Biological Processes

Romelia Clinton

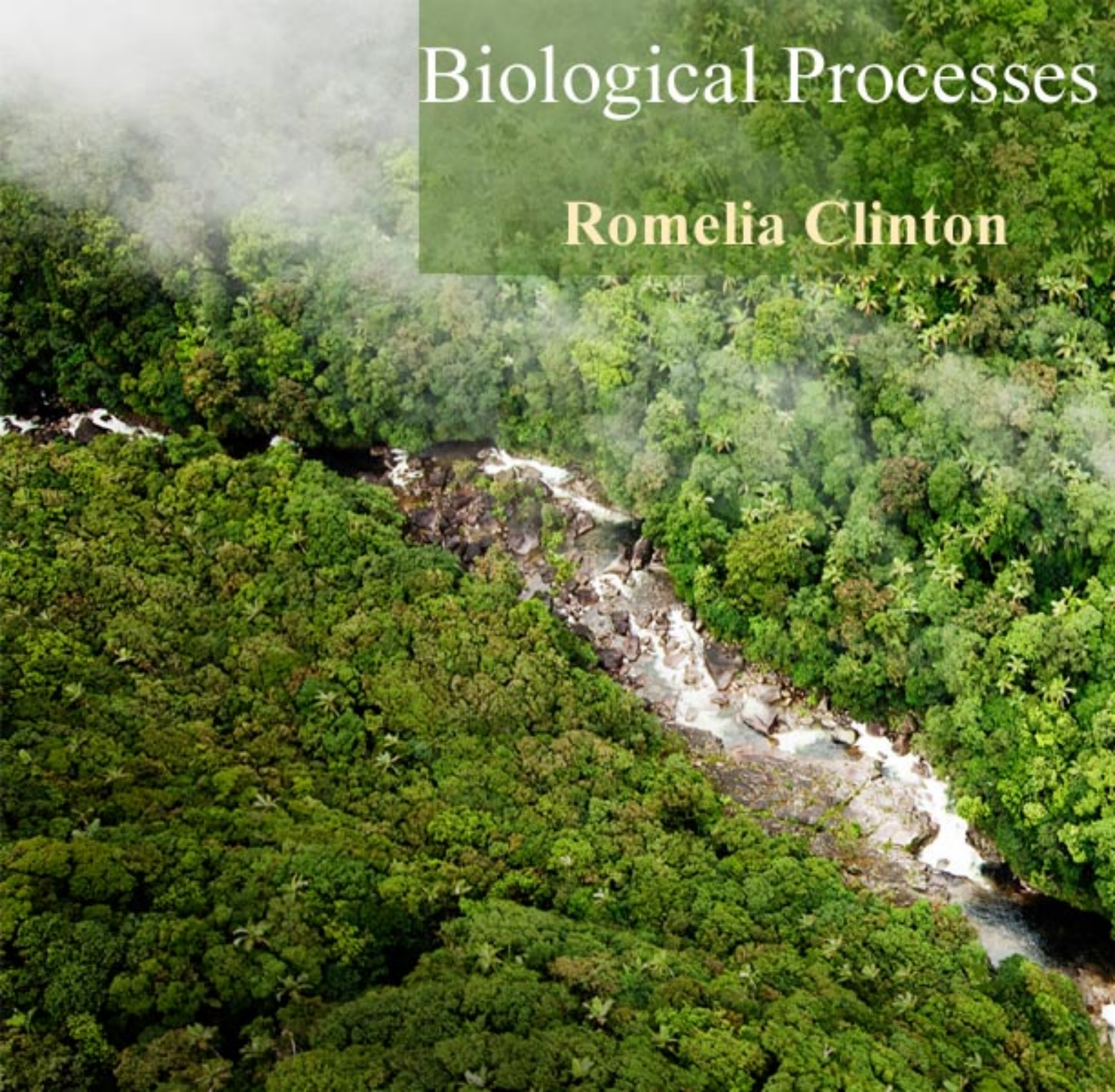

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Table of Contents

Chapter 1

Assimilation (Biology) and Bioerosion

Assimilation (biology)

Biological **assimilation**, or *bioassimilation*, is the combination of two processes to supply animal cells with nutrients. The first is the process of absorbing vitamins, minerals, and other chemicals from food within the gastrointestinal tract. In humans this is done with a chemical breakdown (enzymes and acids) and physical breakdown (oral mastication and stomach churning.) The second process of bioassimilation is the chemical alteration of substances in the bloodstream by the liver or cellular secretions. Although many similar compounds can be absorbed in digestion bioassimilation, the bioavailability of many compounds is dictated by this second process since both the liver and cellular secretions can be very specific in their metabolic action. This second process is where the absorbed food reaches the cells via the liver.

Most foods are composed of largely indigestible components depending on the enzymes and effectiveness of an animal's digestive tract. The most well known of these indigestible compounds is cellulose; the basic chemical polymer in the makeup of plant cell walls. Most animals, however, do not produce cellulase; the enzyme needed to digest cellulose. However some animal species have developed symbiotic relationships with cellulase producing bacteria. This allows termites to use the energy dense cellulose carbohydrate. Other such enzymes are known to significantly improve bioassimilation of nutrients. Because of the use of bacterial derivatives enzymatic dietary supplements now contain such enzymes as Amylase, Glucoamylase, Protease, Invertase, Peptidase, Lipase, Lactase, Phytase, and Cellulase. These enzymes improve the overall bioassimilation in the digestive tract but are still not proven to increase bloodstream bioavailability. Basically the enzymes and other breakdowns make the bigger substances of food smaller so they can go through the rest of their digestion more easily.

Examples of biological assimilation

- Photosynthesis, a process whereby carbon dioxide and water are transformed into a number of organic molecules in plant cells.

- Nitrogen fixation from the soil into organic molecules by symbiotic bacteria which live in the roots of certain plants, such as Leguminosae.
- Magnesium supplements orotate, oxide, sulfate, citrate, and glycerate are all structurally similar. However, oxide and sulfate are not water soluble and do not enter the blood stream while orotate and glycerate have normal exiguous liver conversion. Chlorophyll sources or magnesium citrate are highly bioassimilable.

- The absorption of nutrients into the body after digestion in the intestine and its transformation in biological tissues and fluids.
- Assimilation is occurring in every cell of the body to help develop new cells.

Bioerosion

Sponge borings (*Entobia*) and encrusters on a modern bivalve shell, North Carolina.

Bioerosion describes the erosion of hard ocean substrates – and less often terrestrial substrates – by living organisms. Marine bioerosion can be caused by mollusks, polychaete worms, phoronids, sponges, crustaceans, echinoids, and fish; it can occur on coastlines, on coral reefs, and on ships; its mechanisms include biotic boring, drilling, rasping, and scraping. On dry land, bioerosion is typically performed by pioneer plants or plant-like organisms such as lichen, and mostly chemical (e.g. by acidic secretions on limestone) or mechanical (e.g. by roots growing into cracks) in nature.

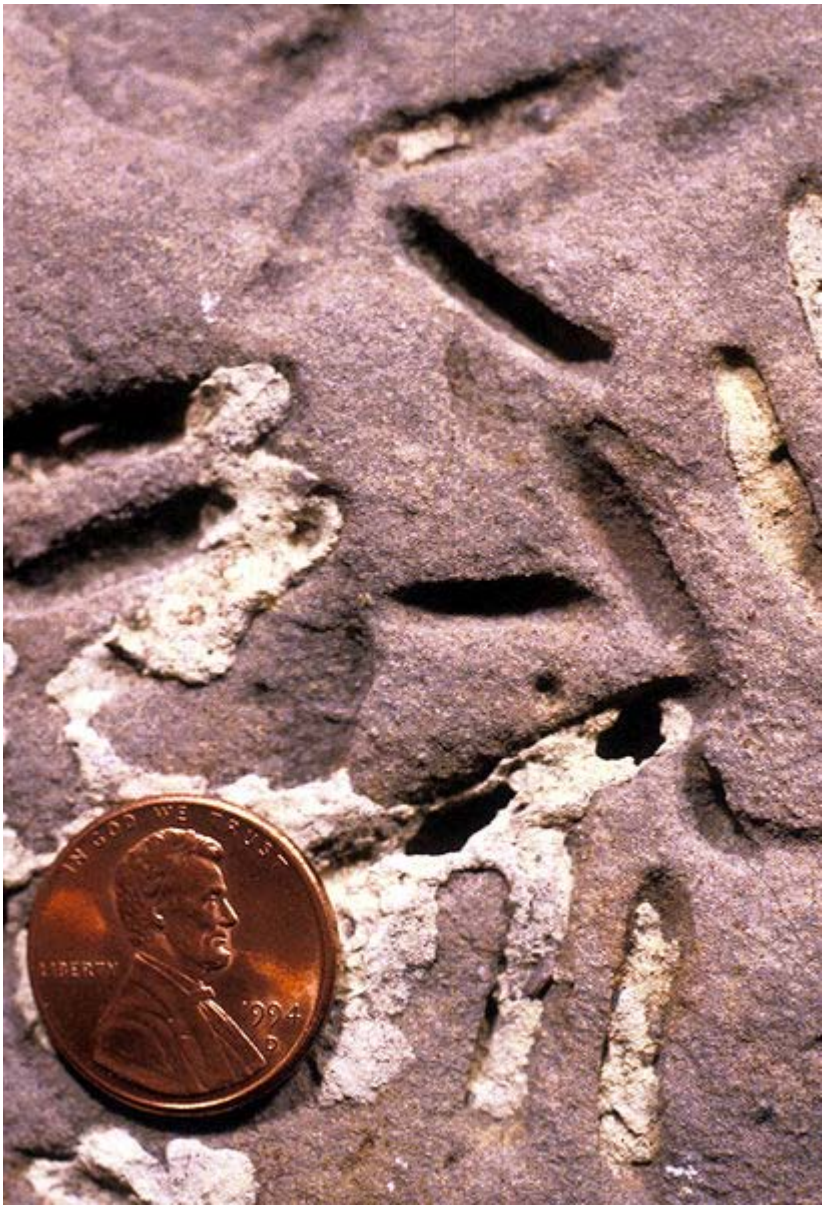
Bioerosion of coral reefs generates the fine and white coral sand characteristic of tropical islands. The coral is converted to sand by internal bioeroders such as algae, fungi,

bacteria (microborers) and sponges (Clionaidae), bivalves (including *Lithophaga*), sipunculans, polychaetes, acrothoracican barnacles and phoronids, generating extremely fine sediment with diameters of 10 to 100 micrometres. External bioeroders include sea urchins (such as *Diadema*) and chitons. These forces in concert produce a great deal of erosion. Sea urchin erosion of calcium carbonate has been reported in some reefs at annual rates exceeding 20 kg/m².

Fish also erode coral while eating algae. Parrotfish cause a great deal of bioerosion using well developed jaw muscles, tooth armature, and a pharyngeal mill, to grind ingested material into sand-sized particles. Bioerosion of coral reef aragonite by parrotfish can range from 1017.7±186.3 kg/yr (0.41±0.07 m³/yr) for *Chlorurus gibbus* and 23.6±3.4 kg/yr (9.7 10^{-3}±1.3 10^{-3} m²/yr) for *Chlorurus sordidus* (Bellwood, 1995).

Bioerosion is also well known in the fossil record on shells and hardgrounds (Bromley, 1970), with traces of this activity stretching back well into the Precambrian (Taylor & Wilson, 2003). Macrobioerosion, which produces borings visible to the naked eye, shows two distinct evolutionary radiations. One was in the Middle Ordovician and the other in the Jurassic. Microbioerosion also has a long fossil record and its own radiations.

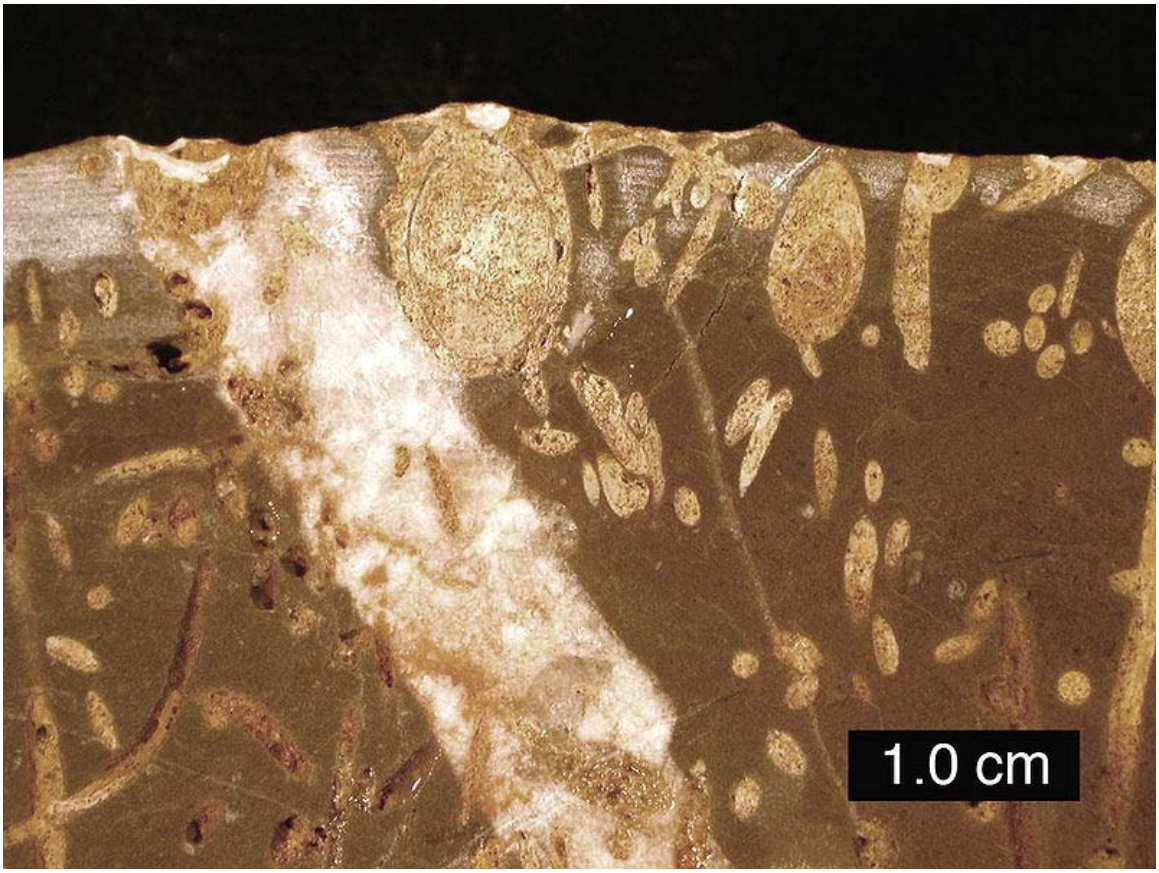
Trypanites borings in an Upper Ordovician hardground, southeastern Indiana



Petroxestes borings in an Upper Ordovician hardground, southern Ohio

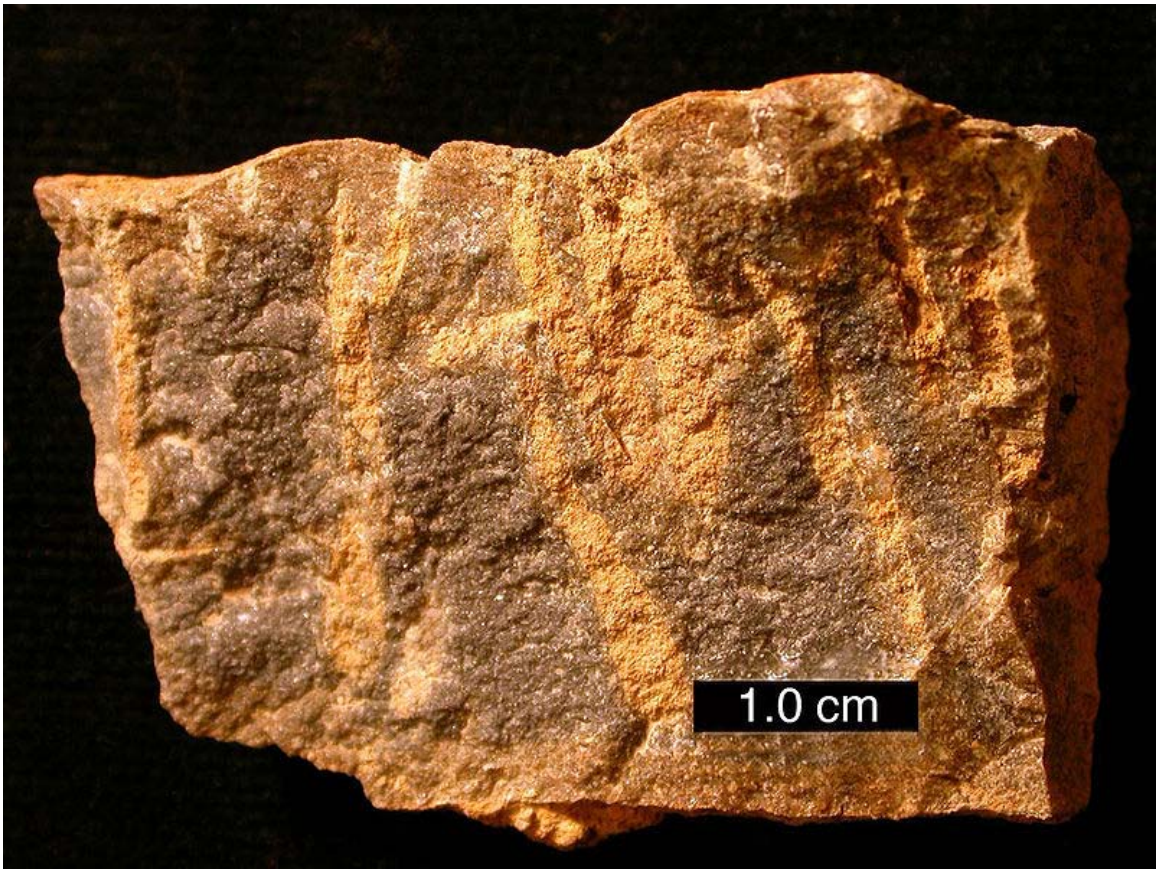
Gastrochaenolites borings in a Middle Jurassic hardground, southern Utah

Numerous borings in a Cretaceous cobble, Faringdon, England

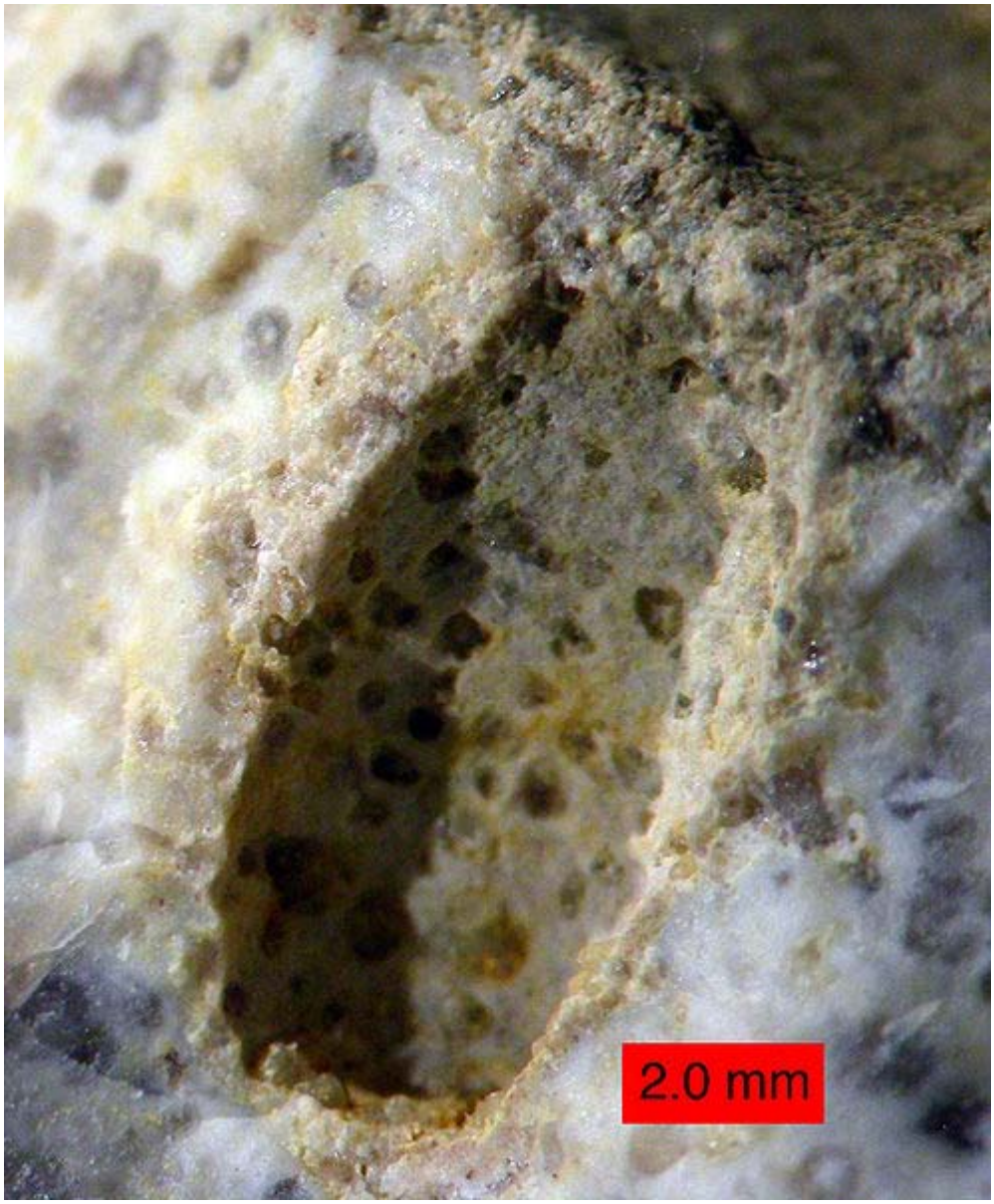


Cross-section of a Jurassic rockground; borings include *Gastrochaenolites* (some with boring bivalves in place) and *Trypanites*; Mendip Hills, England; scale bar = 1 cm.

Teredolites borings in a modern wharf piling; the work of bivalves known as "shipworms".



Ordovician hardground cross-section with Trypanites borings filled with dolomite; southern Ohio.



Gastrochaenolites boring in a recrystallized scleractinian coral, Matmor Formation (Middle Jurassic) of southern Israel.

Chapter 2

Cell Migration

Cell migration is a central process in the development and maintenance of multicellular organisms. Tissue formation during embryonic development, wound healing and immune responses all require the orchestrated movement of cells in particular directions to specific locations. Errors during this process have serious consequences, including mental retardation, vascular disease, tumor formation and metastasis. An understanding of the mechanism by which cells migrate may lead to the development of novel therapeutic strategies for controlling, for example, invasive tumour cells. Cells often migrate in response to, and towards, specific external signals, a process called chemotaxis.

Studying cell migration

The migration of single mammalian cells is usually viewed in the microscope as the cells move randomly on a glass slide. As the actual movement is very slow — usually a few micrometers/minute — time-lapse films are taken so that a speeded up movie can be viewed. This shows that, although the shape of a moving cell varies considerably, its leading front has a characteristic behaviour. This region of the cell is highly active, sometimes spreading forwards quickly, sometimes retracting, sometimes ruffling or bubbling. It is generally accepted that the leading front is the main motor which pulls the cell forward.

Common features

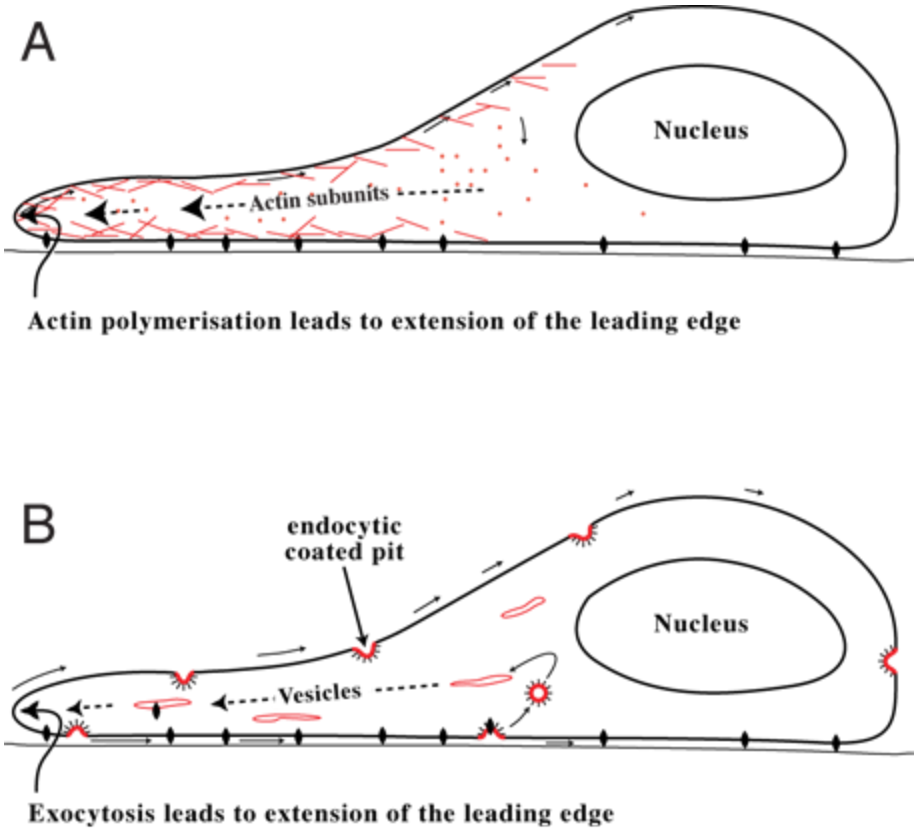
There is still great uncertainty of how cell migration really works. However, because the locomotion of all mammalian cells (except sperm) has several common features, the underlying processes are believed to be similar. The two main constant features are:

1. the behaviour of the leading front and
2. the observation that any debris on the dorsal surface of the cell moves backwards on the cell's surface towards its trailing end. The latter feature is most easily observed when aggregates of a surface molecule are cross-linked with a fluorescent antibody or when small beads become artificially bound to the front of the cell.

Besides mammalian cells, many other eukaryotic cells appear to move in a similar way. One of the most valuable model creatures for studying locomotion and chemotaxis is the amoeba Dictyostelium discoideum because they move more quickly than most mammalian cells grown in the lab and they chemotax towards cyclic AMP. In addition, they have a haploid genome which assists understanding the role of a particular gene product in movement.

Molecular processes at the front

There are two main theories for how the cell advances its front edge: the cytoskeletal model and membrane flow model. It is possible that both underlying processes contribute to cell extension.



Two different models for how cells move. A) Cytoskeletal model. B) Membrane Flow Model

Cytoskeletal model (A)

Experimentally it is found that the cell's front is a site of rapid actin polymerisation: soluble actin monomers polymerise there to form filaments. This has led to the view that it is the formation of these actin filaments which pushes the leading front forward and is the main motile force for advancing the cell’s front. In addition, cytoskeletal elements are able to interact extensively and intimately with a cell's plasma membrane.

Membrane flow model (B)

Studies have also shown that the front is the site at which membrane is returned to the cell surface from internal membrane pools at the end of the endocytic cycle. This has led to the view that extension of the leading edge occurs primarily by addition of membrane at the front of the cell. If so, the actin filaments which form at the front might stabilize the added membrane so that a structured extension, or lamella, is formed rather than the cell blowing bubbles (or "blebs") at its front. For a cell to move, it is necessary to bring a fresh supply of feet — those molecules, called integrins, which attach a cell to the surface on which it is crawling — to the front. It is likely that these feet are endocytosed towards the rear of the cell and brought to the cell's front by exocytosis, to be reused to form new attachments to the substrate.

The nucleus and rear

Given that a cell's front advances, what about the rest of the cell? Is it simply dragged forward, like a sack? We do not know, but there are suggestions that the nucleus and perhaps other large structures inside the cell may also be pulled forward by actin filaments. In addition, it may be that the rear of the cell actively contracts, as it is here that, in some cells, the major contractile protein myosin is found.

Mutants

Insight into how complex biological processes work can often be gleaned from a study of mutations. In the case of the intracellular mechanisms underlying cell movement, this has been largely unsuccessful. Thus, although many mutants are known in Drosophila which affect migratory processes, these tend to fall into two groups: transcription factors (such as *slow border cells* (*slbo*) which affects the migration of the border cells) or key regulator proteins (such as C-Jun N-terminal kinases (JNK) which controls dorsal closure). These, however, tell us little about how cells actually move.

