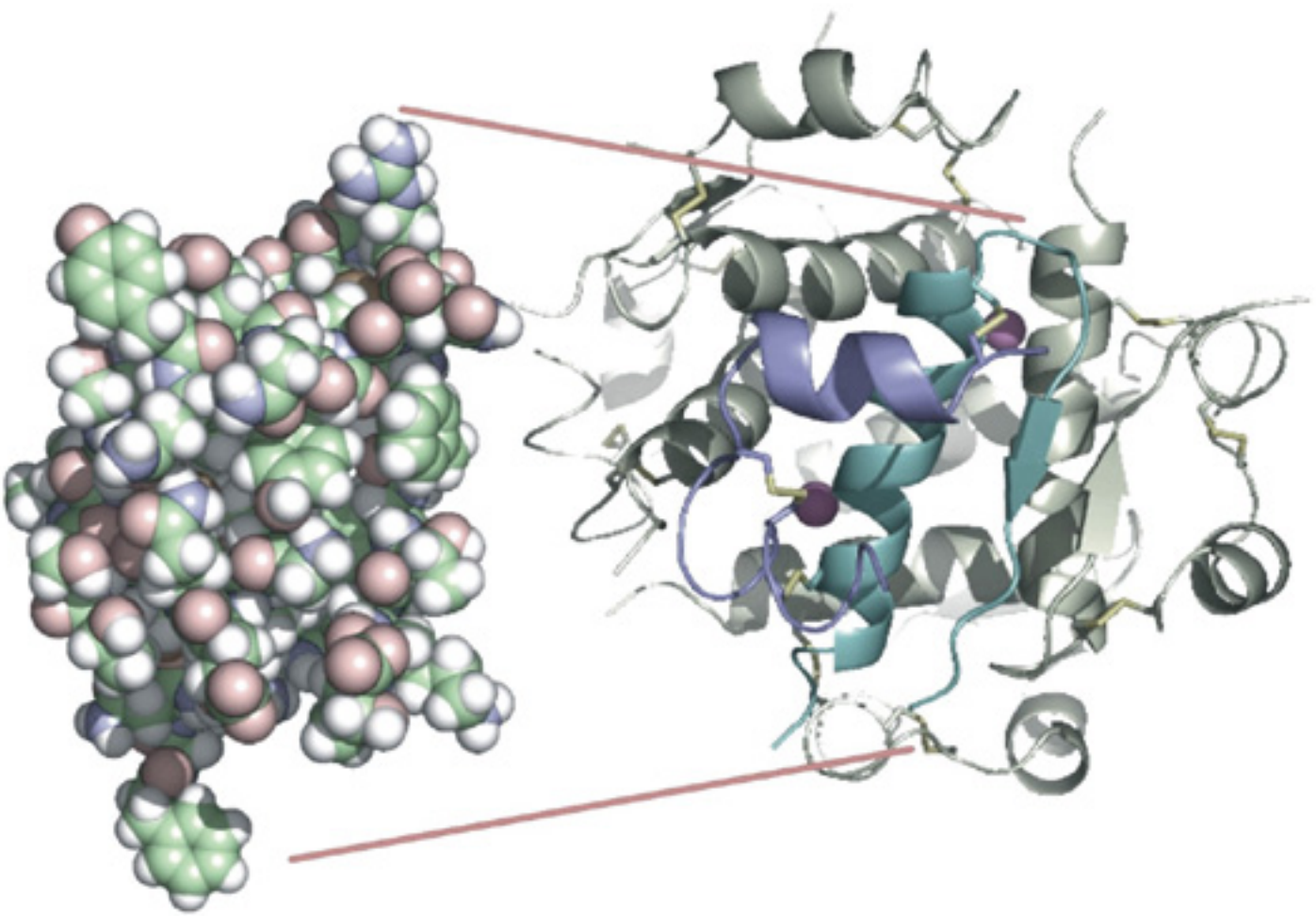
Biotechnology

Christopher Lewis



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Introduction

Where would we have been had it not been for the discovery of the art of collecting seeds and cultivating them for food? Life would surely have been very different!

Early man was a food gatherer, depending on nature for all his needs. He gradually moved on to being a food grower with the discovery of agriculture, and settled down in one place, learning to live in a group. This was the beginning of civilization as we know it today.

Early knowledge of agriculture was an accumulation of experiences that were passed on from father to son. Some of these have been preserved as religious commandments and some in the ancient inscriptions. There is evidence to show that as early as 2000 BC the Egyptian civilization followed particular dates for sowing and reaping. Some Greek and Roman classics give instructions on how to get a higher yield.

The development of agriculture made it apparent that more food could be extracted from a given area of land by encouraging useful and hardy plant and animal species, and discouraging others.

At the turn of the 19th century, a movement began in central Europe to train farmers in specific farming skills. A truly scientific approach was begun by Justine von Liebig of Darmstadt who in his classic work introduced the systematic development of agriculture science. From the 19th century onwards plant production became a scientific discipline.

In the early 20th century, the legendary work of Gregor Mendel laid the foundation of modern day genetics. His work explained the basics of inheritance in terms of the factor we today call genes.

Apart from selection and hybridization, new and innovative techniques such as genetic engineering that aid plant breeders have been developed in the recent past. One example of this is BtCotton. With advances in human and plant biology, more intricate details about the cell – the basic unit of life – were illuminated. The possibility of raising whole plants from various plant tissues, commonly know as tissue culture, has thrown open the doors for expedited evolution both in terms of generation of genetic variability and multiplication of elite plant types. The knowledge of the wonder molecule DNA has also opened a new area of plant breeding research. These new technologies have been collectively referred to as biotechnology. It is a collective effort for plant breeding in the future and will compliment man's crusade for more and better food. In India, the Green Revolution saw the rapid progress of agriculture and the application of different methods to enhance production. Biofertilizers have been proven to be more environmentally friendly fertilizers that do not cause harm to life. Bioremediation methods have been used to clear oil spills using bacteria.

Biotechnology

Biotechnology is short for biological technology. Technology is the ability to better utilize our surroundings. Biotechnology applies the same principles to living organisms as do other technologies. Biotechnology can be defined as the application of our knowledge and understanding of biology to meet practical needs. It is as old as the growing of crops. Today's biotechnology is largely identified with applications in medicine and agriculture based on our knowledge of the genetic code of life. Fermentation, used in making bread, beer, and cheese, is an example of biotechnology. Modern biotechnology simply allows scientists to be more specific in their work.

Different types of crops have been produced using the molecular tools of biotechnology and are beginning to be utilized in agricultural systems all over the world. At the same time, an increasing number of farmers are adopting sustainable cultural practices.

Biotechnology has the potential to assist farmers in reducing on-farm chemical inputs and produce value-added commodities. Conversely, there are concerns about the use of biotechnology in agricultural systems including the possibility that it may lead to greater farmer dependence on the providers of the new technology.

Genetic engineering

Genetically modified plants are created by the process of genetic engineering, which allows scientists to move genetic material between organisms with the aim of changing their characteristics. All organisms are composed of cells that contain the DNA molecule. Molecules of DNA form units of genetic information, known as genes. Each organism has a genetic blueprint made up of DNA that determines the regulatory functions of its cells and thus the characteristics that make it unique.

Prior to genetic engineering, the exchange of DNA material was possible only between individual organisms of the same species. With the advent of genetic engineering in 1972, scientists have been able to identify specific genes associated with desirable traits in one organism and transfer those genes across species boundaries into another organism. For example, a gene from bacteria, virus, or animal may be transferred into plants to produce genetically modified plants having changed characteristics. Thus, this method allows mixing of the genetic material among species that cannot otherwise breed naturally. The success of a genetically improved plant depends on the ability to grow single modified cells into whole plants. Some plants like potato and tomato grow easily from single cell or plant tissue. Others such as corn, soy bean, and wheat are more difficult to grow.

After years of research, plant specialists have been able to apply their knowledge of genetics to improve various crops such as corn, potato, and cotton. They have to be careful to ensure that the basic characteristics of these new plants are the same as the traditional ones, except for the addition of the improved traits.

The world of biotechnology has always moved fast, and now it is moving even faster. More traits are emerging; more land than ever before is being planted with genetically modified varieties of an ever-expanding number of crops. Research efforts are being made to genetically modify most

plants with a high economic value such as cereals, fruits, vegetables, and floriculture and horticulture species.

Public concern

The potential of biotechnology as a method to enhance agricultural productivity in the future has been accepted globally.

However, because of its revolutionary nature, there is a great degree of risk and uncertainty attached to the process of genetic engineering and the resultant genetically modified products.

Risks are also associated with genetically modified plants that are released into the environment. The nature of interactions with other organisms of the natural ecosystems cannot be anticipated without proper scientific testing. For example, modified plants with enhanced resistance to pests or disease threaten to transfer resistance to the wild relatives. This may have implications for biodiversity and ecosystem integrity. These and other numerous doubts plague the minds of common people and the decision-makers.

Some of the many applications for which Plant Biotechnology is currently being used are
developing plants that are resistant to diseases, pests, and stress
keeping fruits and vegetables fresh for longer periods of time, which is extremely important in tropical countries
producing plants that possess healthy fats and oils
producing plants that have increased nutritive value
producing soy beans with a higher expression of the anti-cancer proteins naturally found in soy beans
producing new substances in plants, including biodegradable plastics, and small proteins or peptides such as prophylactic and therapeutic vaccines.

Plant Breeding

As a part of agriculture, man started rearing plants and animals to meet his requirements. This is when humans started to learn how to influence the process of natural evolution so as to breed plant or animals.

Slowly and gradually, this process of expedited evolution, through selection and cultivation of plants, acquired the form of a routine endeavor—what we today call ‘plant breeding’. In this, heredity, which refers to the passage of various characteristic features from the main plant (the parent) to the plantlets (the progeny), plays an important role. The effects of heredity had been apparent to early man and he had taken advantage of them ever since the advent of agriculture.

Various methods have evolved in plant breeding. One of the most important methods is that of selection.

The ability to choose gave birth to the idea of selection. This is the most primitive and by and large the most successful method of plant breeding. Selection as a part of plant breeding started with the domestication of plants by early man. Domestication refers to the process of bringing wild species under human management. Not all selection over the years have been human influenced—many of the important crop species have resulted from the natural selection process, which is an integral part of evolution. As human knowledge of agriculture grew, man started shuffling crop species from one geographical terrain to another, thus making new introductions.

The first prerequisite of selection is the availability of variability, i.e. different types of forms. After a variable population is recognized, individuals that are the best performers for the desired feature, say fruit size in the case of tomatoes, are chosen and the rest of the population is discarded or rejected. The progeny of the selected individuals is grown further and again screened for the desired feature. This process is repeated until a uniform plant population is attained which has the best-desired characters. Eventually, a desired uniform crop variety is produced by this successive selection followed by multiplication of the selected individuals.

Selecting higher yielding plant varieties is no easy task. Various tools have been devised to deal with plant selection. In fact, the birth of genetics as an independent discipline in plant science started with some clever mathematical computations. This brainchild of yesteryears is now an important branch of genetics known as biometrics. Biometrics is defined as the application of statistics in biology. This has contributed greatly to the development of various systems based on which selection of plants is done. There are various methods by which plant selection is carried out, namely selection for uniform plants, known as pure line selection; selection from field-grown plants, known as bulk selection or mass selection; and selection from a well-documented list of parentage, commonly known as the pedigree system. Overall, the hallmark of selection lies in human ability to chose the best plants from a cluster of many.

Hybridization

In traditional terms, hybridization refers to the union of the male and the female gamete to produce a zygote. In plant science, hybridization also refers to the crossing or mating of two plants. The story of scientific hybridization of crop plants started with J G Kolreuter, who in 1761 published his work on the scientific bases of hybridization. Since then, hybridization followed by selection, has been the major tool of plant breeding.

In his quest to find more variability, man started experimenting with hybridization of plants so as to achieve the perfect plant type. This process was actually the beginning of expedited evolution since it led to the formation of new plant types artificially or due to human intervention at a much faster pace than it would have happened in nature. For example, the bread wheat that we eat today has taken about 500 years to evolve to its present form through human intervention. This form of wheat would have taken thousands of years to evolve had it been left to the natural evolution process.

Ways in which hybridization is used

Some of the ways in which hybridization has been exploited in breeding crop plants are given below

- **Combination breeding**: The main aim of combination breeding is to transfer one or more characters into a single variety or plant type from many others. For this, an existing plant variety may be used as the recipient parent while many other crop varieties or wild relatives may contribute as donor parents. The most commonly used method to achieve this goal is known as the backcross method. The plant type in which the character or the trait is being transferred is known as the recipient parent and the other as the donor parent. For this, the two plants are mated or crossed and the progeny is screened for the desired trait. The progeny plants possessing the desired trait are then selected and crossed back to the recipient parent. This process is repeated until the desired plant type having all the characteristics of the recipient in addition to the trait being transferred is finally obtained. This exercise is known as backcrossing. Backcrossing involves both hybridization and selection.

- **Hybrid varieties:** Plant scientists exploit the characteristic feature of better yielding ‘hybrids’ in plants. Hybrid vigour, or hetrosis as it is scientifically known, exploits the fact that some offspring from the progeny of a cross between two known parents would be better than the parents themselves. Many hybrid varieties of several crop species are being grown all over the world today. An example of this is the hybrid tomatoes that we eat commonly. The philosophy of hybridization has been extended from ‘within the same species or genera (the same type of plants)’ to ‘different species or genera (totally different plants)’. This is known as wide or distant hybridization. Wide hybridization has helped breeders to break what is known as the species or genera barrier for gene transfer, i.e. it has helped breeders to transfer beneficial characteristics from wild and weedy plants to the cultivated crop species.

Bt cotton

Cotton and other monocultured crops require an intensive use of pesticides as various types of pests attack these crops causing extensive damage. Over the past 40 years, many pests have developed resistance to pesticides.

So far, the only successful approach to engineering crops for insect tolerance has been the addition of Bt toxin, a family of toxins originally derived from soil bacteria. The Bt toxin contained by the Bt crops is no different from other chemical pesticides, but causes much less damage to the environment. These toxins are effective against a variety of economically important crop pests but pose no hazard to non-target organisms like mammals and fish. Three Bt crops are now commercially available: corn, cotton, and potato.

As of now, cotton is the most popular of the Bt crops: it was planted on about 1.8 million acres (728437 ha) in 1996 and 1997. The Bt gene was isolated and transferred from a bacterium bacillus thurigiensis to American cotton. The American cotton was subsequently crossed with Indian cotton to introduce the gene into native varieties.

The Bt cotton variety contains a foreign gene obtained from bacillus thuringiensis. This bacterial gene, introduced genetically into the cotton seeds, protects the plants from bollworm (A. lepidoptora), a major pest of cotton. The worm feeding on the leaves of a BT cotton plant becomes lethargic and sleepy, thereby causing less damage to the plant.

Field trials have shown that farmers who grew the Bt variety obtained 25%–75% more cotton than those who grew the normal variety. Also, Bt cotton requires only two sprays of chemical pesticide against eight sprays for normal variety. According to the director general of the Indian Council of Agricultural Research, India uses about half of its pesticides on cotton to fight the bollworm menace.

Use of Bt cotton has led to a 3%–27 increase in cotton yield in countries where it is grown.

Plant Tissue Culture

In 1965, French botanist George Morel was attempting to obtain a virus-free orchid plant when he discovered that a millimetre-long shoot could be developed into complete plantlets by micropropagation. This was the beginning of tissue culture. Thereafter, in the 1970s developed countries began commercial exploitation of this technology. It entered the developing world in the 1980s. It was earlier used to develop ornamental plants and flowering plants for export. With tree species, the technique of tissue culture remained confined for many years to the laboratory stage and had generally invited only academic interest. But in most developing countries, the shortage of biomass and the ever-increasing energy requirements created the need to explore possibilities of mass propagation of trees by tissue culture.

Tissue culture or mass cloning methods of elite tree species is done for increasing land productivity. They are being modified or adapted for large-scale modification.

Species are selected for tissue culture on the following basis.

- Species that have regeneration problems, specially because of poor seed set or germination (as in Anogeissus and bamboo). In these cases, seeds collected from superior trees are used for initiating cultures.
- Species that vary markedly in their desirable traits, i.e. Eucalyptus. The selected trees are marked from the variant population for the desirable trait such as disease resistance, straight bole, higher productivity, etc. in consultation with officials from state forest department or growers.
- Species where plants of any one particular sex is of commercial importance, for example female plants of papaya and male plants of asparagus

In tissue culture cells, tissues, and organs of a plant are separated. These separated cells are grown especially in containers with a nutrient media under controlled conditions of temperature and light. The cultured plant requires a source of energy from sugar, salts, a few vitamins, amino acids, etc. that are provided in the nutrient media. From these cultured parts, an embryo or a

shoot bud may develop, which then grows into a whole new plantlet. Similarly, portions of organs or tissues can be cultured in a culture media. Generally, these give rise to an unorganized mass of cells called callus (soft tissue that forms over a cut surface).

Tissue culture plantlets have poor photosynthesis efficiency and lack the proper mechanism to control water loss. They need to be hardened gradually by moving them along a humidity gradient in the greenhouse. Once these plants are in the research fields, they are evaluated under field conditions and the data is collected every 6 months. A large number of tissue culture plants that have grown into trees are remarkably uniform and show an increase in biomass production over the conventionally raised plants.

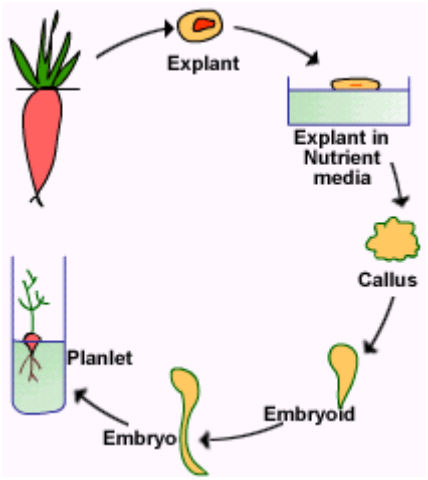


Figure Tissue culture and totipotency

Application of tissue culture

- **Micropropagation**

Rapid vegetative multiplication of valuable plant material for agriculture, horticulture, and forestry.

- **Production of disease-free plants**

When the apex of shoot is used for multiplication by tissue culture, we get disease free plants because the shoot apical meristem, a group of dividing cells at the tip of a stem or root, is free from pathogens.

- **Plant breeding**

Tissue culture has also been successfully used in plant breeding programmes.

- **Production of disease- and pest-resistant plants**

Plants grown from tissue culture usually pass trough callus phase and show many variations. These show some agronomic characteristics like tolerance to pests, diseases, etc.

Cloning

Genetically identical plants derived from an individual are called clones. Processes that produce clones can be put under the term ‘cloning’. This includes all the methods of vegetative propagation such as cutting, layering, and grafting. Propagation by tissue culture also helps in producing clones. Using the shoot tip, it is possible to obtain a large number of plantlets. This technique is used extensively in the commercial field for micropropagation of ornamental plants

like chrysanthemum, gladiolus, etc. and also crops such as sugar cane, tapioca, and potato. Thus an unlimited number of plants that are genetically similar or are clones can be produced in a short span of time by tissue culture.

Large-scale propagation

To bridge the gap between research and application, the Department of Biotechnology, Government of India sponsored the setting-up of two pilot-scale facilities for large-scale propagation of elite planting material of forest trees through tissue culture. One of these facilities has been established at TERI's 36-hectare-campus in Gual Pahari, Haryana with an annual capacity of a million plantlets. Research at these facilities focuses exclusively on developing new protocols for mass cloning of elite planting material, mainly of trees.

Till date, over 4 million plants have been dispatched for field plantation from these facilities. The tissue culture raised plants are presently being evaluated under field conditions. This is being done in tandem with the forest departments of Haryana, Uttar Pradesh, Madhya Pradesh, Bihar, Jammu and Kashmir, and Orissa. For initial screening for phenotypically superior trees only a few hundred plantlets of the same are raised and tested under various agroclimatic zones. The best clones are then mass multiplied and monitored regularly for their performance. Field data suggest a survival percentage of more than 90% even in the harsh conditions of Aravalis without the life-saving irrigation. At half the rotation age some of the selected clones of Eucalyptus are showing a significant increase in productivity as compared to the conventional seed raised progenies.

The Green Revolution

The world's worst recorded food disaster occurred in 1943 in British-ruled India. Known as the Bengal Famine, an estimated 4 million people died of hunger that year in eastern India (which included today's Bangladesh). Initially, this catastrophe was attributed to an acute shortfall in food production in the area. However, Indian economist Amartya Sen (recipient of the Nobel Prize for Economics, 1998) has established that while food shortage was a contributor to the problem, a more potent factor was the result of hysteria related to World War II, which made food supply a low priority for the British rulers.

When the British left India in 1947, India continued to be haunted by memories of the Bengal Famine. It was therefore natural that food security was one of the main items on free India's agenda. This awareness led, on one hand, to the Green Revolution in India and, on the other, legislative measures to ensure that businessmen would never again be able to hoard food for reasons of profit.

The Green Revolution, spreading over the period from1967/68 to 1977/78, changed India's status from a food-deficient country to one of the world's leading agricultural nations. Until 1967 the government largely concentrated on expanding the farming areas. But the population was growing at a much faster rate than food production. This called for an immediate and drastic action to increase yield. The action came in the form of the Green Revolution. The term 'Green Revolution' is a general one that is applied to successful agricultural experiments in many developing countries. India is one of the countries where it was most successful.

There were three basic elements in the method of the Green Revolution

- Continuing expansion of farming areas
- Double-cropping in the existing farmland
- Using seeds with improved genetics.

The area of land under cultivation was being increased from 1947 itself. But this was not enough to meet the rising demand. Though other methods were required, the expansion of cultivable land also had to continue. So, the Green Revolution continued with this quantitative expansion of farmlands.

Double cropping was a primary feature of the Green Revolution. Instead of one crop season per year, the decision was made to have two crop seasons per year. The one-season-per-year practice was based on the fact that there is only one rainy season annually. Water for the second phase now came from huge irrigation projects. Dams were built and other simple irrigation techniques were also adopted.

Using seeds with superior genetics was the scientific aspect of the Green Revolution. The Indian Council for Agricultural Research (which was established by the British in 1929) was reorganized in 1965 and then again in 1973. It developed new strains of high yield variety seeds, mainly wheat and rice and also millet and corn.

The Green Revolution was a technology package comprising material components of improved high yielding varieties of two staple cereals (rice and wheat), irrigation or controlled water supply and improved moisture utilization, fertilizers, and pesticides, and associated management skills.

Benefits

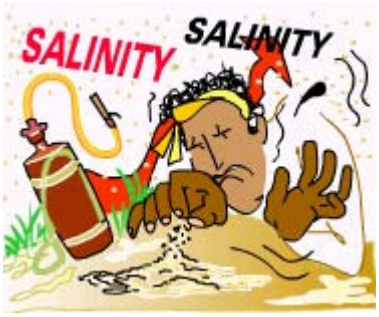
Thanks to the new seeds, tens of millions of extra tonnes of grain a year are being harvested.

The Green Revolution resulted in a record grain output of 131 million tonnes in 1978/79. This established India as one of the world's biggest agricultural producers. Yield per unit of farmland improved by more than 30% between1947 (when India gained political independence) and 1979. The crop area under high yielding varieties of wheat and rice grew considerably during the Green Revolution.

The Green Revolution also created plenty of jobs not only for agricultural workers but also industrial workers by the creation of related facilities such as factories and hydroelectric power stations.

Shortcomings

In spite of this, India's agricultural output sometimes falls short of demand even today. India has failed to extend the concept of high yield value seeds to all crops or all regions. In terms of crops, it remains largely confined to foodgrains only, not to all kinds of agricultural produce.



In regional terms, only the states of Punjab and Haryana showed the best results of the Green Revolution. The eastern plains of the River Ganges in West Bengal also showed reasonably good results. But results were less impressive in other parts of India.

The Green Revolution has created some problems mainly to adverse impacts on the environment. The increasing use of agrochemical-based pest and weed control in some crops has affected the surrounding environment as well as human health. Increase in the area under irrigation has led to rise in the salinity of the land. Although high yielding varieties had their plus points, it has led to significant genetic erosion.

Since the beginning of agriculture, people have been working to improving seed quality and variety. But the term 'Green Revolution' was coined in the 1960s after improved varieties of wheat dramatically increased yields in test plots in northwest Mexico. The reason why these 'modern varieties' produced more than traditional varieties was that they were more responsive to controlled irrigation and to petrochemical fertilizers. With a big boost from the international agricultural research centres created by the Rockefeller and Ford Foundations, the 'miracle' seeds quickly spread to Asia, and soon new strains of rice and corn were developed as well.

By the 1970s the new seeds, accompanied by chemical fertilizers, pesticides, and, for the most part, irrigation, had replaced the traditional farming practices of millions of farmers in developing countries. By the 1990s, almost 75% of the area under rice cultivation in Aisa was growing these new varieties. The same was true for almost half of the wheat planted in Africa and more than half of that in Latin America and Asia, and more than 50% of the world's corn as well. Overall, a very large percentage of farmers in the developing world were using Green Revolution seeds, with the greatest use found in Asia, followed by Latin America.

Biofertilizers

One of the major concerns in today's world is the pollution and contamination of soil. The use of chemical fertilizers and pesticides has caused tremendous harm to the environment. An answer to this is the biofertilizer, an environmentally friendly fertilizer now used in most countries. Biofertilizers are organisms that enrich the nutrient quality of soil. The main sources of biofertilizers are bacteria, fungi, and cynobacteria (blue-green algae). The most striking relationship that these have with plants is symbiosis, in which the partners derive benefits from each other.

Plants have a number of relationships with fungi, bacteria, and algae, the most common of which are with mycorrhiza, rhizobium, and cyanophyceae. These are known to deliver a number of benefits including plant nutrition, disease resistance, and tolerance to adverse soil and climatic conditions. These techniques have proved to be successful biofertilizers that form a health relationship with the roots.

Biofertilizers will help solve such problems as increased salinity of the soil and chemical run-offs from the agricultural fields. Thus, biofertilizers are important if we are to ensure a healthy future for the generations to come.

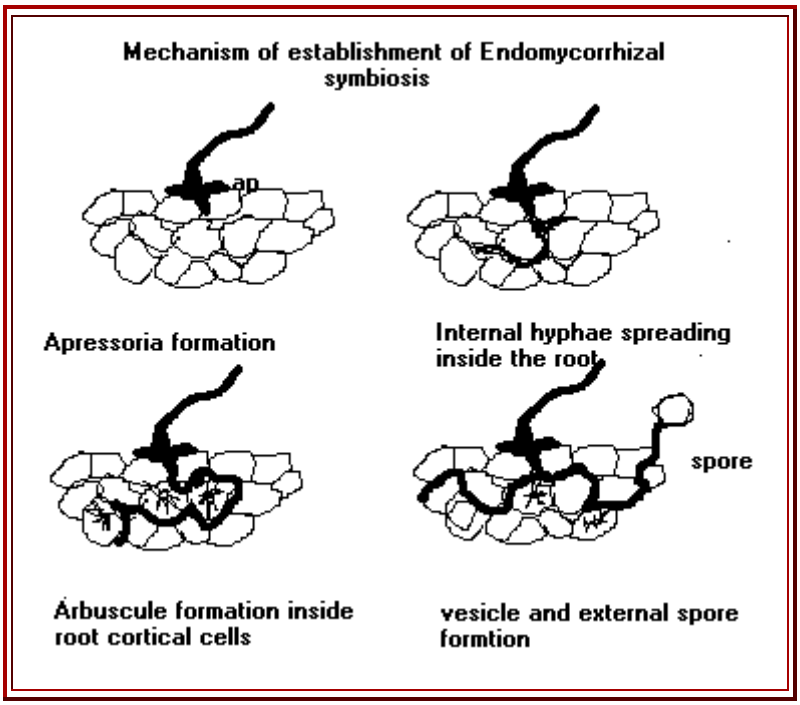
Mycorrhiza

Mycorrhizae are a group of fungi that include a number of types based on the different structures formed inside or outside the root. These are specific fungi that match with a number of favourable parameters of the the host plant on which it grows. This includes soil type, the presence of particular chemicals in the soil types, and other conditions.

These fungi grow on the roots of these plants. In fact, seedlings that have mycorrhizal fungi growing on their roots survive better after transplantation and grow faster. The fungal symbiont gets shelter and food from the plant which, in turn, acquires an array of benefits such as better uptake of phosphorus, salinity and drought tolerance, maintenance of water balance, and overall increase in plant growth and development.

While selecting fungi, the right fungi have to be matched with the plant. There are specific fungi for vegetables, fodder crops, flowers, trees, etc.

Mycorrhizal fungi can increase the yield of a plot of land by 30%-40%. It can absorb phosphorus from the soil and pass it on to the plant. Mycorrhizal plants show higher tolerance to high soil temperatures, various soil- and root-borne pathogens, and heavy metal toxicity.



Legume-rhizobium relationship

Leguminous plants require high quantities of nitrogen compared to other plants. Nitrogen is an inert gas and its uptake is possible only in fixed form, which is facilitated by the rhizobium bacteria present in the nodules of the root system. The bacterium lives in the soil to form root nodules (i.e. outgrowth on roots) in plants such as beans, gram, groundnut, and soybean.

Blue-green algae

Blue-green algae are considered the simplest, living autotrophic plants, i.e. organisms capable of building up food materials from inorganic matter. They are microscopic. Blue-green algae are widely distributed in the aquatic environment. Some of them are responsible for water blooms in stagnant water. They adapt to extreme weather conditions and are found in snow and in hot springs, where the water is 85 °C.

Certain blue-green algae live intimately with other organisms in a symbiotic relationship. Some are associated with the fungi in form of lichens. The ability of blue-green algae tophotosynthesize food and fix atmospheric nitrogen accounts for their symbiotic associations and also for their presence in paddy fields.

Blue-green algae are of immense economic value as they add organic matter to the soil and increase soil fertility. Barren alkaline lands in India have been reclaimed and made productive by inducing the proper growth of certain blue-green algae.

Bioremediation

Enormous quantities of organic and inorganic compounds are released into the environment each year as a result of human

activities. In some cases these releases are deliberate and well regulated (e.g. industrial emissions) while in other cases they are accidental (e.g. chemical or oil spills). Petroleum and its products are one of the most common environmental pollutants. They are a fire hazard, threat to marine life, and a source of air and groundwater pollution. They contaminate land and water bodies by accidental spills like the Alaska Oil spill in 1989 and oil spills during the Gulf War, leakage from pipelines, and other human activities. Detoxification of the contaminated sites is expensive and time consuming by conventional chemical or physical methods.

