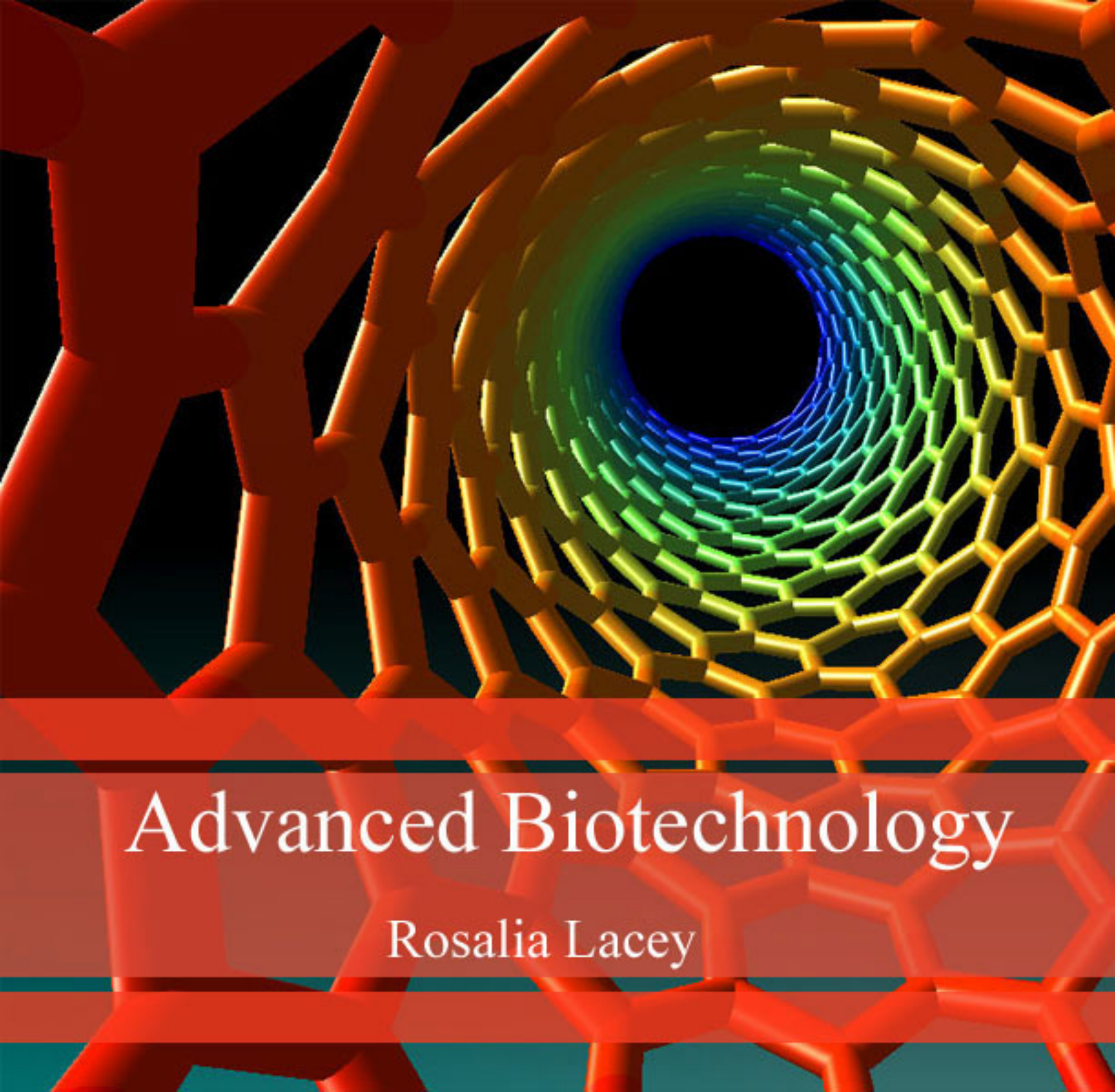

Advanced Biotechnology

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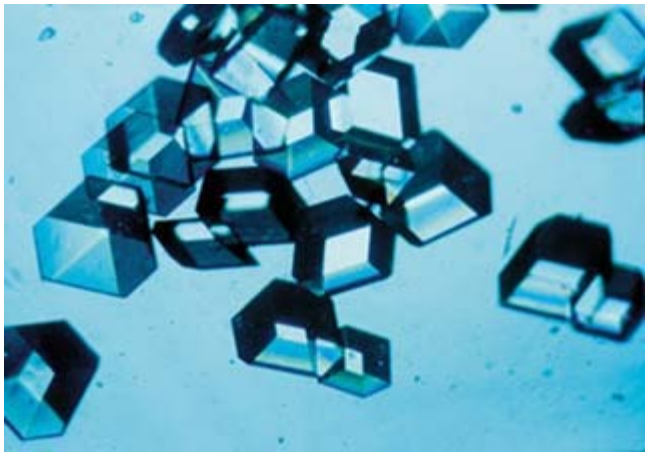
Biotechnology



Insulin crystals.

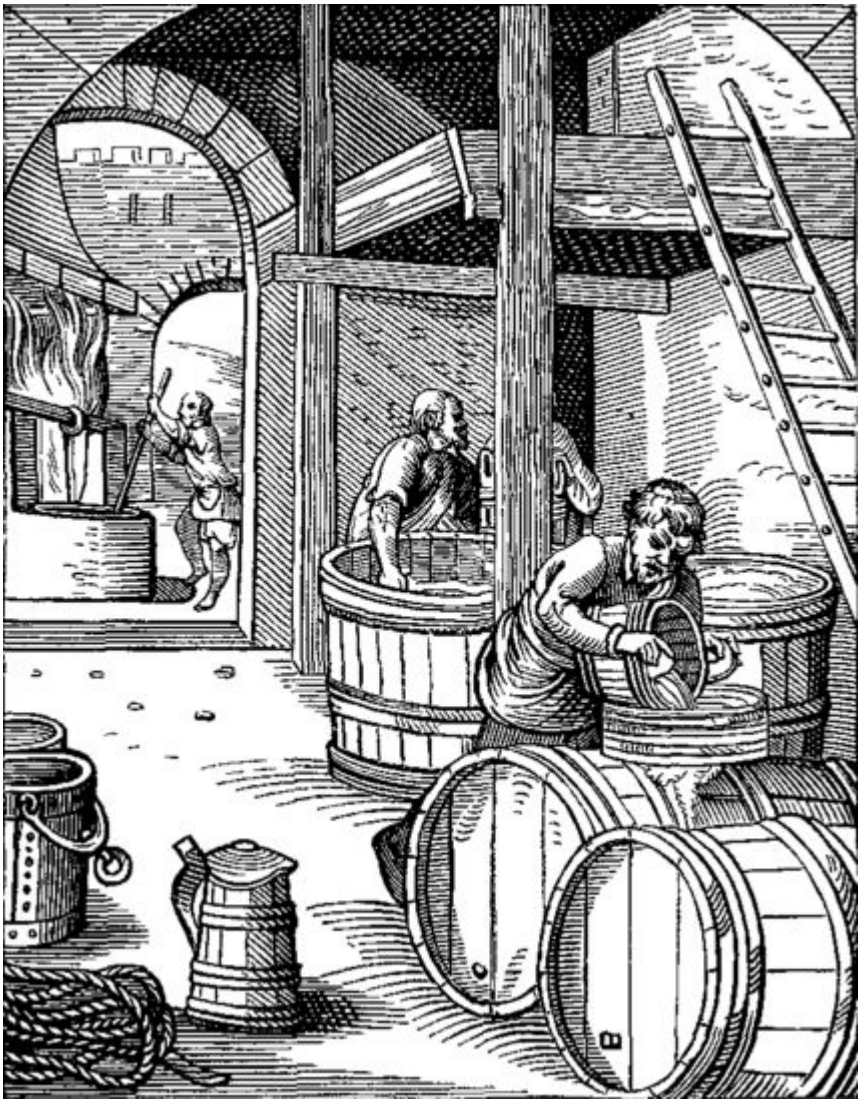
Biotechnology is a field of applied biology that involves the use of living organisms and bioprocesses in engineering, technology, medicine and other fields requiring bioproducts. Modern use of similar terms includes genetic engineering as well as cell- and tissue culture technologies. The concept encompasses a wide range of procedures (and history) for modifying living organisms according to human purposes - going back to domestication of animals, cultivation of plants, and "improvements" to these through breeding programs that employ artificial selection and hybridization. By comparison to biotechnology, bioengineering is generally thought of as a related field with its emphasis more on higher systems approaches (not necessarily altering or using biological materials *directly*) for interfacing with and utilizing living things. The United Nations Convention on Biological Diversity defines biotechnology as:

"Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use."

Biotechnology draws on the pure biological sciences (genetics, microbiology, animal cell culture, molecular biology, biochemistry, embryology, cell biology) and in many

instances is also dependent on knowledge and methods from outside the sphere of biology (chemical engineering, bioprocess engineering, information technology, biorobotics). Conversely, modern biological sciences (including even concepts such as molecular ecology) are intimately entwined and dependent on the methods developed through biotechnology and what is commonly thought of as the life sciences industry.

History



Brewing was an early application of biotechnology

Biotechnology is not limited to medical/health applications (*unlike* Biomedical Engineering, which includes much biotechnology). Although not normally thought of as biotechnology, agriculture clearly fits the broad definition of "*using a biotechnological system to make products*" such that the cultivation of plants may be viewed as the earliest biotechnological enterprise. 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 crops, having the highest yields, to produce enough food to support a growing population. Other uses of biotechnology were required as the crops and fields became increasingly large and difficult to maintain. Specific organisms and organism by-products 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 and breeding them with other plants—one of the first forms of biotechnology. Cultures such as those in Mesopotamia, Egypt, and India developed the process of brewing beer. It is still done by the same basic method of using malted grains (containing enzymes) to convert starch from grains into sugar and then adding specific yeasts to produce beer. In this process the carbohydrates in the grains were broken down into alcohols such as ethanol. Ancient Indians also used the juices of the plant Ephedra vulgaris and used to call it Soma. 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 Pasteur's work in 1857, it is still the first use of biotechnology to convert a food source into another form.

For thousands of years, humans have used selective breeding to improve production of crops and livestock to use them for food. In selective breeding, organisms with desirable characteristics are mated to produce offspring with the same characteristics. For example, this technique was used with corn to produce the largest and sweetest crops.

In the early twentieth century scientists gained a greater understanding of microbiology and explored ways of manufacturing specific products. In 1917, Chaim Weizmann first used a pure microbiological culture in an industrial process, that of manufacturing corn starch using *Clostridium acetobutylicum,* to produce acetone, which the United Kingdom desperately needed to manufacture explosives during World War I.

Biotechnology has led to the development of antibiotics. In 1928, Alexander Fleming discovered the mold Penicillium. His work led to the purification of the antibiotic by Howard Florey, Ernst Boris Chain and Norman Heatley penicillin. In 1940, penicillin became available for medicinal use to treat bacterial infections in humans.

The field of modern biotechnology is thought to have largely begun on June 16, 1980, when the United States Supreme Court ruled that a genetically modified microorganism could be patented in the case of *Diamond v. Chakrabarty*. Indian-born Ananda Chakrabarty, working for General Electric, had developed a bacterium (derived from the *Pseudomonas* genus) capable of breaking down crude oil, which he proposed to use in treating oil spills.

Revenue in the industry is expected to grow by 12.9% in 2008. Another factor influencing the biotechnology sector's success is improved intellectual property rights legislation—and enforcement—worldwide, as well as strengthened demand for medical and pharmaceutical products to cope with an ageing, and ailing, U.S. population.

Rising demand for biofuels is expected to be good news for the biotechnology sector, with the Department of Energy estimating ethanol usage could reduce U.S. petroleum-derived fuel consumption by up to 30% by 2030. The biotechnology sector has allowed the U.S. farming industry to rapidly increase its supply of corn and soybeans—the main inputs into biofuels—by developing genetically modified seeds which are resistant to pests and drought. By boosting farm productivity, biotechnology plays a crucial role in ensuring that biofuel production targets are met.

Applications

A rose plant that began as cells grown in a tissue culture

Biotechnology has applications in four major industrial areas, including health care (medical), crop production and agriculture, non food (industrial) uses of crops and other products (e.g. biodegradable plastics, vegetable oil, biofuels), and environmental uses.

For example, one application of biotechnology is the directed use of organisms for the manufacture of organic products (examples include beer and milk products). Another example is using naturally present bacteria by the mining industry in bioleaching. Biotechnology is also used to recycle, treat waste, clean up sites contaminated by industrial activities (bioremediation), and also to produce biological weapons.

A series of derived terms have been coined to identify several branches of biotechnology, for example:

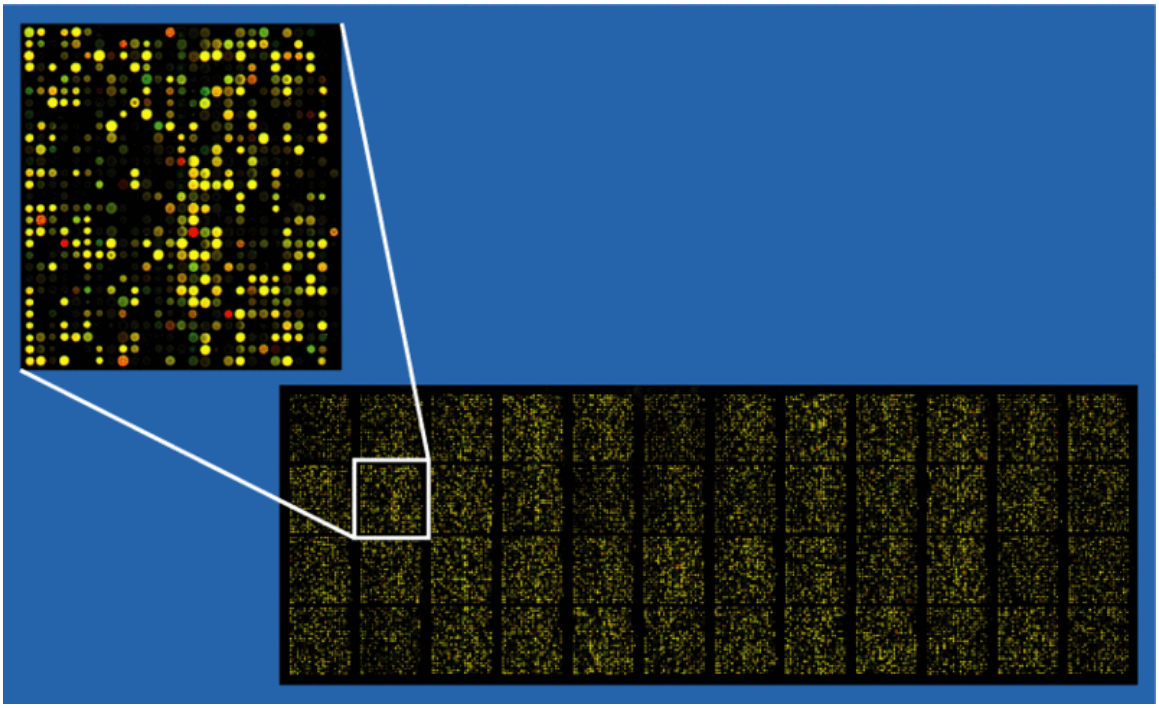
- **Bioinformatics** is an interdisciplinary field which addresses biological problems using computational techniques, and makes the rapid organization and analysis of biological data possible. The field may also be referred to as *computational biology*, and can be defined as, "conceptualizing biology in terms of molecules and then applying informatics techniques to understand and organize the information associated with these molecules, on a large scale." Bioinformatics 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.
- **Blue biotechnology** is a term that has been used to describe the marine and aquatic applications of biotechnology, but its use is relatively rare.
- **Green biotechnology** is biotechnology applied to agricultural processes. An example would be the selection and domestication of plants via micropropagation. Another example is the designing of transgenic plants to grow under specific environments in the presence (or absence) of 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 ending the need of 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.
- **Red biotechnology** is applied to medical processes. Some examples are the designing of organisms to produce antibiotics, and the engineering of genetic cures through genetic manipulation.
- **White biotechnology**, also known as industrial biotechnology, is biotechnology applied to industrial processes. An example is the designing of an organism to produce a useful chemical. Another example is the using of enzymes as industrial catalysts to either produce valuable chemicals or destroy hazardous/polluting chemicals. White biotechnology tends to consume less in resources than traditional processes used to produce industrial goods. The investment and economic output of all of these types of applied biotechnologies is termed as **bioeconomy**.

Medicine

In medicine, modern biotechnology finds promising applications in such areas as

- drug production
- pharmacogenomics
- gene therapy
- genetic testing: techniques in molecular biology detect genetic diseases. To test the developing fetus for Down syndrome, Amniocentesis and chorionic villus sampling can be used.

Pharmacogenomics



DNA Microarray chip – Some can do as many as a million blood tests at once

Pharmacogenomics is the study of how the genetic inheritance of an individual affects his/her body's response to drugs. It is a coined word derived from the words "pharmacology" and "genomics". It is hence the study of the relationship between pharmaceuticals and genetics. The vision of pharmacogenomics is to be able to design and produce drugs that are adapted to each person's genetic makeup.

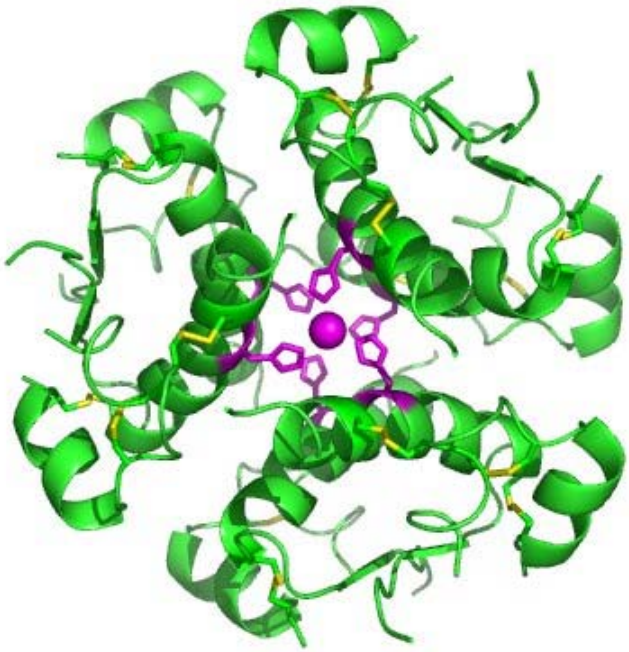
Pharmacogenomics results in the following benefits:

1. Development of tailor-made medicines. Using pharmacogenomics, pharmaceutical companies can create drugs based on the proteins, enzymes and RNA molecules that are associated with specific genes and diseases. These tailor-

made drugs promise not only to maximize therapeutic effects but also to decrease damage to nearby healthy cells.

2. More accurate methods of determining appropriate drug dosages. Knowing a patient's genetics will enable doctors to determine how well his/ her body can process and metabolize a medicine. This will maximize the value of the medicine and decrease the likelihood of overdose.
3. Improvements in the drug discovery and approval process. The discovery of potential therapies will be made easier using genome targets. Genes have been associated with numerous diseases and disorders. With modern biotechnology, these genes can be used as targets for the development of effective new therapies, which could significantly shorten the drug discovery process.
4. Better vaccines. Safer vaccines can be designed and produced by organisms transformed by means of genetic engineering. These vaccines will elicit the immune response without the attendant risks of infection. They will be inexpensive, stable, easy to store, and capable of being engineered to carry several strains of pathogen at once.

Pharmaceutical products



Computer-generated image of insulin hexamers highlighting the threefold symmetry, the zinc ions holding it together, and the histidine residues involved in zinc binding.

Most traditional pharmaceutical drugs are relatively simple molecules that have been found primarily through trial and error to treat the symptoms of a disease or illness. Biopharmaceuticals are large biological molecules known as proteins and these usually

target the underlying mechanisms and pathways of a malady (but not always, as is the case with using insulin to treat type 1 diabetes mellitus, as that treatment merely addresses the symptoms of the disease, not the underlying cause which is autoimmunity); it is a relatively young industry. They can deal with targets in humans that may not be 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 larger molecules are created by living cells such as those found in the human body: for example, bacteria cells, yeast cells, animal or plant 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 synthetic 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 used to manufacture certain pharmaceuticals. Another promising new biotechnology application is the development of plant-made pharmaceuticals.

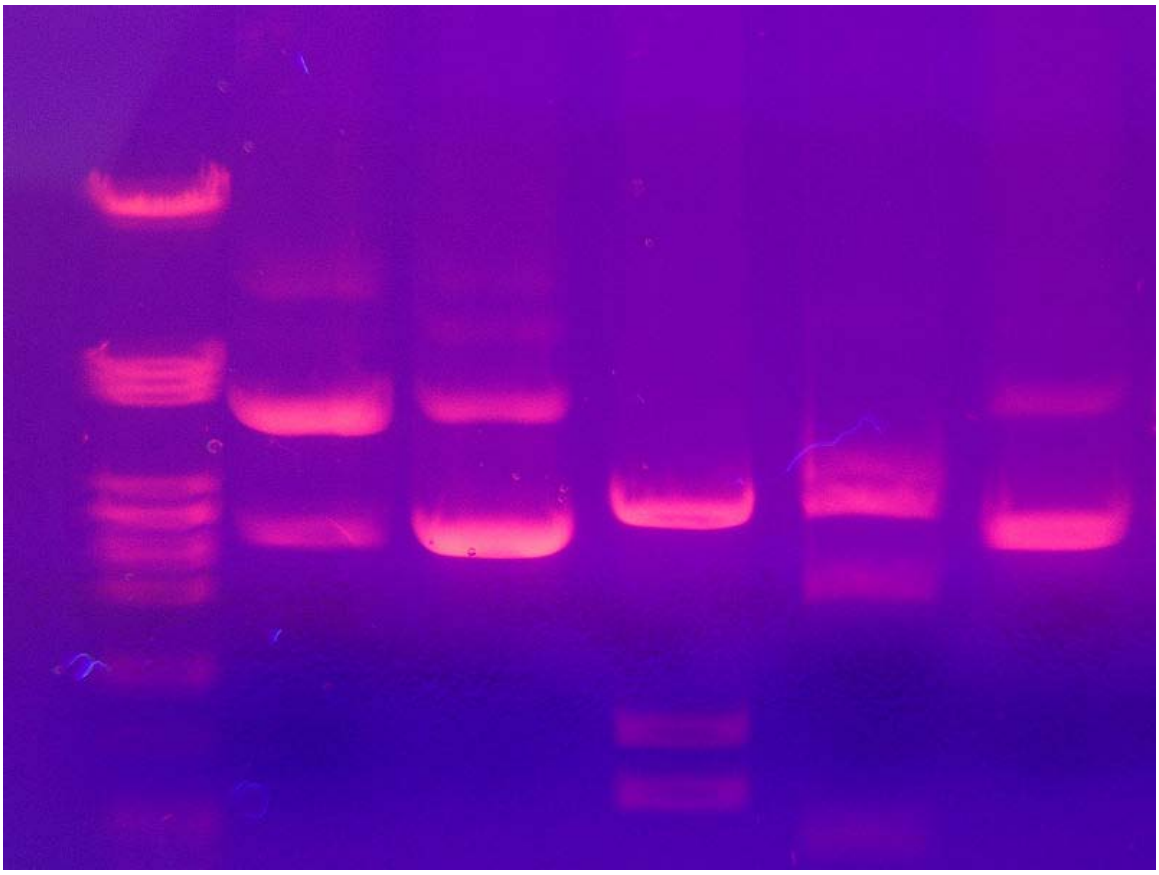
Biotechnology is also commonly associated with landmark breakthroughs in new medical therapies to treat hepatitis B, hepatitis C, cancers, arthritis, haemophilia, bone fractures, multiple sclerosis, and cardiovascular disorders. The biotechnology industry has also been instrumental in developing molecular diagnostic devices that can be used to define the target patient population for a given biopharmaceutical. Herceptin, for example, was 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.

Modern biotechnology can be used to manufacture existing medicines relatively easily and cheaply. The first genetically engineered products were medicines designed to treat human diseases. To cite one example, in 1978 Genentech developed synthetic humanized insulin by joining its gene with a plasmid vector inserted into the bacterium *Escherichia coli*. Insulin, widely used for the treatment of diabetes, was previously extracted from the pancreas of abattoir animals (cattle and/or pigs). The resulting genetically engineered bacterium enabled the production of vast quantities of synthetic human insulin at relatively low cost. According to a 2003 study undertaken by the International Diabetes Federation (IDF) on the access to and availability of insulin in its member countries, synthetic 'human' insulin is considerably more expensive in most countries where both synthetic 'human' and animal insulin are commercially available: e.g. within European countries the average price of synthetic 'human' insulin was twice as high as the price of pork insulin. Yet in its position statement, the IDF writes that "there is no overwhelming evidence to prefer one species of insulin over another" and "[modern, highly purified] animal insulins remain a perfectly acceptable alternative.

Modern biotechnology has evolved, making it possible to produce more easily and relatively cheaply human growth hormone, clotting factors for hemophiliacs, fertility drugs, erythropoietin and other drugs. Most drugs today are based on about 500 molecular targets. Genomic knowledge of the genes involved in diseases, disease

pathways, and drug-response sites are expected to lead to the discovery of thousands more new targets.

Genetic testing



Gel electrophoresis

Genetic testing involves the direct examination of the DNA molecule itself. A scientist scans a patient's DNA sample for mutated sequences.

There are two major types of gene tests. In the first type, a researcher may design short pieces of DNA ("probes") whose sequences are complementary to the mutated sequences. These probes will seek their complement among the base pairs of an individual's genome. If the mutated sequence is present in the patient's genome, the probe will bind to it and flag the mutation. In the second type, a researcher may conduct the gene test by comparing the sequence of DNA bases in a patient's gene to disease in healthy individuals or their progeny.