Another major source of mutants is the haploid amoeba Dictyostelium. Many single copy genes associated with cytoskeletal function have been deleted: these mutants usually have only a weak phenotype, suggesting either that these genes are not required for locomotion or that there are multiple mechanisms by which cells can move. However, temperature-sensitive mutants in the genes for N-ethylmaleimide sensitive fusion protein (NSF) and Sec1 rapidly block cell migration indicating that the NSF protein and Sec1p are both required for some aspects of cell movement. NSF is known to function in intracellular membrane fusion; Sec1p in yeast is required for polarised exocytosis.

Polarity in migrating cells

Migrating cells clearly have a polarity: a front and a back. Without it, they would move in all directions at once, or spread. How this arrow is formulated at a molecular level inside a cell is unknown. In a cell which is meandering in a random way, the front can easily give way to become passive as some other region, or regions, of the cell form(s) a new

front. In chemotaxing cells, the stability of the front appears enhanced as the cell advances towards a higher concentration of the stimulating chemical. This polarity is reflected at a molecular level by a restriction of certain molecules to particular regions of the inner cell surface: thus the phospholipid PIP3 and activated Rac and CDC42 are found at the front of the cell, whereas Rho GTPase and PTEN are found towards the rear.

It is believed that microtubules and filamentous actin are important for establishing and maintaining a cell's polarity. Thus, drugs which destroy microtubules disrupt the polarity of many cells: if the cell is attached to a substratum, they often become round and flat. Drugs which destroy actin filaments have multiple and complex effects, reflecting the wide role that these filaments play in many cell processes. It may be that, as part of the locomotory process, membrane vesicles are transported along these filaments to the cell's front. In chemotaxing cells, the increased persistence of migration towards the target may result from an increased stability of the arrangement of the filamentous structures inside the cell and which determine its polarity. In turn, these filamentous structures may be arranged inside the cell according to how molecules like PIP3 and PTEN are arranged on the inner cell surface. And where these are located appears in turn to be determined by the chemoattractant signals as these impinge on specific receptors on the cell's outer surface.

Chapter 3

Natural Selection

Natural selection is the process by which traits become more or less common in a population due to consistent effects upon the survival or reproduction of their bearers. It is a key mechanism of evolution.

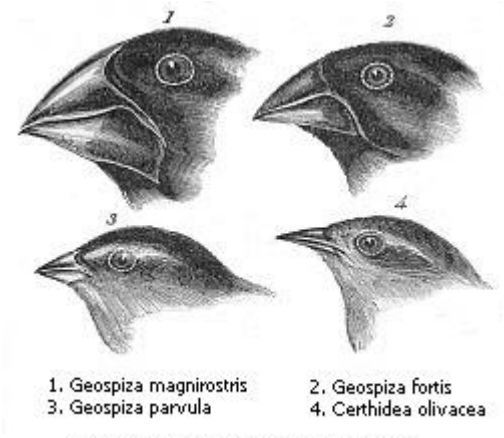
The natural genetic variation within a population of organisms may cause some individuals to survive and reproduce more successfully than others in their current environment. For example, the peppered moth exists in both light and dark colours in the United Kingdom, but during the industrial revolution many of the trees on which the moths rested became blackened by soot, giving the dark-colored moths an advantage in hiding from predators. This gave dark-colored moths a better chance of surviving to produce dark-colored offspring, and in just a few generations the majority of the moths were dark. Factors which affect reproductive success are also important, an issue which Charles Darwin developed in his ideas on sexual selection.

Natural selection acts on the phenotype, or the observable characteristics of an organism, but the genetic (heritable) basis of any phenotype which gives a reproductive advantage will become more common in a population. Over time, this process can result in adaptations that specialize populations for particular ecological niches and may eventually result in the emergence of new species. In other words, natural selection is an important process (though not the only process) by which evolution takes place within a population of organisms. As opposed to artificial selection, in which humans favor specific traits, in natural selection the environment acts as a sieve through which only certain variations can pass.

Natural selection is one of the cornerstones of modern biology. The term was introduced by Darwin in his influential 1859 book *On the Origin of Species*, in which natural selection was described as analogous to artificial selection, a process by which animals and plants with traits considered desirable by human breeders are systematically favored for reproduction. The concept of natural selection was originally developed in the absence of a valid theory of heredity; at the time of Darwin's writing, nothing was known of modern genetics. The union of traditional Darwinian evolution with subsequent

discoveries in classical and molecular genetics is termed the *modern evolutionary synthesis*. Natural selection remains the primary explanation for adaptive evolution.

General principles



Finches from Galapagos Archipelago

Darwin's illustrations of beak variation in the finches of the Galápagos Islands, which hold 13 closely related species that differ most markedly in the shape of their beaks. The beak of each species is suited to its preferred food, suggesting that beak shapes evolved by natural selection.

Natural variation occurs among the individuals of any population of organisms. Many of these differences do not affect survival (such as differences in eye color in humans), but some differences may improve the chances of survival of a particular individual. A rabbit that runs faster than others may be more likely to escape from predators, and algae that are more efficient at extracting energy from sunlight will grow faster. Something that increases an animal's survival will often also include its reproductive rate; however, sometimes there is a trade-off between survival and current reproduction. Ultimately, what matters is total lifetime reproduction of the animal.

If the traits that give these individuals a reproductive advantage are also heritable, that is, passed from parent to child, then there will be a slightly higher proportion of fast rabbits or efficient algae in the next generation. This is known as *differential reproduction*. Even if the reproductive advantage is very slight, over many generations any heritable advantage will become dominant in the population. In this way the natural environment of an organism "selects" for traits that confer a reproductive advantage, causing gradual changes or evolution of life. This effect was first described and named by Charles Darwin.

The concept of natural selection predates the understanding of genetics, the mechanism of heredity for all known life forms. In modern terms, selection acts on an organism's phenotype, or observable characteristics, but it is the organism's genetic make-up or genotype that is inherited. The phenotype is the result of the genotype and the environment in which the organism lives.

This is the link between natural selection and genetics, as described in the modern evolutionary synthesis. Although a complete theory of evolution also requires an account of how genetic variation arises in the first place (such as by mutation and sexual reproduction) and includes other evolutionary mechanisms (such as genetic drift and gene flow), natural selection appears to be the most important mechanism for creating complex adaptations in nature.

Nomenclature and usage

The term *natural selection* has slightly different definitions in different contexts. It is most often defined to operate on heritable traits, because these are the traits that directly participate in evolution. However, natural selection is "blind" in the sense that changes in phenotype (physical and behavioral characteristics) can give a reproductive advantage regardless of whether or not the trait is heritable (non heritable traits can be the result of environmental factors or the life experience of the organism).

Following Darwin's primary usage the term is often used to refer to both the evolutionary consequence of blind selection and to its mechanisms. It is sometimes helpful to explicitly distinguish between selection's mechanisms and its effects; when this distinction is important, scientists define "natural selection" specifically as "those mechanisms that contribute to the selection of individuals that reproduce", without regard to whether the basis of the selection is heritable. This is sometimes referred to as "phenotypic natural selection".

Traits that cause greater reproductive success of an organism are said to be selected for, whereas those that reduce success are selected against. Selection for a trait may also result in the selection of other correlated traits that do not themselves directly influence reproductive advantage. This may occur as a result of pleiotropy or gene linkage.

Fitness

The concept of fitness is central to natural selection. Broadly, individuals which are more "fit" have better potential for survival, as in the well-known phrase "survival of the fittest". However, as with natural selection above, the precise meaning of the term is much more subtle, and Richard Dawkins manages in his later books to avoid it entirely. (He devotes a chapter of his book, *The Extended Phenotype*, to discussing the various senses in which the term is used). Modern evolutionary theory defines fitness not by how long an organism lives, but by how successful it is at reproducing. If an organism lives half as long as others of its species, but has twice as many offspring surviving to adulthood, its genes will become more common in the adult population of the next generation.

Though natural selection acts on individuals, the effects of chance mean that fitness can only really be defined "on average" for the individuals within a population. The fitness of a particular genotype corresponds to the average effect on all individuals with that

genotype. Very low-fitness genotypes cause their bearers to have few or no offspring on average; examples include many human genetic disorders like cystic fibrosis.

Since fitness is an averaged quantity, it is also possible that a favorable mutation arises in an individual that does not survive to adulthood for unrelated reasons. Fitness also depends crucially upon the environment. Conditions like sickle-cell anemia may have low fitness in the general human population, but because the sickle-cell trait confers immunity from malaria, it has high fitness value in populations which have high malaria infection rates.

Types of selection

Natural selection can act on any heritable phenotypic trait, and selective pressure can be produced by any aspect of the environment, including sexual selection and competition with members of the same or other species. However, this does not imply that natural selection is always directional and results in adaptive evolution; natural selection often results in the maintenance of the status quo by eliminating less fit variants.

The unit of selection can be the individual or it can be another level within the hierarchy of biological organisation, such as genes, cells, and kin groups. There is still debate about whether natural selection acts at the level of groups or species to produce adaptations that benefit a larger, non-kin group. Likewise, there is debate as to whether selection at the molecular level prior to gene mutations and fertilization of the zygote should be ascribed to conventional natural selection because traditionally natural selection is an environmental and exterior force that acts upon a phenotype typically after birth. Some science journalists distinguish natural selection from gene selection by informally referencing selection of mutations as "pre-selection."

Selection at a different level such as the gene can result in an increase in fitness for that gene, while at the same time reducing the fitness of the individuals carrying that gene, in a process called intragenomic conflict. Overall, the combined effect of all selection pressures at various levels determines the overall fitness of an individual, and hence the outcome of natural selection.

Natural selection occurs at every life stage of an individual. An individual organism must survive until adulthood before it can reproduce, and selection of those that reach this stage is called *viability selection*. In many species, adults must compete with each other for mates via sexual selection, and success in this competition determines who will parent the next generation. When individuals can reproduce more than once, a longer survival in the reproductive phase increases the number of offspring, called *survival selection*.

The fecundity of both females and males (for example, giant sperm in certain species of *Drosophila*) can be limited via "fecundity selection". The viability of produced gametes can differ, while intragenomic conflicts such as meiotic drive between the haploid gametes can result in gametic or "genic selection". Finally, the union of some

combinations of eggs and sperm might be more compatible than others; this is termed *compatibility selection*.

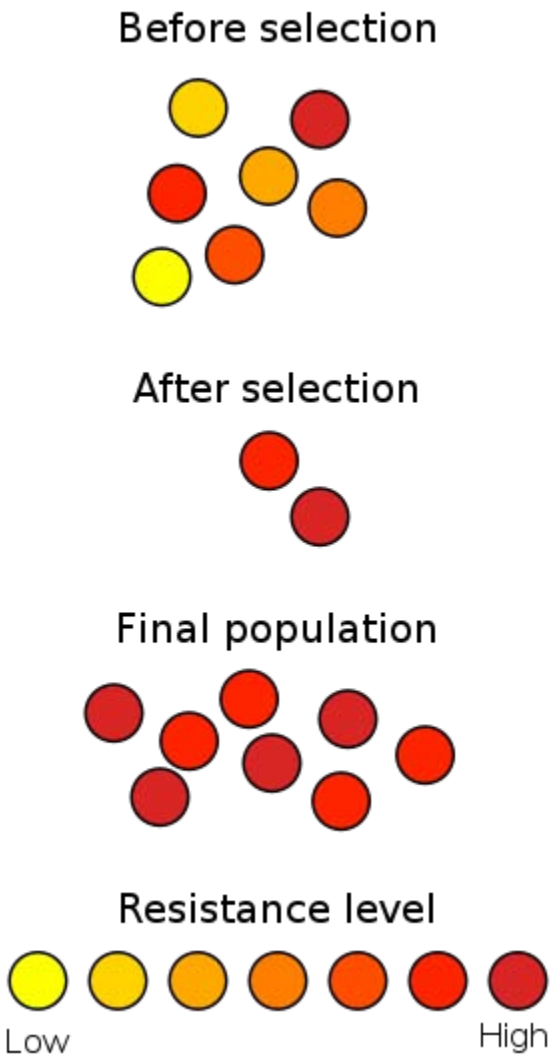
Sexual selection

It is useful to distinguish between "ecological selection" and "sexual selection". Ecological selection covers any mechanism of selection as a result of the environment (including relatives, e.g. kin selection, competition, and infanticide), while "sexual selection" refers specifically to competition for mates.

Sexual selection can be *intrasexual*, as in cases of competition among individuals of the same sex in a population, or *intersexual*, as in cases where one sex controls reproductive access by choosing among a population of available mates. Most commonly, intrasexual selection involves male–male competition and intersexual selection involves female choice of suitable males, due to the generally greater investment of resources for a female than a male in a single offspring. However, some species exhibit sex-role reversed behavior in which it is males that are most selective in mate choice; the best-known examples of this pattern occur in some fishes of the family *Syngnathidae*, though likely examples have also been found in amphibian and bird species.

Some features that are confined to one sex only of a particular species can be explained by selection exercised by the other sex in the choice of a mate, for example, the extravagant plumage of some male birds. Similarly, aggression between members of the same sex is sometimes associated with very distinctive features, such as the antlers of stags, which are used in combat with other stags. More generally, intrasexual selection is often associated with sexual dimorphism, including differences in body size between males and females of a species.

Examples of natural selection



Resistance to antibiotics is increased though the survival of individuals which are immune to the effects of the antibiotic, whose offspring then inherit the resistance, creating a new population of resistant bacteria.

A well-known example of natural selection in action is the development of antibiotic resistance in microorganisms. Since the discovery of penicillin in 1928 by Alexander Fleming, antibiotics have been used to fight bacterial diseases. Natural populations of bacteria contain, among their vast numbers of individual members, considerable variation in their genetic material, primarily as the result of mutations. When exposed to antibiotics, most bacteria die quickly, but some may have mutations that make them slightly less susceptible. If the exposure to antibiotics is short, these individuals will survive the treatment. This selective elimination of maladapted individuals from a population is natural selection.

These surviving bacteria will then reproduce again, producing the next generation. Due to the elimination of the maladapted individuals in the past generation, this population contains more bacteria that have some resistance against the antibiotic. At the same time, new mutations occur, contributing new genetic variation to the existing genetic variation. Spontaneous mutations are very rare, and advantageous mutations are even rarer. However, populations of bacteria are large enough that a few individuals will have beneficial mutations. If a new mutation reduces their susceptibility to an antibiotic, these individuals are more likely to survive when next confronted with that antibiotic.

Given enough time, and repeated exposure to the antibiotic, a population of antibiotic-resistant bacteria will emerge. This new changed population of antibiotic-resistant bacteria is optimally adapted to the context it evolved in. At the same time, it is not necessarily optimally adapted any more to the old antibiotic free environment. The end result of natural selection is two populations that are both optimally adapted to their specific environment, while both perform substandard in the other environment.

The widespread use and misuse of antibiotics has resulted in increased microbial resistance to antibiotics in clinical use, to the point that the methicillin-resistant *Staphylococcus aureus* (MRSA) has been described as a "superbug" because of the threat it poses to health and its relative invulnerability to existing drugs. Response strategies typically include the use of different, stronger antibiotics; however, new strains of MRSA have recently emerged that are resistant even to these drugs.

This is an example of what is known as an evolutionary arms race, in which bacteria continue to develop strains that are less susceptible to antibiotics, while medical researchers continue to develop new antibiotics that can kill them. A similar situation occurs with pesticide resistance in plants and insects. Arms races are not necessarily induced by man; a well-documented example involves the spread of a gene in the butterfly Hypolimnas bolina suppressing male-killing activity by Wolbachia bacteria parasites on the island of Samoa, where the spread of the gene is known to have occurred over a period of just five years

Evolution by means of natural selection

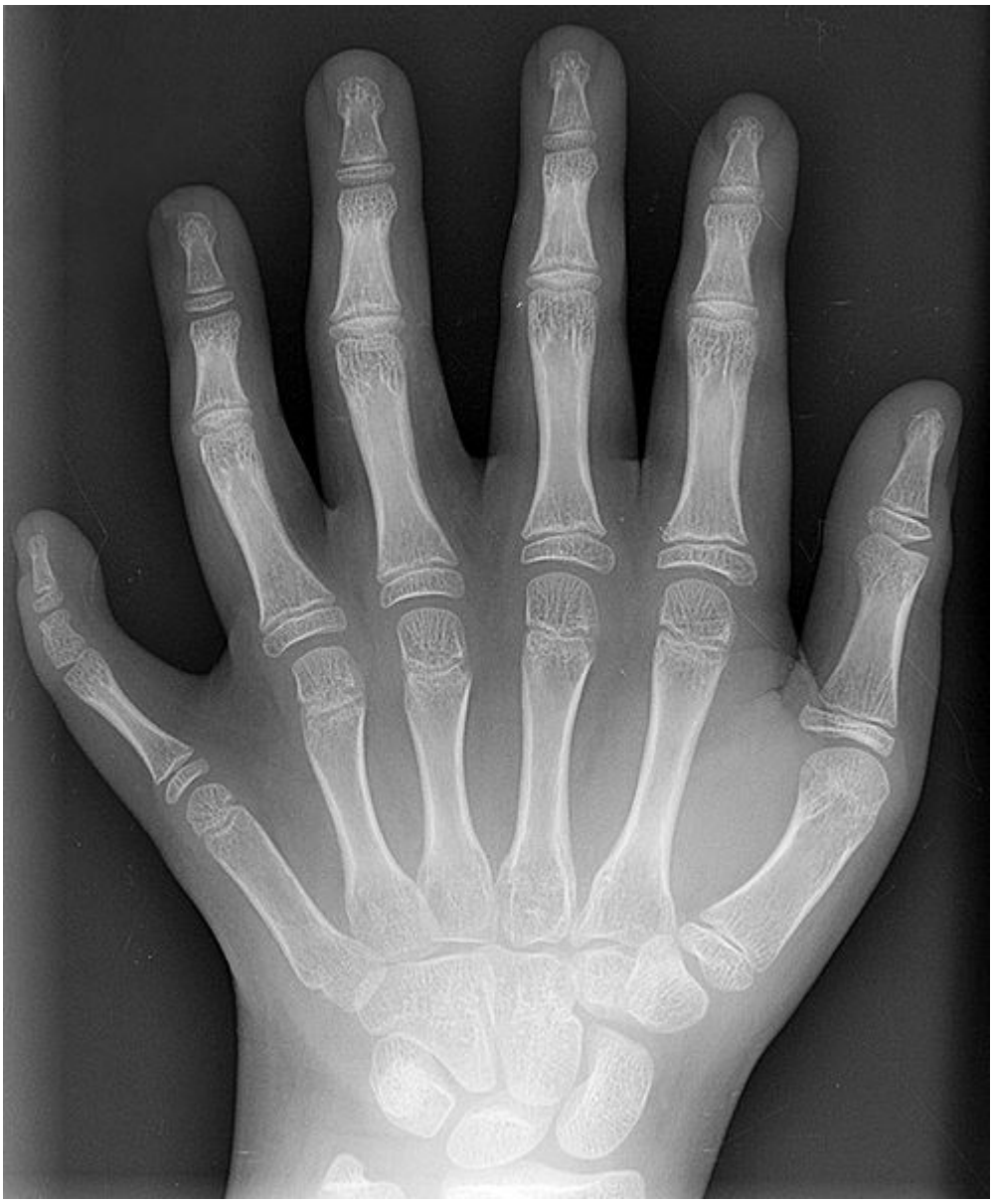
A prerequisite for natural selection to result in adaptive evolution, novel traits and speciation, is the presence of heritable genetic variation that results in fitness differences. Genetic variation is the result of mutations, recombinations and alterations in the karyotype (the number, shape, size and internal arrangement of the chromosomes). Any of these changes might have an effect that is highly advantageous or highly disadvantageous, but large effects are very rare. In the past, most changes in the genetic material were considered neutral or close to neutral because they occurred in noncoding DNA or resulted in a synonymous substitution. However, recent research suggests that many mutations in non-coding DNA do have slight deleterious effects. Although both mutation rates and average fitness effects of mutations are dependent on the organism, estimates from data in humans have found that a majority of mutations are slightly deleterious.

The exuberant tail of the peacock is thought to be the result of sexual selection by females. This peacock is an albino; selection against albinos in nature is intense because they are easily spotted by predators or are unsuccessful in competition for mates.

By the definition of fitness, individuals with greater fitness are more likely to contribute offspring to the next generation, while individuals with lesser fitness are more likely to die early or fail to reproduce. As a result, alleles which on average result in greater fitness become more abundant in the next generation, while alleles which generally reduce fitness become rarer. If the selection forces remain the same for many generations, beneficial alleles become more and more abundant, until they dominate the population, while alleles with a lesser fitness disappear. In every generation, new mutations and re-combinations arise spontaneously, producing a new spectrum of phenotypes. Therefore, each new generation will be enriched by the increasing abundance of alleles that contribute to those traits that were favored by selection, enhancing these traits over successive generations.

Some mutations occur in so-called regulatory genes. Changes in these can have large effects on the phenotype of the individual because they regulate the function of many other genes. Most, but not all, mutations in regulatory genes result in non-viable zygotes. Examples of nonlethal regulatory mutations occur in HOX genes in humans, which can result in a cervical rib or polydactyly, an increase in the number of fingers or toes. When

such mutations result in a higher fitness, natural selection will favor these phenotypes and the novel trait will spread in the population.



X-ray of the left hand of a ten year old boy with polydactyly.

Established traits are not immutable; traits that have high fitness in one environmental context may be much less fit if environmental conditions change. In the absence of natural selection to preserve such a trait, it will become more variable and deteriorate over time, possibly resulting in a vestigial manifestation of the trait, also called evolutionary baggage. In many circumstances, the apparently vestigial structure may retain a limited functionality, or may be co-opted for other advantageous traits in a phenomenon known as preadaptation. A famous example of a vestigial structure, the eye of the blind mole rat, is believed to retain function in photoperiod perception.

Speciation

Speciation requires selective mating, which result in a reduced gene flow. Selective mating can be the result of 1. Geographic isolation, 2. Behavioral isolation, or 3. Temporal isolation. For example, a change in the physical environment (geographic isolation by an extrinsic barrier) would follow number 1, a change in camouflage for number 2 or a shift in mating times (i.e., one species of deer shifts location and therefore changes its "rut") for number 3.

Over time, these subgroups might diverge radically to become different species, either because of differences in selection pressures on the different subgroups, or because different mutations arise spontaneously in the different populations, or because of founder effects – some potentially beneficial alleles may, by chance, be present in only one or other of two subgroups when they first become separated. A lesser-known mechanism of speciation occurs via hybridization, well-documented in plants and occasionally observed in species-rich groups of animals such as cichlid fishes. Such mechanisms of rapid speciation can reflect a mechanism of evolutionary change known as punctuated equilibrium, which suggests that evolutionary change and particularly speciation typically happens quickly after interrupting long periods of stasis.

Genetic changes within groups result in increasing incompatibility between the genomes of the two subgroups, thus reducing gene flow between the groups. Gene flow will effectively cease when the distinctive mutations characterizing each subgroup become fixed. As few as two mutations can result in speciation: if each mutation has a neutral or positive effect on fitness when they occur separately, but a negative effect when they occur together, then fixation of these genes in the respective subgroups will lead to two reproductively isolated populations. According to the biological species concept, these will be two different species.

Historical development

The modern theory of natural selection derives from the work of Charles Darwin in the nineteenth century.

Pre-Darwinian theories

Several ancient philosophers expressed the idea that nature produces a huge variety of creatures, apparently randomly, and that only those creatures survive that manage to provide for themselves and reproduce successfully; well-known examples include Empedocles and his intellectual successor, Lucretius, while related ideas were later refined by Aristotle. The struggle for existence was later described by Al-Jahiz, who argued that environmental factors influence animals to develop new characteristics to ensure survival.

Abu Rayhan Biruni described the idea of artificial selection and argued that nature works in much the same way. Similar ideas were later expressed by Nasir al-Din Tusi and Ibn Khaldun. Such classical arguments were reintroduced in the 18th century by Pierre Louis Maupertuis and others, including Charles Darwin's grandfather Erasmus Darwin. While

these forerunners had an influence on Darwinism, they later had little influence on the trajectory of evolutionary thought after Charles Darwin.

Until the early 19th century, the prevailing view in Western societies was that differences between individuals of a species were uninteresting departures from their Platonic idealism (or typus) of created kinds. However, the theory of uniformitarianism in geology promoted the idea that simple, weak forces could act continuously over long periods of time to produce radical changes in the Earth's landscape. The success of this theory raised awareness of the vast scale of geological time and made plausible the idea that tiny, virtually imperceptible changes in successive generations could produce consequences on the scale of differences between species.

Early 19th century evolutionists such as Jean Baptiste Lamarck suggested the inheritance of acquired characteristics as a mechanism for evolutionary change; adaptive traits acquired by an organism during its lifetime could be inherited by that organism's progeny, eventually causing transmutation of species. This theory has come to be known as Lamarckism and was an influence on the anti-genetic ideas of the Stalinist Soviet biologist Trofim Lysenko.

Darwin's theory

In 1859, Charles Darwin set out his theory of evolution by natural selection as an explanation for adaptation and speciation. He defined natural selection as the "principle by which each slight variation [of a trait], if useful, is preserved". The concept was simple but powerful: individuals best adapted to their environments are more likely to survive and reproduce. As long as there is some variation between them, there will be an inevitable selection of individuals with the most advantageous variations. If the variations are inherited, then differential reproductive success will lead to a progressive evolution of particular populations of a species, and populations that evolve to be sufficiently different eventually become different species.

Darwin's ideas were inspired by the observations that he had made on the *Beagle* voyage, and by the work of a political economist, the Reverend Thomas Malthus, who in *An Essay on the Principle of Population*, noted that population (if unchecked) increases exponentially whereas the food supply grows only arithmetically; thus inevitable limitations of resources would have demographic implications, leading to a "struggle for existence". When Darwin read Malthus in 1838 he was already primed by his work as a naturalist to appreciate the "struggle for existence" in nature and it struck him that as population outgrew resources, "favourable variations would tend to be preserved, and unfavourable ones to be destroyed. The result of this would be the formation of new species."

Here is Darwin's own summary of the idea, which can be found in the fourth chapter of the *Origin*:

If during the long course of ages and under varying conditions of life, organic beings vary at all in the several parts of their organisation, and I think this cannot be disputed; if there be, owing to the high geometrical powers of increase of each species, at some age, season, or year, a severe struggle for life, and this certainly cannot be disputed; then, considering the infinite complexity of the relations of all organic beings to each other and to their conditions of existence, causing an infinite diversity in structure, constitution, and habits, to be advantageous to them, I think it would be a most extraordinary fact if no variation ever had occurred useful to each being's own welfare, in the same way as so many variations have occurred useful to man. But if variations useful to any organic being do occur, assuredly individuals thus characterised will have the best chance of being preserved in the struggle for life; and from the strong principle of inheritance they will tend to produce offspring similarly characterised. This principle of preservation, I have called, for the sake of brevity, Natural Selection.

Once he had his theory "by which to work", Darwin was meticulous about gathering and refining evidence as his "prime hobby" before making his idea public. He was in the process of writing his "big book" to present his researches when the naturalist Alfred Russel Wallace independently conceived of the principle and described it in an essay he sent to Darwin to forward to Charles Lyell. Lyell and Joseph Dalton Hooker decided (without Wallace's knowledge) to present his essay together with unpublished writings which Darwin had sent to fellow naturalists, and *On the Tendency of Species to form Varieties; and on the Perpetuation of Varieties and Species by Natural Means of Selection* was read to the Linnean Society announcing co-discovery of the principle in July 1858. Darwin published a detailed account of his evidence and conclusions in *On the Origin of Species* in 1859. In the 3rd edition of 1861 Darwin acknowledged that others — notably William Charles Wells in 1813, and Patrick Matthew in 1831 — had proposed similar ideas, but had neither developed them nor presented them in notable scientific publications.

Darwin thought of natural selection by analogy to how farmers select crops or livestock for breeding, which he called "artificial selection"; in his early manuscripts he referred to a 'Nature' which would do the selection. At the time, other mechanisms of evolution such as evolution by genetic drift were not yet explicitly formulated, and Darwin believed that selection was likely only part of the story: "I am convinced that [it] has been the main, but not exclusive means of modification." In a letter to Charles Lyell in September 1860, Darwin regretted the use of the term "Natural Selection", preferring the term "Natural Preservation".

