carbon (DOC), which attracts heterotrophic bacteria (some of which might be beneficial nitrogen-fixers, but most of which are not plant symbionts at all). Bacteria-eating protists follow. This microbial migration ends up concentrating nitrogen into the rhizosphere: bacteria have on average more nitrogen per unit of mass (mostly because of their peptidoglycan wall) than eukaryotes, so when protists feed on them, they often excrete the excess nitrogen back into the soil, closer to the plant than it would have otherwise have been, and in a form that can be assimilated through the roots.

5.2 The Microbial Loop

Protist's ecological influence is arguably even more important in aquatic ecosystems. To begin with, microscopic algae (photosynthetic protists) are the ocean's most important autotrophs and the primary producers of most of the biomass in marine environments. Heterotrophic protists feed on these algae and are in turn eaten by zooplankton (larger protists or small aquatic animals, especially crustaceans), passing their carbon up the traditional food chain when larger animals eat the zooplankton.

However, much of the algal biomass never reaches the mouths of predators: algae can be ruptured by waves or rough currents or — very commonly — lysed by viruses and other pathogens, releasing their cell contents into the water column; even when that isn't the case, algae are actually very "leaky", constantly exuding DOC, mostly as sugars. These precious nutrients might leave the traditional marine food web, since protists and animals that prey on algae are unable to efficiently use DOC directly. Instead, the carbon is shunted back into the system by osmotrophic bacteria, that flock to the dissolved organic material on which they thrive, followed by various small protists that specialize in eating bacteria, like choanoflagellates, some haptophytes and mixotrophic green algae, and small ciliates. These bacterial-feeders are in turn eaten by larger protists, like dinoflagellates, radiolarians, and large ciliates (some of which feed on bacteria too), who are big enough to be consumed by small animals, ultimately shunting the nutrients from ruptured and leaking algae back into higher trophic level. Enormous amounts of energy and fixed carbon would not be available to consumers without this process, called the "microbial loop", and its heterotrophic bacteria and protists protagonists.

Taxonomic Chapters



6 Brown Algae

Notable Genera: *Ascophyllum*, *Ectocarpus*, *Fucus*, *Laminaria*, *Macrocystis*, *Sargassum*...

6.1 Overview

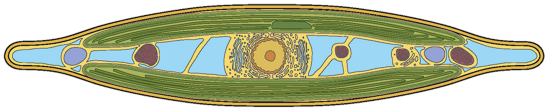
Brown algae are a diverse group of mostly benthic, multicellular algae. They range from feathery, thread-like organisms only a few millimeters in length to towering kelps that form large underwater forests contributing significantly to global carbon fixation. Their colour is due to fucoxanthin, a photosynthetic pigment typical of stramenopile plastids that emits a brownish-green light. All brown algae are multicellular and were initially assumed to be plants due to their large size and leafy appearance, but they are in fact entirely unrelated to land plants and acquired their photosynthesis by secondary endosymbiosis with a red alga.

6.2 Kelp

Kelps are an order of brown algae that play a fundamental role in ocean ecology, acting as a source of both food and habitat for numerous ocean-dwelling organisms. One species, *Macrocystis pyrifera* (giant kelp), grows up to 60 meters tall and forms huge ecosystems that provide home to seals, octopuses, otters, sea urchins, and many other marine animals. Otters and sea urchins have particular importance to kelp forests. Urchins are voracious herbivores that gnaw on the kelp's holdfasts and cleave them from the sediment, which causes immeasurable damage to the forest when left unchecked. Luckily, the kelp's resident sea otters balance the playing field by preying on and controlling the urchin populations; when otter populations are depleted, however, the consequences on the ecosystem are sudden and dire. Kelps provide some practical value to humans too. Chemicals called alginates found in kelps are used commercially to make food products, gels, dyes, and explosives.

7 Diatoms

Notable Genera: *Chaetoceros*, *Nitzschia*, *Oscillaria*, *Phaeodactylum*, *Thalassiosira*...



7.1 Overview

Diatoms are a diverse group of unicellular algae with an estimated 200,000 species that inhabit a wide variety of habitats including oceans, lakes, rivers, waterways, and moist soil. Marine diatoms are one of Earth's fundamental primary producers, fixing 10-20 billion metric tons of CO_2 per year – for reference, the Amazon rainforest fixes roughly 70 billion metric tons of CO_2 per year. Diatoms cover themselves in an elegant, two-piece siliceous skeleton called a frustule, which can come in many different shapes and sizes, mostly with either a central symmetry (centric diatoms) or a bilateral symmetry (pennate diatoms).

7.2 Life Cycle of Centric Diatoms

Like most protists, diatoms reproduce asexually, and trigger sexual processes only in specific circumstances. Centric diatoms have a diplontic life cycle and usually have anisogametes, with non-flagellated female gametes and male gametes that might have a flagellum with mastigonemes. The development of gametes is triggered by an unusual size-related mechanism. A diatom's frustule is composed of two parts called "valves" that fit together like a shoebox and its lid – so one valve must be slightly smaller than the other to fit.

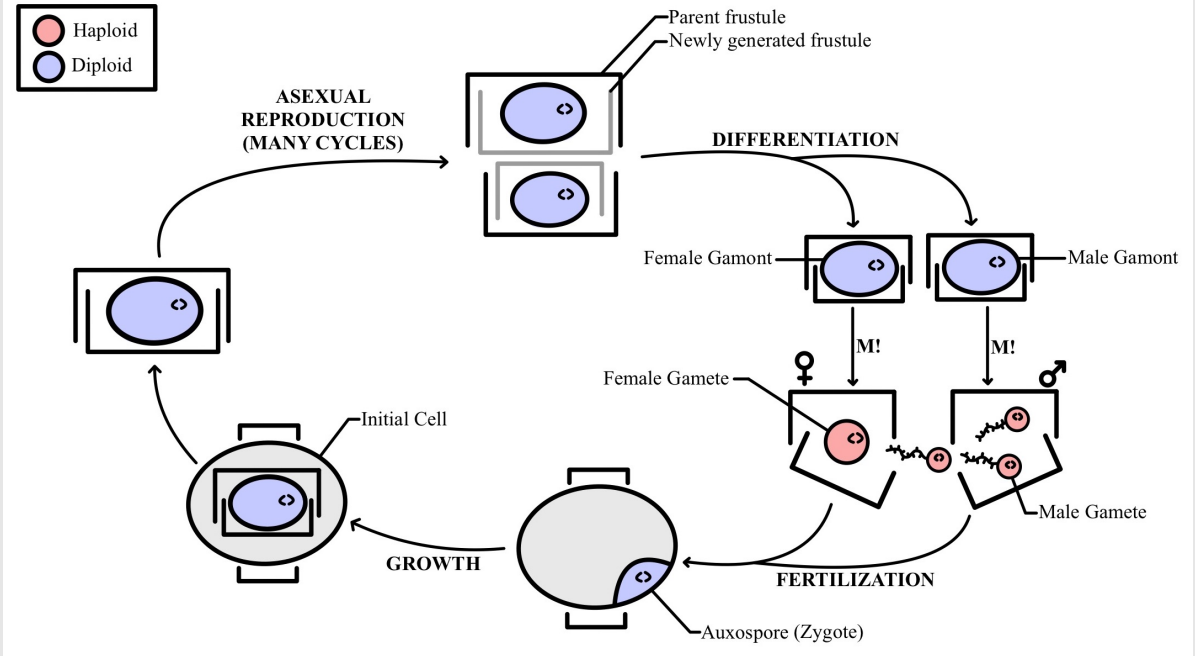


Figure 21: Typical Centric Diatom Life Cycle

Every time a diatom undergoes mitosis, it passes on one valve to each of its daughter cells, that in turn grow a new valve to complete the box, but they always grow a smaller one to fit inside the one they inherit. Diatoms that inherit the smaller valve will therefore grow an even smaller valve, and this cycle will repeat so that most of the population is continuously growing smaller and smaller. This is obviously unsustainable, so to break this ratchet diatoms undergo sex, which results in diatom cells with a full-sized frustule, resetting the cycle.

7.3 Gliding Motility in Pennate Diatoms

The frustule, although essential to diatom survival, provides major physical limitations for the diatom because the alga is (quite literally) trapped in a glass box. Without room for cilia or flagella, most diatoms have no means of motility, but some pennate diatoms implement a different locomotion strategy: gliding. The diatom's frustule contains a longitudinal slit called the raphe; diatoms secrete a sticky polysaccharide called mucilage through the raphe which sticks to surfaces. The mucilage is attached to a transmembrane protein complex, which is itself attached to myosin. Myosin is a molecular motor that moves along actin filaments, and when myosin moves towards the posterior end of the cell, it pulls the diatom forward along the surface the mucilage is attached to.

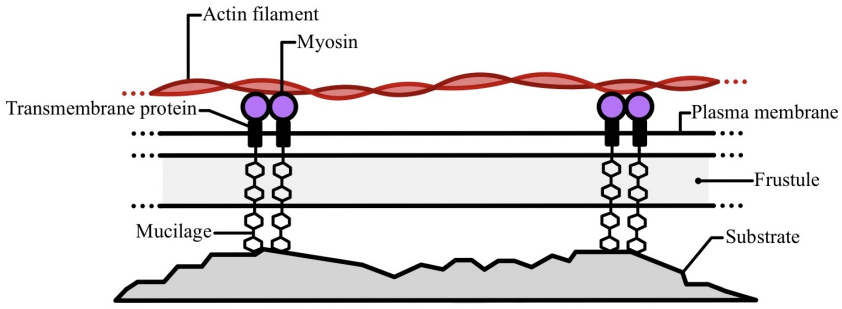


Figure 22: Gliding Motility in Pennate Diatoms - Side View

When the myosin reaches the end of its actin road (at the posterior end of the cell), the corresponding mucilage is cleaved and left stuck to the substrate as a slime trail behind the diatom. The myosin is then moved back to the anterior end of the diatom, where it can attach to another transmembrane protein-mucilage complex and begin the process all over again. The raphe must allow the mucilage to connect the membrane to the substrate without compromising the overall integrity of the frustule, and this is accomplished by the raphe being not a simple slit, but a tight-fitting C-shaped path, preserving the strength of the wall.

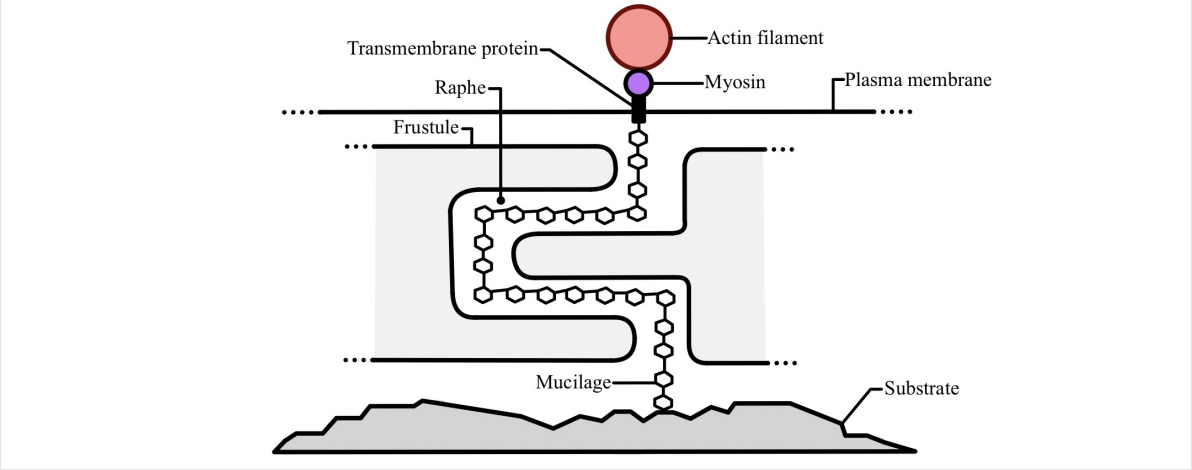
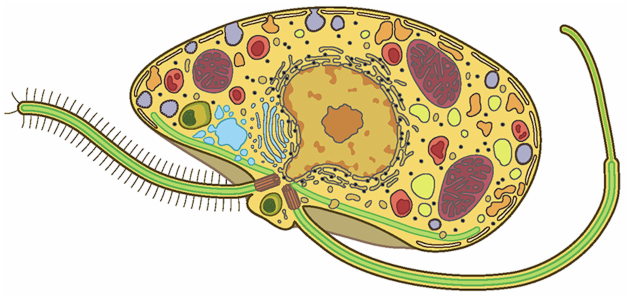


Figure 23: Gliding Motility in Pennate Diatoms - Front View



8 Oomycetes

Notable Genera: *Peronospora*, *Phytophthora*, *Saprolegnia*...

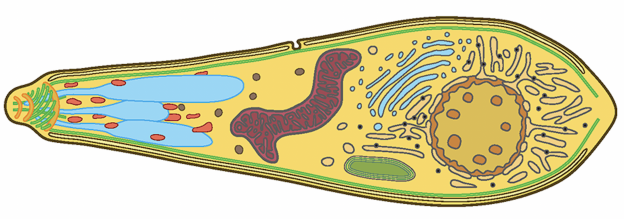
8.1 Overview

Oomycetes are parasitic, fungi-like organisms that infect a variety of plant and animal hosts. The oomycete species *Phytophthora infestans* caused the Irish Famine in the XIX century, withering potato harvests for eight consecutive years which, together with the British "corn law", led to the death of roughly one million people and the emigration of even more. Oomycetes infect plants, release spores, and form hyphae, and on account of these features were originally thought to be fungi. However, molecular data later revealed that oomycetes belong to a completely different branch on the tree of life: stramenopiles. Like most stramenopiles, and unlike any fungus, oomycetes possess an heterokont flagellated life stage, represented by the zoospore in this group. Oomycetes are among the few eukaryotes that rely on osmotrophy for feeding, something they are able to do through a combination of high surface area to maximize diffusion and a rich battery of digestive enzymes acquired from fungi via horizontal gene transfer.

8.2 Oomycete Infection

Oomycetes have complex infection mechanisms that vary from species to species, so we will use the oomycete infection mechanism in plants as an example. First, cold and rainy conditions trigger the sporangium of an established oomycete to release numerous zoospores into the air, which land on neighbouring leaves via raindrops. Once landed, the zoospore releases a barrage of enzymes onto adjacent plant cells, degrading the host's defensive toxins and preparing for further infection. The oomycete then begins to develop its hyphae network inside the leaf tissue to obtain nutrients, grows, and eventually forms a new sporangium to begin the process all over again.

9 Apicomplexans



Notable Genera: *Babesia*, *Cryptosporidium*, *Gregarina*, *Nephromyces*, *Plasmodium*, *Toxoplasma*...

9.1 Overview

Apicomplexans are parasites that cause serious diseases in both vertebrates and invertebrates, the most serious being malaria, which is transmitted via a mosquito vector and kills over 600,000 people each year. Apicomplexans have complex life cycles composed of multiple stages, each with different morphological features adapted for either infection or survival and replication inside their hosts. The group is defined by a cluster of organelles called the apical complex, involving a set of cytoskeletal and endomembrane structures that play a major role in the protist's infection mechanism, which is in fact a specialized form of gliding motility. Apicomplexans also have a non-photosynthetic secondary plastid called apicoplast, a remnant structure from their algal ancestors that has been retained for a number of biosyntheses.

9.2 The Apical Complex

The apical complex is a highly structured cluster of secretory organelles and cytoskeletal elements that play a fundamental role in the apicomplexan infection mechanism. The structure is found at the anterior end of the cell, and consists of an apical vesicle, an apical ring, rhoptries, micronemes, dense granules, and a conoid, a tubulin structure from which coiled microtubules extend throughout the cell.

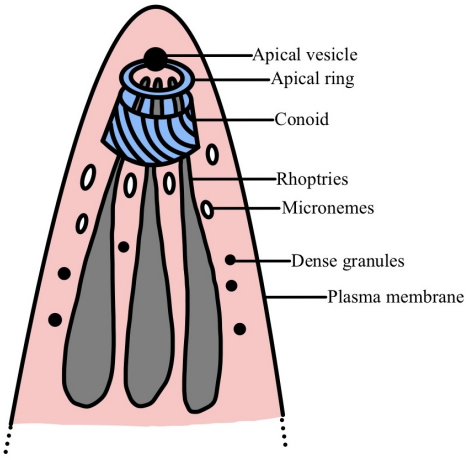


Figure 24: The Apical Complex

9.3 Examples of Human Diseases

Malaria is caused by a genus of apicomplexans called *Plasmodium*, which contains five species causing malaria in humans. The vast majority of deaths are caused by *Plasmodium falciparum*, whereas *Plasmodium ovale* and *Plasmodium malariae* cause a much milder form of the sickness. Symptoms begin roughly 10-15 days after being bitten by a mosquito carrying the parasite and

typically include fatigue, vomiting, anemia, and bouts of fever and chills that come every 36-48 hours. As the disease progresses, infected individuals may begin to suffer from respiratory failure, organ failure, and some may even fall into a coma. Patients who survive often experience a resurgence of the disease roughly three months after recovery.

Toxoplasma gondii is an apicomplexan that can infect various organs of virtually all birds and mammals. 25-30% of humans carry *Toxoplasma* in their brains; most remain asymptomatic, but immunocompromised individuals can develop severe flu-like symptoms (toxoplasmosis), which may lead to death, and in pregnant women a *Toxoplasma* infection can lead to birth defects. *Toxoplasma* requires a feline host to undergo sexual reproduction, and the parasite maximizes its chances of finding one by influencing the behaviour of other hosts. The most famous example is that mice infected with *Toxoplasma* are attracted to the smell of cat urine and display more curious/risky behavior. One study shows that infected humans have increased levels of violent behavior, risk-taking, and are more likely to suffer from schizophrenia, however this conclusion is highly controversial and is not definitively proven.

9.4 Infection Mechanism

Most apicomplexans can only complete their life cycle by infecting an animal cell. The infection process begins when an apicomplexan recognizes a target cell through contact. The parasite then discharges specialized vesicles called micronemes, which release proteins (e.g. TRAPs or MICs) onto the surface of the parasite able to bind receptors on the target cell. The apicomplexan then uses gliding motility to squeeze its way into the cell, forming a membrane vesicle around itself called the parasitophorous vacuole. To prevent being destroyed by the host cell, the invading apicomplexan's rhoptries and dense granules excrete numerous lipids and proteins that change the parasitophorous vacuole, dampening the immune response.

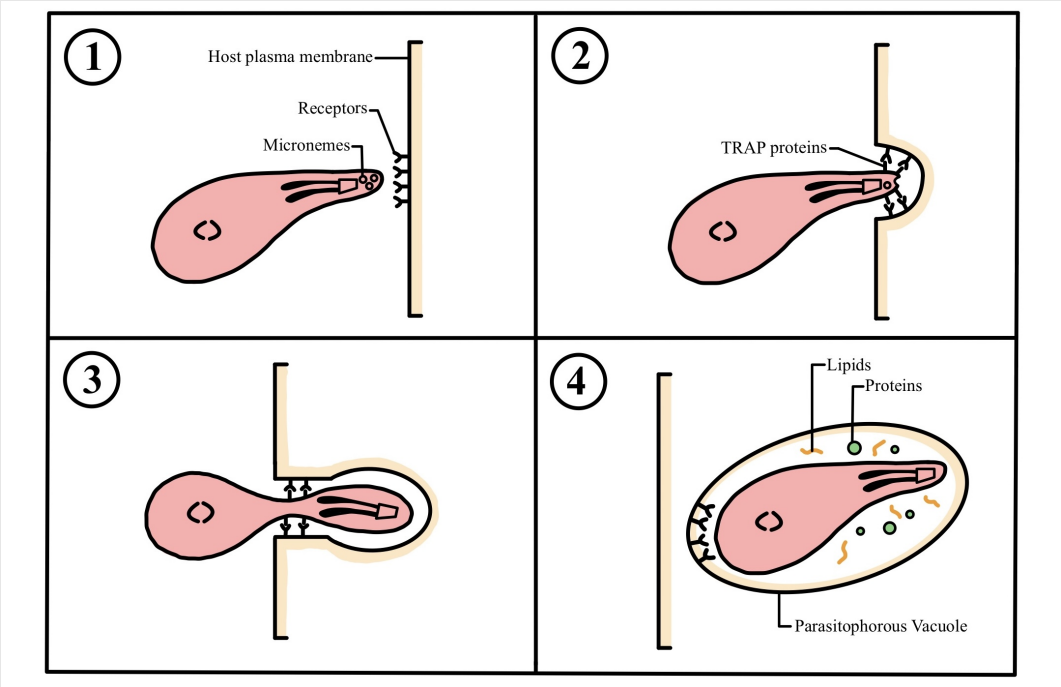


Figure 25: Infection of Host Cells

9.5 Gliding Motility

Gliding motility in apicomplexans shares fundamental features with gliding motility in other protists, but since they evolved independently it differs vastly in the details. Like all gliding motility, it requires a cytoskeletal component, a motor, a transmembrane protein, and a means to stick to a substrate. Apicomplexan gliding is based on actin and myosin, but with the twist that myosin, the motor, is anchored in place in the cell. The myosin is attached to a protein complex that traverses the alveolus and is solidly bound to the cell's cytoskeleton. The actin filaments are short and transient, forming and depolymerizing in the space between the alveolus and the plasma membrane. The transmembrane TRAP or MIC proteins bind the actin via other proteins on one side, and the host cell surface receptors on the other, so when the actin is pushed by the motor, the parasite moves across the surface of the host cell.