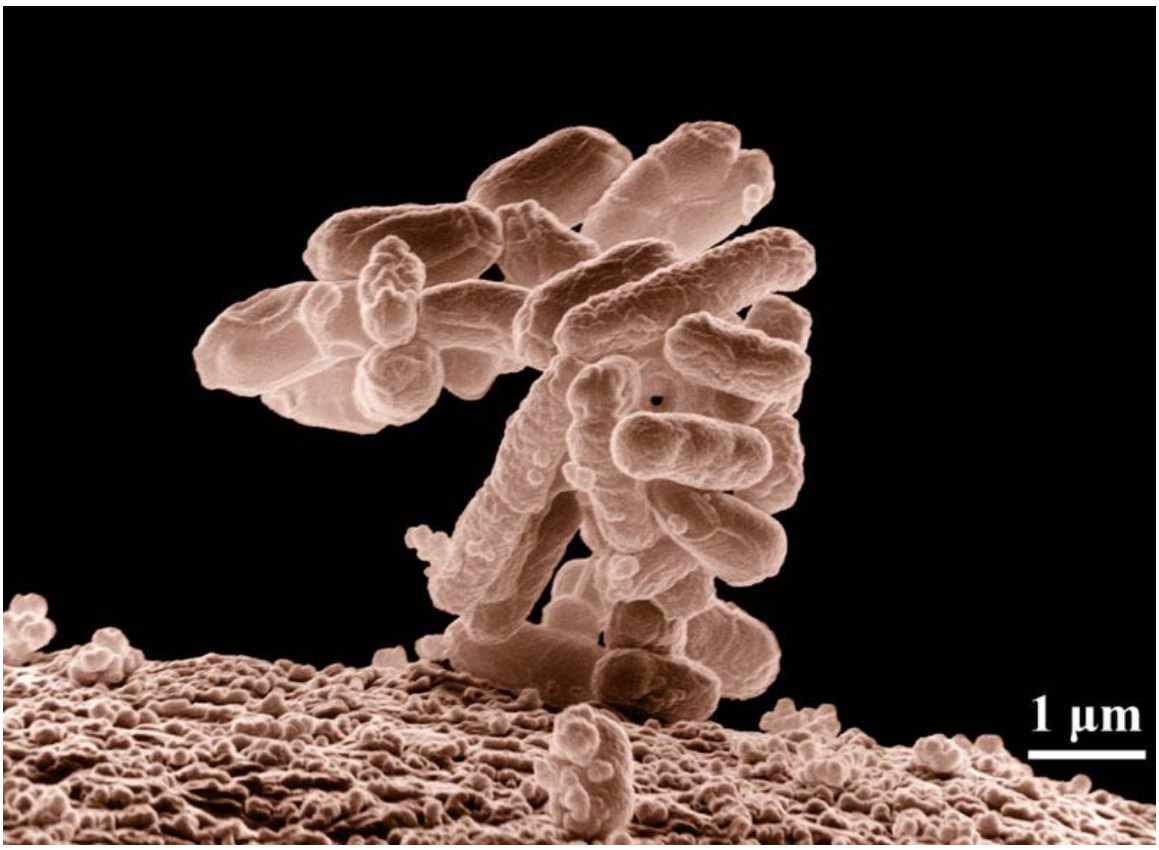
Genetic testing is now used for:

- Carrier screening, or the identification of unaffected individuals who carry one copy of a gene for a disease that requires two copies for the disease to manifest;
- Confirmational diagnosis of symptomatic individuals;

- Determining sex;
- Forensic/identity testing;
- Newborn screening;
- Prenatal diagnostic screening;
- Presymptomatic testing for estimating the risk of developing adult-onset cancers;
- Presymptomatic testing for predicting adult-onset disorders.

Some genetic tests are already available, although most of them are used in developed countries. The tests currently available can detect mutations associated with rare genetic disorders like cystic fibrosis, sickle cell anemia, and Huntington's disease. Recently, tests have been developed to detect mutation for a handful of more complex conditions such as breast, ovarian, and colon cancers. However, gene tests may not detect every mutation associated with a particular condition because many are as yet undiscovered, and the ones they do detect may present different risks to different people and populations.

Controversial questions

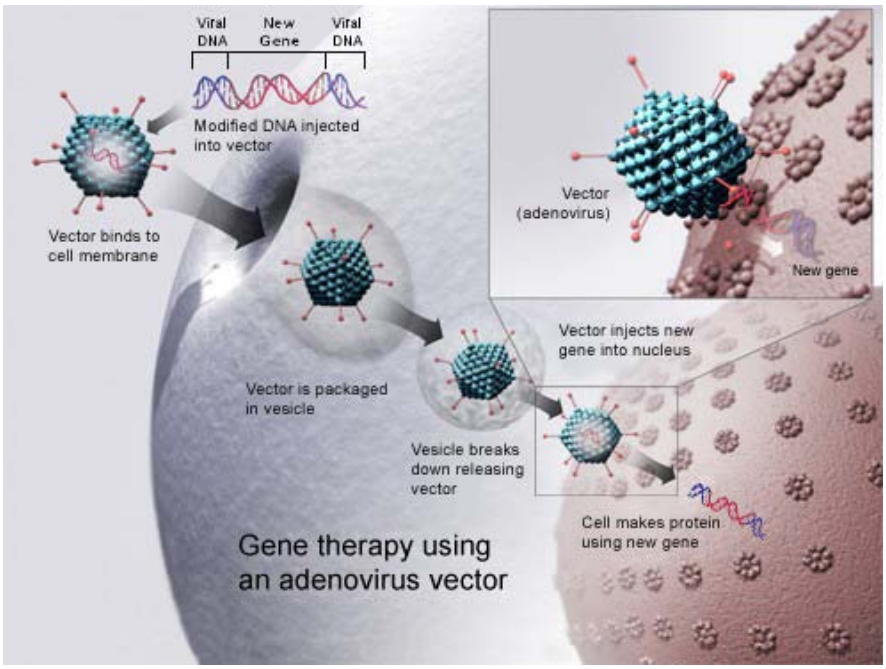


The bacterium *Escherichia coli* is routinely genetically engineered.

The absence of privacy and anti-discrimination legal protections in most countries can lead to discrimination in employment or insurance or other use of personal genetic information. This raises questions such as whether genetic privacy is different from medical privacy.

1. Reproductive issues. These include the use of genetic information in reproductive decision-making and the possibility of genetically altering reproductive cells that may be passed on to future generations. For example, germline therapy changes the genetic make-up of an individual's descendants. Thus, any error in technology or judgment may have far-reaching consequences (though the same can also happen through natural reproduction). Ethical issues like designed babies and human cloning have also given rise to controversies between and among scientists and bioethicists, especially in the light of past abuses with eugenics.
2. Clinical issues. These center on the capabilities and limitations of doctors and other health-service providers, people identified with genetic conditions, and the general public in dealing with genetic information.
3. Effects on social institutions. Genetic tests reveal information about individuals and their families. Thus, test results can affect the dynamics within social institutions, particularly the family.
4. Conceptual and philosophical implications regarding human responsibility, free will vis-à-vis genetic determinism, and the concepts of health and disease.

Gene therapy



Gene therapy using an Adenovirus vector. A new gene is inserted into an adenovirus vector, which is used to introduce the modified DNA into a human cell. If the treatment is successful, the new gene will make a functional protein.

Gene therapy may be used for treating, or even curing, genetic and acquired diseases like cancer and AIDS by using normal genes to supplement or replace defective genes or to bolster a normal function such as immunity. It can be used to target somatic (i.e., body) or gametes (i.e., egg and sperm) cells. In somatic gene therapy, the genome of the recipient is changed, but this change is not passed along to the next generation. In

contrast, in germline gene therapy, the egg and sperm cells of the parents are changed for the purpose of passing on the changes to their offspring.

There are basically two ways of implementing a gene therapy treatment:

1. *Ex vivo*, which means "outside the body" – Cells from the patient's blood or bone marrow are removed and grown in the laboratory. They are then exposed to a virus carrying the desired gene. The virus enters the cells, and the desired gene becomes part of the DNA of the cells. The cells are allowed to grow in the laboratory before being returned to the patient by injection into a vein.
2. *In vivo*, which means "inside the body" – No cells are removed from the patient's body. Instead, vectors are used to deliver the desired gene to cells in the patient's body.

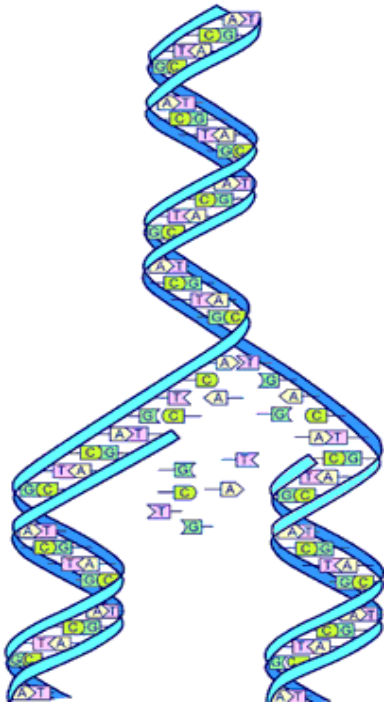
As of June 2001, more than 500 clinical gene-therapy trials involving about 3,500 patients have been identified worldwide. Around 78% of these are in the United States, with Europe having 18%. These trials focus on various types of cancer, although other multigenic diseases are being studied as well. Recently, two children born with severe combined immunodeficiency disorder ("SCID") were reported to have been cured after being given genetically engineered cells.

Gene therapy faces many obstacles before it can become a practical approach for treating disease. At least four of these obstacles are as follows:

1. *Gene delivery tools*. Genes are inserted into the body using gene carriers called vectors. The most common vectors now are viruses, which have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists manipulate the genome of the virus by removing the disease-causing genes and inserting the therapeutic genes. However, while viruses are effective, they can introduce problems like toxicity, immune and inflammatory responses, and gene control and targeting issues. In addition, in order for gene therapy to provide permanent therapeutic effects, the introduced gene needs to be integrated within the host cell's genome. Some viral vectors effect this in a random fashion, which can introduce other problems such as disruption of an endogenous host gene.
2. *High costs*. Since gene therapy is relatively new and at an experimental stage, it is an expensive treatment to undertake. This explains why current studies are focused on illnesses commonly found in developed countries, where more people can afford to pay for treatment. It may take decades before developing countries can take advantage of this technology.
3. *Limited knowledge of the functions of genes*. Scientists currently know the functions of only a few genes. Hence, gene therapy can address only some genes that cause a particular disease. Worse, it is not known exactly whether genes have more than one function, which creates uncertainty as to whether replacing such genes is indeed desirable.

4. *Multigene disorders and effect of environment*. Most genetic disorders involve more than one gene. Moreover, most diseases involve the interaction of several genes and the environment. For example, many people with cancer not only inherit the disease gene for the disorder, but may have also failed to inherit specific tumor suppressor genes. Diet, exercise, smoking and other environmental factors may have also contributed to their disease.

Human Genome Project



DNA Replication image from the Human Genome Project (HGP)

The Human Genome Project is an initiative of the U.S. Department of Energy ("DOE") that aims to generate a high-quality reference sequence for the entire human genome and identify all the human genes.

The DOE and its predecessor agencies were assigned by the U.S. Congress to develop new energy resources and technologies and to pursue a deeper understanding of potential health and environmental risks posed by their production and use. In 1986, the DOE announced its Human Genome Initiative. Shortly thereafter, the DOE and National Institutes of Health developed a plan for a joint Human Genome Project ("HGP"), which officially began in 1990.

The HGP was originally planned to last 15 years. However, rapid technological advances and worldwide participation accelerated the completion date to 2003 (making it a 13 year project). Already it has enabled gene hunters to pinpoint genes associated with more than 30 disorders.

Cloning

Cloning involves the removal of the nucleus from one cell and its placement in an unfertilized egg cell whose nucleus has either been deactivated or removed.

There are two types of cloning:

1. Reproductive cloning. After a few divisions, the egg cell is placed into a uterus where it is allowed to develop into a fetus that is genetically identical to the donor of the original nucleus.
2. Therapeutic cloning. The egg is placed into a Petri dish where it develops into embryonic stem cells, which have shown potentials for treating several ailments.

In February 1997, cloning became the focus of media attention when Ian Wilmut and his colleagues at the Roslin Institute announced the successful cloning of a sheep, named Dolly, from the mammary glands of an adult female. The cloning of Dolly made it apparent to many that the techniques used to produce her could someday be used to clone human beings. This stirred a lot of controversy because of its ethical implications.

Agriculture

Crop yield

Using the techniques of modern biotechnology, one or two genes (Smartstax from Monsanto in collaboration with Dow AgroSciences will use 8, starting in 2010) may be transferred to a highly developed crop variety to impart a new character that would increase its yield. However, while increases in crop yield are the most obvious applications of modern biotechnology in agriculture, it is also the most difficult one. Current genetic engineering techniques work best for effects that are controlled by a single gene. Many of the genetic characteristics associated with yield (e.g., enhanced growth) are controlled by a large number of genes, each of which has a minimal effect on the overall yield. There is, therefore, much scientific work to be done in this area.

Reduced vulnerability of crops to environmental stresses

Crops containing genes that will enable them to withstand biotic and abiotic stresses may be developed. For example, drought and excessively salty soil are two important limiting factors in crop productivity. Biotechnologists are studying plants that can cope with these extreme conditions in the hope of finding the genes that enable them to do so and eventually transferring these genes to the more desirable crops. One of the latest developments is the identification of a plant gene, At-DBF2, from Arabidopsis thaliana, a tiny weed that is often used for plant research because it is very easy to grow and its genetic code is well mapped out. When this gene was inserted into tomato and tobacco cells, the cells were able to withstand environmental stresses like salt, drought, cold and heat, far more than ordinary cells. If these preliminary results prove successful in larger trials, then At-DBF2 genes can help in engineering crops that can better withstand harsh

environments. Researchers have also created transgenic rice plants that are resistant to rice yellow mottle virus (RYMV). In Africa, this virus destroys majority of the rice crops and makes the surviving plants more susceptible to fungal infections.

Increased nutritional qualities

Proteins in foods may be modified to increase their nutritional qualities. Proteins in legumes and cereals may be transformed to provide the amino acids needed by human beings for a balanced diet. A good example is the work of Professors Ingo Potrykus and Peter Beyer in creating Golden rice (discussed below).

Improved taste, texture or appearance of food

Modern biotechnology can be used to slow down the process of spoilage so that fruit can ripen longer on the plant and then be transported to the consumer with a still reasonable shelf life. This alters the taste, texture and appearance of the fruit. More importantly, it could expand the market for farmers in developing countries due to the reduction in spoilage. However, there is sometimes a lack of understanding by researchers in developed countries about the actual needs of prospective beneficiaries in developing countries. For example, engineering soybeans to resist spoilage makes them less suitable for producing tempeh which is a significant source of protein that depends on fermentation. The use of modified soybeans results in a lumpy texture that is less palatable and less convenient when cooking.

The first genetically modified food product was a tomato which was transformed to delay its ripening. Researchers in Indonesia, Malaysia, Thailand, Philippines and Vietnam are currently working on delayed-ripening papaya in collaboration with the University of Nottingham and Zeneca.

Biotechnology in cheese production: enzymes produced by micro-organisms provide an alternative to animal rennet – a cheese coagulant – and an alternative supply for cheese makers. This also eliminates possible public concerns with animal-derived material, although there are currently no plans to develop synthetic milk, thus making this argument less compelling. Enzymes offer an animal-friendly alternative to animal rennet. While providing comparable quality, they are theoretically also less expensive.

About 85 million tons of wheat flour is used every year to bake bread. By adding an enzyme called maltogenic amylase to the flour, bread stays fresher longer. Assuming that 10–15% of bread is thrown away as stale, if it could be made to stay fresh another 5–7 days then perhaps 2 million tons of flour per year would be saved. Other enzymes can cause bread to expand to make a lighter loaf, or alter the loaf in a range of ways.

Reduced dependence on fertilizers, pesticides and other agrochemicals

Most of the current commercial applications of modern biotechnology in agriculture are on reducing the dependence of farmers on agrochemicals. For example, *Bacillus*

thuringiensis (Bt) is a soil bacterium that produces a protein with insecticidal qualities. Traditionally, a fermentation process has been used to produce an insecticidal spray from these bacteria. In this form, the Bt toxin occurs as an inactive protoxin, which requires digestion by an insect to be effective. There are several Bt toxins and each one is specific to certain target insects. Crop plants have now been engineered to contain and express the genes for Bt toxin, which they produce in its active form. When a susceptible insect ingests the transgenic crop cultivar expressing the Bt protein, it stops feeding and soon thereafter dies as a result of the Bt toxin binding to its gut wall. Bt corn is now commercially available in a number of countries to control corn borer (a lepidopteran insect), which is otherwise controlled by spraying (a more difficult process).

Crops have also been genetically engineered to acquire tolerance to broad-spectrum herbicide. The lack of herbicides with broad-spectrum activity and no crop injury was a consistent limitation in crop weed management. Multiple applications of numerous herbicides were routinely used to control a wide range of weed species detrimental to agronomic crops. Weed management tended to rely on preemergence—that is, herbicide applications were sprayed in response to expected weed infestations rather than in response to actual weeds present. Mechanical cultivation and hand weeding were often necessary to control weeds not controlled by herbicide applications. The introduction of herbicide-tolerant crops has the potential of reducing the number of herbicide active ingredients used for weed management, reducing the number of herbicide applications made during a season, and increasing yield due to improved weed management and less crop injury. Transgenic crops that express tolerance to glyphosate, glufosinate and bromoxynil have been developed. These herbicides can now be sprayed on transgenic crops without inflicting damage on the crops while killing nearby weeds.

From 1996 to 2001, herbicide tolerance was the most dominant trait introduced to commercially available transgenic crops, followed by insect resistance. In 2001, herbicide tolerance deployed in soybean, corn and cotton accounted for 77% of the 626,000 square kilometres planted to transgenic crops; Bt crops accounted for 15%; and "stacked genes" for herbicide tolerance and insect resistance used in both cotton and corn accounted for 8%.

Production of novel substances in crop plants

Biotechnology is being applied for novel uses other than food. For example, oilseed can be modified to produce fatty acids for detergents, substitute fuels and petrochemicals. Potatoes, tomatoes, rice tobacco, lettuce, safflowers, and other plants have been genetically engineered to produce insulin and certain vaccines. If future clinical trials prove successful, the advantages of edible vaccines would be enormous, especially for developing countries. The transgenic plants may be grown locally and cheaply. Homegrown vaccines would also avoid logistical and economic problems posed by having to transport traditional preparations over long distances and keeping them cold while in transit. And since they are edible, they will not need syringes, which are not only an additional expense in the traditional vaccine preparations but also a source of infections if contaminated. In the case of insulin grown in transgenic plants, it is well-

established that the gastrointestinal system breaks the protein down therefore this could not currently be administered as an edible protein. However, it might be produced at significantly lower cost than insulin produced in costly bioreactors. For example, Calgary, Canada-based SemBioSys Genetics, Inc. reports that its safflower-produced insulin will reduce unit costs by over 25% or more and approximates a reduction in the capital costs associated with building a commercial-scale insulin manufacturing facility of over $100 million, compared to traditional biomanufacturing facilities.

Criticism

There is another side to the agricultural biotechnology issue. It includes increased herbicide usage and resultant herbicide resistance, "super weeds," residues on and in food crops, genetic contamination of non-GM crops which hurt organic and conventional farmers, etc.

Biological engineering

Biotechnological engineering or biological engineering is a branch of engineering that focuses on biotechnologies and biological science. It includes different disciplines such as biochemical engineering, biomedical engineering, bio-process engineering, biosystem engineering and so on. Because of the novelty of the field, the definition of a bioengineer is still undefined. However, in general it is an integrated approach of fundamental biological sciences and traditional engineering principles.

Biotechnologists are often employed to scale up bio processes from the laboratory scale to the manufacturing scale. Moreover, as with most engineers, they often deal with management, economic and legal issues. Since patents and regulation (e.g., U.S. Food and Drug Administration regulation in the U.S.) are very important issues for biotech enterprises, bioengineers are often required to have knowledge related to these issues.

The increasing number of biotech enterprises is likely to create a need for bioengineers in the years to come. Many universities throughout the world are now providing programs in bioengineering and biotechnology (as independent programs or specialty programs within more established engineering fields).

Bioremediation and biodegradation

Biotechnology is being used to engineer and adapt organisms especially microorganisms in an effort to find sustainable ways to clean up contaminated environments. The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. Biological processes play a major role in the removal of contaminants and biotechnology is taking advantage of the astonishing catabolic versatility of microorganisms to degrade/convert such compounds. New methodological breakthroughs in sequencing, genomics, proteomics, bioinformatics and imaging are producing vast amounts of information. In the field of Environmental Microbiology, genome-based

global studies open a new era providing unprecedented *in silico* views of metabolic and regulatory networks, as well as clues to the evolution of degradation pathways and to the molecular adaptation strategies to changing environmental conditions. Functional genomic and metagenomic approaches are increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds and they will certainly accelerate the development of bioremediation technologies and biotransformation processes.

Marine environments are especially vulnerable since oil spills of coastal regions and the open sea are poorly containable and mitigation is difficult. In addition to pollution through human activities, millions of tons of petroleum enter the marine environment every year from natural seepages. Despite its toxicity, a considerable fraction of petroleum oil entering marine systems is eliminated by the hydrocarbon-degrading activities of microbial communities, in particular by a remarkable recently discovered group of specialists, the so-called hydrocarbonoclastic bacteria (HCCB).

Biotechnology regulations

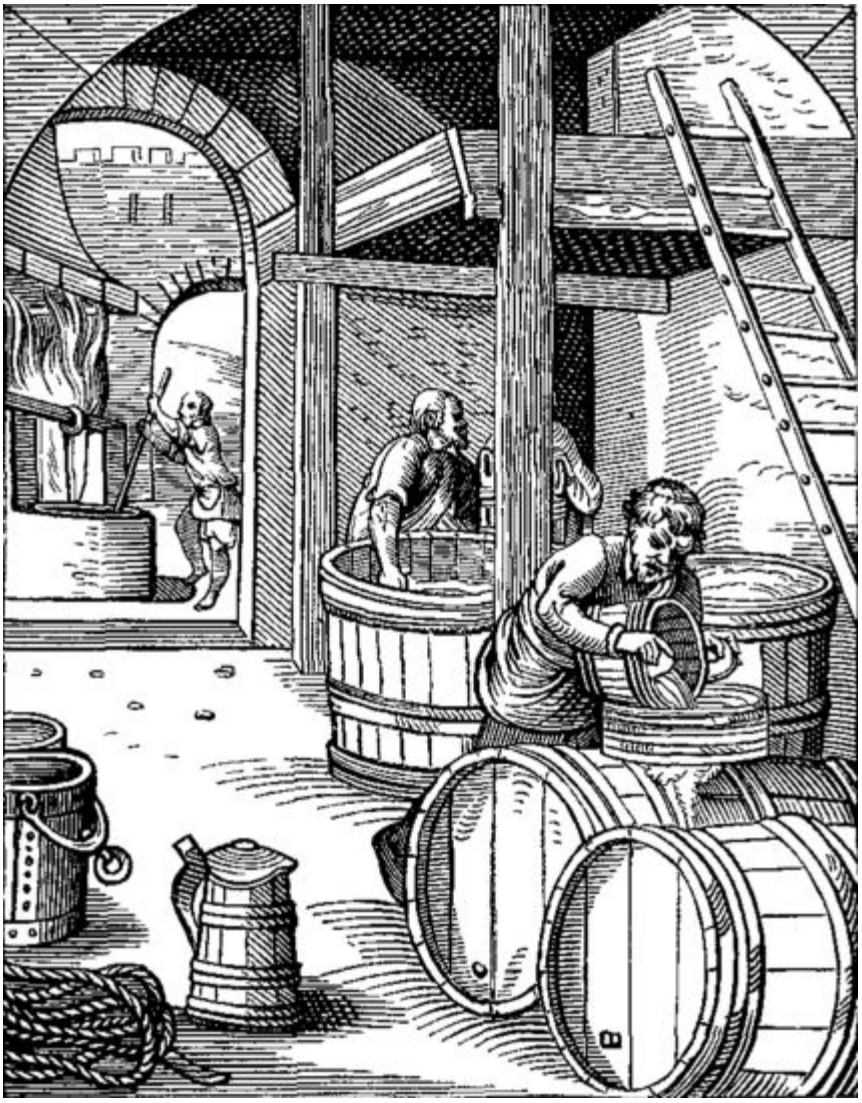
The National Institute of Health was the first federal agency to assume regulatory responsibility in the United States. The Recombinant DNA Advisory Committee of the NIH published guidelines for working with recombinant DNA and recombinant organisms in the laboratory. Nowadays, the agencies that are responsible for the biotechnology regulation are: US Department of Agriculture (USDA) that regulates plant pests and medical preparation from living organisms, Environmental Protection Agency (EPA) that regulates pesticides and herbicides, and the Food and Drug Administration (FDA) which ensures that the food and drug products are safe and effective

Education

In 1988, after prompting from the United States Congress, the National Institute of General Medical Sciences (National Institutes of Health) instituted a funding mechanism for biotechnology training. Universities nationwide compete for these funds to establish Biotechnology Training Programs (BTPs). Each successful application is generally funded for five years then must be competitively renewed. Graduate students in turn compete for acceptance into a BTP; if accepted then stipend, tuition and health insurance support is provided for two or three years during the course of their PhD thesis work. Nineteen institutions offer NIGMS supported BTPs. Biotechnology training is also offered at the undergraduate level and in community colleges.

Chapter- 2

History of Biotechnology



Brewing was an early example of biotechnology

Biotechnology is the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services. From its inception, biotechnology has maintained a close relationship with society. Although now most often associated with the development of remarkable drugs, historically biotechnology has been principally associated with food, addressing such issues as malnutrition and famine. The history of biotechnology begins with zymotechnology, which commenced with a focus on brewing techniques for beer. By World War I, however, zymotechnology would expand to tackle larger industrial issues, and the potential of industrial fermentation gave rise to biotechnology.However, both the single-cell protein and gasohol projects failed to progress due to varying issues including public resistance, a changing economic scene, and shifts in political power.

Yet the formation of a new field, genetic engineering, would soon bring biotechnology to the forefront of science in society, and the intimate relationship between the scientific community, the public, and the government would ensue. These debates gained exposure in 1975 at the Asilomar Conference, where Joshua Lederberg was the most outspoken supporter for this emerging field in biotechnology. By as early as 1978, with the synthesis of synthetic human insulin, Lederberg's claims would prove valid, and the biotechnology industry grew rapidly. Each new scientific advance became a media event designed to capture public support, and by the 1980s, biotechnology grew into a promising real industry. In 1988, only five proteins from genetically engineered cells had been approved as drugs by the United States Food and Drug Administration (FDA), but this number would skyrocket to over 125 by the end of the 1990s.

The field of genetic engineering remains a heated topic of discussion in today's society with the advent of gene therapy, stem cell research, cloning, and genetically-modified food. While it seems only natural nowadays to link pharmaceutical drugs as solutions to health and societal problems, this relationship of biotechnology serving social needs began centuries ago.

Origins of biotechnology

Biotechnology arose from the field of zymotechnology, which began as a search for a better understanding of industrial fermentation, particularly beer. Beer was an important industrial, and not just social, commodity. In late 19th century Germany, brewing contributed as much to the gross national product as steel, and taxes on alcohol proved to be significant sources of revenue to the government. In the 1860s, institutes and remunerative consultancies were dedicated to the technology of brewing. The most famous was the private Carlsberg Institute, founded in 1875, which employed Emil Christian Hansen, who pioneered the pure yeast process for the reliable production of consistent beer. Less well known were private consultancies that advised the brewing industry. One of these, the Zymotechnic Institute, was established in Chicago by the German-born chemist John Ewald Siebel.

The heyday and expansion of zymotechnology came in World War I in response to industrial needs to support the war. Max Delbruck grew yeast on an immense scale

during the war to meet 60 percent of Germany's animal feed needs. Compounds of another fermentation product, lactic acid, made up for a lack of hydraulic fluid, glycerol. On the Allied side the Russian chemist Chaim Weizmann used starch to eliminate Britain's shortage of acetone, a key raw material in explosives, by fermenting maize to acetone. The industrial potential of fermentation was outgrowing its traditional home in brewing, and "zymotechnology" soon gave way to "biotechnology."