For Darwin and his contemporaries, natural selection was essentially synonymous with evolution by natural selection. After the publication of *On the Origin of Species*, educated people generally accepted that evolution had occurred in some form. However, natural selection remained controversial as a mechanism, partly because it was perceived to be too weak to explain the range of observed characteristics of living organisms, and partly because even supporters of evolution balked at its "unguided" and non-progressive nature, a response that has been characterized as the single most significant impediment to the idea's acceptance.

However, some thinkers enthusiastically embraced natural selection; after reading Darwin, Herbert Spencer introduced the term *survival of the fittest*, which became a popular summary of the theory. The fifth edition of *On the Origin of Species* published in 1869 included Spencer's phrase as an alternative to natural selection, with credit given: "But the expression often used by Mr. Herbert Spencer, of the Survival of the Fittest, is more accurate, and is sometimes equally convenient." Although the phrase is still often used by non-biologists, modern biologists avoid it because it is tautological if "fittest" is read to mean "functionally superior" and is applied to individuals rather than considered as an averaged quantity over populations.

Modern evolutionary synthesis

Natural selection relies crucially on the idea of heredity, but it was developed long before the basic concepts of genetics. Although the Austrian monk Gregor Mendel, the father of modern genetics, was a contemporary of Darwin's, his work would lie in obscurity until the early 20th century. Only after the integration of Darwin's theory of evolution with a complex statistical appreciation of Gregor Mendel's 're-discovered' laws of inheritance did natural selection become generally accepted by scientists.

The work of Ronald Fisher (who developed the required mathematical language and The Genetical Theory of Natural Selection), J.B.S. Haldane (who introduced the concept of the "cost" of natural selection), Sewall Wright (who elucidated the nature of selection and adaptation), Theodosius Dobzhansky (who established the idea that mutation, by creating genetic diversity, supplied the raw material for natural selection), William Hamilton (who conceived of kin selection), Ernst Mayr (who recognised the key importance of reproductive isolation for speciation) and many others formed the modern evolutionary synthesis. This synthesis cemented natural selection as the foundation of evolutionary theory, where it remains today.

Impact of the idea

Darwin's ideas, along with those of Adam Smith and Karl Marx, had a profound influence on 19th century thought. Perhaps the most radical claim of the theory of evolution through natural selection is that "elaborately constructed forms, so different from each other, and dependent on each other in so complex a manner" evolved from the simplest forms of life by a few simple principles. This claim inspired some of Darwin's most ardent supporters—and provoked the most profound opposition. The radicalism of natural selection, according to Stephen Jay Gould, lay in its power to "dethrone some of the deepest and most traditional comforts of Western thought". In particular, it challenged long-standing beliefs in such concepts as a special and exalted place for humans in the natural world and a benevolent creator whose intentions were reflected in nature's order and design.

In the words of the philosopher Daniel Dennett, "Darwin's dangerous idea" of evolution by natural selection is a "universal acid" which cannot be kept restricted to any vessel or container, as it soon leaks out, working its way into ever wider surroundings. Thus, in the

last decades the concept of natural selection has spread from evolutionary biology into virtually all disciplines, including evolutionary computation, quantum darwinism, evolutionary economics, evolutionary epistemology, evolutionary psychology and cosmological natural selection. This unlimited applicability has been called Universal Darwinism.

Cell and molecular biology

In the 19th century, Wilhelm Roux, a founder of modern embryology, wrote a book entitled « Der Kampf der Teile im Organismus » (The struggle of parts in the organism) in which he suggested that the development of an organism results from a Darwinian competition between the parts of the embryo, occurring at all levels, from molecules to organs. In recent years, a modern version of this theory has been proposed by Jean-Jacques Kupiec. According to this cellular Darwinism, stochasticity at the molecular level generates diversity in cell types whereas cell interactions impose a characteristic order on the developing embryo.

Social and psychological theory

The social implications of the theory of evolution by natural selection also became the source of continuing controversy. Friedrich Engels, a German political philosopher and co-originator of the ideology of communism, wrote in 1872 that "Darwin did not know what a bitter satire he wrote on mankind when he showed that free competition, the struggle for existence, which the economists celebrate as the highest historical achievement, is the normal state of the animal kingdom". Interpretation of natural selection as necessarily 'progressive', leading to increasing 'advances' in intelligence and civilisation, was used as a justification for colonialism and policies of eugenics, as well as broader sociopolitical positions now described as Social Darwinism. Konrad Lorenz won the Nobel Prize in Physiology or Medicine in 1973 for his analysis of animal behavior in terms of the role of natural selection (particularly group selection). However, in Germany in 1940, in writings that he subsequently disowned, he used the theory as a justification for policies of the Nazi state. He wrote "... selection for toughness, heroism, and social utility...must be accomplished by some human institution, if mankind, in default of selective factors, is not to be ruined by domestication-induced degeneracy. The racial idea as the basis of our state has already accomplished much in this respect." Others have developed ideas that human societies and culture evolve by mechanisms that are analogous to those that apply to evolution of species.

More recently, work among anthropologists and psychologists has led to the development of sociobiology and later evolutionary psychology, a field that attempts to explain features of human psychology in terms of adaptation to the ancestral environment. The most prominent such example, notably advanced in the early work of Noam Chomsky and later by Steven Pinker, is the hypothesis that the human brain is adapted to acquire the grammatical rules of natural language. Other aspects of human behavior and social structures, from specific cultural norms such as incest avoidance to broader patterns such as gender roles, have been hypothesized to have similar origins as adaptations to the early

environment in which modern humans evolved. By analogy to the action of natural selection on genes, the concept of memes – "units of cultural transmission", or culture's equivalents of genes undergoing selection and recombination – has arisen, first described in this form by Richard Dawkins and subsequently expanded upon by philosophers such as Daniel Dennett as explanations for complex cultural activities, including human consciousness. Extensions of the theory of natural selection to such a wide range of cultural phenomena have been distinctly controversial and are not widely accepted.

Information and systems theory

In 1922, Alfred Lotka proposed that natural selection might be understood as a physical principle which could be described in terms of the use of energy by a system, a concept that was later developed by Howard Odum as the maximum power principle whereby evolutionary systems with selective advantage maximise the rate of useful energy transformation. Such concepts are sometimes relevant in the study of applied thermodynamics.

The principles of natural selection have inspired a variety of computational techniques, such as "soft" artificial life, that simulate selective processes and can be highly efficient in 'adapting' entities to an environment defined by a specified fitness function. For example, a class of heuristic optimization algorithms known as genetic algorithms, pioneered by John Holland in the 1970s and expanded upon by David E. Goldberg, identify optimal solutions by simulated reproduction and mutation of a population of solutions defined by an initial probability distribution. Such algorithms are particularly useful when applied to problems whose solution landscape is very rough or has many local minima.

Genetic basis of natural selection

The idea of natural selection predates the understanding of genetics. We now have a much better idea of the biology underlying heritability, which is the basis of natural selection.

Genotype and phenotype

Natural selection acts on an organism's phenotype, or physical characteristics. Phenotype is determined by an organism's genetic make-up (genotype) and the environment in which the organism lives. Often, natural selection acts on specific traits of an individual, and the terms phenotype and genotype are used narrowly to indicate these specific traits.

When different organisms in a population possess different versions of a gene for a certain trait, each of these versions is known as an allele. It is this genetic variation that underlies phenotypic traits. A typical example is that certain combinations of genes for eye color in humans which, for instance, give rise to the phenotype of blue eyes. (On the other hand, when all the organisms in a population share the same allele for a particular trait, and this state is stable over time, the allele is said to be *fixed* in that population.)

Some traits are governed by only a single gene, but most traits are influenced by the interactions of many genes. A variation in one of the many genes that contributes to a trait may have only a small effect on the phenotype; together, these genes can produce a continuum of possible phenotypic values.

Directionality of selection

When some component of a trait is heritable, selection will alter the frequencies of the different alleles, or variants of the gene that produces the variants of the trait. Selection can be divided into three classes, on the basis of its effect on allele frequencies.

Directional selection occurs when a certain allele has a greater fitness than others, resulting in an increase of its frequency. This process can continue until the allele is fixed and the entire population shares the fitter phenotype. It is directional selection that is illustrated in the antibiotic resistance example above.

Far more common is stabilizing selection (which is commonly **confused** with *purifying selection*), which lowers the frequency of alleles that have a deleterious effect on the phenotype – that is, produce organisms of lower fitness. This process can continue until the allele is eliminated from the population. Purifying selection results in functional genetic features, such as protein-coding genes or regulatory sequences, being conserved over time due to selective pressure against deleterious variants.

Finally, a number of forms of balancing selection exist, which do not result in fixation, but maintain an allele at intermediate frequencies in a population. This can occur in diploid species (that is, those that have two pairs of chromosomes) when heterozygote individuals, who have different alleles on each chromosome at a single genetic locus, have a higher fitness than homozygote individuals that have two of the same alleles. This is called heterozygote advantage or overdominance, of which the best-known example is the malarial resistance observed in heterozygous humans who carry only one copy of the gene for sickle cell anemia. Maintenance of allelic variation can also occur through disruptive or diversifying selection, which favors genotypes that depart from the average in either direction (that is, the opposite of overdominance), and can result in a bimodal distribution of trait values. Finally, balancing selection can occur through frequency-dependent selection, where the fitness of one particular phenotype depends on the distribution of other phenotypes in the population. The principles of game theory have been applied to understand the fitness distributions in these situations, particularly in the study of kin selection and the evolution of reciprocal altruism.

Selection and genetic variation

A portion of all genetic variation is functionally neutral in that it produces no phenotypic effect or significant difference in fitness; the hypothesis that this variation accounts for a large fraction of observed genetic diversity is known as the neutral theory of molecular evolution and was originated by Motoo Kimura. When genetic variation does not result in differences in fitness, selection cannot *directly* affect the frequency of such variation. As

a result, the genetic variation at those sites will be higher than at sites where variation does influence fitness. However, after a period with no new mutation, the genetic variation at these sites will be eliminated due to genetic drift.

Mutation selection balance

Natural selection results in the reduction of genetic variation through the elimination of maladapted individuals and consequently of the mutations that caused the maladaptation. At the same time, new mutations occur, resulting in a mutation-selection balance. The exact outcome of the two processes depends both on the rate at which new mutations occur and on the strength of the natural selection, which is a function of how unfavorable the mutation proves to be. Consequently, changes in the mutation rate or the selection pressure will result in a different mutation-selection balance.

Genetic linkage

Genetic linkage occurs when the loci of two alleles are *linked*, or in close proximity to each other on the chromosome. During the formation of gametes, recombination of the genetic material results in reshuffling of the alleles. However, the chance that such a reshuffle occurs between two alleles depends on the distance between those alleles; the closer the alleles are to each other, the less likely it is that such a reshuffle will occur. Consequently, when selection targets one allele, this automatically results in selection of the other allele as well; through this mechanism, selection can have a strong influence on patterns of variation in the genome.

Selective sweeps occur when an allele becomes more common in a population as a result of positive selection. As the prevalence of one allele increases, linked alleles can also become more common, whether they are neutral or even slightly deleterious. This is called *genetic hitchhiking*. A strong selective sweep results in a region of the genome where the positively selected haplotype (the allele and its neighbours) are essentially the only ones that exist in the population.

Whether a selective sweep has occurred or not can be investigated by measuring linkage disequilibrium, or whether a given haplotype is overrepresented in the population. Normally, genetic recombination results in a reshuffling of the different alleles within a haplotype, and none of the haplotypes will dominate the population. However, during a selective sweep, selection for a specific allele will also result in selection of neighbouring alleles. Therefore, the presence of a block of strong linkage disequilibrium might indicate that there has been a 'recent' selective sweep near the center of the block, and this can be used to identify sites recently under selection.

Background selection is the opposite of a selective sweep. If a specific site experiences strong and persistent purifying selection, linked variation will tend to be weeded out along with it, producing a region in the genome of low overall variability. Because background selection is a result of deleterious new mutations, which can occur randomly in any haplotype, it does not produce clear blocks of linkage disequilibrium, although

with low recombination it can still lead to slightly negative linkage disequilibrium overall.

Chapter 4

Biological Pigment

The Blue Morpho butterfly, native to Central America, derives its distinctive blue coloring from iridescence rather than from pigmentation.

Biological pigments, also known simply as **pigments** or **biochromes** are substances produced by living organisms that have a color resulting from selective color absorption.

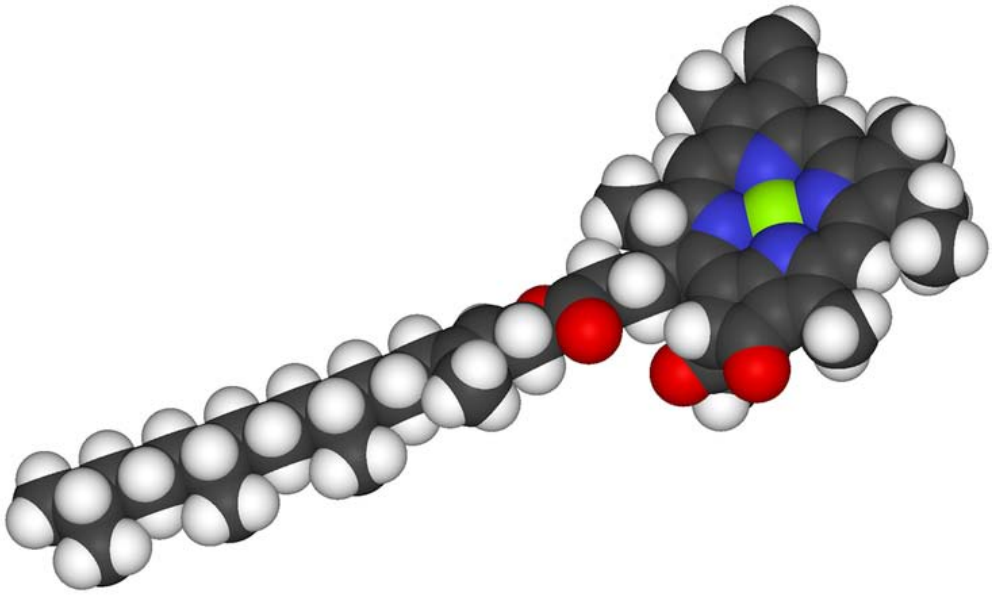
Biological pigments include **plant pigments** and **flower pigments**. Many biological structures, such as skin, eyes, fur and hair contain pigments such as melanin in specialized cells called chromatophores.

Pigment color differs from structural color in that it is the same for all viewing angles, whereas structural color is the result of selective reflection or iridescence, usually because of multilayer structures. For example, butterfly wings typically contain structural color, although many butterflies have cells that contain pigment as well.

Biological pigments

- Heme/porphyrin-based: chlorophyll, bilirubin, hemocyanin, hemoglobin, myoglobin
- Light-emitting: luciferin
- Carotenoids:
    - Hematochromes (algal pigments, mixes of carotenoids and their derivates)
    - Carotenes: alpha and beta carotene, lycopene, rhodopsin
    - Xanthophylls: canthaxanthin, zeaxanthin, lutein
- Proteinaceous: phytochrome, phycobiliproteins
- Polyene enolates: a class of red pigments unique to parrots
- Other: melanin, urochrome, flavonoids

Pigments in plants



Space-filling model of the chlorophyll molecule.

Anthocyanin gives these pansies their purple pigmentation.

Plant pigments include a variety of different kinds of molecules, including porphyrins, carotenoids, anthocyanins and betalains. All biological pigments selectively absorb certain wavelengths of light while reflecting others. The light that is absorbed may be used by the plant to power chemical reactions, while the reflected wavelengths of light determine the color the pigment will appear to the eye. Pigments also serve to attract pollinators.

Chlorophyll is the primary pigment in plants; it is a porphyrin that absorbs yellow and blue wavelengths of light while reflecting green. It is the presence and relative abundance of chlorophyll that gives plants their green color. All land plants and green algae possess two forms of this pigment: chlorophyll *a* and chlorophyll *b*. Kelps,diatoms,and other photosynthetic heterokonts contain chlorophyll *c* instead of *b*, while red algae possess only chlorophyll *a*. All chlorophylls serve as the primary means plants use to intercept light in order to fuel photosynthesis.

Carotenoids are red, orange, or yellow tetraterpenoids. They function as accessory pigments in plants, helping to fuel photosynthesis by gathering wavelengths of light not readily absorbed by chlorophyll. The most familiar carotenoids are carotene (an orange pigment found in carrots), lutein (a yellow pigment found in fruits and vegetables), and lycopene (the red pigment responsible for the color of tomatoes). Carotenoids have been shown to act as antioxidants and to promote healthy eyesight in humans.

Anthocyanins (literally "flower blue") are water-soluble flavonoid pigments that appear red to blue, according to pH. They occur in all tissues of higher plants, providing color in leaves, plant stem, roots, flowers, and fruits, though not always in sufficient quantities to be noticeable. Anthocyanins are most visible in the petals of flowers, where they may make up as much as 30% of the dry weight of the tissue.They are also responsible for the purple color seen on the underside of tropical shade plants such as *Tradescantia zebrina*; in these plants, the anthocyanin catches light that has passed through the leaf and reflects it back towards regions bearing chlorophyll, in order to maximize the use of available light.

Betalains are red or yellow pigments. Like anthocyanins they are water-soluble, but unlike anthocyanins they are indole-derived compounds synthesized from tyrosine. This class of pigments is found only in the Caryophyllales (including cactus and amaranth), and never co-occur in plants with anthocyanins. Betalains are responsible for the deep red color of beets, and are used commercially as food-coloring agents.

Pigments in animals

Pigments in animals may serve to protect tissues from ultraviolet radiation, such as melanin in the skin. Pigments may also aid in sexual reproduction, identifying species and gender of animals to potential mates, or signaling readiness to breed. Some biological structures in animals, such as heme groups, however are colored as a result of their structure and their color does not serve a function.

Some cephalopods use pigmented chromatophores to communicate.

Pigmentation is used by many animals for protection, by means of camouflage, mimicry, or warning coloration. Chameleons use pigments to blend into their surroundings by controlling the absorption levels of the electromagnetic spectrum.

Diseases and conditions

A variety of diseases and abnormal conditions that involve pigmentation arise in humans and animals, either from absence of or loss of pigmentation or pigment cells, or from the excess production of pigment.

- Albinism is an inherited disorder characterized by total or partial loss of melanin. Humans and animals that suffer from albinism are called "albinistic" (the term "albino" is also sometimes used, but may be considered offensive when applied to people).
- Lamellar ichthyosis, also called "fish scale disease", is an inherited condition in which one symptom is excess production of melanin. The skin is darker than normal, and is characterized by darkened, scaly, dry patches.

- Melasma is a condition in which dark brown patches of pigment appear on the face, influenced by hormonal changes. When it occurs during a pregnancy, this condition is called *the mask of pregnancy*.
- *ocular pigmentation* is an accumulation of pigment in the eye, and may be caused by latanoprost medication.
- Vitiligo is a condition in which there is a loss of pigment-producing cells called melanocytes in patches of skin.

Commercial uses

Pigments may be extracted and used as dyes.

Pigments in Marine Animals

Carotenoids/ Carotenoprotein Carotenoids are the most common group of pigments found in nature. Over 600 different kinds of carotenoids are found in animals and plants. In plants, caroteinoids are responsible for photo-protection, light-harvesting, and singlet oxygen scavenging in the process of photosynthesis. This pigment is usually found in the chloroplast of plants and other photosynthetic organism such as algae, fungus, and some bacteria. On the other hand, animals are incapable of making their own carotenoids. Thus, they rely on plants for these pigments.

Carotenoids form complexes with proteins which are known as carotenoproteins. These complexes are common among marine animals. The carotenoprotein complexes are responsible for the various colors (red, purple, blue, green, etc.) to these marine invertebrates for mating rituals and camouflage. There are two main types of carotenoproteins: Type A and Type B. Type A has carotenoids (chromogen) which are stoichiometrically associated with a simple protein (glycoprotein). The second type, Type B, has carotenoids which are associated with a lipo protein and is usually less stable. While Type A is commonly found in the surface (shells and skins) of marine invertebrates, Type B is usually in eggs, ovaries, and blood. The colors and characteristic absorption of these carotenoprotein complexes are based upon the chemical binding of the chromogen and the protein subunits.

For example, the blue carotenoprotein, linckiacyanin has about 100-200 carotenoid molecules per every complex. In addition, the functions of these pigment-protein complexes also change their chemical structure as well. Carotenoproteins that are within the photosynthetic structure are more common, but complicated. Pigment-protein complexes that are outside of the photosynthetic system are less common, but have a simpler structure. For example, there are only two of these blue astaxanthin-proteins in the jellyfish, Velella velella, contains only about 100 carotenoids per complex.

The most common carotenoprotein is astaxanthin, which gives off a purple-blue and green pigment. Astaxanthin's color is formed by creating complexes with proteins in a certain order. For example, the crustochrin has approximately 20 astaxanthin molecules

bonded with protein. When the complexes interact by exciton-exciton interaction, it lowers the absorbance maximum, changing the different color pigments.

In lobsters, there are various types of astaxanthin-protein complexes present. The first one is crustacyanin (max 632 nm), a slate-blue pigment found in the lobster's carapace. The second one is crustochrin (max 409), a yellow pigment which is found on the outer layer of the carapace. Lastly, the lipoglycoprotein and ovoverdin forms a bright green pigment that is usually present in the outer layers of the carapace and the lobster eggs.

Tetrapyrroles

Tetrapyrroles are the next most common group of pigments. They have four pyrrole rings, each ring consisting of C4H4NH. The main role of the tetrapyrroles is their connection in the biological oxidation process. Tetrapyrroles has a major role in electron transport and acts as a replacement for many enzymes. In addition, they also have a role in the pigmentation of the marine organism's tissues.

Melanin

Melanin is a class of compounds that serves as a pigment with different structures responsible for dark, tan, yellowish/ redish pigments in marine animals. It's produced as the amino acid tyrosine is converted into Melanin, which is found in the skin, hair, and eyes. Derived from aerobic oxidation of phenols, they are polymers.

There are several different types of melanins considering that they are an aggregate of smaller component molecules, such as nitrogen containing melanins. There are two classes of pigments: black and brown insoluble eumelanins, which are derived from aerobic oxidation of tyrosine in the presence of tyrosinase, and the alkali-soluble phaeomelanins which range from a yellow to red brown color, arising from the deviation of the eumelanin pathway through the intervention of cysteine and/or glutathione. Eumelanins are usually found in the skin and eyes. Several different melanins include melanoprotein (dark brown melanin that's stored in high concentrations in the ink sac of the cuttlefish Sepia Officianalis), echinoidea (found in sand dollars, and the hearts of sea urchins), holothuroidea (found in sea cucumbers), and ophiuroidea (found in brittle and snake stars). These melanins are possibly polymers which arise from the repeated coupling of simple bi-polyfunctional monomdric intermediates, or of high molecular weights. The compounds benzothiazole and tetrahydroisoquinoline ring systems act as UV-absorbing compounds. There are several different types of melanins considering that they are an aggregate of smaller component molecules, such as nitrogen containing melanins.

Bioluminescence

The only light source in the deep sea, marine animals give off visible light energy called bioluminescence, a subset of chemiluminescence. This is the chemical reaction in which chemical energy is converted to light energy. It is estimated that 90% of deep-sea animals

produce some sort of bioluminescence. Considering that a large proportion of the visible light spectrum is absorbed before reaching the deep sea, most of the emitted light from the sea-animals is blue and green. However, some species may emit a red and infrared light, and there has even been a genus that is found to emit yellow bioluminescence. The organ that is responsible for the emission of bioluminescence is known as photophores. This type is only present in squid and fish, and is used to illuminate their ventral surfaces, which disguise their silhouettes from predators. The uses of the photophores in the sea-animals differ, such as lenses for controlling intensity of color, and the intensity of the light produced. Squids have both photophores and chromatophores which controls both of these intensities. Another thing that is responsible for the emission of bioluminescence, which is evident in the bursts of light that jellyfish emit, start with a luciferin (a photogen) and ends with the light emitter (a photagogikon.) Luciferin, luciferase, salt, and oxygen react and combine to create a single unit called photo-proteins, which can produce light when reacted with another molecule such as Ca+. Jellyfish use this as a defense mechanism; when a smaller predator is attempting to devour a jellyfish, it will flash its lights, which would therefore lure a larger predator and chase the smaller predator away. It is also used as mating behavior.

In reef-building coral and sea anemones, they fluoresce; light is absorbed at one wavelength, and re-emitted at another. These pigments may act as natural sunscreens, aid in photosynthesis, serve as warning coloration, attract mates, warn rivals, or confuse predators.

Chromatophores

Chromatophores are color pigment changing cells that are directly stimulated by central motor neurons. They are primarily used for quick environmental adaptation for camouflaging. The process of changing the color pigment of their skin relies on a single highly developed chromatophore cell and many muscles, nerves, glail and sheath cells. Chromatophores contract and contain vesicles that stores three different liquid pigments. Each color is indicated by the three types of chromatophore cells: erythrophores, melanophores, and xanthophores. The first type is the erythrophores, which contains reddish pigments such as carotenoids and pteridines. The second type is the melanophores, which contains black and brown pigments such as the melanins. The third type is the xanthophores which contains yellow pigments in the forms of carotenoids. The various colors are made by the combination of the different layers of the chromatophores. These cells are usually located beneath the skin or scale the animals. There are two categories of colors generated by the cell – biochrome and schematochromes. Biochromes are colors chemically formed microscopic, natural pigments. Their chemical composition is created to take in some color of light and reflect the rest. In contrast, schematochromes (structural colors) are colors created by light reflections from a colorless surface and refractions by tissues. Schematochromes act like prisms, refracting and dispersing visible light to the surroundings, which will eventually reflect a specific combination of colors. These categories are determined by the movement of pigments within the chromatophores. The physiological color changes are short-term and fast, found in fishes, and are a result from an animal's response to a change in the

environment. In contrast, the morphological color changes are long-term changes, occurs in different stages of the animal, and are due the change of numbers of chromatophores. To change the color pigments, transparency, or opacity, the cells alter in form and size, and stretch or contract their outer covering.

Photo-protective Pigments

Due to damage from UV-A and UV-B, marine animals have evolved to have compounds that absorb UV light and act as sunscreen. Mycosporine-like amino acids (MAAs) can absorb UV rays at 310-360 nm. Melanin is another well-known UV-protector. Carotenoids and photopigments both indirectly act as photo-protective pigments, as they quench oxygen free-radicals. They also supplement photosynthetic pigments that absorb light energy in the blue region.

Defensive role of pigments

It's known that animals use their color patterns to warn off predators, however it has been observed that a sponge pigment mimicked a chemical which involved the regulation of moulting of an amphipod that was known to prey on sponges. So whenever that amphipod eats the sponge, the chemical pigments prevents the moulting, and the amphipod eventually dies.

Environmental Influence on Color

Coloration in invertebrates varies based on the depth, water temperature, food source, currents, geographic location, light exposure, and sedimentation. For example, the amount of carotenoid a certain sea anemone decreases as we go deeper into the ocean. Thus, the marine life that resides on deeper waters is less brilliant than the organisms that live in well-lit areas due to the reduction of pigments. In the colonies of the colonial ascidian-cyanophyte symbiosis Trididemnum solidum, their colors are different depending on the light regime in which they live. The colonies that are exposed to full sunlight are heavily calcified, thicker, and are white. In contrast the colonies that live in shaded areas have more phycoerythrin (pigment that absorbs green) in comparison to phycocyanin (pigment that absorbs red), thinner, and are purple. The purple color in the shaded colonies are mainly due to the phycobilin pigment of the algae, meaning the variation of exposure in light changes the colors of these colonies.

Adaptive Coloration

Aposematism is the warning coloration to signal potential predators to stay away. In many chromodrorid nudibranchs, they take in distasteful and toxic chemicals emitted from sponges and store them in their repugnatorial glands (located around the mantle edge). Predators of nudibranchs have learned to avoid these certain nudibranchs based on their bright color patterns. Preys also protect themselves by their toxic compounds ranging from a variety of organic and inorganic compounds.

Physiological activities of pigment

Pigments of marine animals sever several different purposes, other than defensive roles. Some pigments are known to protect against UV. In the nudibranch Nembrotha Kubaryana, tetrapyrrole pigment 13 has been found to be a potent antimicrobial agent. Also in this creature, tamjamines A, B, C, E, and F (Figure 79a-e) has shown antimicrobial, antitumor, and immunosuppressive activities.