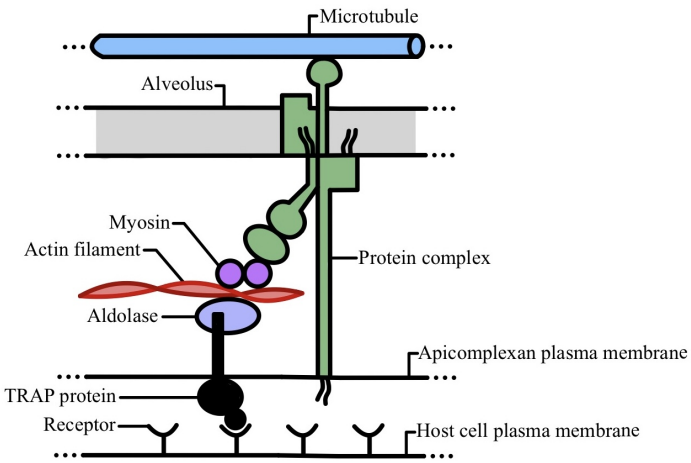


Figure 26: Apicomplexan Gliding Motility

9.6 Gregarines

Gregarines are a group of apicomplexans that infect the guts of a variety of invertebrate hosts. Gregarines have a multistage life-cycle: a gregarine infection begins during the sporozoite stage, when it is ingested. Once the parasite has localized to the intestinal tract, it will attach onto the host's epithelial cells of the gut wall and feed on their contents, entering its trophozoite (feeding) stage. The trophozoites eventually differentiate into male or female gamonts, and pair with a gamont of the opposite sex. These two cells will then encase themselves in a protective cyst in preparation to be excreted. The two gamonts will undergo multiple fission, producing gametes. After fertilization, each zygote forms a smaller cyst (oocyst), within which new sporozoites are produced by meiosis, until they are released after being ingested by another host.

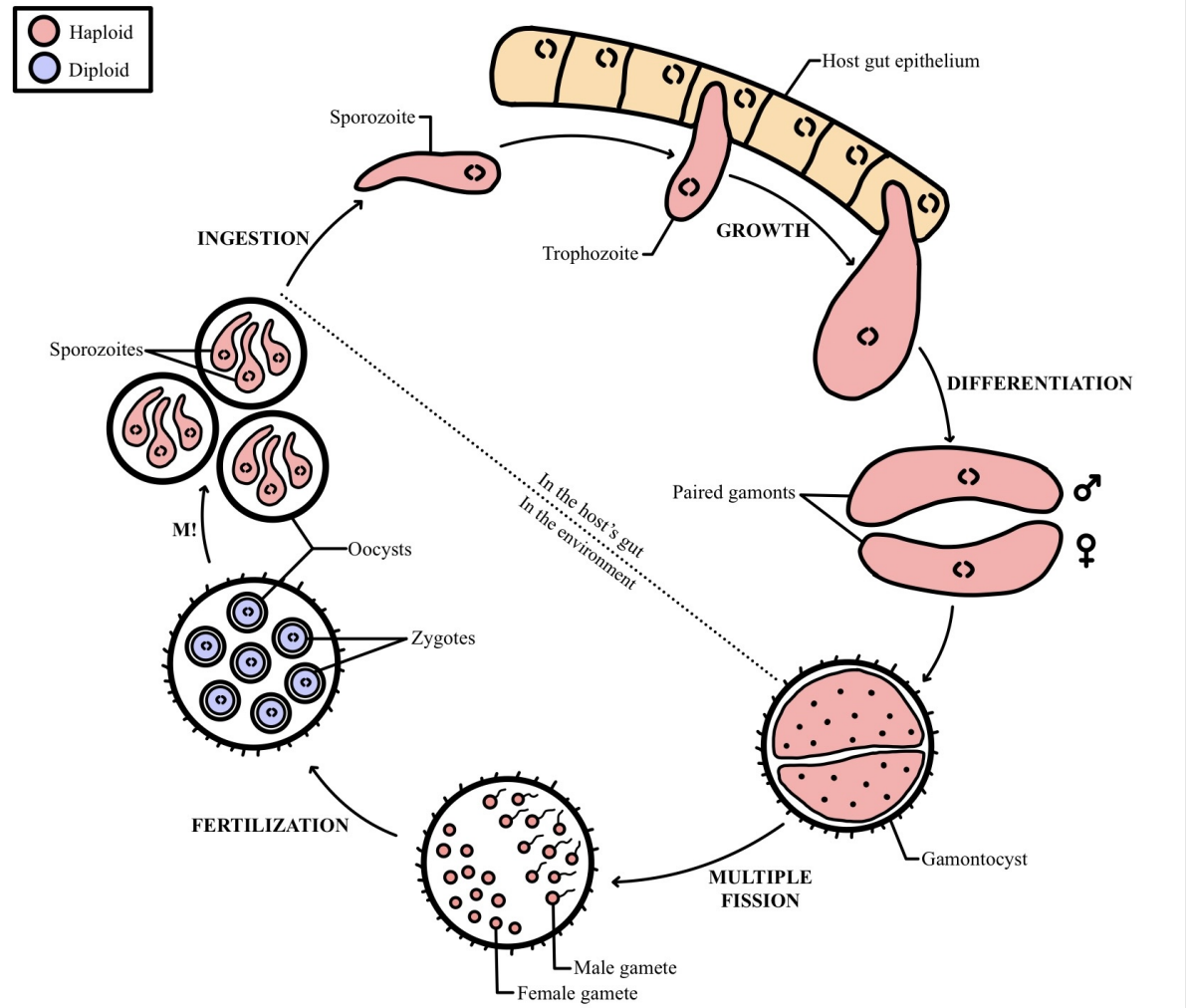


Figure 27: Gregarine Life Cycle

9.7 *Plasmodium*

Symptoms of malaria (the disease caused by *Plasmodium*) were discussed earlier in the chapter, however we will now dive into the life stages of the parasite that induce these symptoms.

Plasmodium infection is initiated by the transfer of a sporozoite from the salivary glands of a mosquito vector into the bloodstream of a human host. The sporozoite drifts through the blood until it reaches a liver cell, which it will burrow into to evade the body's immune response. Once inside the liver, the apicomplexan enters its trophozoite stage and begins to feed on the contents of its host cell, greatly increasing in volume. Once a particular size is reached, the trophozoite rapidly replicates its DNA, forming tens of thousands of new nuclei as it shifts from its trophozoite stage into its meront stage. The meront quickly undergoes multiple fission, creating roughly 40,000 merozoites that flood into the bloodstream and begin to infect red blood cells (RBCs). While most merozoites target RBCs, some reinfect liver cells, only to resurge 3 months later after an individual has recovered.

Merozoites that infect RBCs undergo a similar process to the sporozoites. Once inside a target

cell the merozoite will grow into a trophozoite and then a meront, undergo multiple fission, and burst the RBC, releasing thousands more merozoites back into the bloodstream. Extraordinarily, in a process that is still not fully understood, *Plasmodium* synchronizes the release of its merozoites to overwhelm the host's immune system; millions of merozoites are released into the bloodstream simultaneously only to quickly hide away in new RBCs before the body can mount an immune response. This cycling results in a fever every 36-48 hours, corresponding to a sudden onslaught of parasites emerging into the bloodstream.

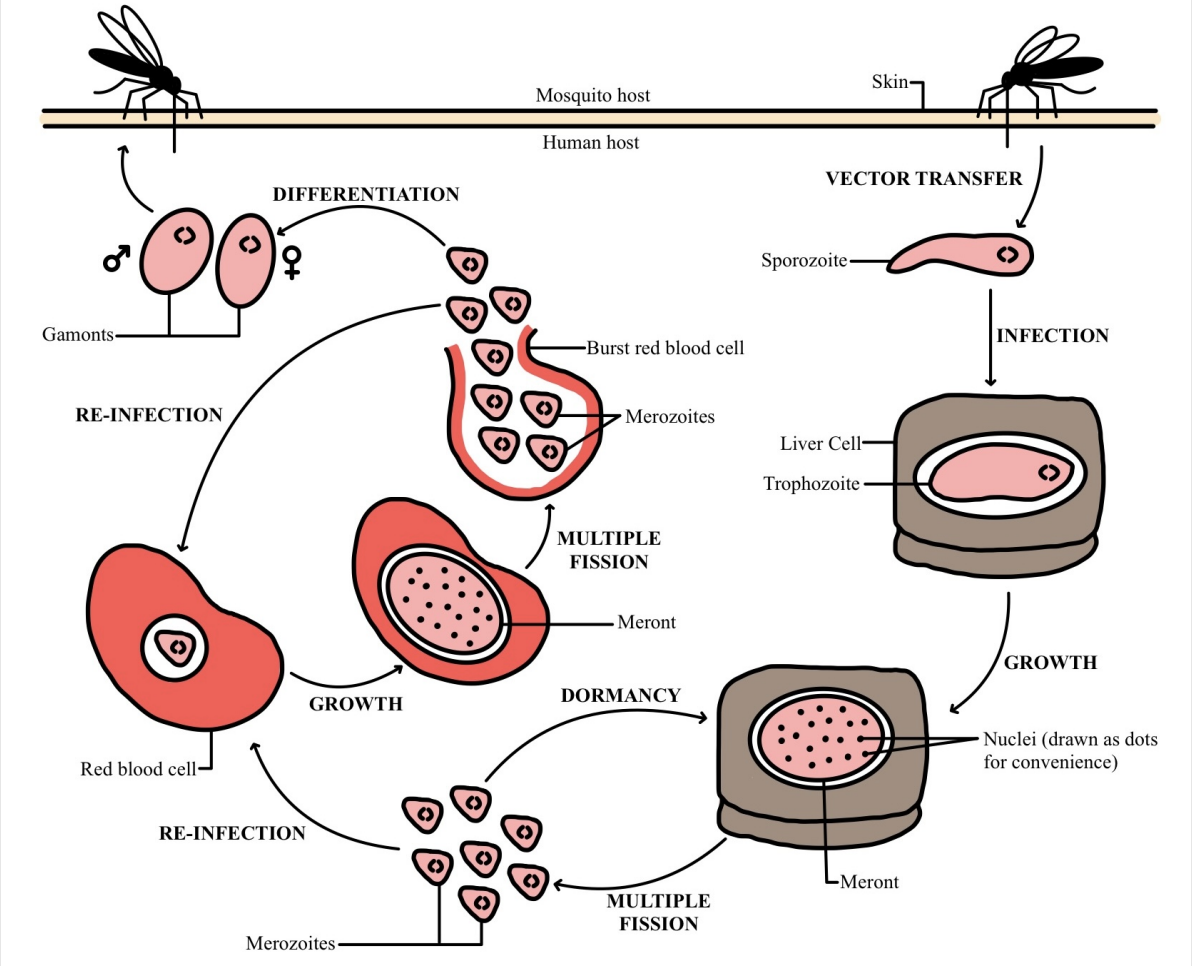


Figure 28: *Plasmodium* Life Cycle in Humans

The repetitive bursting of RBCs not surprisingly causes major health issues for the infected individual: malaria patients often suffer from anemia as they become deficient in RBCs, while the broken cells become so numerous they often cause the spleen to clog and rupture. When functional, the spleen will filter out infected RBCs. To avoid being destroyed by the spleen, merozoites secrete a sticky protein that makes infected RBCs adhere to the walls of the host's blood vessels. In rare cases, sticky RBCs will form clogs in the capillaries of the brain, causing a coma, or in the lungs, causing respiratory failure.

Some merozoites will differentiate into male and female gamonts, in preparation to enter their sexual stage. When a mosquito draws blood from an infected individual, these gamonts will be

sucked up the mosquito's gut. Here, gamonts produce gametes which fuse into zygotes through fertilization. Zygotes will then cross the mosquito gut epithelium into the body cavity, where they generate new sporozoites by meiosis. These sporozoites break free from the zygote cyst and make their way into cells of the mosquito's salivary glands, ready to infect a new human host.

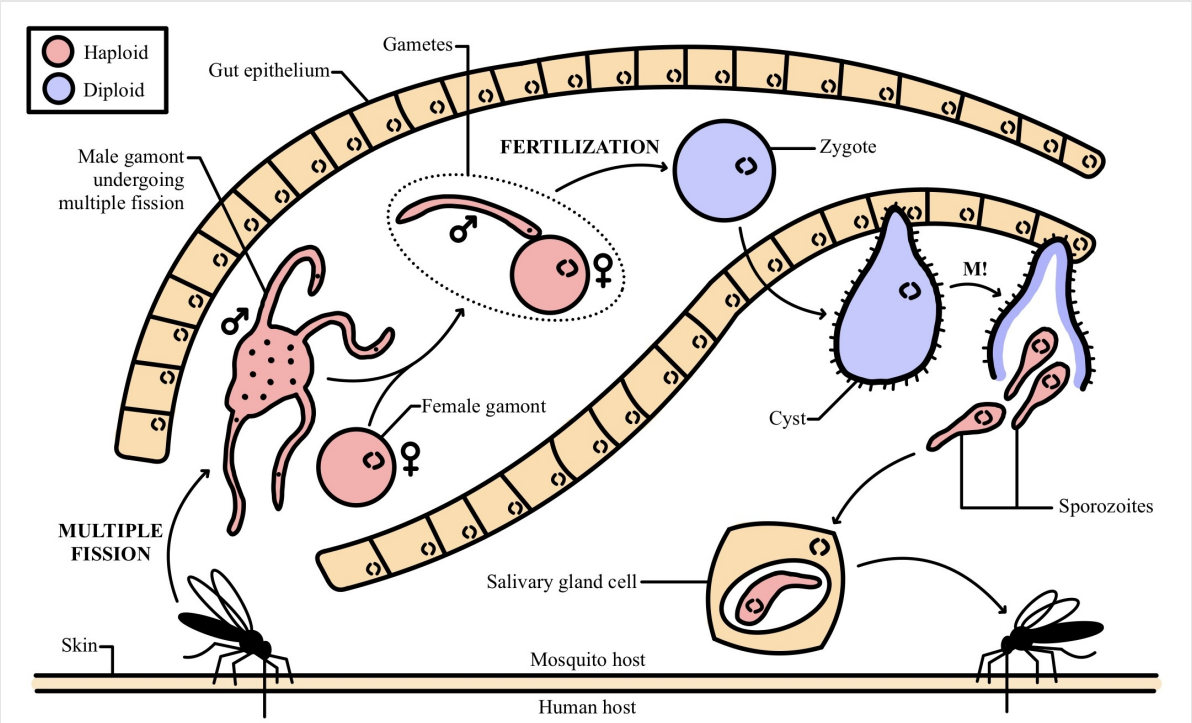
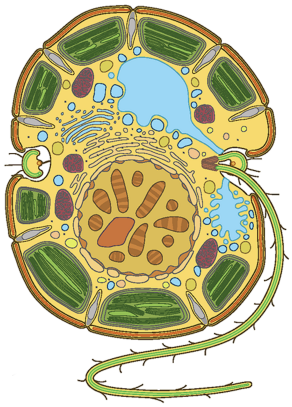


Figure 29: *Plasmodium* Life Cycle in Mosquitoes



10 Dinoflagellates

Notable Genera: *Alexandrium*, *Amphidinium*, *Dinophysis*, *Erythropsidinium*, *Gyrodinium*, *Karenia*, *Noctiluca*, *Protoperidinium*, *Symbiodinium*...

10.1 Overview

Dinoflagellates are a large group of aquatic protists that can be either heterotrophic, mixotrophic, or autotrophic. Roughly 85% of known dinoflagellate species are planktonic and marine, and are deeply important to the marine ecosystem as both primary producers and key players in the microbial loop. Heterotrophic and mixotrophic dinoflagellates consume small protists like choanoflagellates, diatoms, and other algae that are too tiny to be consumed by larger marine predators. Many dinoflagellates have an endoskeleton composed of cellulose plates that rest inside their alveoli, forming a structure called the theca, which gives many dinoflagellates their panelled shape. Dinoflagellates have two flagella: one that trails posteriorly (producing thrust) and another that waggles transversely (probably helping in both propulsion and steering), both of which rest in grooves in the membrane between the underlying thecal plates.

10.2 Life Cycle

Dinoflagellates have a haplontic life cycle, and most of the time reproduce asexually. For example, dinoflagellate blooms are periods of mass asexual reproduction often in response to favourable conditions. Conversely, environmental stress triggers dinoflagellates to sexually differentiate, which increases their genetic variance and induces the formation of a resistance cyst, maximizing their chances of survival.

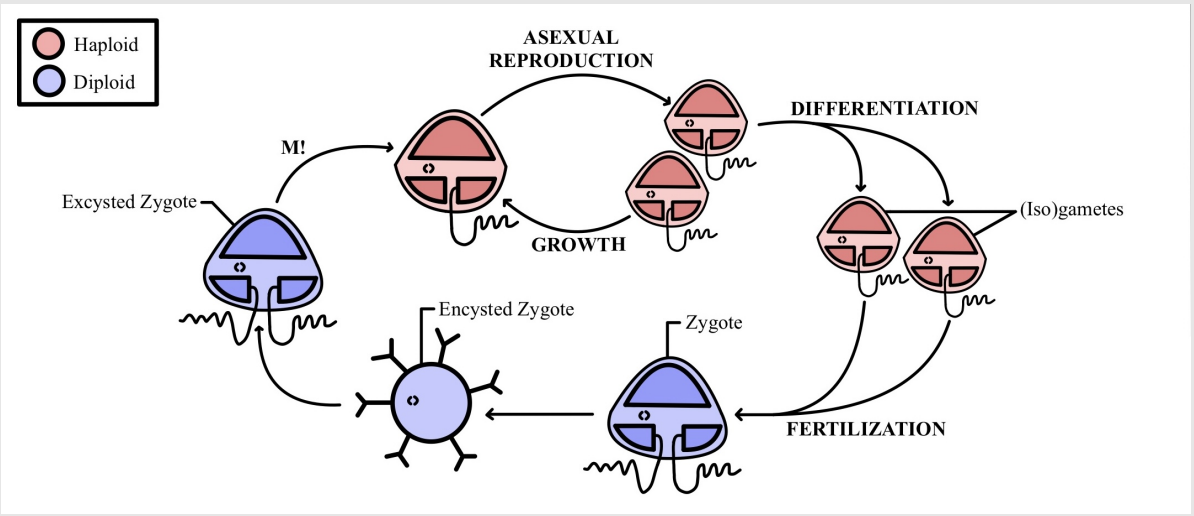


Figure 30: Typical Dinoflagellate Life Cycle

10.3 The Dinokaryon

Dinoflagellates have a unique nuclear organization, resulting in a characteristic-looking nucleus sometimes called “dinokaryon”. The chromosomes are permanently condensed and lack histones,

instead using different proteins acquired from bacteria (histone-like proteins, or HLPs) and viruses (dinoflagellate/viral nucleoproteins, or DVNPs) to organize their DNA. These nucleoproteins pack DNA into a unique paracrystalline structure; since DNA is thought to play a structural role, dinoflagellate genomes can be quite large, with some having orders of magnitude more DNA than human cells.