Bioremediation consists of using naturally occurring or laboratory cultivated micro-organisms to reduce or eliminate toxic pollutants. Petroleum products are a rich source of energy and some organisms are able to take advantage of this and use hydrocarbons as a source of food and energy. This results in the breakdown of these complex compounds into simpler forms such as carbon dioxide and water. Bioremediation thus involves detoxifying hazardous substances instead of merely transferring them from one medium to another. This process is less disruptive and can be carried out at the site which reduces the need of transporting these toxic materials to separate treatment sites.

Using bioremediation techniques, TERI has developed a mixture of bacteria called 'oilzapper' which degrades the pollutants of oil-contaminated sites, leaving behind no harmful residues. This technique is not only environment friendly, but also highly cost-effective.

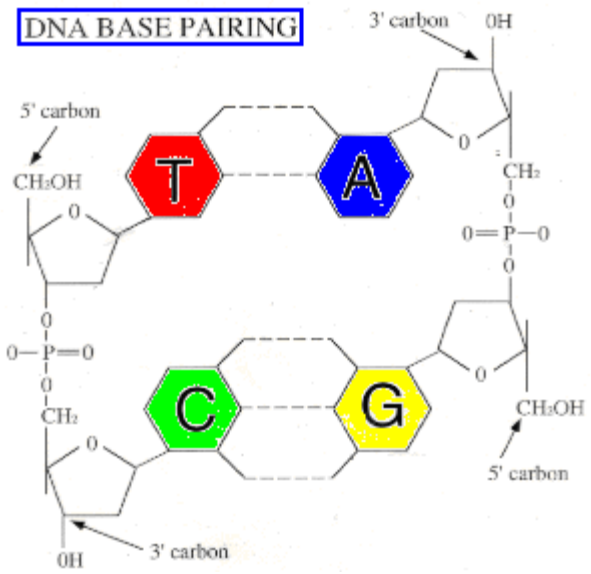
DNA

Since the time Gregor Mendel began studying about inheritance in garden plants some 150 years back, researchers have worked to learn more about the language of life – how characteristics pass from one generation to another. Researchers began to understand DNA from the 1800s when they stated that all living beings, whether plants, humans, animals, or bacteria, comprised cells that have the same basic components.

Living organism are made up of cells, i.e. cells are the basic units of life. For example, each of us is made up of billions of this basic unit. If one closely inspects the structure of the cell, one is likely to find various smaller bodies or organelles like mitochondria that generates the energy required to perform all life processes ('the powerhouse'), chloroplast (only in green plants and responsible for their coloration), the central core – 'the nucleus, to name a few. The nucleus harbours the blueprint of life and the genetic material – DNA or deoxyribonucleic acid – and is the control centre of any cell. The genetic material or the blueprint is contained in all the cells that make up an organism and is transmitted from one generation to another. A child inherits half of the genetic material from each of his/her parents.

The chemical structure of everyone's DNA is the same. Structurally, DNA is a double helix: two strands of genetic material spiraled around each other. Each strand contains a sequence of bases, also called nucleotides. A base is one of four chemicals: adenine, guanine, cytosine, and thymine. The two strands of DNA are connected at each base. Each base will only bond with one other base, as follows: Adenine (A) will only bond with thymine (T), and guanine (G) will only bond with cytosine (C). If one strand of DNA looks like A-A-C-T-G-A-T-A-G-G-T-C-T-A-,the DNA strand bound to it will look like T-T-G-A-C-T-A-T-C-C-A-G-A-T-C.

Together, the section of DNA would be represented as given in Figure



T-T-G-A-C-T-A-T-C-C-A-G-A-T-C

A-A-C-T-G-A-T-A-G-G-T-C-T-A-G

The length of the DNA strand varies from organism to organism but within individuals of a particular species it is nearly constant. For example, a certain virus may have only 50 000 (5×10^4) bases constituting the genetic material whereas a human cell contains nearly 3.2 billion (3.2×10^9) bases in each of the cells (except the germ line cells). The amount and sequence in all the cells of an organism is identical. The DNA is for most part of the time present as condensed body called chromosomes (coloured body) except when it is replicating or dividing. A piece of a chromosome that dictates a particular trait, for example, eye and skin colour in humans, is called a gene. In any cell, the DNA can be classified into two categories – the sequence that codes for traits or genes and the sequence that has no apparent function or the non-coding DNA. The coding sequence (genes) in humans constitutes only five per cent of the total DNA and is identical in all humans. The non-coding sequence, which is nearly 95% in humans, varies from one individual to another, and forms the basis of DNA fingerprinting.

DNA fingerprinting

The only difference between two individuals is the order of the base pairs. Each individual has a different sequence of DNA, specially in the non-coding region. Using these sequences, every person could be identified solely by the sequence of their base pairs. However, because the entire DNA is so huge, the task would be time-consuming and nearly impossible. Instead, scientists are able to use a shorter method.

The steps involved in DNA fingerprinting can be summarized as follows.

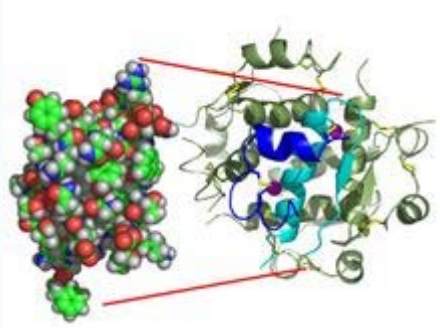
- Isolating the DNA in question from the rest of the cellular material in the nucleus.
- Cutting the DNA into several pieces of different sizes.
- Sorting the DNA pieces by size. The process by which the size separation, or 'size fractionation', is done is called gel electrophoresis.

This is the basic concept behind fingerprinting technique.

DNA fingerprinting in plants

The concept of DNA fingerprinting can also be extended to plants and many institutions in the country are doing it today. TERI has successfully generated fingerprints of various medicinal plants such as neem, ashwagandha, and amla with the objective of determining their identity. With the help of fingerprints one can find out the genetic diversity in India. This knowledge has profound implications. Based on the extent of genetic diversity, one can establish the centre of origin of a particular plant species. And having done that we are better equipped to prevent bio-piracy or the theft of our genetic resources

Biotechnology



The structure of insulin

Biotechnology is technology based on biology, especially when used in agriculture, food science, and medicine. The UN Convention on Biological Diversity has come up with one of many definitions of biotechnology:

> *"Biotechnology means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use."*

This definition is at odds with common usage in the United States, where "biotechnology" generally refers to recombinant DNA based and/or tissue culture based processes that have only been commercialized since the 1970s. Thus, in common usage, modifying plants or animals by breeding, which has been practiced for thousands of years, would not be considered biotechnology. This distinction emphasizes that modern, recombinant DNA based biotechnology is not just a more powerful version of existing technology, but represents something new and different; for instance, theoretically, recombinant DNA biotechnology allows us to take virtually any gene and express it in any organism; we can take the genes that make crimson color in plants and put them into guinea pigs to make pink pets, or, we can take the genes that help arctic fish survive the freezing temperatures and put them into food to increase the amount of time it can grow before it freezes. This sort of gene transfer was virtually impossible with historical processes.

There has been a great deal of talk - and money - poured into biotechnology with the hope that miracle drugs will appear. While there do seem to be a small number of efficacious drugs, in general the Biotech revolution has not happened in the pharmaceutical sector. However, recent progress with monoclonal antibody based drugs, such as Genentech's Avastin ™ suggest that biotech may finally have found a role in pharmaceutical sales.

Biotechnology can also be defined as the manipulation of organisms to do practical things and to provide useful products.

One aspect of biotechnology is the directed use of organisms for the manufacture of organic products (examples include beer and milk products). For another example,

naturally present bacteria are utilized by the mining industry in bioleaching. Biotechnology is also used to recycle, treat waste, clean up sites contaminated by industrial activities (bioremediation), and produce biological weapons.

There are also applications of biotechnology that do not use living organisms. Examples are DNA microarrays used in genetics and radioactive tracers used in medicine.

Red biotechnology is applied to medical processes. Some examples are the designing of organisms to produce antibiotics, and the engineering of genetic cures through genomic manipulation.

White biotechnology, also known as **grey biotechnology**, is biotechnology applied to industrial processes. An example is the designing of an organism to produce a useful chemical. White biotechnology tends to consume less in resources than traditional processes used to produce industrial goods.

Green biotechnology is biotechnology applied to agricultural processes. An example is the designing of transgenic plants to grow under specific environmental conditions or in the presence (or absence) of certain agricultural chemicals. One hope is that green biotechnology might produce more environmentally friendly solutions than traditional industrial agriculture. An example of this is the engineering of a plant to express a pesticide, thereby eliminating the need for external application of pesticides. An example of this would be Bt corn. Whether or not green biotechnology products such as this are ultimately more environmentally friendly is a topic of considerable debate.

Bioinformatics is an interdisciplinary field which addresses biological problems using computational techniques. The field is also often referred to as computational biology. It plays a key role in various areas, such as functional genomics, structural genomics, and proteomics, and forms a key component in the biotechnology and pharmaceutical sector.

The term **blue biotechnology** has also been used to describe the marine and aquatic applications of biotechnology, but its use is relatively rare.

Biotechnology medical products

Traditional pharmaceutical drugs are small chemicals molecules that treat the symptoms of a disease or illness - one molecule directed at a single target. Biopharmaceuticals are large biological molecules known as proteins and these target the underlying mechanisms and pathways of a malady; it is a relatively young industry. They can deal with targets in humans that are not accessible with traditional medicines. A patient typically is dosed with a small molecule *via* a tablet while a large molecule is typically injected.

Small molecules are manufactured by chemistry but large molecules are created by living cells: for example, - bacteria cells, yeast cell,animal cells.

Modern biotechnology is often associated with the use of genetically altered microorganisms such as *E. coli* or yeast for the production of substances like insulin or antibiotics. It can also refer to transgenic animals or transgenic plants, such as Bt corn. Genetically altered mammalian cells, such as Chinese Hamster Ovary (CHO) cells, are also widely used to manufacture pharmaceuticals. Another promising new biotechnology application is the development of plant-made pharmaceuticals.

Biotechnology is also commonly associated with landmark breakthroughs in new medical therapies to treat diabetes, Hepatitis B, Hepatitis C, Cancers, Arthritis, Haemophilia, Bone Fractures, Multiple Sclerosis, Cardiovascular as well as molecular diagnostic devices than can be used to define the patient population. Herceptin, is the first drug approved for use with a matching diagnostic test and is used to treat breast cancer in women whose cancer cells express the protein HER2.

History

History of Biotechnology

Early cultures also understood the importance of using natural processes to breakdown waste products into inert forms. From very early nomadic tribes to pre-urban civilizations it was common knowledge that given enough time organic waste products would be absorbed and eventually integrated into the soil. It was not until the advent of modern microbiology and chemistry that this process was fully understood and attributed to bacteria.

The most practical use of biotechnology, which is still present today, is the cultivations of plants to produce food suitable to humans. Agriculture has been theorized to have become the dominant way of producing food since the Neolithic Revolution. The processes and methods of agriculture have been refined by other mechanical and biological sciences since its inception. Through early biotechnology farmers were able to select the best suited and high-yield crops to produce enough food to support a growing population. Other uses of biotechnology were required as crops and fields became increasingly large and difficult to maintain. Specific organisms and organism byproducts were used to fertilize, restore nitrogen, and control pests. Throughout the use of agriculture farmers have inadvertently altered the genetics of their crops through introducing them to new environments, breeding them with other plants, and by using artificial selection. In modern times some plants are genetically modified to produce specific nutritional values or to be economical.

The process of Ethanol fermentation was also one of the first forms of biotechnology. Cultures such as those in Mesopotamia, Egypt, and Iran developed the process of brewing which consisted of combining malted grains with specifics yeasts to produce alcoholic beverages. In this process the carbohydrates in the grains were broken down into alcohols such as ethanol. Later other cultures produced the process of Lactic acid fermentation which allowed the fermentation and preservation of other forms of food. Fermentation was also used in this time period to produce leavened bread. Although the

process of fermentation was not fully understood until Louis Pasteur's work in 1857, it is still the first use of biotechnology to convert a food source into another form.

Combinations of plants and other organisms were used as medications in many early civilizations. Since as early as 200 BC people began to use disabled or minute amounts of infectious agents to immunize themselves against infections. These and similar processes have been refined in modern medicine and have lead to many developments such as antibiotics, vaccines, and other methods of fighting sickness.

A more recent field in biotechnology is that of genetic engineering. Genetic modification has opened up many new fields of biotechnology and allowed the modification of plants, animals, and even humans on a molecular level.

Global biotechnology trends

According to Burrill and Company, an industry investment bank, over $350 billion has been invested in biotech so far, and global revenues have risen from $23 billion in 2000 to more than $50 billion in 2005. The greatest growth has been in Latin America but all regions of the world have shown strong growth trends.

There has been little innovation in the traditional pharmaceutical industry over the past decade and biopharmaceuticals are now achieving the fastest rates of growth against this background, particularly in breast cancer treatment. Biopharmaceuticals typically treat sub-sets of the total population with a disease whereas traditional drugs are developed to treat the population as a whole. However, one of the great difficulties with traditional drugs are the toxic side effects the incidence of which can be unpredictable in individual patients.

Many have expressed concerns about the safety, environmental impacts, and social impacts of biotechnology. A book by Michael Mehta (2005) entitled *Biotechnology Unglued: Science, Society and Social Cohesion* (UBC Press) examines the two faces of biotechnology, and provides a series of case-studies on how different applications in biotechnology affect the social cohesiveness of different kinds of communities.

Technician: Skills Needed

The Biotech Technician must be a person posessing skills with ability to solve problems and meet the customer in such a way that the translations of what is possible can be made clear. They have to maintian a notebook, one that can be read by someone else. Present results in a clear manner, and work with others to meet objectives.

Laboratory Skills

A technician must use the tools of the trade not unlike any other trade, we are farmers but our herd is tiny tiny wildlife. To take care of our herd we must measure certian aspects of thier environment.

Solute,solvent, and solution

solute = Dry Material
Solvent = What you mix with
Solution = Solute + Solvent

pH

Measurement

1. Probe and meter

most accurate more expensive piece of equipment Store in buffer Check for clogging

1. Litmus paper

very coarse measurement of pH

1. Field kit

The letters pH stand for “power of hydrogen”

Hydrogen the most abundant element in the universe is hydrogen, which makes up about ¾ of all matter!

Stronger acids give up more protons, H+ (hydrogen ions); stronger bases give up more OH- (hydroxide ions). Neutral substances have an even balance of H+ and OH-, Eg. Pure (distilled) water.

>7 base -- 7 Neutral -- <7 Acid

Depending on your definition, an acid is a hydrogen ion or proton donator and a base is a hydrogen ion acceptor, hydroxide ion donator, or electron acceptor.

Acids produce H+ ions in aqueous solutions, whereas bases produce OH- ions in aqueous solutions

pH electrode compared to a battery

Store in buffer not H2O

Mercury tube Good for metals and biologicals and up to 80 degrees C

The common Silver-Silver Chloride reference electrode used with most combination pH electrodes has a Potassium Chloride salt-bridge which is saturated with Silver Chloride.

Works well in most samples, but not in biological samples containing proteins or related materials

Calibration

Span error Difference b/w perfect and actual pH Electrode at 25C produces 59.12 mV/pH unit

Offset error

Difference from Perfect reading from actual reading is the offset error

signal @ pH 7.0 @ 25 C is 0 mV

Three point calibration

pH's 4, 7 and 10

Calibrate W/I range you going to use

Buffer and reagent Prep

Chemist use buffers to moderate the pH of a reaction. Buffers stabilize a solution at a specific pH value. Resist pH change when small amounts of acid or alkali are added.

KPO4

KPO4 buffer is highly recommended for most P450 assays (microsomal or recombinant enzymes) with the exception of CYP 2C9 and and 2A6 where a Tris buffer system is more appropriate.

TRIS buffer

TRIS buffers are used by biochemists to control pH in the physiological range (about 7 to 8 pH) because phosphates cause undesirable side reactions with the biological substances in their test samples.

”Good” buffers

These buffers were well received by the research community because “Good” buffers are nontoxic, easy to purify and their pKa is typically between 6.0 and 8.0, the range at which most biological reactions occur.

The “Good” buffers also feature minimal penetration of membranes, minimal absorbance in the 240-700 nm range and minimal effects due to salt, concentration or temperature.

pKa = dissociation constant

In chemistry and biochemistry, a dissociation constant or an ionization constant is a specific type of equilibrium constant used for dissociation (ionizationation) reactions. Dissociation in chemistry and biochemistry is a general process in which complexes, molecules, or salts separate or split into smaller molecules, ions, or radicals, usually in a reversible manner. Dissociation is the opposite of association and recombination.

Problems

1. Ionic Strength
2. Temperature
3. Counter ion

colony agar plating

What is Agar?

A gelatinous material derived from certian marine algae.

1. Growth Media
2. Carbon Sources
3. Carbohydrates
4. Proteins
5. Nitrogen Sources

Two types:

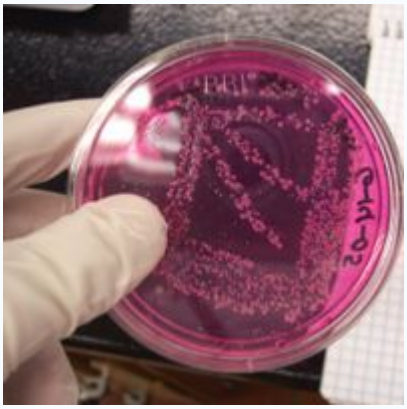
Simple
Complex

Compounents required for preparing a minimal agar

1. minerals
2. H2O
3. vitamins
4. Precursors
5. Componds that may lead to your product - inducers
6. Componds added to induce gene expression

Nutrient Agar. LB (Luria-Bertani) Media

Agar Plates



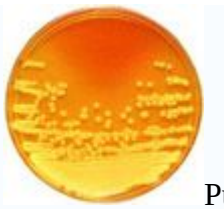
Blood

contains blood cells from an animal (e.g. a sheep). Most bacteria will grow on this medium

Chocolate

This contains lysed blood cells, and is used for growing fastidious (fussy) respiratory bacteria.

Mannitol Salt



Purpose Mannitol salt agar is both a selective and differential growth medium.

Brillant Green

Inhibits Gram+ MacConkey

This type of agar is used since it is one of the most forgiving media available - it is hard to contaminate, and E. coli usually grow up as red colonies.

(Almost all spore forming bacteria are Gram-positive, but these cannot grow on MacConkey agar because of the detergent in it (bile salts), and very few Gram-negative bacteria can tolerate either the initial dryness of the plates, or the boiling temperatures needed to make the MacConkey agar. Also, while fungal spores can tolerate the dryness, they cannot tolerate the boiling.)

This is an agar upon which only Gram-negative bacteria can grow

Starch

An agar plate is a sterile Petri dish that contains agar plus nutrients, and is used to culture bacteria or fungi.

Neomycin agar

contains the antibiotic neomycin.

Sabouraud agar

Used for fungi. It contains gentamicin and has a low pH that will kill most bacteria.

LB

- Complex + pH 7.2

UV/VIS Spectroscopy

Common UV/ VIS spectrophotometers Following is a list of commonly used spectrophotometers: GeneSys 20 HP8452A Diode Array Spectronic 20

Ultraviolet-Visible spectroscopy or Ultraviolet-Visible spectrophotometry (UV/ VIS) involves the spectroscopy of photons (spectrophotometry). It uses light in the visible and

adjacent near ultraviolet (UV) and near infrared (NIR) ranges. In this region of energy space molecules undergo electronic transitions.

A=elc

1. A=absorbance
2. e=Molar Absorbtivity
3. l=path legth (cm)
4. c=concentration (M)

Laboratory calculations

1. + - Prep of Sterile solid and Liquid media

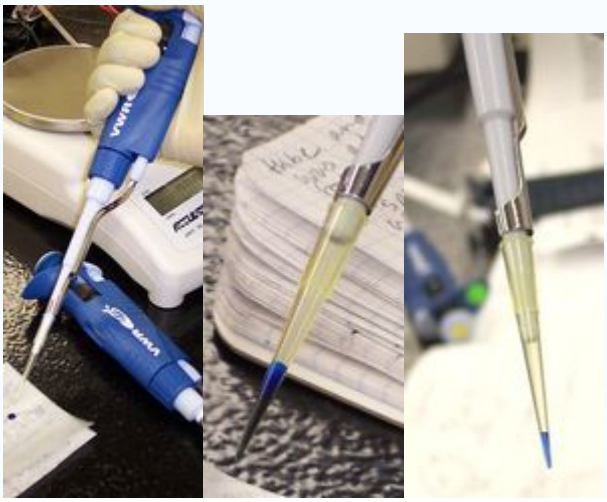
Sterilization Methods

There are different types of Sterilization techniques. Some of them are 1. Physical sterilzation 2. Chemical sterilzation

Under Physical sterilzation a) Heat b) Filteration c) Ionisation Radiation etc., In Heat sterilization i. Temperature above 100 C ii. Temperature at 100 C iii. Temperature below 100 C.

i. Temperature above 100 C There are two methods involved in it a. Moisture heat sterilization b. Dry heat sterilization

Pipetting



1. Growing concerns regarding repetitive strain injuries (RSIs)
2. Adopting good pipetting techniques is critical for the accuracy of your analysis and your health.
3. Contamination Prevention

4. Care of

Measuring / Mixing

Using a balance Calibration / documentation

UV/VIS spectroscopy

- http://www.scienceofspectroscopy.info/
- Use correct Reagent
- Use correct Wavelength

Gel Electrophoresis

Gel electrophoresis is a method that separates macromolecules-either nucleic acids or proteins-on the basis of size, electric charge, and other physical properties.

materials

Two basic types

agarose and polyacrylamide

agarose

Agarose is a natural colloid extracted from sea weed It is very fragile and easily destroyed by handling Agarose gels have very large “pore” size and are used primarily to separate very large molecules wiht a molecular mass greater than 200 kdal Agarose gels can be processed faster than polyacrylamide gels, but their resolution is inferior.

Agarose is a linear polysaccharide (average molecular mas about 12,000) made up of the basic repeat unit agarobiose, which comprises alternating units of galactose and 3,6-anhydrogalactose. Agarose is usually used at concentrations between 1% and 3%. Agarose is a chain of sugar molecules, and is extracted from seaweed.

Perhaps you have seen the terms TBE or TAE.

These are names of two commonly used buffers in electrophoresis.

The “T” stands for Tris, a chemical which helps maintain a consistent pH of the solution.

The “E” stands for EDTA, which itself is another anacronym. EDTA chelates (gobbles up) divalent cations like magnesium. This is important because most nucleases require divalent cations for activity, and you certainly wouldn’t want any stray nucleases degrading your sample while it’s running through the gel, would you?

Finally, the “B” or “A” stand for Boric acid or Acetic acid, which provide the proper ion concentration for the buffer.

polyacrylamide

The polyacrylamide gel electrophoresis (PAGE) technique was introduced by Raymond and Weintraub (1959).

Polyacrylamide is the same material that is used for skin electrodes and in soft contact lenses.

provide a wide variety of electrophoretic conditions:

By controlling the percentage (from 3% to 30%), precise pore sizes can be obtained, usually from 5 to 2,000 kdal. Polyacrylamide gels can be cast in a single percentage or with varying gradients Polyacrylamide gels offer greater flexibility and more sharply defined banding than agarose gels.

Spectrophotometer

Absorbance
Transmission
Colormetric
400 to 700 nm
Pertaining to measurement of concentration of a solution based on its absorption or transmission of light, or on the intensity of color in a liquid.

Centrifugation

- Speed
- Distance from Center of rotation
- RCF= relative Centrifugal Force

factors

```
o Higher RCF the faster the sedimentation
o viscosity
o size of particle
o difference b/w particle and medium
```

Application

o Seperation
o + - Safety
• Never exceed Max speed of rotor
• Never use a cracked tube or bottle
o + - Maintenance
• Clean with mild detergent
• air dry rotor
• Check rotor O-rings

Types

+ - low speed

• rpm<10k
• g<8000

+ - High Speed

• rpm<30k
• g<100k
• refrigeration used

+ - ultracentrifuge

• rpm<120k
• g<700k
• refrigeration used
• vacuum

+ - microfuge

• tabletop
• rpm<15k
• g<21k
• 1-2ml volumes

parts

+ + - rotors
# horizontal
# + - fixed angle
• b/w 15 & 40 degrees
vertical

tubes / bottles

glass
stainless steel
polycarbonate
+ - Teflon

```
•       expensive
# + - polypropylene
•       What most people use
```

Aseptic techniques

Aseptic techniques is defined as a method that keeps undesirable microbes from contaminating a pure culture.

Only a single species of an organism is what one should work with in a microorganismal laboratory all materials that will be used for transfer, growth, and experimentation of the microbe must be sterilized media and glassware most often is sterilized by using an autoclave.

Lab bench needs to be clean with a disinfectant before and after working.

Hands should be washed upon entering and leaving the laboratory.

Inoculation tools and the tops of tubes need to be sterilized over an flame

Inoculating Loop - held like a pencil

fermentor design & application

Employee traits

Computer Skills

1. Popular OS’es and file management
2. Excel
3. PowerPoint
4. Word
5. Linux

People Skills

Energy and interest

WHAT CAN I DO FOR YOU?

How you are qualified

Desire to continue learning

Brainpower:

In today's world, it's "be sharp or die."

Most people use only 5% of their potential cognitive brainpower.

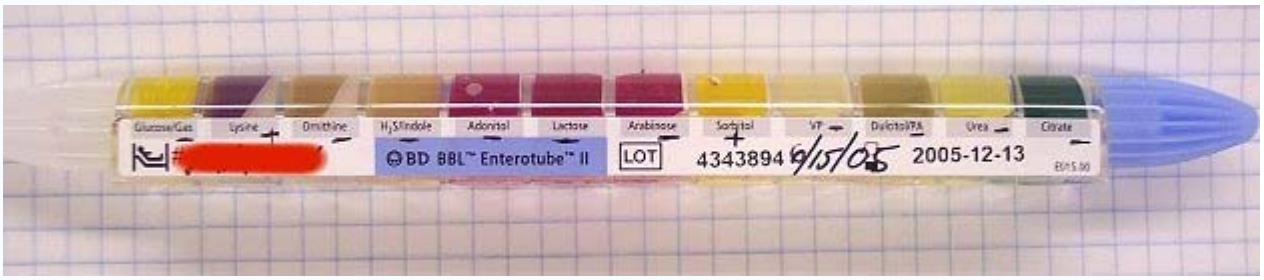
Because we are status quo creatures. We like things just the way they are, thank you. Change unsettles us. If it works (or appears to work, actually), don't fix it.

Ongoing education / cross pollination of other areas:

Basic Microbiology

Basic Microbiology

Cell Identification



cell growth

Log Phase

cell maintenance & storage

Cryopreservation

The protection of biological molecules during freezing and freeze-drying(lyophilization) is a subject of considerable practical importance, particularly in the pharmaceutical industry.

```
wide variety of compounds as cryoprotectants
```

Saccharides are often used in this capacity

They have been found to protect proteins during freezing and drying stresses.

They have also been shown to prevent damage to cells during freezing and drying.

Basic Molecular Biology

Basic Molecular Biology

Organism Design

Genetic Analysis

Strain Validation Applications

Genetic engineering

“Geneticist and science writer Steve Jones argues that humanity does not, and will never have the technology that proponents of transhumanism seek. He once joked that the letters of the genetic code, A, C, G and T should be replaced with the letters H, Y, P and E. Jones claims that technologies like genetic engineering will never be as powerful as is popularly believed.”

E Coli The workhorse of molecular biology

Theodor Escherich isolates a microbe from the colon that is later given the name Escherichia coli in his honor.

1. Ecoli
    1. HB101
        1. Fairly safe
        2. Needs
        3. 37 C
        4. Food
        5. H2O
        6. Vitamins

Escherichia coli, a subgroup of fecal coliform bacteria that is present in the intestinal tracts and feces of warm-blooded animals.

It is used as an indicator of the potential presence of pathogens. There are many different strains of E. coli that are classified into more than 170 serogroups.

Although most strains of E. coli are harmless and live in the intestines of healthy humans and animals, the E. coli O157:H7 strain produces a powerful toxin and can cause severe illness.

Its presence in groundwater is a common indicator of fecal contamination.

(“Enteric” is the adjective that describes organisms that live in the intestines. “Fecal” is the adjective for organisms that live in feces, so it is often a synonym for “enteric.”) The name comes from its discoverer, Theodor Escherich.

Basic Protein Separation

Basic Protein Separation

1. Protein Separation
2. Protein Analysis

Basic Tissue Culture

Basic Tissue Culture

1. Cultures
2. Cell line
3. Culture methods
4. Animal handling

Basic Chromatography

Basic Chromatography

HPLC Systems High Performance Liquid Chromatography (HPLC):

HPLC is a popular method of analysis because it is easy to learn and use and is not limited by the volatility or stability of the sample compound

Modern HPLC has many applications including separation, identification, purification, and quantification of various compounds

GC-MS

Biotech HOT SPOTS in the US

1. RTP - Research Triangle Park, created in 1959, located between Duke University in Durham, North Carolina, the State University in Raleigh, and the University of North Carolina at Chapel Hill.
2. Boston
3. Albany
4. South San Francisco

Acronyms / definitions

Acronyms / definitions

GLP

GLP Good Laboratory Practices

GMP

Biocidol

```
Kills cells
Disinfectant
```

Biostat

```
Chemical that stops cell growth - doesnt kill
```

Supernatants

Liquid removed from a tank once the solids have settled. Usually a clear liquid left after material (like cells) has been precipitated or centrifuged.

The material remaining above the pellet after centrifugation of a suspension.

Biotechnology

What is Biotech?

“Defining “biotechnology””

1. The use of living things to make products.
2. The application of a biological process to produce a useful product.
3. Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific uses.



The use of microorganisms (such as bacteria or yeasts) or biological substances (such as enzymes) to perform specific industrial or manufacturing processes. Applications include the production of certain drugs, synthetic hormones, and bulk foodstuffs, as well as the bioconversion of organic waste and the cleanup of oil spills.

The application of the principles of engineering and technology to the life sciences—bioengineering.

Cloning, genetic manipulation, cell fusion, and mutation.

Essentially, doing “more and faster” what we have known and done for centuries. It is almost as old as agriculture iself.

Farmers and bakers are the biotechs of days gone by. Remember Grandma’s freshly baked bread? How Grandpa kept the seeds of those really big pepper or tomatoes? Your grandparents were practicing biotechnology.

Modifying the genetic material of organisms directly and precisely. It enables the transfer of genes between diverse organisms, allowing combinations unlikely to occur by conventional means. Allowing speedier and more specific results.