With food shortages spreading and resources fading, some dreamed of a new industrial solution. The Hungarian Karl Ereky coined the word "biotechnology" in Hungary during 1919 to describe a technology based on converting raw materials into a more useful product. He built a slaughterhouse for a thousand pigs and also a fattening farm with space for 50,000 pigs, raising over 100,000 pigs a year. The enterprise was enormous, becoming one of the largest and most profitable meat and fat operations in the world. In a book entitled *Biotechnologie*, Ereky further developed a theme that would be reiterated through the 20th century: biotechnology could provide solutions to societal crises, such as food and energy shortages. For Ereky, the term "biotechnologie" indicated the process by which raw materials could be biologically upgraded into socially useful products.

This catchword spread quickly after the First World War, as "biotechnology" entered German dictionaries and was taken up abroad by business-hungry private consultancies as far away as the United States. In Chicago, for example, the coming of prohibition at the end of World War I encouraged biological industries to create opportunities for new fermentation products, in particular a market for nonalcoholic drinks. Emil Siebel, the son of the founder of the Zymotechnic Institute, broke away from his father's company to establish his own called the "Bureau of Biotechnology," which specifically offered expertise in fermented nonalcoholic drinks.

The belief that the needs of an industrial society could be met by fermenting agricultural waste was an important ingredient of the "chemurgic movement." Fermentation-based processes generated products of ever-growing utility. In the 1940s, penicillin was the most dramatic. While it was discovered in England, it was produced industrially in the U.S. using a deep fermentation process originally developed in Peoria, Illinois. The enormous profits and the public expectations penicillin engendered caused a radical shift in the standing of the pharmaceutical industry. Doctors used the phrase "miracle drug", and the historian of its wartime use, David Adams, has suggested that to the public penicillin represented the perfect health that went together with the car and the dream house of wartime American advertising. In the 1950s, steroids were synthesized using fermentation technology. In particular, cortisone promised the same revolutionary ability to change medicine as penicillin had.

Penicillin was viewed as "Maricarmen" that brought enormous profits and public expectations.

Single-cell protein and gasohol projects

Even greater expectations of biotechnology were raised during the 1960s by a process that grew single-cell protein. When the so-called protein gap threatened world hunger, producing food locally by growing it from waste seemed to offer a solution. It was the possibilities of growing microorganisms on oil that captured the imagination of scientists, policy makers, and commerce. Major companies such as British Petroleum (BP) staked their futures on it. In 1962, BP built a pilot plant at Cap de Lavera in Southern France to publicize its product, Toprina. Initial research work at Lavera was done by Alfred Champagnat, In 1963, construction started on BP's second pilot plant at Grangemouth Oil Refinery n Britain.

As there was no well-accepted term to describe the new foods, in 1966 the term "single-cell protein" (SCP) was coined at MIT to provide an acceptable and exciting new title, avoiding the unpleasant connotations of microbial or bacterial.

The "food from oil" idea became quite popular by the 1970s, when facilities for growing yeast fed by n-paraffins were built in a number of countries. The Soviets were particularly enthusiastic, opening large "BVK" (*belkovo-vitaminny kontsentrat*, i.e., "protein-vitamin concentrate") plants next to their oil refineries in Kstovo (1973) and Kirishi (1974).

By the late 1970s, however, the cultural climate had completely changed, as the growth in SCP interest had taken place against a shifting economic and cultural scene (136).

First, the price of oil rose catastrophically in 1974, so that its cost per barrel was five times greater than it had been two years earlier. Second, despite continuing hunger around the world, anticipated demand also began to shift from humans to animals. The program had begun with the vision of growing food for Third World people, yet the product was instead launched as an animal food for the developed world. The rapidly rising demand for animal feed made that market appear economically more attractive. The ultimate downfall of the SCP project, however, came from public resistance.

This was particularly vocal in Japan, where production came closest to fruition. For all their enthusiasm for innovation and traditional interest in microbiologically produced foods, the Japanese were the first to ban the production of single-cell proteins. The Japanese ultimately were unable to separate the idea of their new "natural" foods from the far from natural connotation of oil. These arguments were made against a background of suspicion of heavy industry in which anxiety over minute traces of petroleum was expressed. Thus, public resistance to an unnatural product led to the end of the SCP project as an attempt to solve world hunger.

Also, in 1989 in the USSR, the public environmental concerns made the government decide to close down (or convert to different technilogies) all 8 paraffin-fed-yeast plants that the Soviet Ministry of Microbiological Industry had by that time.

In the late 1970s, biotechnology offered another possible solution to a societal crisis. The escalation in the price of oil in 1974 increased the cost of the Western world's energy tenfold. In response, the U.S. government promoted the production of gasohol, gasoline with 10 percent alcohol added, as an answer to the energy crisis. In 1979, when the Soviet Union sent troops to Afghanistan, the Carter administration cut off its supplies to agricultural produce in retaliation, creating a surplus of agriculture in the U.S. As a result, fermenting the agricultural surpluses to synthesize fuel seemed to be an economical solution to the shortage of oil threatened by the Iran-Iraq war. Before the new direction could be taken, however, the political wind changed again: the Reagan administration came to power in January 1981 and, with the declining oil prices of the 1980s, ended support for the gasohol industry before it was born.

Biotechnology seemed to be the solution for major social problems, including world hunger and energy crises. In the 1960s, radical measures would be needed to meet world starvation, and biotechnology seemed to provide an answer. However, the solutions proved to be too expensive and socially unacceptable, and solving world hunger through SCP food was dismissed. In the 1970s, the food crisis was succeeded by the energy crisis, and here too, biotechnology seemed to provide an answer. But once again, costs proved prohibitive as oil prices slumped in the 1980s. Thus, in practice, the implications of biotechnology were not fully realized in these situations. But this would soon change with the rise of genetic engineering.

Genetic engineering

The origins of biotechnology culminated with the birth of genetic engineering. There were two key events that have come to be seen as scientific breakthroughs beginning the era that would unite genetics with biotechnology. One was the 1953 discovery of the structure of DNA, by Watson and Crick, and the other was the 1973 discovery by Cohen and Boyer of a recombinant DNA technique by which a section of DNA was cut from the plasmid of an E. coli bacterium and transferred into the DNA of another. This approach could, in principle, enable bacteria to adopt the genes and produce proteins of other organisms, including humans. Popularly referred to as "genetic engineering," it came to be defined as the basis of new biotechnology.

Genetic engineering proved to be a topic that thrust biotechnology into the public scene, and the interaction between scientists, politicians, and the public defined the work that was accomplished in this area. Technical developments during this time were revolutionary and at times frightening. In December 1967, the first heart transplant by Christian Barnard reminded the public that the physical identity of a person was becoming increasingly problematic. While poetic imagination had always seen the heart at the center of the soul, now there was the prospect of individuals being defined by other people's hearts. During the same month, Arthur Kornberg announced that he had managed to biochemically replicate a viral gene. "Life had been synthesized," said the head of the National Institutes of Health. Genetic engineering was now on the scientific agenda, as it was becoming possible to identify genetic characteristics with diseases such as beta thalassemia and sickle-cell anemia.

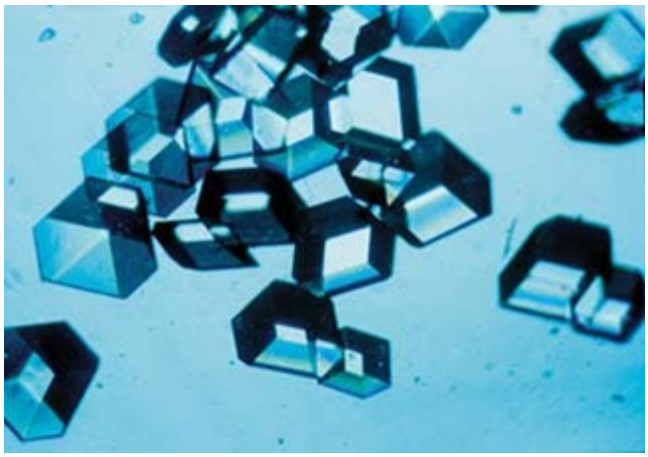
Responses to scientific achievements were colored by cultural skepticism. Scientists and their expertise were looked upon with suspicion. In 1968, an immensely popular work, *The Biological Time Bomb*, was written by the British journalist Gordon Rattray Taylor. The author's preface saw Kornberg's discovery of replicating a viral gene as a route to lethal doomsday bugs. The publisher's blurb for the book warned that within ten years, "You may marry a semi-artificial man or woman…choose your children's sex…tune out pain…change your memories…and live to be 150 if the scientific revolution doesn't destroy us first." The book ended with a chapter called "The Future – If Any." While it is rare for current science to be represented in the movies, in this period of "Star Trek", science fiction and science fact seemed to be converging. "Cloning" became a popular word in the media. Woody Allen satirized the cloning of a person from a nose in his 1973 movie *Sleeper*, and cloning Adolf Hitler from surviving cells was the theme of the 1976 novel by Ira Levin, *The Boys from Brazil*.

In response to these public concerns, scientists, industry, and governments increasingly linked the power of recombinant DNA to the immensely practical functions that biotechnology promised. One of the key scientific figures that attempted to highlight the promising aspects of genetic engineering was Joshua Lederberg, a Stanford professor and Nobel laureate. While in the 1960s "genetic engineering" described eugenics and work involving the manipulation of the human genome, Lederberg stressed research that would involve microbes instead. Lederberg emphasized the importance of focusing on curing

living people. Lederberg's 1963 paper, "Biological Future of Man" suggested that, while molecular biology might one day make it possible to change the human genotype, "what we have overlooked is euphenics, the engineering of human development." Lederberg constructed the word "euphenics" to emphasize changing the phenotype after conception rather than the genotype which would affect future generations.

With the discovery of recombinant DNA by Cohen and Boyer in 1973, the idea that genetic engineering would have major human and societal consequences was born. In July 1974, a group of eminent molecular biologists headed by Paul Berg wrote to *Science* suggesting that the consequences of this work were so potentially destructive that there should be a pause until its implications had been thought through. This suggestion was explored at a meeting in February 1975 at California's Monterey Peninsula, forever immortalized by the location, Asilomar. Its historic outcome was an unprecedented call for a halt in research until it could be regulated in such a way that the public need not be anxious, and it led to a 16-month moratorium until National Institutes of Health (NIH) guidelines were established.

Joshua Lederberg was the leading exception in emphasizing, as he had for years, the potential benefits. At Asilomar, in an atmosphere favoring control and regulation, he circulated a paper countering the pessimism and fears of misuses with the benefits conferred by successful use. He described "an early chance for a technology of untold importance for diagnostic and therapeutic medicine: the ready production of an unlimited variety of human proteins. Analogous applications may be foreseen in fermentation process for cheaply manufacturing essential nutrients, and in the improvement of microbes for the production of antibiotics and of special industrial chemicals." In June 1976, the 16-month moratorium on research expired with the Director's Advisory Committee (DAC) publication of the NIH guidelines of good practice. They defined the risks of certain kinds of experiments and the appropriate physical conditions for their pursuit, as well as a list of things too dangerous to perform at all. Moreover, modified organisms were not to be tested outside the confines of a laboratory or allowed into the environment.



Synthetic insulin crystals synthesized using recombinant DNA technology

Atypical as Lederberg was at Asilomar, his optimistic vision of genetic engineering would soon lead to the development of the biotechnology industry. Over the next two years, as public concern over the dangers of recombinant DNA research grew, so too did interest in its technical and practical applications. Curing genetic diseases remained in the realms of science fiction, but it appeared that producing human simple proteins could be good business. Insulin, one of the smaller, best characterized and understood proteins, had been used in treating type 1 diabetes for a half century. It had been extracted from animals in a chemically slightly different form from the human product. Yet, if one could produce synthetic human insulin, one could meet an existing demand with a product whose approval would be relatively easy to obtain from regulators. In the period 1975 to 1977, synthetic "human" insulin represented the aspirations for new products that could be made with the new biotechnology. Microbial production of synthetic human insulin was finally announced in September 1978 and was produced by a startup company, Genentech., although that company did not commercialize the product themselves, instead, it licensed the production method to Eli Lilly and Company.

The radical shift in the connotation of "genetic engineering" from an emphasis on the inherited characteristics of people to the commercial production of proteins and therapeutic drugs was nurtured by Joshua Lederberg. His broad concerns since the 1960s had been stimulated by enthusiasm for science and its potential medical benefits. Countering calls for strict regulation, he expressed a vision of potential utility. Against a belief that new techniques would entail unmentionable and uncontrollable consequences for humanity and the environment, a growing consensus on the economic value of recombinant DNA emerged.

Biotechnology and industry

A Genentech-sponsored sign declaring South San Francisco to be "The Birthplace of Biotechnology."

With ancestral roots in industrial microbiology that date back centuries, the new biotechnology industry grew rapidly beginning in the mid-1970s. Each new scientific advance became a media event designed to capture investment confidence and public support. Although market expectations and social benefits of new products were frequently overstated, many people were prepared to see genetic engineering as the next great advance in technological progress. By the 1980s, biotechnology characterized a nascent real industry, providing titles for emerging trade organizations such as the Industrial Biotechnology Association.

The main focus of attention after insulin were the potential profit makers in the pharmaceutical industry: human growth hormone and what promised to be a miraculous cure for viral diseases, interferon. Cancer was a central target in the 1970s because increasingly the disease was linked to viruses. By 1980, a new company, Biogen, had produced interferon through recombinant DNA. The emergence of interferon and the possibility of curing cancer raised money in the community for research and increased the enthusiasm of an otherwise uncertain and tentative society. Moreover, to the 1970s plight of cancer was added AIDS in the 1980s, offering an enormous potential market for

a successful therapy, and more immediately, a market for diagnostic tests based on monoclonal antibodies. By 1988, only five proteins from genetically engineered cells had been approved as drugs by the United States Food and Drug Administration (FDA): synthetic insulin, human growth hormone, hepatitis B vaccine, alpha-interferon, and tissue plasminogen activator (TPa), for lysis of blood clots. By the end of the 1990s, however, 125 more genetically engineered drugs would be approved.

Genetic engineering also reached the agricultural front as well. There was tremendous progress since the market introduction of the genetically engineered Flavr Savr tomato in 1994. Ernst and Young reported that in 1998, 30% of the U.S. soybean crop was expected to be from genetically engineered seeds. In 1998, about 30% of the US cotton and corn crops were also expected to be products of genetic engineering.

Genetic engineering in biotechnology stimulated hopes for both therapeutic proteins, drugs and biological organisms themselves, such as seeds, pesticides, engineered yeasts, and modified human cells for treating genetic diseases. From the perspective of its commercial promoters, scientific breakthroughs, industrial commitment, and official support were finally coming together, and biotechnology became a normal part of business. No longer were the proponents for the economic and technological significance of biotechnology the iconoclasts. Their message had finally become accepted and incorporated into the policies of governments and industry.

Global trends

According to Burrill and Company, an industry investment bank, over $350 billion has been invested in biotech since the emergence of the industry, and global revenues rose 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. By 2007 and into 2008, though, a downturn in the fortunes of biotech emerged, at least in the United Kingdom, as the result of declining investment in the face of failure of biotech pipelines to deliver and a consequent downturn in return on investment.

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 where as 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.

Chapter- 3

Biopharmaceutical and Industrial Biotechnology

Biopharmaceutical

Biopharmaceuticals are medical drugs produced using biotechnology. They are proteins (including antibodies), nucleic acids (DNA, RNA or antisense oligonucleotides) used for therapeutic or *in vivo* diagnostic purposes, and are produced by means other than direct extraction from a native (non-engineered) biological source.

The first such substance approved for therapeutic use was biosynthetic 'human' insulin made via recombinant DNA technology. Sometimes referred to as rHI, under the trade name Humulin, was developed by Genentech, but licensed to Eli Lilly and Company, who manufactured and marketed the product starting in 1982.

Bioreactors for producing proteins, NRC Biotechnology Research Institute, Montréal, Canada

The large majority of biopharmaceutical products are pharmaceuticals that are derived from life forms. Small molecule drugs are not typically regarded as biopharmaceutical in nature by the industry. However members of the press and the business and financial community often extend the definition to include pharmaceuticals not created through biotechnology. That is, the term has become an oft-used buzzword for a variety of different companies producing new, apparently high-tech pharmaceutical products. Research and development investment in new medicines by the biopharmaceutical industry stood at $65.2bn in 2008.

When a biopharmaceutical is developed, the company will typically apply for a patent, which is a grant for exclusive manufacturing rights. This is the primary means by which the developer of the drug can recover the investment cost for development of the biopharmaceutical. The patent laws in the United States and Europe differ somewhat on the requirements for a patent, with the European requirements are perceived as more difficult to satisfy. The total number of patents granted for biopharmaceuticals has risen significantly since the 1970s. In 1978 the total patents granted was 30. This had climbed to 15,600 in 1995, and by 2001 there were 34,527 patent applications.

Within the United States, the Food and Drug Administration (FDA) exerts strict control over the commercial distribution of a pharmaceutical product, including biopharmaceuticals. Approval can require several years of clinical trials, including trials with human volunteers. Even after the drug is released, it will still be monitored for performance and safety risks.

The manufacture of the drug must satisfy the "current Good Manufacturing Practices" regulations of the FDA. They are typically manufactured in a clean room environment with set standards for the amount of airborne particles.

Classification of biopharmaceuticals

- Blood factors (Factor VIII and Factor IX)
- Thrombolytic agents (tissue plasminogen activator)
- Hormones (insulin, glucagon, growth hormone, gonadotrophins)
- Haematopoietic growth factors (Erythropoietin, colony stimulating factors)
- Interferons (Interferons-α, -β, -γ)
- Interleukin-based products (Interleukin-2)
- Vaccines (Hepatitis B surface antigen)
- Monoclonal antibodies (Various)
- Additional products (tumour necrosis factor, therapeutic enzymes)

Uses

- Erythropoietin - Treatment of anaemia
- Interferon-α - Treatment of leukaemia
- Interferon-β - Treatment of multiple sclerosis
- Monoclonal antibody - Treatment of rheumatoid arthritis

- Colony stimulating factors - Treatment of neutropenia
- Glucocerebrosidase - Treatment of Gaucher's disease

Large scale production

Biopharmaceuticals may be produced from microbial cells (e.g. recombinant *E. coli* or yeast cultures), mammalian cell lines and plant cell cultures and moss plants in bioreactors of various configurations, including photo-bioreactors.

Important issues of concern are cost of production (a low volume, high purity product is desirable) and microbial contamination (by bacteria, viruses, mycoplasma, etc.). Alternative platforms of production which are being tested include whole plants (plant-made pharmaceuticals).

Transgenics

A potentially controversial method of producing biopharmaceuticals involves transgenic organisms, particularly plants and animals that have been genetically modified to produce drugs. The production of these organisms represents a significant risk on the part of the investor, both in terms of the risk of failure to produce the required organism, and in the risk of non-acceptance by government bodies due to the perceived risks and from ethical issues. Biopharmaceutical crops also represent a risk of cross-contamination with non-engineered crops, or crops engineered for non-medical purposes.

One potential approach to this technology is the creation of a transgenic mammal that can produce the biopharmaceutical in its milk (or blood or urine). Once an animal is produced, typically using the pronuclear microinjection method, it becomes efficacious to use cloning technology to create additional offspring that carry the favorable modified genome. The first such drug manufactured from the milk of a genetically-modified goat was ATryn, but marketing permission was blocked by the European Medicines Agency in February 2006. This decision was reversed in June 2006 and approval was given August 2006.

Industrial biotechnology

An industrial biotechnology plant for the production of modified wheat starch and gluten

Industrial biotechnology (known mainly in Europe as **white biotechnology**) is the application of biotechnology for industrial purposes, including manufacturing, alternative energy (or "bioenergy"), and biomaterials. It includes the practice of using cells or components of cells like enzymes to generate industrially useful products. *The Economist* speculated (as cited in the *Economist* article listed in the "References" section) industrial biotechnology might significantly impact the chemical industry. *The Economist* also suggested it can enable economies to become less dependent on fossil fuels.

The industrial biotechnology community generally accepts an informal divide between industrial and pharmaceutical biotechnology. An example would be that of companies growing fungus to produce antibiotics, e.g. penicillin from the penicillium fungi. One view holds that this is industrial production; the other viewpoint is that it would not strictly lie within the domain of pure industrial production, given its inclusion within medical biotechnology.

This may be better understood by calling to mind the classification by the U.S. biotechnology lobby group, Biotechnology Industry Organization (BIO) of three "waves" of biotechnology. The first wave, Green Biotechnology, refers to agricultural biotechnology. The second wave, Red Biotechnology, refers to pharmaceutical and medical biotechnology. The third wave, White Biotechnology, refers to industrial biotechnology. In actuality, each of the waves may overlap each of the others. Industrial biotechnology, particularly the development of large-scale bioenergy refineries, will

likely involve dedicated genetically modified crops as well as the large-scale bioprocessing and fermentation as is used in some pharmaceutical production.

Industrial biotechnology and climate change

Climate change effects on the developing country populations and its relation to industrial biotechnology present an opportunity of setting a foundation for a transition to and industrial economy via mitigation options set about in the Kyoto protocol. The relationship between industrial biotechnology and climate change cuts across three major spheres of climate change science and policy: impacts, mitigation, and adaptation. The impacts of a changing climate on agriculture and land use will affect the availability of biomass as well as food production. Developing country populations will suffer disproportionately, especially since some of the regions that may be most negatively affected are located in small island states and in already impoverished areas of sub-Saharan Africa.With respect to mitigation, the expansion of industrial biotechnology can offer new opportunities for fossil fuel substitution and carbon sequestration. If genetic modification is employed, the linkages to both mitigation and adaptation would be even more direct. A given crop might be adjusted so as to yield better characteristics for energy production (e.g. more fibre, faster growth, less lignin). With respect to adaptation, varieties might be developed that require less water or are otherwise more suited to the new climate. Biomass and industrial biotechnology can address GHG emissions while at the same time providing a more sustainable foundation for the developing world's transition from an agrarian to an industrial economy.

Novel implementation platforms and identification of existing technologies that are under-utilised or inefficiently utilised will generally be preferred to developing new technologies, particularly in smaller and/or poorer developing countries.

The following options could be considered:

• Improving the efficiency of biomass to energy conversion (e.g. advanced cogeneration, biomass gasification)

• Creating biomass resource options from agricultural or process wastes

• Use of agricultural or process wastes as inputs to industrial processes

• Substitution for products made from fossil sources (e.g. fertilisers, bio-plastics)

The above options tend to have medium-to-large economies-of-scale. Alternatively, in the context of poverty reduction in rural areas, there may be a preference for options aimed at expanding energy services (e.g. biogas for cooking) and/or creating income-generating opportunities (e.g. small-scale agro-industrial plants). At the same time, smaller-scale options with many end-users require more effort for replication and

dissemination, and thus entail higher transaction costs. Detailed analysis of impacts, adaptation, and enhanced sequestration are quite complicated and beyond the scope of this report. Mitigation options through the Kyoto mechanisms (Emissions Trading, Joint Implementation, and CDM) are of greatest near-term interest, not only because of the opportunities to obtain financial support, but also because expanded platforms for industrial biotechnology can address long-term sustainable development goals at the same time that they offer greenhouse gas (GHG) emission reductions. Since only Annex 1 parties have Kyoto obligations, Emissions Trading and JI are only indirectly related to developing country crediting via the linkages from GHG credits that are generated.

Industrial or white biotechnology uses enzymes and micro-organisms to make bio-based products in sectors such as chemicals, food and feed, detergents, paper and pulp, textiles and bioenergy (such as biofuels or biogas). In doing so, biotechnology uses renewable raw materials and is one of the most promising, innovative approaches towards lowering greenhouse gas emissions.

The application of industrial biotechnology has been proven to make quite significant contributions towards mitigating the impacts of climate change in these and other sectors. In addition to environmental benefits, biotechnology can improve industry performance and product value and, as the technology develops and matures, white biotechnology will yield more viable solutions for our environment. These innovative solutions bring added benefits for both our climate and our economy.

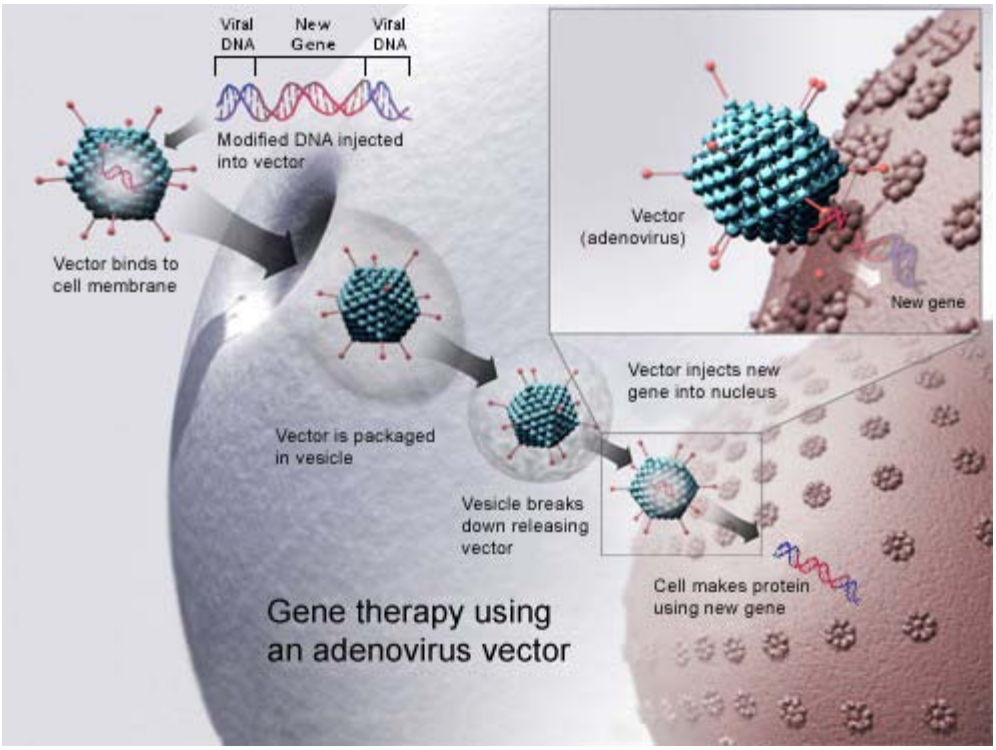
Industrial biotechnology is based on renewable resources, can save energy in production processes, and can significantly reduce CO2 emissions. The impact that biotechnology has on industry is confirmed by scientific studies and reports, such as the OECD's report on the application of biotechnology to industrial sustainability and, most recently, by the World Wide Fund for Nature (WWF) report on the potential of industrial biotechnology to cut CO2 emissions and help build a greener economy.

The WWF report concludes that the full climate change mitigation potential of biotechnology processes and bio-based products ranges from between one billion and 2.5 billion tons CO2 equivalent per year by 2030. This represents more than Germany's total reported emissions in 1990. Many low-carbon technologies are already available, and future innovations offer greater potential. Forward-thinking companies have already discovered the potential of biotechnology to cut greenhouse gas emissions.