Sesquiterpenoids are recognized for their blue and purple colors, but its also been reported to exhibit various bioactivities such as antibacterial, immunoregulating, antimicrobial, and cytotoxic, as well as the inhibitory activity against cell division in the fertilized sea urchin and ascidian eggs. Several other pigments have been shown to be cytotoxic. In fact, two new carotenoids that were isolated from a sponge called Phakellia stelliderma showed mild cytotoxicity against mouse leukemia cells. Other pigments with medical involvements include scytonemin, topsentins, and debromohymenialdisine have several lead compounds in the field of inflammation, rheumatoid arthritis and osteoarthritis respectively. There's evidence that topsentins are potent mediators of immunogenic inflation, and topsentin and scytonemin are potent inhibitors of neurogenic inflammation.

Chapter 5

Reproduction

Production of new individuals along a leaf margin of the air plant, *Kalanchoe pinnata*. The small plant in front is about 1 cm (0.4 in) tall. The concept of "individual" is obviously stretched by this asexual reproductive process.

Reproduction (or **procreation**) is the biological process by which new "offspring" individual organisms are produced from their "parents". Reproduction is a fundamental feature of all known life; each individual organism exists as the result of reproduction.

The known methods of reproduction are broadly grouped into two main types: sexual and asexual.

In asexual reproduction, an individual can reproduce without involvement with another individual of that species. The division of a bacterial cell into two daughter cells is an example of asexual reproduction. Asexual reproduction is not, however, limited to single-celled organisms. Most plants have the ability to reproduce asexually and the ant species Mycocepurus smithii is thought to reproduce entirely by asexual means.

Sexual reproduction typically requires the involvement of two individuals or gametes, one each from opposite type of sex.

Asexual reproduction

Asexual reproduction is the process by which an organism creates a genetically similar or identical copy of itself without a contribution of genetic material from another individual. Bacteria divide asexually via binary fission; viruses take control of host cells to produce more viruses; Hydras (invertebrates of the order *Hydroidea*) and yeasts are able to reproduce by budding. These organisms often do not possess different sexes, and they are capable of "splitting" themselves into two or more individuals. On the other hand, some of these species that are capable of reproducing asexually, like hydra, yeast and jellyfish, may also reproduce sexually. For instance, most plants are capable of vegetative reproduction—reproduction without seeds or spores—but can also reproduce sexually. Likewise, bacteria may exchange genetic information by conjugation. Other ways of asexual reproduction include parthenogenesis, fragmentation and spore formation that involves only mitosis. Parthenogenesis is the growth and development of embryo or seed without fertilization by a male. Parthenogenesis occurs naturally in some species, including lower plants (where it is called apomixis), invertebrates (e.g. water fleas, aphids, some bees and parasitic wasps), and vertebrates (e.g. some reptiles, fish, and, very rarely, birds and sharks). It is sometimes also used to describe reproduction modes in hermaphroditic species which can self-fertilize.

Sexual reproduction

Hoverflies mating in midair flight

Sexual reproduction is a biological process by which organisms create descendants that have a combination of genetic material contributed from two (usually) different members of the species. (Self-fertilization requires only one organism.) Each of two parent organisms contributes half of the offspring's genetic makeup by creating haploid gametes. Most organisms form two different types of gametes. In these ***anisogamous*** species, the two sexes are referred to as male (producing sperm or microspores) and female (producing ova or megaspores). In ***isogamous species***, the gametes are similar or identical in form (isogametes), but may have separable properties and then may be given other different names. For example, in the green alga, *Chlamydomonas reinhardtii*, there are so-called "plus" and "minus" gametes. A few types of organisms, such as ciliates, *Paramecium aurelia*, have more than two types of "sex", called syngens.

Most animals (including humans) and plants reproduce sexually. Sexually reproducing organisms have different sets of genes for every trait (called alleles). Offspring inherit one allele for each trait from each parent, thereby ensuring that offspring have a combination of the parents' genes. Diploid having two copies of every gene within an organism, it is believed that "the masking of deleterious alleles favors the evolution of a dominant diploid phase in organisms that alternate between haploid and diploid phases" where recombination occurs freely.

Bryophyte reproduces sexually but its commonly seen life forms are all haploid, which produce gametes. The zygotes of the gametes develop into sporangium, which produces haploid spores. The diploid stage is relatively short compared with that of haploid stage, i.e. *haploid dominance*. The advantage of diploid, e.g. heterosis, only takes place in diploid life stage. Bryophyte still maintains the sexual reproduction during its evolution despite the fact that the haploid stage does not benefit from heterosis at all. This may be an example that the sexual reproduction has a bigger advantage by itself, since it allows gene shuffling (hybrid or recombination between multiple loci) among different members of the species, that permits natural selection of the fit over these new hybrids or recombinants that are haploid forms.

Allogamy

Allogamy is a term used in the field of biological reproduction describing the fertilization of an ovum from one individual with the spermatozoa of another.

Autogamy

Self-fertilization (also known as autogamy) occurs in hermaphroditic organisms where the two gametes fused in fertilization come from the same individual. They are bound and all the cells merge to form one new gamete.

Mitosis and meiosis

Mitosis and meiosis are an integral part of cell division. Mitosis occurs in somatic cells, while meiosis occurs in gametes.

Mitosis The resultant number of cells in mitosis is twice the number of original cells. The number of chromosomes in the daughter cells is the same as that of the parent cell.

Meiosis The resultant number of cells is four times the number of original cells. This results in cells with half the number of chromosomes present in the parent cell. A diploid cell duplicates itself, then undergoes two divisions (tetraploid to diploid to haploid), in the process forming four haploid cells. This process occurs in two phases, meiosis I and meiosis II.

Same-sex reproduction

In recent decades, developmental biologists have been researching and developing techniques to facilitate same-sex reproduction. The obvious approaches, subject to a growing amount of activity, are female sperm and male eggs, with female sperm closer to being a reality for humans, given that Japanese scientists have already created female sperm for chickens. "However, the ratio of produced W chromosome-bearing (W-bearing) spermatozoa fell substantially below expectations. It is therefore concluded that most of the W-bearing PGC could not differentiate into spermatozoa because of restricted spermatogenesis." In 2004, by altering the function of a few genes involved with

imprinting, other Japanese scientists combined two mouse eggs to produce daughter mice.

Reproductive strategies

There are a wide range of reproductive strategies employed by different species. Some animals, such as the human and Northern Gannet, do not reach sexual maturity for many years after birth and even then produce few offspring. Others reproduce quickly; but, under normal circumstances, most offspring do not survive to adulthood. For example, a rabbit (mature after 8 months) can produce 10–30 offspring per year, and a fruit fly (mature after 10–14 days) can produce up to 900 offspring per year. These two main strategies are known as K-selection (few offspring) and r-selection (many offspring). Which strategy is favoured by evolution depends on a variety of circumstances. Animals with few offspring can devote more resources to the nurturing and protection of each individual offspring, thus reducing the need for many offspring. On the other hand, animals with many offspring may devote fewer resources to each individual offspring; for these types of animals it is common for many offspring to die soon after birth, but enough individuals typically survive to maintain the population. Some organisms such as honey bees and fruit flies retain sperm in a process called sperm storage thereby increasing the duration of their fertility.

Other types of reproductive strategies

Polycyclic animals reproduce intermittently throughout their lives.

Semelparous organisms reproduce only once in their lifetime, such as annual plants (including all grain crops), and certain species of salmon, spiders, bamboos and centru plants. Often, they die shortly after reproduction. This is often associated with r-strategists.

Iteroparous organisms produce offspring in successive (e.g. annual or seasonal) cycles, such as perennial plants. Iteroparous animals survive over multiple seasons (or periodic condition changes). This is more associated with K-strategists.

Asexual vs. sexual reproduction

Organisms that reproduce through asexual reproduction tend to grow in number exponentially. However, because they rely on mutation for variations in their DNA, all members of the species have similar vulnerabilities. Organisms that reproduce sexually yield a smaller number of offspring, but the large amount of variation in their genes makes them less susceptible to disease.

Many organisms can reproduce sexually as well as asexually. Aphids, slime molds, sea anemones, some species of starfish (by fragmentation), and many plants are examples. When environmental factors are favorable, asexual reproduction is employed to exploit suitable conditions for survival such as an abundant food supply, adequate shelter,

favorable climate, disease, optimum pH or a proper mix of other lifestyle requirements. Populations of these organisms increase exponentially via asexual reproductive strategies to take full advantage of the rich supply resources.

When food sources have been depleted, the climate becomes hostile, or individual survival is jeopardized by some other adverse change in living conditions, these organisms switch to sexual forms of reproduction. Sexual reproduction ensures a mixing of the gene pool of the species. The variations found in offspring of sexual reproduction allow some individuals to be better suited for survival and provide a mechanism for selective adaptation to occur. In addition, sexual reproduction usually results in the formation of a life stage that is able to endure the conditions that threaten the offspring of an asexual parent. Thus, seeds, spores, eggs, pupae, cysts or other "over-wintering" stages of sexual reproduction ensure the survival during unfavorable times and the organism can "wait out" adverse situations until a swing back to suitability occurs.

Life without reproduction

The existence of life without reproduction is the subject of some speculation. The biological study of how the origin of life led from non-reproducing elements to reproducing organisms is called abiogenesis. Whether or not there were several independent abiogenetic events, biologists believe that the last universal ancestor to all present life on earth lived about 3.5 billion years ago.

Today, some scientists have speculated about the possibility of creating life non-reproductively in the laboratory. Several scientists have succeeded in producing simple viruses from entirely non-living materials. The virus is often regarded as not alive. Being nothing more than a bit of RNA or DNA in a protein capsule, they have no metabolism and can only replicate with the assistance of a hijacked cell's metabolic machinery.

The production of a truly living organism (*e.g.*, a simple bacterium) with no ancestors would be a much more complex task, but may well be possible according to current biological knowledge. A synthetic genome has been transferred into an existing bacterium where it replaced the native DNA, resulting in the artificial production of a new M. mycoides organism.

Lottery principle

Sexual reproduction has many drawbacks, since it requires far more energy than asexual reproduction and diverts the organisms from other pursuits, and there is some argument about why so many species use it.

George C. Williams used lottery tickets as an analogy in one explanation for the widespread use of sexual reproduction. He argued that asexual reproduction, which produces little or no genetic variety in offspring, was like buying many tickets that all have the same number, limiting the chance of "winning" - that is, producing surviving

offspring. Sexual reproduction, he argued, was like purchasing fewer tickets but with a greater variety of numbers and therefore a greater chance of success.

The point of this analogy is that since asexual reproduction does not produce genetic variations, there is little ability to quickly adapt to a changing environment. The lottery principle is less accepted these days because of evidence that asexual reproduction is more prevalent in unstable environments, the opposite of what it predicts.

Chapter 6

Biotinylation

In biochemistry, **biotinylation** is the process of covalently attaching biotin to a protein, nucleic acid or other molecule. Biotinylation is rapid, specific and is unlikely to perturb the natural function of the molecule due to the small size of biotin (MW = 244.31). Biotin binds to streptavidin and avidin with an extremely high affinity and specificity, and these interactions are exploited in many areas of biotechnology to isolate biotinylated molecules of interest. Biotin-binding to streptavidin and avidin is resistant to extremes of heat, pH and proteolysis, making capture of biotinylated molecules possible in a wide variety of environments. Also, multiple biotin molecules can be conjugated to a protein of interest, which allows binding of multiple streptavidin, avidin or Neutravidin protein molecules and increases the sensitivity of detection of the protein of interest. There is a large number of biotinylation reagents available that exploit the wide range of possible labelling methods.

Labeling methods

Proteins can be biotinylated chemically or enzymatically. Chemical biotinylation utilises various conjugation chemistries to yield nonspecific biotinylation of amines, carboxylates, sulfhydryls and carbohydrates (e.g., NHS-coupling gives biotinylation of any primary amines in the protein). Enzymatic biotinylation results in biotinylation of a specific lysine within a certain sequence by a bacterial biotin ligase. Most biotinylation reagents consist of a reactive group attached via a linker to the valeric acid side chain of biotin. As the biotin binding pocket in avidin / streptavidin is buried beneath the protein surface, biotinylation reagents possessing a longer linker are desirable, as they enable the biotin molecule to be more accessible to binding avidin/streptavidin/Neutravidin protein. This linker can also mediate the solubility of biotinylation reagents; linkers that incorporate poly(ethylene) glycol (PEG) can make water-insoluble reagents soluble or increase the solubility of biotinylation reagents that are already soluble to some extent.

Primary amine biotinylation

The most common targets for modifying protein molecules are primary amine groups that are present as lysine side chain epsilon-amines and N-terminal α-amines. Amine-reactive biotinylation reagents can be divided into two groups based on water solubility.

N-hydroxysuccinimide (NHS) esters have poor solubility in aqueous solutions. For reactions in aqueous solution, they must first be dissolved in an organic solvent, then diluted into the aqueous reaction mixture. The most commonly used organic solvents for this purpose are dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF), which are compatible with most proteins at low concentrations. Because of the hydrophobicity of NHS-esters, NHS biotinylation reagents can also diffuse through the cell membrane, meaning that they will biotinylate both internal and external components of a cell.

Sulfo-NHS esters are more soluble in water and should be dissolved in water just before use because they hydrolyze easily. The water solubility of sulfo-NHS-esters stems from their sulfonate group on the N-hydroxysuccinimide ring and eliminates the need to dissolve the reagent in an organic solvent. Sulfo-NHS-esters of biotin also can be used as cell surface biotinylation reagents, because they do not penetrate the cell membrane.

The chemical reactions of NHS- and sulfo-NHS esters are essentially identical, in that they both react spontaneously with amines to form an amide bond. Because the target for the ester is a deprotonated primary amine, the reaction is favored under basic conditions (above pH 7). Hydrolysis of the NHS ester is a major competing reaction, and the rate of hydrolysis increases with increasing pH. NHS- and sulfo-NHS-esters have a half-life of several hours at pH 7 but only a few minutes at pH 9.

There is some flexibility in the conditions for conjugating NHS-esters to primary amines. Incubation temperatures can range from 4-37°C, pH values in the reaction range from 7-9, and incubation times range from a few minutes to 12 hours. Buffers containing amines (such as Tris or glycine) must be avoided, because they compete with the reaction.

Sulfhydryl biotinylation

An alternative to primary amine biotinylation is to label sulfhydryl groups with biotin. Because free sulfhydryl groups are less prevalent on most proteins compared to primary amines, sulfhydryl biotinylation is useful when primary amines are located in the regulatory domain(s) of the target protein or when a reduced level of biotinylation is required. Sulfhydryl-reactive groups such as [[maleimide]s], haloacetyls and pyridyl disulfides, require free sulfhydryl groups for conjugation; disulfide bonds must first be reduced to free up the sulfhydryl groups for biotinylation. If no free sulfhydryl groups are available, lysines can be modified with various thiolation reagents (Traut's Reagent, SAT(PEG4), SATA and SATP), resulting in the addition of a free sulfhydryl. Sulfhydryl biotinylation is performed at a slightly lower pH (6.5-7.5) than labeling with NHS esters.

Besides whole proteins, biotinylated peptides can be synthesized by introducing a cysteine (Cys) residue during synthesis at the terminus of the amino acid chain to get a site specific and oriented biotinylation. Nucleotides can also be biotinylated by incorporation of biotinylated nucleotides.

Carboxyl biotinylation

Carboxyl groups are found on the C-terminal ends of proteins and on glutamate and aspartate amino acid side chains. Biotinylation reagents that target carboxyl groups do not have a carboxyl-reactive moiety per se but instead rely on a carbodiimide crosslinker such as EDC to bind the primary amine on the biotinylation reagents to the carboxyl group on the target protein.

Biotinylation at carboxyl groups occur at pH 4.5-5.5. To prevent crossreactivity of the crosslinker with buffer constituents, buffers should not contain primary amines (e.g., Tris, glycine) or carboxyls (e.g., acetate, citrate); MES buffer is an ideal choice.

Glycoprotein biotinylation

Glycoproteins can be biotinylated by modifying the carbohydrate residues to aldehydes, which then react with hydrazine- or alkoxyamine-based biotinylation reagents. Sodium periodate oxidizes the sialic acids on glycoproteins to aldehydes to form these stable linkages wat pH 4-6.

Polyclonal antibodies are heavily glycosylated, and because glycosylation does not interfere with the antibody activity, biotinylating the glycosyl groups is an ideal strategy to generate biotinylated antibodies.

Non-specific biotinylation

Photoactivatable biotinylation reagents are ideal when primary amines, sulfhydryls, carboxyls and carbohydrates are not available for labeling. These reagents rely on aryl azides, which become activated by ultraviolet light (UV; >350nm), which then react at C-H and N-H bonds. Because these types of bonds occur independent of the type of amino acid, this type of biotinylation is termed "non-specific".

Photoactivatable biotinylation reagents can also be used to activate biotinylation at specific times in an experiment or during certain reaction conditions, by simply exposing the reaction to UV light at the specific time or condition.

Purpose

Purification

The biotin tag can be used in affinity chromatography together with a column that has avidin (also streptavidin or Neutravidin) bound to it, which is the natural ligand for biotin. However, harsh conditions (e.g., 6M GuHCl at pH 1.5) are needed to break the avidin / streptavidin - biotin interaction, which will most likely denature the protein carrying the biotin tag. If isolation of the tagged protein is needed, it is better to tag the protein with iminobiotin. This biotin analogue gives strong binding to avidin/streptavidin at alkaline pH, but the affinity is reduced upon lowering the pH. Therefore, a

iminobiotin-tagged, functional protein can be released from an avidin/streptavidin column by decreasing the pH (to around pH 4).

Detection

This tag can also be used in detection of the protein via anti-biotin antibodies or avidin/streptavidin-tagged detection strategies such as enzyme reporters (e.g., horseradish peroxidase, alkaline phosphatase) or fluorescent probes. This can be useful in localization, ELISA assays, ELISPOT assays, western blots and other immunoanalytical methods.

Other uses

The non-covalent bond formed between biotin and avidin or streptavidin has a binding affinity that is higher than most antigen and antibody bonds and approaches the strength of a covalent bond. This very tight binding makes labeling proteins with biotin a useful tool for applications such as affinity chromatography using immobilized avidin or streptavidin to separate the biotinylated protein from a mixture of other proteins and biochemicals. Biotinylated protein such as biotinylated bovine serum albumin (BSA) is used in solid-phase assays as a coating on the well surface in multiwell assay plates. Biotinylation of red blood cells has been used as a means of determining total blood volume without the use of radiolabels such as chromium 51, allowing volume determinations in low birth weight infants and pregnant women who could not otherwise be exposed to the required doses of radioactivity.

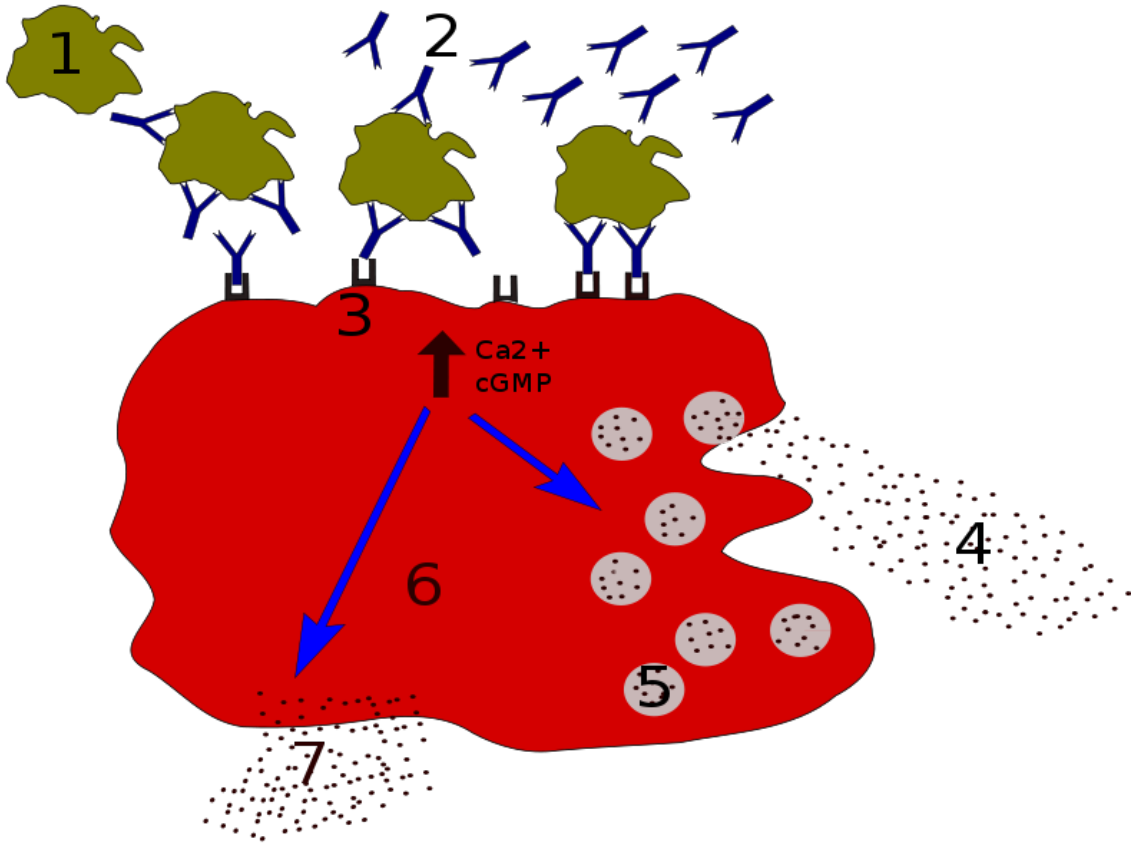
Determining the extent of biotinylation

Reaction conditions for biotinylation are chosen so that the target molecule (e.g., an antibody) is labeled with sufficient biotin molecules to purify or detect the molecule, but not so much that the biotin interferes with the function of the molecule. The HABA dye(2-(4-hydroxyazobenzene) benzoic acid) method is used to determine the extent of biotinylation. HABA dye is bound to avidin and yields a characteristic absorbance. When biotinylated proteins or other molecules are introduced, the biotin displaces the dye, resulting in a change in absorbance at 500 nm. This change is directly proportional to the level of biotin in the sample.

Chapter 7

Degranulation and Immunoglobulin Class Switching

Degranulation



The degranulation process in a Mast cell. 1 = antigen; 2 = IgE; 3 = FcεRI; 4 = preformed mediators (histamine, proteases, chemokines, heparin); 5 = granules; 6 - Mast cell; 7 -

newly formed mediators (prostaglandins, leukotrienes, thromboxanes, platelet-activating factor)

Degranulation is a cellular process that releases antimicrobial cytotoxic molecules from secretory vesicles called granules found inside some cells. It is used by several different cells involved in the immune system, including granulocytes (neutrophils, basophils and eosinophils) and mast cells, and certain lymphocytes such as natural killer (NK) cells and cytotoxic T cells, whose main purpose is to destroy invading microorganisms.

Mast cells

Antigens interact with IgE molecules already bound to high affinity Fc receptors on the surface of mast cells to induce degranulation. The mast cell releases a mixture of compounds, including histamine, proteoglycans and serine proteases, from its cytoplasmic granules.

Eosinophils

In a similar mechanism, activated eosinophils release preformed mediators such as major basic protein, and enzymes such as peroxidase, following interaction between their Fc receptors and IgE molecules that are bound to large parasites like helminths.

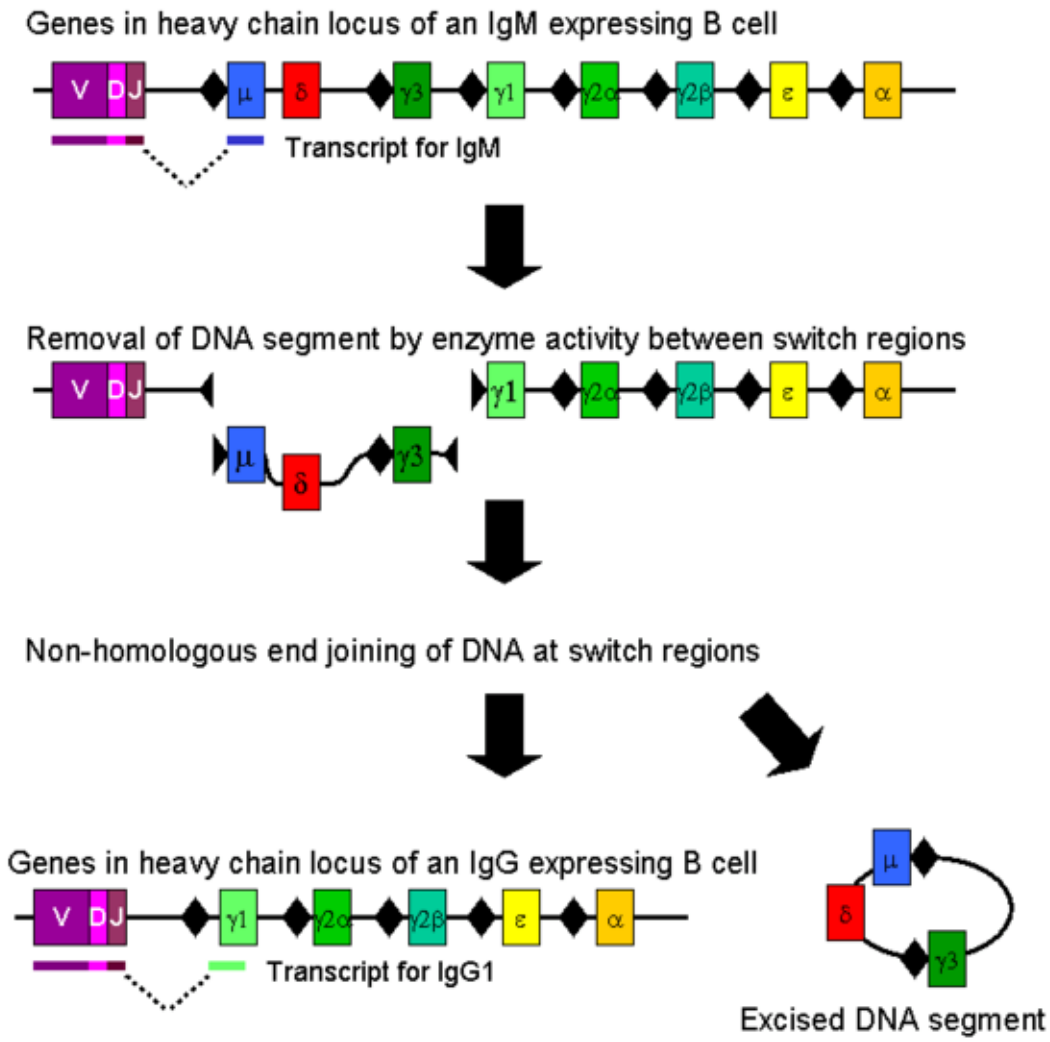
Neutrophils

Four kinds of granules exist in neutrophils that display differences in content and regulation. Secretory vesicles are the most likely to release their contents by degranulation, followed by gelatinase granules, specific granules, and azurophil granules.

Cytotoxic T cells and NK cells

Cytotoxic T cells and NK cells release molecules like perforin and granzymes by a process of directed exocytosis to kill infected target cells.

Immunoglobulin class switching



Mechanism of class switch recombination that allows isotype switching in activated B cells

Immunoglobulin class switching (or **isotype switching** or **isotypic commutation** or **class switch recombination**(CSR)) is a biological mechanism that changes a B cell's production of antibody from one class to another, for example, from an isotype called IgM to an isotype called IgG. During this process, the constant region portion of the antibody heavy chain is changed, but the variable region of the heavy chain stays the same (the terms "constant" and "variable" refer to changes or lack thereof between antibodies that target different epitopes). Since the variable region does not change, class

switching does not affect antigen specificity. Instead, the antibody retains affinity for the same antigens, but can interact with different effector molecules.

Mechanism

Class switching occurs after activation of a mature B cell via its membrane-bound antibody molecule (or B cell receptor) to generate the different classes of antibody, all with the same variable domains as the original antibody generated in the immature B cell during the process of V(D)J recombination, but possessing distinct constant domains in their heavy chains.

Naïve mature B cells produce both IgM and IgD, which are the first two heavy chain segments in the immunoglobulin locus. After activation by antigen, these B cells proliferate. If these activated B cells encounter specific signaling molecules via their CD40 and cytokine receptors (both modulated by T helper cells), they undergo antibody class switching to produce IgG, IgA or IgE antibodies. During class switching, the constant region of the immunoglobulin heavy chain changes but the variable regions, and therefore antigenic specificity, stay the same. This allows different daughter cells from the same activated B cell to produce antibodies of different isotypes or subtypes (e.g. IgG1, IgG2 etc.).