Life- Define:

Moving
Growing
Eating
Breeding
Response to stimuli

Products

Products

Traditional

1. Beer / Wine (See Fermentation)
2. Yeast
3. Ethanol
4. Cheese

Protein Therapeutics

1. Herceptin
2. Neupogen
3. Insulin - 1982 U.S. pharmaceutical manufacturer Eli Lilly the first to market genetically-engineered human insulin

Agriculture

1. Bacillus thuringiensis (Bt) Crops Bt crops
2. Herbicide tolerance
3. Disease resistance

Industrial / Environmental

1. Hazardous Waste
2. Wastewater Treatment
3. Bioremediation

Other

1. Plastics
2. Biopulping
3. GMO (Genetically modified organism)

21 CFR 58

Good laboratory practice for nonclinical laboratory studies:

21 CFR 11

Title 21 Code of Federal Regulations (21 CFR Part 11) Electronic Records; Electronic Signatures

21 Code of Federal Regulations Parts 210 and 211

Part 210 - current good manufacturing practice in manufacturing, processing, packing, or holding of drugs; general

Part 211 - current good manufacturing practice for finished pharmaceuticals

SOPs

SOP’s (Standard Operating Procedures)

1. Approved by management
2. You don’t put data in a SOP
3. Written at 6^{th} grade level
4. Clear, current, complete
5. Simple, Concise, Accurate
6. Archived Where?

Notebooks

Notebook

1. If it is not written, it is not done.
2. pages signed
3. Can you read it?
4. Black or blue ink
5. pages numbered
6. Leave some pages in the front for TOC
7. Required by GLP / GMP regs
8. Signed by witness
9. Tracability

Documentation for Integrity and traceablity

1. What you did
2. Why you did it
3. Results
4. Reference earlier work?
5. Also for Quality

Keys to Successful Biotech products

Keys to Successful Biotech products

1. Availability of labor force
2. Skilled labor force
3. Power
4. Good Universities nearby:

- For retraining
- For new ideas

1. National and international labor pool
2. Raw materials
3. Marketing

- Clear trend and need

1. FDA approval

Record Keeping

```
Inventory control logs
Process Steps
SOP
ect
If it’s not written it’s not done
Love it
Live it
```

- Clinical trails
- GMP apply to:

```
•       Manufacture
•       Processing
•       Packing
•       Storage
•       Validation of each process
•       PAT (Process Anylitical Technology)
Quality should be built in and by desighn
```

1. Evironmental Concerns

```
•       Use of energy
•       Use of water
•       Evironmental Laws
•       Wastestreams
```

1. Do we have the technology?

requirements

```
Safety
Identity and Strength
Quality and purity



The plant:
Can we Measure
•       pH
•       Aeration
•       Baffiling
•       impellers
•       medium
•       Seed train
```

1. Are we Better than our competition?

```
why?
```

processes

Development / Upstream / Downstream processes

Development

1. Need / market analysis
2. Select a solution
3. Proccess Development
4. Understand the growth requirements
5. Calculate yields of biomass
6. Run small scale test
    - Fermentation

Chemical

Yeast

```
High biomass yields
Will secrete the protein
Picha Pastoris
```

Fungi

Mammalian Cells

Upstream

1. Media prep

Fermentation?

Find best conditions

Expensive Labor intensive Open Ended Time Consuming

Constraints

Raw Materials Batch to Batch variations Transportation costs Storage

1. Seed Prep

Find cell line

Composition Growth kenetics Yield Seed Bank

Master Seed Bank MSB

Original Stored Cells

Working Seed Bank WSB

Used in actual fermentation

Downstream

1. Slurry Handling
2. Excreted product
3. Contamination
4. Pruduct purification
5. Seperation / purification / sterilization
   How?

1. Plate and frame
2. Bulk filtration
3. Spiral wound

```
Plugs up easily
Usually not used for bulk filtration
```

1. Storage

Agriculture

Agriculture (a term which encompasses **farming**) is the process of producing food, feed, fiber and other goods by the systematic raising of plants and animals.

Agri is from Latin *ager*, meaning “a field”, and *culture* is from Latin *cultura*, meaning “cultivation” in the strict sense of *tillage of the soil*. A literal reading of the English word yields: *tillage of the soil of a field*. In modern usage, the word *Agriculture* covers all activities essential to food/feed/fiber production, including all techniques for raising and processing livestock. Agriculture is also short for the study of the practice of agriculture—more formally known as agricultural science. The history of agriculture is closely linked to human history, and agricultural developments have been crucial factors in social change, including the specialization of human activity.

42% of the world’s laborers are employed in agriculture, making it by far the most common occupation. However, agricultural production accounts for less than 5% of the Gross World Product (an aggregate of all Gross Domestic Products).

A farmer in Germany working the land with horse and plough.

Overview

Tea plantation in Java, Indonesia.

Farming with a tractor in Sweden.

"Oh Farmers, Pray That Your Summers Be Wet And Your Winters Clear." - Virgil

Farming refers to a wide range agricultural production work, covering a large spectrum of operation scales (acerage, output, etc), practices, and commercial inclination. At one end of this spectrum, the subsistence farmer farms a small area with limited resource inputs, and produces only enough food to meet the needs of his/her family.

At the other end of the spectrum is commercial intensive agriculture, including industrial agriculture. Such farming involves large fields and/or numbers of animals, large resource inputs (pesticides, and fertilizers, etc.), and a high level of mechanization. These operations generally attempt to maximize financial income from produce or livestock.

Modern agriculture extends well beyond the traditional production of food for humans and animal feeds. Other agricultural production goods include cut flowers, ornamental and nursery plants, timber, fertilizers, animal hides, leather, industrial chemicals (starch, sugar, ethanol, alcohols and plastics), fibers (cotton, wool, hemp, and flax), fuels (methane from biomass, biodiesel) and both legal and illegal drugs (biopharmaceuticals, tobacco, marijuana, opium, cocaine).

The 20th Century saw massive changes in agricultural practice, particularly in agricultural chemistry. Agricultural chemistry includes the application of chemical fertilizer, chemical insecticides (see Pest control), and chemical fungicides, soil makeup, analysis of agricultural products, and nutritional needs of farm animals. Beginning in the Western world, the green revolution spread many of these changes to farms throughout the world, with varying success.

Other recent changes in agriculture include hydroponics, plant breeding, hybridization, gene manipulation, better management of soil nutrients, and improved weed control. Genetic engineering has yielded crops which have capabilities beyond those of naturally occurring plants, such as higher yields and disease resistance. Modified seeds germinate faster, and thus can be grown in an extended growing area. Genetic engineering of plants has proven controversial, particularly in the case of herbicide-resistant plants.

Engineers may develop plans for irrigation, drainage, conservation and sanitary engineering, particularly important in normally arid areas which rely upon constant irrigation, and on large scale farms.

The packing, processing, and marketing of agricultural products are closely related activities also influenced by science. Methods of quick-freezing and dehydration have increased the markets for farm products (see Food preservation; Meat packing industry).

Animals, including horses, mules, oxen, camels, llamas, alpacas, and dogs, are often used to cultivate fields, harvest crops and transport farm products to markets. Animal husbandry means breeding and raising animals for meat or to harvest animal products (like milk, eggs, or wool) on a continual basis. Mechanization has enormously increased farm efficiency and productivity in Western agriculture (see Agricultural machinery).

Airplanes, helicopters, trucks and tractors are used in Western agriculture for seeding, spraying operations for insect and disease control, aerial topdressing and transporting perishable products. Radio and television disseminate vital weather reports and other information such as market reports that concern farmers. Computers have become an essential tool for farm management.

Farming, ploughing rice paddy, in Indonesia.

According to the National Academy of Engineering in the US, agricultural mechanization is one of the 20 greatest engineering achievements of the 20th century. Early in the century, it took one American farmer to produce food for 2.5 people. Today, due to advances in agricultural technology, a single farmer can feed over 130 people. This comes at a cost, however. A large energy input, often from fossil fuel, are required to maintain such high levels of output.

In recent years, some aspects of intensive industrial agriculture have been the subject of increasing discussion. The widening sphere of influence held by large seed and chemical companies, meat packers and food processors has been a source of concern both within the farming community and for the general public. Another issue is the type of feed given

to some animals that can cause Bovine Spongiform Encephalopathy in cattle. There has also been concern because of the disastrous effect that intensive agriculture has on the environment. In the US, for example, fertilizer has been running off into the Mississippi for years and has caused a dead spot in the Gulf of Mexico, where the Mississippi empties. Intensive agriculture also depletes the fertility of the land over time, potentially leading to Desertification.

A Field

The patent protection given to companies that develop new types of seed using genetic engineering has allowed seed to be licensed to farmers in much the same way that computer software is licensed to users. This has changed the balance of power in favor of the seed companies, allowing them to dictate terms and conditions previously unheard of. The Indian activist and scientiest Vandana Shiva argues that these companies are guilty of biopiracy.

Soil conservation and nutrient management have been important concerns since the 1950s, with the best farmers taking a stewardship role with the land they operate. However, increasing contamination of waterways and wetlands by nutrients like nitrogen and phosphorus are of concern in many countries.

Increasing consumer awareness of agricultural issues has led to the rise of community-supported agriculture, local food movement, Slow Food, and commercial organic farming.

History

History of agriculture

Ancient Egyptian farmer

Ancient Origins

Developed independently by geographically distant populations, evidence suggests that agriculture first appeared in Southwest Asia, in the Fertile Crescent area of Mesopotamia. Around 9,500 B.C., farmers first began to select and cultivate food plants with specific characteristics. Though there is evidence of earlier use of wild cereals, it wasn't until after 9,500 B.C. that the eight so-called founder crops of agriculture appear: first emmer and einkorn wheat, then hulled barley, peas, lentils, bitter vetch, chick peas and flax.

By 7000 B.C., sowing and harvesting reached Egypt. By 6000 B.C., farming was entrenched on the banks of the Nile River. About this time, agriculture was developed independently in the Far East, with rice, rather than wheat, the primary crop. By 5000 B.C., Sumerians had developed core agricultural techniques including large scale intensive cultivation of land, mono-cropping, organized irrigation, and use of a specialized labour force.

Evidence suggests that Maize was first domesticated in the Americas around 3000-2700 B.C. The potato, the tomato, the pepper, squash, several varieties of bean, and several other plants were also developed in the New World, as was extensive terracing of steep hillsides in much of Andean South America.

Roman agriculture built on techniques pioneered by the Sumerians, with a specific emphasis on the cultivation of crops for trade and export.

Sumerian Harvester's sickle, 3000 BCE. Baked clay. Field Museum.

Agriculture in the Middle Ages

During the Middle Ages, Muslim farmers in North Africa and the Near East developed and disseminated agricultural technologies including irrigation systems based on hydraulic and hydrostatic principles, the use of machines such as norias, and the use of water raising machines, dams, and reservoirs. Muslims also wrote location-specific Farming manuals, and were instrumental in the wider adoption of crops including sugar cane, rice, citrus fruit, apricots, cotton, artichokes, aubergines, and saffron. Muslims also brought lemons, oranges, cotton, almonds, figs and sub-tropical crops such as bananas to Spain.

Renaissance to Present Day

A tractor ploughing an alfalfa field

The invention of a three field system of crop rotation during the Middle Ages, and the importation of the Chinese-invented moldboard plow, vastly improved agricultural efficiency.

After 1492, a global exchange of previously local crops and livestock breeds occurred. Key crops involved in this exchange included the tomato, maize, potato, cocoa, tobacco, and coffee.

By the early 1800s, agricultural practices, particularly careful selection of hardy strains and cultivars, had so improved that yield per land unit was many times that seen in the Middle Ages. With the rapid rise of mechanization in the late 19th and 20th centuries, particularly in the form of the tractor, farming tasks could be done with a speed and on a scale previously impossible. These advances have led to efficiencies enabling certain modern farms in the United States, Argentina, Israel, Germany and a few other nations to output volumes of high quality produce per land unit at what may be the practical limit.

Crops

World production of major crops in 2004

Specific crops are cultivated in distinct growing regions throughout the world. In millions of metric tons, based on FAO estimates.

Meat	259
Oilcrops	133
Fish (2001 estimate)	130
Eggs	63
Pulses	60
Vegetable Fiber	30
Source:	

Food and Agriculture Organization (FA

Top agricultural products, by individual crops (million metric tons) 2004 data	
Sugar Cane	1,324
Maize	721
Wheat	627
Rice	605
Potatoes	328
Sugar Beet	249
Soybean	204
Oil Palm Fruit	162
Barley	154
Tomato	120
Source: *Food and Agriculture Organization (FAO)*	

Crop improvement

Plant breeding

Tractor and Chaser Bin

An agricultural scientist records corn growth

Netting protecting wine grapes from birds

Domestication of plants is done in order to increase yield, improve disease resistance and drought tolerance, ease harvest and to improve the taste and nutritional value and many other characteristics. Centuries of careful selection and breeding have had enormous effects on the characteristics of crop plants. Plant breeders use greenhouses and other techniques to get as many as three generations of plants per year so that they can make improvements all the more quickly.

Plant selection and breeding in the 1920s and '30s improved pasture (grasses and clover) in New Zealand. Extensive radiation mutagenesis efforts (i.e. primitive genetic engineering) during the 1950s produced the modern commercial varieties of grains such as wheat, corn and barley.

For example, average yields of corn (maize) in the USA have increased from around 2.5 tons per hectare (40 bushels per acre) in 1900 to about 9.4 t/ha (150 bushels per acre) in 2001. Similarly, worldwide average wheat yields have increased from less than 1 t/ha in 1900 to more than 2.5 t/ha in 1990. South American average wheat yields are around 2 t/ha, African under 1 t/ha, Egypt and Arabia up to 3.5 to 4 t/ha with irrigation. In contrast, the average wheat yield in countries such as France is over 8 t/ha. Variation in yields are due mainly to variation in climate, genetics, and the use or non-use of intensive farming techniques (use of fertilizers, chemical pest control, growth control to avoid lodging). [Conversion note: 1 bushel of wheat = 60 pounds (lb) ≈ 27.215 kg. 1 bushel of corn = 56 pounds ≈ 25.401 kg]

In industrialized agriculture, crop "improvement" has often reduced nutritional and other qualities of food plants to serve the interests of producers. After mechanical tomato-harvesters were developed in the early 1960s, agricultural scientists bred tomatoes that were harder and less nutritious (Friedland and Barton 1975). In fact, a major longitudinal study of nutrient levels in numerous vegetables showed significant declines in the last 50 years; garden vegetables in the U.S. today contain on average 38 percent less vitamin B2 and 15 percent less vitamin C (Davis and Riordan 2004).

Very recently, genetic engineering has begun to be employed in some parts of the world to speed up the selection and breeding process. The most widely used modification is a herbicide resistance gene that allows plants to tolerate exposure to glyphosate, which is used to control weeds in the crop. A less frequently used but more controversial

modification causes the plant to produce a toxin to reduce damage from insects (c.f. Starlink).

There are specialty producers who raise less common types of livestock or plants. Aquaculture, the farming of fish, shrimp, and algae, is closely associated with agriculture. Apiculture, the culture of bees, traditionally for honey—increasingly for crop pollination.

: botany, List of domesticated plants, List of vegetables, List of herbs, List of fruit

Environmental problems

Severe soil erosion in a wheat field near Washington State University, US (c.2005)

Agriculture may often cause environmental problems because it changes natural environments and produces harmful by-products. Some of the negative effects are:

- Surplus of nitrogen and phosphorus in rivers and lakes
- Detrimental effects of herbicides, fungicides, insecticides, and other biocides
- Conversion of natural ecosystems of all types into arable land
- Consolidation of diverse biomass into a few species
- Soil erosion
- Depletion of minerals in the soil
- Particulate matter, including ammonia and ammonium off-gasing from animal waste contributing to air pollution
- Weeds - feral plants and animals
- Odor from agricultural waste
- Soil salination

Agriculture is cited as a significant adverse impact to biodiversity in many nations' Biodiversity Action Plans, due to reduction of forests and other habitats when new lands are converted to farming. Some critics also include agriculture as a cause of current global climate change.

Policy

Agricultural policy focuses on the goals and methods of agricultural production. At the policy level, common goals of agriculture include:

- Food safety: Ensuring that the food supply is free of contamination.
- Food security: Ensuring that the food supply meets the population's needs.
- Food quality: Ensuring that the food supply is of a consistent and known quality.
- Conservation
- Environmental impact
- Economic stability

Food science

Food science is a discipline concerned with all technical aspects of food, beginning with harvesting or slaughtering, and ending with its cooking and consumption. It is considered one of the agricultural sciences, and is usually considered distinct from the field of nutrition.

Examples of the activities of food scientists include the development of new food products, design of processes to produce these foods, choice of packaging materials, shelf-life studies, sensory evaluation of the product with trained expert panels or potential consumers, as well as microbiological and chemical testing. Food scientists at universities may study more fundamental phenomena that are directly linked to the production of particular food product and its properties. In the U.S., food science is typically studied at land-grant universities.

Food science is a highly interdisciplinary applied science. It incorporates concepts from many different fields including microbiology, chemical engineering, biochemistry, and many others.

Some of the subdisciplines of food science include:

- Food safety or Food microbiology - the causes and prevention of foodborne illness
- Food preservation - the causes and prevention of quality degradation
- Food engineering - the industrial processes used to manufacture food
- Product development - the invention of new food products
- Sensory analysis - the study of how food is perceived by the consumer's senses

- Food chemistry - the molecular composition of food and the involvement of these molecules in chemical reactions
- Food packaging - the study of how food is packaged to preserve the food after it has been processed.
- molecular gastronomy — the application of science to culinary practice and more generally gastronomical phenomena

The main organization in the United States regarding food science and food technology is the Institute of Food Technologists (IFT), headquarted in Chicago, Illinois.

In the October 2006 issue of *Food Technology*, IFT President Dennis R. Heldman noted that the IFT Committee on Higher Education stated that the current definition the “Food Science is the discipline in which the engineering, biological, and physical sciences are used to study the nature of foods, the causes of deterioration, the principles underlying food processing, and the improvement of foods for the consuming public.”

Medicine

This article is about the field and science of medical practice and health care.
For the substances known as medicines, see medication and pharmacology.

Medicine is a branch of health science and the sector of public life concerned with maintaining or restoring human health through the study, diagnosis, treatment and possible prevention of disease and injury. It is both an *area of knowledge* – a science of body systems, their diseases and treatment – and the *applied practice* of that knowledge.

The caduceus is a common symbol for medicine.

Overview

Physician examining a child

Western medical care is shared between *medical professionals* (physicians) and other professionals such as physician assistants, nurses and pharmacists, sometimes known as allied health professionals. Historically, only those with a medical doctorate have been considered to *practice* medicine. *Clinicians* (licensed professionals who deal with patients) can be physicians, nurses, therapists or others. The medical profession is the social and occupational structure of the group of people formally trained and authorized to apply medical knowledge. Many countries and legal jurisdictions have legal limitations on who may practice medicine.

Medicine comprises various specialized sub-branches, such as cardiology, pulmonology, neurology, or other fields such as sports medicine, research or public health.

Human societies have had various different systems of health care practice since at least the beginning of recorded history. Medicine, in the modern period, is the mainstream scientific tradition which developed in the Western world since the early Renaissance (around 1450). Many other traditions of health care are still practiced throughout the world; most of these are separate from Western medicine, which is also called **biomedicine**, **allopathic medicine** or the Hippocratic tradition. The most highly developed of these are traditional Chinese medicine, Tibetan medicine and the Ayurvedic traditions of India and Sri Lanka. Various non-mainstream traditions of health care have also developed in the Western world. These systems are sometimes considered companions to Hippocratic medicine, and sometimes are seen as competition to the Western tradition. Few of them have any scientific confirmation of their tenets, because if they did they would be brought into the fold of Western medicine.

“Medicine” is also often used amongst medical professionals as shorthand for internal medicine. Veterinary medicine is the practice of health care in animal species other than human beings.

History of medicine

Physician treating a patient. Louvre Museum, Paris, France.

The earliest type of medicine in most cultures was the use of plants (Herbalism) and animal parts. This was usually in concert with ‘magic’ of various kinds in which: animism (the notion of inanimate objects having spirits); spiritualism (here meaning an appeal to gods or communion with ancestor spirits); shamanism (the vesting of an individual with mystic powers); and divination (the supposed obtaining of truth by magic means), played a major role.

The practice of medicine developed gradually, and separately, in ancient Egypt, India, China, Greece, Persia and elsewhere. Medicine as it is practiced now developed largely in the late eighteenth century and early nineteenth century in England (William Harvey, seventeenth century), Germany (Rudolf Virchow) and France (Jean-Martin Charcot, Claude Bernard and others). The new, “scientific” medicine (where results are testable and repeatable) replaced early Western traditions of medicine, based on herbalism, the Greek “four humours” and other pre-modern theories. The focal points of development of clinical medicine shifted to the United Kingdom and the USA by the early 1900s

(Canadian-born)Sir William Osler, Harvey Cushing). Possibly the major shift in medical thinking was the gradual rejection in the 1400's of what may be called the 'traditional authority' approach to science and medicine. This was the notion that because some prominent person in the past said something must be so, then that was the way it was, and anything one observed to the contrary was an anomaly (which was paralleled by a similar shift in European society in general - see Copernicus's rejection of Ptolemy's theories on astronomy). People like Vesalius led the way in improving upon or indeed rejecting the theories of great authorities from the past such as Galen, Hippocrates, and Avicenna/Ibn Sina, all of whose theories were in time almost totally discredited. Such new attitudes were also only made possible by the weakening of the Roman Catholic church's power in society, especially in the Republic of Venice.

Evidence-based medicine is a recent movement to establish the most effective algorithms of practice (ways of doing things) through the use of the scientific method and modern global information science by collating all the evidence and developing standard protocols which are then disseminated to healthcare providers. One problem with this 'best practice' approach is that it could be seen to stifle novel approaches to treatment.

Genomics and knowledge of human genetics is already having some influence on medicine, as the causative genes of most monogenic genetic disorders have now been identified, and the development of techniques in molecular biology and genetics are influencing medical practice and decision-making.

Pharmacology has developed from herbalism and many drugs are still derived from plants (atropine, ephedrine, warfarin, aspirin, digoxin, vinca alkaloids, taxol, hyoscine, etc). The modern era really began with Koch's discoveries around 1880 of the transmission of disease by bacteria, and then the discovery of antibiotics shortly thereafter around 1900. The first major class of antibiotics was the sulfa drugs, derived originally from azo dyes. Throughout the twentieth century, major advances in the treatment of infectious diseases were observable in (Western) societies. The medical establishment is now developing drugs that are targeted towards one particular disease process. Thus drugs are being developed to minimise the side effects of prescribed drugs, to treat cancer, geriatric problems, long-term problems (such as high cholesterol), chronic diseases type 2 diabetes, lifestyle and degenerative diseases such as arthritis and Alzheimer's disease.

Practice of medicine

The practice of medicine combines both science as the evidence base and art in the application of this medical knowledge in combination with intuition and clinical judgement to determine the treatment plan for each patient.

Central to medicine is the patient-physician relationship established when a person with a health concern seeks a physician's help; the 'medical encounter'. Other health professionals similarly establish a relationship with a patient and may perform various interventions, e.g. nurses, radiographers and therapists.

As part of the medical encounter, the healthcare provider needs to:

- develop a relationship with the patient
- gather data (medical history, systems enquiry, and physical examination, combined with laboratory or imaging studies (investigations))
- analyze and synthesize that data (assessment and/or differential diagnoses), and then:
- develop a treatment plan (further testing, therapy, watchful observation, referral and follow-up)
- treat the patient accordingly
- assess the progress of treatment and alter the plan as necessary (management).

The medical encounter is documented in a medical record, which is a legal document in many jurisdictions.

Health care delivery systems

Medicine is practiced within the medical system, which is a legal, credentialing and financing framework, established by a particular culture or government. The characteristics of a health care system have significant effect on the way medical care is delivered.

Financing has a great influence as it defines who pays the costs. Aside from tribal cultures, the most significant divide in developed countries is between universal health care and *market-based health care* (such as practiced in the U.S.). Universal health care might allow or ban a parallel private market. The latter is described as single-payor system.

Transparency of information is another factor defining a delivery system. Access to information on conditions, treatments, quality and pricing greatly affects the choice by patients / consumers and therefore the incentives of medical professionals. While US health care system has come under fire for lack of openness, new legislation may encourage greater openness. There is a perceived tension between the need for transparency on the one hand and such issues as patient confidentiality and the possible exploitation of information for commercial gain on the other.

Health care delivery

See also: clinic, hospital, and hospice

Paint of Henriette Browne

Medical care delivery is classified into primary, secondary and tertiary care.

Primary care medical services are provided by physicians or other health professionals who has first contact with a patient seeking medical treatment or care. These occur in physician's office, clinics, nursing homes, schools, home visits and other places close to patients. About 90% of medical visits can be treated by the primary care provider. These include treatment of acute and chronic illnesses, preventive care and health education for all ages and both sexes.

Secondary care medical services are provided by medical specialists in their offices or clinics or at local community hospitals for a patient referred by a primary care provider who first diagnosed or treated the patient. Referrals are made for those patients who required the expertise or procedures performed by specialists. These include both ambulatory care and inpatient services, emergency rooms, intensive care medicine, surgery services, physical therapy, labor and delivery, endoscopy units, diagnostic laboratory and medical imaging services, hospice centers, etc. Some primary care providers may also take care of hospitalized patients and deliver babies in a secondary care setting.

Tertiary care medical services are provided by specialist hospitals or regional centers equipped with diagnostic and treatment facilities not generally available at local hospitals. These include trauma centers, burn treatment centers, advanced neonatology unit services, organ transplants, high-risk pregnancy, radiation oncology, etc.

Modern medical care also depends on information - still delivered in many health care settings on paper records, but increasingly nowadays by electronic means.

Physician-patient relationship

The physician-patient relationship and interaction is a central process in the practice of medicine. There are many perspectives from which to understand and describe it.

An idealized physician's perspective, such as is taught in medical school, sees the core aspects of the process as the physician learning the patient's symptoms, concerns and values; in response the physician examines the patient, interprets the symptoms, and formulates a diagnosis to explain the symptoms and their cause to the patient and to propose a treatment. The job of a physician is similar to a human biologist: that is, to know the human frame and situation in terms of normality. Once the physician knows what is normal and can measure the patient against those norms, he or she can then determine the particular departure from the normal and the degree of departure. This is called the diagnosis.

The four great cornerstones of diagnostic medicine are anatomy (structure: what is there), physiology (how the structure/s work), pathology (what goes wrong with the anatomy and physiology) and psychology (mind and behaviour). In addition, the physician should consider the patient in their 'well' context rather than simply as a walking medical condition. This means the socio-political context of the patient (family, work, stress, beliefs) should be assessed as it often offers vital clues to the patient's condition and

further management. In more detail, the patient presents a set of complaints (the symptoms) to the physician, who then obtains further information about the patient's symptoms, previous state of health, living conditions, and so forth. The physician then makes a *review of systems* (ROS) or *systems enquiry*, which is a set of ordered questions about each major body system in order: general (such as weight loss), endocrine, cardio-respiratory, etc. Next comes the actual physical examination; the findings are recorded, leading to a list of possible diagnoses. These will be in order of probability. The next task is to enlist the patient's agreement to a management plan, which will include treatment as well as plans for follow-up. Importantly, during this process the healthcare provider educates the patient about the causes, progression, outcomes, and possible treatments of his ailments, as well as often providing advice for maintaining health. This teaching relationship is the basis of calling the physician *doctor*, which originally meant "teacher" in Latin. The patient-physician relationship is additionally complicated by the patient's suffering (*patient* derives from the Latin *patior*, "suffer") and limited ability to relieve it on his/her own. The physician's expertise comes from his knowledge of what is healthy and normal contrasted with knowledge and experience of other people who have suffered similar symptoms (unhealthy and abnormal), and the proven ability to relieve it with medicines (pharmacology) or other therapies about which the patient may initially have little knowledge, although the latter may be better performed by a pharmacist.

The physician-patient relationship can be analyzed from the perspective of ethical concerns, in terms of how well the goals of non-maleficence, beneficence, autonomy, and justice are achieved. Many other values and ethical issues can be added to these. In different societies, periods, and cultures, different values may be assigned different priorities. For example, in the last 30 years medical care in the Western World has increasingly emphasized patient autonomy in decision making.

The relationship and process can also be analyzed in terms of social power relationships (e.g., by Michel Foucault), or economic transactions. Physicians have been accorded gradually higher status and respect over the last century, and they have been entrusted with control of access to prescription medicines as a public health measure. This represents a concentration of power and carries both advantages and disadvantages to particular kinds of patients with particular kinds of conditions. A further twist has occurred in the last 25 years as costs of medical care have risen, and a third party (an insurance company or government agency) now often insists upon a share of decision-making power for a variety of reasons, reducing freedom of choice of healthcare providers and patients in many ways.

The quality of the patient-physician relationship is important to both parties. The better the relationship in terms of mutual respect, knowledge, trust, shared values and perspectives about disease and life, and time available, the better will be the amount and quality of information about the patient's disease transferred in both directions, enhancing accuracy of diagnosis and increasing the patient's knowledge about the disease. Where such a relationship is poor the physician's ability to make a full assessment is compromised and the patient is more likely to distrust the diagnosis and proposed treatment. In these circumstances and also in cases where there is genuine divergence of medical opinions, a *second opinion* from another physician may be sought.

In some settings, e.g. the hospital ward, the patient-physician relationship is much more complex, and many other people are involved when somebody is ill: relatives, neighbors, rescue specialists, nurses, technical personnel, social workers and others.

Clinical skills

Main articles: Medical history and Physical examination

A complete medical evaluation includes a medical history, a systems enquiry, a physical examination, appropriate laboratory or imaging studies, analysis of data and medical decision making to obtain diagnoses, and a treatment plan.

The components of the medical history are:

- Chief complaint (CC): the reason for the current medical visit. These are the 'symptoms.' They are in the patient's own words and are recorded along with the duration of each one. Also called 'presenting complaint.'
- History of present illness / complaint (HPI): the chronological order of events of symptoms and further clarification of each symptom.
- Current activity: occupation, hobbies, what the patient actually does.
- Medications: what drugs the patient takes including over-the-counter, and home remedies, as well as herbal medicines/herbal remedies such as St. John's Wort. Allergies are recorded.
- Past medical history (PMH/PMHx): concurrent medical problems, past hospitalizations and operations, injuries, past infectious diseases and/or vaccinations, history of known allergies.
- Social history (SH): birthplace, residences, marital history, social and economic status, habits (including diet, medications, tobacco, alcohol).
- Family history (FH): listing of diseases in the family that may impact the patient. A family tree is sometimes used.
- Review of systems (ROS) or *systems enquiry*: an set of additional questions to ask which may be missed on HPI, generally following the body's main organ systems (heart, lungs, digestive tract, urinary tract, etc).