However, in order to fully realise the potential of biotechnology it will be critical that international policy creates a fully supportive biotechnology legislative framework.

Chapter- 4

Gene Therapy



Gene therapy

Gene therapy is the insertion, alteration, or removal of genes within an individual's cells and biological tissues to treat disease. It is a technique for correcting defective genes that are responsible for disease development. The most common form of gene therapy involves the insertion of functional genes into an unspecified genomic location in order to replace a mutated gene, but other forms involve directly correcting the mutation or modifying normal gene that enables a viral infection. Although the technology is still in its infancy, it has been used with some success.

Gene therapy using an Adenovirus vector. A new gene is inserted into an adenovirus vector, which is used to introduce the modified DNA into a human cell. If the treatment is successful, the new gene will make a functional protein.

Approach

Scientists have taken the logical step of trying to introduce genes directly into human cells, focusing on diseases caused by single-gene defects, such as cystic fibrosis, haemophilia, muscular dystrophy and sickle cell anemia. However, this has proven more difficult than modifying bacteria, primarily because of the problems involved in carrying large sections of DNA and delivering them to the correct site on the gene. Today, most gene therapy studies are aimed at cancer and hereditary diseases linked to a genetic defect. Antisense therapy is not strictly a form of gene therapy, but is a related, genetically-mediated therapy.

The most common form of genetic engineering involves the insertion of a functional gene at an unspecified location in the host genome. This is accomplished by isolating and copying the gene of interest, generating a construct containing all the genetic elements for correct expression, and then inserting this construct into a random location in the host organism. Other forms of genetic engineering include gene targeting and knocking out specific genes via engineered nucleases such as zinc finger nucleases, engineered I-CreI homing endonucleases, or nucleases generated from TAL effectors. An example of gene-knockout mediated gene therapy is the knockout of the human CCR5 gene in T-cells in order to control HIV infection. This approach is currently being used in several human clinical trials.

The biology of human gene therapy remains complex and many techniques need further development. Many diseases and their strict genetic link need to be understood more fully before gene therapy can be used appropriately. The public policy debate surrounding the possible use of genetically engineered material in human subjects has been equally complex. Major participants in the debate have come from the fields of biology, government, law, medicine, philosophy, politics, and religion, each bringing different views to the discussion.

Types of gene therapy

Gene therapy may be classified into the two following types:

Germ line gene therapy

In the case of germ line gene therapy, germ cells, i.e., sperm or eggs, are modified by the introduction of functional genes, which are integrated into their genomes. Therefore, the change due to therapy would be heritable and would be passed on to later generations. This new approach, theoretically, should be highly effective in counteracting genetic disorders and hereditary diseases. However, many jurisdictions prohibit this for

application in human beings, at least for the present, for a variety of technical and ethical reasons.

Somatic gene therapy

In the case of somatic gene therapy, the therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only, and will not be inherited by the patient's offspring or later generations.

Vectors in gene therapy

Viruses

All viruses bind to their hosts and introduce their genetic material into the host cell as part of their replication cycle. This genetic material contains basic 'instructions' of how to produce more copies of these viruses, hijacking the body's normal production machinery to serve the needs of the virus. The host cell will carry out these instructions and produce additional copies of the virus, leading to more and more cells becoming infected. Some types of viruses insert their genes into the host's genome, but do not actually enter the cell. Others penetrate the cell membrane disguised as protein molecules and enter the cell.

There are two main types of virus infection: lytic and lysogenic. Shortly after inserting its DNA, viruses of the lytic cycle quickly produce more viruses, burst from the cell and infect more cells. Lysogenic viruses integrate their DNA into the DNA of the host cell and may live in the body for many years before responding to a trigger. The virus reproduces as the cell does and does not inflict bodily harm until it is triggered. The trigger releases the DNA from that of the host and employs it to create new viruses. HIV is a lysogenic infection. Some scientists believe that if they find the origin of its trigger, they will be able to stop the virus from ever reproducing throughout the body.

Retroviruses

The genetic material in retroviruses is in the form of RNA molecules, while the genetic material of their hosts is in the form of DNA. When a retrovirus infects a host cell, it will introduce its RNA together with some enzymes, namely reverse transcriptase and integrase, into the cell. This RNA molecule from the retrovirus must produce a DNA copy from its RNA molecule before it can be integrated into the genetic material of the host cell. The process of producing a DNA copy from an RNA molecule is termed reverse transcription. It is carried out by one of the enzymes carried in the virus, called reverse transcriptase. After this DNA copy is produced and is free in the nucleus of the host cell, it must be incorporated into the genome of the host cell. That is, it must be inserted into the large DNA molecules in the cell (the chromosomes). This process is done by another enzyme carried in the virus called integrase.

Now that the genetic material of the virus has been inserted, it can be said that the host cell has been modified to contain new genes. If this host cell divides later, its descendants will all contain the new genes. Sometimes the genes of the retrovirus do not express their information immediately.

One of the problems of gene therapy using retroviruses is that the integrase enzyme can insert the genetic material of the virus into any arbitrary position in the genome of the host; it randomly inserts the genetic material into a chromosome. If genetic material happens to be inserted in the middle of one of the original genes of the host cell, this gene will be disrupted (insertional mutagenesis). If the gene happens to be one regulating cell division, uncontrolled cell division (i.e., cancer) can occur. This problem has recently begun to be addressed by utilizing zinc finger nucleases or by including certain sequences such as the beta-globin locus control region to direct the site of integration to specific chromosomal sites.

Gene therapy trials using retroviral vectors to treat X-linked severe combined immunodeficiency (X-SCID) represent the most successful application of gene therapy to date. More than twenty patients have been treated in France and Britain, with a high rate of immune system reconstitution observed. Similar trials were restricted or halted in the USA when leukemia was reported in patients treated in the French X-SCID gene therapy trial. To date, four children in the French trial and one in the British trial have developed leukemia as a result of insertional mutagenesis by the retroviral vector. All but one of these children responded well to conventional anti-leukemia treatment. Gene therapy trials to treat SCID due to deficiency of the Adenosine Deaminase (ADA) enzyme continue with relative success in the USA, Britain, Italy and Japan.

Adenoviruses

Adenoviruses are viruses that carry their genetic material in the form of double-stranded DNA. They cause respiratory, intestinal, and eye infections in humans (especially the common cold). When these viruses infect a host cell, they introduce their DNA molecule into the host. The genetic material of the adenoviruses is not incorporated (transient) into the host cell's genetic material. The DNA molecule is left free in the nucleus of the host cell, and the instructions in this extra DNA molecule are transcribed just like any other gene. The only difference is that these extra genes are not replicated when the cell is about to undergo cell division so the descendants of that cell will not have the extra gene. As a result, treatment with the adenovirus will require readministration in a growing cell population although the absence of integration into the host cell's genome should prevent the type of cancer seen in the SCID trials. This vector system has been promoted for treating cancer and indeed the first gene therapy product to be licensed to treat cancer, Gendicine, is an adenovirus. Gendicine, an adenoviral p53-based gene therapy was approved by the Chinese FDA in 2003 for treatment of head and neck cancer. Advexin, a similar gene therapy approach from Introgen, was turned down by the US FDA in 2008.

Concerns about the safety of adenovirus vectors were raised after the 1999 death of Jesse Gelsinger while participating in a gene therapy trial. Since then, work using adenovirus vectors has focused on genetically crippled versions of the virus.

Adeno-associated viruses

Adeno-associated viruses, from the parvovirus family, are small viruses with a genome of single stranded DNA. The wild type AAV can insert genetic material at a specific site on chromosome 19 with near 100% certainty. But the recombinant AAV, which does not contain any viral genes and only the therapeutic gene, does not integrate into the genome. Instead the recombinant viral genome fuses at its ends via the ITR (inverted terminal repeats) recombination to form circular, episomal forms which are predicted to be the primary cause of the long term gene expression. There are a few disadvantages to using AAV, including the small amount of DNA it can carry (low capacity) and the difficulty in producing it. The production problem however has recently been solved by Amsterdam Molecular Therapeutics. This type of virus is being used, however, because it is non-pathogenic (most people carry this harmless virus). In contrast to adenoviruses, most people treated with AAV will not build an immune response to remove the virus and the cells that have been successfully treated with it. Several trials with AAV are on-going or in preparation, mainly trying to treat muscle and eye diseases; the two tissues where the virus seems particularly useful. However, clinical trials have also been initiated where AAV vectors are used to deliver genes to the brain. This is possible because AAV viruses can infect non-dividing (quiescent) cells, such as neurons in which their genomes are expressed for a long time.

Envelope protein pseudotyping of viral vectors

The viral vectors described above have natural host cell populations that they infect most efficiently. Retroviruses have limited natural host cell ranges, and although adenovirus and adeno-associated virus are able to infect a relatively broader range of cells efficiently, some cell types are refractory to infection by these viruses as well. Attachment to and entry into a susceptible cell is mediated by the protein envelope on the surface of a virus. Retroviruses and adeno-associated viruses have a single protein coating their membrane, while adenoviruses are coated with both an envelope protein and fibers that extend away from the surface of the virus. The envelope proteins on each of these viruses bind to cell-surface molecules such as heparin sulfate, which localizes them upon the surface of the potential host, as well as with the specific protein receptor that either induces entry-promoting structural changes in the viral protein, or localizes the virus in endosomes wherein acidification of the lumen induces this refolding of the viral coat. In either case, entry into potential host cells requires a favorable interaction between a protein on the surface of the virus and a protein on the surface of the cell. For the purposes of gene therapy, one might either want to limit or expand the range of cells susceptible to transduction by a gene therapy vector. To this end, many vectors have been developed in which the endogenous viral envelope proteins have been replaced by either envelope proteins from other viruses, or by chimeric proteins. Such chimera would consist of those parts of the viral protein necessary for incorporation into the virion as well as sequences

meant to interact with specific host cell proteins. Viruses in which the envelope proteins have been replaced as described are referred to as pseudotyped viruses. For example, the most popular retroviral vector for use in gene therapy trials has been the lentivirus Simian immunodeficiency virus coated with the envelope proteins, G-protein, from Vesicular stomatitis virus. This vector is referred to as VSV G-pseudotyped lentivirus, and infects an almost universal set of cells. This tropism is characteristic of the VSV G-protein with which this vector is coated. Many attempts have been made to limit the tropism of viral vectors to one or a few host cell populations. This advance would allow for the systemic administration of a relatively small amount of vector. The potential for off-target cell modification would be limited, and many concerns from the medical community would be alleviated. Most attempts to limit tropism have used chimeric envelope proteins bearing antibody fragments. These vectors show great promise for the development of "magic bullet" gene therapies.

Replication-Competent Vectors

A replication-competent vector called ONYX-015 is used in replicating tumor cells. It was found that in the absence of the E1B-55Kd viral protein, adenovirus caused very rapid apoptosis of infected, p53(+) cells, and this results in dramatically reduced virus progeny and no subsequent spread. Apoptosis was mainly the result of the ability of EIA to inactivate p300. In p53(-) cells, deletion of E1B 55kd has no consequence in terms of apoptosis, and viral replication is similar to that of wild-type virus, resulting in massive killing of cells.

A replication-defective vector deletes some essential genes. These deleted genes are still necessary in the body so they are replaced with either a helper virus or a DNA molecule.

Cis and trans-acting elements

Replication-defective vectors always contain a “transfer construct”. The transfer construct carries the gene to be transduced or “transgene”. The transfer construct also carries the sequences which are necessary for the general functioning of the viral genome: packaging sequence, repeats for replication and, when needed, priming of reverse transcription. These are denominated cis-acting elements, because they need to be on the same piece of DNA as the viral genome and the gene of interest. Trans-acting elements are viral elements, which can be encoded on a different DNA molecule. For example, the viral structural proteins can be expressed from a different genetic element than the viral genome.

Herpes Semplex Virus

Herpes Semplex Virus is a human neurotropic virus. This is mostly examined for gene transfer in the nervous system. The wild type HSV-1 virus is able to infect neurons. Infected neurones are not rejected by the immune system. Though the latent virus is not transcriptionally apparent, it does possess neurone specific promoters that can continue to

function normally. Antibodies to HSV-1 are common in humans, however complications due to herpes infection are somewhat rare.

Non-viral methods

Non-viral methods present certain advantages over viral methods, with simple large scale production and low host immunogenicity being just two. Previously, low levels of transfection and expression of the gene held non-viral methods at a disadvantage; however, recent advances in vector technology have yielded molecules and techniques with transfection efficiencies similar to those of viruses.

Injection of Naked DNA

This is the simplest method of non-viral transfection. Clinical trials carried out of intramuscular injection of a naked DNA plasmid have occurred with some success; however, the expression has been very low in comparison to other methods of transfection. In addition to trials with plasmids, there have been trials with naked PCR product, which have had similar or greater success. Cellular uptake of naked DNA is generally inefficient. Research efforts focusing on improving the efficiency of naked DNA uptake have yielded several novel methods, such as electroporation, sonoporation, and the use of a "gene gun", which shoots DNA coated gold particles into the cell using high pressure gas.

Physical Methods to Enhance Delivery

Electroporation

Electroporation is a method that uses short pulses of high voltage to carry DNA across the cell membrane. This shock is thought to cause temporary formation of pores in the cell membrane, allowing DNA molecules to pass through. Electroporation is generally efficient and works across a broad range of cell types. However, a high rate of cell death following electroporation has limited its use, including clinical applications.

More recently a newer method of electroporation, termed electron-avalanche transfection, has been used in gene therapy experiments. By using a high-voltage plasma discharge, DNA was efficiently delivered following very short (microsecond) pulses. Compared to electroporation, the technique resulted in greatly increased efficiency and less cellular damage.

Gene Gun

The use of particle bombardment, or the gene gun, is another physical method of DNA transfection. In this technique, DNA is coated with gold particles and loaded into a device which generates a force to achieve penetration of DNA/gold into the cells.

Sonoporation

Sonoporation uses ultrasonic frequencies to deliver DNA into cells. The process of acoustic cavitation is thought to disrupt the cell membrane and allow DNA to move into cells.

Magnetofection

In a method termed magnetofection, DNA is complexed to a magnetic particles, and a magnet is placed underneath the tissue culture dish to bring DNA complexes into contact with a cell monolayer.

Chemical Methods to Enhance Delivery

Oligonucleotides

The use of synthetic oligonucleotides in gene therapy is to inactivate the genes involved in the disease process. There are several methods by which this is achieved. One strategy uses antisense specific to the target gene to disrupt the transcription of the faulty gene. Another uses small molecules of RNA called siRNA to signal the cell to cleave specific unique sequences in the mRNA transcript of the faulty gene, disrupting translation of the faulty mRNA, and therefore expression of the gene. A further strategy uses double stranded oligodeoxynucleotides as a decoy for the transcription factors that are required to activate the transcription of the target gene. The transcription factors bind to the decoys instead of the promoter of the faulty gene, which reduces the transcription of the target gene, lowering expression. Additionally, single stranded DNA oligonucleotides have been used to direct a single base change within a mutant gene. The oligonucleotide is designed to anneal with complementarity to the target gene with the exception of a central base, the target base, which serves as the template base for repair. This technique is referred to as oligonucleotide mediated gene repair, targeted gene repair, or targeted nucleotide alteration.

Lipoplexes and polyplexes

To improve the delivery of the new DNA into the cell, the DNA must be protected from damage and (positively charged). Initially, anionic and neutral lipids were used for the construction of lipoplexes for synthetic vectors. However, in spite of the facts that there is little toxicity associated with them, that they are compatible with body fluids and that there was a possibility of adapting them to be tissue specific; they are complicated and time consuming to produce so attention was turned to the cationic versions.

Cationic lipids, due to their positive charge, were first used to condense negatively charged DNA molecules so as to facilitate the encapsulation of DNA into liposomes. Later it was found that the use of cationic lipids significantly enhanced the stability of lipoplexes. Also as a result of their charge, cationic liposomes interact with the cell membrane, endocytosis was widely believed as the major route by which cells uptake lipoplexes. Endosomes are formed as the results of endocytosis, however, if genes can not be released into cytoplasm by breaking the membrane of endosome, they will be sent to lysosomes where all DNA will be destroyed before they could achieve their functions.

It was also found that although cationic lipids themselves could condense and encapsulate DNA into liposomes, the transfection efficiency is very low due to the lack of ability in terms of "endosomal escaping". However, when helper lipids (usually electroneutral lipids, such as DOPE) were added to form lipoplexes, much higher transfection efficiency was observed. Later on, it was figured out that certain lipids have the ability to destabilize endosomal membranes so as to facilitate the escape of DNA from endosome, therefore those lipids are called fusogenic lipids. Although cationic liposomes have been widely used as an alternative for gene delivery vectors, a dose dependent toxicity of cationic lipids were also observed which could limit their therapeutic usages.

The most common use of lipoplexes has been in gene transfer into cancer cells, where the supplied genes have activated tumor suppressor control genes in the cell and decrease the activity of oncogenes. Recent studies have shown lipoplexes to be useful in transfecting respiratory epithelial cells, so they may be used for treatment of genetic respiratory diseases such as cystic fibrosis.

Complexes of polymers with DNA are called polyplexes. Most polyplexes consist of cationic polymers and their production is regulated by ionic interactions. One large difference between the methods of action of polyplexes and lipoplexes is that polyplexes cannot release their DNA load into the cytoplasm, so to this end, co-transfection with endosome-lytic agents (to lyse the endosome that is made during endocytosis, the process by which the polyplex enters the cell) such as inactivated adenovirus must occur. However, this isn't always the case, polymers such as polyethylenimine have their own method of endosome disruption as does chitosan and trimethylchitosan.

Dendrimers

A dendrimer is a highly branched macromolecule with a spherical shape. The surface of the particle may be functionalized in many ways and many of the properties of the resulting construct are determined by its surface.

In particular it is possible to construct a cationic dendrimer, i.e. one with a positive surface charge. When in the presence of genetic material such as DNA or RNA, charge complimentarity leads to a temporary association of the nucleic acid with the cationic dendrimer. On reaching its destination the dendrimer-nucleic acid complex is then taken into the cell via endocytosis.

In recent years the benchmark for transfection agents has been cationic lipids. Limitations of these competing reagents have been reported to include: the lack of ability to transfect a number of cell types, the lack of robust active targeting capabilities, incompatibility with animal models, and toxicity. Dendrimers offer robust covalent construction and extreme control over molecule structure, and therefore size. Together these give compelling advantages compared to existing approaches.

Producing dendrimers has historically been a slow and expensive process consisting of numerous slow reactions, an obstacle that severely curtailed their commercial development. The Michigan based company Dendritic Nanotechnologies discovered a

method to produce dendrimers using kinetically driven chemistry, a process that not only reduced cost by a magnitude of three, but also cut reaction time from over a month to several days. These new "Priostar" dendrimers can be specifically constructed to carry a DNA or RNA payload that transfects cells at a high efficiency with little or no toxicity.

Hybrid methods

Due to every method of gene transfer having shortcomings, there have been some hybrid methods developed that combine two or more techniques. Virosomes are one example; they combine liposomes with an inactivated HIV or influenza virus. This has been shown to have more efficient gene transfer in respiratory epithelial cells than either viral or liposomal methods alone. Other methods involve mixing other viral vectors with cationic lipids or hybridising viruses.

Major developments in gene therapy

1970s and earlier

In 1972 Friedmann and Roblin authored a paper in Science titled "Gene therapy for human genetic disease?" They cite Rogers S for proposing "that exogenous 'good'" DNA be used to replace the defective DNA in those who suffer from genetic defects. They also cite the first attempt to perform gene therapy as [New York Times, 20 September 1970].

1990s

The first approved gene therapy case in the United States took place on September 14, 1990, at the National Institute of Health. It was performed on a four year old girl named Ashanti DeSilva. It was a treatment for a genetic defect that left her with an Immune System deficiency. The effects were only temporary, but successful (Boylan 313).

New gene therapy approach repairs errors in messenger RNA derived from defective genes. This technique has the potential to treat the blood disorder thalassaemia, cystic fibrosis, and some cancers. Researchers at Case Western Reserve University and Copernicus Therapeutics are able to create tiny liposomes 25 nanometers across that can carry therapeutic DNA through pores in the nuclear membrane.

Sickle cell disease is successfully treated in mice.

in 1992 Doctor Claudio Bordignon working at the Vita-Salute San Raffaele University, Milan, Italy performed the first procedure of gene therapy using hematopoietic stem cells as vectors to deliver genes intended to correct hereditary diseases. In 2002 this work led to the publication of the first successful gene therapy treatment for adenosine deaminase-deficiency (SCID). The success of a multi-center trial for treating children with SCID (severe combined immune deficiency or "bubble boy" disease) held from 2000 and 2002 was questioned when two of the ten children treated at the trial's Paris center developed a leukemia-like condition. Clinical trials were halted temporarily in 2002, but resumed

after regulatory review of the protocol in the United States, the United Kingdom, France, Italy, and Germany.

In 1993 Andrew Gobea was born with severe combined immunodeficiency (SCID). Genetic screening before birth showed that he had SCID. Blood was removed from Andrew's placenta and umbilical cord immediately after birth, containing stem cells. The allele that codes for ADA was obtained and was inserted into a retrovirus. Retroviruses and stem cells were mixed, after which they entered and inserted the gene into the stem cells' chromosomes. Stem cells containing the working ADA gene were injected into Andrew's blood system via a vein. Injections of the ADA enzyme were also given weekly. For four years T-cells (white blood cells), produced by stem cells, made ADA enzymes using the ADA gene. After four years more treatment was needed.

The 1999 death of Jesse Gelsinger in a gene therapy experiment resulted in a significant setback to gene therapy research in the United States. The pivotal event resulted in the FDA's suspension of several clinical trials as ethical and procedural practices in the field were reevaluated.

2000s

In 2003 a University of California, Los Angeles research team inserted genes into the brain using liposomes coated in a polymer called polyethylene glycol. The transfer of genes into the brain is a significant achievement because viral vectors are too big to get across the blood-brain barrier. This method has potential for treating Parkinson's disease.

RNA interference or gene silencing may be a new way to treat Huntington's disease. Short pieces of double-stranded RNA (short, interfering RNAs or siRNAs) are used by cells to degrade RNA of a particular sequence. If a siRNA is designed to match the RNA copied from a faulty gene, then the abnormal protein product of that gene will not be produced.

Scientists at the National Institutes of Health (Bethesda, Maryland) have successfully treated metastatic melanoma in two patients using killer T cells genetically retargeted to attack the cancer cells. This study constitutes one of the first demonstrations that gene therapy can be effective in treating cancer.

In March 2006 an international group of scientists announced the successful use of gene therapy to treat two adult patients for a disease affecting myeloid cells. The study, published in Nature Medicine, is believed to be the first to show that gene therapy can cure diseases of the myeloid system.

In May 2006 a team of scientists led by Dr. Luigi Naldini and Dr. Brian Brown from the San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET) in Milan, Italy reported a breakthrough for gene therapy in which they developed a way to prevent the immune system from rejecting a newly delivered gene. Similar to organ transplantation, gene therapy has been plagued by the problem of immune rejection. So far, delivery of the

'normal' gene has been difficult because the immune system recognizes the new gene as foreign and rejects the cells carrying it. To overcome this problem, the HSR-TIGET group utilized a newly uncovered network of genes regulated by molecules known as microRNAs. Dr. Naldini's group reasoned that they could use this natural function of microRNA to selectively turn off the identity of their therapeutic gene in cells of the immune system and prevent the gene from being found and destroyed. The researchers injected mice with the gene containing an immune-cell microRNA target sequence, and the mice did not reject the gene, as previously occurred when vectors without the microRNA target sequence were used. This work will have important implications for the treatment of hemophilia and other genetic diseases by gene therapy.

In November 2006 Preston Nix from the University of Pennsylvania School of Medicine reported on VRX496, a gene-based immunotherapy for the treatment of human immunodeficiency virus (HIV) that uses a lentiviral vector for delivery of an antisense gene against the HIV envelope. In the Phase I trial enrolling five subjects with chronic HIV infection who had failed to respond to at least two antiretroviral regimens, a single intravenous infusion of autologous CD4 T cells genetically modified with VRX496 was safe and well tolerated. All patients had stable or decreased viral load; four of the five patients had stable or increased CD4 T cell counts. In addition, all five patients had stable or increased immune response to HIV antigens and other pathogens. This was the first evaluation of a lentiviral vector administered in U.S. Food and Drug Administration-approved human clinical trials for any disease. Data from an ongoing Phase I/II clinical trial were presented at CROI 2009.

On 1 May 2007 Moorfields Eye Hospital and University College London's Institute of Ophthalmology announced the world's first gene therapy trial for inherited retinal disease. The first operation was carried out on a 23 year-old British male, Robert Johnson, in early 2007. Leber's congenital amaurosis is an inherited blinding disease caused by mutations in the RPE65 gene. The results of the Moorfields/UCL trial were published in New England Journal of Medicine in April 2008. They researched the safety of the subretinal delivery of recombinant adeno associated virus (AAV) carrying RPE65 gene, and found it yielded positive results, with patients having modest increase in vision, and, perhaps more importantly, no apparent side-effects.

In September 2009, the journal Nature reported that researchers at the University of Washington and University of Florida were able to give trichromatic vision to squirrel monkeys using gene therapy, a hopeful precursor to a treatment for color blindness in humans. In November 2009, the journal Science reported that researchers succeeded at halting a fatal brain disease, adrenoleukodystrophy, using a vector derived from HIV to deliver the gene for the missing enzyme.

A paper by Komáromy *et* al. published in April 2010, deals with gene therapy for a form of achromatopsia in dogs. Achromatopsia, or complete color blindness, is presented as an ideal model to develop gene therapy directed to cone photoreceptors. Cone function and day vision have been restored for at least 33 months in two young dogs with achromatopsia. However, the therapy was less efficient for older dogs.

Problems and ethics

For the safety of gene therapy, the Weismann barrier is fundamental in the current thinking. Soma-to-germline feedback should therefore be impossible. However, there are indications that the Weismann barrier can be breached. One way it might possibly be breached is if the treatment were somehow misapplied and spread to the testes and therefore would infect the germline against the intentions of the therapy.

Some of the problems of gene therapy include:

- Short-lived nature of gene therapy – Before gene therapy can become a permanent cure for any condition, the therapeutic DNA introduced into target cells must remain functional and the cells containing the therapeutic DNA must be long-lived and stable. Problems with integrating therapeutic DNA into the genome and the rapidly dividing nature of many cells prevent gene therapy from achieving any long-term benefits. Patients will have to undergo multiple rounds of gene therapy.
- Immune response – Anytime a foreign object is introduced into human tissues, the immune system has evolved to attack the invader. The risk of stimulating the immune system in a way that reduces gene therapy effectiveness is always a possibility. Furthermore, the immune system's enhanced response to invaders that it has seen before makes it difficult for gene therapy to be repeated in patients.
- Problems with viral vectors – Viruses, the carrier of choice in most gene therapy studies, present a variety of potential problems to the patient —toxicity, immune and inflammatory responses, and gene control and targeting issues. In addition, there is always the fear that the viral vector, once inside the patient, may recover its ability to cause disease.
- Multigene disorders – Conditions or disorders that arise from mutations in a single gene are the best candidates for gene therapy. Unfortunately, some of the most commonly occurring disorders, such as heart disease, high blood pressure, Alzheimer's disease, arthritis, and diabetes, are caused by the combined effects of variations in many genes. Multigene or multifactorial disorders such as these would be especially difficult to treat effectively using gene therapy.
- Chance of inducing a tumor (insertional mutagenesis) - If the DNA is integrated in the wrong place in the genome, for example in a tumor suppressor gene, it could induce a tumor. This has occurred in clinical trials for X-linked severe combined immunodeficiency (X-SCID) patients, in which hematopoietic stem cells were transduced with a corrective transgene using a retrovirus, and this led to the development of T cell leukemia in 3 of 20 patients.