10.4 Feeding

Mixotrophic and heterotrophic dinoflagellates consume their prey in a variety of ways. Like most heterotrophic protists, dinoflagellates do phagocytosis, but for species with a theca the plates create a structural impediment. For smaller prey, dinoflagellates widen a groove (the sulcus) between their thecal plates, creating an open area of the plasma membrane where phagocytosis can occur. To consume larger prey, some dinoflagellates use pallium feeding, extending a projection of cytoplasm (technically, a pseudopodium) to surround and digest prey outside of the plated body. Another method called myzocytosis involves a tube-like organelle called the peduncle that pierces the membrane of the prey and "sucks" up its cytoplasm, although the actual mechanism used to transport the material is unclear.

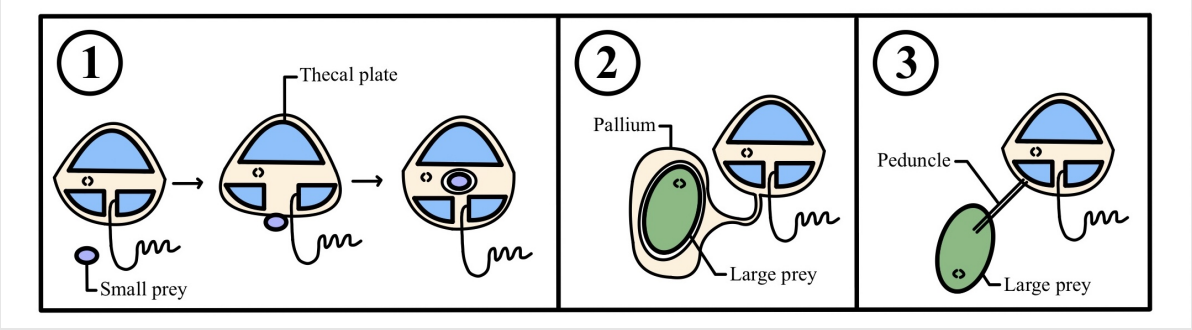


Figure 31: Dinoflagellate Feeding Strategies

10.5 Bioluminescence

Night swimmers often find themselves entranced by the magical display of bioluminescence, as their movements through the water cause it to glow and shimmer. This spectacle is caused by dinoflagellates, who according to some hypotheses use light as a defence system to ward off predators. If a dinoflagellate is agitated by predators, it oxidizes an organic compound called luciferin which emits light in a mechanism reminiscent of the one responsible for fireflies' glow. The sudden flashes of light have been shown to interfere with predators and reduce the number of dinoflagellates consumed. Additionally, the light might attract larger organisms that prey on the dinoflagellate's predator.

10.6 Red Tides

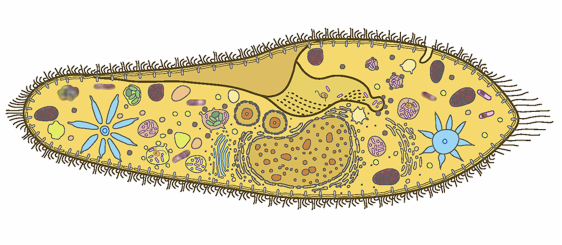
In nutrient-rich conditions, some dinoflagellates can reproduce so quickly that they form a "bloom", with a population density so high that they discolour the water in a phenomenon known as a "red tide". Some bloom-forming dinoflagellates also produce potent toxins like saxitoxin, a neurotoxin synthesized in such large quantities during a bloom that it can accumulate in shellfish, making them poisonous to eat. Others produce ciguatoxin, which is absorbed by tropical fish and causes ciguatera, a type of food poisoning and a major cause of seafood-related health problems. During

blooms, the abundance of mixotrophic algae can also lead to decreased oxygen in the water, killing some of the more sensitive marine life.

10.7 Symbiosis with Coral - *Symbiodinium*

Symbiodinium is a genus of dinoflagellates that live symbiotically with coral. Corals build their skeleton from calcium and bicarbonate in the water, each generation adding over time to a megastructure of huge ecological importance, the reef. This process is energetically demanding, and the coral can only do it because of a symbiosis with dinoflagellate partners (commonly called "zooxanthellae"). The algae provide the coral with sugars, accounting for 99% of the coral's energy, and photosynthesis performed by the dinoflagellates also consumes CO_2 byproducts from the coral's calcification process, accelerating the reaction.

11 Ciliates



Notable Genera: *Didinium*, *Euplotes*, *Lacrymaria*, *Loxodes*, *Mesodinium*, *Oxytricha*, *Paramecium*, *Stentor*, *Tetrahymena*, *Vorticella*...

11.1 Overview

Ciliates are a diverse group of heterotrophic (or, occasionally, mixotrophic) protists, with roughly 8,000 known species. Although ciliates come in many different shapes and sizes, they share three ancestral characteristics. First, ciliates have rows of cilia which they use to swim, feed, sense the environment, and sometimes "walk". Second, ciliates have nuclear dimorphism, meaning each cell has two types of nuclei: germline micronuclei and a somatic macronucleus. Third, ciliates undergo a sexual process called conjugation, where two individual cells partially fuse and exchange DNA through micronuclear karyogamy. Ciliates are found everywhere there is water and are deeply important ecologically as they provide a trophic link between animals and smaller protists. Most protists and bacteria are too small for animal predators, but are readily consumed by ciliates which are then eaten by small zooplanktonic or grazing animals. Ciliates are also essential in sewage treatment plants, where they control the bacterial population to prevent it from overgrowing.

11.2 Feeding and Digestion

Ciliates are typically voracious predators eating bacteria or other protists. Predation strategies vary widely. Ciliates can detect chemical signals to chase after their prey (raptorial feeding) or use their cilia to create water currents that drive bacteria and smaller protists into a groove of the cell leading to the site where phagocytosis occurs (filter-feeding). Others have more niche methods, like using specialized extrusomes that inject poison to stun larger and more challenging prey (including small multicellular animals), or extremely complex cytoskeletal structures strengthening their oral apparatus to "chew" on hardy food. When food is scarce, a few species of ciliates turn to an entirely new strategy: they become cannibalistic, grow into much larger forms called "giants", and hunt down ciliates of their own species.

Usually, cilia cover the majority of a ciliate's surface area, and are supported by an array of sub-membrane cytoskeletal structures (including basal bodies, microtubule bundles, and fibers) that would obstruct phagocytosis. In addition, ciliates have alveoli lined beneath their plasma membrane that act as yet another barrier. As a result, ciliates, like many other protists, have a designated area of their surface that is adapted to engulfing prey, called the cytostome.

Paramecium is a model ciliate that filter-feeds using a pit-like structure called an oral groove to trap prey near its cytostome. The beating cilia on the surface of the cell create a water current that drives prey into the oral groove, where they are filtered from the water by specialized rows of cilia, and phagocytosed. The captured prey in the feeding vacuole are subjected to a series of digestive treatments, similar to digestion in an animal gut. First, small organelles called acidosomes fuse with the feeding vacuole, lowering its pH. Next, lysosomes fuse and release digestive enzymes into the vacuole. Once all organic material has been broken down, nutrient-containing vesicles bud from the vacuole to be transferred for storage or for use. Any remaining waste is directed to the cytoproct – another cortical area with fewer underlying structures – where it is exocytosed.

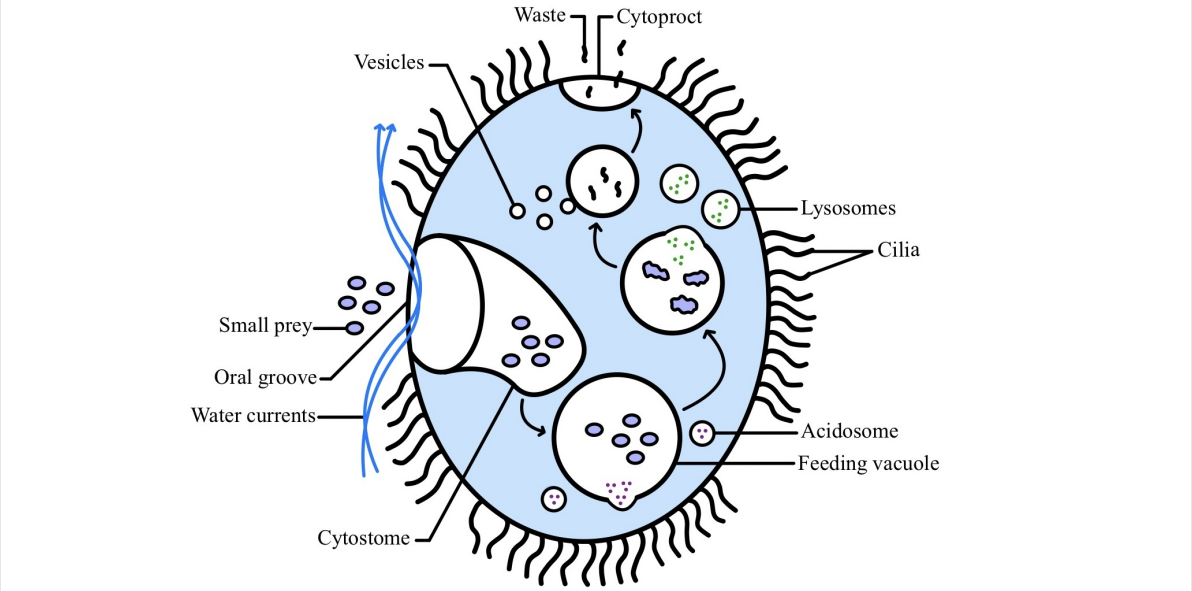


Figure 32: Digestion in *Paramecium*

11.3 The Macronucleus and Micronucleus

Ciliates have two types of nuclei, micronuclei (MICs) and macronuclei (MACs). The MICs are the germline, meaning that they are usually NOT actively expressed but their DNA is passed down to the next generation after sex, whereas the MAC is somatic, meaning it is actively expressed throughout the ciliate life cycle, but its DNA is NOT passed down to the next generation after sex. There are genetic discrepancies between these two types of nuclei. The MIC contains more unique DNA sequence but much less DNA than the MAC, and has larger chromosomes (but fewer of them). The MIC is normally diploid, whereas the MAC's ploidy varies from species to species AND from chromosome to chromosome, but is always more than 2. Finally, most of the genes in the MIC have insertions that would prevent them from expressing functional proteins. Below we will describe how a MIC can develop into a MAC, and how all these genetic changes take place, all of which is linked to sex.

11.4 Conjugation

Most of the time, ciliates reproduce asexually. However, when they are exposed to environmental stress or other cues, they undergo a sexual process, presumably to diversify their gene pool (although more complex reasons related to rejuvenation have also been advanced). The exchange and recombination of DNA is mediated by meiosis and karyogamy of the micronuclei during a process called conjugation. Conjugation begins when two compatible ciliates partially fuse and perform a series of actions that vary in details between different species, but overall include meiosis, the exchange of haploid MICs, and the replacement of the parental MAC with a new one. The model for this process has been best studied in the genus *Tetrahymena* and can be broken down into these 12 steps:

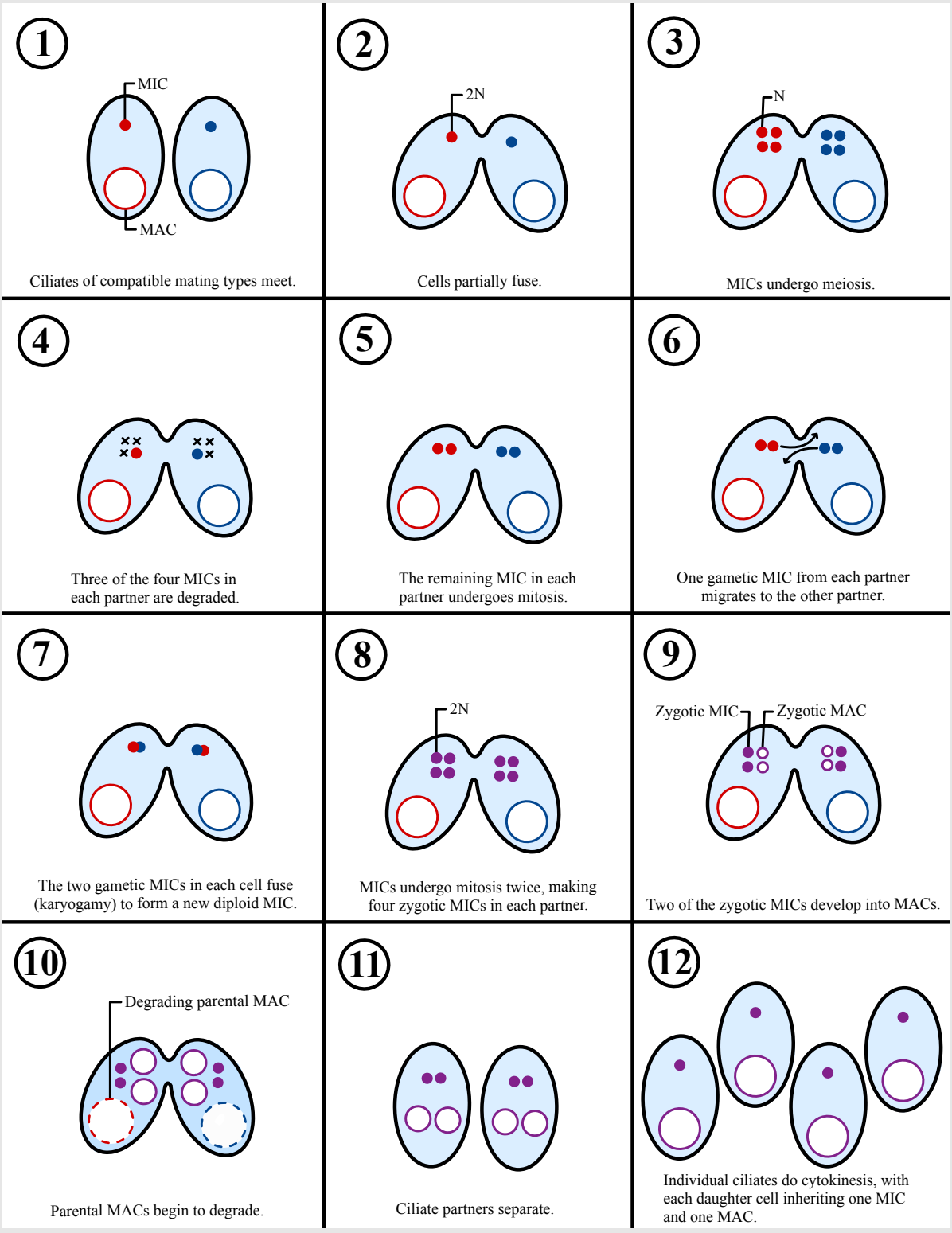


Figure 33: Steps of Conjugation

11.5 Overview of Macronuclear Development

The MIC contains many sequences of non-coding DNA called internal eliminated sequences (IESs), which are scattered throughout the MIC chromosomes, interrupting many genes and making them non-functional. During conjugation, a new MAC is derived from a MIC and a major part of this development is the removal of all the IESs from its genome. The MIC also has a few larger chromosomes whereas the MAC has many small chromosomes. This is because during MAC development the chromosomes are also fragmented, and the smaller chromosomes are then replicated multiple times. Maturation from a MIC to a MAC therefore involves three mechanisms:

1. **Deletion**: Deletion of MIC-specific, non-functional DNA sequences (IESs). Deletion involves scan RNAs (scnRNAs), which are specialized RNA molecules that help the ciliate determine which DNA sequences are IESs and which are not.
2. **Fragmentation**: Fragmentation of chromosomes into chromosomes of smaller sizes.
3. **Amplification**: Extensive replication of each fragmented chromosome. The MAC uses many copies of the same chromosome to maximize the copy number of genes to ensure there is enough expression.

11.6 Macronuclear Development in *Tetrahymena*

MAC development is different in different ciliates, but one major model is the genus *Tetrahymena*. Here are a few important notes on its overall MAC development:

- Deletion: Roughly 20% of the MIC genome is deleted.
- Fragmentation: The MIC has 5 chromosomes, each roughly 24,000 kbp long, whereas the MAC has 200 chromosomes, each about 300-800 kbp long.
- Amplification: Total DNA amount is increased by 60 times.

IESs are deleted during MAC development in *Tetrahymena* in the following series of events. Each step is quite complex and mediated by many proteins and enzymes, but we won't go into much mechanistic detail:

1. In the parental MIC, the entire genome is transcribed. The transcripts are then cut up into many small fragments called scnRNAs.
2. The scnRNAs are complexed with proteins and moved to the old parental MAC.
3. The scnRNAs base pair with the genome in the parental MAC, binding to complementary sequences, if they exist. But scnRNAs that correspond to IES sequences will not bind, as those sequences are not present in the MAC.
4. scnRNAs that successfully bind in the parental MAC are degraded. The remaining scnRNAs, which correspond to IESs, are then moved again, this time to the developing zygotic MAC.
5. In the developing MAC, scnRNAs base pair with the genome again, binding to IESs, and marking them for (protein-mediated) deletion.

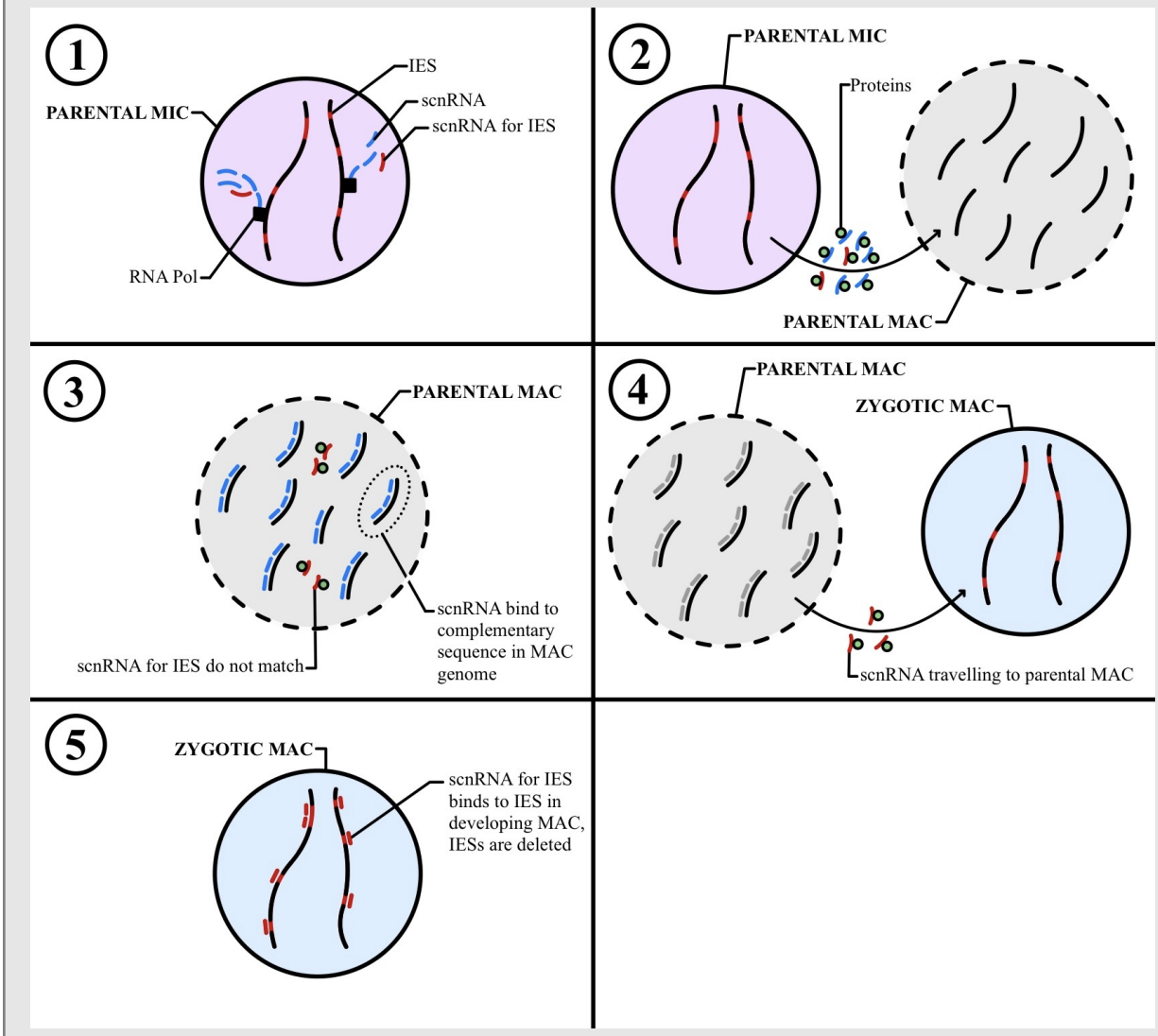


Figure 34: Macronucleus Development in *Tetrahymena*

11.7 Macronuclear Development in *Oxytricha*

To illustrate how variable the process of MAC development is, we show here how it works in another genus of ciliates, *Oxytricha*. Here are a few important notes on its overall MAC development:

- **Deletion**: Roughly 95% of the MIC genome is deleted.
- **Fragmentation**: The MIC has 3,000 chromosomes, each roughly 800 kbp long, whereas the MAC has 60,000 chromosomes, each about 2 kbp long (just 1 gene per chromosome).
- **Amplification**: Total DNA amount is increased by 1,000 times.