The physical examination is the examination of the patient looking for signs of disease ('Symptoms' are what the patient volunteers, 'signs' are what the healthcare provider detects by examination). The healthcare provider uses the senses of sight, hearing, touch, and sometimes smell (taste has been made redundant by the availability of modern lab tests). Four chief methods are used: inspection, palpation (feel), percussion (tap to determine resonance characteristics), and auscultation (listen); smelling may be useful (e.g. infection, uremia, diabetic ketoacidosis). The clinical examination involves study of:

- Vital signs including height, weight, body temperature, blood pressure, pulse, respiration rate, hemoglobin oxygen saturation
- General appearance of the patient and specific indicators of disease (nutritional status, presence of jaundice, pallor or clubbing)
- Skin
- Head, eye, ear, nose, and throat (HEENT)
- Cardiovascular (heart and blood vessels)

- Respiratory (large airways and lungs)
- Abdomen and rectum
- Genitalia (and pregnancy if the patient is or could be pregnant)
- Musculoskeletal (spine and extremities)
- Neurological (consciousness, awareness, brain, cranial nerves, spinal cord and peripheral nerves)
- Psychiatric (orientation, mental state, evidence of abnormal perception or thought)

Laboratory and imaging studies results may be obtained, if necessary.

The medical decision-making (MDM) process involves analysis and synthesis of all the above data to come up with a list of possible diagnoses (the differential diagnoses), along with an idea of what needs to be done to obtain a definitive diagnosis that would explain the patient's problem.

The treatment plan may include ordering additional laboratory tests and studies, starting therapy, referral to a specialist, or watchful observation. Follow-up may be advised.

This process is used by primary care providers as well as specialists. It may take only a few minutes if the problem is simple and straightforward. On the other hand, it may take weeks in a patient who has been hospitalized with bizarre symptoms or multi-system problems, with involvement by several specialists.

On subsequent visits, the process may be repeated in an abbreviated manner to obtain any new history, symptoms, physical findings, and lab or imaging results or specialist consultations.

Branches of medicine

Working together as an interdisciplinary team, many highly trained health professionals besides medical practitioners are involved in the delivery of modern health care. Some examples include: nurses, laboratory scientists, pharmacists, physiotherapists, respiratory therapists, speech therapists, occupational therapists, dietitians and bioengineers.

The scope and sciences underpinning human medicine overlap many other fields. Dentistry and psychology, while separate disciplines from medicine, are considered medical fields.

Midlevel Practitioners

Nurse practitioners, midwives and physician assistants, treat patients and prescribe medication in many legal jurisdictions.

Veterinary Medicine

Veterinarians applies similar techniques as physicians to the care of animals.

Physicians have many specializations and subspecializations which are listed below. There are variations from country to country regarding which specialities certain subspecialities are in.

Diagnostic specialties

- *Clinical laboratory sciences* are the clinical diagnostic services which apply laboratory techniques to diagnosis and management of patients. In the United States these services are supervised by a pathologist. The personnel that work in these medical laboratory departments are technically trained staff, each of whom usually hold a medical technology degree, who actually perform the tests, assays, and procedures needed for providing the specific services.
- *Pathology* is the branch of medicine that deals with the study of diseases and the morphologic, physiologic changes produced by them. As a diagnostic specialty, pathology can be considered the basis of modern scientific medical knowledge and plays a large rôle in evidence-based medicine. Many modern molecular tests such as flow cytometry, polymerase chain reaction (PCR), immunohistochemistry, cytogenetics, gene rearragements studies and fluorescent in situ hybridization (FISH) fall within the territory of pathology.
- *Radiology* is concerned with imaging of the human body, e.g. by x-rays, x-ray computed tomography, ultrasonography, and nuclear magnetic resonance tomography.

Clinical disciplines

- *Anesthesiology* (AE) or *anaesthesia* (BE) is the clinical discipline concerned with providing anesthesia. Pain medicine is often practiced by specialised anesthesiologists.
- *Dermatology* is concerned with the skin and its diseases. In the UK, dermatology is a subspeciality of general medicine.
- *Emergency medicine* is concerned with the diagnosis and treatment of acute or life-threatening conditions, including trauma, surgical, medical, pediatric, and psychiatric emergencies.
- *General practice*, *family practice*, *family medicine* or *primary care* is, in many countries, the first port-of-call for patients with non-emergency medical problems. Family practitioners are usually able to treat over 90% of all complaints without referring to specialists.
- *Hospital medicine* is the general medical care of hospitalized patients. Physicians whose primary professional focus is hospital medicine are called hospitalists in the USA.
- *Internal medicine* is concerned with systemic diseases of adults, i.e. those diseases that affect the body as a whole (restrictive, current meaning), or with all adult non-operative somatic medicine (traditional, inclusive meaning), thus excluding pediatrics, surgery, gynecology and obstetrics, and psychiatry. There are several subdisciplines of internal medicine:
    - Cardiology

    - Endocrinology
    - Gastroenterology
    - Hematology
    - Infectious Diseases
    - Intensive care medicine
    - Nephrology
    - Oncology
    - Pulmonology
    - Rheumatology
- *Neurology* is concerned with the diagnosis and treatment of nervous system diseases. It is a subspeciality of general medicine in the UK.
- *Obstetrics and gynecology* (often abbreviated as *Ob/Gyn*) are concerned respectively with childbirth and the female reproductive and associated organs. Reproductive medicine and fertility medicine are generally practiced by gynecological specialists.
- *Palliative care* is a relatively modern branch of clinical medicine that deals with pain and symptom relief and emotional support in patients with terminal illnesses including cancer and heart failure.
- *Pediatrics* (AE) or *paediatrics* (BE) is devoted to the care of infants, children, and adolescents. Like internal medicine, there are many pediatric subspecialities for specific age ranges, organ systems, disease classes, and sites of care delivery. Most subspecialities of adult medicine have a pediatric equivalent such as pediatric cardiology, pediatric endocrinology, pediatric gastroenterology, pediatric hematology, pediatric oncology, pediatric ophthalmology, and neonatology.
- *Physical medicine and rehabilitation* (or *physiatry*) is concerned with functional improvement after injury, illness, or congenital disorders.
- *Preventive medicine* is the branch of medicine concerned with preventing disease.
- *Psychiatry* is the branch of medicine concerned with the bio-psycho-social study of the etiology, diagnosis, treatment and prevention of cognitive, perceptual, emotional and behavioral disorders. Related non-medical fields include psychotherapy and clinical psychology.
- *Radiation therapy* is concerned with the therapeutic use of ionizing radiation and high energy elementary particle beams in patient treatment.
- *Radiology* is concerned with the interpretation of imaging modalities including x-rays, ultrasound, radioisotopes, and MRI (Magnetic Resonance Imaging). A newer branch of radiology, interventional radiology, is concerned with using medical devices to access areas of the body with minimally invasive techniques.
- *Surgical specialties* employ operative treatment. These include Orthopedics, Urology, Ophthalmology, Neurosurgery, Plastic Surgery, Otolaryngology and various subspecialties such as transplant and cardiothoracic. Some disciplines are highly specialized and are often not considered subdisciplines of surgery, although their naming might suggest so.
- *Urgent care* focuses on delivery of unscheduled, walk-in care outside of the hospital emergency department for injuries and illnesses that are not severe enough to require care in an emergency department.

- *Gender-based medicine* studies the biological and physiological differences between the human sexes and how that affects differences in disease.

Interdisciplinary fields

Interdisciplinary sub-specialties of medicine are:

- *Aerospace medicine* deals with medical problems related to flying and space travel.
- *Bioethics* is a field of study which concerns the relationship between biology, science, medicine and ethics, philosophy and theology.
- *Biomedical Engineering* is a field dealing with the application of engineering principles to medical practice.
- *Clinical pharmacology* is concerned with how systems of therapeutics interact with patients.
- *Conservation medicine* studies the relationship between human and animal health, and environmental conditions. Also known as ecological medicine, environmental medicine, or medical geology.
- *Diving medicine* (or hyperbaric medicine) is the prevention and treatment of diving-related problems.
- *Evolutionary medicine* is a perspective on medicine derived through applying evolutionary theory.
- *Forensic medicine* deals with medical questions in legal context, such as determination of the time and cause of death.
- *Medical humanities* includes the humanities (literature, philosophy, ethics, history and religion), social science (anthropology, cultural studies, psychology, sociology), and the arts (literature, theater, film, and visual arts) and their application to medical education and practice.
- *eHealth*, *Medical informatics*, and *medical computer science* are relatively recent fields that deal with the application of computers and information technology to medicine.
- *Naturopathic medicine* is concerned with primary care, natural remedies, patient education and disease prevention.
- *Nosology* is the classification of diseases for various purposes.
- Pharmacogenomics is a form of individualized medicine.
- *PanVascular Medicine* is an approach to deal with the problems of highly specialised but both, medical and economical inefficiently arranged human resources and medical equipment in today's vascular care facilities
- *Sports medicine* deals with the treatment and preventive care of athletics, amateur and professional. The team includes specialty physicians and surgeons, athletic trainers, physical therapists, coaches, other personnel, and, of course, the athlete.
- *Therapeutics* is the field, more commonly referenced in earlier periods of history, of the various remedies that can be used to treat disease and promote health .
- *Travel medicine* or *emporiatrics* deals with health problems of international travelers or travelers across highly different environments.

Medical education

An image of a 1901 examination in the faculty of medicine.

Main articles: Medical education and Medical school

Medical education is education related to the practice of being a medical practitioner, either the initial training to become a physician or further training thereafter.

Medical education and training varies considerably across the world, however typically involves entry level education at a university medical school, followed by a period of supervised practice (Internship and/or Residency) and possibly postgraduate vocational training. Continuing medical education is a requirement of many regulatory authorities.

Various teaching methodologies have been utilised in medical education, which is an active area of educational research.

Legal restrictions

In most countries, it is a legal requirement for medical doctors to be licensed or registered. In general, this entails a medical degree from a university and accreditation by a medical board or an equivalent national organization, which may ask the applicant to pass exams. This restricts the considerable legal authority of the medical profession to physicians that are trained and qualified by national standards. It is also intended as an assurance to patients and as a safeguard against charlatans that practice inadequate medicine for personal gain. While the laws generally require medical doctors to be trained in “evidence based”, Western, or Hippocratic Medicine, they are not intended to discourage different paradigms of health and healing, such as alternative medicine or faith healing.

Recombinant DNA

Recombinant DNA (sometimes **rDNA**) is an artificial DNA sequence resulting from the combining of two other DNA sequences in a plasmid. A **recombinant protein** is a protein produced by an organism after the relevant DNA is inserted into its genome (that is, by a genetically modified organism). This *recombines* the DNA of two different organisms.

Recombinant DNA technique was discovered by Stanley Cohen and Herbert Boyer in 1973, Nov 1973 publication of “Construction of Biologically Functional Bacterial Plasmids in vitro”, this paper described a technique to isolate and amplify genes, or DNA segments, and insert them into another cell with precision. Recombinant DNA technology was made possible by the discovery of restriction endonucleases by Werner Arber, Daniel Nathans, and Hamilton Smith, for which they received the 1978 Nobel Prize in Medicine.

The term **recombinant DNA** refers to a new combination of DNA molecules that are not found together naturally. Although processes such as crossing over technically produce recombinant DNA, the term is generally reserved for DNA produced by joining molecules derived from different biological sources.

Uses

Recombinant DNA is used for genetic transformation to produce genetically modified organisms. Some examples of recombinant DNA products are peptide hormone medications including insulin, growth hormone, and oxytocin. Vaccines can also be produced using recombinant processes. The organism most commonly used is *Escherichia coli*.

Plasmids and recombinant DNA technology

Plasmids are extranuclear fragments of DNA present in some bacteria. A plasmid can transfer genetic material to another bacterium, allowing it to express the transmitted gene(s). Restriction enzymes which cut sequences of DNA at certain spots are used to splice into a plasmid the DNA sequence for the desired gene. The plasmid is then inserted into a bacterium in order to express the gene and produce the protein coded for by the gene. Large amounts of the protein can be produced in a factory with vats of the genetically engineered bacteria. Plasmid is extrachromosomal self replicating circular DNA. Most of the plasmid is used for the production of antibiotics. Most eukaryotes cannot use circular DNA such as that present in a plasmid. Yeasts are the only (known) exception. In the others, other systems, such as transfection with viruses, are used instead.

Tissue culture

Tissue culture refers to the growth of tissues and/or cells separate from the organism. In 1907 the American zoologist Ross Granville Harrison demonstrated the growth of frog nerve cell processes in a medium of clotted lymph. This term usually is used in the context of animal tissue culture, while the more specific term plant tissue culture is used for plants.

Modern Usage

> *cell culture*

In modern usage, “tissue culture” often refers to the growth of animal or plant cells *in vitro*. In particular, the term is often used interchangeably with **cell culture** to specifically describe the *in vitro* culturing of mammalian cells.

However, “tissue culture” can also be used to refer to the culturing of tissue pieces, i.e. explant culture or whole organs, i.e. organ culture.

Fermentation (biochemistry)

Fermentation in progress

Fermentation is a process of making energy in a cell with no oxygen present.

Typical examples of fermentation products are ethanol, lactic acid, and hydrogen. However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone.

Common Example

Fermentation can be simply defined, in this context, as the conversion of sugar molecules, into ethanol and carbon dioxide by yeast.

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$

History

French chemist Louis Pasteur was the first *zymologist*, when in 1857 he connected yeast to fermentation. Pasteur originally defined fermentation as *respiration without air*.

Pasteur performed careful research and concluded, *"I am of the opinion that alcoholic fermentation never occurs without simultaneous organization, development and multiplication of cells.... If asked, in what consists the chemical act whereby the sugar is decomposed ... I am completely ignorant of it."*.

The German Eduard Buchner, winner of the 1907 Nobel Prize in chemistry, later determined that fermentation was actually caused by a yeast secretion that he termed *zymase*.

The research efforts undertaken by the Danish Carlsberg scientists greatly accelerated the gain of knowledge about yeast and brewing. The Carlsberg scientists are generally acknowledged with jump-starting the entire field of molecular biology.

Reaction

The reaction differs according to the sugar being used in the process of anaerobic respiration, below, the sugar will be glucose ($C_6H_{12}O_6$) the simplest sugar.

Chemical Equation

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 2$ ATP (Energy Released:118 kJ mol^{-1})

Word Equation

Sugar (glucose, fructose, or sucrose) → Alcohol (ethanol) + Carbon Dioxide + Energy (ATP)

Energy source in anaerobic conditions

Fermentation is thought to have been the primary means of energy production in earlier organisms before oxygen was at high concentration in the atmosphere and thus would represent a more ancient form of energy production in cells.

Fermentation products contain chemical energy (they are not fully oxidized) but are considered waste products since they cannot be metabolised further without the use of

oxygen (or other more highly-oxidized electron acceptors). A consequence is that the production of ATP by fermentation is less efficient than oxidative phosphorylation, where pyruvate is fully oxidized to carbon dioxide. Fermentation produces two ATP molecules per molecule of glucose compared to approximately 36 by aerobic respiration.

Aerobic glycolysis is a method employed by muscle cells for the production of lower-intensity energy over a longer period of time when oxygen is plentiful. Under low-oxygen conditions, however, vertebrates use the less-efficient but faster *anaerobic glycolysis* to produce ATP. The speed at which ATP is produced is about 100 times that of oxidative phosphorylation. While fermentation is helpful during short, intense periods of exertion, it is not sustained over extended periods in complex aerobic organisms. In humans, for example, lactic acid fermentation provides energy for a period ranging from 30 seconds to 2 minutes.

The final step of fermentation, the conversion of pyruvate to fermentation end-products, does not produce energy. However, it is critical for an anaerobic cell since it regenerates nicotinamide adenine dinucleotide (NAD^+), which is required for glycolysis. This is important for normal cellular function, as glycolysis is the only source of ATP in anaerobic conditions.

Products

Products produced by fermentation are actually waste products produced during the reduction of pyruvate to regenerate NAD+ in the absence of oxygen. Bacteria generally produce acids. Vinegar (acetic acid) is the direct result of bacterial metabolism (Bacteria need oxygen to convert the alcohol to acetic acid). In milk, the acid coagulates the casein, producing curds. In pickling, the acid preserves the food from pathogenic and putrefactive bacteria.

When yeast ferments, it breaks down the glucose ($C_6H_{12}O_6$) into exactly two molecules of ethanol (C_2H_6O) and two molecules of carbon dioxide (CO_2).

- Ethanol fermentation (performed by yeast and some types of bacteria) breaks the pyruvate down into ethanol and carbon dioxide. It is important in bread-making, brewing, and wine-making. When the ferment has a high concentration of pectin, minute quantities of methanol can be produced. Usually only one of the products is desired; in bread the alcohol is baked out, and in alcohol production the carbon dioxide is released into the atmosphere.
- Lactic acid fermentation breaks down the pyruvate into lactic acid. It occurs in the muscles of animals when they need energy faster than the blood can supply oxygen. It also occurs in some bacteria and some fungi. It is this type of bacteria that convert lactose into lactic acid in yogurt, giving it its sour taste.

In vertebrates, during intense exercise, cellular respiration will deplete oxygen in the muscles faster than it can be replenished. An associated burning sensation in muscles has been attributed lactic acid causing a decrease in the pH during a shift to **anaerobic**

glycolysis. While this does partially explain acute muscle soreness, lactic acid may also help delay muscle fatigue, although, eventually the lower pH will inhibit enzymes involved in glycolysis. Contrary to currently popular belief, the lactic acid is not the primary causes for the drop in pH, but rather ATP-derived hydrogen ions.

Delayed onset muscle soreness cannot be attributed to the lactic acid and other waste products as they are quickly removed after exercise. It is actually due to microtrauma of the muscle fibres. Eventually the liver metabolises the lactic acid back to pyruvate.

Zymology

Zymology is the scientific term for fermentation. It deals with the biochemical processes involved in fermentation, with yeast selection and physiology, and with the practical issues of brewing. Zymology is occasionally known as *zymurgy*.

Biological warfare

*"Germ Warfare" redirects here. For the episode of M*A*S*H, see Germ Warfare (M*A*S*H episode).*

Biological warfare, also known as **germ warfare**, is the use of any pathogen (bacteria, virus or other disease-causing organism) or toxin found in nature, as a weapon of war. It is meant to incapacitate or kill an adversary. It may also be defined as the material; or defense against such employment.

The creation and stockpiling of biological weapons was outlawed by the 1972 Biological Weapons Convention (BWC), signed by over 100 countries. The BWC remains in force. The rationale behind the agreement is to avoid the devastating impact of a successful biological attack which could conceivably result in thousands, possibly even millions, of deaths and cause severe disruptions to societies and economies. Oddly enough, the convention prohibits only creation and storage, but not usage, of these weapons. However, the consensus among military analysts is that, except in the context of bioterrorism, biological warfare is of little military use.

As a tactical weapon, the main military problem with a biological warfare attack is that it would take days to be effective, and therefore, unlike a nuclear or chemical attack, would not immediately stop an opposing force. As a strategic weapon, biological warfare is again militarily problematic, because it is difficult to prevent the attack from spreading, either to allies or to the attacker, and while an attack is taking effect, the opponent can undertake massive retaliation.

History of biological warfare

The use of biological agents is not new, but before the 20th century, biological warfare took three main forms:

- deliberate poisoning of food and water with infectious material
- use of microorganisms, toxins or animals, living or dead, in a weapon system
- use of biologically inoculated fabrics

Biological warfare has been practiced repeatedly throughout history. During the 6th Century B.C., the Assyrians poisoned enemy wells with a fungus that would make the enemy delusional. In 184 BC, Hannibal of Carthage had clay pots filled with venomous snakes and instructed his soldiers to throw the pots onto the decks of Pergamene ships.

In 1521, the Aztecs were defeated by the Spaniards during the siege of Tenochtitlán in part because of the smallpox epidemic among the Aztecs. Though the Spaniards did not infect the Aztecs intentionally, the disease was endemic within the European population and arrived with the Spaniards. As the Aztecs and other local populations had never been exposed to this disease, the epidemic caused many casualties. The disease ultimately contributed to the downfall of one of the greatest empires in ancient America.

Historical accounts from medieval Europe detail the use of infected animal carcasses, by Mongols, Turks and other groups, to infect enemy water supplies. Prior to the bubonic

plague epidemic known as the Black Death, Mongol and Turkish armies were reported to have catapulted diseased corpses into besieged cities.

During the Middle Ages, victims of the bubonic plague were used for biological attacks, often by flinging their corpses and excrement over castle walls using catapults. The last known incident of using plague corpses for biological warfare occurred in 1710, when Russian forces attacked the Swedes by flinging plague-infected corpses over the city walls of Reval (Tallinn).

The Native American population was decimated after contact with the Old World due to the introduction of many different fatal diseases. The British army at least once used smallpox as a weapon, when British soldiers at Fort Pitt in what is now Pittsburgh, Pennsylvania gave contaminated blankets to the Lenape during Pontiac's War. It is suspected that biological warfare was used against the Native Americans at other times as well.

Native peoples in Aptos gave Spaniards gifts of freshly cut flowers wrapped in leaves of poison oak.

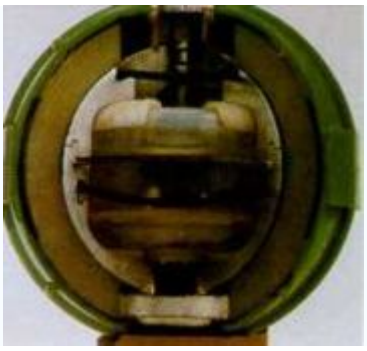
During the United States Civil War, General Sherman reported that Confederate forces shot farm animals in ponds upon which the Union depended for drinking water.

Use of such weapons was banned in international law by the Geneva Protocol of 1925. The 1972 Biological and Toxin Weapons Convention extended the ban to almost all production, storage and transport. However, the Soviet Union continued research and production of offensive biological weapons in a program called biopreparat, despite having signed the Biological and Toxin Weapons Convention. The United States was unaware of the program until Dr. Kanatjan Alibekov, the first deputy director of biopreparat defected in 1992. It is, however, believed that since the signing of the convention the number of countries capable of producing such weapons has increased.

During the Sino-Japanese War (1937-1945) and World War II, Unit 731 of the Imperial Japanese Army conducted human experimentation on thousands, mostly Chinese and Korean. In military campaigns, the Japanese army used biological weapons on Chinese soldiers and civilians. This employment was largely viewed as ineffective due to inefficient delivery systems. However, new information has surfaced within the last decade, which alleges a more active Japanese usage. For example, firsthand accounts testify the Japanese infected civilians through the distribution of plagued foodstuffs, such as dumplings and vegetables. There are also reports of contaminated water supplies. Such estimates report over 580,000 victims, largely due to plague and cholera outbreaks. In addition, repeated seasonal outbreaks after the conclusion of the war bring the death toll much higher.

In response to suspected biological weapons development in Germany and Japan, the United States, United Kingdom, and Canada initiated a BW development program in 1941 that resulted in the weaponization of anthrax, brucellosis, and botulism toxin. The center for U.S. military BW research was Fort Detrick, Maryland. Some biological and chemical weapons research was also conducted at "Dugway Proving Grounds" in Utah. Research carried out in the United Kingdom during World War II left Gruinard island in Scotland contaminated with anthrax for the next 48 years. During WWII, US

conscientious objectors were used as test subjects for biological agents in a program known as Operation Whitecoat



E120 biological bomblet, developed before the U.S. signed the Biological and Toxic Weapons Convention

Considerable research on the topic was performed by the United States, the Soviet Union (see Biopreparat), and probably other major nations throughout the Cold War era, though it is generally believed that such weapons were never used. This view was challenged by China and North Korea, who accused the United States of large-scale field testing of biological weapons, including the use of disease-carrying insects, against them during the Korean War (1950-1953). Their accusation is substantiated by Stephen Endicott and Edward Hagerman in 'The United States and Biological Warfare: secrets of the early Cold War and Korea' (Bloomington, Indiana University Press, 1998). In 1972, the U.S. signed the Biological and Toxic Weapons Convention, which banned "development, production and stockpiling of microbes or their poisonous products except in amounts necessary for protective and peaceful research." By 1996, 137 countries had signed the treaty.

In 1986, the U.S. government spent US$42 million on research for developing infectious diseases and toxins, ten times more money than was spent in 1981. The money went to 24 U.S. universities in hopes of developing strains of anthrax, Rift Valley fever, Japanese encephalitis, tularemia, shigella, botulin, and Q fever. When the Biology Department at MIT voted to refuse Pentagon funds for biotech research, the Reagan administration forced it to reverse its decision by threatening to cut off other funds.

There have been reports that the United States Army has been developing weapons-grade anthrax spores at Dugway Proving Ground, a chemical and biological defense testing facility in Utah, since at least as early as 1992. Under the BWC, nations are permitted to develop small amounts of BW agents for the purpose of defensive research. The United States has maintained a stated national policy of never using biological weapons under any circumstances since November 1969 President Nixon.

Beginning on September 18, 2001, several letters were received by members of the U.S. Congress and media outlets containing anthranx. The attack killed five people. The identity of the perpetrator remains unknown as of 2006.

Biological weapons characteristics

The international biological hazard symbol. It represents a mature cellular organism in the background which has produced three partially formed offspring in the foreground

Ideal characteristics of biological weapons are high infectivity, high potency, availability of vaccines, and delivery as an aerosol.

Diseases most likely to be considered for use as biological weapons are contenders because of their lethality (if delivered efficiently), and robustness (making aerosol delivery feasible).

The biological agents used in biological weapons can often be manufactured quickly and easily. The primary difficulty is not the production of the biological agent but delivery in an infective form to a vulnerable target.

For example, anthrax is considered an effective agent for several reasons. First, it forms hardy spores, perfect for dispersal aerosols. Second, pneumonic (lung) infections of anthrax usually do not cause secondary infections in other people. Thus, the effect of the agent is usually confined to the target. A pneumonic anthrax infection starts with ordinary "cold" symptoms and quickly becomes lethal, with a fatality rate that is 80% or higher. Finally, friendly personnel can be protected with suitable antibiotics.

A mass attack using anthrax would require the creation of aerosol particles of 1.5 to 5 micrometres. Too large and the aerosol would be filtered out by the respiratory system. Too small and the aerosol would be inhaled and exhaled. Also, at this size, nonconductive powders tend to clump and cling because of electrostatic charges. This hinders dispersion. So, the material must be treated with silica to insulate and discharge the charges. The aerosol must be delivered so that rain and sun does not rot it, and yet the human lung can be infected. There are other technological difficulties as well.

Diseases considered for weaponization, or known to be weaponized include anthrax, Ebola, Bubonic Plague, Cholera, Tularemia, Brucellosis, Q fever, Machupo, Coccidioides mycosis, Glanders, Melioidosis, Shigella, Rocky Mountain Spotted Fever, Typhus, Psittacosis, Yellow Fever, Japanese B Encephalitis, Rift Valley Fever, and Smallpox. Naturally-occurring toxins that can be used as weapons include Ricin, SEB, Botulism toxin, Saxitoxin, and many Mycotoxins. The organisms causing these diseases are known as select agents. Their possession, use, and transfer are regulated by the Centers for Disease Control and Prevention's Select Agent Program.

Attacking crops and animals

Biological warfare can also specifically target plants to destroy crops or defoliate vegetation. The United States and Britain discovered plant growth regulators (i.e., herbicides) during the Second World War, and initiated a Herbicidal Warfare program that was eventually used in Malaya and Vietnam in counter insurgency. Though herbicides are chemicals, they are often grouped with biological warfare as bioregulators in a similar manner as biotoxins.Scorched earth tactics or destroying livestock and farmland were carried out in the Vietnam war and Eelam War in Sri Lanka.

The United States developed an anti-crop capability during the Cold War that used plant diseases (bioherbicides, or mycoherbicides) for destroying enemy agriculture. It was believed that destruction of enemy agriculture on a strategic scale could thwart Sino-Soviet aggression in a general war. Diseases such as wheat blast and rice blast were weaponized in aerial spray tanks and cluster bombs for delivery to enemy water sheds in agricultural regions to initiate epiphytotics (epidemics among plants). When the United States renounced its offensive biological warfare program in 1969 and 1970, the vast majority of its biological arsenal was composed of these plant diseases.

Attacking animals is another area of biological warfare intended to eliminate animal resources for transportation and food. In the First World War German agents were arrested attempting to inoculate draft animals with anthrax, and believed responsible for outbreaks of glanders in horses and mules. The British tainted small feed cakes with anthrax in the Second World War as a potential means of attacking German cattle for food denial, but never employed the weapon. In the 1950s the United States had a field trial with hog cholera.

The role of public health departments and disease surveillance

It is important to note that all of the classical and modern biological weapons organisms are animal diseases, the only exception being smallpox. Thus, in any use of biological weapons, it is highly likely that animals will become ill either simultaneously with, or perhaps earlier than humans. Indeed, in the largest biological weapons “accident” known—the anthrax outbreak in Sverdlovsk (now Yekaterinburg) in the Soviet Union in 1979, sheep became ill with anthrax as far as 200 kilometers from the release point of the organism from a military facility in the southeastern portion of the city (known as Compound 15 and still off limits to visitors today).

Thus, a robust surveillance system involving human clinicians and veterinarians may identify a bioweapons attack early in the course of an epidemic, permitting the prophylaxis of disease in the vast majority of people (and/or animals) exposed but not yet ill. For example in the case of anthrax, it is likely that by 24 - 36 hours after an attack, some small percentage of individuals (those with compromised immune system or who had received a large dose of the organism due to proximity to the release point) will become ill with classical symptoms and signs (including a virtually unique chest X-ray finding, often recognized by public health officials if they receive timely reports). By making these data available to local public health officials in real time, most models of anthrax epidemics indicate that more than 80% of an exposed population can receive antibiotic treatment before becoming symptomatic, and thus avoid the high mortality of the disease.

Identification of bioweapons

The growing threat of biowarfare agents and bioterrorism has led to the development of specific field tools that perform on-the-spot analysis and identification of encountered suspect materials. One such technology, being developed by researchers from the Lawrence Livermore National Laboratory (LLNL), employs a “sandwich immunoassay”, in which fluorescent dye-labeled antibodies aimed at specific pathogens are attached to silver and gold nanowires. Researchers at Ben Gurion University in Israel are developing a different device called the BioPen, essentially a “Lab-in-a-Pen”, which can detect known biological agents in under 20 minutes using an adaptation of the ELISA, a similar widely employed immunological technique, that in this case incorporates fiber optics .

Allele

In genetics, an **allele** (pronounced al-eel or al-e-ul) is the different formes of a trait that make up a gene pair. Usually alleles are DNA (deoxyribonucleic acid) sequences that code for a gene, but sometimes the term is used to refer to a non-gene sequence. An individual's genotype for that gene is the set of alleles it happens to possess. In a diploid organism, one that has two copies of each chromosome, two alleles make up the individual's genotype.

An example is the gene for blossom color in many species of flower—a single gene controls the color of the petals, but there may be several different versions (or alleles) of the gene. One version might result in red petals, while another might result in white petals. The resulting color of an individual flower will depend on which two alleles it possesses for the gene and how the two interact.