The order of the heavy chain exons are as follows:

- μ - IgM
- δ - IgD
- γ3 - IgG3
- γ1 - IgG1
- pseudogene similar to ε gene that is not used
- α1 - IgA1
- γ2 - IgG2
- γ4 - IgG4
- ε - IgE
- α2 - IgA2

Class switching occurs by a mechanism called class switch recombination (CSR) binding. Class switch recombination is a biological mechanism that allows the class of antibody produced by an activated B cell to change during a process known as isotype or class switching. During CSR, portions of the antibody heavy chain locus are removed from the chromosome, and the gene segments surrounding the deleted portion are rejoined to retain a functional antibody gene that produces antibody of a different isotype. Double-stranded breaks are generated in DNA at conserved nucleotide motifs, called switch (S) regions, which are upstream from gene segments that encode the constant regions of antibody heavy chains; these occur adjacent to all heavy chain constant region genes with the exception of the δ-chain. DNA is nicked and broken at two selected S-regions by the activity of a series of enzymes, including Activation-Induced (Cytidine) Deaminase (AID), uracil DNA glycosylase and apyrimidic/apurinic (AP)-endonucleases. The

intervening DNA between the S-regions is subsequently deleted from the chromosome, removing unwanted μ or δ heavy chain constant region exons and allowing substitution of a γ, α or ε constant region gene segment. The free ends of the DNA are rejoined by a process called non-homologous end joining (NHEJ) to link the variable domain exon to the desired downstream constant domain exon of the antibody heavy chain. In the absence of non-homologous end joining, free ends of DNA may be rejoined by an alternative pathway biased toward microhomology joins. With the exception of the μ and δ genes, only one antibody class is expressed by a B cell at any point in time.

Cytokines responsible for class switching

T cell cytokines are responsible for class switching in mouse (Table 1) and human (Table 2). These cytokines may have suppressive effect on production of IgM.

Table 1. Class switching in mouse

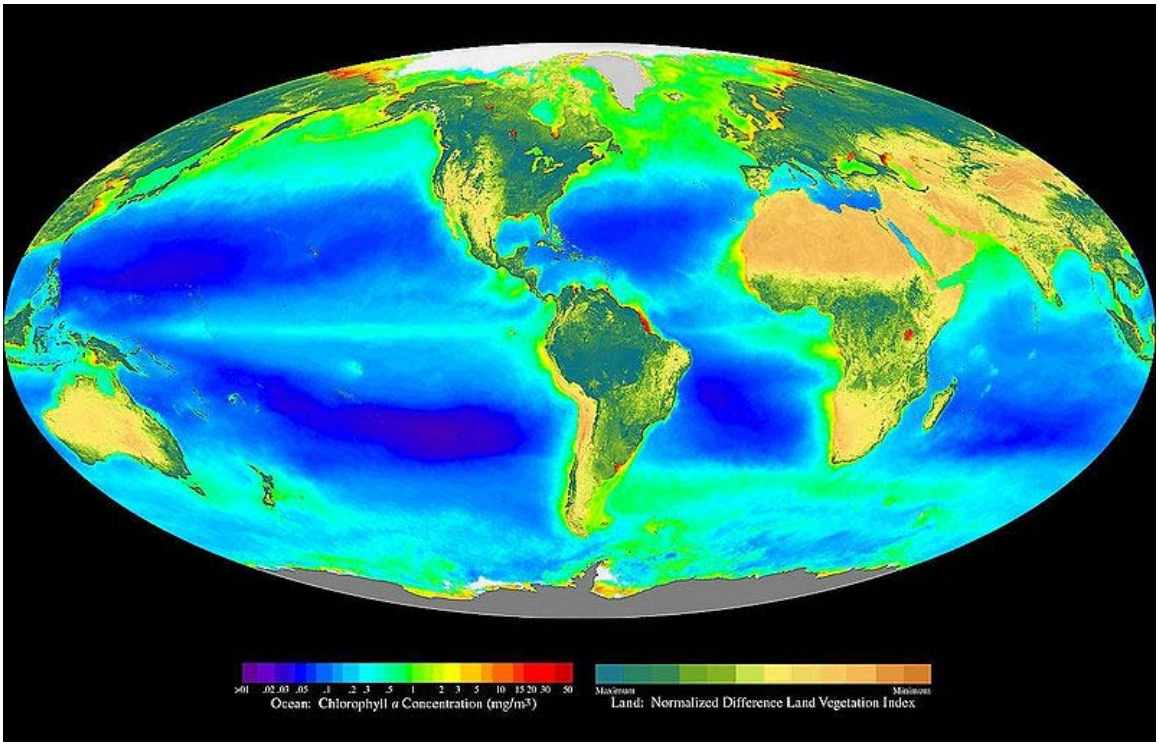
T cells	Cytokines	Immunoglobulin classes					
		IgG1	IgG2a	IgG2b	IgG3	IgA	IgE
Th2	IL-4	↑	↓	↓	↓	↓	↑
	IL-5					↑	
Th1	IFNγ	↓	↑	↓	↓	↓	↓
Treg	TGFβ			↑	↓	↑	

Table 2. Class switching in human

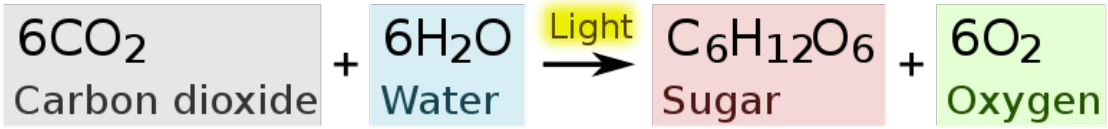
T cells	Cytokines	Immunoglobulin classes					
		IgG1	IgG2	IgG3	IgG4	IgA	IgE
Th2	IL-4				↑		↑
	IL-5					↑	
Th1	IFNγ						
Treg	TGFβ					↑	

Chapter 8

Photosynthesis



Composite image showing the global distribution of photosynthesis, including both oceanic phytoplankton and vegetation

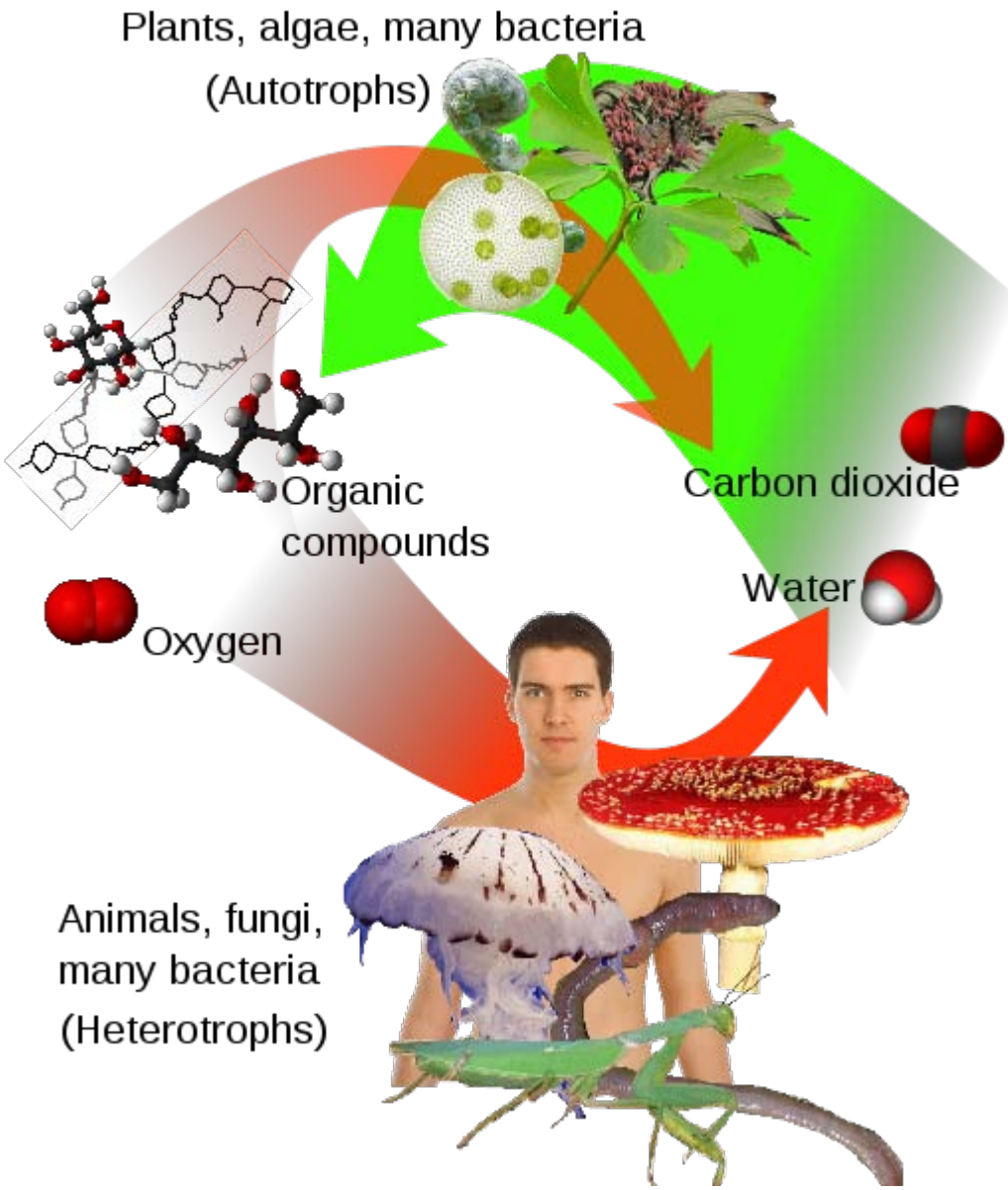


Overall equation for the type of photosynthesis that occurs in plants

Photosynthesis is a process that converts carbon dioxide into organic compounds, especially sugars, using the energy from sunlight. Photosynthesis occurs in plants, algae,

and many species of bacteria, but not in archaea. Photosynthetic organisms are called *photoautotrophs*, since they can create their own food. In plants, algae, and cyanobacteria, photosynthesis uses carbon dioxide and water, releasing oxygen as a waste product. Photosynthesis is vital for all aerobic life on Earth. As well as maintaining the normal level of oxygen in the atmosphere, nearly all life either depends on it directly as a source of energy, or indirectly as the ultimate source of the energy in their food (the exceptions are chemoautotrophs that live in rocks or around deep sea hydrothermal vents). The rate of energy capture by photosynthesis is immense, approximately 100 terawatts, which is about six times larger than the power consumption of human civilization. As well as energy, photosynthesis is also the source of the carbon in all the organic compounds within organisms' bodies. In all, photosynthetic organisms convert around 100–115 teragrams of carbon into biomass per year.

Although photosynthesis can happen in different ways in different species, some features are always the same. For example, the process always begins when energy from light is absorbed by proteins called photosynthetic reaction centers that contain chlorophylls. In plants, these proteins are held inside organelles called chloroplasts, while in bacteria they are embedded in the plasma membrane. Some of the light energy gathered by chlorophylls is stored in the form of adenosine triphosphate (ATP). The rest of the energy is used to remove electrons from a substance such as water. These electrons are then used in the reactions that turn carbon dioxide into organic compounds. In plants, algae and cyanobacteria, this is done by a sequence of reactions called the Calvin cycle, but different sets of reactions are found in some bacteria, such as the reverse Krebs cycle in *Chlorobium*. Many photosynthetic organisms have adaptations that concentrate or store carbon dioxide. This helps reduce a wasteful process called photorespiration that can consume part of the sugar produced during photosynthesis.

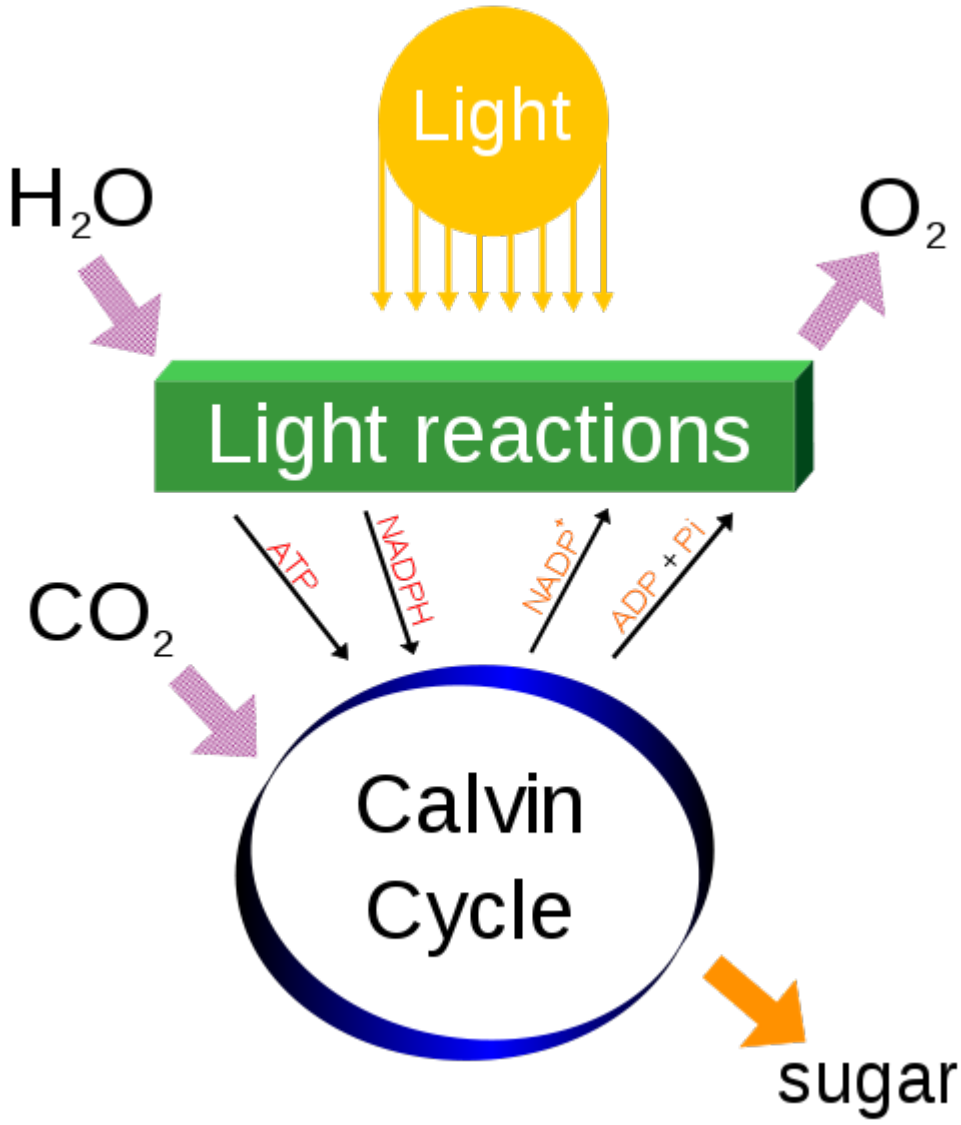


Overview of cycle between autotrophs and heterotrophs. Photosynthesis is the main means by which plants, algae and many bacteria produce organic compounds and oxygen from carbon dioxide and water (green arrow).

The first photosynthetic organisms probably evolved about 3,500 million years ago, early in the evolutionary history of life, when all forms of life on Earth were microorganisms and the atmosphere had much more carbon dioxide. They most likely used hydrogen or hydrogen sulfide as sources of electrons, rather than water. Cyanobacteria appeared later, around 3,000 million years ago, and drastically changed the Earth when they began to oxygenate the atmosphere, beginning about 2,400 million years ago. This new atmosphere allowed the evolution of complex life such as protists. Eventually, no later than a billion years ago, one of these protists formed a symbiotic relationship with a

cyanobacterium, producing the ancestor of many plants and algae. The chloroplasts in modern plants are the descendants of these ancient symbiotic cyanobacteria.

Overview



Photosynthesis changes the energy from the sun into chemical energy, splits water to liberate O_2, and fixes CO_2 into sugar.

Photosynthetic organisms are photoautotrophs, which means that they are able to synthesize food directly from carbon dioxide using energy from light. However, not all organisms that use light as a source of energy carry out photosynthesis, since *photoheterotrophs* use organic compounds, rather than carbon dioxide, as a source of carbon. In plants, algae and cyanobacteria, photosynthesis releases oxygen. This is called *oxygenic photosynthesis*. Although there are some differences between oxygenic

photosynthesis in plants, algae and cyanobacteria, the overall process is quite similar in these organisms. However, there are some types of bacteria that carry out anoxygenic photosynthesis, which consumes carbon dioxide but does not release oxygen.

Carbon dioxide is converted into sugars in a process called carbon fixation. Carbon fixation is a redox reaction, so photosynthesis needs to supply both a source of energy to drive this process, and the electrons needed to convert carbon dioxide into carbohydrate, which is a reduction reaction. In general outline, photosynthesis is the opposite of cellular respiration, where glucose and other compounds are oxidized to produce carbon dioxide, water, and release chemical energy. However, the two processes take place through a different sequence of chemical reactions and in different cellular compartments.

The general equation for photosynthesis is therefore:

$$2n\ CO_2 + 2n\ H_2O + \text{photons} \rightarrow 2(CH_2O)_n + n\ O_2 + 2n\ A$$

Carbon dioxide + electron donor + light energy → carbohydrate + oxygen + oxidized electron donor

Since water is used as the electron donor in oxygenic photosynthesis, the equation for this process is:

$$2n\ CO_2 + 2n\ H_2O + \text{photons} \rightarrow 2(CH_2O)_n + 2n\ O_2$$

carbon dioxide + water + light energy → carbohydrate + oxygen

Other processes substitute other compounds (such as arsenite) for water in the electron-supply role; the microbes use sunlight to oxidize arsenite to arsenate: The equation for this reaction is:

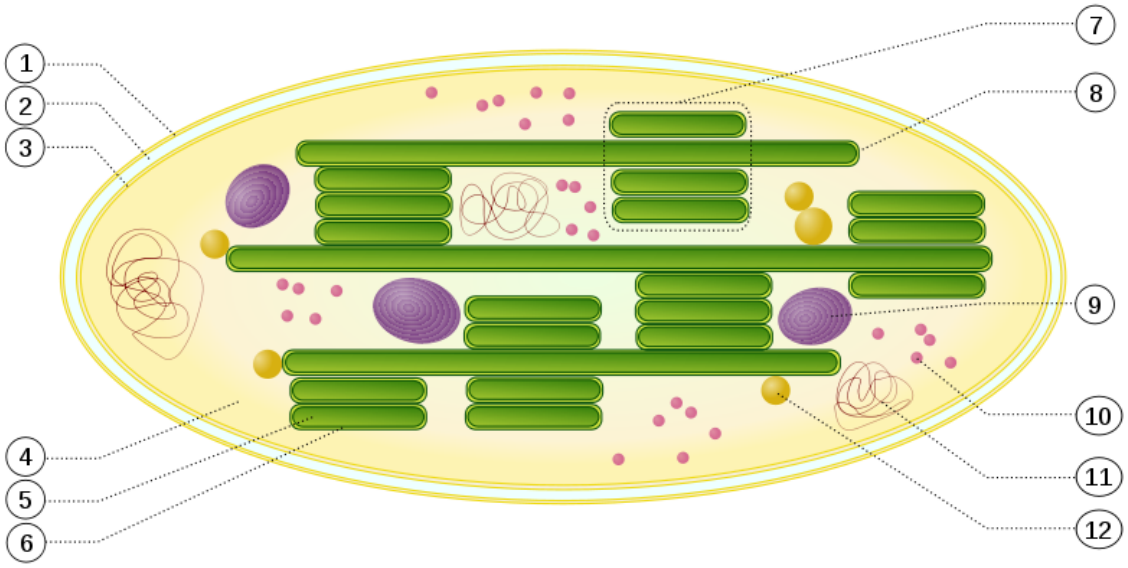
$$(AsO_3^{3-}) + CO_2 + \text{photons} \rightarrow CO + (AsO_4^{3-})$$

carbon dioxide + arsenite + light energy → arsenate + carbon monoxide (used to build other compounds in subsequent reactions)

Photosynthesis occurs in two stages. In the first stage, *light-dependent reactions* or *light reactions* capture the energy of light and use it to make the energy-storage molecules ATP and NADPH. During the second stage, the *light-independent reactions* use these products to capture and reduce carbon dioxide.

Most organisms that utilize photosynthesis to produce oxygen use visible light to do so, although at least three use infrared radiation.

Photosynthetic membranes and organelles



Chloroplast ultrastructure:
1. outer membrane
2. intermembrane space
3. inner membrane (1+2+3: envelope)
4. stroma (aqueous fluid)
5. thylakoid lumen (inside of thylakoid)
6. thylakoid membrane
7. granum (stack of thylakoids)
8. thylakoid (lamella)
9. starch
10. ribosome
11. plastidial DNA
12. plastoglobule (drop of lipids)

The proteins that gather light for photosynthesis are embedded within cell membranes. The simplest way these are arranged is in photosynthetic bacteria, where these proteins are held within the plasma membrane. However, this membrane may be tightly folded into cylindrical sheets called thylakoids, or bunched up into round vesicles called *intracytoplasmic membranes*. These structures can fill most of the interior of a cell, giving the membrane a very large surface area and therefore increasing the amount of light that the bacteria can absorb.

In plants and algae, photosynthesis takes place in organelles called chloroplasts. A typical plant cell contains about 10 to 100 chloroplasts. The chloroplast is enclosed by a membrane. This membrane is composed of a phospholipid inner membrane, a phospholipid outer membrane, and an intermembrane space between them. Within the membrane is an aqueous fluid called the stroma. The stroma contains stacks (grana) of thylakoids, which are the site of photosynthesis. The thylakoids are flattened disks,

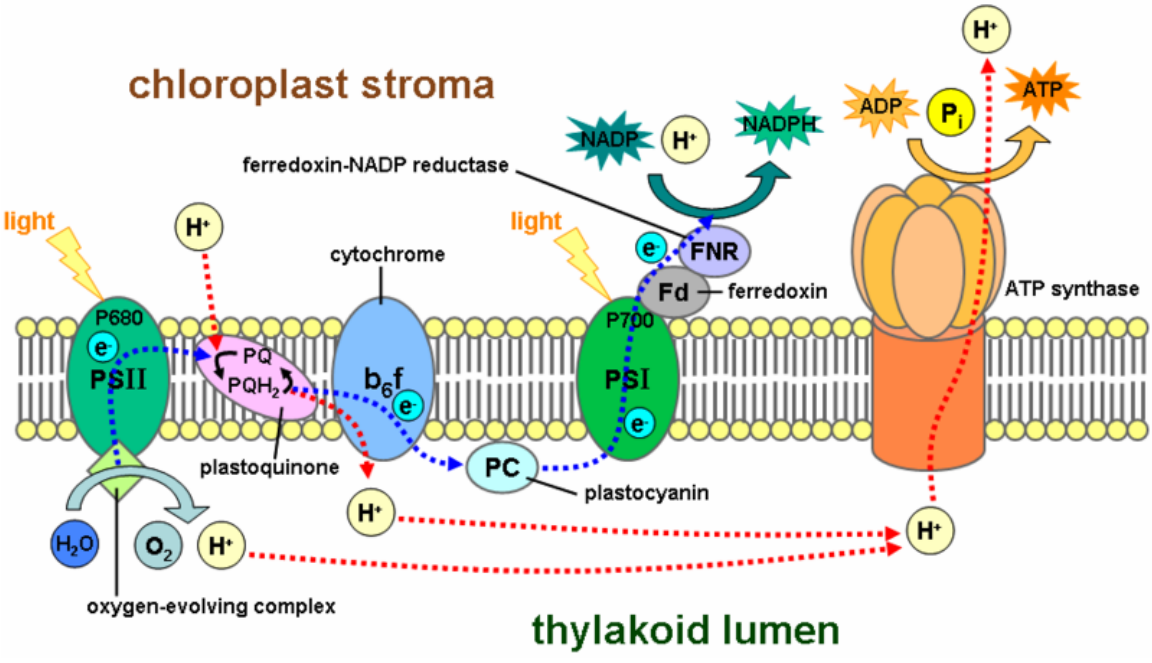
bounded by a membrane with a lumen or thylakoid space within it. The site of photosynthesis is the thylakoid membrane, which contains integral and peripheral membrane protein complexes, including the pigments that absorb light energy, which form the photosystems.

Plants absorb light primarily using the pigment chlorophyll, which is the reason that most plants have a green color. Besides chlorophyll, plants also use pigments such as carotenes and xanthophylls. Algae also use chlorophyll, but various other pigments are present as phycocyanin, carotenes, and xanthophylls in green algae, phycoerythrin in red algae (rhodophytes) and fucoxanthin in brown algae and diatoms resulting in a wide variety of colors.

These pigments are embedded in plants and algae in special antenna-proteins. In such proteins all the pigments are ordered to work well together. Such a protein is also called a light-harvesting complex.

Although all cells in the green parts of a plant have chloroplasts, most of the energy is captured in the leaves. The cells in the interior tissues of a leaf, called the mesophyll, can contain between 450,000 and 800,000 chloroplasts for every square millimeter of leaf. The surface of the leaf is uniformly coated with a water-resistant waxy cuticle that protects the leaf from excessive evaporation of water and decreases the absorption of ultraviolet or blue light to reduce heating. The transparent epidermis layer allows light to pass through to the palisade mesophyll cells where most of the photosynthesis takes place.

Light reactions



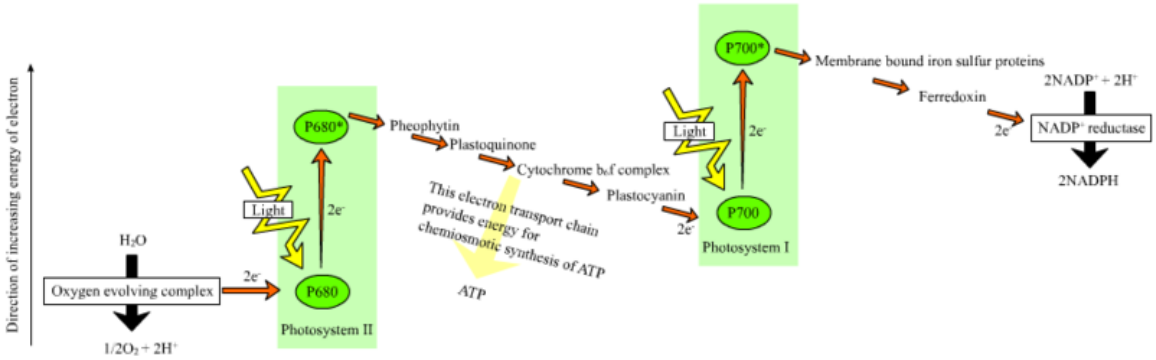
Light-dependent reactions of photosynthesis at the thylakoid membrane

In the light reactions, one molecule of the pigment chlorophyll absorbs one photon and loses one electron. This electron is passed to a modified form of chlorophyll called pheophytin, which passes the electron to a quinone molecule, allowing the start of a flow of electrons down an electron transport chain that leads to the ultimate reduction of NADP to NADPH. In addition, this creates a proton gradient across the chloroplast membrane; its dissipation is used by ATP synthase for the concomitant synthesis of ATP. The chlorophyll molecule regains the lost electron from a water molecule through a process called photolysis, which releases a dioxygen (O_2) molecule. The overall equation for the light-dependent reactions under the conditions of non-cyclic electron flow in green plants is:

$$2\ H_2O + 2\ NADP^+ + 3\ ADP + 3\ P_i + light \rightarrow 2\ NADPH + 2\ H^+ + 3\ ATP + O_2$$

Not all wavelengths of light can support photosynthesis. The photosynthetic action spectrum depends on the type of accessory pigments present. For example, in green plants, the action spectrum resembles the absorption spectrum for chlorophylls and carotenoids with peaks for violet-blue and red light. In red algae, the action spectrum overlaps with the absorption spectrum of phycobilins for blue-green light, which allows these algae to grow in deeper waters that filter out the longer wavelengths used by green plants. The non-absorbed part of the light spectrum is what gives photosynthetic organisms their color (e.g., green plants, red algae, purple bacteria) and is the least effective for photosynthesis in the respective organisms.