The following steps outline the deletion phase of MAC development in *Oxytricha*:

1. In the parental MAC, the entire genome is transcribed, resulting in long scnRNAs that correspond to entire MAC chromosomes (remember that the chromosomes in *Oxytricha* are short and mostly contain one gene each).
2. The scnRNAs are moved to the developing zygotic MAC.
3. In the zygotic MAC, scnRNAs base pair with the DNA.
4. Because the scnRNAs originated in the parental MAC, they do not base pair with IESs, so any DNA that does not bind to the scnRNAs is marked for deletion.

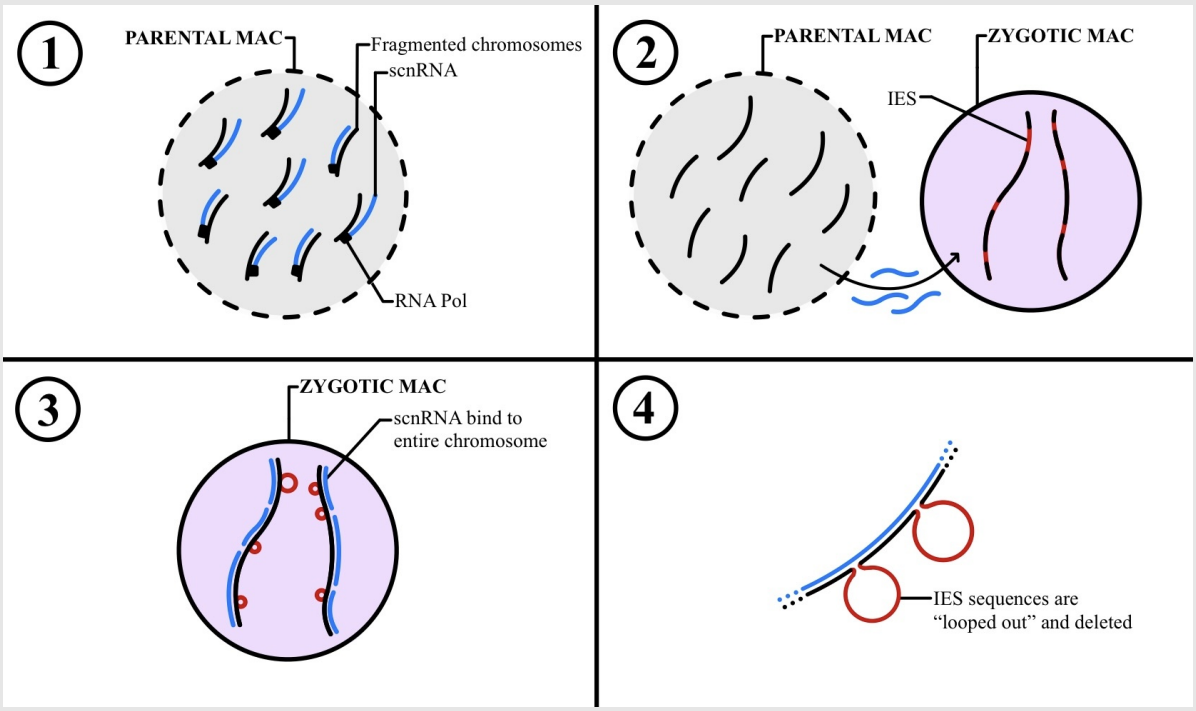


Figure 35: Macronucleus Development in *Oxytricha*

12 Foraminiferans

Notable Genera: *Allogromia, Elphidium, Globigerina, Peneroplis, Reticulomyxa. . .*

12.1 Overview

Foraminiferans — also called forams — are large, abundant, conspicuous protists. Forams typically live on the ocean floor where they feast on marine detritus; however, a few planktonic species exist that also serve important ecological roles. Many forams live in calcareous exoskeletons called tests, which serve as protection from environmental threats (but both naked forams and forams with other types of exoskeletons, especially proteinaceous and agglutinated, exist). The most complex tests are constructed from a series of hollow chambers, which are continuously added to the growing skeleton in a spiral-like pattern. Starting with a few chambers, a foram will build a new chamber off of the last, increasing the size of their test. The final chamber of a test has a small opening, through which the foram extends its reticulopodia which branch out like a net and catch (by suspension feeding) falling detritus and smaller organisms, from bacteria to protists and even small animals. Many forams host intracellular symbiotic algae, making them functionally mixotrophic. Due to their long-lasting skeletons, forams are extremely common in the fossil record. They are in fact so prevalent that the limestone bricks of the great pyramids are predominantly composed of foram tests. Because calcium carbonate structures can only form under certain conditions, these tests can be used to determine the conditions of Earth millions of years ago.

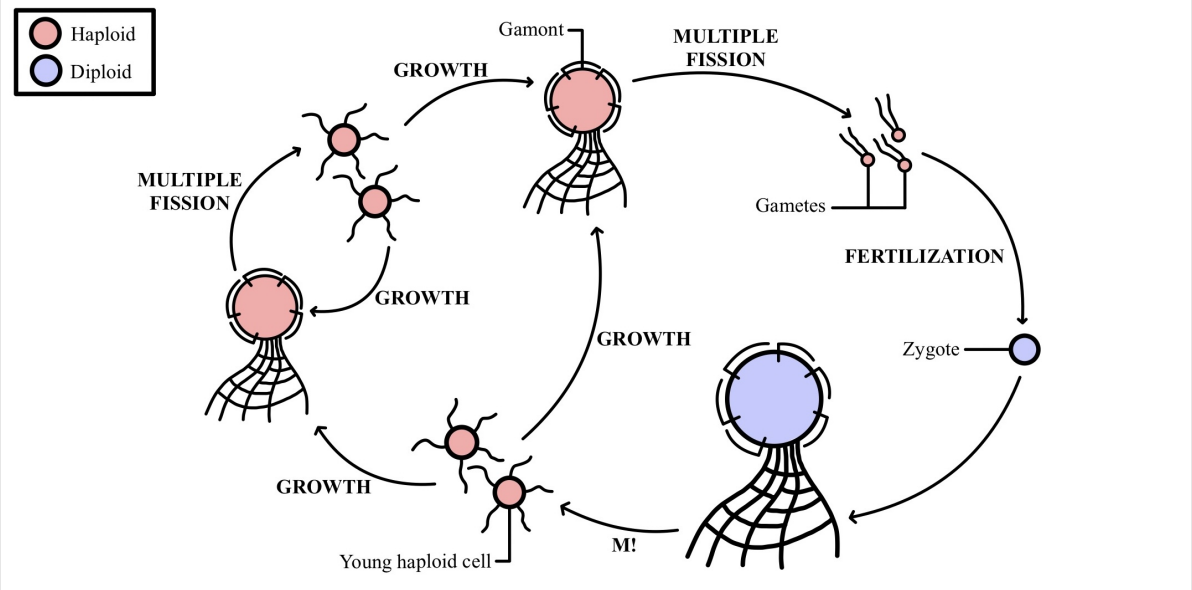
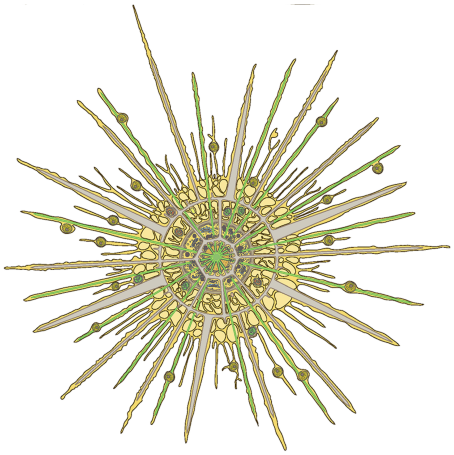


Figure 36: Example of a Foraminiferan Life Cycle (nuclei not shown)



13 Radiolarians

Notable Genera: *Collozoum*, *Dictyocoryne*, *Heteracon*, *Lithomelissa*, *Oroscena*...

13.1 Overview

Radiolarians are structurally beautiful protists with intricate skeletons built from minerals in the water column. They are often mixotrophic, consuming prey caught on their axopodia while also using photosynthetic products harvested from symbiotic algae and cyanobacteria that live in their endoplasm. Radiolarians consume a variety of prey, including bacteria, microinvertebrates, algae, and other protists. They have an organic, bilayered exoskeleton made from glycoproteins and cellulose fibres, which contains perforations that allow strands of cytoplasm to poke through and rest between the layers. These cytoplasmic protrusions form the radiolarian ectoplasm, which is important for the protist's feeding and buoyancy. Radiolarians usually have multiple nuclei.

13.2 Radiolarian Skeletons

Radiolarians have both an exoskeleton and an endoskeleton. The exoskeleton is organic, consisting of two continuous parts that form an inner and outer layer: the central capsule and the periplasmic cortex, respectively. The endoskeleton is mineral and composed of long spines that reside below the plasma membrane, although the kind of mineral varies between the major subgroups of radiolarian.

Acantharians are the most common group of radiolarians, and their spines (usually twenty per cell) are composed of strontium sulfate (also called celestite). Strontium sulphate is not especially common and is also one of the heaviest marine minerals, so it is an unexpected material for planktonic protists that rely on buoyancy to remain in the water column.

Polycystines are another group of radiolarians; their skeletons are siliceous, in the form of either spines or complex lattices with many different shapes and sizes. These lattices are commonly bell-shaped or spherical, although they can be a variety of other shapes. Polycystines are extremely abundant in the fossil record, and their skeletons make up large portions of marine sediment.

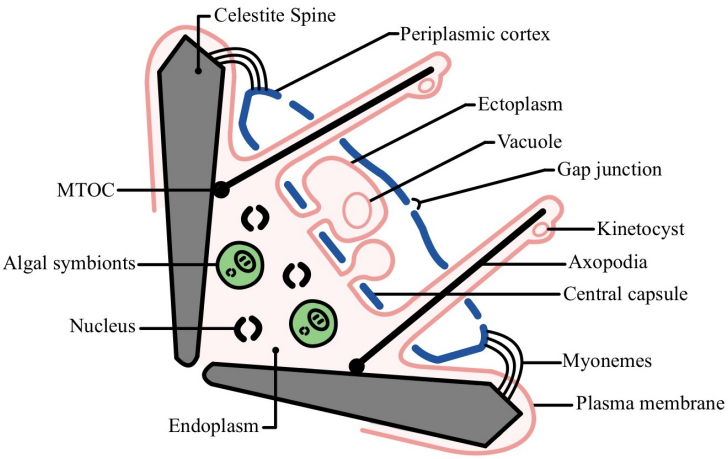


Figure 37: Acantharian Cellular Structures and Anatomy

13.3 Acantharian Feeding and Buoyancy

Acantharians use axopodia (long stiff pseudopodia supported by microtubules) to catch their prey. Bacteria, and sometimes much bigger organisms, that drift through the water column become stuck to the sticky substances extruded by kinetocysts in the axopodia, and are then shifted down the axopod until they reach the periplasmic cortex. The periplasmic cortex is divided into 20 plates (one around each celestite spine), which can be raised by contractile fibres called myonemes, opening gaps for ectoplasmic pseudopodia to reach through, contact the prey, and phagocytose it.

Acantharians counter the weight from their heavy strontium spines with numerous ectoplasmic vacuoles filled with a solution that is less dense than seawater, produced by actively pumping out heavier ions. Some acantharians periodically raise and lower all their periplasmic cortex plates together (rather than individual ones, as described for feeding), probably to refresh the seawater in closer contact with their ectoplasm. Sinking can be achieved quickly, by simply exocytosing the light solution in the vacuoles.

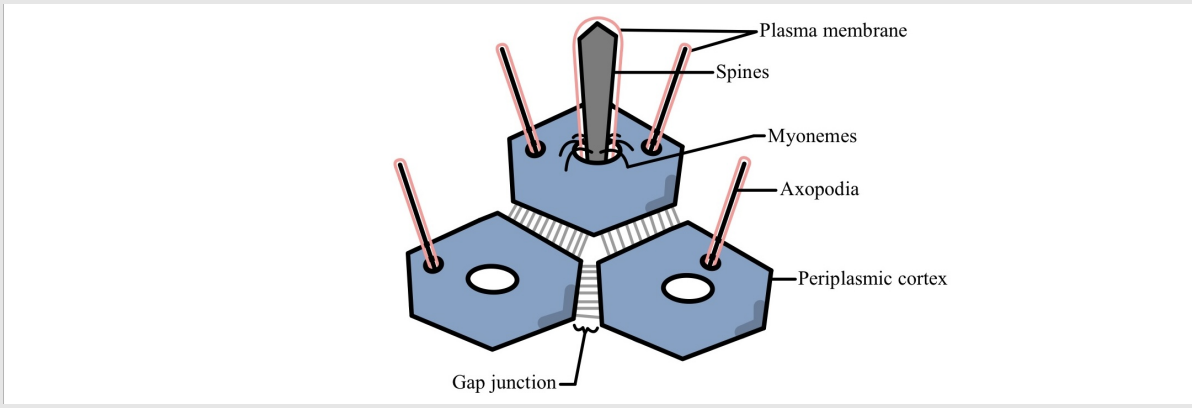


Figure 38: Acantharian Surface Morphology

14 Cercozoans

Notable Genera:

Chlorarachniophytes: *Bigelowiella*, *Lotharella*...
Vampyrellids: *Vampyrella*...
Euglyphids: *Euglypha*, *Paulinella*...

14.1 Overview

Cercozoans are a group of protists that lack any common morphological features and are united solely by molecular characters and molecular phylogenetic analyses. They are accordingly very morphologically and ecologically diverse, but are generally made up of a variety of flagellates and amoeboflagellates. They tend to be small and inconspicuous, so are not well studied, but are very abundant in marine and freshwater ecosystems where they mostly eat bacteria by phagocytosis. But other cercozoans are parasites of animals or plants, photosynthetic algae, filose amoebae with hard shells sometimes mistaken for amoebozoans, and large axopodial amoebae with elaborate skeletons that were once assumed to be related to radiolarians.

14.2 Chlorarachniophytes

Chlorarachniophytes are small mixotrophic algae. There are only a few known species, and they are marine, living in tropical or temperate waters across the globe. Different species of chlorarachniophytes have one or more of three different life stages: an amoeboid form with filopodia, a flagellated stage with a single flagellum that sometimes wraps around their body propelling them up to 10 times their cell length per second (roughly five times faster than the speedy ciliate *Paramecium*), or a coccoid cyst with a thick wall.

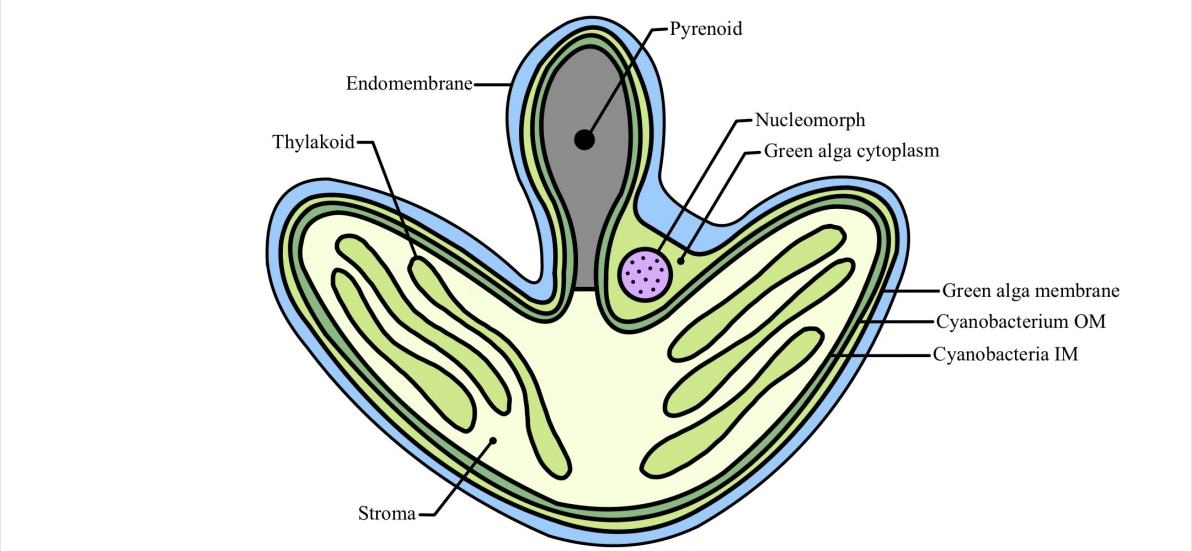


Figure 39: Chlorarachniophyte Plastid

Chlorarachniophytes are primarily studied as a model for plastid evolution. Their plastids originate from a secondary endosymbiosis with a green alga, and are distinctive because they

contain an internal organelle called the nucleomorph. The nucleomorph is the vestigial nucleus of the green algal endosymbiont that has an extremely tiny, reduced genome with as few as 300 genes and 380 kb of DNA. Surprisingly, there are many hundreds of introns in the nucleomorph of chlorarachniophytes, and they are all extremely short (18-21 bp). Nucleomorphs were important clues that helped make sense of secondary endosymbiosis and are only known to exist associated with the plastids of chlorarachniophytes and cryptomonads (see the corresponding chapter for the different characteristics of cryptomonad nucleomorphs).

14.3 The Vampire Amoeba, *Vampyrella*

Vampyrella is a genus of free-living, heterotrophic amoebae. They live in aquatic environments, lurking in the sediment where they hunt various types of algae. *Vampyrella* are famous for the unique way in which they feed: when attacking much larger multicellular, filamentous algae, *Vampyrella* pierce the wall of each individual algal cell, in turn, then scoop out the cell inside using a pseudopodium and ingest it whole. Their piercing ability has earned *Vampyrella* the nickname "the vampire amoeba".

14.4 *Paulinella chromatophora* and Chromatophores

Paulinella chromatophora is a free-living amoeba with a hard shell, or test, made from extracellular siliceous plates. It uses long filopodia to drag itself along the substrate. Unlike other testate amoebae, however, they are autotrophs that live by photosynthesis in lower light conditions, and their plastid is indeed truly special.

That's because *P. chromatophora* has undergone primary endosymbiosis with a cyanobacterium, wholly independently from the more famous event in the ancestor of archaeplastids. There have only been three known primary endosymbiosis events: one resulted in the mitochondria, one resulted in the plastid in archaeplastids, and one resulted in the chromatophore, a unique plastid found only in *P. chromatophora* (see the haptophyte chapter for a very recently discovered fourth candidate). The chromatophore is a much younger organelle than the plastid in archaeplastids. Whereas the original cyanobacterium in archaeplastids was taken up ∼1.6 billion years ago, the chromatophore is relatively young, ∼100 million years old. Chromatophores have undergone significant gene transfer to their host nucleus, but have retained many bacterial structures. For example, chromatophores have a vestigial peptidoglycan wall between their membranes and still use phycobiliproteins for their antennae.

15 Haptophytes

Notable Genera: *Braarudosphaera*, *Emiliania*, *Prymnesium*...

15.1 Overview

Haptophytes are a group of marine algae that inhabit oceans across the globe and can form large blooms, some of which are toxic. Haptophytes have a special organelle called haptonema, which is used to consume prey. Coccolithophorids, a group of haptophytes, play an important role in the global carbon cycle and include a species that is the only known eukaryote able to fix nitrogen.