Deaths have occurred due to gene therapy, including that of Jesse Gelsinger.

Chapter- 5

Genetic Testing

Genetic Testing : Gene tests (also called DNA-based tests), the newest and most sophisticated of the techniques used to test for genetic disorders, involve direct examination of the DNA molecule itself. Other genetic tests include biochemical tests for such gene products as enzymes and other proteins and for microscopic examination of stained or fluorescent chromosomes. Genetic tests are used for several reasons, including:

- carrier screening, which involves identifying unaffected individuals who carry one copy of a gene for a disease that requires two copies for the disease to be expressed
- preimplantation genetic diagnosis
- prenatal diagnostic testing
- newborn screening
- presymptomatic testing for predicting adult-onset disorders such as Huntington's disease
- presymptomatic testing for estimating the risk of developing adult-onset cancers and Alzheimer's disease
- confirmational diagnosis of a symptomatic individual
- forensic/identity testing

Genetic testing allows the genetic diagnosis of vulnerabilities to inherited diseases, and can also be used to determine a child's paternity (genetic father) or a person's ancestry. Normally, every person carries two copies of every gene (with the exception of genes related to sex-linked traits, which are only inherited from the mother by males), one inherited from their mother, one inherited from their father. The human genome is believed to contain around 20,000 - 25,000 genes. In addition to studying chromosomes to the level of individual genes, genetic testing in a broader sense includes biochemical tests for the possible presence of genetic diseases, or mutant forms of genes associated with increased risk of developing genetic disorders. Genetic testing identifies changes in chromosomes, genes, or proteins. Most of the time, testing is used to find changes that are associated with inherited disorders. The results of a genetic test can confirm or rule out a suspected genetic condition or help determine a person's chance of developing or

passing on a genetic disorder. Several hundred genetic tests are currently in use, and more are being developed.

Since genetic testing may open up ethical or psychological problems, genetic testing is often accompanied by genetic counseling.

Types

Genetic testing is "the analysis of, chromosomes (DNA), proteins, and certain metabolites in order to detect heritable disease-related genotypes, mutations, phenotypes, or karyotypes for clinical purposes." It can provide information about a person's genes and chromosomes throughout life. Available types of testing include:

- Newborn screening: Newborn screening is used just after birth to identify genetic disorders that can be treated early in life. The routine testing of infants for certain disorders is the most widespread use of genetic testing—millions of babies are tested each year in the United States. All states currently test infants for phenylketonuria (a genetic disorder that causes mental illness if left untreated) and congenital hypothyroidism (a disorder of the thyroid gland).
- Diagnostic testing: Diagnostic testing is used to diagnose or rule out a specific genetic or chromosomal condition. In many cases, genetic testing is used to confirm a diagnosis when a particular condition is suspected based on physical mutations and symptoms. Diagnostic testing can be performed at any time during a person's life, but is not available for all genes or all genetic conditions. The results of a diagnostic test can influence a person's choices about health care and the management of the disease.
- Carrier testing: Carrier testing is used to identify people who carry one copy of a gene mutation that, when present in two copies, causes a genetic disorder. This type of testing is offered to individuals who have a family history of a genetic disorder and to people in ethnic groups with an increased risk of specific genetic conditions. If both parents are tested, the test can provide information about a couple's risk of having a child with a genetic condition.
- Prenatal testing: Prenatal testing is used to detect changes in a fetus's genes or chromosomes before birth. This type of testing is offered to couples with an increased risk of having a baby with a genetic or chromosomal disorder. In some cases, prenatal testing can lessen a couple's uncertainty or help them decide whether to abort the pregnancy. It cannot identify all possible inherited disorders and birth defects, however.
- Preimplantation genetic diagnosis: Genetic testing procedures that are performed on human embryos prior to the implantation as part of an in vitro fertilization procedure.
- Predictive and presymptomatic testing: Predictive and presymptomatic types of testing are used to detect gene mutations associated with disorders that appear after birth, often later in life. These tests can be helpful to people who have a family member with a genetic disorder, but who have no features of the disorder themselves at the time of testing. Predictive testing can identify mutations that

increase a person's chances of developing disorders with a genetic basis, such as certain types of cancer. For example, an individual with a mutation in *BRCA1* has a 65% cumulative risk of breast cancer . Presymptomatic testing can determine whether a person will develop a genetic disorder, such as hemochromatosis (an iron overload disorder), before any signs or symptoms appear. The results of predictive and presymptomatic testing can provide information about a person's risk of developing a specific disorder and help with making decisions about medical care.

- Forensic testing: Forensic testing uses DNA sequences to identify an individual for legal purposes. Unlike the tests described above, forensic testing is not used to detect gene mutations associated with disease. This type of testing can identify crime or catastrophe victims, rule out or implicate a crime suspect, or establish biological relationships between people (for example, paternity).
- Parental testing: This type of genetic test uses special DNA markers to identify the same or similar inheritance patterns between related individuals. Based on the fact that we all inherit half of our DNA from the father, and half from the mother, DNA scientists test individuals to find the match of DNA sequences at some highly differential markers to draw the conclusion of relatedness.
- Research testing: Research testing includes finding unknown genes, learning how genes work and advancing our understanding of genetic conditions. The results of testing done as part of a research study are usually not available to patients or their healthcare providers.
- Pharmacogenomics: type of genetic testing that determines the influence of genetic variation on drug response.

Medical procedure

Genetic testing is often done as part of a genetic consultation and as of mid-2008 there were more than 1,200 clinically applicable genetic tests available. Once a person decides to proceed with genetic testing, a medical geneticist, genetic counselor, primary care doctor, or specialist can order the test after obtaining informed consent.

Genetic tests are performed on a sample of blood, hair, skin, amniotic fluid (the fluid that surrounds a fetus during pregnancy), or other tissue. For example, a medical procedure called a buccal smear uses a small brush or cotton swab to collect a sample of cells from the inside surface of the cheek. Alternatively, a small amount of saline mouthwash may be swished in the mouth to collect the cells. The sample is sent to a laboratory where technicians look for specific changes in chromosomes, DNA, or proteins, depending on the suspected disorder. The laboratory reports the test results in writing to a person's doctor or genetic counselor.

Routine newborn screening tests are done on a small blood sample obtained by pricking the baby's heel with a lancet.

Interpreting results

The results of genetic tests are not always straightforward, which often makes them challenging to interpret and explain. When interpreting test results, healthcare professionals consider a person's medical history, family history, and the type of genetic test that was done.

A positive test result means that the laboratory found a change in a particular gene, chromosome, or protein of interest. Depending on the purpose of the test, this result may confirm a diagnosis, indicate that a person is a carrier of a particular genetic mutation, identify an increased risk of developing a disease (such as cancer) in the future, or suggest a need for further testing. Because family members have some genetic material in common, a positive test result may also have implications for certain blood relatives of the person undergoing testing. It is important to note that a positive result of a predictive or presymptomatic genetic test usually cannot establish the exact risk of developing a disorder. Also, health professionals typically cannot use a positive test result to predict the course or severity of a condition.

A negative test result means that the laboratory did not find a dangerous copy of the gene, chromosome, or protein under consideration. This result can indicate that a person is not affected by a particular disorder, is not a carrier of a specific genetic mutation, or does not have an increased risk of developing a certain disease. It is possible, however, that the test missed a disease-causing genetic alteration because many tests cannot detect all genetic changes that can cause a particular disorder. Further testing may be required to confirm a negative result.

In some cases, a negative result might not give any useful information. This type of result is called uninformative, indeterminate, inconclusive, or ambiguous. Uninformative test results sometimes occur because everyone has common, natural variations in their DNA, called polymorphisms, that do not affect health. If a genetic test finds a change in DNA that has not been associated with a disorder in other people, it can be difficult to tell whether it is a natural polymorphism or a disease-causing mutation. An uninformative result cannot confirm or rule out a specific diagnosis, and it cannot indicate whether a person has an increased risk of developing a disorder. In some cases, testing other affected and unaffected family members can help clarify this type of result.

Risks and limitations

The physical risks associated with most genetic tests are very small, particularly for those tests that require only a blood sample or buccal smear (a procedure that samples cells from the inside surface of the cheek). The procedures used for prenatal testing carry a small but real risk of losing the pregnancy (miscarriage) because they require a sample of amniotic fluid or tissue from around the fetus.

Many of the risks associated with genetic testing involve the emotional, social, or financial consequences of the test results. People may feel angry, depressed, anxious, or

guilty about their results. In some cases, genetic testing creates tension within a family because the results can reveal information about other family members in addition to the person who is tested. The possibility of genetic discrimination in employment or insurance is also a concern. Some individuals avoid genetic testing out of fear it will affect their ability to purchase insurance or find a job. Health insurers do not currently require applicants for coverage to undergo genetic testing, and when insurers encounter genetic information, it is subject to the same confidentiality protections as any other sensitive health information. In the United States, the use of genetic information is governed by the Genetic Information Nondiscrimination Act (GINA).

Genetic testing can provide only limited information about an inherited condition. The test often can't determine if a person will show symptoms of a disorder, how severe the symptoms will be, or whether the disorder will progress over time. Another major limitation is the lack of treatment strategies for many genetic disorders once they are diagnosed.

A genetics professional can explain in detail the benefits, risks, and limitations of a particular test. It is important that any person who is considering genetic testing understand and weigh these factors before making a decision.

Direct-to-Consumer genetic testing

Direct-to-Consumer (DTC) genetic testing is a type of genetic test that is accessible directly to the consumer without having to go through a health care professional. Usually, to obtain a genetic test, health care professionals such as doctors acquire the permission of the patient and order the desired test. DTC genetic tests, however, allow consumers to bypass this process and order one themselves. There are a variety of DTC tests, ranging from testing for breast cancer alleles to mutations linked to cystic fibrosis. Benefits of DTC testing are the accessibility of tests to consumers, promotion of proactive healthcare and the privacy of genetic information. Possible additional risks of DTC testing are the lack of governmental regulation and the potential misinterpretation of genetic information.

Controversy

DTC genetic testing has been controversial due to outspoken opposition within the scientific community. Critics of DTC testing argue against the risks involved, the unregulated advertising and marketing claims, and the overall lack of governmental oversight.

DTC testing involves many of the same risks associated with any genetic test. One of the more obvious and dangerous of these is the possibility of severe misreading of test results. Without professional guidance, consumers can potentially misinterpret genetic information, causing them to be deluded about their personal health.

Some advertising for direct-to-consumer genetic testing has been criticized as conveying an exaggerated and inaccurate message about the connection between genetic information and disease risk, utilizing emotions as a selling factor. An advertisement for a BRCA-predictive genetic test for breast cancer stated: “There is no stronger antidote for fear than information.”

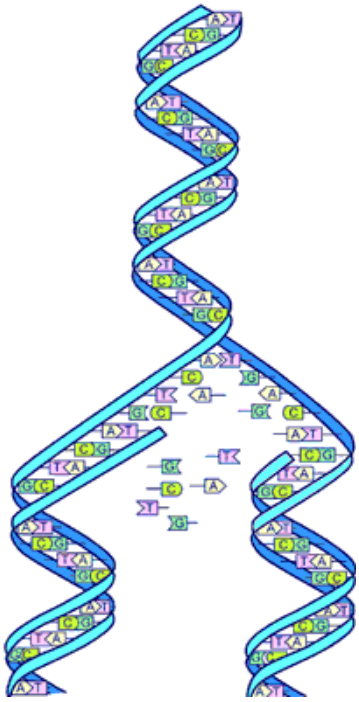
Government regulation in the United States

Currently, the U.S. has no strong Federal regulation moderating the DTC market. Though there are several hundred tests available, only a handful are approved by the Food and Drug Administration (FDA); these are sold as at-home test kits, and are therefore considered "medical devices" over which the FDA may assert jurisdiction. Other types of DTC tests require customers to mail in DNA samples for testing; it is difficult for the FDA to exercise jurisdiction over these types of tests, because the actual testing is completed in the laboratories of providers. As of 2007, the FDA had not yet officially substantiated with scientific evidence the claimed accuracy of the majority of direct-to-consumer genetic tests.

With regard to genetic testing and information in general, legislation in the United States called the Genetic Information Nondiscrimination Act prohibits group health plans and health insurers from denying coverage to a healthy individual or charging that person higher premiums based solely on a genetic predisposition to developing a disease in the future. The legislation also bars employers from using individuals’ genetic information when making hiring, firing, job placement, or promotion decisions. The legislation, the first of its kind in the U.S., was passed by the United States Senate on April 24, 2008, on a vote of 95-0, and was signed into law by President George W. Bush on May 21, 2008. It went into effect on November 21, 2009.

Chapter- 6

Human Genome Project



DNA Replication.

The **Human Genome Project (HGP)** is an international scientific research project with a primary goal of determining the sequence of chemical base pairs which make up DNA and to identify and map the approximately 20,000–25,000 genes of the human genome from both a physical and functional standpoint.

The project began in 1990 and was initially headed by Ari Patrinos, head of the Office of Biological and Environmental Research in the U.S. Department of Energy's Office of Science. Francis Collins directed the National Institutes of Health National Human Genome Research Institute efforts. A working draft of the genome was announced in 2000 and a complete one in 2003, with further, more detailed analysis still being

published. A parallel project was conducted outside of government by the Celera Corporation, which was formally launched in 1998. Most of the government-sponsored sequencing was performed in universities and research centers from the United States, the United Kingdom, Japan, France, Germany, and China. The mapping of human genes is an important step in the development of medicines and other aspects of health care.

While the objective of the Human Genome Project is to understand the genetic makeup of the human species, the project has also focused on several other nonhuman organisms such as *E. coli*, the fruit fly, and the laboratory mouse. It remains one of the largest single investigative projects in modern science.

The Human Genome Project originally aimed to map the nucleotides contained in a human haploid reference genome (more than three billion). Several groups have announced efforts to extend this to diploid human genomes including the International HapMap Project, Applied Biosystems, Perlegen, Illumina, JCVI, Personal Genome Project, and Roche-454.

The "genome" of any given individual (except for identical twins and cloned organisms) is unique; mapping "the human genome" involves sequencing multiple variations of each gene. The project did not study the entire DNA found in human cells; some heterochromatic areas (about 8% of the total genome) remain un-sequenced.

Project

Background

The project began with the culmination of several years of work supported by the United States Department of Energy, in particular workshops in 1984 and 1986 and a subsequent initiative of the US Department of Energy. This 1987 report stated boldly, "The ultimate goal of this initiative is to understand the human genome" and "knowledge of the human as necessary to the continuing progress of medicine and other health sciences as knowledge of human anatomy has been for the present state of medicine." Candidate technologies were already being considered for the proposed undertaking at least as early as 1985.

James D. Watson was head of the National Center for Human Genome Research at the National Institutes of Health (NIH) in the United States starting from 1988. Largely due to his disagreement with his boss, Bernadine Healy, over the issue of patenting genes, Watson was forced to resign in 1992. He was replaced by Francis Collins in April 1993, and the name of the Center was changed to the National Human Genome Research Institute (NHGRI) in 1997.

The $3-billion project was formally founded in 1990 by the United States Department of Energy and the U.S. National Institutes of Health, and was expected to take 15 years. In addition to the United States, the international consortium comprised geneticists in the United Kingdom, France, Germany, Japan, China, and India.

Due to widespread international cooperation and advances in the field of genomics (especially in sequence analysis), as well as major advances in computing technology, a 'rough draft' of the genome was finished in 2000 (announced jointly by then US president Bill Clinton and the British Prime Minister Tony Blair on June 26, 2000). This first available rough draft assembly of the genome was completed by the UCSC Genome Bioinformatics Group, primarily led by then graduate student Jim Kent. Ongoing sequencing led to the announcement of the essentially complete genome in April 2003, 2 years earlier than planned. In May 2006, another milestone was passed on the way to completion of the project, when the sequence of the last chromosome was published in the journal Nature.

State of completion

There are multiple definitions of the "complete sequence of the human genome". According to some of these definitions, the genome has already been completely sequenced, and according to other definitions, the genome has yet to be completely sequenced. There have been multiple popular press articles reporting that the genome was "complete." The genome has been completely sequenced using the definition employed by the International Human Genome Project. A graphical history of the human genome project shows that most of the human genome was complete by the end of 2003. However, there are a number of regions of the human genome that can be considered unfinished:

- First, the central regions of each chromosome, known as centromeres, are highly repetitive DNA sequences that are difficult to sequence using current technology. The centromeres are millions (possibly tens of millions) of base pairs long, and for the most part these are entirely un-sequenced.
- Second, the ends of the chromosomes, called telomeres, are also highly repetitive, and for most of the 46 chromosome ends these too are incomplete. It is not known precisely how much sequence remains before the telomeres of each chromosome are reached, but as with the centromeres, current technological restraints are prohibitive.
- Third, there are several loci in each individual's genome that contain members of multigene families that are difficult to disentangle with shotgun sequencing methods – these multigene families often encode proteins important for immune functions.
- Other than these regions, there remain a few dozen gaps scattered around the genome, some of them rather large, but there is hope that all these will be closed in the next couple of years.

In summary: the best estimates of total genome size indicate that about 92.3% of the genome has been completed and it is likely that the centromeres and telomeres will remain un-sequenced until new technology is developed that facilitates their sequencing. Most of the remaining DNA is highly repetitive and unlikely to contain genes, but it cannot be truly known until it is entirely sequenced. Understanding the functions of all the genes and their regulation is far from complete. The roles of junk DNA, the evolution

of the genome, the differences between individuals, and many other questions are still the subject of intense interest by laboratories all over the world.

Goals

The sequence of the human DNA is stored in databases available to anyone on the Internet. The U.S. National Center for Biotechnology Information (and sister organizations in Europe and Japan) house the gene sequence in a database known as GenBank, along with sequences of known and hypothetical genes and proteins. Other organizations such as the University of California, Santa Cruz, and Ensembl present additional data and annotation and powerful tools for visualizing and searching it. Computer programs have been developed to analyze the data, because the data itself is difficult to interpret without such programs.

The process of identifying the boundaries between genes and other features in a raw DNA sequence is called genome annotation and is the domain of bioinformatics. While expert biologists make the best annotators, their work proceeds slowly, and computer programs are increasingly used to meet the high-throughput demands of genome sequencing projects. The best current technologies for annotation make use of statistical models that take advantage of parallels between DNA sequences and human language, using concepts from computer science such as formal grammars.

Another, often overlooked, goal of the HGP is the study of its ethical, legal, and social implications. It is important to research these issues and find the most appropriate solutions before they become large dilemmas whose effect will manifest in the form of major political concerns.

All humans have unique gene sequences. Therefore the data published by the HGP does not represent the exact sequence of each and every individual's genome. It is the combined "reference genome" of a small number of anonymous donors. The HGP genome is a scaffold for future work in identifying differences among individuals. Most of the current effort in identifying differences among individuals involves single-nucleotide polymorphisms and the HapMap.

Findings

Key findings of the draft (2001) and complete (2004) genome sequences include

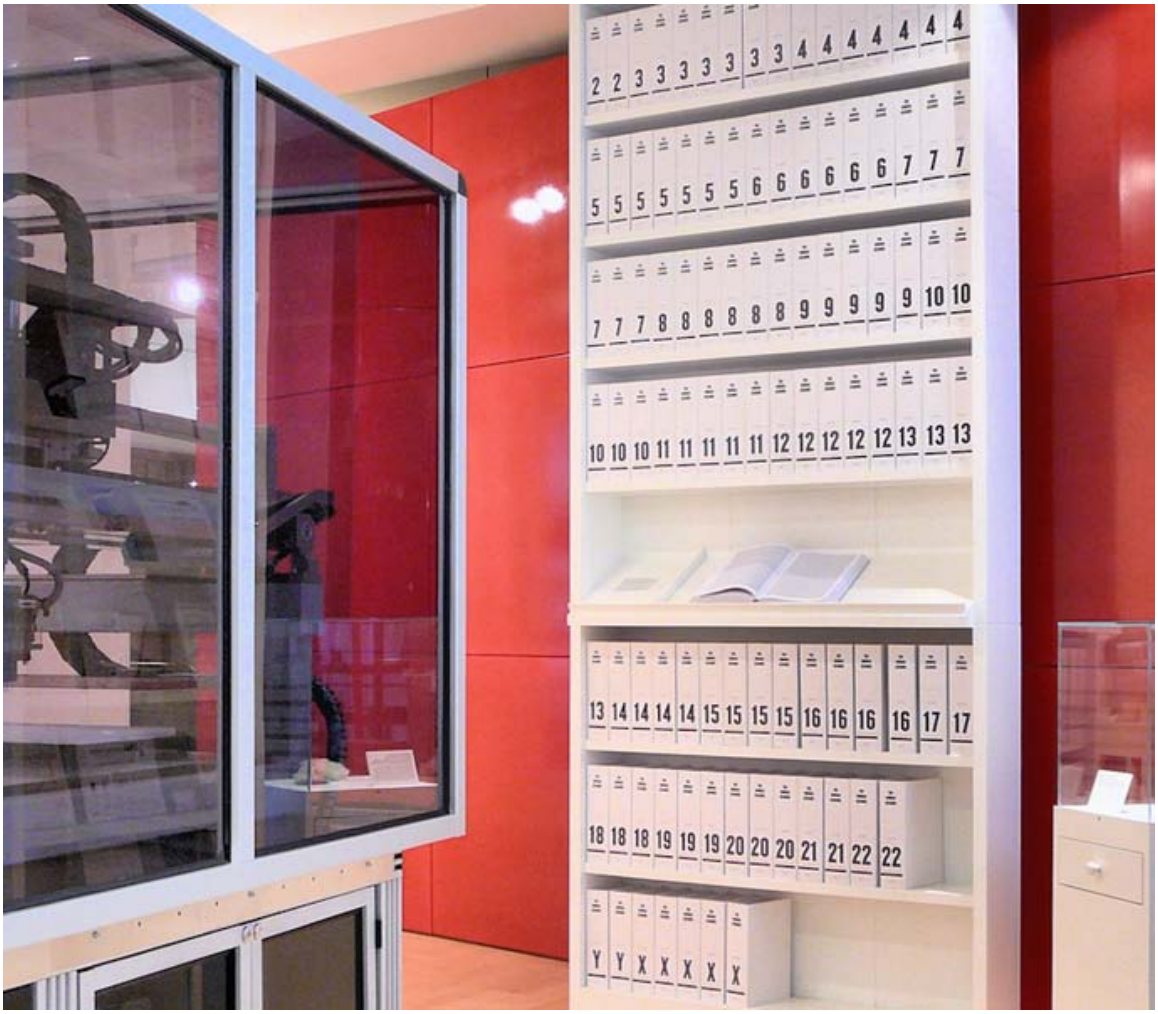
1. There are approximately 20,500 genes in human beings, the same range as in mice and twice that of roundworms. Understanding how these genes express themselves will provide clues to how diseases are caused.

2. Between 1.1% to 1.4% of the genome's sequence codes for proteins

3. The human genome has significantly more segmental duplications (nearly identical, repeated sections of DNA) than other mammalian genomes. These sections may underlie the creation of new primate-specific genes

4. At the time when the draft sequence was published less than 7% of protein families appeared to be vertebrate specific

How it was accomplished



The first printout of the human genome to be presented as a series of books, displayed at the Wellcome Collection, London

The Human Genome Project was started in 1989 with the goal of sequencing and identifying all three billion chemical units in the human genetic instruction set, finding the genetic roots of disease and then developing treatments. With the sequence in hand, the next step was to identify the genetic variants that increase the risk for common diseases like cancer and diabetes.

It was far too expensive at that time to think of sequencing patients' whole genomes. So the National Institutes of Health embraced the idea for a "shortcut", which was to look just at sites on the genome where many people have a variant DNA unit. The theory behind the shortcut was that since the major diseases are common, so too would be the genetic variants that caused them. Natural selection keeps the human genome free of variants that damage health before children are grown, the theory held, but fails against variants that strike later in life, allowing them to become quite common. (In 2002 the National Institutes of Health started a $138 million project called the HapMap to catalog the common variants in European, East Asian and African genomes.)

The genome was broken into smaller pieces; approximately 150,000 base pairs in length. These pieces were then ligated into a type of vector known as "bacterial artificial chromosomes", or BACs, which are derived from bacterial chromosomes which have been genetically engineered. The vectors containing the genes can be inserted into bacteria where they are copied by the bacterial DNA replication machinery. Each of these pieces was then sequenced separately as a small "shotgun" project and then assembled. The larger, 150,000 base pairs go together to create chromosomes. This is known as the "hierarchical shotgun" approach, because the genome is first broken into relatively large chunks, which are then mapped to chromosomes before being selected for sequencing.

Funding came from the US government through the National Institutes of Health in the United States, and a UK charity organization, the Wellcome Trust, as well as numerous other groups from around the world. The funding supported a number of large sequencing centers including those at Whitehead Institute, the Sanger Centre, Washington University in St. Louis, and Baylor College of Medicine.

The Human Genome Project is considered a Mega Project because the human genome has approximately 3.3 billion base-pairs; if the cost of sequencing is US $3 per base-pair, then the approximate cost will be US $10 billion.

If the sequence obtained was to be stored in book form, and if each page contained 1000 base-pairs recorded and each book contained 1000 pages, then 3300 such books would be needed in order to store the complete genome. However, if expressed in units of computer data storage, 3.3 billion base-pairs recorded at 2 bits per pair would equal 786 megabytes of raw data. This is comparable to a fully data loaded CD.

Public versus private approaches

In 1998, a similar, privately funded quest was launched by the American researcher Craig Venter, and his firm Celera Genomics. Venter was a scientist at the NIH during the early 1990s when the project was initiated. The $300,000,000 Celera effort was intended to proceed at a faster pace and at a fraction of the cost of the roughly $3 billion publicly funded project.

Celera used a technique called whole genome shotgun sequencing, employing pairwise end sequencing, which had been used to sequence bacterial genomes of up to six million

base pairs in length, but not for anything nearly as large as the three billion base pair human genome.

Celera initially announced that it would seek patent protection on "only 200–300" genes, but later amended this to seeking "intellectual property protection" on "fully-characterized important structures" amounting to 100–300 targets. The firm eventually filed preliminary ("place-holder") patent applications on 6,500 whole or partial genes. Celera also promised to publish their findings in accordance with the terms of the 1996 "Bermuda Statement," by releasing new data annually (the HGP released its new data daily), although, unlike the publicly funded project, they would not permit free redistribution or scientific use of the data. The publicly funded competitor UC Santa Cruz was compelled to publish the first draft of the human genome before Celera for this reason. On July 7, 2000, the UCSC Genome Bioinformatics Group released a first working draft on the web. The scientific community downloaded one-half trillion bytes of information from the UCSC genome server in the first 24 hours of free and unrestricted access to the first ever assembled blueprint of our human species.