Z scheme



The "Z scheme"

In plants, light-dependent reactions occur in the thylakoid membranes of the chloroplasts and use light energy to synthesize ATP and NADPH. The light-dependent reaction has two forms: cyclic and non-cyclic. In the non-cyclic reaction, the photons are captured in the light-harvesting antenna complexes of photosystem II by chlorophyll and other accessory pigments. When a chlorophyll molecule at the core of the photosystem II reaction center obtains sufficient excitation energy from the adjacent antenna pigments, an electron is transferred to the primary electron-acceptor molecule, pheophytin, through a process called photoinduced charge separation. These electrons are shuttled through an electron transport chain, the so called ***Z-scheme*** shown in the diagram, that initially

functions to generate a chemiosmotic potential across the membrane. An ATP synthase enzyme uses the chemiosmotic potential to make ATP during photophosphorylation, whereas NADPH is a product of the terminal redox reaction in the *Z-scheme*. The electron enters a chlorophyll molecule in Photosystem I. The electron is excited due to the light absorbed by the photosystem. A second electron carrier accepts the electron, which again is passed down lowering energies of electron acceptors. The energy created by the electron acceptors is used to move hydrogen ions across the thylakoid membrane into the lumen. The electron is used to reduce the co-enzyme NADP, which has functions in the light-independent reaction. The cyclic reaction is similar to that of the non-cyclic, but differs in the form that it generates only ATP, and no reduced NADP (NADPH) is created. The cyclic reaction takes place only at photosystem I. Once the electron is displaced from the photosystem, the electron is passed down the electron acceptor molecules and returns back to photosystem I, from where it was emitted, hence the name *cyclic reaction*.

Water photolysis

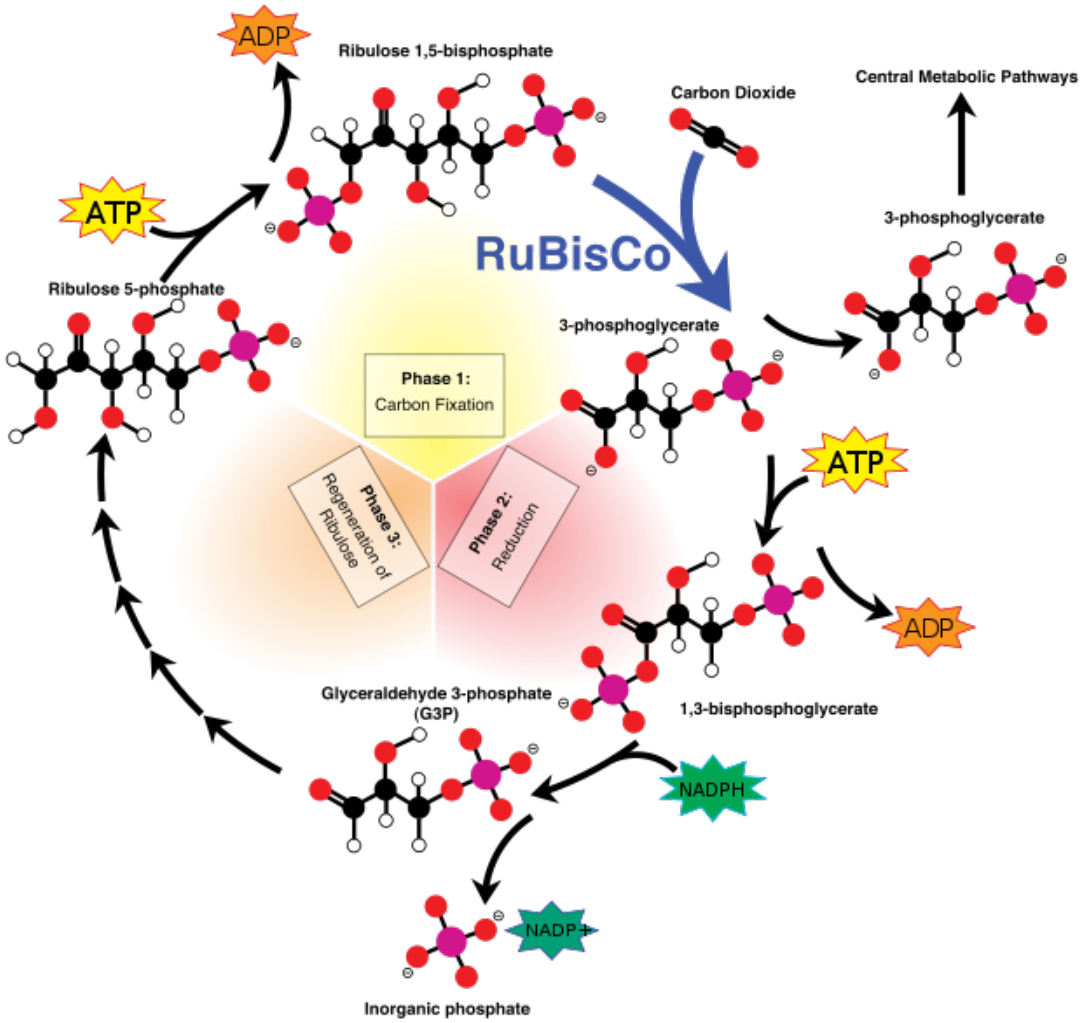
The NADPH is the main reducing agent in chloroplasts, providing a source of energetic electrons to other reactions. Its production leaves chlorophyll with a deficit of electrons (oxidized), which must be obtained from some other reducing agent. The excited electrons lost from chlorophyll in photosystem I are replaced from the electron transport chain by plastocyanin. However, since photosystem II includes the first steps of the *Z-scheme*, an external source of electrons is required to reduce its oxidized **chlorophyll *a*** molecules. The source of electrons in green-plant and cyanobacterial photosynthesis is water. Two water molecules are oxidized by four successive charge-separation reactions by photosystem II to yield a molecule of diatomic oxygen and four hydrogen ions; the electron yielded in each step is transferred to a redox-active tyrosine residue that then reduces the photoxidized paired-chlorophyll *a* species called P680 that serves as the primary (light-driven) electron donor in the photosystem II reaction center. The oxidation of water is catalyzed in photosystem II by a redox-active structure that contains four manganese ions and a calcium ion; this oxygen-evolving complex binds two water molecules and stores the four oxidizing equivalents that are required to drive the water-oxidizing reaction. Photosystem II is the only known biological enzyme that carries out this oxidation of water. The hydrogen ions contribute to the transmembrane chemiosmotic potential that leads to ATP synthesis. Oxygen is a waste product of light-dependent reactions, but the majority of organisms on Earth use oxygen for cellular respiration, including photosynthetic organisms.

Light-independent reactions

The Calvin Cycle

In the Light-independent or dark reactions the enzyme RuBisCO captures CO_2 from the atmosphere and in a process that requires the newly formed NADPH, called the Calvin-Benson Cycle, releases three-carbon sugars, which are later combined to form sucrose and starch. The overall equation for the light-independent reactions in green plants is:

$3\ CO_2 + 9\ ATP + 6\ NADPH + 6\ H^+ \rightarrow C_3H_6O_3\text{-phosphate} + 9\ ADP + 8\ P_i + 6\ NADP^+ + 3\ H_2O$



Overview of the Calvin cycle and carbon fixation

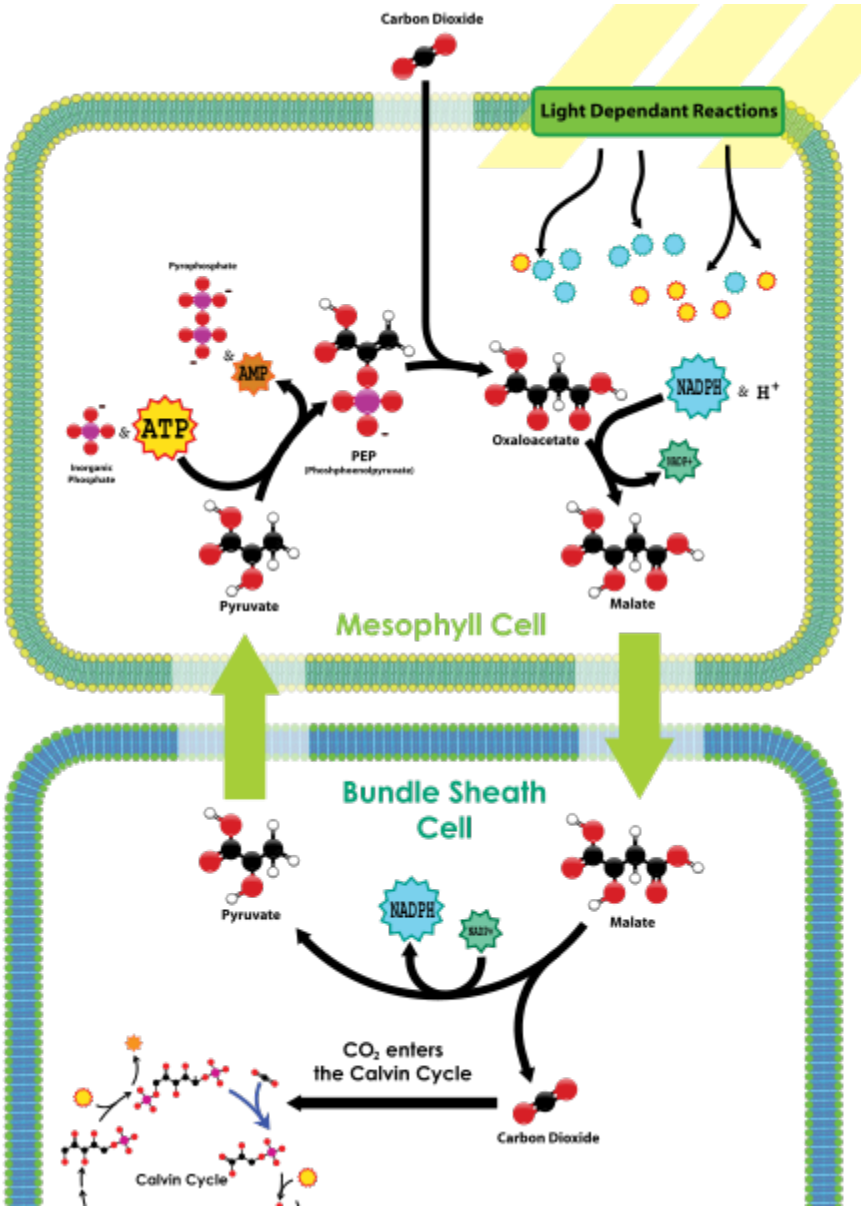
To be more specific, carbon fixation produces an intermediate product, which is then converted to the final carbohydrate products. The carbon skeletons produced by photosynthesis are then variously used to form other organic compounds, such as the building material cellulose, as precursors for lipid and amino acid biosynthesis, or as a fuel in cellular respiration. The latter occurs not only in plants but also in animals when the energy from plants gets passed through a food chain.

The fixation or reduction of carbon dioxide is a process in which carbon dioxide combines with a five-carbon sugar, ribulose 1,5-bisphosphate (RuBP), to yield two molecules of a three-carbon compound, glycerate 3-phosphate (GP), also known as 3-phosphoglycerate (PGA). GP, in the presence of ATP and NADPH from the light-dependent stages, is reduced to glyceraldehyde 3-phosphate (G3P). This product is also referred to as 3-phosphoglyceraldehyde (PGAL) or even as triose phosphate. Triose is a

3-carbon sugar. Most (5 out of 6 molecules) of the G3P produced is used to regenerate RuBP so the process can continue. The 1 out of 6 molecules of the triose phosphates not "recycled" often condense to form hexose phosphates, which ultimately yield sucrose, starch and cellulose. The sugars produced during carbon metabolism yield carbon skeletons that can be used for other metabolic reactions like the production of amino acids and lipids.

Carbon concentrating mechanisms

On land



Overview of C4 carbon fixation

In hot and dry conditions, plants close their stomata to prevent the loss of water. Under these conditions, CO_2 will decrease, and oxygen gas, produced by the light reactions of photosynthesis, will decrease in the stem, not leaves, causing an increase of photorespiration by the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase and decrease in carbon fixation. Some plants have evolved mechanisms to increase the CO_2 concentration in the leaves under these conditions.

C_4 plants chemically fix carbon dioxide in the cells of the mesophyll by adding it to the three-carbon molecule phosphoenolpyruvate (PEP), a reaction catalyzed by an enzyme called PEP carboxylase, creating the four-carbon organic acid oxaloacetic acid. Oxaloacetic acid or malate synthesized by this process is then translocated to specialized bundle sheath cells where the enzyme RuBisCO and other Calvin cycle enzymes are located, and where CO_2 released by decarboxylation of the four-carbon acids is then fixed by RuBisCO activity to the three-carbon sugar 3-phosphoglyceric acids. The physical separation of RuBisCO from the oxygen-generating light reactions reduces photorespiration and increases CO_2 fixation and, thus, photosynthetic capacity of the leaf. C_4 plants can produce more sugar than C_3 plants in conditions of high light and temperature. Many important crop plants are C_4 plants, including maize, sorghum, sugarcane, and millet. Plants that do not use PEP-carboxylase in carbon fixation are called C_3 plants because the primary carboxylation reaction, catalyzed by RuBisCO, produces the three-carbon sugar 3-phosphoglyceric acids directly in the Calvin-Benson cycle. Over 90% of plants use C_3 carbon fixation, compared to 3% that use C_4 carbon fixation.

Xerophytes, such as cacti and most succulents, also use PEP carboxylase to capture carbon dioxide in a process called Crassulacean acid metabolism (CAM). In contrast to C_4 metabolism, which *physically* separates the CO_2 fixation to PEP from the Calvin cycle, CAM *temporally* separates these two processes. CAM plants have a different leaf anatomy from C_3 plants, and fix the CO_2 at night, when their stomata are open. CAM plants store the CO_2 mostly in the form of malic acid via carboxylation of phosphoenolpyruvate to oxaloacetate, which is then reduced to malate. Decarboxylation of malate during the day releases CO_2 inside the leaves, thus allowing carbon fixation to 3-phosphoglycerate by RuBisCO. Sixteen thousand species of plants use CAM.

In water

Cyanobacteria possess carboxysomes, which increase the concentration of CO_2 around RuBisCO to increase the rate of photosynthesis. This operates by carbonic anhydrase, producing hydrocarbonate ions (HCO_3^-), which are then pumped into the carboxysome, before being processed by a different carbonic anhydrase to produce CO_2. Pyrenoids in algae and hornworts also act to concentrate CO_2 around rubisco.

Order and kinetics

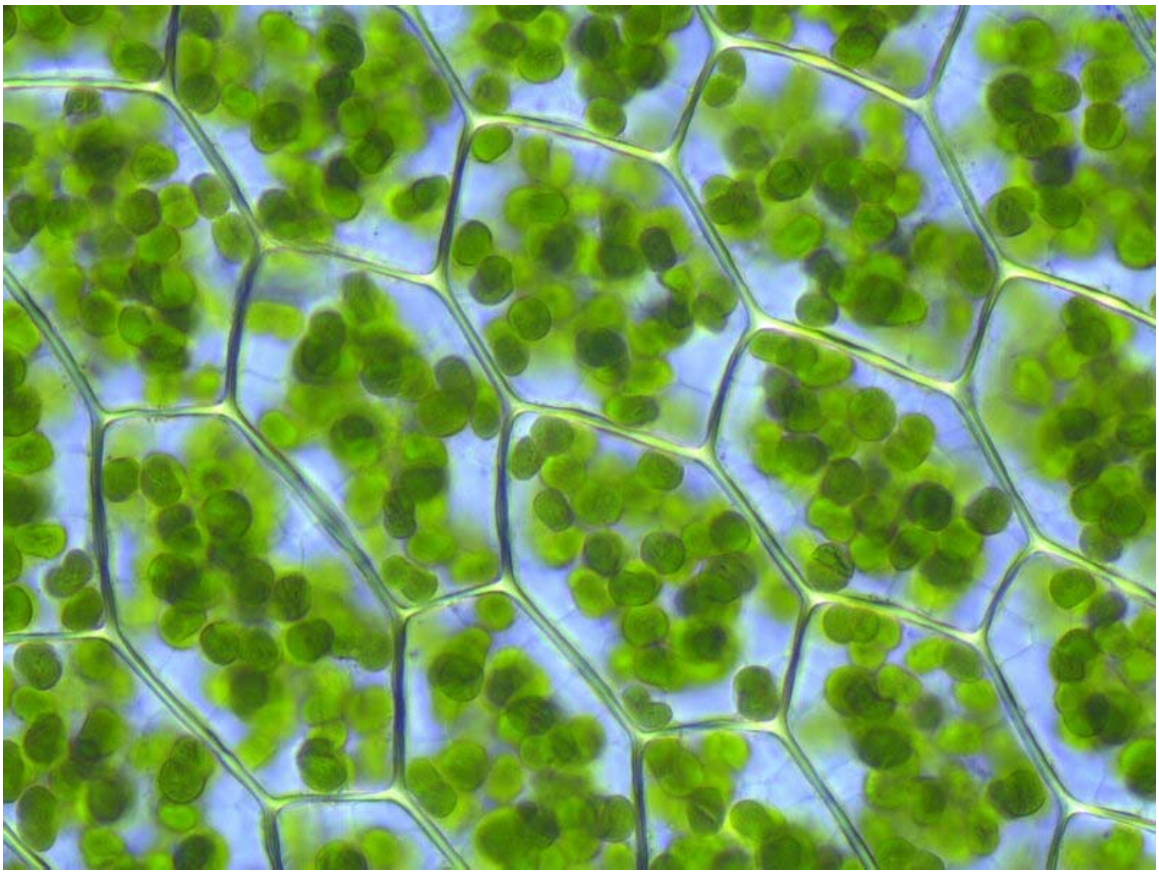
The overall process of photosynthesis takes place in four stages. The first, energy transfer in antenna chlorophyll takes place in the femtosecond (1 femtosecond (fs) = 10^{-15} s) to

picosecond (1 picosecond (ps) = 10^{-12} s) time scale. The next phase, the transfer of electrons in photochemical reactions, takes place in the picosecond to nanosecond time scale (1 nanosecond (ns) = 10^{-9} s). The third phase, the electron transport chain and ATP synthesis, takes place on the microsecond (1 microsecond (μs) = 10^{-6} s) to millisecond (1 millisecond (ms) = 10^{-3} s) time scale. The final phase is carbon fixation and export of stable products, which takes place in the millisecond-to-second time scale. The first three stages occur in the thylakoid membranes.

Efficiency

Plants usually convert light into chemical energy with a photosynthetic efficiency of 3–6%. Actual plants' photosynthetic efficiency varies with the frequency of the light being converted, light intensity, temperature and proportion of carbon dioxide in the atmosphere, and can vary from 0.1% to 8%. By comparison, solar panels convert light into electric energy at an efficiency of approximately 6–20% for mass-produced panels, and up to 41% in a research laboratory.

Evolution



Plant cells with visible chloroplasts (from a moss, *Plagiomnium affine*)

Early photosynthetic systems, such as those from green and purple sulfur and green and purple nonsulfur bacteria, are thought to have been anoxygenic, using various molecules as electron donors. Green and purple sulfur bacteria are thought to have used hydrogen and sulfur as an electron donor. Green nonsulfur bacteria used various amino and other organic acids. Purple nonsulfur bacteria used a variety of nonspecific organic molecules. The use of these molecules is consistent with the geological evidence that the atmosphere was highly reduced at that time.

Fossils of what are thought to be filamentous photosynthetic organisms have been dated at 3.4 billion years old.

The main source of oxygen in the atmosphere is oxygenic photosynthesis, and its first appearance is sometimes referred to as the oxygen catastrophe. Geological evidence suggests that oxygenic photosynthesis, such as that in cyanobacteria, became important during the Paleoproterozoic era around 2 billion years ago. Modern photosynthesis in plants and most photosynthetic prokaryotes is oxygenic. Oxygenic photosynthesis uses water as an electron donor, which is oxidized to molecular oxygen (O_2) in the photosynthetic reaction center.

Symbiosis and the origin of chloroplasts

Several groups of animals have formed symbiotic relationships with photosynthetic algae. These are most common in corals, sponges and sea anemones. It is presumed that this is due to the particularly simple body plans and large surface areas of these animals compared to their volumes. In addition, a few marine mollusks *Elysia viridis* and *Elysia chlorotica* also maintain a symbiotic relationship with chloroplasts they capture from the algae in their diet and then store in their bodies. This allows the molluscs to survive solely by photosynthesis for several months at a time. Some of the genes from the plant cell nucleus have even been transferred to the slugs, so that the chloroplasts can be supplied with proteins that they need to survive.

An even closer form of symbiosis may explain the origin of chloroplasts. Chloroplasts have many similarities with photosynthetic bacteria, including a circular chromosome, prokaryotic-type ribosomes, and similar proteins in the photosynthetic reaction center. The endosymbiotic theory suggests that photosynthetic bacteria were acquired (by endocytosis) by early eukaryotic cells to form the first plant cells. Therefore, chloroplasts may be photosynthetic bacteria that adapted to life inside plant cells. Like mitochondria, chloroplasts still possess their own DNA, separate from the nuclear DNA of their plant host cells and the genes in this chloroplast DNA resemble those in cyanobacteria. DNA in chloroplasts codes for redox proteins such as photosynthetic reaction centers. The CoRR Hypothesis proposes that this **Co**-location is required for **R**edox **R**egulation.

Cyanobacteria and the evolution of photosynthesis

The biochemical capacity to use water as the source for electrons in photosynthesis evolved once, in a common ancestor of extant cyanobacteria. The geological record

indicates that this transforming event took place early in Earth's history, at least 2450–2320 million years ago (Ma), and, it is speculated, much earlier. Available evidence from geobiological studies of Archean (>2500 Ma) sedimentary rocks indicates that life existed 3500 Ma, but the question of when oxygenic photosynthesis evolved is still unanswered. A clear paleontological window on cyanobacterial evolution opened about 2000 Ma, revealing an already-diverse biota of blue-greens. Cyanobacteria remained principal primary producers throughout the Proterozoic Eon (2500–543 Ma), in part because the redox structure of the oceans favored photoautotrophs capable of nitrogen fixation. Green algae joined blue-greens as major primary producers on continental shelves near the end of the Proterozoic, but only with the Mesozoic (251–65 Ma) radiations of dinoflagellates, coccolithophorids, and diatoms did primary production in marine shelf waters take modern form. Cyanobacteria remain critical to marine ecosystems as primary producers in oceanic gyres, as agents of biological nitrogen fixation, and, in modified form, as the plastids of marine algae.

A 2010 study by researchers at Tel Aviv University discovered that the Oriental hornet (*Vespa orientalis*) converts sunlight into electric power using a pigment called xanthopterin. This is the first scientific evidence of a member of the animal kingdom engaging in photosynthesis.

Discovery

Although some of the steps in photosynthesis are still not completely understood, the overall photosynthetic equation has been known since the 19th century.

Jan van Helmont began the research of the process in the mid-17th century when he carefully measured the mass of the soil used by a plant and the mass of the plant as it grew. After noticing that the soil mass changed very little, he hypothesized that the mass of the growing plant must come from the water, the only substance he added to the potted plant. His hypothesis was partially accurate — much of the gained mass also comes from carbon dioxide as well as water. However, this was a signaling point to the idea that the bulk of a plant's biomass comes from the inputs of photosynthesis, not the soil itself.

Joseph Priestley, a chemist and minister, discovered that, when he isolated a volume of air under an inverted jar, and burned a candle in it, the candle would burn out very quickly, much before it ran out of wax. He further discovered that a mouse could similarly "injure" air. He then showed that the air that had been "injured" by the candle and the mouse could be restored by a plant.

In 1778, Jan Ingenhousz, court physician to the Austrian Empress, repeated Priestley's experiments. He discovered that it was the influence of sunlight on the plant that could cause it to revive a mouse in a matter of hours.

In 1796, Jean Senebier, a Swiss pastor, botanist, and naturalist, demonstrated that green plants consume carbon dioxide and release oxygen under the influence of light. Soon afterward, Nicolas-Théodore de Saussure showed that the increase in mass of the plant as

it grows could not be due only to uptake of CO_2 but also to the incorporation of water. Thus, the basic reaction by which photosynthesis is used to produce food (such as glucose) was outlined.

Cornelis Van Niel made key discoveries explaining the chemistry of photosynthesis. By studying purple sulfur bacteria and green bacteria he was the first scientist to demonstrate that photosynthesis is a light-dependent redox reaction, in which hydrogen reduces carbon dioxide.

Robert Emerson discovered two light reactions by testing plant productivity using different wavelengths of light. With the red alone, the light reactions were suppressed. When blue and red were combined, the output was much more substantial. Thus, there were two photosystems, one aborbing up to 600 nm wavelengths, the other up to 700. The former is known as PSII, the latter is PSI. PSI contains only chlorophyll a, PSII contains primarily chlorophyll a with most of the available chlorophyll b, among other pigments.

Further experiments to prove that the oxygen developed during the photosynthesis of green plants came from water, were performed by Robert Hill in 1937 and 1939. He showed that isolated chloroplasts give off oxygen in the presence of unnatural reducing agents like iron oxalate, ferricyanide or benzoquinone after exposure to light. The Hill reaction is as follows:

$$2\ H_2O + 2\ A + (\text{light, chloroplasts}) \rightarrow 2\ AH_2 + O_2$$

where A is the electron acceptor. Therefore, in light, the electron acceptor is reduced and oxygen is evolved. Cyt b_6, now known as a plastoquinone, is one electron acceptor.

Samuel Ruben and Martin Kamen used radioactive isotopes to determine that the oxygen liberated in photosynthesis came from the water.

Melvin Calvin and Andrew Benson, along with James Bassham, elucidated the path of carbon assimilation (the photosynthetic carbon reduction cycle) in plants. The carbon reduction cycle is known as the Calvin cycle, which ignores the contribution of Bassham and Benson. Many scientists refer to the cycle as the Calvin-Benson Cycle, Benson-Calvin, and some even call it the Calvin-Benson-Bassham (or CBB) Cycle.

Nobel Prize-winning scientist Rudolph A. Marcus was able to discover the function and significance of the electron transport chain.

Otto Heinrich Warburg and Dean Burk discovered the I-quantum photosynthesis reaction that splits the CO2, activated by the respiration.

Factors

The leaf is the primary site of photosynthesis in plants.

There are three main factors affecting photosynthesis and several corollary factors. The three main are:

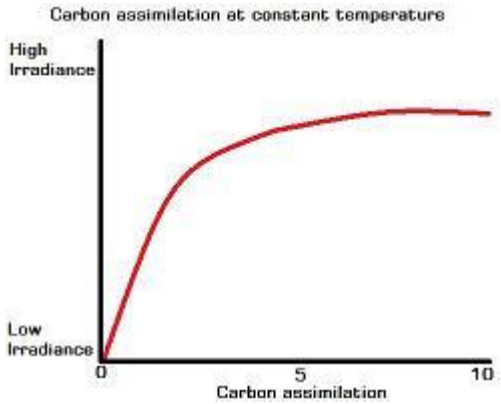
- Light irradiance and wavelength
- Carbon dioxide concentration
- Temperature.

Light intensity (irradiance), wavelength and temperature

In the early 20th century, Frederick Frost Blackman along with Albert Einstein investigated the effects of light intensity (irradiance) and temperature on the rate of carbon assimilation.

- At constant temperature, the rate of carbon assimilation varies with irradiance, initially increasing as the irradiance increases. However, at higher irradiance, this relationship no longer holds and the rate of carbon assimilation reaches a plateau.
- At constant irradiance, the rate of carbon assimilation increases as the temperature is increased over a limited range. This effect is seen only at high irradiance levels.

At low irradiance, increasing the temperature has little influence on the rate of carbon assimilation.



Carbon assimilation at a constant temperature.

These two experiments illustrate vital points: First, from research it is known that, in general, photochemical reactions are not affected by temperature. However, these experiments clearly show that temperature affects the rate of carbon assimilation, so there must be two sets of reactions in the full process of carbon assimilation. These are, of course, the light-dependent 'photochemical' stage and the light-independent, temperature-dependent stage. Second, Blackman's experiments illustrate the concept of limiting factors. Another limiting factor is the wavelength of light. Cyanobacteria, which reside several meters underwater, cannot receive the correct wavelengths required to cause photoinduced charge separation in conventional photosynthetic pigments. To combat this problem, a series of proteins with different pigments surround the reaction center. This unit is called a phycobilisome.

Carbon dioxide levels and photorespiration

As carbon dioxide concentrations rise, the rate at which sugars are made by the light-independent reactions increases until limited by other factors. RuBisCO, the enzyme that captures carbon dioxide in the light-independent reactions, has a binding affinity for both carbon dioxide and oxygen. When the concentration of carbon dioxide is high, RuBisCO will fix carbon dioxide. However, if the carbon dioxide concentration is low, RuBisCO will bind oxygen instead of carbon dioxide. This process, called photorespiration, uses energy, but does not produce sugars.