15.2 Haptonema

The haptonema is an organelle unique to haptophytes that looks like a flagellum at a glance but differs in its composition, function, and structure. Haptonemata are usually supported by 6-7 microtubules, surrounded by a cylinder of endoplasmic reticulum that is continuous with the peripheral ER (a network of endoplasmic reticulum that is spread throughout the cell). The haptonema is often, but not exclusively, used for feeding: nutrient particles (prey) adhere to its surface and are transported down its length to a spot slightly above the insertion point, where particles aggregate. The aggregate is then moved to the tip of the haptonema, which bends towards the cytostome to facilitate phagocytosis.

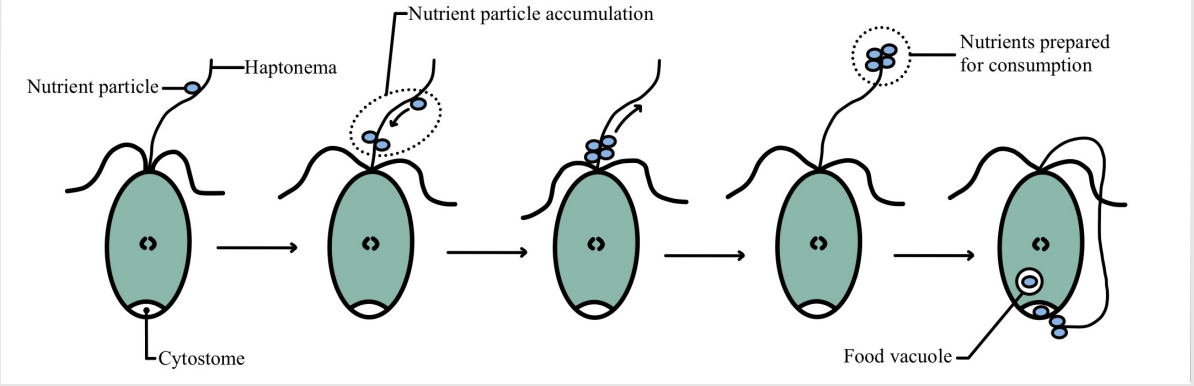


Figure 40: Haptonema Feeding Mechanism

15.3 Coccolithophorids - Life Cycle

Coccolithophorids are an abundant group of haptophytes that wear scaled armor made from calcareous plates called coccoliths that are built assembly-line fashion in the endomembrane system before being exported to the outside of the cell. These coccoliths are shed and rebuilt in different forms based on the life cycle phase the coccolithophorid is currently in: holococcoliths are usually flat, oval, and non-overlapping and are expressed during the haploid phase, whereas heterococcoliths are large, sturdy, often interlocking and/or with ridges, and are expressed during the diploid phase. Coccolithophorids have a haplodiplontic life cycle, meaning that they undergo multiple asexual reproduction cycles in both their haploid and diploid phase.

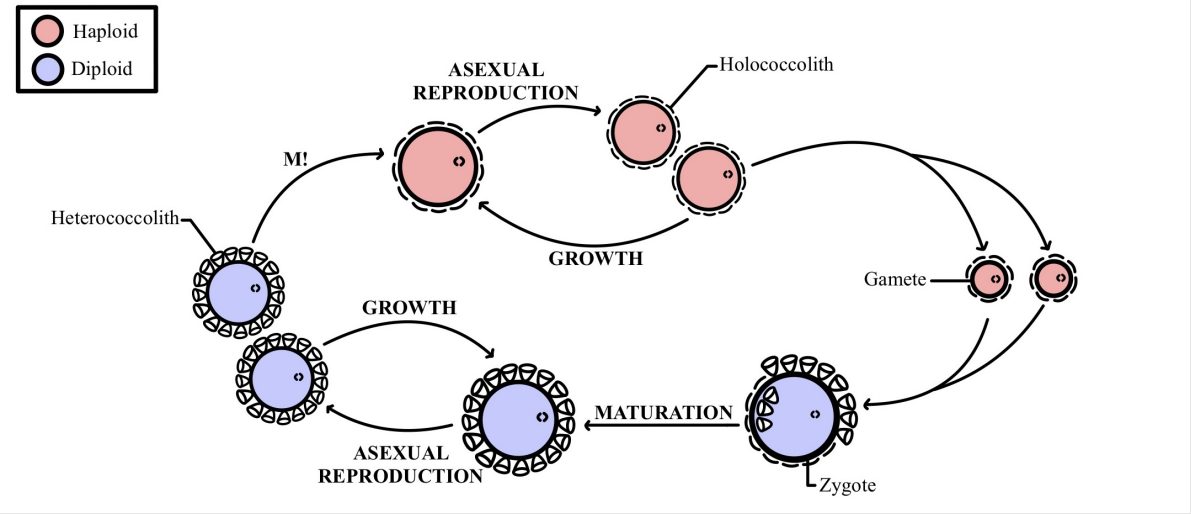
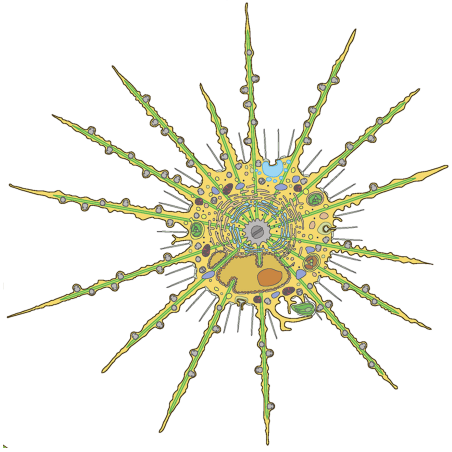


Figure 41: Typical Coccolithophorid Life Cycle

15.4 Nitroplasts

One species of coccolithophorid, *Braarudosphaera bigelowii*, is the only known nitrogen-fixing eukaryote. Their nitrogen fixation process uses a specialized organelle called the nitroplast, derived from an endosymbiotic cyanobacterium named UCYN-A, which has become genetically integrated with the coccolithophorid nucleus and is now considered to be an organelle.



16 Centrohelids

Notable Genera: *Acanthocystis*, *Raphidiophrys*...

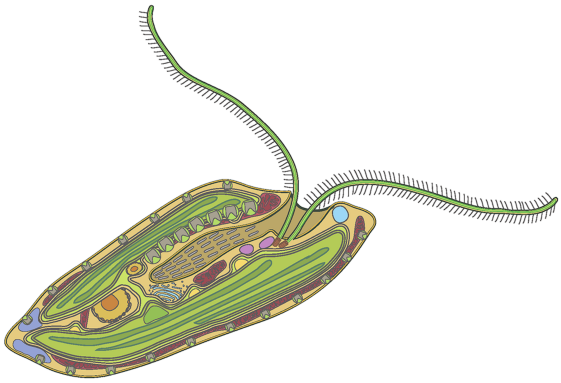
16.1 Overview

Centrohelids are aquatic, heterotrophic protists, usually 10-30 μm in size but in some instances big enough to be visible with the naked eye. The ray-like axopodia of these organisms inspired their first name, "sun animalcules", a motif that was retained when they were later renamed "centro-helids" after Helios, the Greek god of the sun. Centrohelids have a notoriously confusing taxonomic history. Initially included in the so-called "heliozoans" – a group defined as all protists with axopodia except for radiolarians – modern phylogenomic analysis has helped determine that centrohelids are the sister group of haptophytes instead. Centrohelids have some practical importance to humans. Their axopodia, which can extend and retract at rapid speed, are used as a model in microtubule polymerization and depolymerization research.

16.2 Feeding

Centrohelids are entirely heterotrophic, and use a diffusion-feeding strategy to eat smaller protists, although a few species eat bacteria. Centrohelids are mostly benthic and drift along the bottom of ponds, sometimes anchoring themselves to the sediment. When prey in the water bumps into a centrohelid, their impact triggers the release of sticky mucus excreted by kinetocysts lining the centrohelid's axopodia. Stuck to the axopodium, the prey is shifted down towards the cell body, snagged by extending pseudopodia and phagocytosed.

17 Cryptomonads



Notable Genera: *Cryptomonas, Geminigera, Guillardia...*

17.1 Overview

Cryptomonads are ubiquitous and common mixotrophic algae that live in both freshwater and marine environments. Cryptomonad cells are asymmetrical, with a deep feeding apparatus on one side and two anterior unequal flagella: one has mastigonemes on one side, while the other has mastigonemes on both sides. Cryptomonads have a unique type of extrusomes called ejectosomes, found in different parts of the cell and likely involved in different processes (e.g. feeding and predator avoidance). When cryptomonad cells are mechanically triggered, ejectosomes suddenly discharge their content, two coiled proteinaceous ribbons, producing enough force to alter the alga's swimming pattern.

17.2 Cryptomonad Plastids

Cryptomonad plastids originated from a secondary endosymbiosis with a red alga, and are distinguished in two ways. First, cryptomonad plastids have four membranes, the outermost of which is connected to the outer layer of the nuclear membrane (as is also the case in plastids from stramenopiles and haptophytes). Second, cryptomonad plastids also contain a vestigial red alga nucleus called a nucleomorph. The nucleomorph genome is severely reduced in size and numbers of genes, although slightly less so than the nucleomorph of chlorarachniophytes (see corresponding chapter), and has few if any introns.

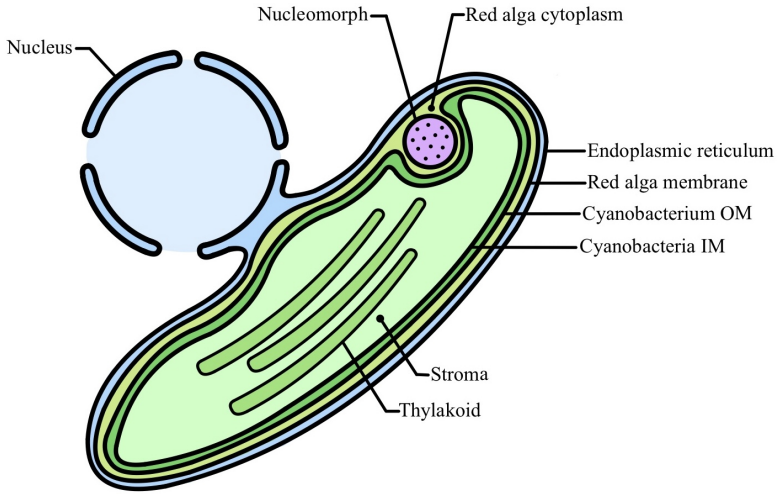
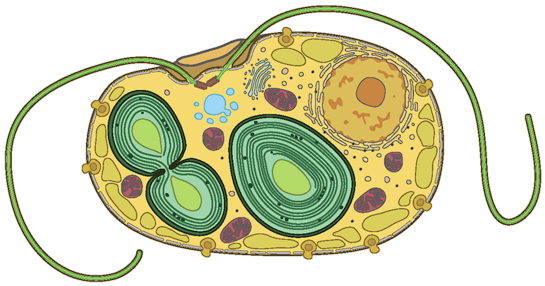


Figure 42: Cryptomonad Plastid

18 Glaucophytes



Notable Genera: *Glaucocystis*, *Gloeochaete*...

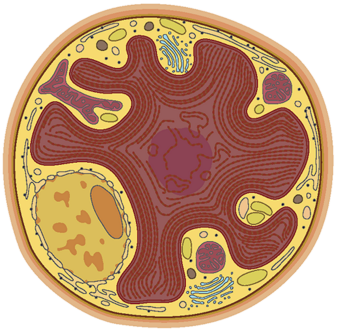
18.1 Overview

Glaucophytes are a small group of unicellular photosynthetic protists, with only about 15 known species that live in freshwater environments such as lakes, ponds, ditches, or moist soil. They are found worldwide but are usually present at low abundance. Glaucophytes have two flagella, which can sometimes be covered in small, hair-like projections (mastigonemes).

18.2 Muroplasts - Primary Plastids with Peptidoglycan

Glaucophytes have mostly been studied because they are, with red and green algae, the third algal lineage with primary plastids – i.e., members of Archaeplastida, the descendants of the original primary symbiotic event. The plastids of glaucophytes are termed "muroplasts" or "cyanelles", and are distinguished by retaining a vestigial peptidoglycan wall between their inner and outer membrane. Peptidoglycan is a compound found almost exclusively in the cell walls of bacteria, and its presence supports the theory that primary plastids originated from the endosymbiosis with cyanobacteria.

19 Red Algae



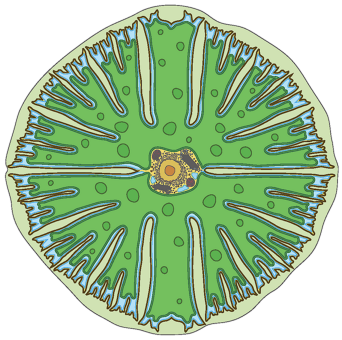
Notable Genera: *Bangia*, *Corallina*, *Cyanidioschizon*, *Gelidium*, *Porphyra*, *Pyropia*...

19.1 Overview

Red algae, or rhodophytes, are autotrophic protists that range from tiny single-celled cocci, to thin rock-hard crusts, to large leafy seaweeds. Red algae are primarily marine; however, some extremophile red algae live in sulphuric hot springs or other mildly acidic environments. Large multicellular red algae play a fundamental ecological role in intertidal zones and the understories of kelp forests, where they provide food and shelter for a variety of marine animals, and also in coral reefs, where crustose coralline red algae act as "glue" helping to hold the reef together. Red algae are surrounded by thick cell walls and coated in sticky mucilage composed of complex sugars called agar and carrageenans. Agar and carrageenans have some practical uses for humans: carrageenan is used as a thickening agent in food products, and agar is used by microbiologists as a culture medium, and as a food gelling agent in warmer climates. Red algae have a few unique features, such as reduced nuclear genomes and a complete lack of flagella in any stage of their life cycles.

19.2 Plastids

Red algae have primary plastids that are distinctive in their huge light-harvesting protein complexes called phycobilisomes, which are embedded in the thylakoid membranes and are so large that they impede the thylakoids from folding into the stacked structures seen in the plastids of other algae and land plants. Phycobilisomes are composed of phycobilins – proteins derived from the original endosymbiotic cyanobacterium – that emit red light and give red algae their distinct colour. Red algae gave origin to the secondary plastids of cryptomonads, dinoflagellates, apicomplexans, photosynthetic stramenopiles, and haptophytes.



20 Green Algae

Notable Genera: *Acetabularia*, *Chlamydomonas*, *Chlorella*, *Cladogonium*, *Helicosporidium*, *Micromonas*, *Ulva*, *Volvox*...

20.1 Overview

Green algae are autotrophic eukaryotes and the closest living relatives of land plants (technically, plants evolved from an ancestor *within* the green algae clade). They are morphologically very diverse, with unicellular forms ranging from featureless coccoid cells to flagellates with two or four flagella, and multicellular forms ranging from filaments invisible to the naked eye to large, structurally complex seaweeds in tide pools, coral reefs, and the intertidal zone. Green algae can be found in many types of marine, freshwater, and terrestrial environments. Some species specialize in niche habitats; one you might be familiar with here is *Chlamydomonas nivalis*, which grows on snowfields in cold climates, giving rise to distinctive "red snow" due to its photoprotective proteins. The secondary plastids of euglenids and chlorarachniophytes originate from secondary endosymbioses with green algae.

20.2 Ecological Importance

Some green algae act as foundation species in ecosystems, providing food and habitat for many small fish and marine invertebrates. Others – such as the cold-living snow algae, lichen symbionts, or *Chlorella ohadii* which live in desert crusts – can be important producers in extreme environments where land plants are unable to grow. Probably their main ecological impact, however, is through photosynthesis in the ocean, since a few species of very small, simple green algae are extremely widespread and reach high population sizes, fixing massive amounts of CO_2 every year.

20.3 Flagellar Gliding in *Chlamydomonas*

We usually think of flagella being used to swim, but they are also involved in a different kind of locomotion called flagellar gliding. Flagellar gliding is employed by many flagellated protists, but is best studied in *Chlamydomonas*, a unicellular green alga used as a model in genetics and cell biology.

To understand flagellar gliding, we must first understand intraflagellar transport (IFT) trains, a universal feature of eukaryotic flagella. IFT trains are complexes used to transport proteins up and down the flagellum; the transport of tubulin is especially important because the polymerization of the axoneme happens at the flagellar tip. IFT trains are carried along the axoneme by two motor proteins: kinesin (which moves towards the tip of the flagellum in what is called anterograde movement) and dynein (which moves towards the base of the flagellum in what is called retrograde movement).

Flagellar gliding uses modified IFT trains that are fused to glycoproteins in the plasma membrane surrounding the flagellum. Glycoproteins are naturally "sticky", so in *Chlamydomonas*, when dyneins act on the modified IFT trains, the glycoproteins cling to the substrate and create traction. As the dyneins move towards the base of the flagellum, this motion pulls the entire cell in the opposite direction.

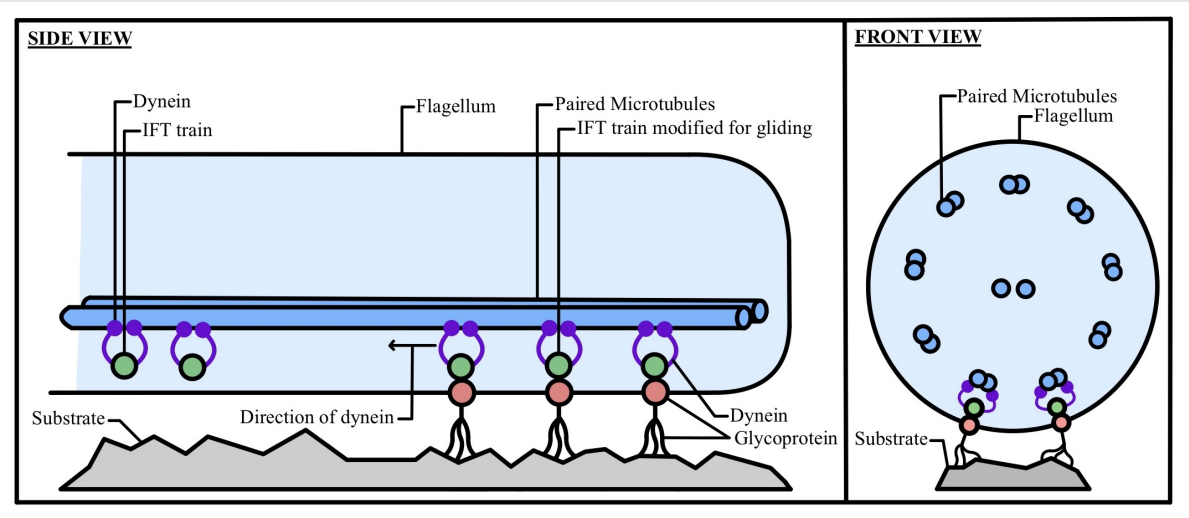
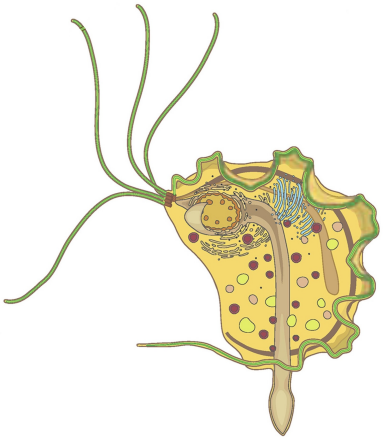


Figure 43: Flagellar Gliding in *Chlamydomonas*



21 Parabasalians

Notable Genera: *Dientamoeba*, *Mixotricha*, *Pseudotrichonympha*, *Trichomitopsis*, *Trichomonas*, *Trichonympha*...

21.1 Overview

Parabasalians are a diverse group of anaerobic protists distinguished by specialized metabolic organelles known as hydrogenosomes and the unique arrangement of their Golgi apparatus. Many species of parabasalians are symbionts that live in the gut of wood-eating insects like termites. They use their specialized metabolism to digest lignocellulose, a complex material found in wood that is indigestible for most animals. Other species are parasitic, such as *Trichomonas vaginalis*, which causes the most prevalent non-viral STD in humans. Although these two types of parabasalians differ greatly in size and morphology, they are united not only by phylogeny but by unique cellular structures.

A key structural feature of parabasalians is the "parabasal body", an organelle only found in this group that consists of long parabasal fibres (cytoskeletal elements that are made of neither tubulin nor actin) supporting the Golgi apparatus.