Introduction

Diploid organisms, such as humans, have paired homologous chromosomes in their somatic cells, and these contain two copies of each gene. An organism in which the two copies of the gene are identical — that is, have the same allele — is called homozygous for that gene. An organism which has two different alleles of the gene is called heterozygous. Phenotypes (the expressed characteristics) associated with a certain allele can sometimes be dominant or recessive, but often they are neither. A dominant phenotype will be expressed when at least one allele of its associated type is present, whereas a recessive phenotype will only be expressed when both alleles are of its associated type.

However, there are exceptions to the way heterozygotes express themselves in the phenotype. One exception is incomplete dominance (sometimes called blending inheritance) when alleles blend their traits in the phenotype. An example of this would be seen if, when crossing Antirrhinums — flowers with incompletely dominant "red" and "white" alleles for petal color — the resulting offspring had pink petals. Another exception is co-dominance, where both alleles are active and both traits are expressed at the same time; for example, both red and white petals in the same bloom or red and white flowers on the same plant. Codominance is also apparent in human blood types. A person with one "A" blood type allele and one "B" blood type allele would have a blood type of "AB".

A wild type allele is an allele which is considered to be "normal" for the organism in question, as opposed to a mutant allele which is usually a relatively new modification.

(Note that with the advent of neutral genetic markers, the term 'allele' is now often used to refer to DNA sequence variants in non-functional, or junk DNA. For example, allele frequency tables are often presented for genetic markers, such as the DYS markers.)

Equations

There are two equations for the frequency of two alleles of a given gene (see Hardy-Weinberg principle).

Equation 1: $p + q = 1$,

Equation 2: $p^2 + 2pq + q^2 = 1$

where p is the frequency of one allele and q is the frequency of the other allele. Under appropriate conditions, subject to numerous limitations regarding the applicability of the Hardy-Weinberg principle, p^2 is the population fraction that is homozygous for the p allele, $2pq$ is the frequency of heterozygotes and q^2 is the population fraction that is homozygous for the q allele.

Natural selection can act on p and q in Equation 1, and obviously affect the frequency of alleles seen in Equation 2.

Equation 2 is a consequence of Equation 1, obtained by squaring both sides and applying the binomial theorem to the left-hand side. Conversely, $p^2 + 2pq + q^2 = 1$ implies $p + q = 1$ since p and q are positive numbers.

The following equation (commonly termed the Lee equation) can be used to calculate the number of possible genotypes in a diploid organism for a specific gene with a given number of alleles.

G = 0.5a^2 + 0.5a

where ‘a’ is the number of different alleles for the gene being dealt with and ‘G’ is the number of possible genotypes. For example, the human ABO blood group gene has three alleles; A (for blood group A), B (for blood group B) and i (for blood group O). As such, (using the equation) the number of possible genotypes a human may have with respect to the ABO gene are 6 (AA, Ai, AB, BB, Bi, ii). Take care when using the equation though as it does not in any way calculate the number of possible phenotypes. Such an equation would be quite impossible as the number of possible phenotypes varies amongst different genes and their alleles. For example, in a diploid heterozygote some genotypes may show complete dominance, incomplete dominance etc., depending of the gene involved.

Artificial selection

This Chihuahua mix and Great Dane show the wide range of dog breed sizes created using artificial selection.

Carrots selectively bred to produce different colors

Artificial selection is the breeding of certain traits over others. It was originally defined by Charles Darwin in contrast to the process of natural selection, in which the differential reproduction of organisms with certain traits is attributed to improved survival and reproductive ability in the natural habitat of the organism. Artificial selection that produces an undesirable outcome from a human perspective is sometimes called negative selection (but note that this term has a better-established meaning as a type of natural selection).

Historical development

Charles Darwin originally coined the term as an illustration of his proposed wider process of natural selection. He noted that many domesticated animals and plants had special

properties that were developed by intentionally encouraging the breeding potential of individuals who both possessed desirable characteristics, and discouraging the breeding of individuals who had less desirable characteristics.

He then postulated that a similar process occurs naturally; individuals in the wild who possess characteristics that enhance their prospects for having offspring would then undergo a similar process of change over time; although in this case “desirable” characteristics would be not those which specifically satisfy human needs, but those which enhance survivability. This natural process forms the basis of the theory of Darwinian evolution.

Contrast to natural selection

The difference between natural and artificial selection centers on the difference in environment among organisms subject to the two processes. Essentially, in artificial selection, the fitness which is the amount of offsping an individual contributes to a population relative to other individuals in that same population of an organism is defined in part by its display of the traits being selected for by humans. Since humans either intentionally or unintentionally exert control over which organisms in a population reproduce or how many offspring they produce, the distribution of traits in the organisms’ population will change.

It should be emphasized that there is no real difference in the genetic processes underlying artificial and natural selection, and that the concept of artificial selection was first introduced as an illustration of the wider process of natural selection. The selection process is termed “artificial” when human preferences or influences have a significant effect on the evolution of a particular population or species.

Examples of artificial selection

Most examples of artificial selection fall into the category of selective breeding, in which particular individuals are selected for breeding because they possess desired characteristics or excluded from breeding because their traits are undesirable. Both processes have contributed to the domestication of animals and plants by humans.

The most obvious examples of artificial selection can be found in the range of specialised body shapes and even personality types in bred in domesticated dogs. The wide range of sizes and shapes, from Dachshund to Wolfhound, shows the power of artificial selection through selective breeding. Systematic selective breeding has led to extreme traits such as the large size and eating habits of the Great Dane versus the small size of the Chihuahua. It is possible for traits to be selected for under artificial selection - for example, aggressive behavior in small dogs - that would be selected against in the natural environment absent human influence. An even more illustrative example is the domestication of corn, which has been bred so that it no longer disperses its seeds,

instead relying on human intervention to disseminate them. Because both organisms derive significant benefits from the other, this could be termed a symbiotic relationship.

Certain characteristics may unintentionally be encouraged while intentionally selecting for a desired result. For example, the domestic chicken has been bred to reach a large size relatively quickly (compared to its feral ancestors). The resulting changes in the chicken's gut have come at the expense of a reduced brain size and relatively smaller leg bones; these latter changes were not intentional artificial selections, but through a parallel process sometimes called "unconscious selection".

This 1845 painting of a Shorthorn bull by J. Loader shows how animals can be bred for size.

It is also possible for humans to exert artificial selection pressures on our own species, either unintentionally through social pressures, or intentionally. Eugenics efforts, in which those with "undesirable" characteristics are prevented from reproducing and those with "desirable" characteristics are encouraged to reproduce, form the most extreme such example.

Biochemical engineering

Biochemical engineering is a branch of chemical engineering that mainly deals with the design and construction of unit processes that involve biological organisms or molecules. Biochemical engineering is often taught as a supplementary option to chemical engineering due to the similarities in both the background subject curriculum and problem-solving techniques used by both professions. Its applications are used in the pharmaceutical, biotechnology, and water treatment industries.

The Bioreactor

Bioreactors

A **bioreactor** may refer to any device or system that supports a biologically active environment. In one case, a bioreactor is a vessel in which is carried out a chemical process which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from some liter to cube meters, and are often made of stainless steel.

A bioreactor may also refer to a device or system meant to grow cells or tissues in the context of cell culture. These devices are being developed for use in tissue engineering.

On the basis of **mode of operation**, a bioreactor may be classified as batch, fed batch or continuous (e.g. Continuous stirred-tank reactor model). An example of a bioreactor is the chemostat.

Organism growing in bioreactor may be suspended or immobilized . The simplest, where cells are immobilized, is a Petri dish with agar gel. Large scale immobilized cell bioreactors are:

- packed bed
- fibrous bed
- membrane

Bioreactor design

Bioreactor design is quite a complex engineering task. Under optimum conditions the microorganisms or cells are able to perform their desired function with great efficiency. The bioreactor's environmental conditions like gas (i.e., air, oxygen, nitrogen, carbon dioxide) flowrates, temperature, pH and dissolved oxygen levels, and agitation speed/circulation rate need to be closely monitored and controlled.

Most industrial bioreactor manufacturers use vessels, sensors, controllers, and a control system, networked together for their bioreactor system, see programmable logic controller (PLC).

Fouling can harm the overall sterility and efficiency of the bioreactor, especially the heat exchangers. To avoid it the bioreactor must be easily cleanable and must be as smooth as possible (therefore the round shape).

A heat exchanger is needed to maintain the bioprocess at a constant temperature. Biological fermentation is a major source of heat, therefore in most cases bioreactors need water refrigeration. They can be refrigerated with an external jacket or, for very large vessels, with internal coils.

In an aerobic process, optimal oxygen transfer is perhaps the most difficult task to accomplish. Oxygen is poorly soluble in water -and even less in fermentation broths- and is relatively scarce in air (20.8%). Oxygen transfer is usually helped by agitation, that is also needed to mix nutrients and to keep the fermentation homogeneous. There are however limits to the speed of agitation, due both to high power consumption (which is proportional to the cube of the speed of the electric motor) and the damage to organisms due to excessive tip speed.

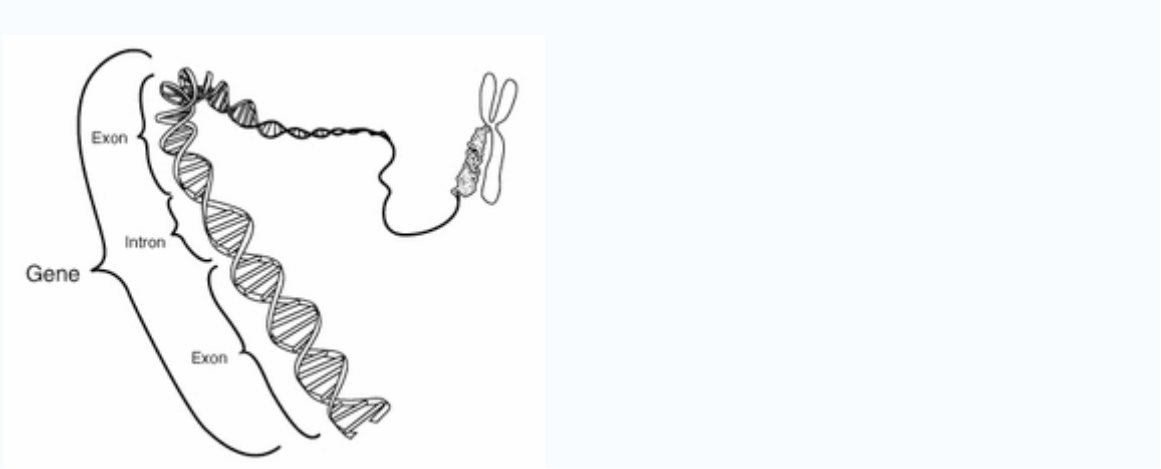
Industrial bioreactors usually employ bacteria or other simple organisms that can withstand the forces of agitation. They are also simple to sustain, requiring only simple nutrient solutions and can grow at astounding rates.

In bioreactors where the goal is grow cells or tissues for experimental or therapeutic purposes, the design is significantly different from industrial bioreactors. Many cells and tissues, especially mammalian, must have a surface or other structural support in order to grow, and agitated environments are often destructive to these cell types and tissues. Higher organisms also need more complex growth medium.

NASA Tissue Cloning Bioreactor

NASA has developed a new type of bioreactor that artificially grows tissue in cell cultures. NASA's tissue bioreactor can grow heart tissue, skeletal tissue, ligaments, cancer tissue for study, and other types of tissue

Gene



This stylistic schematic diagram shows a gene in relation to the double helix structure of DNA and to a chromosome (right). Introns are regions often found in eukaryote genes which are removed in the splicing process: only the exons encode the protein. This diagram labels a region of only 40 or so bases as a gene. In reality many genes are much larger, as are introns and exons.

A **gene** is the unit of heredity in every living organism. Genes are encoded in an organism's genome, composed of DNA or RNA, and direct the physical development and behavior of the organism. Most genes encode proteins, which are biological macromolecules comprising linear chains of amino acids that affect most of the chemical reactions carried out by the cell. Some genes do not encode proteins, but produce non-coding RNA molecules that play key roles in protein biosynthesis and gene regulation. Molecules that result from gene expression, whether RNA or protein, are collectively known as gene products.

Most genes contain non-coding regions, that do not code for the gene products, but often regulate gene expression. A critical non-coding region is the promoter, a short DNA sequence that is required for initiation of gene expression. The genes of eukaryotic organisms often contain non-coding regions called introns which are removed from the messenger RNA in a process known as splicing. The regions that actually encode the gene product, which can be much smaller than the introns, are known as exons.

The total complement of genes in an organism or cell is known as its genome. The genome size of an organism is loosely dependent on its complexity; prokaryotes such as bacteria and archaea have generally smaller genomes, in both number of base pairs and number of genes, than even single-celled eukaryotes. However, the largest known genome belongs to the single-celled amoeba *Amoeba duria*, with over 6 billion base pairs. The estimated number of genes in the human genome has been repeatedly revised downward since the completion of the Human Genome Project; current estimates place the human genome at just under 3 billion base pairs and about 20,000-25,000 genes. The gene density of a genome is a measure of the number of genes per million base pairs (called a megabase, Mb); prokaryotic genomes have much higher gene densities than

eukaryotes due to the absence of introns in prokaryotic genomes. The gene density of the human genome is roughly 12-15 genes/Mb.

Mendelian inheritance and classical genetics

Mendelian inheritance and Classical genetics

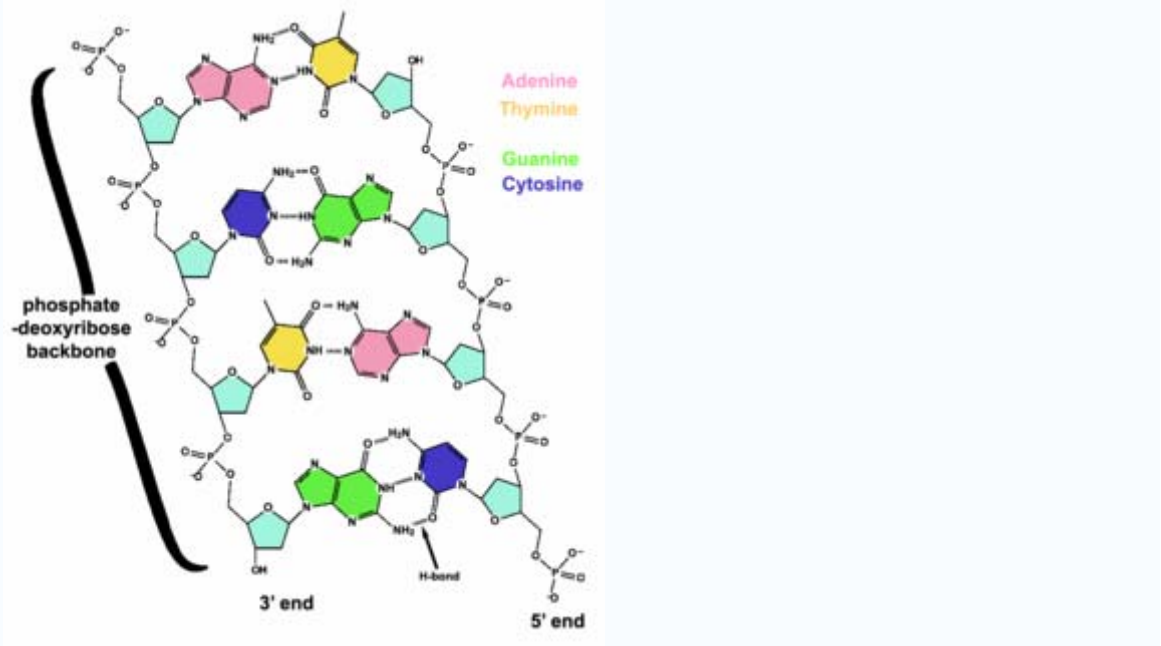
The modern conception of the gene originated with work by Gregor Mendel, a 19th century Austrian monk who systematically studied heredity in pea plants. Mendel's work was the first to illustrate particulate inheritance, or the theory that inherited traits are passed from one generation to the next in discrete units that interact in well-defined ways. Danish botanist Wilhelm Johannsen coined the word "gene" in 1909 to describe these fundamental physical and functional units of heredity. The word was derived from Hugo De Vries' term *pangen*, itself a derivative of the word *pangenesis* coined by Darwin (1868). The word pangenesis is made from the Greek words *pan* (a prefix meaning "whole", "encompassing") and *genesis* ("birth") or *genos* ("origin").

According to the theory of Mendelian inheritance, variations in phenotype - the observable physical and behavioral characteristics of an organism - are due to variations in genotype, or the organism's particular set of genes, each of which specifies a particular trait. Different genes for the same trait, which give rise to different phenotypes, are known as alleles. Organisms such as the pea plants Mendel worked on, along with many plants and animals, have two alleles for each trait, one inherited from each parent. Alleles may be dominant or recessive; dominant alleles give rise to their corresponding phenotypes when paired with any other allele for the same trait, while recessive alleles give rise to their corresponding phenotype only when paired with another copy of the same allele. For example, if the allele specifying tall stems in pea plants is dominant over the allele specifying short stems, then pea plants that inherit one tall allele from one parent and one short allele from the other parent will also have tall stems. Mendel's work found that alleles assort independently in the production of gametes, or germ cells, ensuring variation in the next generation.

Prior to Mendel's work, the dominant theory of heredity was one of blending inheritance, which proposes that the traits of the parents blend or mix in a smooth, continuous gradient in the offspring. Although Mendel's work was largely unrecognized after its first publication in 1866, it was rediscovered in 1900 by three European scientists, Hugo de Vries, Carl Correns, and Erich von Tschermak, who had reached similar conclusions from their own research. However, these scientists were not yet aware of the identity of the 'discrete units' on which genetic material resides.

A series of subsequent discoveries led to the realization that chromosomes within cells are the carriers of genetic material, and that they are made of DNA (deoxyribonucleic acid), a polymeric molecule found in all cells on which the 'discrete units' of Mendelian inheritance are encoded. The modern study of genetics at the level of DNA is known as molecular genetics and the synthesis of molecular genetics with traditional Darwinian evolution is known as the modern evolutionary synthesis.

The physical gene



The chemical structure of a four-base fragment of a DNA double helix.

The vast majority of living organisms encode their genes in long polymeric strands of DNA. DNA consists of four monomeric subunits known as nucleotides (sometimes called ‘bases’): adenine, cytosine, guanine, and thymine. Each nucleotide within the polymer consists of three components: a phosphate group, a deoxyribose sugar ring, and a nucleobase. Adenine and guanine are purines and cytosine and thymine are pyrimidines. The most common form of DNA in a cell is in a double helix structure, in which two individual DNA strands are paired; this pairing is mediated by hydrogen bonds between the constituent bases. The Watson-Crick base pairing rules, which are by far the most common forms of pairing in DNA, specify that guanine pairs with cytosine and adenine pairs with thymine (thus, each pair contains one purine and one pyrimidine). The two strands that make up a double helix must of course be *complementary*; that is, their bases must align such that only adenines of one strand are paired with thymines of the other strand, and so on. The structure of the DNA double helix was discovered by James Watson and Francis Crick based on X-ray crystallography studies by Rosalind Franklin.

Due to the structure of the bases, DNA strands have directionality. One end of a DNA polymer contains an exposed hydroxyl group on the deoxyribose; this is known as the 3’ end of the molecule. The other end contains an exposed phosphate group; this is the 5’ end. The directionality of DNA is vitally important to many cellular processes, since double helices are necessarily directional (a strand running 5’-3’ pairs with a strand running 3’-5’) and processes such as DNA replication occur in only one direction. All nucleic acid synthesis in a cell occurs in the 5’-3’ direction, because new monomers are added via a dehydration reaction that uses the exposed 3’ hydroxyl as a nucleophile.

The expression of genes encoded in DNA begins by transcribing the gene into RNA, a second type of nucleic acid that is very similar to DNA, but whose monomers contain the sugar ribose rather than deoxyribose. RNA also contains the base uracil in place of thymine. RNA molecules are less stable than DNA and are typically single-stranded. Genes that encode proteins are composed of a series of three-nucleotide sequences called

codons, which serve as the “words” in the genetic “language”. The genetic code specifies the correspondence during protein translation between codons and amino acids. The genetic code is nearly the same for all known organisms.

RNA genes

In most cases, RNA is an intermediate product in the process of manufacturing proteins from genes. However, for some gene sequences, the RNA molecules are the actual functional products. For example, RNAs known as ribozymes are capable of enzymatic function, and miRNAs have a regulatory role. The DNA sequences from which such RNAs are transcribed are known as non-coding DNA, or RNA genes.

Some viruses store their entire genomes in the form of RNA, and contain no DNA at all. Because they use RNA to store genes, their cellular hosts may synthesize their proteins as soon as they are infected and without the delay in waiting for transcription. On the other hand, RNA retroviruses, such as HIV, require the reverse transcription of their genome from RNA into DNA before their proteins can be synthesized. In 2006, French researcher came across a puzzling example of RNA-mediated inheritance in mouse. Mice with a loss-of-function mutation in the gene Kit have white tails. Offspring of these mutants can have white tails despite having only normal Kit genes. The research team traced this effect back to mutated Kit RNA . While RNA is common as genetic storage material in viruses, in mammals in particular RNA inheritance has been observed very rarely.

Functional structure of a gene

All genes have regulatory regions in addition to regions that explicitly code for a protein or RNA product. A universal regulatory region shared by all genes is known as the promoter, which provides a position that is recognized by the transcription machinery when a gene is about to be transcribed and expressed. Although promoter regions have a consensus sequence that is the most common sequence at this position, some genes have “strong” promoters that bind the transcription machinery well, and others have “weak” promoters that bind poorly. These weak promoters usually permit a lower rate of transcription than the strong promoters, because the transcription machinery binds to them and initiates transcription less frequently. Other possible regulatory regions include enhancers, which can compensate for a weak promoter. Most regulatory regions are “upstream” - that is, toward the 5’ end of - the transcription initiation site. Eukaryotic promoter regions are much more complex and difficult to identify than prokaryotic promoters.

Many prokaryotic genes are organized into operons, or groups of genes whose products have related functions and which are transcribed as a unit. By contrast, eukaryotic genes are transcribed only one at a time, but may include long stretches of DNA called introns which are transcribed but never translated into protein.

Chromosomes

The total complement of genes in an organism or cell is known as its genome, which may be stored on one or more chromosomes; the region of the chromosome at which a particular gene is located is called its locus. A chromosome consists of a single, very long DNA helix on which thousands of genes are encoded. Prokaryotes - bacteria and archaea - typically store their genomes on a single large, circular chromosome, sometimes supplemented by additional small circles of DNA called plasmids, which usually encode only a few genes and are easily transferable between individuals. For example, the genes for antibiotic resistance are usually encoded on bacterial plasmids and can be passed between individual cells, even those of different species, via horizontal gene transfer. Although some simple eukaryotes also possess plasmids with small numbers of genes, the majority of eukaryotic genes are stored on multiple linear chromosomes, which are packed within the nucleus in complex with storage proteins called histones. The manner in which DNA is stored on the histone, as well as chemical modifications of the histone itself, are regulatory mechanisms governing whether a particular region of DNA is accessible for gene expression. The ends of eukaryotic chromosomes are capped by long stretches of repetitive sequences called telomeres, which do not code for any gene product but are present to prevent degradation of coding and regulatory regions during DNA replication. The length of the telomeres tends to decrease each time the genome is replicated in preparation for cell division; the loss of telomeres has been proposed as an explanation for cellular senescence, or the loss of the ability to divide, and by extension for the aging process in organisms.

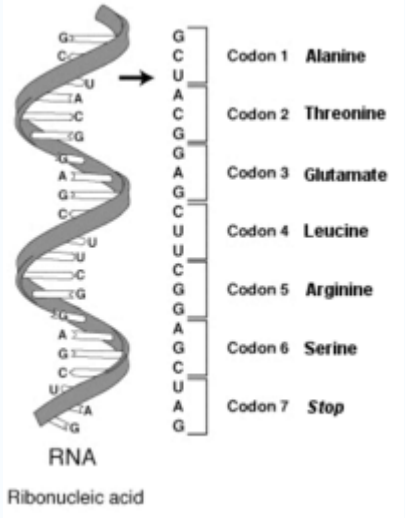
While the chromosomes of prokaryotes are relatively gene-dense, those of eukaryotes often contain so-called “junk DNA”, or regions of DNA that serve no obvious function. Simple single-celled eukaryotes have relatively small amounts of such DNA, while the genomes of complex multicellular organisms, including humans, contain an absolute majority of DNA without an identified function.

Gene expression

Gene expression

In all organisms, there are two major steps separating a protein-coding gene from its protein: first, the DNA on which the gene resides must be *transcribed* from DNA to messenger RNA (mRNA), and second, it must be *translated* from mRNA to protein. RNA-coding genes must still go through the first step, but are not translated into protein. The process of producing a biologically functional molecule of either RNA or protein is called gene expression, and the resulting molecule itself is called a gene product.

The genetic code



Schematic diagram of a single-stranded RNA molecule illustrating the position of three-base codons.

Genetic code

The genetic code is the set of rules by which a gene is translated into a functional protein. Each gene consists of a specific sequence of nucleotides encoded in a DNA (or sometimes RNA) strand; a correspondence between nucleotides, the basic building blocks of genetic material, and amino acids, the basic building blocks of proteins, must be established for genes to be successfully translated into functional proteins. Sets of three nucleotides, known as codons, each correspond to a a specific amino acid or to a signal; three codons are known as “stop codons” and, instead of specifying a new amino acid, alert the translation machinery that the end of the gene has been reached. There are 64 possible codons (four possible nucleotides at each of three positions, hence 4^3 possible codons) and only 20 standard amino acids; hence the code is redundant and multiple codons can specify the same amino acid. The correspondence between codons and amino acids is nearly universal among all known living organisms.

Transcription

The process of genetic transcription produces a single-stranded RNA molecule known as messenger RNA, whose nucleotide sequence is complementary to the DNA from which it was transcribed. The DNA strand whose sequence matches that of the RNA is known as the coding strand and the strand from which the RNA was synthesized is the template strand. Transcription is performed by an enzyme called an RNA polymerase, which reads the template strand in the 3’ to 5’ direction and synthesizes the RNA from 5’ to 3’. To initiate transcription,the polymerase first recongizes and binds a promoter region of the gene. Thus a major mechanism of gene regulation is the blocking or sequestering of the promoter region, either by tight binding by repressor molecules that physically block the polymerase, or by organizing the DNA such that the promoter region is not accessible.

In prokaryotes, transcription occurs in the cytoplasm; for very long transcripts, translation may begin at the 5’ end of the RNA while the 3’ end is still being transcribed. In

eukaryotes, transcription necessarily occurs in the nucleus, where the cell's DNA is sequestered; the RNA molecule produced by the polymerase is known as the primary transcript and must undergo post-transcriptional modifications before being exported to the cytoplasm for translation. The splicing of introns present within the transcribed region is a modification unique to eukaryotes; alternative splicing mechanisms can result in mature transcripts from the same gene having different sequences and thus coding for different proteins. This is a major form of regulation in eukaryotic cells.

Translation

Translation is the process by which a mature mRNA molecule is used as a template for synthesizing a new protein. Translation is carried out by ribosomes, large complexes of RNA and protein responsible for carrying out the chemical reactions to add new amino acids to a growing polypeptide chain by the formation of peptide bonds. The genetic code is read three nucleotides at a time, in units called codons, via interactions with specialized RNA molecules called transfer RNA (tRNA). Each tRNA has three unpaired bases known as the anticodon that are complementary to the codon it reads; the tRNA is also covalently attached to the amino acid specified by the complementary codon. When the tRNA binds to its complementary codon in an mRNA strand, the ribosome ligates its amino acid cargo to the new polypeptide chain, which is synthesized from amino terminus to carboxyl terminus. During and after its synthesis, the new protein must fold to its active three-dimensional structure before it can carry out its cellular function.

DNA replication and inheritance

DNA replication

DNA replication

The growth, development, and reproduction of organisms relies on cell division, or the process by which a single cell divides into two usually identical daughter cells. This requires first making a duplicate copy of every gene in the genome in a process called DNA replication. The copies are made by specialized enzymes known as DNA polymerases, which "read" one strand of the double-helical DNA, known as the template strand, and synthesize a new complementary strand. Because the DNA double helix is held together by base pairing, the sequence of one strand completely specified the sequence of its complement; hence only one strand needs to be read by the enzyme to produce a faithful copy. In order to read the DNA sequence, the existing double helical strands must first be separated, which is done by proteins called helicases. The site of this separation is called a replication fork.

DNA replication is a directional process; since the DNA polymerase can only add new nucleotide bases to a growing DNA strand at the 3' end, the new strands grows in the 5' to 3' direction. For the template strand that runs in the 3'-5' direction, known as the *leading strand* this is simple, and allows the enzyme to synthesize a single long DNA molecule. The opposite template strand that runs in the 5'-3' direction, known as the

lagging strand, presents a greater challenge; these strands' complements are synthesized in a series of fragments known as Okazaki fragments, which are then stitched together (or "ligated") by a different type of DNA polymerase. The replication process produces two new DNA molecules, each of which is paired in a double helix to one of the original DNA molecules that was used as a template. Thus each daughter cell inherits a copy of the genome that consists of one original and one newly synthesized strand. This is known as semiconservative replication, famously and convincingly demonstrated by the Meselson-Stahl experiment. In order to complete the replication of the entire genome, the DNA polymerase enzyme must be highly processive; that is, it must add many nucleotides to the growing strand before "falling off" the template strand. DNA polymerases alone typically add only a small number of nucleotides at a time; they must associate with other proteins called DNA clamps which prevent them from dissociating from the template strand.

Cell division

Main articles: Cell division and Cell cycle

After DNA replication is complete, the cell must divide into two daughter cells. In prokaryotes - bacteria and archaea - this usually occurs via a relatively simple process called binary fission, in which each circular genome attaches to the cell membrane and is separated into the daughter cells as the membrane invaginates to split the cytoplasm into two mmebrane-bound portions. This process is extremely fast compared to the rates of cell division in eukaryotes.

Eukaryotic cell division is a more complex process known as the cell cycle. The phase of the cell cycle in which DNA replication occurs is known (largely for historical reasons) as S phase. This phase is followed by G2 phase, in which the cell increases in volume and prepares for the division that will take place in M phase. M phase incorporates both mitosis, or the segregation of chromosomes into two opposite poles within the dividing cell, and cytokinesis, or the separation of the cytoplasm. In many single-celled eukaryotes such as yeast, reproduction by budding is common, which results in asymmetrical portions of cytoplasm in the two daughter cells.