In March 2000, President Clinton announced that the genome sequence could not be patented, and should be made freely available to all researchers. The statement sent Celera's stock plummeting and dragged down the biotechnology-heavy Nasdaq. The biotechnology sector lost about $50 billion in market capitalization in two days.

Although the working draft was announced in June 2000, it was not until February 2001 that Celera and the HGP scientists published details of their drafts. Special issues of *Nature* (which published the publicly funded project's scientific paper) and *Science* (which published Celera's paper) described the methods used to produce the draft sequence and offered analysis of the sequence. These drafts covered about 83% of the genome (90% of the euchromatic regions with 150,000 gaps and the order and orientation of many segments not yet established). In February 2001, at the time of the joint publications, press releases announced that the project had been completed by both groups. Improved drafts were announced in 2003 and 2005, filling in to ≈92% of the sequence currently.

The competition proved to be very good for the project, spurring the public groups to modify their strategy in order to accelerate progress. The rivals at UC Santa Cruz initially agreed to pool their data, but the agreement fell apart when Celera refused to deposit its data in the unrestricted public database GenBank. Celera had incorporated the public data into their genome, but forbade the public effort to use Celera data.

HGP is the most well known of many international genome projects aimed at sequencing the DNA of a specific organism. While the human DNA sequence offers the most tangible benefits, important developments in biology and medicine are predicted as a result of the sequencing of model organisms, including mice, fruit flies, zebrafish, yeast, nematodes, plants, and many microbial organisms and parasites.

In 2004, researchers from the International Human Genome Sequencing Consortium (IHGSC) of the HGP announced a new estimate of 20,000 to 25,000 genes in the human genome. Previously 30,000 to 40,000 had been predicted, while estimates at the start of the project reached up to as high as 2,000,000. The number continues to fluctuate and it is now expected that it will take many years to agree on a precise value for the number of genes in the human genome.

History

In 1976, the genome of the RNA virus Bacteriophage MS2 was the first complete genome to be determined, by Walter Fiers and his team at the University of Ghent (Ghent, Belgium). The idea for the shotgun technique came from the use of an algorithm that combined sequence information from many small fragments of DNA to reconstruct a genome. This technique was pioneered by Frederick Sanger to sequence the genome of the Phage Φ-X174, a virus (bacteriophage) that primarily infects bacteria that was the first fully sequenced genome (DNA-sequence) in 1977. The technique was called shotgun sequencing because the genome was broken into millions of pieces as if it had been blasted with a shotgun. In order to scale up the method, both the sequencing and genome assembly had to be automated, as they were in the 1980s.

Those techniques were shown applicable to sequencing of the first free-living bacterial genome (1.8 million base pairs) of *Haemophilus influenzae* in 1995 and the first animal genome (~100 Mbp) It involved the use of automated sequencers, longer individual sequences using approximately 500 base pairs at that time. Paired sequences separated by a fixed distance of around 2000 base pairs which were critical elements enabling the development of the first genome assembly programs for reconstruction of large regions of genomes (aka 'contigs').

Three years later, in 1998, the announcement by the newly-formed Celera Genomics that it would scale up the pairwise end sequencing method to the human genome was greeted with skepticism in some circles. The shotgun technique breaks the DNA into fragments of various sizes, ranging from 2,000 to 300,000 base pairs in length, forming what is called a DNA "library". Using an automated DNA sequencer the DNA is read in 800bp lengths from both ends of each fragment. Using a complex genome assembly algorithm and a supercomputer, the pieces are combined and the genome can be reconstructed from the millions of short, 800 base pair fragments. The success of both the public and privately funded effort hinged upon a new, more highly automated capillary DNA sequencing machine, called the Applied Biosystems 3700, that ran the DNA sequences through an extremely fine capillary tube rather than a flat gel. Even more critical was the development of a new, larger-scale genome assembly program, which could handle the 30–50 million sequences that would be required to sequence the entire human genome with this method. At the time, such a program did not exist. One of the first major projects at Celera Genomics was the development of this assembler, which was written in parallel with the construction of a large, highly automated genome sequencing factory. Development of the assembler was led by Brian Ramos. The first version of this assembler was demonstrated in 2000, when the Celera team joined forces with Professor

Gerald Rubin to sequence the fruit fly Drosophila melanogaster using the whole-genome shotgun method. At 130 million base pairs, it was at least 10 times larger than any genome previously shotgun assembled. One year later, the Celera team published their assembly of the three billion base pair human genome.

The Human Genome Project was a 13 year old mega project, that was launched in the year 1990 and completed in 2003. This project is closely associated to the branch of biology called Bio-informatics. The human genome project international consortium announced the publication of a draft sequence and analysis of the human genome—the genetic blueprint for the human being. An American company—Celera, led by Craig Venter and the other huge international collaboration of distinguished scientists led by Francis Collins, director, National Human Genome Research Institute, U.S., both published their findings.

This Mega Project is co-ordinated by the U.S. Department of Energy and the National Institute of Health. During the early years of the project, the Wellcome Trust (U.K.) became a major partner, other countries like Japan, Germany, China and France contributed significantly. Already the atlas has revealed some starting facts. The two factors that made this project a success are:

1. Genetic Engineering Techniques, with which it is possible to isolate and clone any segment of DNA.
2. Availability of simple and fast technologies, to determining the DNA sequences.

Being the most complex organisms, human beings were expected to have more than 100,000 genes or combination of DNA that provides commands for every characteristics of the body. Instead their studies show that humans have only 30,000 genes – around the same as mice, three times as many as flies, and only five times more than bacteria. Scientist told that not only are the numbers similar, the genes themselves, baring a few, are alike in mice and men. In a companion volume to the Book of Life, scientists have created a catalogue of 1.4 million single-letter differences, or single-nucleotide polymorphisms (SNPs) – and specified their exact locations in the human genome. This SNP map, the world's largest publicly available catalogue of SNP's, promises to revolutionize both mapping diseases and tracing human history. The sequence information from the consortium has been immediately and freely released to the world, with no restrictions on its use or redistribution. The information is scanned daily by scientists in academia and industry, as well as commercial database companies, providing key information services to bio-technologists. Already, many genes have been identified from the genome sequence, including more than 30 that play a direct role in human diseases. By dating the three millions repeat elements and examining the pattern of interspersed repeats on the Y-chromosome, scientists estimated the relative mutation rates in the X and the Y chromosomes and in the male and the female germ lines. They found that the ratio of mutations in male Vs female is 2:1. Scientists point to several possible reasons for the higher mutation rate in the male germ line, including the fact that there are a greater number of cell divisions involved in the formation of sperm than in the formation of eggs.

Methods

The IHGSC used pair-end sequencing plus whole-genome shotgun mapping of large (≈100 Kbp) plasmid clones and shotgun sequencing of smaller plasmid sub-clones plus a variety of other mapping data to orient and check the assembly of each human chromosome.

The Celera group emphasized the importance of the "whole-genome shotgun" sequencing method, relying on sequence information to orient and locate their fragments within the chromosome. However they used the publicly available data from HGP to assist in the assembly and orientation process, raising concerns that the Celera sequence was not independently derived.

Genome donors

In the IHGSC international public-sector Human Genome Project (HGP), researchers collected blood (female) or sperm (male) samples from a large number of donors. Only a few of many collected samples were processed as DNA resources. Thus the donor identities were protected so neither donors nor scientists could know whose DNA was sequenced. DNA clones from many different libraries were used in the overall project, with most of those libraries being created by Dr. Pieter J. de Jong. It has been informally reported, and is well known in the genomics community, that much of the DNA for the public HGP came from a single anonymous male donor from Buffalo, New York (code name RP11).

HGP scientists used white blood cells from the blood of two male and two female donors (randomly selected from 20 of each) -- each donor yielding a separate DNA library. One of these libraries (RP11) was used considerably more than others, due to quality considerations. One minor technical issue is that male samples contain just over half as much DNA from the sex chromosomes (one X chromosome and one Y chromosome) compared to female samples (which contain two X chromosomes). The other 22 chromosomes (the autosomes) are the same for both genders.

Although the main sequencing phase of the HGP has been completed, studies of DNA variation continue in the International HapMap Project, whose goal is to identify patterns of single-nucleotide polymorphism (SNP) groups (called haplotypes, or "haps"). The DNA samples for the HapMap came from a total of 270 individuals: Yoruba people in Ibadan, Nigeria; Japanese people in Tokyo; Han Chinese in Beijing; and the French Centre d'Etude du Polymorphisms Humain (CEf) resource, which consisted of residents of the United States having ancestry from Western and Northern Europe.

In the Celera Genomics private-sector project, DNA from five different individuals were used for sequencing. The lead scientist of Celera Genomics at that time, Craig Venter, later acknowledged (in a public letter to the journal Science) that his DNA was one of 21 samples in the pool, five of which were selected for use.

On September 4, 2007, a team led by Craig Venter published his complete DNA sequence, unveiling the six-billion-nucleotide genome of a single individual for the first time.

Benefits

The work on interpretation of genome data is still in its initial stages. It is anticipated that detailed knowledge of the human genome will provide new avenues for advances in medicine and biotechnology. Clear practical results of the project emerged even before the work was finished. For example, a number of companies, such as Myriad Genetics started offering easy ways to administer genetic tests that can show predisposition to a variety of illnesses, including breast cancer, disorders of hemostasis, cystic fibrosis, liver diseases and many others. Also, the etiologies for cancers, Alzheimer's disease and other areas of clinical interest are considered likely to benefit from genome information and possibly may lead in the long term to significant advances in their management.

There are also many tangible benefits for biological scientists. For example, a researcher investigating a certain form of cancer may have narrowed down his/her search to a particular gene. By visiting the human genome database on the World Wide Web, this researcher can examine what other scientists have written about this gene, including (potentially) the three-dimensional structure of its product, its function(s), its evolutionary relationships to other human genes, or to genes in mice or yeast or fruit flies, possible detrimental mutations, interactions with other genes, body tissues in which this gene is activated, diseases associated with this gene or other datatypes.

Further, deeper understanding of the disease processes at the level of molecular biology may determine new therapeutic procedures. Given the established importance of DNA in molecular biology and its central role in determining the fundamental operation of cellular processes, it is likely that expanded knowledge in this area will facilitate medical advances in numerous areas of clinical interest that may not have been possible without them.

The analysis of similarities between DNA sequences from different organisms is also opening new avenues in the study of evolution. In many cases, evolutionary questions can now be framed in terms of molecular biology; indeed, many major evolutionary milestones (the emergence of the ribosome and organelles, the development of embryos with body plans, the vertebrate immune system) can be related to the molecular level. Many questions about the similarities and differences between humans and our closest relatives (the primates, and indeed the other mammals) are expected to be illuminated by the data from this project.

The Human Genome Diversity Project (HGDP), spinoff research aimed at mapping the DNA that varies between human ethnic groups, which was rumored to have been halted, actually did continue and to date has yielded new conclusions. In the future, HGDP could possibly expose new data in disease surveillance, human development and anthropology. HGDP could unlock secrets behind and create new strategies for managing the

vulnerability of ethnic groups to certain diseases. It could also show how human populations have adapted to these vulnerabilities.

Advantages of Human Genome Project:

1. Knowledge of the effects of variation of DNA among individuals can revolutionize the ways to diagnose, treat and even prevent a number of diseases that affects the human beings.
2. It provides clues to the understanding of human biology.

Ethical, legal and social issues

The project's goals included not only identifying all of the approximately 24,000 genes in the human genome, but also to address the ethical, legal, and social issues (ELSI) that might arise from the availability of genetic information. Five percent of the annual budget was allocated to address the ELSI arising from the project.

Debra Harry, Executive Director of the U.S group Indigenous Peoples Council on Biocolonialism (IPCB), says that despite a decade of ELSI funding, the burden of genetics education has fallen on the tribes themselves to understand the motives of Human genome project and its potential impacts on their lives. Meanwhile, the government has been busily funding projects studying indigenous groups without any meaningful consultation with the groups.

The main criticism of ELSI is the failure to address the conditions raised by population-based research, especially with regard to unique processes for group decision-making and cultural worldviews. Genetic variation research such as HGP is group population research, but most ethical guidelines, according to Harry, focus on individual rights instead of group rights. She says the research represents a clash of culture: indigenous people's life revolves around collectivity and group decision making whereas the Western culture promotes individuality. Harry suggests that one of the challenges of ethical research is to include respect for collective review and decision making, while also upholding the Western model of individual rights.

Chapter- 7

Cloning

The sea anemone, *Anthopleura elegantissima* in process of cloning

Cloning in biology is the process of producing similar populations of genetically identical individuals that occurs in nature when organisms such as bacteria, insects or plants reproduce asexually. Cloning in biotechnology refers to processes used to create copies of DNA fragments (molecular cloning), cells (cell cloning), or organisms. The term also refers to the production of multiple copies of a product such as digital media or software.

The term *clone* is derived from *κλῶνος*, the Greek word for "trunk, branch", referring to the process whereby a new plant can be created from a twig. In horticulture, the spelling *clon* was used until the twentieth century; the final *e* came into use to indicate the vowel is a "long o" instead of a "short o". Since the term entered the popular lexicon in a more general context, the spelling *clone* has been used exclusively.

Molecular cloning

Molecular cloning refers to the process of making multiple molecules. Cloning is commonly used to amplify DNA fragments containing whole genes, but it can also be used to amplify any DNA sequence such as promoters, non-coding sequences and randomly fragmented DNA. It is used in a wide array of biological experiments and practical applications ranging from genetic fingerprinting to large scale protein production. Occasionally, the term cloning is misleadingly used to refer to the identification of the chromosomal location of a gene associated with a particular phenotype of interest, such as in positional cloning. In practice, localization of the gene to a chromosome or genomic region does not necessarily enable one to isolate or amplify the relevant genomic sequence. To amplify any DNA sequence in a living organism, that sequence must be linked to an origin of replication, which is a sequence of DNA capable of directing the propagation of itself and any linked sequence. However, a number of other features are needed and a variety of specialised cloning vectors (small piece of DNA into which a foreign DNA fragment can be inserted) exist that allow protein expression, tagging, single stranded RNA and DNA production and a host of other manipulations.

Cloning of any DNA fragment essentially involves four steps

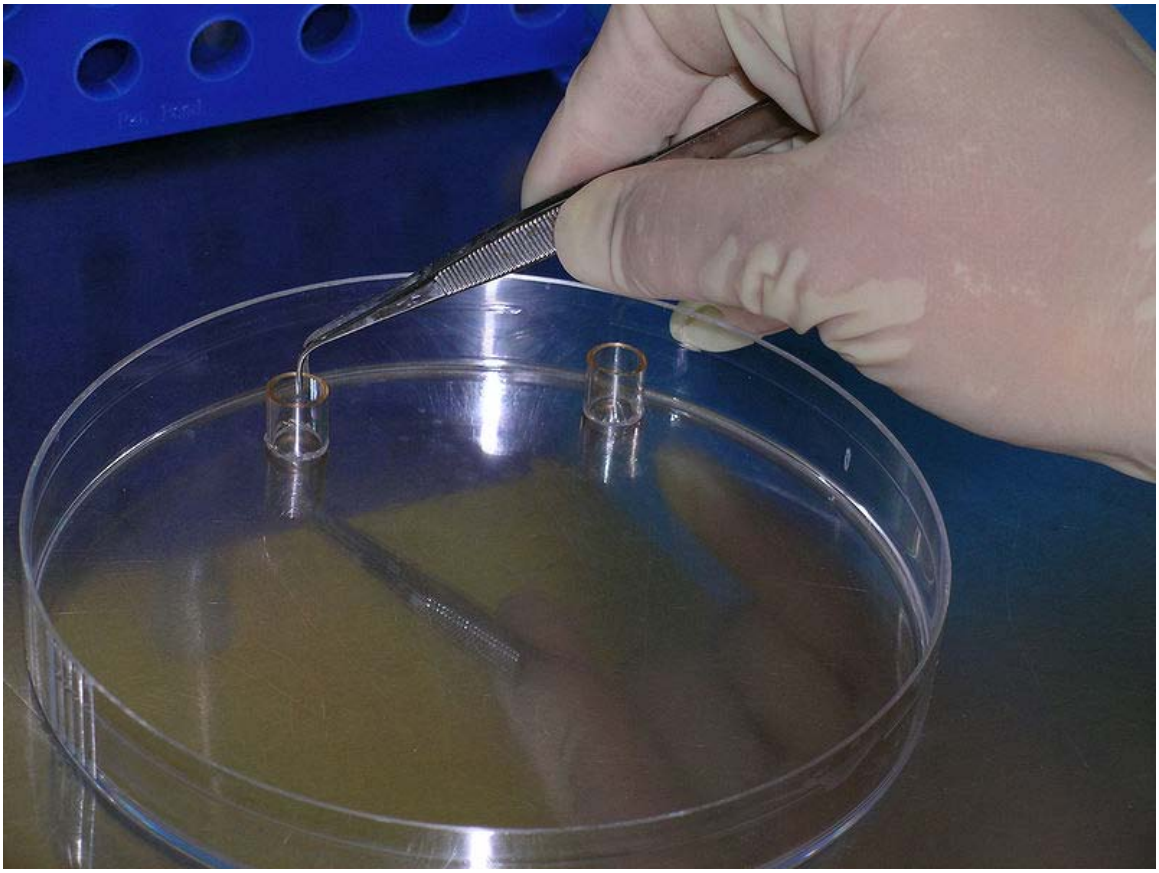
1. fragmentation - breaking apart a strand of DNA
2. ligation - gluing together pieces of DNA in a desired sequence
3. transfection - inserting the newly formed pieces of DNA into cells
4. screening/selection - selecting out the cells that were successfully transfected with the new DNA

Although these steps are invariable among cloning procedures a number of alternative routes can be selected, these are summarized as a 'cloning strategy'.

Initially, the DNA of interest needs to be isolated to provide a DNA segment of suitable size. Subsequently, a ligation procedure is used where the amplified fragment is inserted into a vector (piece of DNA). The vector (which is frequently circular) is linearised using restriction enzymes, and incubated with the fragment of interest under appropriate conditions with an enzyme called DNA ligase. Following ligation the vector with the insert of interest is transfected into cells. A number of alternative techniques are available, such as chemical sensitivation of cells, electroporation, optical injection and biolistics. Finally, the transfected cells are cultured. As the aforementioned procedures are of particularly low efficiency, there is a need to identify the cells that have been successfully transfected with the vector construct containing the desired insertion sequence in the required orientation. Modern cloning vectors include selectable antibiotic resistance markers, which allow only cells in which the vector has been transfected, to grow. Additionally, the cloning vectors may contain colour selection markers, which provide blue/white screening (??-factor complementation) on X-gal medium. Nevertheless, these selection steps do not absolutely guarantee that the DNA insert is present in the cells obtained. Further investigation of the resulting colonies must be

required to confirm that cloning was successful. This may be accomplished by means of PCR, restriction fragment analysis and/or DNA sequencing.

Unicellular organisms



Cloning cell-line colonies using cloning rings

Cloning a cell means to derive a population of cells from a single cell. In the case of unicellular organisms such as bacteria and yeast, this process is remarkably simple and essentially only requires the inoculation of the appropriate medium. However, in the case of cell cultures from multi-cellular organisms, cell cloning is an arduous task as these cells will not readily grow in standard media.

A useful tissue culture technique used to clone distinct lineages of cell lines involves the use of cloning rings (cylinders). According to this technique, a single-cell suspension of cells that have been exposed to a mutagenic agent or drug used to drive selection is plated at high dilution to create isolated colonies; each arising from a single and potentially clonal distinct cell. At an early growth stage when colonies consist of only a few of cells, sterile polystyrene rings (cloning rings), which have been dipped in grease are placed over an individual colony and a small amount of trypsin is added. Cloned cells are collected from inside the ring and transferred to a new vessel for further growth.

Cloning in stem cell research

Somatic cell nuclear transfer, known as SCNT, can also be used to create embryos for research or therapeutic purposes. The most likely purpose for this is to produce embryos for use in stem cell research. This process is also called "research cloning" or "therapeutic cloning." The goal is not to create cloned human beings (called "reproductive cloning"), but rather to harvest stem cells that can be used to study human development and to potentially treat disease. While a clonal human blastocyst has been created, stem cell lines are yet to be isolated from a clonal source.

Organism cloning

Organism cloning (also called reproductive cloning) refers to the procedure of creating a new multicellular organism, genetically identical to another. In essence this form of cloning is an asexual method of reproduction, where fertilization or inter-gamete contact does not take place. Asexual reproduction is a naturally occurring phenomenon in many species, including most plants and some insects. Scientists have made some major achievements with cloning, including the asexual reproduction of sheep and cows. There is a lot of ethical debate over whether or not cloning should be used. However, cloning, or asexual propagation, has been common practice in the horticultural world for hundreds of years.

Horticultural

The term *clone* is used in horticulture to mean all descendants of a single plant, produced by vegetative reproduction or apomixis. Many horticultural plant cultivars are clones, having been derived from a single individual, multiplied by some process other than sexual reproduction. As an example, some European cultivars of grapes represent clones that have been propagated for over two millennia. Other examples are potato and banana. Grafting can be regarded as cloning, since all the shoots and branches coming from the graft are genetically a clone of a single individual, but this particular kind of cloning has not come under ethical scrutiny and is generally treated as an entirely different kind of operation.

Many trees, shrubs, vines, ferns and other herbaceous perennials form clonal colonies. Parts of a large clonal colony often become detached from the parent, termed fragmentation, to form separate individuals. Some plants also form seeds is asexually, termed apomixis, e.g. dandelion.

Parthenogenesis

Clonal derivation exists in nature in some animal species and is referred to as parthenogenesis (reproduction of an organism by itself without a mate). This is an asexual form of reproduction that is only found in females of some insects, crustaceans and lizards. The growth and development occurs without fertilization by a male. In plants, parthenogenesis means the development of an embryo from an unfertilized egg

cell, and is a component process of apomixis. In species that use the XY sex-determination system, the offspring will always be female. An example is the "Little Fire Ant" (*Wasmannia auropunctata*), which is native to Central and South America but has spread throughout many tropical environments.

Artificial cloning of organisms

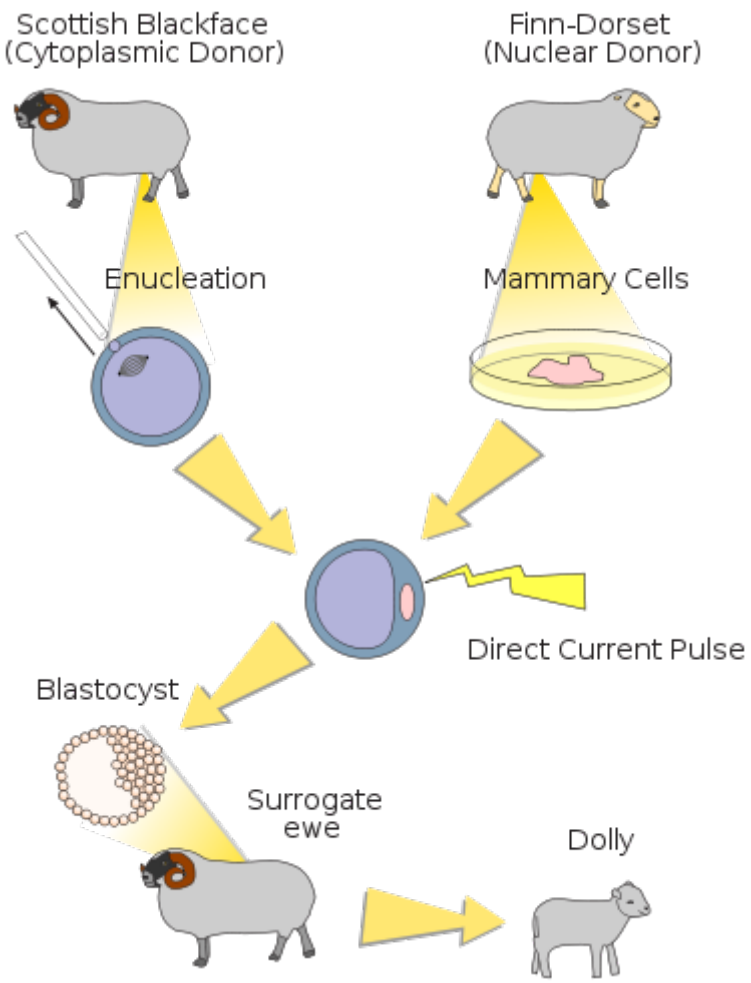
Artificial cloning of organisms may also be called reproductive cloning.

Methods

Reproductive cloning generally uses "somatic cell nuclear transfer" (SCNT) to create animals that are genetically identical. This process entails the transfer of a nucleus from a donor adult cell (somatic cell) to an egg that has no nucleus. If the egg begins to divide normally it is transferred into the uterus of the surrogate mother. Such clones are not strictly identical since the somatic cells may contain mutations in their nuclear DNA. Additionally, the mitochondria in the cytoplasm also contains DNA and during SCNT this DNA is wholly from the donor egg, thus the mitochondrial genome is not the same as that of the nucleus donor cell from which it was produced. This may have important implications for cross-species nuclear transfer in which nuclear-mitochondrial incompatibilities may lead to death.

Artificial *embryo splitting* or *embryo twinning* may also be used as a method of cloning, where an embryo is split in the maturation before embryo transfer. It is optimally performed at the 6- to 8-cell stage, where it can be used as an expansion of IVF to increase the number of available embryos. If both embryos are successful, it gives rise to monozygotic (identical) twins.

Dolly the Sheep



Dolly, a Finn-Dorset ewe, was the first mammal to have been successfully cloned from an adult cell. She was cloned at the Roslin Institute in Scotland and lived there from her birth in 1996 until her death in 2003 when she was six. Her stuffed remains were placed at Edinburgh's Royal Museum, part of the National Museums of Scotland.

Dolly was publicly significant because the effort showed that the genetic material from a specific adult cell, programmed to express only a distinct subset of its genes, can be reprogrammed to grow an entirely new organism. Before this demonstration, it had been shown by John Gurdon that nuclei from differentiated cells could give rise to an entire organism after transplantation into an enucleated egg. However, this concept was not yet demonstrated in a mamallian system.

Cloning Dolly the sheep had a low success rate per fertilized egg; she was born after 237 eggs were used to create 29 embryos, which only produced three lambs at birth, only one of which lived. Seventy calves have been created and one third of them died young;

Prometea took 277 attempts. Notably, although the first clones were frogs, no adult cloned frog has yet been produced from a somatic adult nucleus donor cell.

There were early claims that Dolly the Sheep had pathologies resembling accelerated aging. Scientists speculated that Dolly's death in 2003 was related to the shortening of telomeres, DNA-protein complexes that protect the end of linear chromosomes. However, other researchers, including Ian Wilmut who led the team that successfully cloned Dolly, argue that Dolly's early death due to respiratory infection was unrelated to deficiencies with the cloning process.