Some parabasalians have additional structures that are not common to the whole group: the costa, an undulating membrane, the pelta, or an axostyle are all large cytoskeletal features that can be seen in certain subgroups of parabasalians. As is the case in many flagellated protists, the nuclei of parabasalians are usually in close connection to the flagellar apparatus, in a morphological unit called "karyomastigont". Number, detailed features, or absence of karyomastigonts (i.e., cases where the nucleus and the flagella lost their tight connection) are another trait that allows to distinguish different parabasalian subgroups.

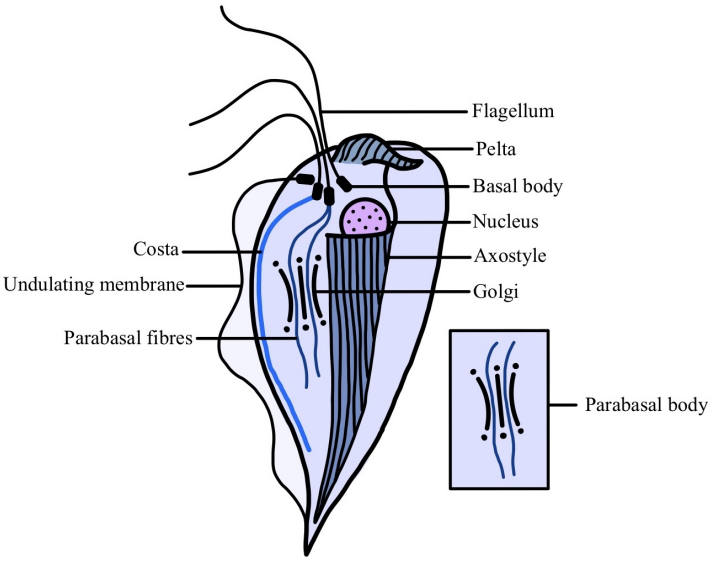


Figure 44: Anatomy of a Small Parabasalian - *Trichomonas*

21.2 Metabolism and Symbiosis

Parabasalians are often symbionts of animals, most famously in the hindgut of termites, where parabasalians play a crucial role in the digestion of wood. The digestion process begins with the parabasalian symbiont endocytosing small particles of wood that have been ground up by the chewing of their termite host. The lignocellulose in the wood is then broken down into pyruvate, which is transferred to the protist's hydrogenosome. The hydrogenosome is a reduced and highly specialized derivative of the mitochondrion, which instead of producing CO_2 and H_2O, as seen in cellular respiration, instead produces CO_2, H_2, and acetate. Like mitochondria, hydrogenosomes provide energy to the cell, but the biochemical processes involved are quite different.

Parabasalians secrete acetate, which is a high-energy compound. Termites take advantage of this, and metabolize the acetate to CO_2. Nothing goes to waste in this system, as the CO_2 made by both the animal and the symbionts and the H_2 made by the hydrogenosome are taken up by intracellular methanogens (archaea) living inside the parabasalians, who harvest these compounds for energy, with the added benefit for the parabasalian of speeding up their metabolism by taking up the end products of the hydrogenosome. Bacteria in the termite gut called acetogens (either free-living in the gut lumen or ectosymbionts on parabasalians) fix CO_2 into additional acetate for the termite (using H_2 as reducing power), also speeding up the hydrogenosome metabolism. This complex web of interdependencies is an excellent case of symbiosis in microbial communities.

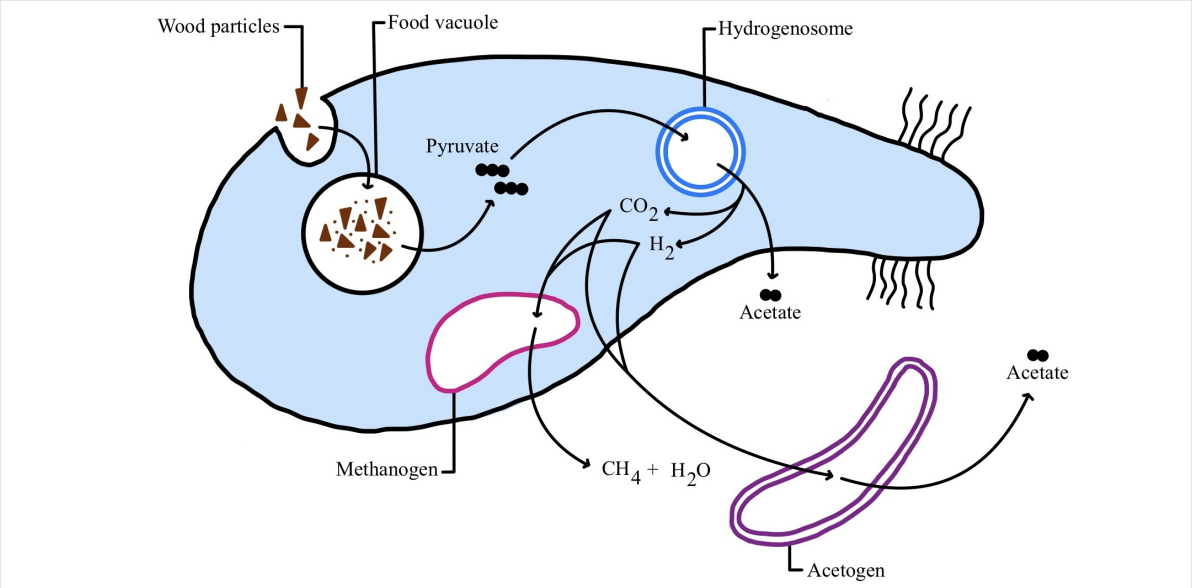
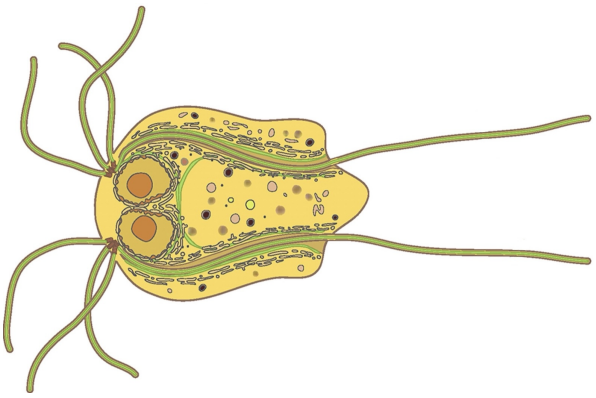


Figure 45: "Symbiotic Metabolism" of Large Parabasalians in Termites - *Trichonympha*



22 Diplomonads

Notable Genera: *Giardia*, *Spironucleus*...

22.1 Overview

Diplomonads are anaerobic and mostly parasitic protists that infect the intestinal tract of many vertebrates, with a few free-living species feeding on bacteria in anoxic habitats. Diplomonads are distinguished by having karyomastigonts with four flagella that are in most species symmetrically duplicated, resulting in mirror-image cells with two nuclei that appear to be functionally identical.

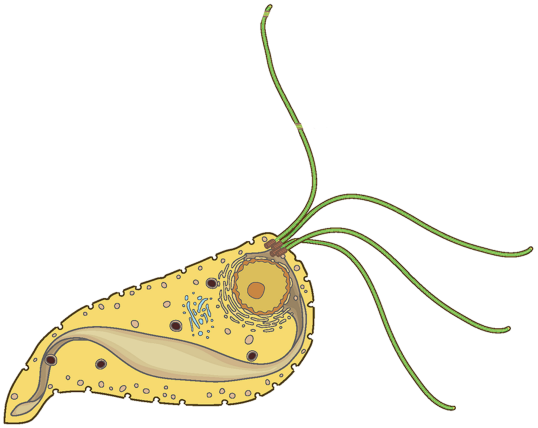
22.2 Mitosomes

Diplomonads have an anaerobic energy metabolism that relies on substrate-level phosphorylation in the cytoplasm. Their mitochondria are highly reduced "mitosomes" with no role in energy metabolism, but still able to synthesize iron-sulfur clusters required by cytosolic enzymes. Diplomonads were thought to be "ancestrally amitocondriate" archezoans, before mitosomes were conclusively proven to be relict mitochondria.

22.3 Beaver Fever

The diplomonad genus *Giardia* is a pathogen of humans and other animals that attaches to the villi of the intestine and feeds on content of the intestinal lumen, causing the disease known as "beaver fever". *Giardia* adhere to the host using a modified area of their surface, often called a "sucker", that looks like a giant suction cup on the bottom of the cell. Patients suffering from beaver fever experience weeks of diarrhea, stomach cramps, gas, and diminished nutrient absorption due to the large number of parasites blocking the uptake of nutrients. In some cases, infected individuals can develop lactose intolerance that persists long after the *Giardia* has been cleared. *Giardia* trophozoites eventually form cysts, which are excreted into the environment to infect new hosts via contaminated water.

23 Oxymonads

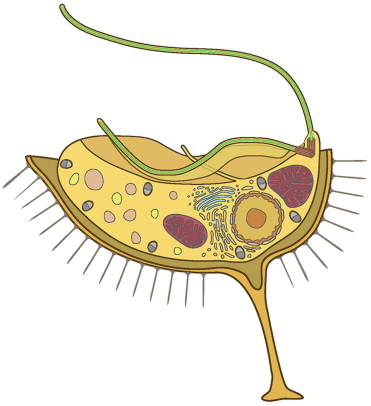


Notable Genera: *Monocercomonoides*, *Pyrsonympha*, *Saccinobaculus*, *Streblomastix*...

23.1 Overview

Oxymonads are anaerobic protists who are usually symbionts of animals. They live primarily in the hindguts of termites and wood-eating cockroaches, where they help digest the lignocellulose contained in ground-up particles of wood. Oxymonads harbour their own symbionts; they have bacteria clinging to their plasma membrane, and archaea living within their cytosol. Oxymonads are amitochondriate, but unlike the other so-called "Archezoa" they do not have mitosomes or hydrogenosomes – their mitochondria and mitochondrial genes have been completely lost. Oxymonads generate ATP exclusively from glycolysis, and have acquired alternative, cytosolic biosynthetic pathways for iron-sulphur clusters via horizontal gene transfer with bacteria. Oxymonads also have a reduced and modified Golgi apparatus: genomics has identified multiple Golgi-specific genes, but they lack any visible structure resembling a typical Golgi and are hypothesized to have other membranes perform the same tasks. Many oxymonads have a cytoskeletal structure called an axostyle, which is different and possibly unrelated to the axostyle of parabasalians, and in some species is highly contractile, causing the cell to wiggle and undulate.

24 Jakobids

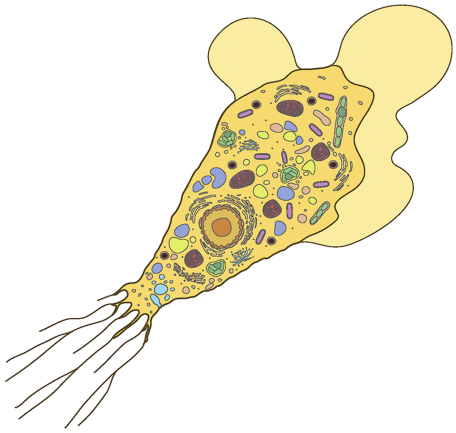


Notable Genera: *Jakoba*, *Reclinomonas*...

24.1 Overview

Jakobids are free-living, heterotrophic protists. There are only 20 known species, most of which are small in size (less than 15 μm long) and low in abundance in nature. Jakobids have two flagella: an anterior flagellum that beats freely, and a recurrent flagellum with a flattened vane that rests in a feeding groove and generates water currents for filter-feeding. Thought to specialize in environments with high densities of bacteria, jakobids sometimes anchor themselves to the substrate, occasionally particles of detritus, and waggle their recurrent flagellum, harvesting aquatic bacteria. Jakobid mitochondria have attracted attention because they possess an unusually large number of genes, with almost 3 times more mitochondrial genes than human mitochondria, and in particular retain the ancestral bacterial RNA polymerase that has been replaced with a viral polymerase in the mitochondrion of all other eukaryotes. Based on this, the jakobids were proposed to be an "ancient" lineage with ancestral mitochondrial genome structure, but they are now known to be related to euglenozoans (the clade including euglenids, kinetoplastids, and diplonemids), and other eukaryotes with large mitochondrial genomes have also since been found.

25 Heterolobosean



Notable Genera: *Acrasis*, *Naegleria*, *Vahlkampfia*...

25.1 Overview

Heterolobosean are usually amoeboflagellate, heterotrophic protists. Most have a multistage life cycle with distinct amoeboid and flagellated stages. In their amoeboid stage, heterolobosean usually have lobose pseudopodia that branch rapidly and abruptly, and long trailing uroids. The amoeboid stage can transform into a cyst or a flagellate, both of which are usually non-feeding. There are a few exceptions: some genera have flagellate forms that feed, while others lack a flagellate phase completely. For example, one heterolobosean subgroup called acrasids lives only as an amoeba, and has a multicellular stage that forms stalked fruiting bodies, like the more famous cellular slime moulds in Amoebozoa. The multistage life cycles of heterolobosean serve as a great model system for the development of flagella.

25.2 The Brain-Eating Amoeba, *Naegleria fowleri*

Naegleria fowleri are small heterolobosean that live in warm freshwater. They thrive in high temperatures, living in hot springs, lakes, and sometimes poorly maintained pools. In their amoeboid trophozoite stage, *N. fowleri* hunt small bacteria, which they grab with their pseudopodia and phagocytose. However, in rare cases they can infect humans – and when they do, the implications are serious. Infections of *N. fowleri* are lethal in more than 95% of cases. Every Summer, a few individuals diving and swimming in warm lake water accidentally inhale the amoeba through the nose, from which it makes its way into the brain. Symptoms start between one and nine days after the infection and can include fever, sore throat, and stiffness of the neck. These symptoms rapidly escalate to mental confusion, coma, and death roughly seven to ten days after infection.

The infection begins when water containing *N. fowleri* splashes into the nasal cavity. Most pathogens are overwhelmed by mucus and immune cells, while these amoebae are mostly unbothered and slide through the mucus. The olfactory nerve releases a neurotransmitter called acetylcholine – which through sheer bad luck *N. fowleri* have a wild affinity for. When the amoebae detect acetylcholine, they move toward the source, chasing increasing concentrations of the neurotransmitter in between the nerve cells and up to the olfactory bulb in the brain. Once inside the brain, the amoebae are immersed in a sea of acetylcholine, causing them to attack and eat the brain's neurons.

N. fowleri begin releasing various enzymes called proteases, which break down neurons so that they can be consumed in "bite-sized" pieces. As they feed, the amoebae begin to reproduce, rapidly increasing in number and causing damage to the brain. Although some microglia (brain immune cells) try to combat the onslaught of amoebae, *N. fowleri* quickly overwhelm the brain's natural defences. The body is forced to launch a massive immune response, directing fluid from the bloodstream containing neutrophils and other immune cells/chemicals into the brain. Unfortunately, immune cells are not perfectly precise, and they inflict serious damage to healthy neurons, meanwhile, the robust amoebae continue to consume and destroy the brain. Soon, the combination of damage from the amoebae, immune cells, and the cranial swelling causes meningitis. The brain stem, which controls breathing and heart rate, begins to fail and the infected individual will either fall into a coma or die.

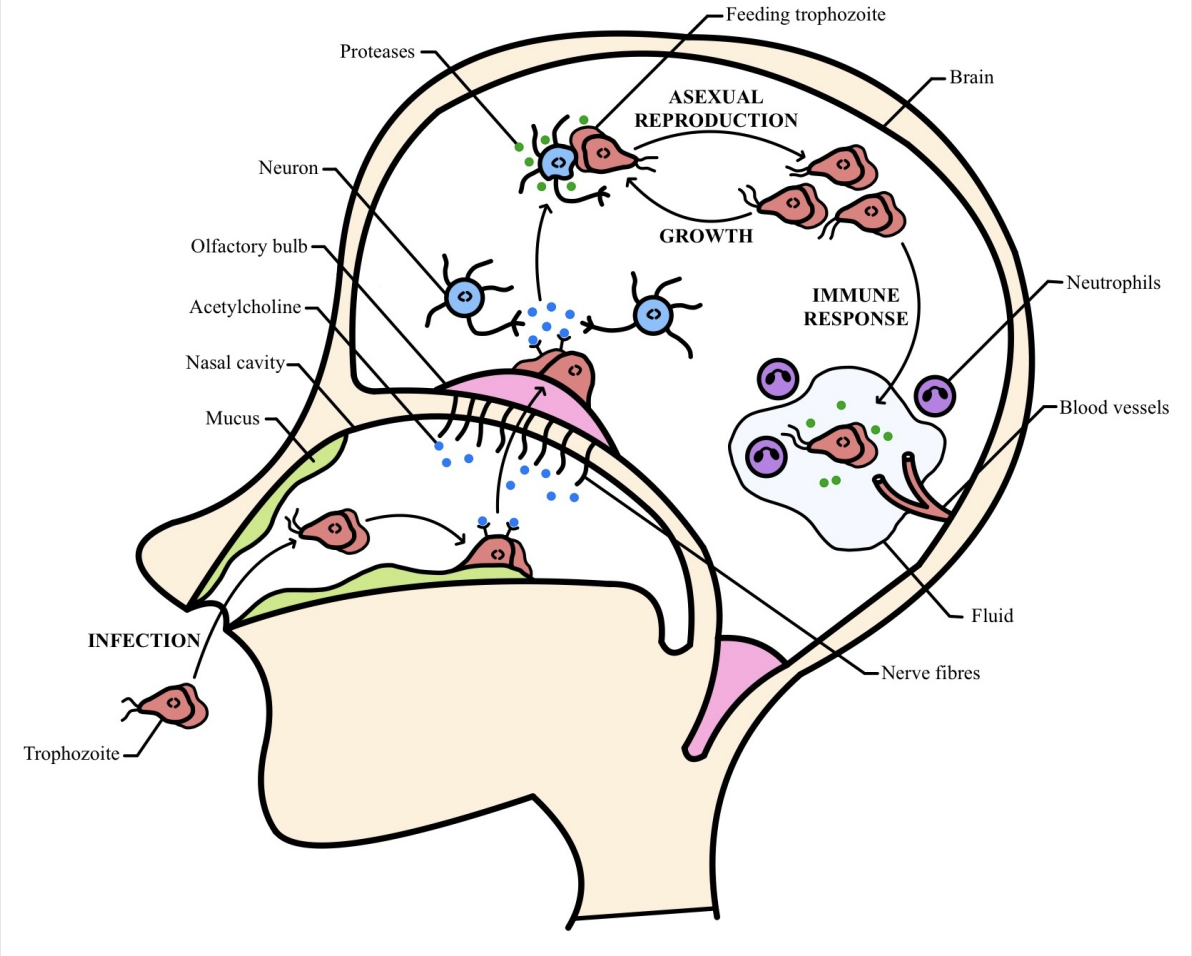
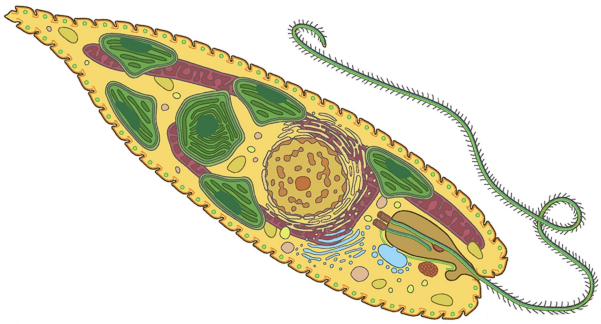


Figure 46: Infection Mechanism of *Naegleria fowleri* - Human Head Cross Section

It is important to remember that *N. fowleri* is not truly a parasite, as it does not benefit from exploiting its host. It is only by accident that these amoebae end up in the human brain, and they always end up dying with the host, as they lack the capability for transmission to another host, or back to the environment.



26 Euglenids

Notable Genera: *Euglena*, *Peranema*, *Petalomonas*, *Phacus*, *Rapaza*...

26.1 Overview

Euglenids are unicellular flagellates that can be photosynthetic, heterotrophic, or mixotrophic. They are mostly known from freshwater environments, often seen on the surface of muddy sediments or gliding between sand particles and debris, but are also common in the ocean and in the soil. Most heterotrophic euglenids eat bacteria or small protists by phagocytosis, and some feed osmotically on the cytoplasm of ruptured cells. One species, *Dolium sedentarium*, anchors itself to the substrate and is theorized to "ambush" wandering diatoms. One clade is photosynthetic, having secondary plastids originating from the symbiosis with a green alga. Euglenids have one or two flagella arising from a deep invagination of the cell, one of which often bearing mastigonemes, that can be free-beating, capable of flagellar gliding, or both. These protists also have their own unique type of movement called metaboly, which involves long proteinaceous strips that slide against one another, making the euglenid flex and elongate.