Because the genomes of eukaryotes are divided among multiple chromosomes consisting of linear DNA, the assortment of chromosomes into daughter cells is complex; each daughter cell must end up with one and only one copy of each chromosome. A series of mitotic checkpoints, or molecular events that stall the cell division process if certain conditions are not met, exist to prevent the cell from attempting to divide before the chromosomes are properly organized. During must of the cell cycle, eukaryotic DNA is organized as diffuse chromatin within the nucleus; only during mitosis do the chromosomes condense to the 'X' shape familar from a karyotype. Each of these chromosomes is composed of two sister chromatids attached at their center by a centromere, which align along the center of the cell and attach to microtubules that extend from the center of the cell to two opposite poles. Force exerted by the microtubules splits the chromatids so that each pole receives one; after cytokinesis, this results in two individual cells that each once again contain two copies of each gene.

Molecular inheritance

Meiosis

Meiosis is a specialized form of cell division that some cells in sexually reproducing organisms undergo. Meiosis results in cells called gametes or germ cells that are haploid, or contain only one copy of each gene. In humans, the gametes produced by females are called eggs or ova and those produced by males are called sperm. Two gametes fuse to produce a fertilized egg, a single cell that once again has a diploid number of genes - each with one copy from the mother and one copy from the father. While the two sister chromatids are close to one another, a process called genetic recombination or *crossing-over* can sometimes occur, in which a length of DNA on one chromatid is swapped with a length of DNA on the sister chromatid. This has no effect if the alleles on the chromatids were the same, but results in reassortment of otherwise linked alleles otherwise.

The Mendelian principle of independent assortment asserts that each of the parent's two genes for each trait will sort independently into gametes. That is, if there is a gene controlling stem length and a gene controlling leaf width in pea plants, the presence of a gene for tall stems in a particular gamete is independent of the presence of a gene for narrow leaves. This is in fact only true for genes that do not reside on the same chromosome, or are located very far from one another on the same chromosome. The closer two genes lie on the same chromosome, the more closely they will be associated in gametes and the more often they will appear together; genes that are very close are essentially never separated because it is extremely unlikely that a crossover point will occur between them. This is known as genetic linkage.

Mutation

Mutation

DNA replication is for the most part extremely accurate, with an error rate per site of around 10^{-6} to 10^{-10} in eukaryotes. Rare, spontaneous alterations in the base sequence of a particular gene arise from a number of sources, such as errors in DNA replication and the aftermath of DNA damage. These errors are called mutations. The cell contains many DNA repair mechanisms for preventing mutations and maintaining the integrity of the genome; however, in some cases - such as breaks in both DNA strands of a chromosome - repairing the physical damage to the molecule is a higher priority than producing an exact copy. Due to the degeneracy of the genetic code, some mutations in protein-coding genes are *silent*, or produce no change in the amino acid sequence of the protein for which they code; for example, the codons UCU and UUC both code for serine, so the U↔C mutation has no effect on the protein. Mutations that do have phenotypic effects are most often neutral or deleterious to the organism, but sometimes they confer benefits to the organism's fitness.

Mutations propagated to the next generation lead to variations within a species' population. Variants of a single gene are known as alleles, and differences in alleles may give rise to differences in traits, for example eye colour. Although it is rare for the

variants in a single gene to have clearly distinguishable phenotypic effects, certain well-defined traits are in fact controlled by single genetic loci. A gene's most common allele is called the wild type allele, and rare alleles are called mutants. However, this does not imply that the wild-type allele is the ancestor from which the mutants are descended.

The genome

Chromosomal organization

All the genes and intervening DNA together make up the genome of an organism, which in many species is divided among several chromosomes and typically present in two or more copies. The location (or locus) of a gene and the chromosome on which it is situated is in a sense arbitrary. Genes that appear together on the chromosomes of one species, such as humans, may appear on separate chromosomes in another species, such as mice. Two genes positioned near one another on a chromosome may encode proteins that figure in the same cellular process or in completely unrelated processes. As an example of the former, many of the genes involved in spermatogenesis reside together on the Y chromosome.

Many species carry more than one copy of their genome within each of their somatic cells. These organisms are called diploid if they have two copies or polyploid if they have more than two copies. In such organisms, the copies are practically never identical. With respect to each gene, the copies that an individual possesses are liable to be distinct alleles, which may act synergistically or antagonistically to generate a trait or phenotype. The ways that gene copies interact are explained by chemical dominance relationships (see the articles on genetics, allele).

In the case of viruses the term chromosome is rarely used. Here the most common term is RNA or DNA genome.

Composition of the genome

Gene content and genome size of various organisms

organism	genes	base pairs
Plant	<50,000	$<10^{11}$
Human, mouse or rat	25,000	3×10^{9}
Fugu fish	40,000	$4x10^{8}$
Fruit Fly	13,767	1.3×10^{8}

Worm	19,000	9.7×10^7
Fungus	6,000	1.3×10^7
Bacterium	500–6,000	5×10^5–10^7
Mycoplasma genitalium	500	580,000
DNA virus	10–900	5,000–800,000
RNA virus	1–25	1,000–23,000
Viroid	0–1	~500

The attached table gives typical numbers of genes and genome size for some organisms. Estimates of the number of genes in an organism are somewhat controversial because they depend on gene finding algorithms that are susceptible to false negatives, especially when the gene is marked by a promoter region whose sequence is very different from the consensus sequence. These methods are still much more sensitive than the traditional methods used in the early development of molecular genetics, which could identify only genes with multiple alleles represented in a population.

In most eukaryotic species, very little of the DNA in the genome encodes proteins, and the genes may be separated by vast regions of non-coding DNA, much of which has been labeled “junk DNA” due to its apparent lack of function in the modern organism. A commonly studied type of “junk DNA” is the pseudogenes, or region of non-coding DNA that resembles expressed genes but usually lacks appropriate promoters and other control sequences; such regions are hypothesized to be the results of gene duplication events in a lineage’s evolutionary past. Moreover, the genes are often fragmented internally by non-coding sequences called introns, which can be many times longer than the coding sequence. Introns are removed on the heels of transcription by splicing. In the primary molecular sense, they represent parts of a gene, however.

Most organisms have more than one storage site for their genes. Bacteria, for example, store most of their genes in a circular double-stranded piece of DNA while some genes are stored in small plasmids. Usually the term bacterial genome does not include these plasmids. Eukaryotic cells store most of their genes in the nuclear genome composed of chromosomes while a few genes reside in the stripped-down DNA repositories of organelles like mitochondria.

Genetic and genomic nomenclature

For each known human gene the HUGO Gene Nomenclature Committee (HGNC) approve a gene name and symbol (short-form abbreviation). All approved symbols are stored in the HGNC Database. Each symbol is unique and each gene is only given one

approved gene symbol. It is necessary to provide a unique symbol for each gene so that people can talk about them. This also facilitates electronic data retrieval from publications. In preference each symbol maintains parallel construction in different members of a gene family and can be used in other species, especially the mouse.

Evolutionary concept of a gene

George C. Williams first explicitly advocated the gene-centric view of evolution in his 1966 book *Adaptation and Natural Selection*. He proposed an evolutionary concept of gene to be used when we are talking about natural selection favoring some genes. The definition is: "that which segregates and recombines with appreciable frequency." According to this definition, even an asexual genome could be considered a gene, insofar it have an appreciable permanency through many generations.

The difference is: the molecular gene *transcribes* as a unit, and the evolutionary gene *inherits* as a unit.

Richard Dawkins' *The Selfish Gene* and *The Extended Phenotype* defended the idea that the gene is the only replicator in living systems. This means that only genes transmit their structure largely intact and are potentially immortal in the form of copies. So, genes should be the unit of selection. In River Out of Eden, Dawkins further refined the idea of gene-centric selection by describing life as a river of compatible genes flowing through geological time. Scoop up a bucket of genes from the river of genes, and we have an organism serving as temporary bodies or survival machines. A river of genes may fork into two branches representing two non-interbreeding species as a result of geographical separation.

History

History of genetics

The existence of genes was first suggested by Gregor Mendel (1822-1884), who, in the 1860s, studied inheritance in pea plants and hypothesized a factor that conveys traits from parent to offspring. He spent over 10 years or his life on one experiment. Although he did not use the term *gene*, he explained his results in terms of inherited characteristics. Mendel was also the first to hypothesize independent assortment, the distinction between dominant and recessive traits, the distinction between a heterozygote and homozygote, and the difference between what would later be described as genotype and phenotype. Mendel's concept was finally named when Wilhelm Johannsen coined the word *gene* in 1909.

In the early 1900s, Mendel's work received renewed attention from scientists. In 1910, Thomas Hunt Morgan showed that genes reside on specific chromosomes. He later showed that genes occupy specific locations on the chromosome. With this knowledge, Morgan and his students began the first chromosomal map of the fruit fly *Drosophila*. In 1928, Frederick Griffith showed that genes could be transferred. In what is now known as Griffith's experiment, injections into a mouse of a deadly strain of bacteria that had been

heat-killed transferred genetic information to a safe strain of the same bacteria, killing the mouse.

In 1941, George Wells Beadle and Edward Lawrie Tatum showed that mutations in genes caused errors in certain steps in metabolic pathways. This showed that specific genes code for specific proteins, leading to the “one gene, one enzyme” hypothesis. Oswald Avery, Collin Macleod, and Maclyn McCarty showed in 1944 that DNA holds the gene’s information. In 1953, James D. Watson and Francis Crick demonstrated the molecular structure of DNA. Together, these discoveries established the central dogma of molecular biology, which states that proteins are translated from RNA which is transcribed from DNA. This dogma has since been shown to have exceptions, such as reverse transcription in retroviruses.

In 1972, Walter Fiers and his team at the Laboratory of Molecular Biology of the University of Ghent (Ghent, Belgium) were the first to determine the sequence of a gene: the gene for Bacteriophage MS2 coat protein. Richard Roberts and Phillip Sharp discovered in 1977 that genes can be split into segments. This leads to the idea that one gene can make several proteins. Recently (as of 2003-2006), biological results let the notion of gene appear more slippery. In particular, genes do not seem to sit side by side on DNA like discrete beads. Instead, regions of the DNA producing distinct proteins may overlap, so that the idea emerges that “genes are one long continuum”.

Plasmid

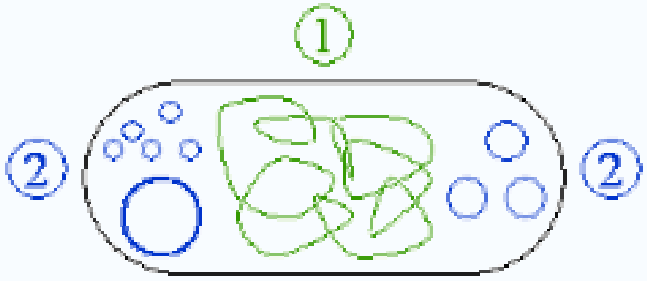


Figure 1: Schematic drawing of a bacterium with plasmids enclosed. 1 Chromosomal DNA. 2 Plasmids.

A **plasmid** is a DNA molecule separate from the chromosomal DNA and capable of autonomous replication. It is typically circular and double-stranded. It usually occurs in bacteria, sometimes in eukaryotic organisms (e.g., the *2-micrometre-ring* in *Saccharomyces cerevisiae*). Size of plasmids varies from 1 to over 400 kilobase pairs (kbp). There may be one copy, for large plasmids, to hundreds of copies of the same plasmid in a single cell, or even thousands of copies, for certain artificial plasmids selected for high copy number (such as the **pUC** series of plasmids).

The term *plasmid* was first introduced by the American molecular biologist Joshua Lederberg in 1952.

Antibiotic resistance

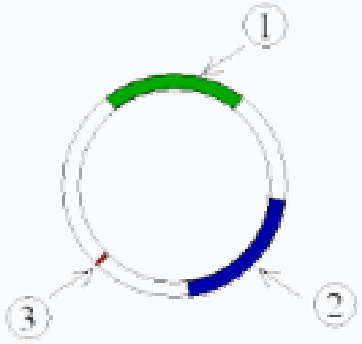


Figure 2: Schematic drawing of a plasmid with antibiotic resistances. 1 & 2 Genes that code for resistance. 3 Ori.

Plasmids often contain genes or gene cassettes that confer a selective advantage to the bacterium harboring them, such as the ability to make the bacterium antibiotic resistant.

Every plasmid contains at least one DNA sequence that serves as an *origin of replication*, or *ori* (a starting point for DNA replication), which enables the plasmid DNA to be duplicated independently from the chromosomal DNA (Figure 2). The chromosomes of

most bacteria are circular, like the plasmid depicted Figure 2, but linear plasmids are also known, which superficially resemble the chromosomes of most eukaryotes.

Episomes

An **episome** is a plasmid that can integrate itself into the chromosomal DNA of the host organism (Fig. 3). For this reason, it can stay intact for a long time, be duplicated with every cell division of the host, and become a basic part of its genetic makeup. This term is no longer commonly used for plasmids, since it is now clear that a region of homology with the chromosome such as a transposon makes a plasmid into an episome. In mammalian systems, the term episome refers to a circular DNA (such as a viral genome) that is maintained by noncovalent tethering to the host cell chromosome.

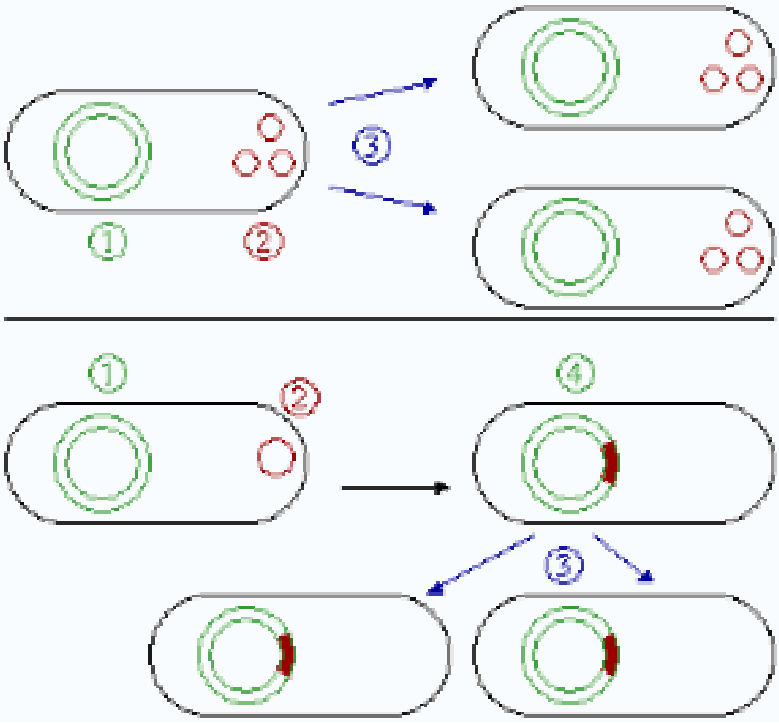


Figure 3: Comparison of non-integrating plasmids (*top*) and episomes (*bottom*). 1 Chromosomal DNA. 2 Plasmids. 3 Cell division. 4 Chromosomal DNA with integrated plasmids.

Vectors

Plasmids used in genetic engineering are called vectors. They are used to transfer genes from one organism to another and typically contain a genetic marker conferring a phenotype that can be selected for or against. Most also contain a polylinker or multiple cloning site (MCS), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments at this location. See Applications below.

Types

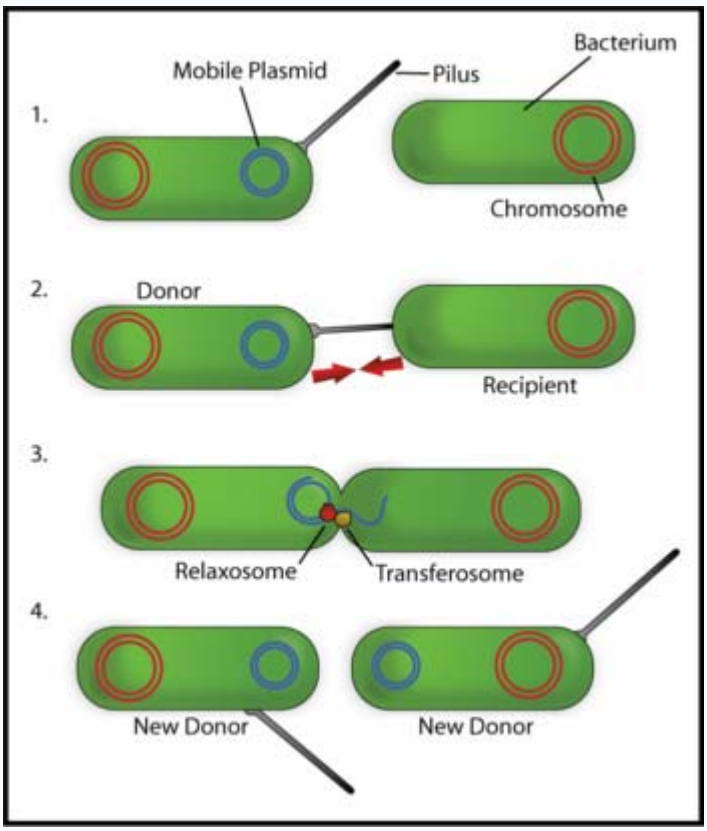


Figure 4: Overview of Bacterial conjugation

One way of grouping plasmids is by their ability to transfer to other bacteria. *Conjugative* plasmids contain so-called *tra-genes*, which perform the complex process of *conjugation*, the sexual transfer of plasmids to another bacterium (Fig. 4). *Non-conjugative* plasmids are incapable of initiating conjugation, hence they can only be transferred with the assistance of conjugative plasmids, by 'accident'. An intermediate class of plasmids are *mobilizable*, and carry only a subset of the genes required for transfer. They can 'parasitise' a conjugative plasmid, transferring at high frequency only in its presence.

It is possible for plasmids of different types to coexist in a single cell. Seven different plasmids have been found in *E. coli*. But *related* plasmids are often incompatible, in the sense that only one of them survives in the cell line, due to the regulation of vital plasmid functions. Therefore, plasmids can be assigned into *compatibility groups*.

Another way to classify plasmids is by function. There are five main classes:

- *Fertility-F-plasmids*, which contain tra-genes. They are capable of conjugation.

- *Resistance-®plasmids*, which contain genes that can build a resistance against antibiotics or poisons. Historically known as R-factors, before the nature of plasmids was understood.
- *Col-plasmids*, which contain genes that *code for* (determine the production of) colicines, proteins that can kill other bacteria.
- *Degrative plasmids*, which enable the digestion of unusual substances, e.g., toluene or salicylic acid.
- *Virulence plasmids*, which turn the bacterium into a pathogen.

Plasmids can belong to more than one of these functional groups.

Plasmids that exist only as one or a few copies in each bacterium are, upon cell division, in danger of being lost in one of the segregating bacteria. Such single-copy plasmids have systems which attempt to actively distribute a copy to both daughter cells.

Some plasmids include an *addiction system* or "postsegregational killing system (PSK)". They produce both a long-lived poison and a short-lived antidote. Daughter cells that retain a copy of the plasmid survive, while a daughter cell that fails to inherit the plasmid dies or suffers a reduced growth-rate because of the lingering poison from the parent cell. This is an example of plasmids as selfish DNA.

Applications

Plasmids serve as important tools in genetics and biochemistry labs, where they are commonly used to multiply (make many copies of) or *express* particular genes. Many plasmids are commercially available for such uses.

The gene to be replicated is inserted into copies of a plasmid which contains genes that make cells resistant to particular antibiotics. Next, the plasmids are inserted into bacteria by a process called *transformation*. Then, the bacteria are exposed to the particular antibiotics. Only bacteria which take up copies of the plasmid survive the antibiotic, since the plasmid makes them resistant. In particular, the protecting genes are expressed (used to make a protein) and the expressed protein breaks down the antibiotics. In this way the antibiotics act as a filter to select only the modified bacteria. Now these bacteria can be grown in large amounts, harvested and lysed to isolate the plasmid of interest.

Another major use of plasmids is to make large amounts of proteins. In this case you grow bacteria containing a plasmid harboring the gene of interest. Just as the bacteria produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing a gene or the protein it then codes for, for example, insulin or even antibiotics.

Plasmid DNA extraction

As alluded to above, plasmids are often used to purify a specific sequence, since they can easily be purified away from the rest of the genome. For their use as vectors, and for molecular cloning, plasmids often need to be isolated.

There are several methods to isolate plasmid DNA from bacteria, the archaetypes of which are the **miniprep** and the **maxiprep**. The former can be used to quickly find out whether the plasmid is correct in any of several bacterial clones. The yield is a small amount of impure plasmid DNA, which is sufficient for analysis by restriction digest and for some cloning techniques.

In the latter, much larger volumes of bacterial suspension are grown from which a maxi-prep can be performed. Essentially this is a scaled-up miniprep followed by additional purification. This results in relatively large amounts (several ug) of very pure plasmid DNA.

In recent times many commercial kits have been created to perform plasmid extraction at various scales, purity and levels of automation.

Conformations

Plasmid DNA may appear in one of five conformations, which (for a given size) run at different speeds in a gel during electrophoresis. The conformations are listed below in order of electrophoretic mobility (speed for a given applied voltage) from slowest to fastest:

- "Nicked Open-Circular" DNA has one strand cut.
- "Linear" DNA has free ends, either because both strands have been cut, or because the DNA was linear *in vivo*. You can model this with an electrical extension cord that is not plugged into itself.
- "Relaxed Circular" DNA is fully intact with both strands uncut, but has been enzymatically "relaxed" (supercoils removed). You can model this by letting an extension cord relax and then plugging it into itself.
- "Supercoiled Denatured" DNA is like **supercoiled DNA** (see below), but has unpaired regions that make it slightly less compact; this can result from excessive alkalinity during plasmid preparation. You can model this by twisting a badly frayed extension cord and then plugging it into itself.
- "Supercoiled" (or "Covalently Closed-Circular") DNA is fully intact with both strands uncut, and with a twist built in, resulting in a compact form. You can model this by twisting an extension cord in good condition and then plugging it into itself.

The rate of migration for small linear fragments is directly proportional to the voltage applied at low voltages. At higher voltages, larger fragments migrate at continually increasing yet different rates. Therefore the resolution of a gel decreases with increased voltage.

At a specified, low voltage, the migration rate of small linear DNA fragments is a function of their length. Large linear fragments (over 20kb or so) migrate at a certain fixed rate regardless of length. This is because the molecules ‘reptate’, with the bulk of the molecule following the leading end through the gel matrix. Restriction digests are frequently used to analyse purified plasmids. These enzymes specifically break the DNA at certain short sequences. The resulting linear fragments form ‘bands’ after gel electrophoresis. It is possible to purify certain fragments by cutting the bands out of the gel and dissolving the gel to release the DNA fragments.

Protein

A representation of the 3D structure of myoglobin, showing coloured alpha helices. This protein was the first to have its structure solved by X-ray crystallography.

Proteins are large organic compounds made of amino acids arranged in a linear chain and joined together between the carboxyl atom of one amino acid and the amine nitrogen of another. This bond is called a peptide bond. The sequence of amino acids in a protein is defined by a gene and encoded in the genetic code. Although this genetic code specifies 20 “standard” amino acids, the residues in a protein are often chemically altered in post-translational modification: either before the protein can function in the cell, or as part of control mechanisms. Proteins can also work together to achieve a particular function, and they often associate to form stable complexes.

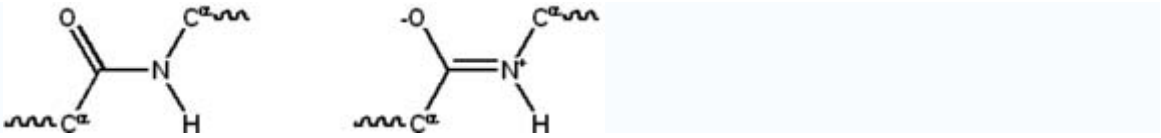
Like other biological macromolecules such as polysaccharides and nucleic acids, proteins are essential parts of all living organisms and participate in every process within cells. Many proteins are enzymes that catalyze biochemical reactions, and are vital to metabolism. Other proteins have structural or mechanical functions, such as the proteins in the cytoskeleton, which forms a system of scaffolding that maintains cell shape. Proteins are also important in cell signaling, immune responses, cell adhesion, and the cell cycle. Protein is also a necessary component in our diet, since animals cannot synthesise all the amino acids and must obtain essential amino acids from food. Through the process of digestion, animals break down ingested protein into free amino acids that can be used for protein synthesis.

The name *protein* comes from the Greek *πρώτα* (“prota”), meaning “*of primary importance*” and were first described and named by Jöns Jakob Berzelius in 1838. However, their central role in living organisms was not fully appreciated until 1926, when James B. Sumner showed that the enzyme urease was a protein. The first protein structures to be solved included insulin and myoglobin; the first was by Sir Frederick Sanger who won a 1958 Nobel Prize for it, and the second by Max Perutz and Sir John

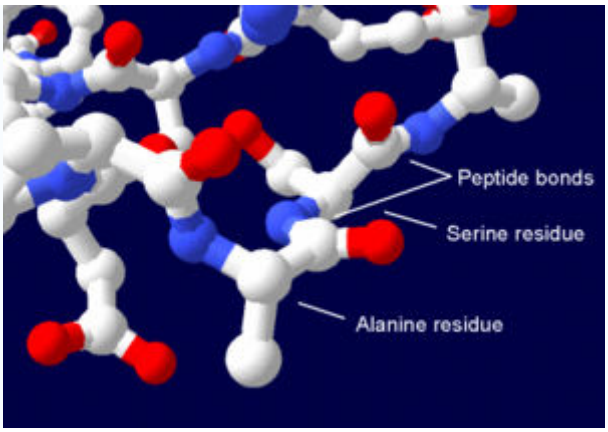
Cowdery Kendrew in 1958. Both proteins' three-dimensional structures were amongst the first determined by x-ray diffraction analysis; the myoglobin structure won the Nobel Prize in Chemistry for its discoverers.

Biochemistry

Main articles: Amino acid and peptide bond



Resonance structures of the peptide bond that links individual amino acids to form a protein polymer.



Section of a protein structure showing serine and alanine residues linked together by peptide bonds. Carbons are shown in white and hydrogens are omitted for clarity.

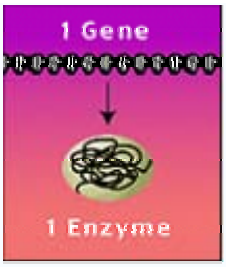
Proteins are linear polymers built from 20 different L-alpha-amino acids. All amino acids share common structural features including an alpha carbon to which an amino group, a carboxyl group, and a variable side chain are bonded. Only proline shows little difference in a fashion by containing an unusual ring to the N-end amine group, which forces the CO-NH amide sequence into a fixed conformation. The side chains of the standard amino acids, detailed in the list of standard amino acids, have varying chemical properties that produce proteins' three-dimensional structure and are therefore critical to protein function. The amino acids in a polypeptide chain are linked by peptide bonds formed in a dehydration reaction. Once linked in the protein chain, an individual amino acid is called a *residue* and the linked series of carbon, nitrogen, and oxygen atoms are known as the *main chain* or *protein backbone*. The peptide bond has two resonance forms that contribute some double bond character and inhibit rotation around its axis, so that the alpha carbons are roughly coplanar. The other two dihedral angles in the peptide bond determine the local shape assumed by the protein backbone.

Due to the chemical structure of the individual amino acids, the protein chain has directionality. The end of the protein with a free carboxyl group is known as the C-terminus or carboxy terminus, while the end with a free amino group is known as the N-terminus or amino terminus.

There is some ambiguity between the usage of the words *protein*, *polypeptide*, and *peptide*. *Protein* is generally used to refer to the complete biological molecule in a stable conformation, while *peptide* is generally reserved for a short amino acid oligomers often lacking a stable 3-dimensional structure. However, the boundary between the two is ill-defined and usually lies near 20-30 residues. *Polypeptide* can refer to any single linear chain of amino acids, usually regardless of length, but often implies an absence of a single defined conformation.

Synthesis

Protein biosynthesis



Proteins are assembled from amino acids using information encoded in genes. Each protein has its own unique amino acid sequence that is specified by the nucleotide sequence of the gene encoding this protein. The genetic code is a set of three-nucleotide sets called codons and each three-nucleotide combination stands for an amino acid, for example ATG stands for methionine. Because DNA contains four nucleotides, the total number of possible codons is 64; hence, there is some redundancy in the genetic code and some amino acids are specified by more than one codon. Genes encoded in DNA are first transcribed into pre-messenger RNA (mRNA) by proteins such as RNA polymerase. Most organisms then process the pre-mRNA (also known as a *primary transcript*) using various forms of post-transcriptional modification to form the mature mRNA, which is then used as a template for protein synthesis by the ribosome. In prokaryotes the mRNA may either be used as soon as it is produced, or be bound by a ribosome after having moved away from the nucleoid. In contrast, eukaryotes make mRNA in the cell nucleus and then translocate it across the nuclear membrane into the cytoplasm, where protein synthesis then takes place. The rate of protein synthesis is higher in prokaryotes than eukaryotes and can reach up to 20 amino acids per second.

The process of synthesizing a protein from an mRNA template is known as translation. The mRNA is loaded onto the ribosome and is read three nucleotides at a time by matching each codon to its base pairing anticodon located on a transfer RNA molecule, which carries the amino acid corresponding to the codon it recognizes. The enzyme aminoacyl tRNA synthetase “charges” the tRNA molecules with the correct amino acids. The growing polypeptide is often termed the *nascent chain*. Proteins are always biosynthesized from N-terminus to C-terminus.

The size of a synthesized protein can be measured by the number of amino acids it contains and by its total molecular mass, which is normally reported in units of *daltons*

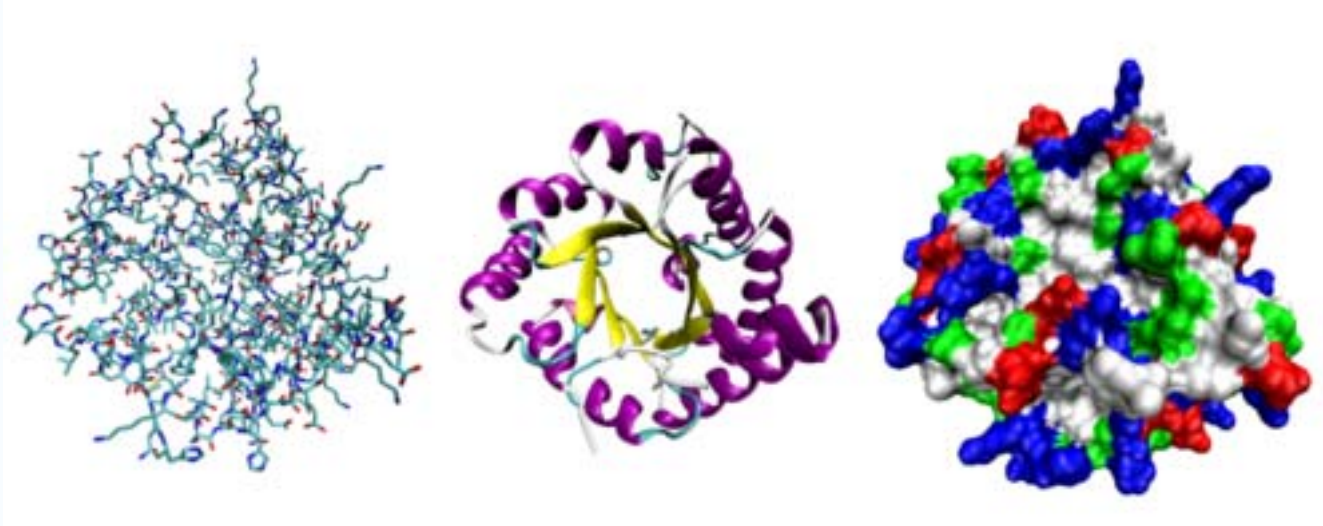
(synonymous with atomic mass units), or the derivative unit kilodalton (kDa). Yeast proteins are on average 466 amino acids long and 53 kDa in mass. The largest known proteins are the titins, a component of the muscle sarcomere, with a molecular mass of almost 3,000 kDa and a total length of almost 27,000 amino acids.