RuBisCO oxygenase activity is disadvantageous to plants for several reasons:

1. One product of oxygenase activity is phosphoglycolate (2 carbon) instead of 3-phosphoglycerate (3 carbon). Phosphoglycolate cannot be metabolized by the Calvin-Benson cycle and represents carbon lost from the cycle. A high oxygenase activity, therefore, drains the sugars that are required to recycle ribulose 5-bisphosphate and for the continuation of the Calvin-Benson cycle.

2. Phosphoglycolate is quickly metabolized to glycolate that is toxic to a plant at a high concentration; it inhibits photosynthesis.
3. Salvaging glycolate is an energetically expensive process that uses the glycolate pathway, and only 75% of the carbon is returned to the Calvin-Benson cycle as 3-phosphoglycerate. The reactions also produce ammonia (NH_3), which is able to diffuse out of the plant, leading to a loss of nitrogen.

   A highly simplified summary is:
   2 glycolate + ATP → 3-phosphoglycerate + carbon dioxide + ADP +NH_3

The salvaging pathway for the products of RuBisCO oxygenase activity is more commonly known as photorespiration, since it is characterized by light-dependent oxygen consumption and the release of carbon dioxide.

Chapter 9

Cellular Differentiation

In developmental biology, **cellular differentiation** is the process by which a less specialized cell becomes a more specialized cell type. Differentiation occurs numerous times during the development of a multicellular organism as the organism changes from a simple zygote to a complex system of tissues and cell types. Differentiation is a common process in adults as well: adult stem cells divide and create fully-differentiated daughter cells during tissue repair and during normal cell turnover. Differentiation dramatically changes a cell's size, shape, membrane potential, metabolic activity, and responsiveness to signals. These changes are largely due to highly-controlled modifications in gene expression. With a few exceptions, cellular differentiation almost never involves a change in the DNA sequence itself. Thus, different cells can have very different physical characteristics despite having the same genome.

A cell that is able to differentiate into all cell types of the adult organism is known as *pluripotent*. Such cells are called embryonic stem cells in animals and meristematic cells in higher plants. A cell that is able to differentiate into all cell types, including the placental tissue, is known as *totipotent*. In mammals, only the zygote and subsequent blastomeres are totipotent, while in plants many differentiated cells can become totipotent with simple laboratory techniques. In cytopathology, the level of cellular differentiation is used as a measure of cancer progression. "Grade" is a marker of how differentiated a cell in a tumor is.

Mammalian cell types

Three basic categories of cells make up the mammalian body: germ cells, somatic cells, and stem cells. Each of the approximately 100 trillion (10^{14}) cells in an adult human has its own copy or copies of the genome except certain cell types, such as red blood cells, that lack nuclei in their fully differentiated state. Most cells are diploid; they have two copies of each chromosome. Such cells, called somatic cells, make up most of the human body, such as skin and muscle cells. Cells differentiate to specialize for different functions.

Germ line cells are any line of cells that give rise to gametes—eggs and sperm—and thus are continuous through the generations. Stem cells, on the other hand, have the ability to

divide for indefinite periods and to give rise to specialized cells. They are best described in the context of normal human development.

Development begins when a sperm fertilizes an egg and creates a single cell that has the potential to form an entire organism. In the first hours after fertilization, this cell divides into identical cells. In humans, approximately four days after fertilization and after several cycles of cell division, these cells begin to specialize, forming a hollow sphere of cells, called a blastocyst. The blastocyst has an outer layer of cells, and inside this hollow sphere, there is a cluster of cells called the inner cell mass. The cells of the inner cell mass go on to form virtually all of the tissues of the human body. Although the cells of the inner cell mass can form virtually every type of cell found in the human body, they cannot form an organism. These cells are referred to as pluripotent.

Pluripotent stem cells undergo further specialization into multipotent progenitor cells that then give rise to functional cells. Examples of stem and progenitor cells include:

- *Hematopoietic stem cells* (adult stem cells) from the bone marrow that give rise to red blood cells, white blood cells, and platelets
- *Mesenchymal stem cells* (adult stem cells) from the bone marrow that give rise to stromal cells, fat cells, and types of bone cells
- *Epithelial stem cells* (progenitor cells) that give rise to the various types of skin cells
- *Muscle satellite cells* (progenitor cells) that contribute to differentiated muscle tissue

Dedifferentiation

Dedifferentiation is a cellular process often seen in more basal life forms such as worms and amphibians in which a partially or terminally differentiated cell reverts to an earlier developmental stage, usually as part of a regenerative process. Dedifferentiation also occurs in plants. Cells in cell culture can lose properties they originally had, such as protein expression, or change shape. This process is also termed dedifferentiation.

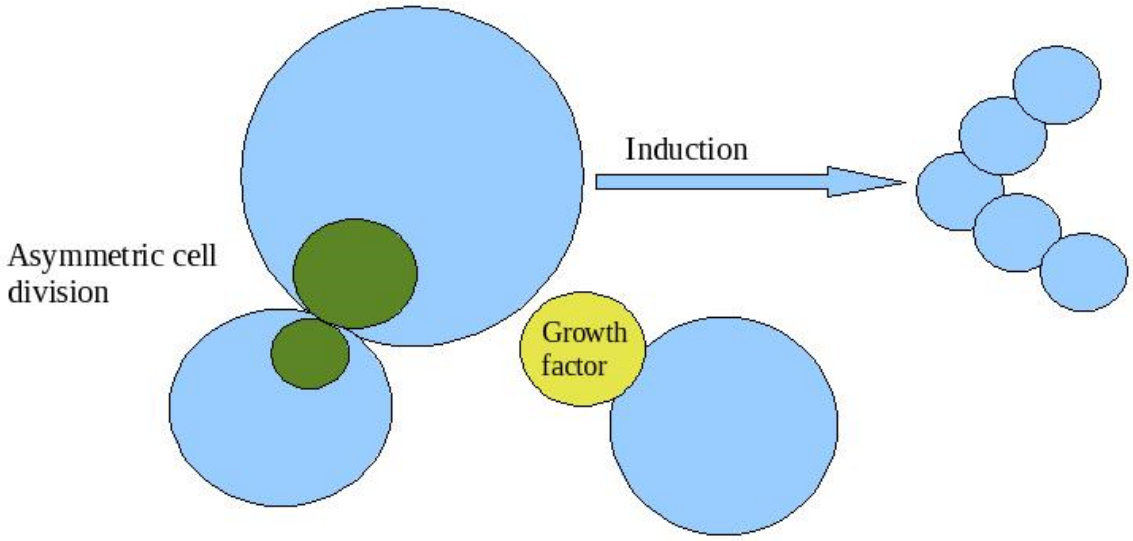
Some believe dedifferentiation is an aberration of the normal development cycle that results in cancer, whereas others believe it to be a natural part of the immune response lost by humans at some point as a result of evolution.

A small molecule dubbed reversine, a purine analog, has been discovered that has proven to induce dedifferentiation in myotubes. These dedifferentiated cells were then able to redifferentiate into osteoblasts and adipocytes.

Dedifferentiation to totipotency or pluripotency: an overview of methods. Various methods exist to revert adult somatic cells to pluripotency or totipotency. In the case of totipotency, reprogramming is mediated through a mature metaphase II oocyte as in somatic cell nuclear transfer (Wilmut et al., 1997). Recent work has demonstrated the feasibility of enucleated zygotes or early blastomeres chemically arrested during mitosis,

such that nuclear envelope break down occurs, to support reprogramming to totipotency in a process called chromosome transfer (Egli and Eggan, 2010). Direct reprogramming methods support reversion to pluripotency; though, vehicles and biotypes vary considerably in efficiencies (Takahashi and Yamanaka, 2006). Viral-mediated transduction robustly supports dedifferentiation to pluripotency through retroviral or DNA-viral routes but carries the onus of insertional inactivation. Additionally, epigenetic reprogramming by enforced expression of OSKM through DNA routes exists such as plasmid DNA, minicircles, transposons, episomes and DNA mulicistronic construct targeting by homologous recombination has also been demonstrated; however, these methods suffer from the burden to potentially alter the recipient genome by gene insertion (Ho et al., 2010). While protein-mediated transduction supports reprogramming adult cells to pluripotency, the method is cumbersome and requires recombinant protein expression and purification expertise, and reprograms albeit at very low frequencies (Kim et al., 2009). A major obstacle of using RNA for reprogramming is its lability and that single-stranded RNA biotypes trigger innate antiviral defense pathways such as interferon and NF-kB-dependent pathways. In vitro transcribed RNA, containing stabilizing modifications such as 5-methylguanosine capping or substituted ribonucleobases, e.g. pseudouracil, is 35-fold more efficient than viral transduction and has the additional benefit of not altering the somatic genome (Warren et al., 2010).

Mechanisms



Mechanisms of cellular differentiation

Each specialized cell type in an organism expresses a subset of all the genes that constitute the genome of that species. Each cell type is defined by its particular pattern of regulated gene expression. Cell differentiation is thus a transition of a cell from one cell type to another and it involves a switch from one pattern of gene expression to another. Cellular differentiation during development can be understood as the result of a gene regulatory network. A regulatory gene and its cis-regulatory modules are nodes in a gene

regulatory network; they receive input and create output elsewhere in the network. The systems biology approach to developmental biology emphasizes the importance of investigating how developmental mechanisms interact to produce predictable patterns (morphogenesis). (However, an alternative view has been proposed recently. Based on stochastic gene expression, cellular differentiation is the result of a Darwinian selective process occurring among cells. In this frame, protein and gene networks are the result of cellular processes and not their cause.)

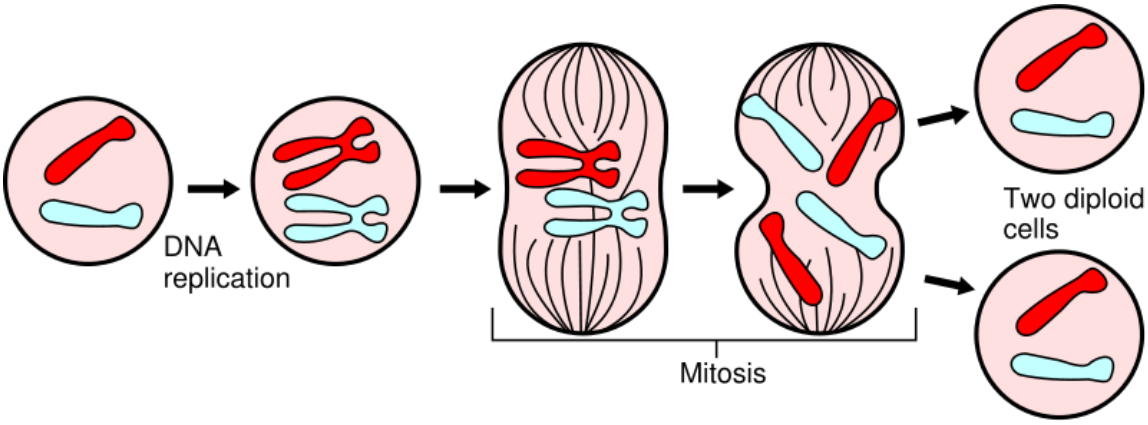
A few evolutionarily conserved types of molecular processes are often involved in the cellular mechanisms that control these switches. The major types of molecular processes that control cellular differentiation involve cell signaling. Many of the signal molecules that convey information from cell to cell during the control of cellular differentiation are called growth factors. Although the details of specific signal transduction pathways vary, these pathways often share the following general steps. A ligand produced by one cell binds to a receptor in the extracellular region of another cell, inducing a conformational change in the receptor. The shape of the cytoplasmic domain of the receptor changes, and the receptor acquires enzymatic activity. The receptor then catalyzes reactions that phosphorylate other proteins, activating them. A cascade of phosphorylation reactions eventually activates a dormant transcription factor or cytoskeletal protein, thus contributing to the differentiation process in the target cell. Cells and tissues can vary in competence, their ability to respond to external signals.

Induction refers to cascades of signaling events, during which a cell or tissue signals to another cell or tissue to influence its developmental fate. Yamamoto and Jeffery investigated the role of the lens in eye formation in cave- and surface-dwelling fish, a striking example of induction. Through reciprocal transplants, Yamamoto and Jeffery found that the lens vesicle of surface fish can induce other parts of the eye to develop in cave- and surface-dwelling fish, while the lens vesicle of the cave-dwelling fish cannot.

Other important mechanisms fall under the category of asymmetric cell divisions, divisions that give rise to daughter cells with distinct developmental fates. Asymmetric cell divisions can occur because of segregation of cytoplasmic determinants or because of signaling. In the former mechanism, distinct daughter cells are created during cytokinesis because of an uneven distribution of regulatory molecules in the parent cell; the distinct cytoplasm that each daughter cell inherits results in a distinct pattern of differentiation for each daughter cell. A well-studied example of pattern formation by asymmetric divisions is body axis patterning in Drosophila. RNA molecules are an important type of intracellular differentiation control signal. The molecular and genetic basis of asymmetric cell divisions has also been studied in green algae of the genus *Volvox*, a model system for studying how unicellular organisms can evolve into multicellular organisms. In *Volvox carteri*, the 16 cells in the anterior hemisphere of a 32-celled embryo divide asymmetrically, each producing one large and one small daughter cell. The size of the cell at the end of all cell divisions determines whether it becomes a specialized germ or somatic cell.

Chapter 10

Cell Cycle

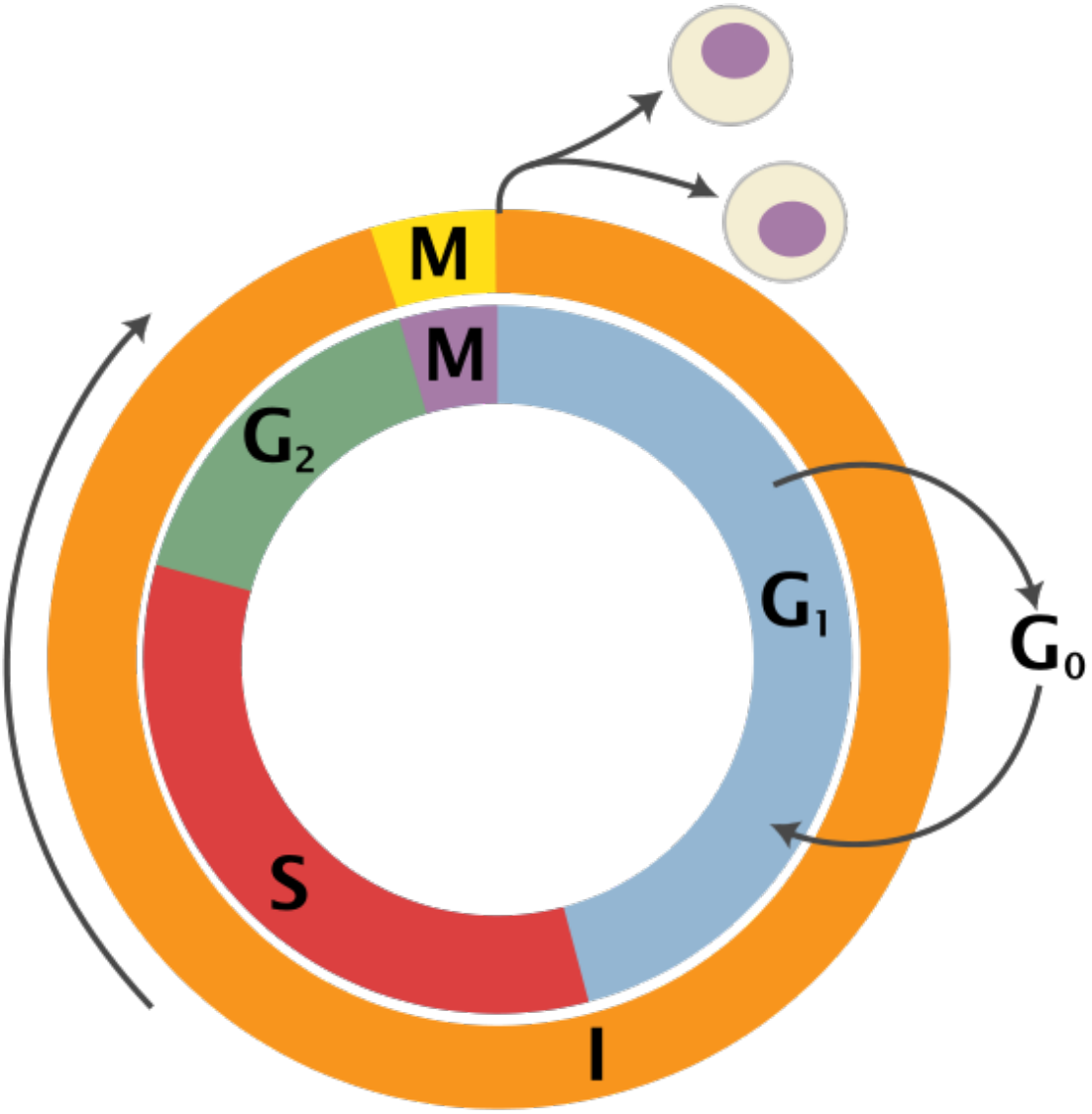


Each turn of the cell cycle divides the chromosomes in a cell nucleus.

The **cell cycle**, or **cell-division cycle**, is the series of events that takes place in a cell leading to its division and duplication (replication). In cells without a nucleus (prokaryotic), the cell cycle occurs via a process termed binary fission. In cells with a nucleus (eukaryotes), the cell cycle can be divided in two brief periods: interphase—during which the cell grows, accumulating nutrients needed for mitosis and duplicating its DNA—and the mitosis (M) phase, during which the cell splits itself into two distinct cells, often called "daughter cells". The cell-division cycle is a vital process by which a single-celled fertilized egg develops into a mature organism, as well as the process by which hair, skin, blood cells, and some internal organs are renewed.

Phases

The cell cycle consists of four distinct phases: G_1 phase, S phase (synthesis), G_2 phase (collectively known as interphase) and M phase (mitosis). M phase is itself composed of two tightly coupled processes: mitosis, in which the cell's chromosomes are divided between the two daughter cells, and cytokinesis, in which the cell's cytoplasm divides in half forming distinct cells. Activation of each phase is dependent on the proper progression and completion of the previous one. Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called G_0 phase.



Schematic of the cell cycle. outer ring: I = Interphase, M = Mitosis; inner ring: M = Mitosis, G_1 = Gap 1, G_2 = Gap 2, S = Synthesis; not in ring: G_0 = Gap 0/Resting.

State	Phase	Abbreviation	Description
quiescent/ senescent	Gap 0	**G_0**	A resting phase where the cell has left the cycle and has stopped dividing.
Interphase	Gap 1	**G_1**	Cells increase in size in Gap 1. The *G_1 checkpoint* control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	**S**	DNA replication occurs during this phase.

	Gap 2	**G_2**	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The *G_2 checkpoint* control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.
Cell division	Mitosis	**M**	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis (*Metaphase Checkpoint*) ensures that the cell is ready to complete cell division.

After cell division, each of the daughter cells begin the interphase of a new cycle. Although the various stages of interphase are not usually morphologically distinguishable, each phase of the cell cycle has a distinct set of specialized biochemical processes that prepare the cell for initiation of cell division.

Resting (G_0 phase)

The term "post-mitotic" is sometimes used to refer to both quiescent and senescent cells. Nonproliferative cells in multicellular eukaryotes generally enter the quiescent G_0 state from G_1 and may remain quiescent for long periods of time, possibly indefinitely (as is often the case for neurons). This is very common for cells that are fully differentiated. Cellular senescence is a state that occurs in response to DNA damage or degradation that would make a cell's progeny nonviable; it is often a biochemical alternative to the self-destruction of such a damaged cell by apoptosis.

Interphase

Before a cell can enter cell division, it needs to take in nutrients. All of the preparations are done during the interphase. Interphase proceeds in three stages, G1, S, and G2. Cell division operates in a cycle. Therefore, interphase is preceded by the previous cycle of mitosis and cytokinesis.

G_1 phase

The first phase within interphase, from the end of the previous M phase until the beginning of DNA synthesis is called G_1 (G indicating *gap*). It is also called the growth phase. During this phase the biosynthetic activities of the cell, which had been considerably slowed down during M phase, resume at a high rate. This phase is marked by synthesis of various enzymes that are required in S phase, mainly those needed for DNA replication. Duration of G_1 is highly variable, even among different cells of the same species.

S phase

The ensuing S phase starts when DNA synthesis commences; when it is complete, all of the chromosomes have been replicated, i.e., each chromosome has two (sister) chromatids. Thus, during this phase, the amount of DNA in the cell has effectively doubled, though the ploidy of the cell remains the same. Rates of RNA transcription and protein synthesis are very low during this phase. An exception to this is histone production, most of which occurs during the S phase.

G_2 phase

The cell then enters the G_2 phase, which lasts until the cell enters mitosis. Again, significant biosynthesis occurs during this phase, mainly involving the production of microtubules, which are required during the process of mitosis. Inhibition of protein synthesis during G_2 phase prevents the cell from undergoing mitosis.

Mitosis (M Phase/Mitotic phase)

The relatively brief M phase consists of nuclear division (karyokinesis). The M phase has been broken down into several distinct phases, sequentially known as:

- prophase,
- metaphase,
- anaphase,
- telophase
- cytokinesis (strictly speaking, cytokinesis is not part of mitosis but is an event that directly follows mitosis in which cytoplasm is divided into two daughter cells)

Mitosis is the process by which a eukaryotic cell separates the chromosomes in its cell nucleus into two identical sets in two nuclei. It is generally followed immediately by cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the **mitotic (M) phase** of the cell cycle - the division of the mother cell into two daughter cells, genetically identical to each other and to their parent cell. This accounts for approximately 10% of the cell cycle.

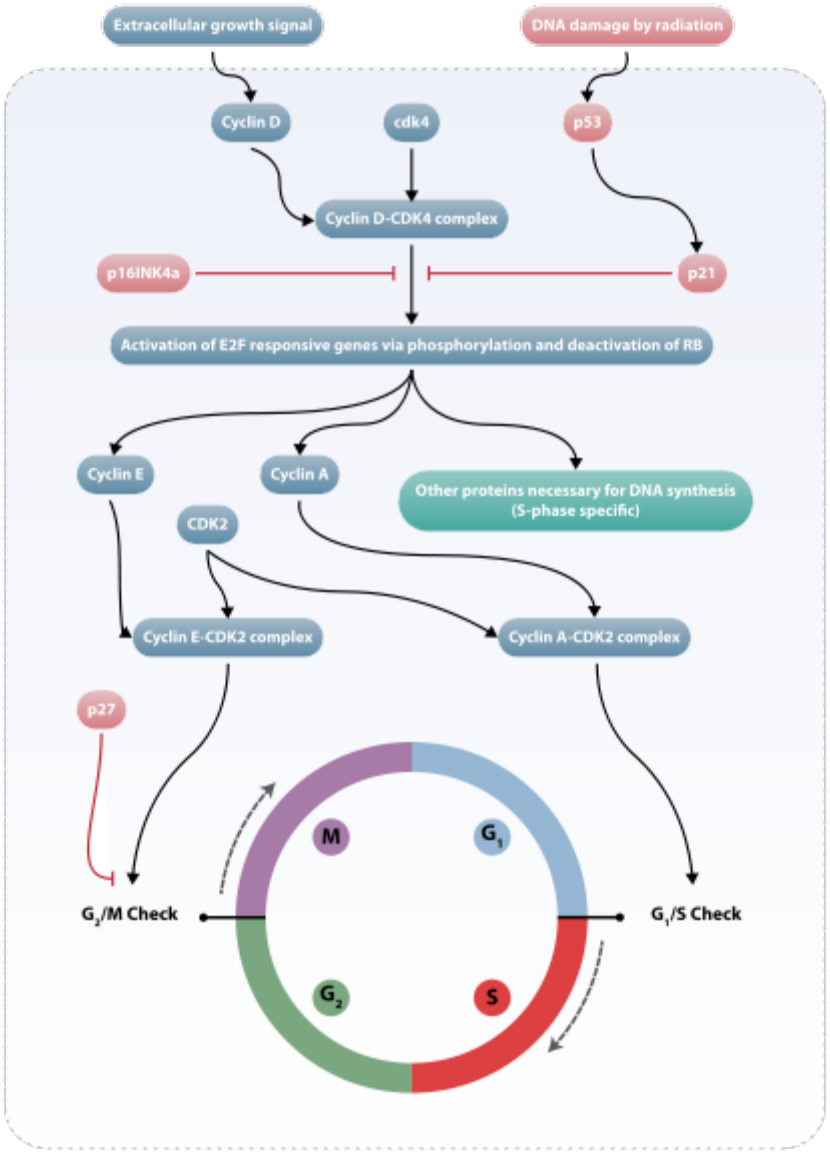
Mitosis occurs exclusively in eukaryotic cells, but occurs in different ways in different species. For example, animals undergo an "open" mitosis, where the nuclear envelope breaks down before the chromosomes separate, while fungi such as *Aspergillus nidulans* and *Saccharomyces cerevisiae* (yeast) undergo a "closed" mitosis, where chromosomes divide within an intact cell nucleus. Prokaryotic cells, which lack a nucleus, divide by a process called binary fission.

The process of mitosis is complex and highly regulated. The sequence of events is divided into phases, corresponding to the completion of one set of activities and the start of the next. These stages are prophase, prometaphase, metaphase, anaphase and

telophase. During the process of mitosis the pairs of chromosomes condense and attach to fibers that pull the sister chromatids to opposite sides of the cell. The cell then divides in cytokinesis, to produce two identical daughter cells.

Because cytokinesis usually occurs in conjunction with mitosis, "mitosis" is often used interchangeably with "M phase". However, there are many cells where mitosis and cytokinesis occur separately, forming single cells with multiple nuclei. This occurs most notably among the fungi and slime moulds, but is found in various different groups. Even in animals, cytokinesis and mitosis may occur independently, for instance during certain stages of fruit fly embryonic development. Errors in mitosis can either kill a cell through apoptosis or cause mutations that may lead to cancer.

Regulation of eukaryotic cell cycle



Regulation of cell cycle: Schematic

Regulation of the cell cycle involves processes crucial to the survival of a cell, including the detection and repair of genetic damage as well as the prevention of uncontrolled cell division. The molecular events that control the cell cycle are ordered and directional; that is, each process occurs in a sequential fashion and it is impossible to "reverse" the cycle.

Role of cyclins and CDKs

Two key classes of regulatory molecules, cyclins and cyclin-dependent kinases (CDKs), determine a cell's progress through the cell cycle. Leland H. Hartwell, R. Timothy Hunt, and Paul M. Nurse won the 2001 Nobel Prize in Physiology or Medicine for their discovery of these central molecules. Many of the genes encoding cyclins and CDKs are conserved among all eukaryotes, but in general more complex organisms have more elaborate cell cycle control systems that incorporate more individual components. Many of the relevant genes were first identified by studying yeast, especially *Saccharomyces cerevisiae*; genetic nomenclature in yeast dubs many these genes *cdc* (for "cell division cycle") followed by an identifying number, e.g., *cdc25* or *cdc20*.

Cyclins form the regulatory subunits and CDKs the catalytic subunits of an activated heterodimer; cyclins have no catalytic activity and CDKs are inactive in the absence of a partner cyclin. When activated by a bound cyclin, CDKs perform a common biochemical reaction called phosphorylation that activates or inactivates target proteins to orchestrate coordinated entry into the next phase of the cell cycle. Different cyclin-CDK combinations determine the downstream proteins targeted. CDKs are constitutively expressed in cells whereas cyclins are synthesised at specific stages of the cell cycle, in response to various molecular signals.

General mechanism of cyclin-CDK interaction

Upon receiving a pro-mitotic extracellular signal, G_1 cyclin-CDK complexes become active to prepare the cell for S phase, promoting the expression of transcription factors that in turn promote the expression of S cyclins and of enzymes required for DNA replication. The G_1 cyclin-CDK complexes also promote the degradation of molecules that function as S phase inhibitors by targeting them for ubiquitination. Once a protein has been ubiquitinated, it is targeted for proteolytic degradation by the proteasome.

Active S cyclin-CDK complexes phosphorylate proteins that make up the pre-replication complexes assembled during G_1 phase on DNA replication origins. The phosphorylation serves two purposes: to activate each already-assembled pre-replication complex, and to prevent new complexes from forming. This ensures that every portion of the cell's genome will be replicated once and only once. The reason for prevention of gaps in replication is fairly clear, because daughter cells that are missing all or part of crucial genes will die. However, for reasons related to gene copy number effects, possession of extra copies of certain genes is also deleterious to the daughter cells.