Species cloned

The modern cloning techniques involving nuclear transfer have been successfully performed on several species. Landmark experiments in chronological order:

- Tadpole: (1952) Many scientists questioned whether cloning had actually occurred and unpublished experiments by other labs were not able to reproduce the reported results.
- Carp: (1963) In China, embryologist Tong Dizhou produced the world's first cloned fish by inserting the DNA from a cell of a male carp into an egg from a female carp. He published the findings in a Chinese science journal.
- Mice: (1986) A mouse was the first mammal successfully cloned from an early embryonic cell. Soviet scientists Chaylakhyan, Veprencev, Sviridova, and Nikitin had the mouse "Masha" cloned. Research was published in the magazine "Biofizika" volume XXXII, issue 5 of 1987.
- Sheep: (1996) From early embryonic cells by Steen Willadsen. Megan and Morag cloned from differentiated embryonic cells in June 1995 and Dolly the sheep from a somatic cell in 1997.
- Rhesus Monkey: Tetra (January 2000) from embryo splitting
- Gaur: (2001) was the first endangered species cloned.
- Cattle: Alpha and Beta (males, 2001) and (2005) Brazil
- Cat: CopyCat "CC" (female, late 2001), Little Nicky, 2004, was the first cat cloned for commercial reasons
- Dog: Snuppy, a male Afghan hound was the first cloned dog (2005).
- Rat: Ralph, the first cloned rat (2003)
- Mule: Idaho Gem, a john mule born 4 May 2003, was the first horse-family clone.
- Horse: Prometea, a Haflinger female born 28 May 2003, was the first horse clone.
- Water Buffalo: Samrupa was the first cloned water buffalo. It was born on February 6, 2009, at India's Karnal National Diary Research Institute but died five days later due to lung infection.
- Camel: (2009) Injaz, is the first cloned camel.

Human cloning

Human cloning is the creation of a genetically identical copy of an existing or previously existing human. The term is generally used to refer to *artificial* human cloning; human

clones in the form of identical twins are commonplace, with their cloning occurring during the natural process of reproduction. There are two commonly discussed types of human cloning: therapeutic cloning and reproductive cloning. Therapeutic cloning involves cloning adult cells for use in medicine and is an active area of research. Reproductive cloning would involve making cloned humans. A third type of cloning called replacement cloning is a theoretical possibility, and would be a combination of therapeutic and reproductive cloning. Replacement cloning would entail the replacement of an extensively damaged, failed, or failing body through cloning followed by whole or partial brain transplant.

The various forms of human cloning are controversial. There have been numerous demands for all progress in the human cloning field to be halted. Most scientific, governmental and religious organizations oppose reproductive cloning. The American Association for the Advancement of Science (AAAS) and other scientific organizations have made public statements suggesting that human reproductive cloning be banned until safety issues are resolved. Serious ethical concerns have been raised by the future possibility of harvesting organs from clones. Some people have considered the idea of growing organs separately from a human organism - in doing this, a new organ supply could be established without the moral implications of harvesting them from humans. Research is also being done on the idea of growing organs that are biologically acceptable to the human body inside of other organisms, such as pigs or cows, then transplanting them to humans, a form of xenotransplantation.

The first hybrid human clone was created in November 1998, by American Cell Technologies. It was created from a man's leg cell, and a cow's egg whose DNA was removed. It was destroyed after 12 days. Since a normal embryo implants at 14 days, Dr Robert Lanza, ACT's director of tissue engineering, told the Daily Mail newspaper that the embryo could not be seen as a person before 14 days. While making an embryo, which may have resulted in a complete human had it been allowed to come to term, according to ACT: "[ACT's] aim was 'therapeutic cloning' not 'reproductive cloning'"

On January, 2008, Wood and Andrew French, Stemagen's chief scientific officer in California, announced that they successfully created the first 5 mature human embryos using DNA from adult skin cells, aiming to provide a source of viable embryonic stem cells. Dr. Samuel Wood and a colleague donated skin cells, and DNA from those cells was transferred to human eggs. It is not clear if the embryos produced would have been capable of further development, but Dr. Wood stated that if that were possible, using the technology for reproductive cloning would be both unethical and illegal. The 5 cloned embryos, created in Stemagen Corporation lab, in La Jolla, were destroyed.

Ethical issues of cloning

Because of recent technological advancements, the cloning of animals (and potentially humans) has been an issue. The Catholic Church and many religious organizations oppose all forms of cloning, on the grounds that life begins at conception. Judaism does not equate life with conception and, though some question the wisdom of cloning,

Orthodox rabbis generally find no firm reason in Jewish law and ethics to object to cloning. From the standpoint of classical liberalism, concerns also exist regarding the protection of the identity of the individual and the right to protect one's genetic identity.

Gregory Stock is a scientist and outspoken critic against restrictions on cloning research. Bioethicist Gregory Pence also attacks the idea of criminalizing attempts to clone humans.

The social implications of an artificial human production scheme were famously explored in Aldous Huxley's novel *Brave New World.*

On December 28, 2006, the U.S. Food and Drug Administration (FDA) approved the consumption of meat and other products from cloned animals. Cloned-animal products were said to be virtually indistinguishable from the non-cloned animals. Furthermore, companies would not be required to provide labels informing the consumer that the meat comes from a cloned animal.

Critics have raised objections to the FDA's approval of cloned-animal products for human consumption, arguing that the FDA's research was inadequate, inappropriately limited, and of questionable scientific validity. Several consumer-advocate groups are working to encourage a tracking program that would allow consumers to become more aware of cloned-animal products within their food.

Joseph Mendelson, legal director of the Center for Food Safety, said that cloned food still should be labeled since safety and ethical issues about it remain questionable.

Carol Tucker Foreman, director of food policy at the Consumer Federation of America, stated that FDA does not consider the fact that the results of some studies revealed that cloned animals have increased rates of mortality and deformity at birth.

Cloning extinct and endangered species

Cloning, or more precisely, the reconstruction of functional DNA from extinct species has, for decades, been a dream of some scientists. The possible implications of this were dramatized in the best-selling novel by Michael Crichton and high budget Hollywood thriller *Jurassic Park*. In real life, one of the most anticipated targets for cloning was once the Woolly Mammoth, but attempts to extract DNA from frozen mammoths have been unsuccessful, though a joint Russo-Japanese team is currently working toward this goal.

In 2001, a cow named Bessie gave birth to a cloned Asian gaur, an endangered species, but the calf died after two days. In 2003, a banteng was successfully cloned, followed by three African wildcats from a thawed frozen embryo. These successes provided hope that similar techniques (using surrogate mothers of another species) might be used to clone extinct species. Anticipating this possibility, tissue samples from the last *bucardo* (Pyrenean Ibex) were frozen in liquid nitrogen immediately after it died in 2000.

Researchers are also considering cloning endangered species such as the giant panda, ocelot, and cheetah. The "Frozen Zoo" at the San Diego Zoo now stores frozen tissue from the world's rarest and most endangered species.

In 2002, geneticists at the Australian Museum announced that they had replicated DNA of the Thylacine (Tasmanian Tiger), extinct about 65 years previous, using polymerase chain reaction. However, on February 15, 2005 the museum announced that it was stopping the project after tests showed the specimens' DNA had been too badly degraded by the (ethanol) preservative. On 15 May 2005 it was announced that the Thylacine project would be revived, with new participation from researchers in New South Wales and Victoria.

In January 2009, for the first time, an extinct animal, the Pyrenean ibex mentioned above was cloned, at the Centre of Food Technology and Research of Aragon, using the preserved DNA of the skin samples from 2001 and domestic goat egg-cells. (The ibex died shortly after birth due to physical defects in its lungs.) One of the continuing obstacles in the attempt to clone extinct species is the need for nearly perfect DNA. Cloning from a single specimen could not create a viable breeding population in sexually reproducing animals. Furthermore, even if males and females were to be cloned, the question would remain open whether they would be viable at all in the absence of parents that could teach or show them their natural behavior.

Cloning endangered species is a highly ideological issue. Many conservation biologists and environmentalists vehemently oppose cloning endangered species — mainly because they think it may deter donations to help preserve natural habitat and wild animal populations. The "rule-of-thumb" in animal conservation is that, if it is still feasible to conserve habitat and viable wild populations, breeding in captivity should not be undertaken in isolation.

In a 2006 review, David Ehrenfeld concluded that cloning in animal conservation is an experimental technology that, at its state in 2006, could not be expected to work except by pure chance and utterly failed a cost-benefit analysis. Furthermore, he said, it is likely to siphon funds from established and working projects and does not address any of the issues underlying animal extinction (such as habitat destruction, hunting or other overexploitation, and an impoverished gene pool). While cloning technologies are well-established and used on a regular basis in plant conservation, care must be taken to ensure genetic diversity. He concluded:

Vertebrate cloning poses little risk to the environment, but it can consume scarce conservation resources, and its chances of success in preserving species seem poor. To date, the conservation benefits of transgenics and vertebrate cloning remain entirely theoretical, but many of the risks are known and documented. Conservation biologists should devote their research and energies to the established methods of conservation, none of which require transgenics or vertebrate cloning.

Chapter- 8

Genetically Modified Food

Genetically modified (GM) foods are foods derived from genetically modified organisms. Genetically modified organisms have had specific changes introduced into their DNA by genetic engineering techniques. These techniques are much more precise than mutagenesis (mutation breeding) where an organism is exposed to radiation or chemicals to create a non-specific but stable change. Other techniques by which humans modify food organisms include selective breeding (plant breeding and animal breeding), and somaclonal variation.

GM foods were first put on the market in the early 1990s. Typically, genetically modified foods are transgenic plant products: soybean, corn, canola, and cotton seed oil. Animal products have also been developed, although as of July 2010 none are currently on the market. In 2006 a pig was controversially engineered to produce omega-3 fatty acids through the expression of a roundworm gene. Researchers have also developed a genetically-modified breed of pigs that are able to absorb plant phosphorus more efficiently, and as a consequence the phosphorus content of their manure is reduced by as much as 60%.

Critics have objected to GM foods on several grounds, including safety issues, ecological concerns, and economic concerns raised by the fact that these organisms are subject to intellectual property law.

Method

Genetic modification involves the insertion or deletion of genes. In the process of cisgenesis, genes are artificially transferred between organisms that could be conventionally bred. In the process of transgenesis, genes from a different species are inserted, which is a form of horizontal gene transfer. In nature this can occur when exogenous DNA penetrates the cell membrane for any reason. To do this artificially may require attaching genes to a virus or just physically inserting the extra DNA into the nucleus of the intended host with a very small syringe, or with very small particles fired from a gene gun. However, other methods exploit natural forms of gene transfer, such as

the ability of Agrobacterium to transfer genetic material to plants, and the ability of lentiviruses to transfer genes to animal cells.

Development

The first commercially grown genetically modified whole food crop was a tomato (called FlavrSavr), which was modified to ripen without softening, by Calgene, later a subsidiary of Monsanto. Calgene took the initiative to obtain FDA approval for its release in 1994 without any special labeling, although legally no such approval was required. It was welcomed by consumers who purchased the fruit at a substantial premium over the price of regular tomatoes. However, production problems and competition from a conventionally bred, longer shelf-life variety prevented the product from becoming profitable. A tomato produced using similar technology to the Flavr Savr was used by Zeneca to produce tomato paste which was sold in Europe during the summer of 1996. The labeling and pricing were designed as a marketing experiment, which proved, at the time, that European consumers would accept genetically engineered foods. Currently, there are a number of food species in which a genetically modified version exists (percent modified are mostly 2009/2010 data).

Food	Properties of the genetically modified variety	Modification	Percent Modified in US	Percent Modified in world
Soybeans	Resistant to glyphosate or glufosinate herbicides	Herbicide resistant gene taken from bacteria inserted into soybean	93%	77%
Corn, field	Resistant to glyphosate or glufosinate herbicides. Insect resistance via producing Bt proteins, some previously used as pesticides in organic crop production. Vitamin-enriched corn derived from South African white corn variety M37W has bright orange kernels, with 169x increase in beta carotene, 6x the vitamin C and 2x folate.	New genes, some from the bacterium Bacillus thuringiensis, added/transferred into plant genome.	86%	26%
Cotton (cottonseed oil)	Pest-resistant cotton	Bt crystal protein gene added/transferred into plant genome	93%	49%

Alfalfa	Resistant to glyphosate or glufosinate herbicides	New genes added/transferred into plant genome.	Planted in the US from 2005–2007; no longer planted currently due to court decisions	
Hawaiian papaya	Variety is resistant to the papaya ringspot virus.	New gene added/transferred into plant genome	80%	
Tomatoes	Variety in which the production of the enzyme polygalacturonase (PG) is suppressed, retarding fruit softening after harvesting.	A reverse copy (an antisense gene) of the gene responsible for the production of PG enzyme added into plant genome	Taken off the market due to commercial failure.	Small quantities grown in China
Rapeseed (Canola)	Resistance to herbicides (glyphosate or glufosinate), high laurate canola	New genes added/transferred into plant genome	93%	21%
Sugar cane	Resistance to certain pesticides, high sucrose content.	New genes added/transferred into plant genome		
Sugar beet	Resistance to glyphosate, glufosinate herbicides	New genes added/transferred into plant genome	95% (2010); planting in the US is halted as of 13 Aug. 2010 by court order	9%
Rice	Genetically modified to contain high amounts of Vitamin A (beta-carotene)	"Golden rice" Three new genes implanted: two from daffodils and the third from a bacterium	Forecast to be on the market in 2012	
Squash (Zucchini)	Resistance to watermelon, cucumber and zucchini yellow	Contains coat protein genes of viruses.	13%	

	mosaic viruses			
Sweet Peppers	Resistance to virus	Contains coat protein genes of the virus.		Small quantities grown in China

In addition, various genetically engineered micro-organisms are routinely used as sources of enzymes for the manufacture of a variety of processed foods. These include alpha-amylase from bacteria, which converts starch to simple sugars, chymosin from bacteria or fungi that clots milk protein for cheese making, and pectinesterase from fungi which improves fruit juice clarity.

Growing GM crops

Between 1997 and 2009, the total surface area of land cultivated with GMOs had increased by a factor of 80, from 17,000 km^2 (4.2 million acres) to 1,340,000 km^2 (331 million acres).

Although most GM crops are grown in North America, in recent years there has been rapid growth in the area sown in developing countries. For instance in 2009 the largest increase in crop area planted to GM crops (soybeans) was in Brazil (214,000 km^2 in 2009 versus 158,000 km^2 in 2008.) There has also been rapid and continuing expansion of GM cotton varieties in India since 2002. (Cotton is a major source of vegetable cooking oil and animal feed.) In 2009 84,000 km^2 of GM cotton were harvested in India.

In India, GM cotton yields in Andhra Pradesh were no better than non-GM cotton in 2002, the first year of commercial GM cotton planting. This was because there was a severe drought in Andhra Pradesh that year and the parental cotton plant used in the genetic engineered variant was not well suited to extreme drought. Maharashtra, Karnataka, and Tamil Nadu had an average 42% increase in yield with GM cotton in the same year. Drought resistant variants were developed and, with the substantially reduced losses to insect predation, by 2009 87% of Indian cotton was GM. Though disputed the economic and environmental benefits of GM cotton in India to the individual farmer have been documented.

In 2009, countries that grew 95% of the global transgenic crops were the United States (46%), Brazil (16%), Argentina (15%), India (6%), Canada (6%), China (3%), Paraguay (2%) and South Africa (2%). The Grocery Manufacturers of America estimate that 75% of all processed foods in the U.S. contain a GM ingredient. In particular, Bt corn, which produces the pesticide within the plant itself, is widely grown, as are soybeans genetically designed to tolerate glyphosate herbicides. These constitute "input-traits" are aimed to financially benefit the producers, have indirect environmental benefits and marginal cost benefits to consumers.

In the US, by 2009/2010, 93% of the planted area of soybeans, 93% of cotton, 86% of corn and 95% of the sugar beet were genetically modified varieties. Genetically modified soybeans carried herbicide-tolerant traits only, but maize and cotton carried both herbicide tolerance and insect protection traits (the latter largely the *Bacillus thuringiensis* Bt insecticidal protein). In the period 2002 to 2006, there were significant increases in the area planted to Bt protected cotton and maize, and herbicide tolerant maize also increased in sown area.

Legal issues in the US

Alfalfa

On 21 June 2010, the US Supreme Court issued its first ruling in regard to a GM crop. This was a ruling in regard to Roundup Ready alfalfa. The case goes back to 2006, when organic farmers, concerned about the impact of GM alfalfa on their crops, sued Monsanto. In response, the California Northern District Court ruled that the United States Department of Agriculture (USDA) was in error when it approved the planting of Roundup Ready alfalfa. According to the presiding judge, the law required the USDA to first conduct a full environmental study, which it had not done. It was the concern of the organic growers that the GM alfalfa could cross-pollinate with their organic alfalfa, making their crops unsalable in countries that forbid the growing of GM crops.

The impact of the current US Supreme Court ruling is somewhat unclear, with both sides appearing to claim victory. While Monsanto can claim technical victory in the case, various other issues still remain open, and will likely be litigated in the future. Meanwhile, the planting of GM alfalfa currently remains halted in the US, and it is unclear when it may resume.

Sugar beets

Between 2009 and 2011, the United States District Court for the Northern District of California considered the case involving the planting of genetically modified sugar beets. This case involves Monsanto's breed of pesticide-resistant sugar beets. Earlier in 2010, Judge Jeffrey S. White allowed the planting of GM sugar beets to continue, but he also warned that this may be blocked in the future while an environmental review was taking place. Finally, on 13 August 2010, Judge White ordered a halt to the planting of the genetically modified sugar beets in the US. He indicated that "the Agriculture Department had not adequately assessed the environmental consequences before approving them for commercial cultivation." The decision was the result of a lawsuit organised by the Center for Food Safety, a US non-governmental organisation that is a critic of biotech crops.

Crop yields

A 1999 study by Charles Benbrook, Chief Scientist of the Organic Center, found that genetically engineered Roundup Ready soybeans did not increase yields. The report

reviewed over 8,200 university trials in 1998 and found that Roundup Ready soybeans had a yield drag of 5.3% across all varieties tested. In addition, the same study found that farmers used 2-5 times more herbicide (Roundup) on Roundup Ready soybeans compared to other popular weed management systems.

However research published in Science in 2003 has shown that the use of genetically modified Bt cotton in India increased yields by 60% over the period 1998-2001 while the number of applications of insecticides against bollworm were three times less on average.

A 2008 Soil Association report found that some scientific studies claimed that genetically modified varieties of plants do not produce higher crop yields than normal plants.

In 2009 the Union of Concerned Scientists summarized numerous peer-reviewed studies on the yield contribution of genetic engineering in the United States. This report examined the two most widely grown engineered crops—soybeans and maize (corn). Unlike many other studies, this work separated the yield contribution of the engineered gene from that of the many naturally occurring yield genes in crops.

The report found that engineered herbicide tolerant soy and maize did not increase yield at the national, aggregate level. Maize engineered with Bt insect resistance genes increased national yield by about 3 to 4 percent. Engineered crops increased net yield in all cases.

The study concluded that in the United States, other agricultural methods have made a much greater contribution to national crop yield increases in recent years than genetic engineering. United States Department of Agriculture data record maize yield increases of about 28 percent since engineered varieties were first commercialized in the mid 1990s. The yield contribution of engineered genes has therefore been a modest fraction—about 14 percent—of the maize yield increase since the mid 1990s.

A 2010 article summarised the results of 49 peer reviewed studies on GM crops worldwide. On average, farmers in developed countries experienced increase in yield of 6% and in underdeveloped countries of 29%. Tillage was decreased by 25-58% on herbicide resistant soybeans, insecticide applications on Bt crops were reduced by 14-76% and 72% of farmers worldwide experienced positive economic results.

Coexistence and traceability

The United States and Canada do not require labeling of genetically modified foods. However in certain other regions, such as the European Union, Japan, Malaysia and Australia, governments have required labeling so consumers can exercise choice between foods that have genetically modified, conventional or organic origins. This requires a labeling system as well as the reliable separation of GM and non-GM organisms at production level and throughout the whole processing chain.

For traceability, the OECD has introduced a "unique identifier" which is given to any GMO when it is approved. This unique identifier must be forwarded at every stage of processing. Many countries have established labeling regulations and guidelines on coexistence and traceability. Research projects such as Co-Extra, SIGMEA and Transcontainer are aimed at investigating improved methods for ensuring coexistence and providing stakeholders the tools required for the implementation of coexistence and traceability.

Detection

Testing on GMOs in food and feed is routinely done using molecular techniques like DNA microarrays or qPCR. These tests can be based on screening genetic elements (like p35S, tNos, pat, or bar) or event-specific markers for the official GMOs (like Mon810, Bt11, or GT73). The array-based method combines multiplex PCR and array technology to screen samples for different potential GMOs, combining different approaches (screening elements, plant-specific markers, and event-specific markers).

The qPCR is used to detect specific GMO events by usage of specific primers for screening elements or event-specific markers. Controls are necessary to avoid false positive or false negative results. For example, a test for CaMV is used to avoid a false positive in the event of a virus contaminated sample.

PLU codes

A Price Look-Up code beginning with the digit *8* indicates genetically modified food.

Controversy

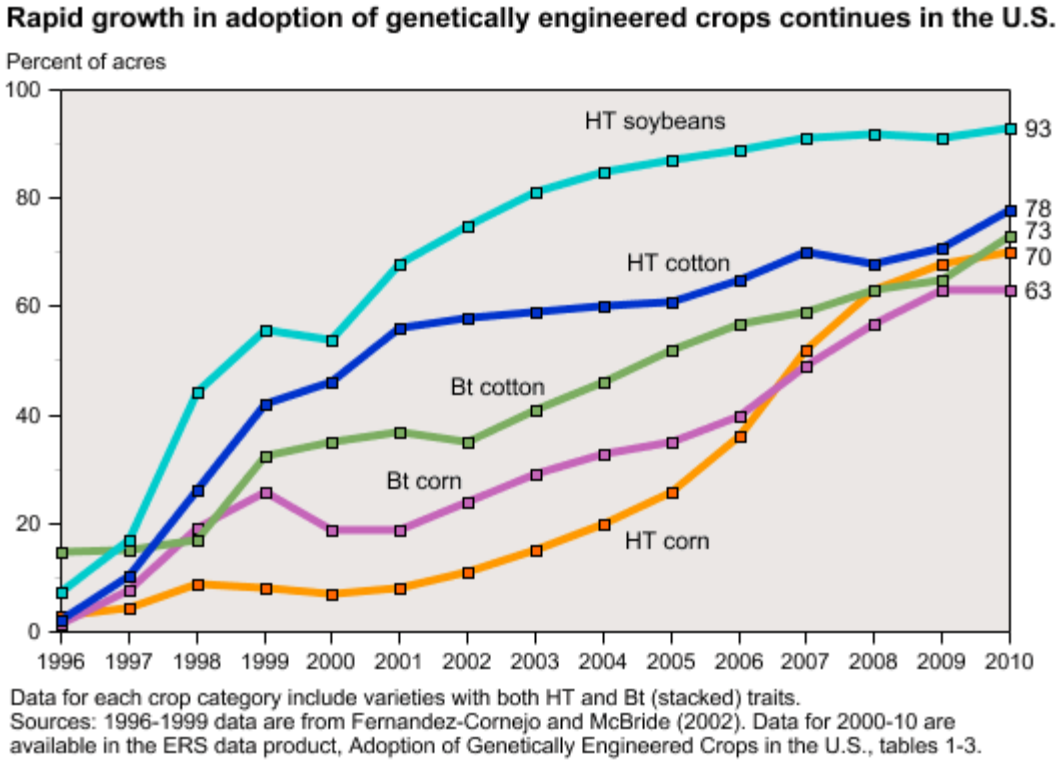
While it is evident that there is a food supply issue, the question is whether GM can solve world hunger problems, or even if that would be the best way to address the issue. Several scientists argue that in order to meet the demand for food in the developing world, a second Green Revolution with increased use of GM crops is needed. Others argue that there is more than enough food in the world and that the hunger crisis is caused by problems in food distribution and politics, not production. Recently some critics and environmentalists have changed their minds on the issue with respect to the need for additional food supplies. Further, it has been widely noted that there are those who consider over-population the real issue here, and that food production is adequate for any reasonable population size.

“Genetic modification is analogous to nuclear power: nobody loves it, but climate change has made its adoption imperative,” says economist Paul Collier of Oxford University. "Declining genetic modification makes a complicated issue more complex. Genetic modification offers both faster crop adaptation and a biological, rather than chemical, approach to yield increases."

On the other hand, many believe that GM food has not been a success and that we should devote our efforts and money into another solution. “We need biodiversity intensification that works with nature’s nutrient and water cycles, not against them,” says Vandana Shiva, the founder of Navdanya, the movement of 500,000 seed keepers and organic farmers in India, argues that GMF’s have not increased yields. Recently, Doug Gurian-Sherman, a member of the Union of Concerned Scientists, a nonprofit science advocacy group, published a report called “Failure to Yield”, in which he stated that in a nearly 20 year record, genetically engineered crops have not increased yields substantially of food and livestock feed crops in the United States.

Some claim that genetically modified food help farmers produce, despite the odds or any environmental barriers. “While new technology must be tested before it is commercially released, we should be mindful of the risks of not releasing it at all,” says Per Pinstrup-Andersen, professor of Food, Nutrition and Public Policy at Cornell University. Per Pinstrup-Anderson argues, “Misguided anti-science ideology and failure by governments to prioritize agricultural and rural development in developing countries brought us the food crisis.” He clearly states the challenge we face is not the challenge of whether we have enough resources to produce, but whether we will change our behavior.

Economic and environmental effects



Adoption of genetically-engineered crops in the United States.

- Many proponents of genetically engineered crops claim they lower pesticide usage and have brought higher yields and profitability to many farmers, including

those in developing nations. For example, a 2010 study by US scientists, found that the economic benefit of Bt corn to farmers in five mid-west states was $6.9 billion over the previous 14 years. They were surprised that the majority ($4.3 billion) of the benefit accrued to non-Bt corn. This was speculated to be because the European Corn Borers that attack the Bt corn die and there are fewer left to attack the non-GM corn nearby.

- The United States has seen a widespread adoption of genetically-engineered corn, cotton and soybean crops since 1996.

- In 2010, the U.S. National Academy of Sciences reported that genetically engineered crops had resulted in reduced pesticide application and reduced soil erosion from tilling. The report also stated that the advent of glyphosate-herbicide resistant weeds—that have developed because of the use of engineered crops—could cause the genetically engineered crops to lose their effectiveness unless farmers also use other established weed management strategies.

- In a study by Scientists at the University of Arkansas published in 2010 showed that about 83 percent of wild or weedy canola they tested contained genetically modified herbicide resistance genes, and they also found some plants that contained resistance to both herbicides, a combination of transgenic traits that had not been developed in canola crops. That leads us to believe that these wild populations that contain modified genes have become established populations.