Chemical synthesis

Short proteins can also be synthesized chemically in the laboratory by a family of methods known as peptide synthesis, which rely on organic synthesis techniques such as chemical ligation to produce peptides in high yield. Chemical synthesis allows for the introduction of non-natural amino acids into polypeptide chains, such as attachment of fluorescent probes to amino acid side chains. These methods are useful in laboratory biochemistry and cell biology, though generally not for commercial applications. Chemical synthesis is inefficient for polypeptides longer than about 300 amino acids, and the synthesized proteins may not readily assume their native tertiary structure. Most chemical synthesis methods proceed from C-terminus to N-terminus, opposite the biological reaction.

Structure of proteins

Protein structure



Three possible representations of the three-dimensional structure of the protein triose phosphate isomerase. Left: all-atom representation colored by atom type. Middle: “cartoon” representation illustrating the backbone conformation, colored by secondary structure. Right: Solvent-accessible surface representation colored by residue type (acidic residues red, basic residues blue, polar residues green, nonpolar residues white).

Most proteins fold into unique 3-dimensional structures. The shape into which a protein naturally folds is known as its native state. Although many proteins can fold unassisted simply through the structural propensities of their component amino acids, others require

the aid of molecular chaperones to efficiently fold to their native states. Biochemists often refer to four distinct aspects of a protein's structure:

- *Primary structure*: the amino acid sequence
- *Secondary structure*: regularly repeating local structures stabilized by hydrogen bonds. The most common examples are the alpha helix and beta sheet. Because secondary structures are local, many regions of different secondary structure can be present in the same protein molecule.
- *Tertiary structure*: the overall shape of a single protein molecule; the spatial relationship of the secondary structures to one another. Tertiary structure is generally stabilized by nonlocal interactions, most commonly the formation of a hydrophobic core, but also through salt bridges, hydrogen bonds, disulfide bonds, and even post-translational modifications. The term "tertiary structure" is often used as synonymous with the term *fold*.
- *Quaternary structure*: the shape or structure that results from the interaction of more than one protein molecule, usually called *protein subunits* in this context, which function as part of the larger assembly or protein complex.

In addition to these levels of structure, proteins may shift between several related structures in performing their biological function. In the context of these functional rearrangements, these tertiary or quaternary structures are usually referred to as "conformations," and transitions between them are called *conformational changes.* Such changes are often induced by the binding of a substrate molecule to an enzyme's active site, or the physical region of the protein that participates in chemical catalysis.

Molecular surface of several proteins showing their comparative sizes. From left to right are: Antibody (IgG), Hemoglobin, Insulin (a hormone), Adenylate kinase (an enzyme), and Glutamine synthetase (an enzyme).

Proteins can be informally divided into three main classes, which correlate with typical tertiary structures: globular proteins, fibrous proteins, and membrane proteins. Almost all globular proteins are soluble and many are enzymes. Fibrous proteins are often structural; membrane proteins often serve as receptors or provide channels for polar or charged molecules to pass through the cell membrane.

A special case of intramolecular hydrogen bonds within proteins, poorly shielded from water attack and hence promoting their own dehydration, are called dehydrons.

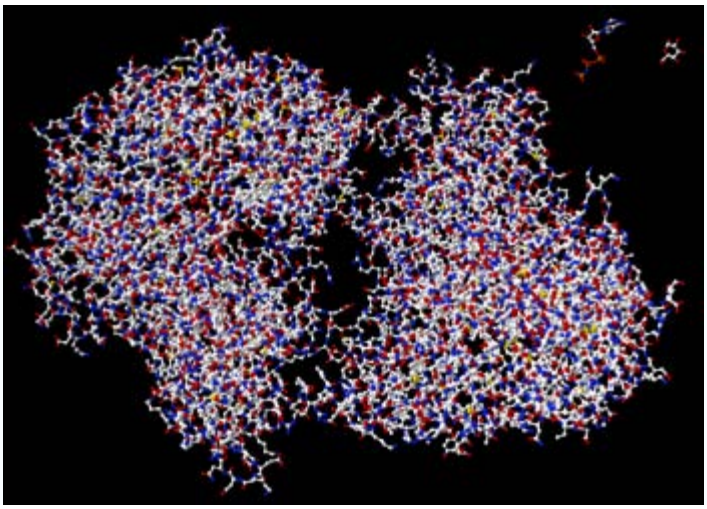
Structure determination

Discovering the tertiary structure of a protein, or the quaternary structure of its complexes, can provide important clues about how the protein performs its function. Common experimental methods of structure determination include X-ray crystallography and NMR spectroscopy, both of which can produce information at atomic resolution. Cryoelectron microscopy is used to produce lower-resolution structural information about very large protein complexes, including assembled viruses; a variant known as electron crystallography can also produce high-resolution information in some cases, especially for two-dimensional crystals of membrane proteins. Solved structures are usually deposited in the Protein Data Bank (PDB), a freely available resource from which structural data about thousands of proteins can be obtained in the form of Cartesian coordinates for each atom in the protein.

There are many more known gene sequences than there are solved protein structures. Further, the set of solved structures is biased toward those proteins that can be easily subjected to the experimental conditions required by one of the major structure determination methods. In particular, globular proteins are comparatively easy to crystallize in preparation for X-ray crystallography, which remains the oldest and most common structure determination technique. Membrane proteins, by contrast, are difficult to crystallize and are underrepresented in the PDB. Structural genomics initiatives have attempted to remedy these deficiencies by systematically solving representative structures of major fold classes. Protein structure prediction methods attempt to provide a means of generating a plausible structure for a proteins whose structures have not been experimentally determined.

Cellular functions

Proteins are the chief actors within the cell, said to be carrying out the duties specified by the information encoded in genes. With the exception of certain types of RNA, most other biological molecules are relatively inert elements upon which proteins act. Proteins make up half the dry weight of an E. coli cell, while other macromolecules such as DNA and RNA make up only 3% and 20% respectively. The total complement of proteins expressed in a particular cell or cell type at a given time point or experimental condition is known as its proteome.



The enzyme hexokinase is shown as a simple ball-and-stick molecular model. To scale in the top right-hand corner are its two substrates, ATP and glucose.

The chief characteristic of proteins that enables them to carry out their diverse cellular functions is their ability to bind other molecules specifically and tightly. The region of the protein responsible for binding another molecule is known as the binding site and is often a depression or "pocket" on the molecular surface. This binding ability is mediated by the tertiary structure of the protein, which defines the binding site pocket, and by the chemical properties of the surrounding amino acids' side chains. Protein binding can be extraordinarily tight and specific; for example, the ribonuclease inhibitor protein binds to human angiogenin with a sub-femtomolar dissociation constant ($<10^{-15}$ M) but does not bind at all to its amphibian homolog onconase (>1 M). Extremely minor chemical changes such as the addition of a single methyl group to a binding partner can sometimes suffice to nearly eliminate binding; for example, the aminoacyl tRNA synthetase specific to the amino acid valine discriminates against the very similar side chain of the amino acid isoleucine.

Proteins can bind to other proteins as well as to small-molecule substrates. When proteins bind specifically to other copies of the same molecule, they can oligomerize to form fibrils; this process occurs often in structural proteins that consist of globular monomers that self-associate to form rigid fibers. Protein-protein interactions also regulate enzymatic activity, control progression through the cell cycle, and allow the assembly of large protein complexes that carry out many closely related reactions with a common biological function. Proteins can also bind to, or even be integrated into, cell membranes. The ability of binding partners to induce conformational changes in proteins allows the construction of enormously complex signaling networks.

Enzymes

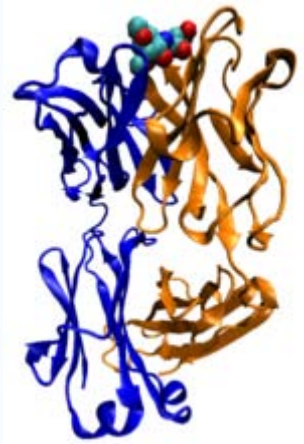
Enzyme

The best-known role of proteins in the cell is their duty as enzymes, which catalyze chemical reactions. Enzymes are usually highly specific catalysts that accelerate only one or a few chemical reactions. Enzymes effect most of the reactions involved in metabolism

and catabolism as well as DNA replication, DNA repair, and RNA synthesis. Some enzymes act on other proteins to add or remove chemical groups in a process known as post-translational modification. About 4,000 reactions are known to be catalyzed by enzymes. The rate acceleration conferred by enzymatic catalysis is often enormous - as much as 10^{17}-fold increase in rate over the uncatalyzed reaction in the case of orotate decarboxylase.

The molecules bound and acted upon by enzymes are known as substrates. Although enzymes can consist of hundreds of amino acids, it is usually only a small fraction of the residues that come in contact with the substrate and an even smaller fraction - 3-4 residues on average - that are directly involved in catalysis. The region of the enzyme that binds the substrate and contains the catalytic residues is known as the active site.

Cell signalling and ligand transport



A mouse antibody against cholera that binds a carbohydrate antigen.

Many proteins are involved in the process of cell signaling and signal transduction. Some proteins, such as insulin, are extracellular proteins that transmit a signal from the cell in which they were synthesized to other cells in distant tissues. Others are membrane proteins that act as receptors whose main function is to bind a signaling molecule and induce a biochemical response in the cell. Many receptors have a binding site exposed on the cell surface and an effector domain within the cell, which may have enzymatic activity or may undergo a conformational change detected by other proteins within the cell.

Antibodies are protein components of adaptive immune system whose main function is to bind antigens, or foreign substances in the body, and target them for destruction. Antibodies can be secreted into the extracellular environment or anchored in the membranes of specialized B cells known as plasma cells. While enzymes are limited in their binding affinity for their substrates by the necessity of conducting their reaction, antibodies have no such constraints. An antibody’s binding affinity to its target is extraordinarily high.

Many ligand transport proteins bind particular small biomolecules and transport them to other locations in the body of a multicellular organism. These proteins must have a high

binding affinity when their ligand is present in high concentrations but must also release the ligand when it is present at low concentrations in the target tissues. The canonical example of a ligand-binding protein is haemoglobin, which transports oxygen from the lungs to other organs and tissues in all vertebrates and has close homologs in every biological kingdom.

Transmembrane proteins can also serve as ligand transport proteins that alter the permeability of the cell's membrane to small molecules and ions. The membrane alone has a hydrophobic core through which polar or charged molecules cannot diffuse. Membrane proteins contain internal channels that allow such molecules to enter and exit the cell. Many ion channel proteins are specialized to select for only a particular ion; for example, potassium and sodium channels often discriminate for only one of the two ions.

Structural proteins

Structural proteins confer stiffness and rigidity to otherwise fluid biological components. Most structural proteins are fibrous proteins; for example, actin and tubulin are globular and soluble as monomers but polymerize to form long, stiff fibers that comprise the cytoskeleton, which allows the cell to maintain its shape and size. Collagen and elastin are critical components of connective tissue such as cartilage, and keratin is found in hard or filamentous structures such as hair, nails, feathers, hooves, and some animal shells.

Other proteins that serve structural functions are motor proteins such as myosin, kinesin, and dynein, which are capable of generating mechanical forces. These proteins are crucial for cellular motility of single-celled organisms and the sperm of many sexually reproducing multicellular organisms. They also generate the forces exerted by contracting muscles.

Methods of study

Protein methods

As some of the most commonly studied biological molecules, the activities and structures of proteins are examined both *in vitro* and *in vivo*. *In vitro* studies of purified proteins in controlled environments are useful for learning how a protein carries out its function: for example, enzyme kinetics studies explore the chemical mechanism of an enzyme's catalytic activity and its relative affinity for various possible substrate molecules. By contrast, *in vivo* experiments on proteins' activities within cells or even within whole organisms can provide complementary information about where a protein functions and how it is regulated.

Protein purification

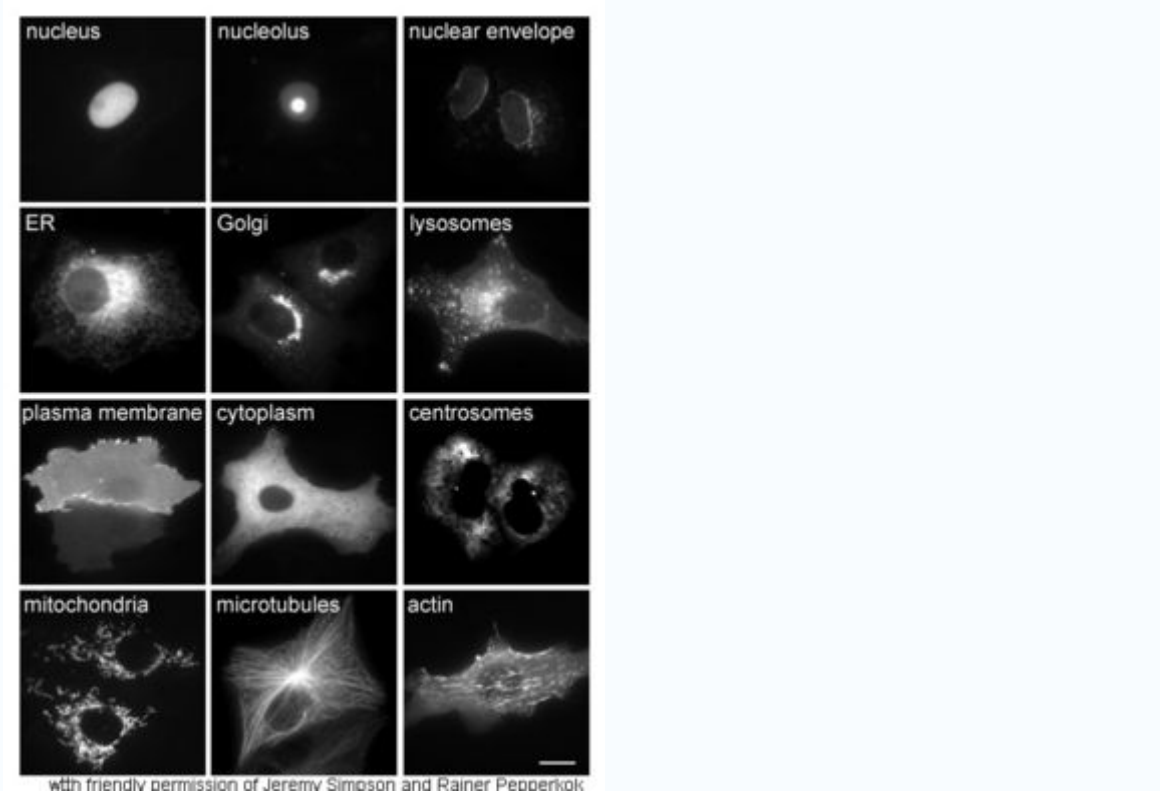
Protein purification

In order to perform *in vitro* analyses, a protein must be purified away from other cellular components. This process usually begins with cell lysis, in which a cell's membrane is

disrupted and its internal contents released into a solution known as a crude lysate. The resulting mixture can be purified using ultracentrifugation, which fractionates the various cellular components into fractions containing soluble proteins; membrane lipids and proteins; cellular organelles, and nucleic acids. Precipitation by a method known as salting out can concentrate the proteins from this lysate. Various types of chromatography are then used to isolate the protein or proteins of interest based on properties such as molecular weight, net charge and binding affinity. The level of purification can be monitored using gel electrophoresis if the desired protein's molecular weight is known, by spectroscopy if the protein has distinguishable spectroscopic features, or by enzyme assays if the protein has enzymatic activity.

For natural proteins, a series of purification steps may be necessary to obtain protein sufficiently pure for laboratory applications. To simplify this process, genetic engineering is often used to add chemical features to proteins that make them easier to purify without affecting their structure or activity. Here, a "tag" consisting of a specific amino acid sequence, often a series of histidine residues (a "His-tag"), is attached to one terminus of the protein. As a result, when the lysate is passed over a chromatography column containing nickel, the histidine residues ligate the nickel and attach to the column while the untagged components of the lysate pass unimpeded.

Cellular localization



Proteins in different cellular compartments and structures tagged with green fluorescent protein.

The study of proteins *in vivo* is often concerned with the synthesis and localization of the protein within the cell. Although many intracellular proteins are synthesized in the cytoplasm and membrane-bound or secreted proteins in the endoplasmic reticulum, the specifics of how proteins are targeted to specific organelles or cellular structures is often unclear. A useful technique for assessing cellular localization uses genetic engineering to express in a cell a fusion protein or chimera consisting of the natural protein of interest linked to a "reporter" such as green fluorescent protein (GFP). The fused protein's position within the cell can be cleanly and efficiently visualized using microscopy, as shown in the figure opposite.

Through another genetic engineering application known as site-directed mutagenesis, researchers can alter the protein sequence and hence its structure, cellular localization, and susceptibility to regulation, which can be followed *in vivo* by GFP tagging or *in vitro* by enzyme kinetics and binding studies.

Proteomics and bioinformatics

Proteomics and Bioinformatics

The total complement of proteins present in a cell or cell type is known as its proteome, and the study of such large-scale data sets defines the field of proteomics, named by analogy to the related field of genomics. Key experimental techniques in proteomics include protein microarrays, which allow the detection of the relative levels of a large number of proteins present in a cell, and two-hybrid screening, which allows the systematic exploration of protein-protein interactions. The total complement of biologically possible such interactions is known as the interactome. A systematic attempt to determine the structures of proteins representing every possible fold is known as structural genomics.

The large amount of genomic and proteomic data available for a variety of organisms, including the human genome, allows researchers to efficiently identify homologous proteins in distantly related organisms by sequence alignment. Sequence profiling tools can perform more specific sequence manipulations such as restriction enzyme maps, open reading frame analyses for nucleotide sequences, and secondary structure prediction. From this data phylogenetic trees can be constructed and evolutionary hypotheses developed using special software like ClustalW regarding the ancestry of modern organisms and the genes they express. The field of bioinformatics seeks to assemble, annotate, and analyze genomic and proteomic data, applying computational techniques to biological problems such as gene finding and cladistics.

Structure prediction and simulation

Complementary to the field of structural genomics, protein structure prediction seeks to develop efficient ways to provide plausible models for proteins whose structures have not yet been determined experimentally. The most successful type of structure prediction, known as homology modeling, relies on the existence of a "template" structure with sequence similarity to the protein being modeled; structural genomics' goal is to provide sufficient representation in solved structures to model most of those that remain.

Although producing accurate models remains a challenge when only distantly related template structures are available, it has been suggested that sequence alignment is the bottleneck in this process, as quite accurate models can be produced if a “perfect” sequence alignment is known. Many structure prediction methods have served to inform the emerging field of protein engineering, in which novel protein folds have already been designed. A more complex computational problem is the prediction of intermolecular interactions, such as in molecular docking and protein-protein interaction prediction.

The processes of protein folding and binding can be simulated using techniques derived from molecular dynamics, which increasingly take advantage of distributed computing as in the Folding@Home project. The folding of small alpha-helical protein domains such as the villin headpiece and the HIV accessory protein have been successfully simulated *in silico*, and hybrid methods that combine standard molecular dynamics with quantum mechanics calculations have allowed exploration of the electronic states of rhodopsins.

Nutrition

Protein in nutrition

Most microorganisms and plants can biosynthesize all 20 standard amino acids, while animals must obtain some of the amino acids from the diet. Key enzymes in the biosynthetic pathways that synthesize certain amino acids - such as aspartokinase, which catalyzes the first step in the synthesis of lysine, methionine, and threonine from aspartate - are not present in animals. The amino acids that an organism cannot synthesize on its own are referred to as essential amino acids. (This designation is often used to specifically identify those essential to humans.) If amino acids are present in the environment, most microorganisms can conserve energy by taking up the amino acids from the environment and downregulating their own biosynthetic pathways. Bacteria are often engineered in the laboratory to lack the genes necessary for synthesizing a particular amino acid, providing a selectable marker for the success of transfection, or the introduction of foreign DNA.

In animals, amino acids are obtained through the consumption of foods containing protein. Ingested proteins are broken down through digestion, which typically involves denaturation of the protein through exposure to acid and degradation by the action of enzymes called proteases. Ingestion of essential amino acids is critical to the health of the organism, since the biosynthesis of proteins that include these amino acids is inhibited by their low concentration. Amino acids are also an important dietary source of nitrogen. Some ingested amino acids, especially those that are not essential, are not used directly for protein biosynthesis. Instead, they are converted to carbohydrates through gluconeogenesis, which is also used under starvation conditions to generate glucose from the body’s own proteins, particularly those found in muscle.

History

Proteins were recognized as a distinct class of biological molecules in the eighteenth century by Antoine Fourcroy and others. Members of this class (called the

"albuminoids", *Eiweisskörper*, or *matières albuminoides*) were recognized by their ability to coagulate or flocculate under various treatments such as heat or acid; well-known examples at the start of the nineteenth century included albumen from egg whites, blood serum albumin, fibrin, and wheat gluten. The similarity between the cooking of egg whites and the curdling of milk was recognized even in ancient times; for example, the name *albumen* for the egg-white protein was coined by Pliny the Elder from the Latin *albus ovi* (egg white).

With the advice of Jöns Jakob Berzelius, the Dutch chemist Gerhardus Johannes Mulder carried out elemental analyses of common animal and plant proteins. To everyone's surprise, all proteins had nearly the same empirical formula, roughly $C_{400}H_{620}N_{100}O_{120}$ with individual sulfur and phosphorus atoms. Mulder published his findings in two papers (1837,1838) and hypothesized that there was one basic substance (*Grundstoff*) of proteins, and that it was synthesized by plants and absorbed from them by animals in digestion. Berzelius was an early proponent of this theory and proposed the name "protein" for this substance in a letter dated 10 July 1838

The name protein that I propose for the organic oxide of fibrin and albumin, I wanted to derive from [the Greek word] πρωτειος, because it appears to be the primitive or principal substance of animal nutrition.

Mulder went on to identify the products of protein degradation such as the amino acid, leucine, for which he found a (nearly correct) molecular weight of 131 Da.

The minimum molecular weight suggested by Mulder's analyses was roughly 9 kDa, hundreds of times larger than other molecules being studied. Hence, the chemical structure of proteins (their primary structure) was an active area of research until 1949, when Fred Sanger sequenced insulin. The (correct) theory that proteins were linear polymers of amino acids linked by peptide bonds was proposed independently and simultaneously by Franz Hofmeister and Emil Fischer at the same conference in 1902. However, some scientists were sceptical that such long macromolecules could be stable in solution. Consequently, numerous alternative theories of the protein primary structure were proposed, e.g., the colloidal hypothesis that proteins were assemblies of small molecules, the cyclol hypothesis of Dorothy Wrinch, the diketopiperazine hypothesis of Emil Abderhalden and the pyrrol/piperidine hypothesis of Troensgard (1942). Most of these theories had difficulties in accounting for the fact that the digestion of proteins yielded peptides and amino acids. Proteins were finally shown to be macromolecules of well-defined composition (and not colloidal mixtures) by Theodor Svedberg using analytical ultracentrifugation. The possibility that some proteins are non-covalent associations of such macromolecules was shown by Gilbert Smithson Adair (by measuring the osmotic pressure of hemoglobin) and, later, by Frederic M. Richards in his studies of ribonuclease S. The mass spectrometry of proteins has long been a useful technique for identifying posttranslational modifications and, more recently, for probing protein structure.

Most proteins are difficult to purify in more than milligram quantities, even using the most modern methods. Hence, early studies focused on proteins that could be purified in large quantities, e.g., those of blood, egg white, various toxins, and digestive/metabolic enzymes obtained from slaughterhouses. Many techniques of protein purification were

developed during World War II in a project led by Edwin Joseph Cohn to purify blood proteins to help keep soldiers alive. In the late 1950's, the Armour Hot Dog Co. purified 1 kg (= one million milligrams) of pure bovine pancreatic ribonuclease A and made it freely available to scientists around the world. This generous act made RNase A the main protein for basic research for the next few decades, resulting in several Nobel Prizes.

The study of protein folding began in 1910 with a famous paper by Henrietta Chick and C. J. Martin, in which they showed that the flocculation of a protein was composed of two distinct processes: the precipitation of a protein from solution was *preceded* by another process called denaturation, in which the protein became much less soluble, lost its enzymatic activity and became more chemically reactive. In the mid-1920's, Tim Anson and Alfred Mirsky proposed that denaturation was a reversible process, a correct hypothesis that was initially lampooned by some scientists as "unboiling the egg". Anson also suggested that denaturation was a two-state ("all-or-none") process, in which one fundamental molecular transition resulted in the drastic changes in solubility, enzymatic activity and chemical reactivity; he further noted that the free energy changes upon denaturation were much smaller than those typically involved in chemical reactions. In 1929, Hsien Wu hypothesized that denaturation was protein folding, a purely conformational change that resulted in the exposure of amino acid side chains to the solvent. According to this (correct) hypothesis, exposure of aliphatic and reactive side chains to solvent rendered the protein less soluble and more reactive, whereas the loss of a specific conformation caused the loss of enzymatic activity. Although considered plausible, Wu's hypothesis was not immediately accepted, since so little was known of protein structure and enzymology and other factors could account for the changes in solubility, enzymatic activity and chemical reactivity. In the early 1960's, Chris Anfinsen showed that the folding of ribonuclease A was fully reversible with no external cofactors needed, verifying the "thermodynamic hypothesis" of protein folding that the folded state represents the global minimum of free energy for the protein.

The hypothesis of protein folding was followed by research into the physical interactions that stabilize folded protein structures. The crucial role of hydrophobic interactions was hypothesized by Dorothy Wrinch and Irving Langmuir, as a mechanism that might stabilize her cyclol structures. Although supported by J. D. Bernal and others, this (correct) hypothesis was rejected along with the cyclol hypothesis, which was disproven in the 1930's by Linus Pauling (among others). Instead, Pauling championed the idea that protein structure was stabilized mainly by hydrogen bonds, an idea advanced initially by William Astbury (1933). Remarkably, Pauling's incorrect theory about H-bonds resulted in his *correct* models for the secondary structure elements of proteins, the alpha helix and the beta sheet. The hydrophobic interaction was restored to its correct prominence by a famous article in 1959 by Walter Kauzman on denaturation, based partly on work by Kaj Linderstrom-Lang. The ionic nature of proteins was demonstrated by Bjerrum, Weber and Arne Tiselius, but Linderstrom-Lang showed that the charges were generally accessible to solvent and not bound to each other (1949).

The secondary and low-resolution tertiary structure of globular proteins was investigated initially by hydrodynamic methods, such as analytical ultracentrifugation and flow birefringence. Spectroscopic methods to probe protein structure (such as circular dichroism, fluorescence, near-ultraviolet and infrared absorbance) were developed in the

1950’s. The first atomic-resolution structures of proteins were solved by X-ray crystallography in the 1960’s and by NMR in the 1980’s. As of 2006, the Protein Data Bank has nearly 40,000 atomic-resolution structures of proteins. In more recent times, cryo-electron microscopy of large macromolecular assemblies and computational protein structure prediction of small protein domains are two methods approaching atomic resolution.

RNA

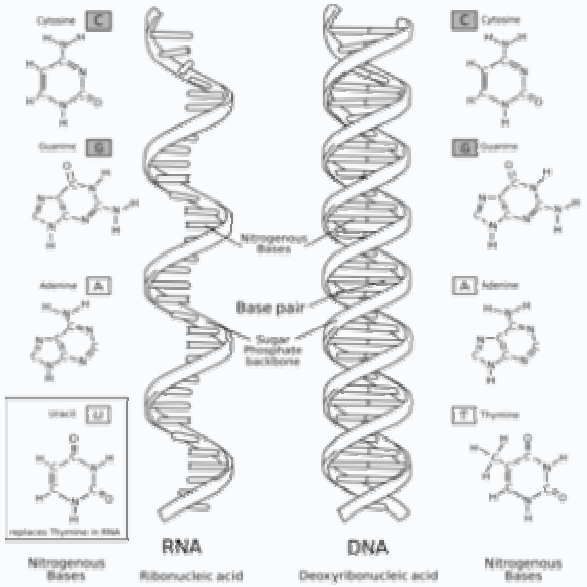
RNA may also refer to the Republic of New Afrika

Ribonucleic acid (**RNA**) is a nucleic acid polymer consisting of nucleotide monomers. RNA nucleotides contain ribose rings and uracil unlike deoxyribonucleic acid (DNA), which contains deoxyribose and thymine. It is transcribed (synthesized) from DNA by enzymes called RNA polymerases and further processed by other enzymes. RNA serves as the template for translation of genes into proteins, transferring amino acids to the ribosome to form proteins, and also translating the transcript into proteins.

History

Nucleic acids were discovered in 1869 by Johann Friedrich Miescher (1844-1895), who called the material 'nuclein' since it was found in the nucleus. It was later discovered that prokaryotic cells, which do not have a nucleus, also contain nucleic acids. The role of RNA in protein synthesis had been suspected since 1939, based on experiments carried out by Torbjörn Caspersson, Jean Brachet and Jack Schultz. Hubert Chantrenne elucidated the messenger role played by RNA in the synthesis of proteins in ribosome. The sequence of the 77 nucleotides of a yeast RNA was found by Robert W. Holley in 1964, winning Holley the 1968 Nobel Prize for Medicine. In 1976, Walter Fiers and his team at the University of Ghent determine the complete nucleotide-sequence of bacteriophage MS2-RNA

Chemical structure



RNA with its nitrogenous bases to the left and DNA to the right.

RNA is primarily made up of four different bases: adenine, guanine, cytosine, and uracil. The first three are the same as those found in DNA, but in DNA thymine replaces uracil as the base complementary to adenine. This base is also a pyrimidine and is very similar to thymine. Uracil is energetically less expensive to produce than thymine, which may account for its use in RNA. In DNA, however, uracil is readily produced by chemical degradation of cytosine, so having thymine as the normal base makes detection and repair of such incipient mutations more efficient. Thus, uracil is appropriate for RNA, where quantity is important but lifespan is not, whereas thymine is appropriate for DNA where maintaining sequence with high fidelity is more critical.