Mitotic cyclin-CDK complexes, which are synthesized but inactivated during S and G_2 phases, promote the initiation of mitosis by stimulating downstream proteins involved in

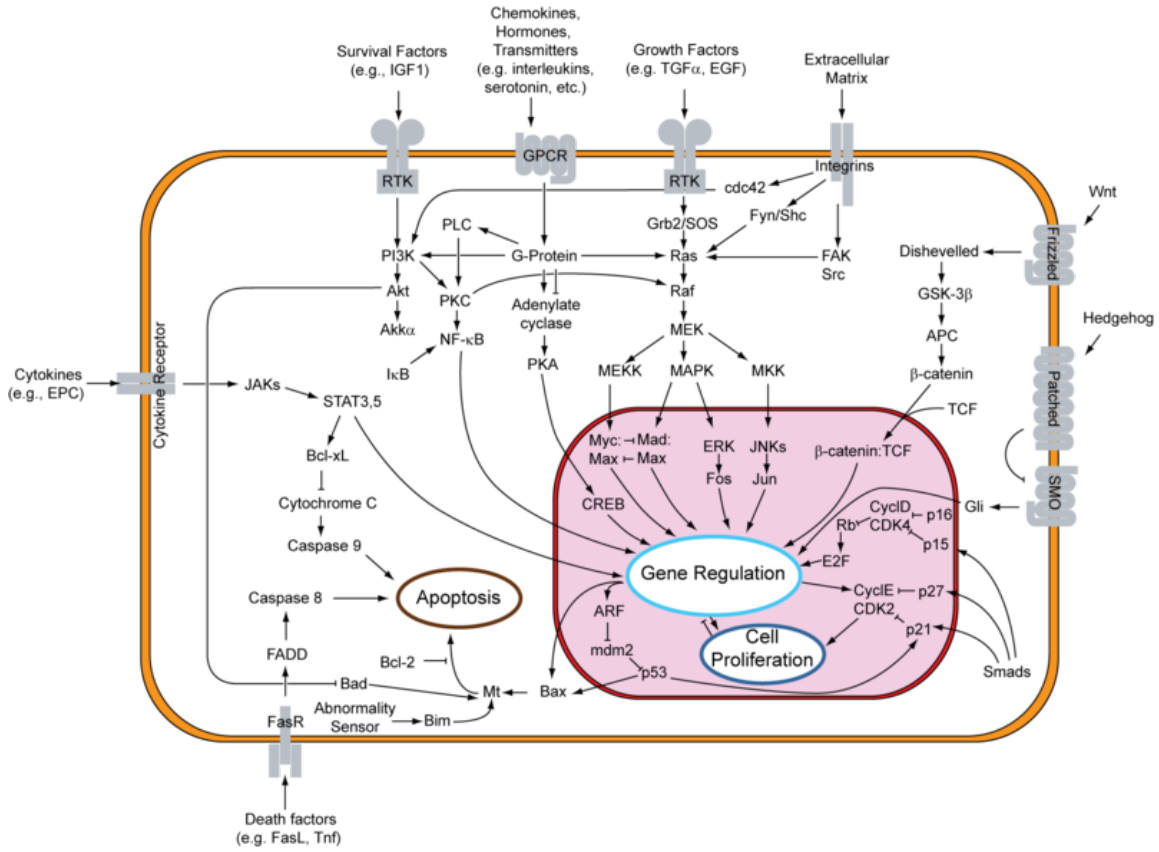
chromosome condensation and mitotic spindle assembly. A critical complex activated during this process is a ubiquitin ligase known as the anaphase-promoting complex (APC), which promotes degradation of structural proteins associated with the chromosomal kinetochore. APC also targets the mitotic cyclins for degradation, ensuring that telophase and cytokinesis can proceed.

Interphase: Interphase generally lasts at least 12 to 24 hours in mammalian tissue. During this period, the cell is constantly synthesizing RNA, producing protein and growing in size. By studying molecular events in cells, scientists have determined that interphase can be divided into 4 steps: Gap 0 (G_0), Gap 1 (G_1), S (synthesis) phase, Gap 2 (G_2).

Specific action of cyclin-CDK complexes

Cyclin D is the first cyclin produced in the cell cycle, in response to extracellular signals (e.g. growth factors). Cyclin D binds to existing CDK4, forming the active cyclin D-CDK4 complex. Cyclin D-CDK4 complex in turn phosphorylates the retinoblastoma susceptibility protein (Rb). The hyperphosphorylated Rb dissociates from the E2F/DP1/Rb complex (which was bound to the E2F responsive genes, effectively "blocking" them from transcription), activating E2F. Activation of E2F results in transcription of various genes like cyclin E, cyclin A, DNA polymerase, thymidine kinase, etc. Cyclin E thus produced binds to CDK2, forming the cyclin E-CDK2 complex, which pushes the cell from G_1 to S phase (G_1/S transition). Cyclin B along with cdc2 (cdc2 - fission yeasts (CDK1 - mammalia)) forms the cyclin B-cdc2 complex, which initiates the G_2/M transition. Cyclin B-cdc2 complex activation causes breakdown of nuclear envelope and initiation of prophase, and subsequently, its deactivation causes the cell to exit mitosis.

Inhibitors



Overview of signal transduction pathways involved in apoptosis, also known as "programmed cell death".

Two families of genes, the *cip/kip* family and the INK4a/ARF (*In*hibitor of *K*inase 4/*A*lternative *R*eading *F*rame) prevent the progression of the cell cycle. Because these genes are instrumental in prevention of tumor formation, they are known as tumor suppressors.

The ***cip/kip* family** includes the genes p21, p27 and p57. They halt cell cycle in G_1 phase, by binding to, and inactivating, cyclin-CDK complexes. p21 is activated by p53 (which, in turn, is triggered by DNA damage e.g. due to radiation). p27 is activated by Transforming Growth Factor β (TGF β), a growth inhibitor.

The **INK4a/ARF family** includes p16INK4a, which binds to CDK4 and arrests the cell cycle in G_1 phase, and p14arf which prevents p53 degradation.

Synthetic inhibitors of Cdc25 could also be useful for the arrest of cell cycle and therefore be useful as antineoplastic and anticancer agents.

Transcriptional Regulatory Network

Evidence suggests that a semi-autonomous transcriptional network acts in concert with the CDK-cyclin machinery to regulate the cell cycle. Several gene expression studies in *Saccharomyces cerevisiae* have identified approximately 800 to 1200 genes that change expression over the course of the cell cycle; they are transcribed at high levels at specific points in the cell cycle, and remain at lower levels throughout the rest of the cell cycle. While the set of identified genes differs between studies due to the computational methods and criterion used to identify them, each study indicates that a large portion of yeast genes are temporally regulated.

Many periodically expressed genes are driven by transcription factors that are also periodically expressed. One screen of single-gene knockouts identified 48 transcription factors (about 20% of all non-essential transcription factors) that show cell cycle progression defects. Genome-wide studies using high throughput technologies have identified the transcription factors that bind to the promoters of yeast genes, and correlating these findings with temporal expression patterns have allowed the identification of transcription factors that drive phase-specific gene expression. The expression profiles of these transcription factors are driven by the transcription factors that peak in the prior phase, and computational models have shown that a CDK-autonomous network of these transcription factors is sufficient to produce steady-state oscillations in gene expression).

Experimental evidence also suggests that gene expression can oscillate with the period seen in dividing wild-type cells independently of the CDK machinery. Orlando et. al. used microarrays to measure the expression of a set of 1,271 genes that they identified as periodic in both wild type cells and cells lacking all S-phase and mitotic cyclins (*clb1,2,3,4,5,6*). Of the 1,271 genes assayed, 882 continued to be expressed in the cyclin-deficient cells at the same time as in the wild type cells, despite the fact that the cyclin-deficient cells arrest at the border between G1 and S phase. However, 833 of the genes assayed changed behavior between the wild type and mutant cells, indicating that these genes are likely directly or indirectly regulated by the CDK-cyclin machinery. Some genes that continued to be expressed on time in the mutant cells were also expressed at different levels in the mutant and wild type cells. These findings suggest that while the transcriptional network may oscillate independently of the CDK-cyclin oscillator, they are coupled in a manner that requires both to ensure the proper timing of cell cycle events. Other work indicates that phosphorylation, a post-translational modification, of cell cycle transcription factors by Cdk1 may alter the localization or activity of the transcription factors in order to tightly control timing of target genes (Ubersax et al 2003; Sidorova et al 1995; White et al 2009).

While oscillatory transcription plays a key role in the progression of the yeast cell cycle, the CDK-cyclin machinery operates independently in the early embryonic cell cycle. Before the midblastula transition, zygotic transcription does not occur and all needed proteins, such as the B-type cyclins, are translated from maternally loaded mRNA.

Checkpoints

Cell cycle checkpoints are used by the cell to monitor and regulate the progress of the cell cycle. Checkpoints prevent cell cycle progression at specific points, allowing verification of necessary phase processes and repair of DNA damage. The cell cannot proceed to the next phase until checkpoint requirements have been met.

Several checkpoints are designed to ensure that damaged or incomplete DNA is not passed on to daughter cells. Two main checkpoints exist: the G_1/S checkpoint and the G_2/M checkpoint. G_1/S transition is a rate-limiting step in the cell cycle and is also known as restriction point. An alternative model of the cell cycle response to DNA damage has also been proposed, known as the postreplication checkpoint.

p53 plays an important role in triggering the control mechanisms at both G_1/S and G_2/M checkpoints.

Role in tumor formation

A disregulation of the cell cycle components may lead to tumor formation. As mentioned above, some genes like the cell cycle inhibitors, RB, p53 etc., when they mutate, may cause the cell to multiply uncontrollably, forming a tumor. Although the duration of cell cycle in tumor cells is equal to or longer than that of normal cell cycle, the proportion of cells that are in active cell division (versus quiescent cells in G_0 phase) in tumors is much higher than that in normal tissue. Thus there is a net increase in cell number as the number of cells that die by apoptosis or senescence remains the same.

The cells which are actively undergoing cell cycle are targeted in cancer therapy as the DNA is relatively exposed during cell division and hence susceptible to damage by drugs or radiation. This fact is made use of in cancer treatment; by a process known as debulking, a significant mass of the tumor is removed which pushes a significant number of the remaining tumor cells from G_0 to G_1 phase (due to increased availability of nutrients, oxygen, growth factors etc.). Radiation or chemotherapy following the debulking procedure kills these cells which have newly entered the cell cycle.

The fastest cycling mammalian cells in culture, crypt cells in the intestinal epithelium, have a cycle time as short as 9 to 10 hours. Stem cells in resting mouse skin may have a cycle time of more than 200 hours. Most of this difference is due to the varying length of G_1, the most variable phase of the cycle. M and S do not vary much.

In general, cells are most radiosensitive in late M and G_2 phases and most resistant in late S.

For cells with a longer cell cycle time and a significantly long G_1 phase, there is a second peak of resistance late in G_1

The pattern of resistance and sensitivity correlates with the level of sulfhydryl compounds in the cell. Sulfhydryls are natural radioprotectors and tend to be at their highest levels in S and at their lowest near mitosis.

Synchronization of cell cultures

Several methods can be used to synchronise cell cultures by halting the cell cycle at a particular phase. For example, serum starvation and treatment with thymidine or aphidicolin halt the cell in the G_1 phase, mitotic shake-off, treatment with colchicine and treatment with nocodazole halt the cell in M phase and treatment with 5-fluorodeoxyuridine halts the cell in S phase.

Chapter 11

Speciation

Speciation is the evolutionary process by which new biological species arise. The biologist Orator F. Cook seems to have been the first to coin the term 'speciation' for the splitting of lineages or 'cladogenesis,' as opposed to 'anagenesis' or 'phyletic evolution' occurring within lineages. Whether genetic drift is a minor or major contributor to speciation is the subject matter of much ongoing discussion.

There are four geographic modes of speciation in nature, based on the extent to which speciating populations are geographically isolated from one another: allopatric, peripatric, parapatric, and sympatric. Speciation may also be induced artificially, through animal husbandry or laboratory experiments. Observed examples of each kind of speciation are provided throughout.

Natural speciation

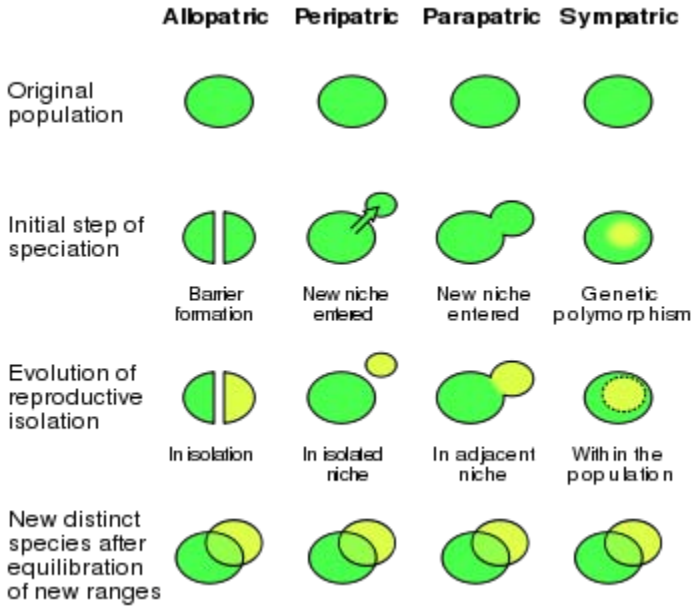
All forms of natural speciation have taken place over the course of evolution; however it still remains a subject of debate as to the relative importance of each mechanism in driving biodiversity.

The three-spined stickleback (*Gasterosteus aculeatus*)

One example of natural speciation is the diversity of the three-spined stickleback, a marine fish that, after the last ice age, has undergone speciation into new freshwater colonies in isolated lakes and streams. Over an estimated 10,000 generations, the sticklebacks show structural differences that are greater than those seen between different genera of fish including variations in fins, changes in the number or size of their bony plates, variable jaw structure, and color differences.

There is debate as to the rate at which speciation events occur over geologic time. While some evolutionary biologists claim that speciation events have remained relatively constant over time, some palaeontologists such as Niles Eldredge and Stephen Jay Gould have argued that species usually remain unchanged over long stretches of time, and that speciation occurs only over relatively brief intervals, a view known as *punctuated equilibrium*.

Allopatric



Comparison of allopatric, peripatric, parapatric and sympatric speciation.

During allopatric speciation, a population splits into two geographically isolated populations (for example, by habitat fragmentation due to geographical change such as mountain building or social change such as emigration). The isolated populations then undergo genotypic and/or phenotypic divergence as: (a) they become subjected to dissimilar selective pressures; (b) they independently undergo genetic drift; (c) different mutations arise in the two populations. When the populations come back into contact, they have evolved such that they are reproductively isolated and are no longer capable of exchanging genes.

Observed instances

Island genetics, the tendency of small, isolated genetic pools to produce unusual traits, has been observed in many circumstances, including insular dwarfism and the radical changes among certain famous island chains, for example on Komodo. The Galápagos islands are particularly famous for their influence on Charles Darwin. During his five weeks there he heard that Galápagos tortoises could be identified by island, and noticed that Mockingbirds differed from one island to another, but it was only nine months later that he reflected that such facts could show that species were changeable. When he returned to England, his speculation on evolution deepened after experts informed him that these were separate species, not just varieties, and famously that other differing Galápagos birds were all species of finches. Though the finches were less important for Darwin, more recent research has shown the birds now known as Darwin's finches to be a classic case of adaptive evolutionary radiation.

Peripatric

In peripatric speciation, a subform of allopatric speciation, new species are formed in isolated, smaller peripheral populations that are prevented from exchanging genes with the main population. It is related to the concept of a founder effect, since small populations often undergo bottlenecks. Genetic drift is often proposed to play a significant role in peripatric speciation.

Observed instances

- Mayr bird fauna
- The Australian bird *Petroica multicolor*
- Reproductive isolation occurs in populations of *Drosophila* subject to population bottlenecking

The London Underground mosquito is a variant of the mosquito *Culex pipiens* that entered in the London Underground in the nineteenth century. Evidence for its speciation include genetic divergence, behavioral differences, and difficulty in mating.

Parapatric

In parapatric speciation, there is only partial separation of the zones of two diverging populations afforded by geography; individuals of each species may come in contact or cross habitats from time to time, but reduced fitness of the heterozygote leads to selection for behaviours or mechanisms that prevent their inter-breeding. Parapatric speciation is modelled on continuous variation within a 'single', connected habitat acting as a source of natural selection rather than the effects of isolation of habitats produced in peripatric and allopatric speciation.

Ecologists refer to parapatric and peripatric speciation in terms of ecological niches. A niche must be available in order for a new species to be successful.

Observed instances

- Ring species
  - The *Larus* gulls form a ring species around the North Pole.
  - The *Ensatina* salamanders, which form a ring round the Central Valley in California.
  - The Greenish Warbler (*Phylloscopus trochiloides*), around the Himalayas.
- the grass *Anthoxanthum* has been known to undergo parapatric speciation in such cases as mine contamination of an area.

Sympatric

Sympatric speciation refers to the formation of two or more descendant species from a single ancestral species all occupying the same geographic location.

In sympatric speciation, species diverge while inhabiting the same place. Often-cited examples of sympatric speciation are found in insects that become dependent on different host plants in the same area. However, the existence of sympatric speciation as a mechanism of speciation is still hotly contested. People have argued that the evidences of sympatric speciation are in fact examples of micro-allopatric, or heteropatric speciation. The most widely accepted example of sympatric speciation is that of the cichlids of Lake Nabugabo in East Africa, which is thought to be due to **sexual selection**.

Until recently, there has a been a dearth of strong evidence that supports this form of speciation, with a general feeling that interbreeding would soon eliminate any genetic differences that might appear. But there has been at least one recent study that suggests that sympatric speciation has occurred in Tennessee cave salamanders.

The three-spined sticklebacks, freshwater fishes, that have been studied by Dolph Schluter (who received his Ph.D. for his work on Darwin's finches with Peter J. Grant) and his current colleagues in British Columbia, were once thought to provide an intriguing example best explained by sympatric speciation. Schluter and colleagues found:

- Two different species of three-spined sticklebacks in each of five different lakes
  - a large benthic species with a large mouth that feeds on large prey in the littoral zone
  - a smaller limnetic species — with a smaller mouth — that feeds on the small plankton in open water
- DNA analysis indicates that each lake was colonized independently, presumably by a marine ancestor, after the last ice age
- DNA analysis also shows that the two species in each lake are more closely related to each other than they are to any of the species in the other lakes
- The two species in each lake are reproductively isolated; neither mates with the other.

- However, aquarium tests showed:
    - the benthic species from one lake will spawn with the benthic species from the other lakes and
    - likewise the limnetic species from the different lakes will spawn with each other.
    - These benthic and limnetic species even display their mating preferences when presented with sticklebacks from Japanese lakes; that is, a Canadian benthic prefers a Japanese benthic over its close limnetic cousin from its own lake.
- Their conclusion: in each lake, what began as a single population faced such competition for limited resources that:
    - disruptive selection — competition favoring fishes at either extreme of body size and mouth size over those nearer the mean — coupled with:
    - assortative mating — each size preferred mates like it — favored a divergence into two subpopulations exploiting different food in different parts of the lake.
    - The fact that this pattern of speciation occurred the same way on three separate occasions suggests strongly that ecological factors in a sympatric population can cause speciation.

However, the DNA evidence cited above is from mitochondrial DNA (mtDNA), which can often move easily between closely related species ("introgression") when they hybridize. A more recent study, using genetic markers from the nuclear genome, shows that limnetic forms in different lakes are more closely related to each other (and to marine lineages) than to benthic forms in the same lake. The three-spine stickleback is now usually considered an example of "double invasion" (a form of allopatric speciation) in which repeated invasions of marine forms have subsequently differentiated into benthic and limnetic forms. The three-spine stickleback provides an example of how molecular biogeographic studies that rely solely on mtDNA can be misleading, and that consideration of the genealogical history of alleles from multiple unlinked markers (i.e. nuclear genes) is necessary to infer speciation histories.

Sympatric speciation driven by ecological factors may also account for the extraordinary diversity of crustaceans living in the depths of Siberia's Lake Baikal.

Speciation via polyploidization

Polyploidy is a mechanism that has caused many rapid speciation events in sympatry because offspring of, for example, tetraploid x diploid matings often result in triploid sterile progeny. However, not all polyploids are reproductively isolated from their parental plants, and gene flow may still occur for example through triploid hybrid x diploid matings that produce tetraploids, or matings between meiotically unreduced gametes from diploids and gametes from tetraploids.

It has been suggested that many of the existing plant and most animal species have undergone an event of polyploidization in their evolutionary history. Reproduction of

successful polyploid species is sometimes asexual, by parthenogenesis or apomixis, as for unknown reasons many asexual organisms are polyploid. Rare instances of polyploid mammals are known, but most often result in prenatal death.

Hawthorn fly

One example of evolution at work is the case of the hawthorn fly, *Rhagoletis pomonella*, also known as the apple maggot fly, which appears to be undergoing sympatric speciation. Different populations of hawthorn fly feed on different fruits. A distinct population emerged in North America in the 19th century some time after apples, a non-native species, were introduced. This apple-feeding population normally feeds only on apples and not on the historically preferred fruit of hawthorns. The current hawthorn feeding population does not normally feed on apples. Some evidence, such as the fact that six out of thirteen allozyme loci are different, that hawthorn flies mature later in the season and take longer to mature than apple flies; and that there is little evidence of interbreeding (researchers have documented a 4-6% hybridization rate) suggests that sympatric speciation is occurring. The emergence of the new hawthorn fly is an example of evolution in progress.

Speciation via hybrid formation

Reinforcement (Wallace effect)

Reinforcement is the process by which natural selection increases reproductive isolation. It may occur after two populations of the same species are separated and then come back into contact. If their reproductive isolation was complete, then they will have already developed into two separate incompatible species. If their reproductive isolation is incomplete, then further mating between the populations will produce hybrids, which may or may not be fertile. If the hybrids are infertile, or fertile but less fit than their ancestors, then there will be no further reproductive isolation and speciation has essentially occurred (e.g., as in horses and donkeys.) The reasoning behind this is that if the parents of the hybrid offspring each have naturally selected traits for their own certain environments, the hybrid offspring will bear traits from both, therefore would not fit either ecological niche as well as either parent. The low fitness of the hybrids would cause selection to favor assortative mating, which would control hybridization. This is sometimes called the Wallace effect after the evolutionary biologist Alfred Russel Wallace who suggested in the late 19th century that it might be an important factor in speciation. If the hybrid offspring are more fit than their ancestors, then the populations will merge back into the same species within the area they are in contact.

Reinforcement is required for both parapatric and sympatric speciation. Without reinforcement, the geographic area of contact between different forms of the same species, called their "hybrid zone," will not develop into a boundary between the different species. Hybrid zones are regions where diverged populations meet and interbreed. Hybrid offspring are very common in these regions, which are usually created by diverged species coming into secondary contact. Without reinforcement the two species

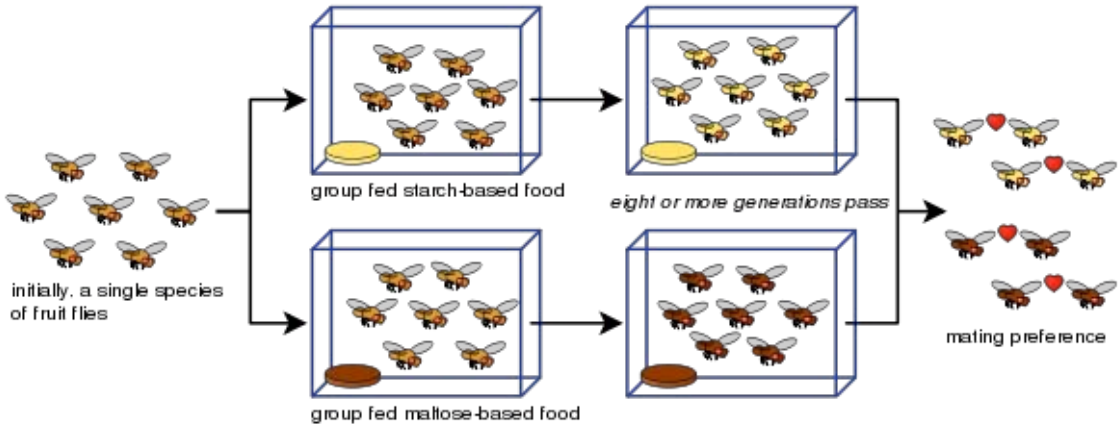
would have uncontrollable inbreeding. Reinforcement may be induced in artificial selection experiments as described below.

Artificial speciation

New species have been created by domesticated animal husbandry, but the initial dates and methods of the initiation of such species are not clear. For example, domestic sheep were created by hybridisation, and no longer produce viable offspring with *Ovis orientalis*, one species from which they are descended. Domestic cattle, on the other hand, can be considered the same species as several varieties of wild ox, gaur, yak, etc., as they readily produce fertile offspring with them.

The best-documented creations of new species in the laboratory were performed in the late 1980s. William Rice and G.W. Salt bred fruit flies, *Drosophila melanogaster,* using a maze with three different choices of habitat such as light/dark and wet/dry. Each generation was placed into the maze, and the groups of flies that came out of two of the eight exits were set apart to breed with each other in their respective groups. After thirty-five generations, the two groups and their offspring were isolated reproductively because of their strong habitat preferences: they mated only within the areas they preferred, and so did not mate with flies that preferred the other areas. The history of such attempts is described in Rice and Hostert (1993).

Diane Dodd was also able to show how reproductive isolation can develop from mating preferences in *Drosophila pseudoobscura* fruit flies after only eight generations using different food types, starch and maltose.



Dodd's experiment has been easy for many others to replicate, including with other kinds of fruit flies and foods.

Genetics

Few speciation genes have been found. They usually involve the reinforcement process of late stages of speciation. In 2008 a speciation gene causing reproductive isolation was reported. It causes hybrid sterility between related subspecies.

Hybrid speciation

Hybridization between two different species sometimes leads to a distinct phenotype. This phenotype can also be fitter than the parental lineage and as such natural selection may then favor these individuals. Eventually, if reproductive isolation is achieved, it may lead to a separate species. However, reproductive isolation between hybrids and their parents is particularly difficult to achieve and thus hybrid speciation is considered an extremely rare event. The Mariana Mallard is known to have arisen from hybrid speciation.

Hybridisation is an important means of speciation in plants, since polyploidy (having more than two copies of each chromosome) is tolerated in plants more readily than in animals. Polyploidy is important in hybrids as it allows reproduction, with the two different sets of chromosomes each being able to pair with an identical partner during meiosis. Polyploids also have more genetic diversity, which allows them to avoid inbreeding depression in small populations.

Hybridization without change in chromosome number is called homoploid hybrid speciation. It is considered very rare but has been shown in *Heliconius* butterflies and sunflowers. Polyploid speciation, which involves changes in chromosome number, is a more common phenomenon, especially in plant species.

Gene transposition as a cause

Theodosius Dobzhansky, who studied fruit flies in the early days of genetic research in 1930s, speculated that parts of chromosomes that switch from one location to another might cause a species to split into two different species. He mapped out how it might be possible for sections of chromosomes to relocate themselves in a genome. Those mobile sections can cause sterility in inter-species hybrids, which can act as a speciation pressure. In theory, his idea was sound, but scientists long debated whether it actually happened in nature. Eventually a competing theory involving the gradual accumulation of mutations was shown to occur in nature so often that geneticists largely dismissed the moving gene hypothesis.

However, 2006 research shows that jumping of a gene from one chromosome to another can contribute to the birth of new species. This validates the reproductive isolation mechanism, a key component of speciation.

Interspersed repeats

Interspersed repetitive DNA sequences function as isolating mechanisms. These repeats protect newly evolving gene sequences from being overwritten by gene conversion, due to the creation of non-homologies between otherwise homologous DNA sequences. The non-homologies create barriers to gene conversion. This barrier allows nascent novel genes to evolve without being overwritten by the progenitors of these genes. This uncoupling allows the evolution of new genes, both within gene families and also allelic forms of a gene. The importance is that this allows the splitting of a gene pool without requiring physical isolation of the organisms harboring those gene sequences.

Human speciation

Humans have genetic similarities with chimpanzees and gorillas, suggesting common ancestors. Analysis of genetic drift and recombination using a Markov model suggests humans and chimpanzees speciated apart 4.1 million years ago.