26.2 Photoreception

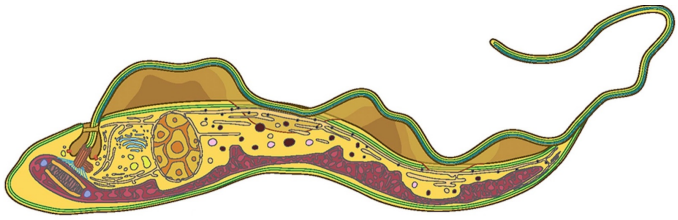
Photosynthetic euglenids can detect the direction and intensity of light using two cellular features, the stigma and a photoreceptor. These give euglenids the ability to move toward light sources and maximize photosynthesis, or conversely, to move away from harmful bright light. The stigma is composed of various orange carotenoids that absorb or reflect incoming light. It is positioned in the cytoplasm, in close proximity to the photoreceptor, which is at the base of one flagellum. As the euglenid moves around, the stigma will periodically be positioned in such a way to block incoming light from reaching the photoreceptor, similar to how the moon blocks the sun during an eclipse. This alignment tells the euglenid which direction the light is coming from relative to the cell, allowing it to change its behaviour accordingly. Many other protists, including most algae, have photoreceptors, but few are as adept at sensing the light's direction as the euglenids.

26.3 Metaboly

Euglenids have an endoskeleton called the pellicle, which rests directly below the plasma membrane and consists of long proteinaceous strips, S-shaped in cross-section, that run the length of the cell and are precisely interlocked. These strips are rigid and are used in combination with other cytoskeletal elements to drive euglenids' distinctive form of movement—metaboly. In metaboly, the euglenid cell elongates and contracts repeatedly, sort of like a slinky, sometimes in a rapid and jerky fashion, sometimes slowly and lethargically. These movements are powered by microtubules and motor proteins, which are integrated with the pellicle and slide the proteinaceous strips against each other, causing the cell to extend or contract. In some genera, the strips of the pellicle are fused, unable to slide, and in these genera metaboly is not observed. The purpose of metaboly remains uncertain, although it is theorized to play a small role in locomotion for some species, and it apparently aids in phagocytosing larger prey.

27 Trypanosomes

Notable Genera: *Leishmania*, *Phytomonas*, *Trypanosoma*,. . .



27.1 Overview

Trypanosomes are flagellated parasites with complex gene expression and a unique mitochondrial genome structure. The name trypanosome originates from the Greek "trypano", meaning borer, as they swim through fluids in a corkscrew motion using an undulating membrane. In their most commonly observed life cycle stage, trypanosomes have a single flagellum that emerges near the posterior end of the cell, extends anteriorly, forming the undulating membrane connected to the cell, and ultimately protrudes freely at the anterior end of the cell.

27.2 Examples of Diseases

Trypanosomes cause a variety of diseases in different vertebrates. The infection of *Trypanosoma brucei* in humans causes sleeping sickness, which is transmitted via a tsetse fly vector that gets into contact with the host's blood. During the chronic stage of the disease, infected individuals display symptoms of irregular sleep patterns and poor coordination. Chagas disease is caused by *Trypanosoma cruzi*, which enters the body via a bite from an insect called "the kissing bug," causing a small intracellular infection at its entry site. However, if the parasite manages to enter the bloodstream, the disease becomes life-threatening. Trypanosomes are not exclusive to humans: *T. brucei*, the same species that causes sleeping sickness in humans, also causes a neurological illness called wasting disease (or "nagana") in cows.

A different genus of trypanosomes, *Leishmania*, is carried by sand flies and causes leishmaniasis. The most deadly form of the disease, visceral leishmaniasis (also called "kala-azar" or "the Black Fever"), occurs when *Leishmania* infects macrophages. Symptoms of visceral leishmaniasis include extreme enlargement of the liver and spleen, and blackening of the skin that etches its way across the back of the infected individual. Visceral leishmaniasis has a near 100% death rate, although deaths are usually caused by co-morbidities such as tuberculosis or AIDS.

27.3 Protein Coat

Species of trypanosomes that live in the bloodstream of their host, such as *T. brucei*, cover their plasma membrane in a dense coating made by many copies of a single protein, which is frequently replaced with a new variant. This prevents the host from making conjugate antibodies to target the parasite, as antibodies matching the current coat protein variant cannot be produced fast enough before the trypanosome creates a new coat. These proteins are called Variant Surface Glycoproteins (VSGs), and to avoid running out of unique variants, trypanosomes recombine their VSG genes with thousands of pseudogenes to express new, never seen before coat proteins.

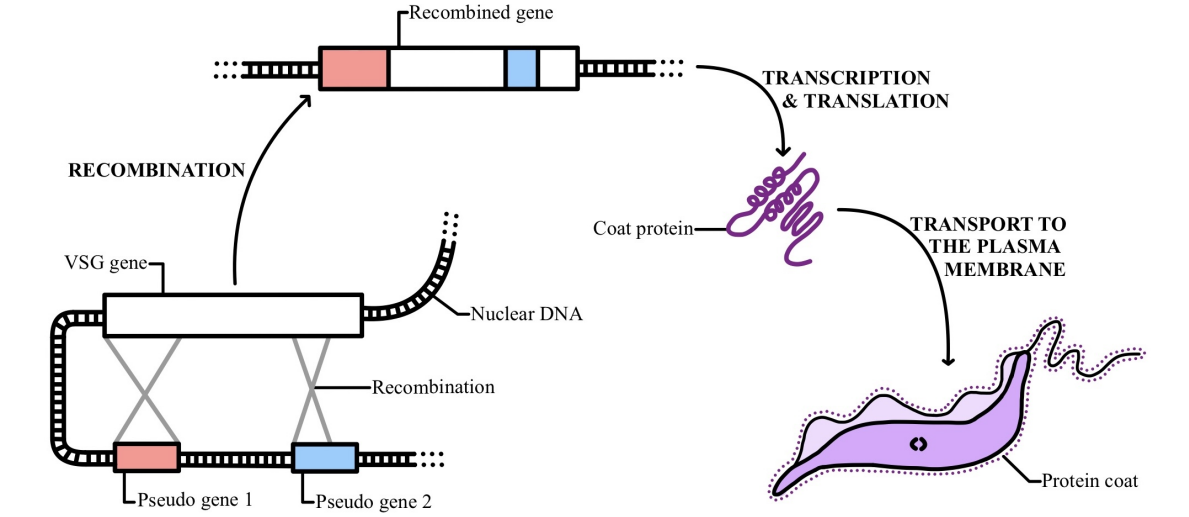


Figure 47: Recombination of VSGs with Pseudogenes - Nuclear Genome

27.4 Trans Splicing in the Nuclear Genome

Unlike most eukaryotes, the organization of genes on trypanosome chromosomes is not random; genes are arranged in large clusters on the same strand of DNA, sometimes occupying entire chromosome arms. These clusters do not have typical promoters; their transcription is initiated at locations where histones have specific modifications, and the genes in the cluster are all transcribed together to form one long polycistronic pre-mRNA. Contrary to bacterial ribosomes, eukaryotic ribosomes cannot translate polycistronic mRNA into multiple proteins. Trypanosomes get around this by something called "trans-splicing". Their genomes contain short genes that code for spliced leaders, which are small fragments of RNA that are spliced onto the 5' end of every transcribed gene by the spliceosome; the process breaks the polycistronic mRNA up into monocistronic mRNAs that can be translated.

This works much like "normal" (spliceosomal) splicing. Between each pair of genes in the pre-mRNA is a non-coding sequence that contains a central adenine (A), and a downstream adenine-guanine (AG). The spliced leader contains a central guanine-uracil (GU) sequence. Trans splicing is initiated by the central adenine nucleotide of the pre-mRNA reacting with the guanine nucleotide in the GU of the spliced leader, in a reaction called transesterification. The nucleotide preceding the guanine on the spliced leader then reacts with the nucleotide following the guanine in the AG on the pre-mRNA, covalently attaching the spliced leader upstream of an expressed gene. At the 3' end, the mRNA processing is completed by an enzyme that cleaves the unnecessary RNA; a poly-A tail is added at the cleavage site. The result is that each expressed gene is encoded on a mature mRNA equipped with the required 5' cap and a poly-A tail.

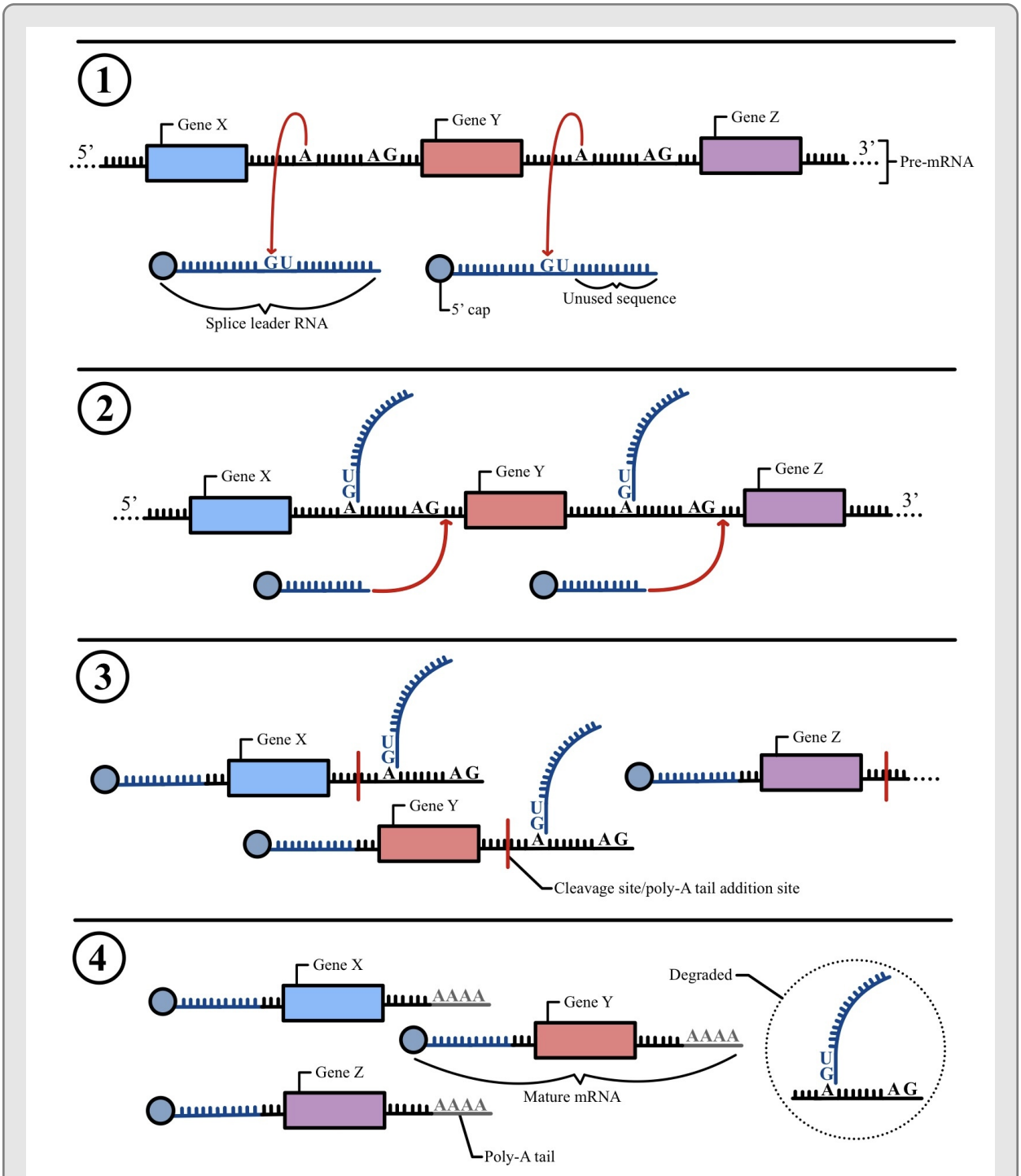


Figure 48: Trans Splicing Mechanism - Nuclear Genome

27.5 Trypanosome Mitochondrial Genome & RNA Editing

The mitochondrial DNA of trypanosomes, or kDNA, is arranged in a unique structure, called the kinetoplast. The kDNA consists mainly of a large number of small circular DNA molecules called

"mini circles" organized in a hockey puck-shaped structure, as well as a small number of larger "maxi circles". Maxi-circles encode genes, which however have mutations (sometimes many, many of them) that make them unreadable. Mini circles encode tiny "guide RNAs", or gRNAs, that are required to edit the maxi circle transcripts.

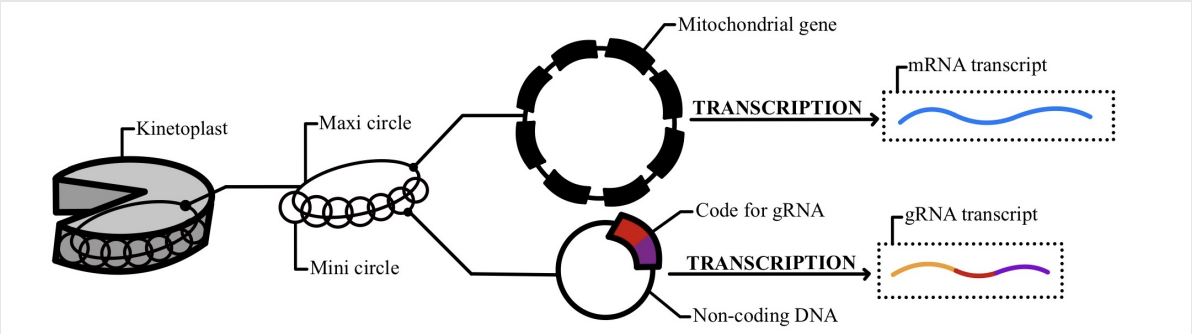


Figure 49: Flow of Information - Mitochondrial Genome

This RNA editing process in trypanosomes defies what Crick called the Central Dogma of Biology: whereas in most genetic systems information in DNA is expressed through RNA to proteins with no added information, in trypanosomes two sources of information are required to form a translatable mRNA, and that second source of information is the gRNA. gRNAs consist of three parts: from 5' to 3', they have an anchor sequence that binds to a specific region of mRNA, an editing zone that is the template for changes made to the mRNA, and a poly-U tail that attaches loosely and non-specifically to non-edited mRNA to provide stability.

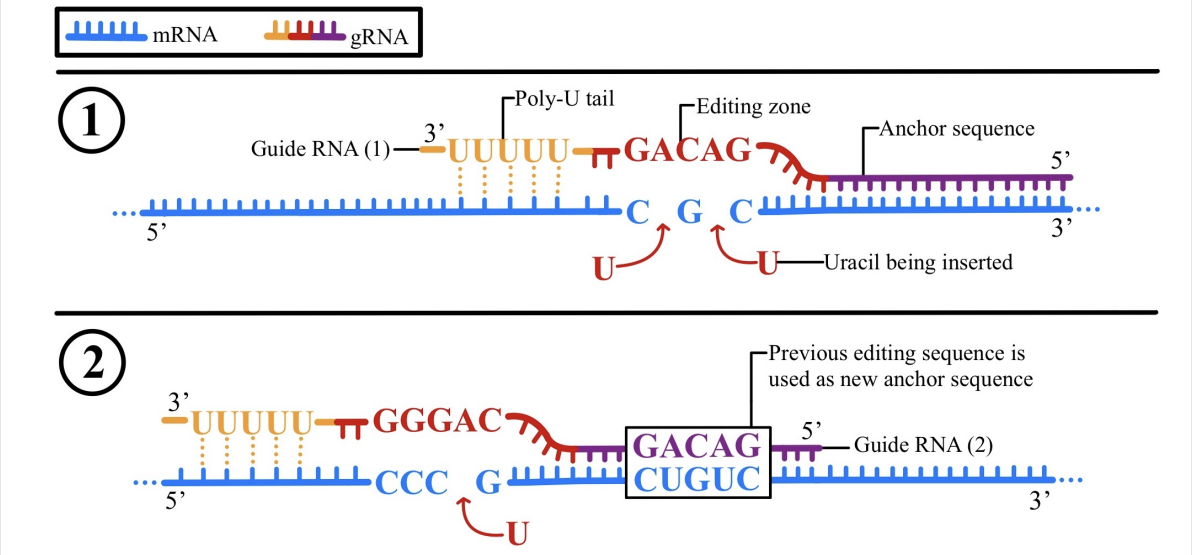


Figure 50: mRNA Editing (Insertions) - Mitochondrial Genome

1. The process begins with the first gRNA binding its anchor sequence somewhere around the 3' end of a pre-edited (or correct to begin with) portion of the mRNA. The gRNA's short

editing zone then base-pairs with the mRNA, and whenever there is a mismatch enzymes will either insert or delete a uracil residue in the mRNA until it matches the gRNA.

2. Once the editing zone is completely base-paired, the gRNA detaches and the next gRNA binds, using the newly edited section of mRNA as its anchor sequence. This process is repeated many times from the 3' end of each mRNA towards the 5' end, until the entire transcript has been edited.

27.6 DNA Replication in the Mitochondrial Genome

Trypanosome kDNA is very complex, with a few maxi circles and thousands of different mini circles that are all essential to express their mitochondrial genes. To replicate this genome, trypanosomes use a unique organizational system that requires a variety of enzymes. For example, the topoisomerase TOPO II enzyme is responsible for breaking mini circles from their chain-linked structure and reforming the chain link after the mini circles have been replicated. To determine which mini circles have been replicated and which have not, nicks left in the DNA after replication are used as an indicator to mark DNA that has already replicated. Mitochondrial mini circle DNA replication requires the following steps:

1. TOPO II releases mini circles from the kDNA network.
2. DNA in mini circles is primed by a primase, then replicated.
3. The primer is removed by a single-stranded endonuclease.
4. Primer gaps are filled by the PolB DNA polymerase.
5. TOPO II re-anneals DNA and inserts it back into the kDNA.
6. After replication is complete, all nicks are fixed simultaneously.

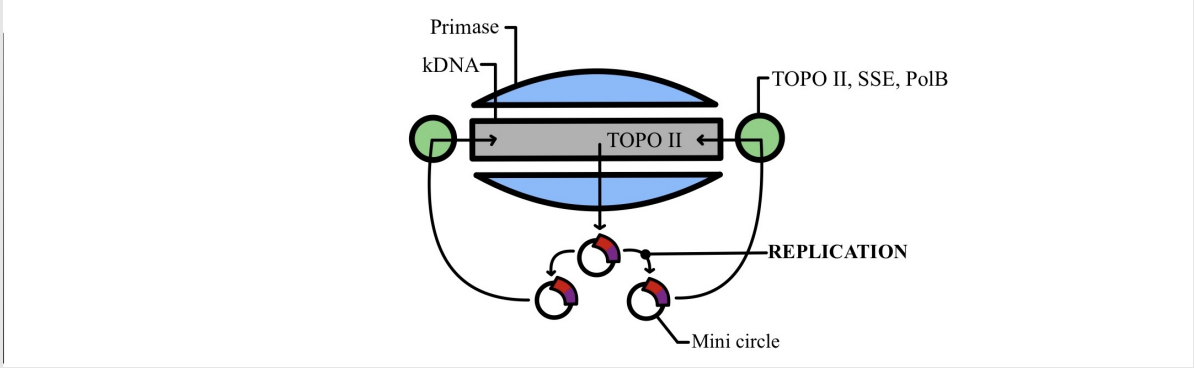


Figure 51: kDNA Replication - Mitochondrial Genome

28 Diplonemids

Notable Genera: *Diplonema*, *Rhynchopus*...