There are also numerous modified bases found in RNA that serve many different roles. Pseudouridine (Ψ) and the DNA nucleoside thymidine are found in various places (most notably in the TΨC loop of every tRNA). Another notable modified base is Inosine (a deaminated Guanine base), which allows a “wobble codon” sequence in tRNA. There are nearly 100 other naturally occurring modified bases, many of which are not fully understood.

Single stranded RNA exhibits a right handed stacking pattern that is stabilized by base stacking.

Comparison with DNA

Unlike DNA, RNA is almost always a single-stranded molecule and has a much shorter chain of nucleotides. RNA contains ribose, rather than the deoxyribose found in DNA (there is a hydroxyl group attached to the pentose ring in the 2’ position whereas RNA has two hydroxyl groups). These hydroxyl groups make RNA less stable than DNA because it is more prone to hydrolysis. Several types of RNA (tRNA, rRNA) contain a great deal of secondary structure, which help promote stability.

Like DNA, most biologically active RNAs including tRNA, rRNA, snRNAs and other non-coding RNAs (such as the SRP RNAs) are extensively base paired to form double stranded helices. Structural analysis of these RNAs have revealed that they are not, “single-stranded” but rather highly structured. Unlike DNA, this structure is not just limited to long double-stranded helices but rather collections of short helices packed together into structures akin to proteins. In this fashion, RNAs can achieve chemical catalysis, like enzymes. For instance, determination of the structure of the ribosome in 2000 revealed that the active site of this enzyme that catalyzes peptide bond formation is composed entirely of RNA.

Synthesis

Synthesis of RNA is usually catalyzed by an enzyme - RNA polymerase, using DNA as a template. Initiation of synthesis begins with the binding of the enzyme to a promoter sequence in the DNA (usually found “upstream” of a gene). The DNA double helix is unwound by the helicase activity of the enzyme. The enzyme then progresses along the

template strand in the 3’ -> 5’ direction, synthesizing a complementary RNA molecule with elongation occurring in the 5’ -> 3’ direction. The DNA sequence also dictates where termination of RNA synthesis will occur.

Biological roles

Messenger RNA (mRNA)

Messenger RNA

Messenger RNA is RNA that carries information from DNA to the ribosome sites of protein synthesis in the cell. Once mRNA has been transcribed from DNA, it is exported from the nucleus into the cytoplasm (in eukaryotes mRNA is “processed” before being exported), where it is bound to ribosomes and translated into protein. After a certain amount of time the message degrades into its component nucleotides, usually with the assistance of RNA polymerases.

Transfer RNA (tRNA)

Transfer RNA

Transfer RNA is a small RNA chain of about 74-93 nucleotides that transfers a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation. It has sites for amino-acid attachment and an anticodon region for codon recognition that binds to a specific sequence on the messenger RNA chain through hydrogen bonding. It is a type of non-coding RNA.

Ribosomal RNA (rRNA)

Ribosomal RNA

Ribosomal RNA is a component of the ribosomes, the protein synthetic factories in the cell. Eukaryotic ribosomes contain four different rRNA molecules: 18S, 5.8S, 28S, and 5S rRNA. Three of the rRNA molecules are synthesized in the nucleolus, and one is synthesized elsewhere. rRNA molecules are extremely abundant and make up at least 80% of the RNA molecules found in a typical eukaryotic cell.

In the cytoplasm, ribsomal RNA and protein combine to form a nucleoprotein called a ribosome. The ribosome binds mRNA and carries out protein synthesis. Several ribosomes may be attached to a single mRNA at any time.

Non-coding RNA or “RNA genes”

Non-coding RNA

RNA genes (sometimes referred to as non-coding RNA or small RNA) are genes that encode RNA that is not translated into a protein. The most prominent examples of RNA genes are transfer RNA (tRNA) and ribosomal RNA (rRNA), both of which are involved in the process of translation. However, since the late 1990s, many new RNA genes have been found, and thus RNA genes may play a much more significant role than previously thought.

In the late 1990s and early 2000, there has been persistent evidence of more complex transcription occurring in mammalian cells (and possibly others). This could point towards a more widespread use of RNA in biology, particularly in gene regulation. A particular class of non-coding RNA, micro RNA, has been found in many metazoans (from *Caenorhabditis elegans* to *Homo sapiens*) and clearly plays an important role in regulating other genes.

First proposed in 2004 by Rassoulzadegan and published in Nature 2006 [Rassoulzadegan M., et al. Nature, doi:10.1038/nature04674 , 2006], RNA is implicated as being part of the germline. If confirmed, this result would significantly alter the present understanding of genetics and lead to many question on DNA-RNA roles and interactions.

Catalytic RNA

Ribozyme

Although RNA contains only four bases, in comparison to the twenty amino acids commonly found in proteins, some RNAs are still able to catalyse chemical reactions. These include cutting and ligating other RNA molecules and also the catalysis of peptide bond formation in the ribosome.

Double-stranded RNA

Double-stranded RNA (or dsRNA) is RNA with two complementary strands, similar to the DNA found in all “higher” cells. dsRNA forms the genetic material of some viruses. In eukaryotes, it acts as a trigger to initiate the process of RNA interference and is present as an intermediate step in the formation of siRNAs (small interfering RNAs). siRNAs are often confused with miRNAs; siRNAs are double-stranded, whereas miRNAs are single-stranded. Although initially single stranded there are regions of intra-molecular association causing hairpin structures in pre-miRNAs; immature miRNAs.

RNA world hypothesis

The RNA world hypothesis proposes that the earliest forms of life relied on RNA both to carry genetic information (like DNA does now) and to catalyze biochemical reactions like an enzyme. According to this hypothesis, descendants of these early lifeforms gradually integrated DNA and proteins into their metabolism.

RNA secondary structures

The functional form of single stranded RNA molecules (like proteins) frequently requires a specific tertiary structure. The scaffold for this structure is provided by secondary structural elements which are hydrogen bonds within the molecule. This leads to several recognizable “domains” of secondary structure like hairpin loops, bulges and internal loops. The secondary structure of RNA molecules can be predicted computationally by calculating the minimum free energies (MFE) structure for all different combinations of hydrogen bondings and domains.

Online tools for MFE structure prediction from single sequences are provided by MFOLD and RNAfold.

Comparative studies of conserved RNA structures are significantly more accurate and provide evolutionary information. Computationally reasonable and accurate online tools for alignment folding are provided by RNAalifold and Pfold.

Glossary of terms

A

Abatement is the reduction or elimination of the degree or intensity of emissions i.e. pollution.

Abiotic Resources are the resources which are considered abiotic and therefore not renewable. Zinc ore and crude oil are examples of abiotic resources.

Acceptable Daily Intake is the highest daily amount of a substance that may be consumed over a lifetime without adverse effects.

Acid Deposition is a comprehensive term for the various ways acidic compounds precipitate from the atmosphere and deposit onto surfaces. It can include:

- wet deposition by means of acid rain, fog, and snow; and
- dry deposition of acidic particles (aerosols).

Acid Rain is rain mixed mainly with nitric and sulphuric acid, that arise from emissions released during the burning of fossil fuels.

Acute Exposure is one or a series of short-term exposures generally lasting less than 24 hours.

Adaptability refers to the degree to which adjustments are possible in practices, processes, or structures of systems to projected or actual changes of climate. Adaptation can be spontaneous or planned, and be carried out in response to or in anticipation of changes in conditions.

Aerobic composting is a method of composting organic waste using bacteria that need oxygen. This requires that the waste be exposed to air either by turning or by forcing air through pipes that pass through the material.

Aerosols are particles of solid or liquid matter that can remain suspended in air from a few minutes to many months depending on the particle size and weight.

Air is a mixture of gases containing about 78 percent nitrogen; 21 percent oxygen; less than 1 percent of carbon dioxide argon, and other gases; and varying amounts of water vapor.

Air Monitoring is the sampling for and measuring of pollutants present in the atmosphere.

Air Pollution is the degradation of air quality resulting from unwanted chemicals or other materials occurring in the air.

Air Pollutants are amounts of foreign and/or natural substances occurring in the atmosphere that may result in adverse effects to humans, animals, vegetation, and/or materials.

Air Quality Standard (AQS) is the prescribed level of a pollutant in the outside air that should not be exceeded during a specific time period to protect public health.

Alternative Fuel are fuels such as methanol, ethanol, natural gas, and liquid petroleum gas that are cleaner and help to meet mobile and stationary emission standards. These fuels may be used in place of less clean fuels for powering motor vehicles.

Ambient Air is the air occurring at a particular time and place outside of structures. Often used interchangeably with outdoor air.

Ambient Air Quality Standards (AAQS) are health and welfare-based standards for outdoor air which identify the maximum acceptable average concentrations of air pollutants during a specified period of time.

Ammonia is a pungent colorless gaseous compound of nitrogen and hydrogen that is very soluble in water and can easily be condensed into a liquid by cold and pressure. Ammonia reacts with NOx to form ammonium nitrate—a major PM2.5 component in the Western United States.

Asbestos is a mineral fiber that can pollute air or water and cause cancer or asbestosis when inhaled. The U.S. EPA has banned or severely restricted its use in manufacturing and construction and the ARB has imposed limits on the amount of asbestos in serpentine rock that is used for surfacing applications.

Atmosphere is the gaseous mass or envelope of air surrounding the Earth. From ground-level up, the atmosphere is further subdivided into the troposphere, stratosphere, mesosphere, and the thermosphere.

Aquaculture, or pisciculture is the breeding or rearing of freshwater or marine fish in captivity, fish farming.

top

B

Binding targets refers to environmental standards that are to be met in the future.

Biodegradable material are any organic material that can be broken down by microorganisms into simpler, more stable compounds. Most organic waste such as foods, paper, etc are biodegradable.

Biogenic Source are biological sources such as plants and animals that emit air pollutants such as volatile organic compounds Examples of biogenic sources include animal management operations, and oak and pine tree forests.

Biomass is the living materials (wood, vegetation, etc.) grown or produced expressly for use as fuel.

Biomass burning is the burning of organic matter for energy production, forest clearing and agricultural purposes. Carbon dioxide is a bi-product of biomass burning

Biomass fuels is wood and forest residues, animal manure and waste, grains, crops and aquatic plants are some common biomass fuels.

Biome is a climatic region characterised by its dominant vegetation.

Bioreserve are the areas with rich ecosystems and species diversity are reserved for conservation.

Biota is the flora and fauna of an area.

Biotic are the resources which are considered biotic and therefore renewable. The rainforests and tigers are examples of biotic resources.

BOD is the biochemical oxygen demand.

Brackish water contains 500 to 3000ppm of sodium chloride.

top

C

Calorie Metric thermal unit is a measure of heat energy; the amount needed to raise the temperature of one kilogram of water by one degree Centigrade. This is the large Calorie (used relating to food energy content) definition. The "small" calorie of fuel research is the amount of energy needed to raise the temperature of one gram of water by one degree Centigrade.

Carbon cycle is the process of removal and uptake of carbon on a global scale. This involves components in food chains, in the atmosphere as carbon dioxide, in the hydrosphere and in the geosphere. The major movement of carbon results from photosynthesis and from respiration. sink and source.

Carbon Dioxide (CO2) is a colorless, odorless gas that occurs naturally in the Earth's atmosphere. Significant quantities are also emitted into the air by fossil fuel combustion and deforestation. It is a greenhouse gas of major concern in the study of global warming. It is estimated that the amount in the air is increasing by 0.27% annually.

Carbon Monoxide (CO) is a colorless, odorless gas resulting from the incomplete combustion of hydrocarbon fuels. CO interferes with the blood's ability to carry oxygen to the body's tissues and results in numerous adverse health effects. Over 80% of the CO emitted in urban areas is contributed by motor vehicles. CO is a criteria air pollutant.

Carbon sequestration generally refers to capturing carbon—in a carbon sink, such as the oceans, or a terrestrial sink such as forests or soils—so as to keep the carbon out of the atmosphere.

Carbon sink is a pool (reservoir) that absorbs or takes up released carbon from another part of the carbon cycle. For example, if the net exchange between the biosphere and the atmosphere is toward the atmosphere, the biosphere is the source, and the atmosphere is the sink.

Carnivore are the flesh eating species.

Carrying capacity is the maximum number of organisms that can use a given area of habitat without degrading the habitat and without causing social stresses that result in the population being reduced.

Catalyst is a substance that can increase or decrease the rate of a chemical reaction between the other chemical species without being consumed in the process.

Catalytic converter is a motor vehicle pollution control device designed to reduce emissions such as oxides of nitrogen hydrocarbons carbon monoxide. Catalytic converters have been required equipment on all new motor vehicles sold in India.

Chlorofluorocarbons (CFCs) is a synthetically produced compounds containing varying amounts of chlorine, fluorine and carbon. Used in industrial processes, refrigeration and as a propellant for gases and sprays. In the atmosphere they are responsible for the depletion of ozone and can destroy as many as 10,000 molecules of ozone in their long lifetime. Their use is now currently restricted under the Montreal Protocol.

Chronic health effect is a health effect that occurs over a relatively long period of time (e.g., months or years).

Climate is the prevalent long term weather conditions in a particular area. Climatic elements include precipitation, temperature, humidity, sunshine and wind velocity and phenomena such as fog, frost, and hail storms.

Climate change can be caused by an increase in the atmospheric concentration of greenhouse gases which inhibit the transmission of some of the sun's energy from the earth's surface to outer space. These gases include carbon dioxide, water vapor, methane, chlorofluorocarbons (CFCs), and other chemicals. The increased concentrations of greenhouse gases result in part from human activity—deforestation; the burning of fossil fuels such as gasoline, oil, coal and natural gas; and the release of CFCs from refrigerators, air conditioners, etc

COD is the chemical oxygen demand.

Combustion is the act or instance of burning some type of fuel such as gasoline to produce energy. Combustion is typically the process that powers automobile engines and power plant generators.

Community is a group of organisms living in a common environment and interdependent.

Compost is the material resulting from composting, which is the natural process of decomposition of organic waste that yields manure or compost, which is very rich in nutrients. Compost, also called humus, is a soil conditioner and a very good fertilizer.

Concentration is the measure of the atmospheric content of a gas, defined in terms of the proportion of the total volume that it accounts for. Greenhouse gases are trace gases in the atmosphere and are usually measured in parts per million by volume (ppmv), parts per billion by volume (ppbv) or parts per trillion (million million) by volume (pptv).

Conservation is the planning and management of resources to secure their long term use and continuity and better their quality, value and diversity. It is the use of less energy, either by using more efficient technologies or by changing wasteful habits.

top

D

Deforestation is the practice or process that result in the long-term change in land-use to non-forest uses. This is often cited as one of the major causes of the enhanced greenhouse effect for two reasons:

- the burning or decomposition of the wood releases carbon dioxide; and
- trees that once removed carbon dioxide from the atmosphere in the process of photosynthesis are lost.

Depletion is the result of the extraction of abiotic resources (non-renewable) from the environmentor the extraction of biotic resources (renewable) faster than they can be renewed.

Desertification are the progressive destruction or degradation of existing vegetative cover to form desert. This can occur due to overgrazing, deforestation, drought and the burning of extensive areas. Once formed, desert can only support a sparse range of vegetation. Climatic effects associated with this phenomenon include increased albedo, reduced atmospheric humidity and greater atmospheric dust loading, which can cause wind erosion and/or atmospheric pollution.

Diversity is the number of species in an area i.e. a community has a high degree of diversity if it contains many species of equal abundance.

top

E

Ecology is the study of the interrelationships between and among organisms and environment.

Efficiency is the ration of desired work-type output to the necessary energy input, in any given energy transformation devide. An efficient LIGHT bulb for example uses most of the input electrical energy to produce light, not heat. An efficient HEAT bulb uses most of its input to produce heat, not light.

El Niño is a climatic phenomenon occurring every 5 to 7 years during Christmas (El Niño means Christ child) in the surface oceans of the SE Pacific. The phenomenon involves seasonal

changes in the direction of Pacific winds and abnormally warm surface ocean temperatures. The changes normally only effect the Pacific region, but major events can disrupt weather patterns over much of the globe. The relationship between these events and global weather patterns are poorly understood and are currently the subject of much research.

Emission is the release of a substance (usually a gas when referring to the subject of climate change) into the atmosphere.

Emission factor is the relationship between the amount of pollution produced and the amount of raw material processed or burned. For mobile sources, the relationship between the amount of pollution produced and the number of vehicle miles traveled. By using the emission factor of a pollutant and specific data regarding quantities of materials used by a given source, it is possible to compute emissions for the source. This approach is used in preparing an emissions inventory.

Endangered species are the plant and animal species in danger of extinction.

Endemic species are the species which are native, restricted or peculiar to an area.

Energy-efficient is electrical lighting devices which produce the same amount of light (lumens) using less electrical energy than incandescent electric light bulbs. Such devices are usually of the fluorescent type, which produce little heat, and may have reflectors to concentrate or direct the light ouput.

Energy efficiency is the amount of fuel needed to sustain a particular level of production or consumption, in an industrial or domestic enterprise. Energy efficiency measures are designed to reduce the amount of fuel consumed, either through greater insulation, less waste, or improved mechanical efficiencies, without losing any of the value of the product or process. Improving energy efficiency is a technological means to reduce emissions of greenhouse gases without increasing production costs.

Energy sources are:

- fossil fuels (coal, oil, gas);
- nuclear (fission and fusion);
- renewables (solar, wind, geothermal, biomass, hydro).

Environment is the surroundings in which an organization operates, including air, water, land, natural resources, flora, fauna, humans, and their interrelations. This definition extends the view from a company focus to the global system.

Environmental effect is any direct or indirect impingement of activities, products and services of an organization upon the environment, whether adverse or beneficial. An environmental effect is the consequence of an environmental intervention in an environmental system.

Environmental impact is any change to the environment, whether adverse or beneficial, wholly or partially resulting from an organization's activities, products or services. An environmental impact addresses an environmental problem.

Estuary is a region where fresh water from a river mixes with salt water from the sea.

Ethanol is Ethyl-alcohol, a volatile alcohol containing two carbon groups. For fuel use, ethanol is produced by fermentation of corn or other plant products.

Evaporative emissions are the emissions from evaporating gasoline, which canoccur during vehicle refueling, vehicle operation, and even when the vehicle is parked. Evaporative emissions can account for two-thirds of the hydrocarbon emissions from gasoline-fueled vehicles on hot summer days.

Exposure is the concentration of the pollutant in the air multiplied by the population exposed to that concentration over a specified time period.

top

F

Fauna is the total animal life in an area.

Flora is the total plant life in an area.

Fluorescent light is a device which uses the glow discharge of an electrified gas for the illuminating element rather than an electrically heated glowing conductive filament.

Fly ash are air-borne solid particles that result from the burning of coal and other solid fuel.

Food chain is a sequence of organisms through which energy is transferred from its ultimate source in a green plant; the predator-prey pathway in which organism eats the next link below and is eaten by the link above.

Food web is a group of interconnecting food chains.

Fossil fuel is any hydrocarbon deposit that can be burned for heat or power such as coal, oil or natural gas. Fossil fuels are formed from the decomposition of ancient animal and plant remains. A major concern is that they emit carbon dioxide into the atmosphere when burnt, a major contributor to the enhanced greenhouse effect.

or

Fossil fuels are the fuels formed eons ago from decayed plants and animals. Oil, coal and natural gas are such fuels.

or

Fossil fuels such as coal, oil, and natural gas are so-called because they are the remains of ancient plant and animal life.

Fuel is a material which is consumed, giving up its molecularly stored energy which is then used for other purposes. e.g. to do work (run a machine).

Fuel efficiency is the amount of work obtained for the amount of fuel consumed. In cars, an efficient fuels allows more miles per gallon of gas than an inefficient fuel.

Fuel cell is an electrochemical cell, which captures the electrical energy of a chemical reaction between fuels such as liquid hydrogen and liquid oxygen and converts it directly and continuously into the energy of a direct electrical current.

Fumes are solid particles under 1 micron in diameter formed as vapors condense, or as chemical reactions take place.

Furnace is combustion chamber; an enclosed structure in which fuel is burned to heat air or material.

top

G

Garbage is the waste that is generated whether in the household, commercial areas, industries, etc.

Gene is a section of a chromosome containing enough DNA to control the formation of a protein; a gene controls the transmission of a hereditary character.

Geothermal is pertaining to heat energy extracted from reservoirs in the earth's interior, as is the use of geysers, molten rock and steam spouts.

Geothermal energy is the heat generated by natural processes within the earth. Chief energy resources are hot dry rock, magma (molten rock), hydrothermal (water/steam from geysers and fissures) and geopressure (water satured with methane under tremendous pressure at great depths).

Global warming is an increase in the temperature of the Earth's troposphere. Global warming has occurred in the past as a result of natural influences, but the term is most often used to refer to the warming predicted by computer models to occur as a result of increased emissions of greenhouse gases.

Greenhouse effect is the progressive, gradual warming of the earth's atmospheric temperature, caused by the insulating effect of carbon dioxide and other greenhouse gases that have proportionately increased in the atmosphere. The greenhouse effect disturbs the way the Earth's climate maintains the balance between incoming and outgoing energy by allowing short-wave radiation from the sun to penetrate through to warm the earth, but preventing the resulting long-wave radiation from escaping back into the atmosphere.
The heat energy is then trapped by the atmosphere, creating a situation similar to that which occurs in a car with its windows rolled up.

Greenhouse gases (GHGs) include the common gases of carbon dioxide and water vapor, but also rarer gases such as methane and chlorofluorocarbons (CFCs) whose properties relate to the transmission or reflection of different types of radiation. The increase in such gases in the atmosphere, which contributes to global warming, is a result of the burning of fossil fuels, the emission of pollutants into the atmosphere, and deforestation.

top

H

Habitat is the natural area in which a species or organism is found.

Hazardous waste is waste that is reactive, toxic, corrosive, or otherwise dangerous to living things and to the environment. Many industrial by products are hazardous.

Haze (Hazy) is a phenomenon that results in reduced visibility due to the scattering of light caused by aerosols. Haze is caused in large part by man-made air pollutants.

Herbivore is an animal that eats plants or parts of plants.

Hydro is that which is produced by or derived from water or the movement of water, as in hydroelectricity.

Hydrocarbons are compounds containing various combinations of hydrogen and carbon atoms. They may be emitted into the air by natural sources (e.g., trees) and as a result of fossil and vegetative fuel combustion, fuel volatilization, and solvent use. Hydrocarbons are a major contributor to smog.

top

I

Incineration is the process of burning solid waste and other material, under controlled conditions, to ash.

Indoor air pollution occur within buildings or other enclosed spaces, as opposed to those occurring in outdoor, or ambient air. Some examples of indoor air pollutants are nitrogen oxides, smoke, asbestos, formaldehyde, and carbon monoxide.

Inorganic waste is waste consisting of materials other than plant or animal matter, such as sand, glass, or any other synthetics.

Insolation is the solar radiant energy received by the earth.

top

J

Joint implementation is a concept where industrialized countries meet their obligations for reducing their greenhouse gas emissions by receiving credits for investing in emissions reductions in developing countries.

top

L

Leachate is the liquid that has seeped through a landfill or a compost pile. If uncontrolled it can contaminate both ground water and surface water.

Lead is a gray-white metal that is soft, malleable, ductile, and resistant to corrosion. Sources of lead resulting in concentrations in the air include industrial sources and crustal weathering of soils followed by fugitive dust emissions. Health effects from exposure to lead include brain and kidney damage and learning disabilities. Lead is the only substance that is currently listed as both a criteria air pollutant and a toxic air contaminant.

top

M

Methane (CH4) is a greenhouse gas, consisting of four molecules of hydrogen and one of carbon. It is produced by anaerobically decomposing solid waste at landfills, paddy fields, etc.

Migration is the regular movements of animals, often between breeding places and winter feeding grounds.

Mudflats are area of mud that do not support any vegetation and are often covered by water.

top

N

Natural resources include renewable (forest, water, soil, wildlife, etc) and nonrenewable (oil, coal, iron ore etc.) resources that are natural assets.

Natural sources are the non-manmade emission sources, including biological and geological sources, wildfires, and windblown dust.

Nitrogen oxides (Oxides of Nitrogen, Nox) is a general term pertaining to compounds of nitric oxide (NO), nitrogen dioxide and other oxides of nitrogen. Nitrogen oxides are typically created during combustion, combustion processes, and are major contributors to smog formation and acid deposition. NO2 is a criteria air pollutant and may result in numerous adverse health effects. They are produced in the emissions of vehicle exhausts and from power stations.

Nitrous oxide (N2O) is a greenhouse gas, consisting of two molecules of nitrogen and one of oxygen.

top

O

Organic Compounds are a large group of chemical compounds containing mainly carbon, hydrogen, nitrogen, and oxygen. All living organisms are made up of organic compounds.

Organic waste is the material that is more directly derived from plant and animal sources, which can generally be decomposed by microorganisms.

Organisms are living thing, animal or plant, that is capable of carrying out life processes.

OTEC - Ocean Thermal Energy Conversion Technology, which uses the temperature differential between warm surface water and cold deep water to run heat engines to produce electrical power.

Oxidant is a substance that brings about oxidation in other substances. Oxidizing agents(oxidants) contain atoms that have suffered electron loss. In oxidizing other substances, these atoms gain electrons. Ozone, which is a primary component of smog is an example of an oxidant.

Oxidation is the chemical reaction of a substance with oxygen or a reaction in which the atoms in an element lose electrons and its valence is correspondingly increased.

Ozone (O3) it consists of three atoms of oxygen bonded together in contrast to normal atmospheric oxygen which consists of two atoms of oxygen. Ozone is formed in the atmosphere and is extremely reactive and thus has a short lifetime. In the stratosphere ozone is both an effective greenhouse gas (absorber of infra-red radiation) and a filter for solar ultra-violet radiation. Ozone in the troposphere can be dangerous since it is toxic to human beings and living matter. Elevated levels of ozone in the troposphere exist in some areas, especially large cities as a result of photochemical reactions of hydrocarbons and nitrogen oxides, released from vehicle emissions and power stations.

Ozone depletion is the reduction in the stratospheric ozone layer. Stratospheric ozone shields the Earth from ultraviolet radiation. The breakdown of certain chlorine and/or bromine-containing compounds that catalytically destroy ozone molecules in the stratosphere can cause a reduction in the ozone layer.

Ozone layer is the ozone in the stratosphere is very diffuse, occupying a region many kilometres in thickness, but is conventionally described as a layer to aid understanding.

top

P

Parasite is an organism that lives upon and at the expense of another organism.

Particulate matter (PM) is any material, except pure water, that exists in the solid or liquid state in the atmosphere. The size of particulate matter can vary from coarse, wind-blown dust particles to fine particle combustion products.

Percolation is the movement of water downwards and radially through the subsurface soil layers, usually continuing downward to the ground water.

Poaching is illegal hunting.

Pollution is the residual discharges of emissions to the air or water following application of emission control devices.

Population is a group of closely related and interbreeding organisms.

Precipitation is any or all form of liquid or solid water particles that fall from the atmosphere and reach the earth's surface. It includes drizzle, rain, snow and hail.

Predator is a animal that feeds on other animals.

Prey is an animal that is eaten by another animal.

Propellant is a gas with a high vapor pressure used to force formulations out of aerosol spraycans. Among the gases used are butanes, propanes and nitrogen ozone hydrocarbons nitrogen oxides, and other chemically reactive compounds which, under certain conditions of weather and sunlight, may result in a murky brown haze that causes adverse health effects. The primary source of smog in California is motor vehicle.

Protected area is any area of land that has legal measures limiting human use of the plants and animals within that area; it includes national parks, game reserves, biosphere reserves, etc.

top

R

Range is the portion of the earth in which a given species is found.

Recharge is the process by which water is added to a reservoir or zone of saturation, often by runoff or percolation from the soil surface.

Recycling is the process of transforming materials (mainly waste) into raw materials for manufacturing new products.

Renewable energy is the energy resource that does not use exhaustible fuels. It is the energy from sources that cannot be used up: sunshine, water flow, wind and vegetation and geothermal energy, as well as some combustible materials, such as landfill gas, biomass, and municipal solid waste.

Resources are the materials found in the environment that can be extracted from the environment in an economic process. There are abiotic resources (non-renewable) and biotic resources (renewable).

Reservoir is any natural or artificial holding area used to store, regulate, or control a substance.

Runoff is that part of precipitation, snow or ice melt or irrigation water that flows from the land to the streams or other water surfaces.

Reuse is when we can use a product more than once in its original form.

top

S

Salinity is the degree of salt in the water or soil.

Smoke is a form of air pollution consisting primarily of particulate matter (i.e., particles released by combustion. Other components of smoke include gaseous air pollutants such as hydrocarbons oxides of nitrogen, and carbon monoxide. Sources of smoke may include fossil fuel combustion, agricultural burning, and other combustion processes.

Sulfur dioxide (SO2) is a strong smelling, colorless gas that is formed by the combustion of fossil fuels. Power plants, which may use coal or oil high in sulfur content, can be major sources of SO2. SO2 and other sulfur oxides contribute to the problem of acid deposition. SO2is a criteria air pollutant.

Surface water is all water naturally open to the atmosphere.

Sustainable development implies economic growth together with the protection of environmental quality, each reinforcing the other. The essence of this form of development is a stable relationship between human activities and the natural world, which does not diminish the prospects for future generations to enjoy a quality of life at least as good as our own.

Swamp is an area that is saturated with water for much of the time but in which soil surface is not deeply submerged.

Symbiosis is the living together in more or less close association of two dissimilar organisms, in which one or both derive benefit from the relationship.

top

T

TDS is the total dissolved solids.

Terrestrial is that which is of, or related to the land.

Tidal marsh is a low, flat, marshland traversed by inter laced channels and subject to tidal inundation. The only vegetation present is halo-tolerant bushes and grasses.

Turbidity is the cloudiness of a liquid caused by suspended matter.

top

V

Vapor is the gaseous phase of liquids or solids at atmospheric temperature and pressure.

Vertebrate is any of a major group of animals (fish, amphibians, reptiles, birds and mammals) with a segmented spinal column (backbone).

Volatile organic compounds(VOCs) are the carbon-containing compounds that evaporate into the air (with a few exceptions). VOCs contribute to the formation of smog and/or may themselves be toxic. VOCs often have an odor, and some examples include gasoline, alcohol, and the solvents used in paints.

top

W

Wetland is temporarily or permanently inundated terrestrial systems which border aquatic systems. It also includes the shallow systems such as estuaries, swamps, salt marshes, flood plains and the lagoons and coastal lakes.

Weathering is the physical and chemical breakdown of rocks due to natural process.

Water table is the level of ground water.

Weather is the result of unequal heating of the earth's atmosphere, as a function of terrain, latitude, time-of-year and other secondary factors.