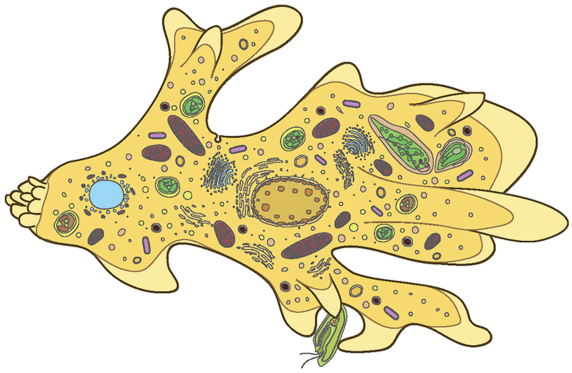
28.1 Overview

Diplonemids are marine and (less commonly) freshwater heterotrophic protists which consume bacteria by phagocytosis. They have two flagella, which sprout from a groove at one end of their smooth and otherwise unremarkable surface. Diplonemids are extremely understudied, with less than a dozen described species, but marine environmental surveys, especially of the deep ocean, suggest that a particular group of diplonemids is actually very abundant and diverse, with thousands or tens of thousands of mysterious species living in this seldom-sampled habitat. This discovery has led many to hypothesize that diplonemids play a fundamental role in deep ocean ecology. Some studies also suggest that certain diplonemids have parasitic characters, infecting protists such as dinoflagellates and other planktonic organisms.

28.2 Mitochondrial Genome and Scrambled Genes

Diplonemids have huge mitochondrial genomes – comparable in DNA amount to their nuclear genomes despite having a "conventional" small set of genes – which are organized into mini circles in a similar way to kinetoplasts (e.g., trypanosomes). Diplonemids are the sister group of kinetoplasts, which may account for their unusual mitochondrial DNA; however, the mini circles in diplonemids have a different structure, and they also have an additional layer of complexity, since all their genes are heavily fragmented, without any "full-length" version in a maxi circle. Each diplonemid mitochondrial gene is scattered amongst many minicircles, with some gene fragments even overlapping on a single minicircle. Diplonemids implement a complex form of trans-splicing that uses biomolecular machinery unseen in any other eukaryote to piece their genes back together post-transcriptionally. After re-assemblage, the transcript undergoes uracil substitutions – like in kinetoplasts, but with a different mechanism – as well as other kinds of RNA editing unique to diplonemids' mitochondria.

29 Amoebozoans



Notable Genera: *Acanthamoeba*, *Amoeba*, *Arcella*, *Chaos*, *Dictyostelium*, *Entamoeba*, *Pelomyxa*, *Physarum*...

29.1 Overview

First, we need to distinguish the term "amoebae" from the "ameobozoans". The word "amoeba" (or "amoeboid") refers to cell with pseudopodia. The "amoebozoans", on the other hand, are a specific taxonomic group that contains many, but not all, amoebae.

Amoebozoans are heterotrophic amoeboid protists, usually bearing multiple thick pseudopodia. Many are naked, while others have scaled organic exoskeletons or mineral shells with an opening for their pseudopodia to protrude. Amoebozoans can be very large: some species of the genera *Chaos* (also called the giant amoeba) grow up to 5 mm in length, while their cousins *Fuligo septica* form giant multinucleate slime moulds that cover several square meters of forest floor. Amoebozoans are diverse and occur in a variety of habitats, including the ocean, freshwater systems, and the soil, where they are among the most important heterotrophic protists. Most amoebozoans require a substrate to "crawl" on, so they are generally benthic, although a few species are amoeboflagellates and can swim in the water column. Some amoebozoans are parasitic, including some of the anaerobic archamoebae who were historically included among the "Archezoa". Archamoebae have highly reduced mitochondria called mitosomes whose only function is the synthesis of Fe-S clusters. Amoebozoans use their pseudopodia to move, but also catch prey, which can be bacteria, eukaryotes, or both. Pseudopodia are extended outwards from the body and wrapped around their prey, which is phagocytosed. Naked amoebozoans (like other amoeboid organisms) don't have complex sub-membrane structures and can ingest their prey from most of their body surface, making them among the few protists who do not require a specialized feeding area (i.e., a cytostome).

29.2 Amoeboid Anatomy and Pseudopodia

Amoebozoans have two types of cytoplasm: endoplasm, which is granular, fluid, and contains the organelles, and ectoplasm, which is thick and gel-like. The ectoplasm acts as a firm but flexible casing lining the watery endoplasm. The difference in viscosity between the two kinds of cytoplasm plays a major role in the locomotion process of amoebozoans, but there is no membrane separating the ectoplasm and endoplasm.

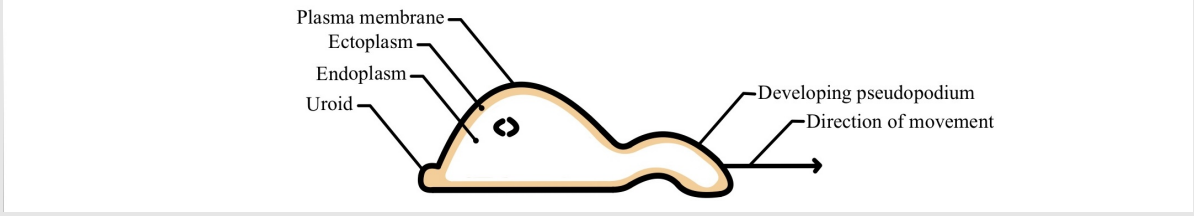


Figure 52: Anatomy of a Naked Amoebozoan

All amoebae have pseudopodia (in fact, amoebae are defined as eukaryotes who change their shape with the formation or retraction of pseudopodia). Amoebozoans usually have lobose pseudopodia (or lobopodia), which are blunt, round finger-like projections of the cell used for movement and food collection, supported by actin microfilaments.

29.3 Amoeboid Locomotion

Most protists are somewhat distinguished by their characteristic shape: acantharians are spherical and spined, dinoflagellates are plated and pointy, and trypanosomes resemble worms with a fin and a tail. Even simple disks or spheres tend to stay being disks or spheres. Amoebozoans are different, particularly naked amoebozoans, because they are almost always shapeshifters and fluid in their form. These protists move by flowing into their growing pseudopodia, with each new pseudopodium expanding and sprouting new projections, while the trailing cell membrane is collected by vesicles and transported to the front of the cell.

Amoeboid locomotion relies on four mechanisms. These mechanisms do not necessarily happen in the order listed here, and different amoebozoans may use exclusively or preferentially only some of them.

1. **Branching actin**: As described before, actin polymerizes at a specific site below the plasma membrane, pushing the membrane outwards forming a blunt projection – a lobopodium.
2. **Endoplasmic flow**: As the pseudopodium develops, watery endoplasm flows into it. This flow is driven by the posterior ectoplasm contracting, which squeezes the endoplasm like a tube of toothpaste into the new pseudopodium.
3. **Membrane transport via vesicles**: A growing pseudopodium requires new membrane material. Vesicles are formed at the uroid (the posterior "tail-end" of an amoebozoan) and travel through the endoplasm, fusing to the front of the pseudopodium.
4. **Anchor proteins**: Amoebozoans use anchor proteins to attach to the substrate. Anchor proteins are membrane proteins that adhere to the substrate, stabilizing the amoebozoan as it moves across the surface. Anchor proteins are detached from the substrate when their section of the amoeba needs to move.

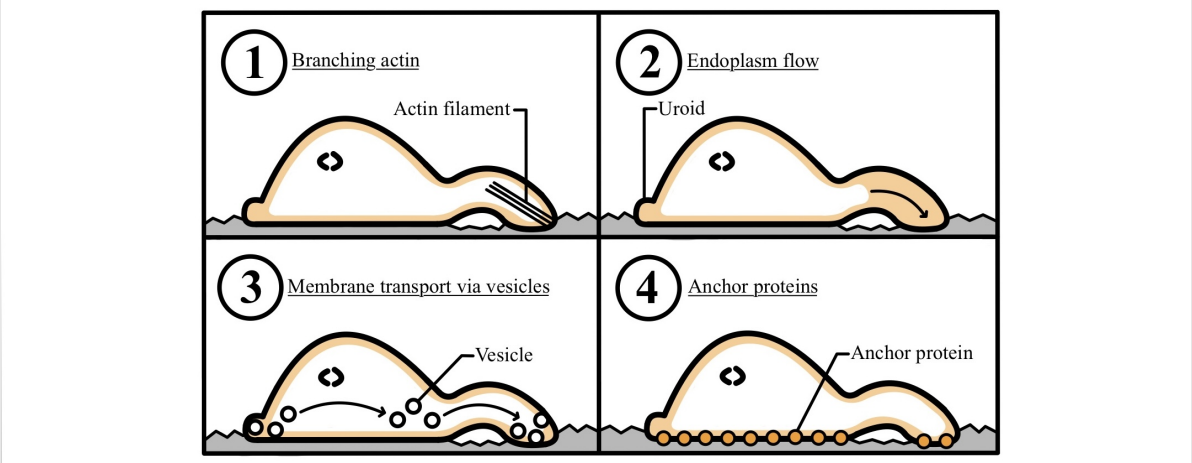


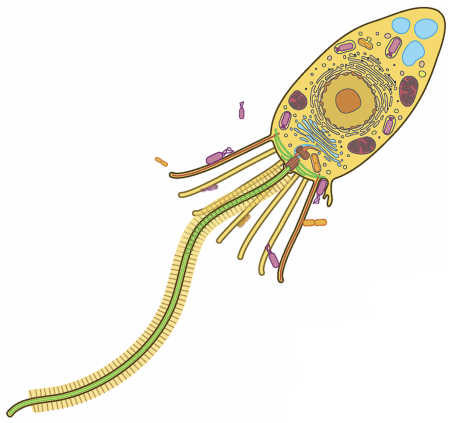
Figure 53: The Four Mechanisms of Amoeboid Locomotion

29.4 Slime Moulds

Some amoebozoans can form a plasmodium. A plasmodium is a cell that replicates its nuclei and increases in size without undergoing cytokinesis, not to be confused with the apicomplexan genus

Plasmodium. Plasmodial slime moulds can look different depending on the species: *Physarum polycephalum* spreads out in a webbed array over decomposing logs, while *Fuligo septica* forms foamy blobs that resemble piles of dog vomit. Slime moulds are not detritivores, instead they harvest bacterial decomposers that themselves infest the rotting wood. Plasmodial slime moulds are proficient at detecting chemical signals from the most nutritious food and are extremely efficient at plotting their webbed networks. A team of researchers inoculated *Physarum polycephalum* on a map of Tokyo with food sources at the site of each major subway station. When they came back the next day, the slime mould had grown a web that matched the Tokyo subway system.

Other amoebozoans, such as *Dictyostelium*, are called "cellular" slime moulds and are not plasmodial. *Dictyostelium* are usually free-living and unicellular soil amoebae, but during food scarcities, they join together to form an aggregate multicellular mass that even undergoes tissue differentiation. Initially, a few hundred amoebae will form "slugs" (or "grex") that move quickly through the soil, finding other aggregates to form an even larger mass made of thousands of amoebae. When an adequate size is reached they become stationary, growing fruiting bodies where a small fraction of the cells have sex, form cysts, and disperse spores in the wind. Notably, most of the *Dictyostelium* sacrifice themselves in the formation of the fruiting body stalks, helping their amoebae comrades but failing to reproduce in the process.



30 Choanoflagellates

Notable Genera: *Acanthoeca*, *Codosiga*, *Salpingoeca*...

30.1 Overview

Choanoflagellates are a small group of mostly marine, heterotrophic protists that feed on bacteria. Since bacteria are not readily consumed by higher trophic levels, choanoflagellates play an important role in the microbial loop by bridging the gap between bacteria and larger heterotrophic protists. The choanoflagellate morphology is reminiscent of that of sponge cells called choanocytes: both have a round cell body, a microvillar cone (called collar), and a single flagellum. Sponges also feed in an identical fashion to choanoflagellates, but on a multicellular scale, with clusters of filter-feeding cells. This similarity hints at the choanoflagellates' placement on the tree of life: they are the closest known relatives of animals.

30.2 Feeding

Choanoflagellates are filter feeders that harvest free-swimming bacteria. Whereas most eukaryotes use their flagella for motility, some choanoflagellates anchor themselves with a stalk and beat their flagellum to create water currents that direct the prey into their collar. The choanoflagellate collar consists of many tightly packed microvilli which filter bacteria and other nutritious debris from the water column. Captured particles are then shifted down the microvilli, where they are phagocytosed. Other choanoflagellates form colonies that play a function similar to that of the stalk; with each flagellum pointing outwards, the currents generated by their beating cancel out, allowing the colony to remain stationary and attract prey to its center.

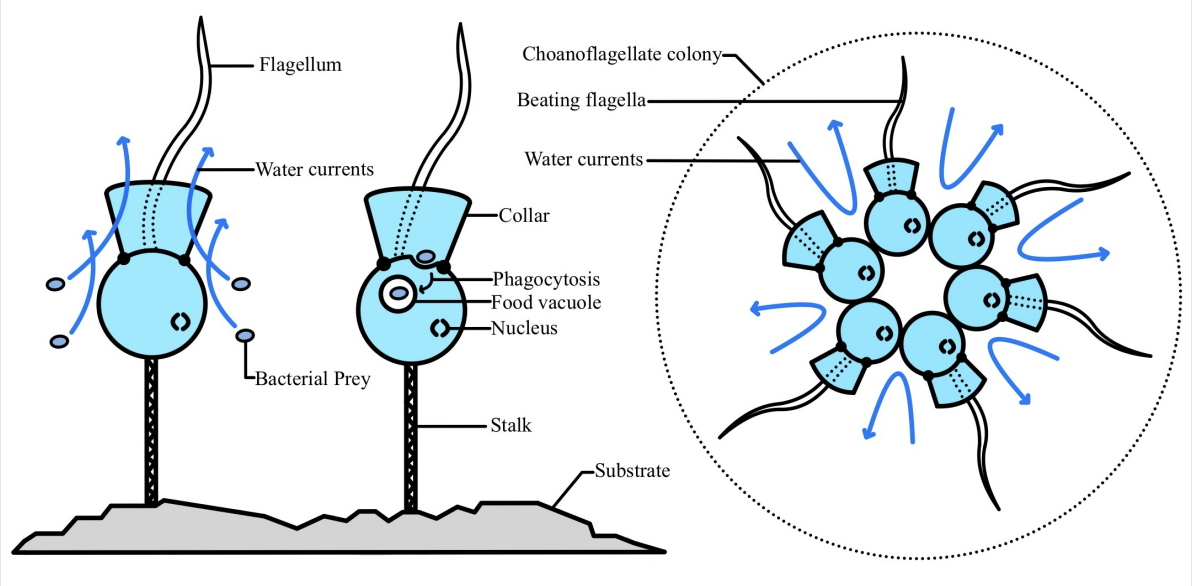
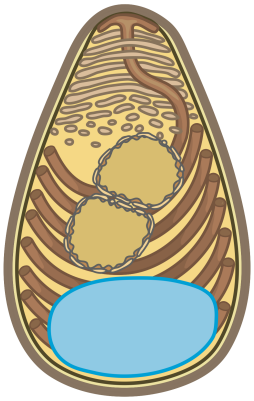


Figure 54: Choanoflagellate Feeding and Colonies



31 Microsporidians

Notable Genera: *Edhazardia*, *Encephalitozoon*, *Enterocytozoon*, *Mitosporidium*, *Nosema*...

31.1 Overview

Microsporidians are a group of obligate intracellular parasites. They can only grow and divide inside another cell, and infect a wide variety of animals. A few microsporidians infect non-animal hosts, and some are called hyperparasites because they infect and live inside apicomplexans, who are themselves parasites of animals. Microsporidians are highly reduced in many ways; for example, they have modified mitochondria called mitosomes which have lost all energy-related functions and are maintained only to synthesize Fe-S clusters. Indeed, some microsporidians have lost all ATP-generating pathways, and have evolved the ability to steal ATP directly from their host. But in their infection mechanism, microsporidians are incredibly complex; they have a life cycle with a proliferative phase focused on growth and reproduction, a sporogonic stage during which they form spores, and an environmental stage where they leave their host and infect new ones. In their environmental stage, microsporidians exist as durable spores that can be found in terrestrial, marine, and freshwater environments. Microsporidian cells are so unusual that for a long time it was difficult to see how they were related to other eukaryotes: historically they were classified with various other kinds of parasites or with other anaerobes (in the "Archezoa" hypothesis). Molecular phylogenetics has now shown that microsporidians are closely related to fungi.

31.2 Infection Mechanism

The best known infection mechanism of microsporidians involves a number of prominent structures in the microsporidian spore. Infectious spores are protected by a double-layered wall around their plasma membrane: the outer protein layer is called the exospore, and a thick inner layer made of chitin is called the endospore. The endospore is thinner at the anterior pole. The cytoplasm is packed with a variety of infectious structures and organelles, the most prominent of which is the polar filament, a long, thin membrane-bound organelle that is coiled inside the spore like a spring and is attached to an anchoring disk near the apical end. Also in the anterior part of the spore is the polaroplast, which is composed of stacks of membranes; at the other end there is a large posterior vacuole.

Microsporidian spores are triggered to germinate by cues that are mostly unknown but likely variable, since these parasites can infect many different kinds of tissue. Germination begins when the parasite pumps water into its posterior vacuole, which swells and raises the intracellular pressure. Held together by the thick spore casing, the vacuole continues expanding, building pressure until the wall finally ruptures, invariably at the thinnest part of the wall, at the apex of the spore.

Within a fraction of a second, the wall fails at the apex, the internal pressure forces the polar filament to rapidly emerge from the spore, everting (turning inside out) as it does. As it everts, the polar filament becomes a polar tube, and because it ejects so quickly it can pierce the plasma membrane of a nearby cell, potentially a host cell. The pressure then drives the microsporidian cytoplasm – including the nucleus and most of its other organelles – through the polar tube. Because the polaroplast lays closest to the apex it is pushed into the tube and emerges from its end first, becoming the plasma membrane of the parasite cell when the rest of the cytoplasm is forced through the tube and emerges inside the host. It is not unlike blowing a soap bubble with your breath: the polaroplast membrane is the film of soap and the cytoplasm fills it like air fills the soap

bubble. The microsporidian is now intracellular and can live out the rest of its life cycle, growing, reproducing, forming new spores, and exiting the host to start the process all over again.

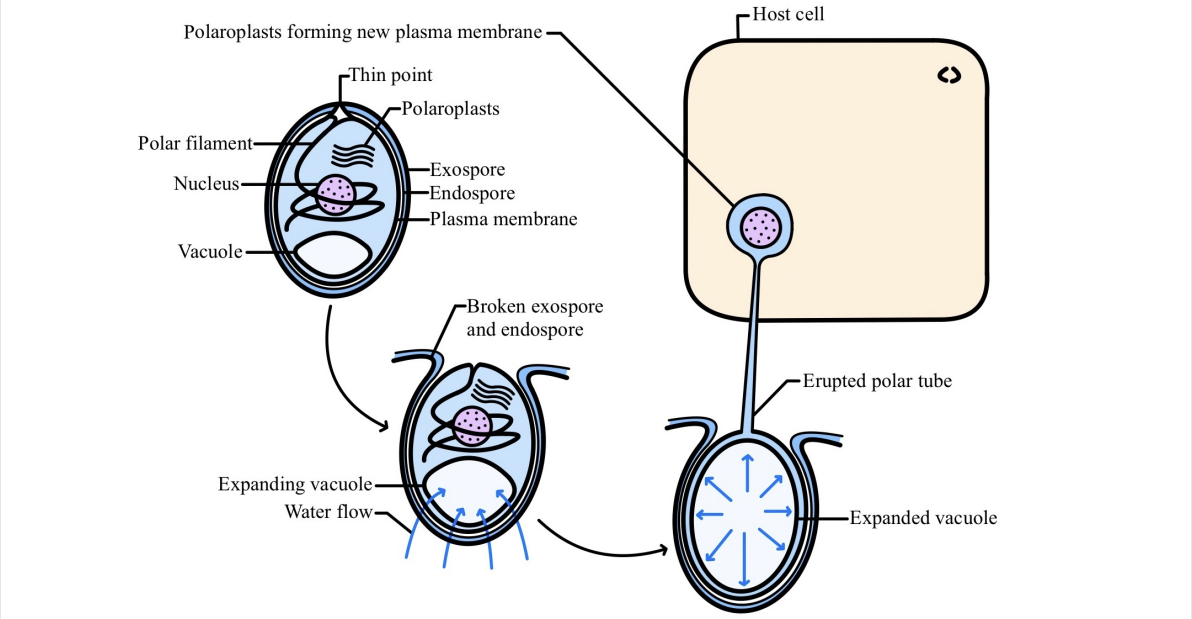


Figure 55: Infection Mechanism of Microsporidians