Bans

- In 2002, Zambia cut off the flow of Genetically Modified Food (mostly maize) from UN's World Food Programme. This left a famine-stricken population without food aid.
- In December 2005 the Zambian government changed its mind in the face of further famine and allowed the importation of GM maize. However, the Zambian Minister for Agriculture Mundia Sikatana has insisted that the ban on genetically modified maize remains, saying "We do not want GM (genetically modified) foods and our hope is that all of us can continue to produce non-GM foods."
- In April 2004 Hugo Chávez announced a total ban on genetically modified seeds in Venezuela.
- In January 2005, the Hungarian government announced a ban on importing and planting of genetic modified maize seeds, which was subsequently authorized by the EU.
- On August 18, 2006, American exports of rice to Europe were interrupted when much of the U.S. crop was confirmed to be contaminated with unapproved engineered genes, possibly caused by cross-pollination with conventional crops.
- On February 9, 2010, Indian Environment Minister, Jairam Ramesh, imposed a moratorium on the cultivation of GMF "for as long as it is needed to establish public trust and confidence". His decision was made after protest from several

groups responding to regulatory approval of the cultivation of Bt brinjal, a GM eggplant in October, 2009.

Intellectual property

Traditionally, farmers in all nations saved their own seed from year to year. It should be noted that this does not apply in more agriculturally developed countries for some crops. Corn is one example where producers generally have not saved seed since the early 1900s with the advent of hybrid corn through selective breeding. Seed producers grow the seed corn instead due to the effort needed to produce hybrids. The offspring of the hybrid corn, while still viable, lose the beneficial traits of the parents, resulting in the loss of hybrid vigor. In these cases, the use of hybrid plants has been the primary reason for growers not saving seed, not intellectual property issues, and has been in practice well before genetically-modified seed was developed. However, the practice of not saving seed in non-hybrid crops, such as soybean, is mainly due to intellectual property regulations. Allowing to follow this practice with genetically modified seed would result in seed developers losing the ability to profit from their breeding work. Therefore, genetically-modified seed is subject to licensing by their developers in contracts that are written to prevent farmers from following this practice.

Enforcement of patents on genetically modified plants is often contentious, especially because of gene flow. In 1998, 95-98 percent of about 10 km^2 planted with canola by Canadian farmer Percy Schmeiser were found to contain Monsanto Company's patented Roundup Ready gene although Schmeiser had never purchased seed from Monsanto. The initial source of the plants was undetermined, and could have been through either gene flow or intentional theft. However, the overwhelming predominance of the trait implied that Schmeiser must have intentionally selected for it. The court determined that Schmeiser had saved seed from areas on and adjacent to his property where Roundup had been sprayed, such as ditches and near power poles.

Although unable to prove direct theft, Monsanto sued Schmeiser for piracy since he knowingly grew *Roundup Ready* plants without paying royalties (Ibid). The case made it to the Canadian Supreme Court, which in 2004 ruled 5 to 4 in Monsanto’s favor. The dissenting judges focused primarily on the fact that Monsanto's patents covered only the gene itself and glyphosate resistant *cells*, and failed to cover transgenic plants in their entirety. All of the judges agreed that Schmeiser would not have to pay any damages since he had not benefited from his use of the genetically modified seed.

In response to criticism, Monsanto Canada's Director of Public Affairs stated that "It is not, nor has it ever been Monsanto Canada's policy to enforce its patent on Roundup Ready crops when they are present on a farmer's field by accident...Only when there has been a knowing and deliberate violation of its patent rights will Monsanto act."

Future developments

Future envisaged applications of GMOs are diverse and include drugs in food, bananas that produce human vaccines against infectious diseases such as Hepatitis B, metabolically engineered fish that mature more quickly, fruit and nut trees that yield years earlier, foods no longer containing properties associated with common intolerances, and plants that produce new plastics with unique properties. While their practicality or efficacy in commercial production has yet to be fully tested, the next decade may see exponential increases in GM product development as researchers gain increasing access to genomic resources that are applicable to organisms beyond the scope of individual projects. Safety testing of these products will also, at the same time, be necessary to ensure that the perceived benefits will indeed outweigh the perceived and hidden costs of development. Plant scientists, backed by results of modern comprehensive profiling of crop composition, point out that crops modified using GM techniques are less likely to have unintended changes than are conventionally bred crops.

Health risks

In the United States, the FDA Center for Food Safety and Applied Nutrition reviews summaries of food safety data developed and voluntarily submitted by developers of engineered foods, in part on the basis of comparability to conventionally-produced foods. There are no specific tests required by FDA to determine safety. FDA does not approve the safety of engineered foods, but after its review, acknowledges that the developer of the food has asserted that it is safe. The table below shows the foods that have been reviewed by FDA as of 2002.

Table 1: GM Foods for Human Consumption Evaluated by FDA

Modified Attribute	Insect Resistance	Viral Resistance	Herbicide Tolerance	Modified Oil	Plant Reproductive Sterility	Delayed Ripening/ Softening
GM Plant Product—# of Plant Varieties	Corn—8 Tomato—1 Potato—4 Cotton—2	Squash—2 Papaya—1 Potato—2	Corn—9 Rice—1 Canola—8 Sugar Beet—2 Flax—1 Cotton[a]—4 Radish—1 Soybean—2	Soybean—1 Canola—1	Corn—3 Canola—3 Radish—1	Cantaloupe—1 Tomato—4
Total[b]	**15**	**5**	**28**	**2**	**7**	**5**

[a]Cotton seed has been used as a protein source in candy.

[b]Fifty products have been evaluated, as of April 2002. The total number of modified attributes is 62 because several products were modified with multiple attributes.

Source: GAO analysis of FDA data.

A 2008 review published by the Royal Society of Medicine noted that GM foods have been eaten by millions of people worldwide for over 15 years, with no reports of ill effects. Similarly a 2004 report from the US National Academies of Sciences stated: "To date, no adverse health effects attributed to genetic engineering have been documented in the human population." There have, however, been no epidemiological studies to determine whether engineered crops have caused any harm to the public. Without such

studies, it is unlikely that harm, if it occurred, would be detected or attributed to engineered foods. Worldwide, there are a range of perspectives within non-governmental organizations on the safety of GM foods. For example, the US pro-GM pressure group AgBioWorld has argued that GM foods have been proven safe, while other pressure groups and consumer rights groups, such as the Organic Consumers Association, and Greenpeace claim the long term health risks which GM could pose, or the environmental risks associated with GM, have not yet been adequately investigated.

In 1998 Rowett Research Institute scientist Árpád Pusztai reported that consumption of potatoes genetically modified to contain lectin had adverse intestinal effects on rats. Pusztai eventually published a paper, co-authored by Stanley Ewen, in the journal, The Lancet. The paper claimed to show that rats fed on potatoes genetically modified with the snowdrop lectin had unusual changes to their gut tissue when compared with rats fed on non modified potatoes. The experiment modified potatoes to add a toxin (snowdrop lectin), but the experiment failed to include a control for the toxin alone or a control for genetic modifications alone (without added toxin); therefore, no conclusion could be made about the safety of the genetic engineering. The experiment has been criticised by other scientists on the grounds that the unmodified potatoes were not a fair control diet and that all the rats may have been sick, due to them being fed a diet of only potatoes.

In 2009 three scientists (Vendômois et al) published a statistical re-analysis of three feeding trials that had previously been published by others as establishing the safety of genetically modified corn. The new article claimed that their statistics instead showed that the three patented crops (Mon 810, Mon 863, and NK 603) developed and owned by Monsanto cause liver, kidney, and heart damage in mammals. A 2007 analysis of part of this data by the same group of scientists funded by Greenpeace was assessed by a panel of independent toxicologists in a study funded by Monsanto and published in the journal *Food and chemical toxicology*. The reviewers reported that the study was statistically flawed and providing no evidence of adverse effects. The French High Council of Biotechnologies Scientific Committee reviewed the 2009 Vendômois et al study and concluded that it "..presents no admissible scientific element likely to ascribe any haematological, hepatic or renal toxicity to the three re-analysed GMOs." An evaluation by the European Food Safety Authority of the 2009 and 2007 studies noted that most of the results were within natural variation and they did not consider any of the effects reported biologically relevant. A review by Food Standards Australia New Zealand of the 2009 Vendômois et al study concluded that the results were due to chance alone.

Gene transfer

As of January 2009 there has only been one human feeding study conducted on the effects of genetically modified foods. The study involved seven human volunteers who had previously had their large intestines removed. These volunteers were to eat GM soy to see if the DNA of the GM soy transferred to the bacteria that naturally lives in the human gut. Researchers identified that three of the seven volunteers had transgenes from GM soya transferred into the bacteria living in their gut before the start of the feeding experiment. As this low-frequency transfer did not increase after the consumption of GM

Soy, the researchers concluded that gene transfer did not occur during the experiment. In volunteers with complete digestive tracts, the transgene did not survive passage through intact gastrointestinal tract. Anti-GM advocates believe the study should prompt additional testing to determine its significance.. Other studies have found DNA from M13 virus, GFP and even ribulose-1,5-bisphosphate carboxylase (Rubisco) genes in the blood and tissue of ingesting animals (reviewed by

Two studies on the possible effects of feeding genetically modified feeds to animals found that there was no significant differences in the safety and nutritional value of feedstuffs containing material derived from genetically modified plants. Specifically, the studies noted that no residues of recombinant DNA or novel proteins have been found in any organ or tissue samples obtained from animals fed with GMP plants.

Allergies

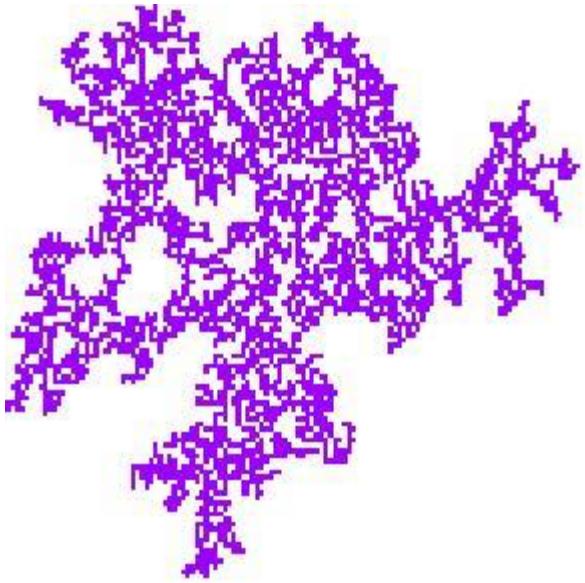
In the mid 1990s Pioneer Hi-Bred tested the allergenicity of a transgenic soybean that expressed a Brazil nut seed storage protein in hope that the seeds would have increased levels of the amino acid methionine. The tests (radioallergosorbent testing, immunoblotting, and skin-prick testing) showed that individuals allergic to Brazil nuts were also allergic to the new GM soybean. Pioneer has indicated that it will not develop commercial cultivars containing Brazil nut protein because the protein is likely to be an allergen.

Traceability

In a January 2010 paper by Costa et al. the extraction and detection of DNA along a complete industrial soybean oil processing chain was described to monitor the presence of Roundup Ready (RR) soybean: "The amplification of soybean lectin gene by end-point polymerase chain reaction (PCR) was successfully achieved in all the steps of extraction and refining processes, until the fully refined soybean oil. The amplification of RR soybean by PCR assays using event-specific primers was also achieved for all the extraction and refining steps, except for the intermediate steps of refining (neutralisation, washing and bleaching) possibly due to sample instability. The real-time PCR assays using specific probes confirmed all the results and proved that it is possible to detect and quantify genetically modified organisms in the fully refined soybean oil. To our knowledge, this has never been reported before and represents an important accomplishment regarding the traceability of genetically modified organisms in refined oils."

Chapter- 9

Biological Engineering



Modeling of the Spread of Disease Using Cellular Automata and Nearest Neighbor Interactions

Biological engineering, **biotechnological engineering** or **bioengineering** (including **biological systems engineering**) is the application of concepts and methods of physics and mathematics to solve problems in life sciences, using engineering's own analytical and synthetical methodologies. In this context, while traditional engineering applies physical and mathematical sciences to analyze, design and manufacture inanimate tools, structures and processess, bioengineering uses the same sciences to study many aspects of living organisms. Usually it is used to analyze and solve problems related to human health.

Biological engineering is a science based discipline founded upon the biological sciences in the same way that chemical engineering, electrical engineering, and mechanical

engineering are based upon chemistry, electricity and magnetism, and statics, respectively.

Biological Engineering can be differentiated from its roots of pure biology or classical engineering in the following way. Biological studies often follow a reductionist approach in viewing a system on its smallest possible scale which naturally leads toward tools such as functional genomics. Engineering approaches, using classical design perspectives, are constructionist, building new devices, approaches, and technologies from component concepts. Biological engineering utilizes both of these methods in concert relying on reductionist approaches to define the fundamental units which are then commingled to generate something new. Although engineered biological systems have been used to manipulate information, construct materials, process chemicals, produce energy, provide food, and help maintain or enhance human health and our environment, our ability to quickly and reliably engineer biological systems that behave as expected remains less well developed than our mastery over mechanical and electrical systems.

The differentiation between Biological Engineering and overlap with Biomedical Engineering can be unclear, as many universities now use the terms "bioengineering" and "biomedical engineering" interchangeably . Some contend that Biological Engineering (like biotechnology) has a broader base which spans molecular methods (tends to emphasize the using of biological substances - applying engineering principles to molecular biology, biochemistry, microbiology, pharmacology, protein chemistry, cytology, immunology, neurobiology and neuroscience, cellular and tissue based methods (including devices and sensors), whole organisms (plants, animals), and up increasing length scales to ecosystems. Neither biological engineering nor biomedical engineering is wholly contained within the other, as there are non-biological products for medical needs and biological products for non-medical needs.

ABET , the U.S. based accreditation board for engineering B.S. programs, makes a distinction between Biomedical Engineering and Biological Engineering; however, the differences are quite small. Biomedical engineers must have life science courses that include human physiology and have experience in performing measurements on living systems while biological engineers must have life science courses (which may or may not include physiology) and experience in making measurements not specifically on living systems. Foundational engineering courses are often the same and include thermodynamics, fluid and mechanical dynamics, kinetics, electronics, and materials properties.

The word bioengineering was coined by British scientist and broadcaster Heinz Wolff in 1954. The term bioengineering is also used to describe the use of vegetation in civil engineering construction. The term bioengineering may also be applied to environmental modifications such as surface soil protection, slope stabilisation, watercourse and shoreline protection, windbreaks, vegetation barriers including noise barriers and visual screens, and the ecological enhancement of an area. The first biological engineering program was created at Mississippi State University in 1967, making it the first

Biological Engineering curriculum in the United States. More recent programs have been launched at MIT and Utah State University .

Biological Engineers or *bioengineers* are engineers who use the principles of biology and the tools of engineering to create usable, tangible products. Biological Engineering employs knowledge and expertise from a number of pure and applied sciences, such as mass and heat transfer, kinetics, biocatalysts, biomechanics, bioinformatics, separation and purification processes, bioreactor design, surface science, fluid mechanics, thermodynamics, and polymer science. It is used in the design of medical devices, diagnostic equipment, biocompatible materials, renewable bioenergy, ecological engineering, and other areas that improve the living standards of societies.

In general, biological engineers attempt to either mimic biological systems in order to create products or modify and control biological systems so that they can replace, augment, or sustain chemical and mechanical processes. Bioengineers can apply their expertise to other applications of engineering and biotechnology, including genetic modification of plants and microorganisms, bioprocess engineering, and biocatalysis.

Because other engineering disciplines also address living organisms (e.g., prosthetics in mechanical engineering), the term biological engineering can be applied more broadly to include agricultural engineering and biotechnology. In fact, many old agricultural engineering departments in universities over the world have rebranded themselves as **agricultural and biological engineering** or **agricultural and biosystems engineering**. Biological engineering is also called bioengineering by some colleges and Biomedical engineering is called Bioengineering by others, and is a rapidly developing field with fluid categorization. The Main Fields of Bioengineering may be categorised as:

- **Bioprocess Engineering**: Bioprocess Design, Biocatalysis, Bioseparation, Bioinformatics, Bioenergy
- **Genetic Engineering**: Synthetic Biology, Horizontal gene transfer.
- **Cellular Engineering**: Cell Engineering, Tissue Culture Engineering, Metabolic engineering.
- **Biomedical Engineering**: Biomedical technology, Biomedical Diagnostics, Biomedical Therapy, Biomechanics, Biomaterials.

Chapter- 10

Microbial Biodegradation

Interest in the **microbial biodegradation** of pollutants has intensified in recent years as humanity strives to find sustainable ways to cleanup contaminated environments. These bioremediation and biotransformation methods endeavour to harness the astonishing, naturally occurring, ability of microbial xenobiotic metabolism to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), heterocyclic compounds (such as pyridine or quinoline), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in recent years have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of organisms to adapt to changing environmental conditions.

The elimination of a wide range of pollutants and wastes from the environment is an absolute requirement to promote a sustainable development of our society with low environmental impact. Biological processes play a major role in the removal of contaminants and they take advantage of the astonishing catabolic versatility of microorganisms to degrade/convert such compounds. New methodological breakthroughs in sequencing, genomics, proteomics, bioinformatics and imaging are producing vast amounts of information. In the field of Environmental Microbiology, genome-based global studies open a new era providing unprecedented *in silico* views of metabolic and regulatory networks, as well as clues to the evolution of degradation pathways and to the molecular adaptation strategies to changing environmental conditions. Functional genomic and metagenomic approaches are increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds and they will certainly accelerate the development of bioremediation technologies and biotransformation processes.

Aerobic biodegradation of pollutants

The burgeoning amount of bacterial genomic data provides unparalleled opportunities for understanding the genetic and molecular bases of the degradation of organic pollutants.

Aromatic compounds are among the most recalcitrant of these pollutants and lessons can be learned from the recent genomic studies of *Burkholderia xenovorans* LB400 and *Rhodococcus* sp. strain RHA1, two of the largest bacterial genomes completely sequenced to date. These studies have helped expand our understanding of bacterial catabolism, non-catabolic physiological adaptation to organic compounds, and the evolution of large bacterial genomes. First, the metabolic pathways from phylogenetically diverse isolates are very similar with respect to overall organization. Thus, as originally noted in pseudomonads, a large number of "peripheral aromatic" pathways funnel a range of natural and xenobiotic compounds into a restricted number of "central aromatic" pathways. Nevertheless, these pathways are genetically organized in genus-specific fashions, as exemplified by the b-ketoadipate and Paa pathways. Comparative genomic studies further reveal that some pathways are more widespread than initially thought. Thus, the Box and Paa pathways illustrate the prevalence of non-oxygenolytic ring-cleavage strategies in aerobic aromatic degradation processes. Functional genomic studies have been useful in establishing that even organisms harboring high numbers of homologous enzymes seem to contain few examples of true redundancy. For example, the multiplicity of ring-cleaving dioxygenases in certain rhodococcal isolates may be attributed to the cryptic aromatic catabolism of different terpenoids and steroids. Finally, analyses have indicated that recent genetic flux appears to have played a more significant role in the evolution of some large genomes, such as LB400's, than others. However, the emerging trend is that the large gene repertoires of potent pollutant degraders such as LB400 and RHA1 have evolved principally through more ancient processes. That this is true in such phylogenetically diverse species is remarkable and further suggests the ancient origin of this catabolic capacity.

Anaerobic biodegradation of pollutants

Anaerobic microbial mineralization of recalcitrant organic pollutants is of great environmental significance and involves intriguing novel biochemical reactions. In particular, hydrocarbons and halogenated compounds have long been doubted to be degradable in the absence of oxygen, but the isolation of hitherto unknown anaerobic hydrocarbon-degrading and reductively dehalogenating bacteria during the last decades provided ultimate proof for these processes in nature. Many novel biochemical reactions were discovered enabling the respective metabolic pathways, but progress in the molecular understanding of these bacteria was rather slow, since genetic systems are not readily applicable for most of them. However, with the increasing application of genomics in the field of environmental microbiology, a new and promising perspective is now at hand to obtain molecular insights into these new metabolic properties. Several complete genome sequences were determined during the last few years from bacteria capable of anaerobic organic pollutant degradation. The ~4.7 Mb genome of the facultative denitrifying *Aromatoleum aromaticum* strain EbN1 was the first to be determined for an anaerobic hydrocarbon degrader (using toluene or ethylbenzene as substrates). The genome sequence revealed about two dozen gene clusters (including several paralogs) coding for a complex catabolic network for anaerobic and aerobic degradation of aromatic compounds. The genome sequence forms the basis for current detailed studies on regulation of pathways and enzyme structures. Further genomes of

anaerobic hydrocarbon degrading bacteria were recently completed for the iron-reducing species *Geobacter metallireducens* (accession nr. NC_007517) and the perchlorate-reducing *Dechloromonas aromatica* (accession nr. NC_007298), but these are not yet evaluated in formal publications. Complete genomes were also determined for bacteria capable of anaerobic degradation of halogenated hydrocarbons by halorespiration: the ~1.4 Mb genomes of *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* sp. strain CBDB1 and the ~5.7 Mb genome of *Desulfitobacterium hafniense* strain Y51. Characteristic for all these bacteria is the presence of multiple paralogous genes for reductive dehalogenases, implicating a wider dehalogenating spectrum of the organisms than previously known. Moreover, genome sequences provided unprecedented insights into the evolution of reductive dehalogenation and differing strategies for niche adaptation.

Recently, it has become apparent that some organisms, including *Desulfitobacterium chlororespirans*, originally evaluated for halorespiration on chlorophenols, can also use certain brominated compounds, such as the herbicide bromoxynil and its major metabolite as electron acceptors for growth. Iodinated compounds may be dehalogenated as well, though the process may not satisfy the need for an electron acceptor.

Bioavailability, chemotaxis, and transport of pollutants

Bioavailability, or the amount of a substance that is physiochemically accessible to microorganisms is a key factor in the efficient biodegradation of pollutants. O'Loughlin et al (2000) showed that, with the exception of kaolinite clay, most soil clays and cation exchange resins attenuated biodegradation of 2-picoline by *Arthrobacter* sp. strain R1, as a result of adsorption of the substrate to the clays. Chemotaxis, or the directed movement of motile organisms towards or away from chemicals in the environment is an important physiological response that may contribute to effective catabolism of molecules in the environment. In addition, mechanisms for the intracellular accumulation of aromatic molecules via various transport mechanisms are also important.

Oil biodegradation

Petroleum oil contains aromatic compounds that are toxic for most life forms. Episodic and chronic pollution of the environment by oil causes major ecological perturbations. Marine environments are especially vulnerable since oil spills of coastal regions and the open sea are poorly containable and mitigation is difficult. In addition to pollution through human activities, about 250 million liters of petroleum enter the marine environment every year from natural seepages. Despite its toxicity, a considerable fraction of petroleum oil entering marine systems is eliminated by the hydrocarbon-degrading activities of microbial communities, in particular by a remarkable recently discovered group of specialists, the so-called hydrocarbonoclastic bacteria (HCB). *Alcanivorax borkumensis* was the first HCB to have its genome sequenced. In addition to hydrocarbons, crude oil often contains various heterocyclic compounds, such as pyridine, which appear to be degraded by similar, though separate mechanisms than hydrocarbons.

Cholesterol biodegradation

Cholesterol is a steroid highly abundant in the environment that plays a major role in the global carbon cycle. Many synthetic steroidic compounds like some sexual hormones frequently appear in municipal and industrial wastewaters, acting as environmental pollutants with strong metabolic activities negatively affecting the ecosystems. Since these compounds are common carbon sources for many different microorganisms their aerobic and anaerobic mineralization has been extensively studied. The interest of these studies lies on the biotechnological applications of sterol transforming enzymes for the industrial synthesis of sexual hormones and corticoids. Very recently the catabolism of cholesterol has acquired a high relevance because it is involved in the infectivity of *Mycobacterium tuberculosis*.

Analysis of waste biotreatment

Sustainable development requires the promotion of environmental management and a constant search for new technologies to treat vast quantities of wastes generated by increasing anthropogenic activities. Biotreatment, the processing of wastes using living organisms, is an environmentally friendly, relatively simple and cost-effective alternative to physico-chemical clean-up options. Confined environments, such as bioreactors, have been engineered to overcome the physical, chemical and biological limiting factors of biotreatment processes in highly controlled systems. The great versatility in the design of confined environments allows the treatment of a wide range of wastes under optimized conditions. To perform a correct assessment, it is necessary to consider various microorganisms having a variety of genomes and expressed transcripts and proteins. A great number of analyses are often required. Using traditional genomic techniques, such assessments are limited and time-consuming. However, several high-throughput techniques originally developed for medical studies can be applied to assess biotreatment in confined environments.

Metabolic engineering and biocatalytic applications

The study of the fate of persistent organic chemicals in the environment has revealed a large reservoir of enzymatic reactions with a large potential in preparative organic synthesis, which has already been exploited for a number of oxygenases on pilot and even on industrial scale. Novel catalysts can be obtained from metagenomic libraries and DNA sequence based approaches. Our increasing capabilities in adapting the catalysts to specific reactions and process requirements by rational and random mutagenesis broadens the scope for application in the fine chemical industry, but also in the field of biodegradation. In many cases, these catalysts need to be exploited in whole cell bioconversions or in fermentations, calling for system-wide approaches to understanding strain physiology and metabolism and rational approaches to the engineering of whole cells as they are increasingly put forward in the area of systems biotechnology and synthetic biology.

Fungal biodegradation

In the ecosystem, different substrates are attacked at different rates by consortia of organisms from different kingdoms. *Aspergillus* and other moulds play an important role in these consortia because they are adept at recycling starches, hemicelluloses, celluloses, pectins and other sugar polymers. Some aspergilli are capable of degrading more refractory compounds such as fats, oils, chitin, and keratin. Maximum decomposition occurs when there is sufficient nitrogen, phosphorus and other essential inorganic nutrients. Fungi also provide food for many soil organisms.

For *Aspergillus* the process of degradation is the means of obtaining nutrients. When these moulds degrade human-made substrates, the process usually is called biodeterioration. Both paper and textiles (cotton, jute, and linen) are particularly vulnerable to *Aspergillus* degradation. Our artistic heritage is also subject to *Aspergillus* assault. To give but one example, after Florence in Italy flooded in 1969, 74% of the isolates from a damaged Ghirlandaio fresco in the Ognissanti church were *Aspergillus versicolor*.

Chapter- 11

Genetic Engineering

Genetic engineering, also called **genetic modification**, is the direct human manipulation of an organism's genetic material in a way that does not occur under natural conditions. It involves the use of recombinant DNA techniques, but does not include traditional animal and plant breeding or mutagenesis. Any organism that is generated using these techniques is considered to be a genetically modified organism. The first organisms genetically engineered were bacteria in 1973 and then mice in 1974. Insulin producing bacteria were commercialized in 1982 and genetically modified food has been sold since 1994.

The most common form of genetic engineering involves the insertion of new genetic material at an unspecified location in the host genome. This is accomplished by isolating and copying the genetic material of interest, generating a construct containing all the genetic elements for correct expression, and then inserting this construct into the host organism. Other forms of genetic engineering include gene targeting and knocking out specific genes via engineered nucleases such as zinc finger nucleases or engineered homing endonucleases.

Genetic engineering techniques have been applied in numerous fields including research, biotechnology, and medicine. Medicines such as insulin and human growth hormone are now produced in bacteria, experimental mice such as the oncomouse and the knockout mouse are being used for research purposes and insect resistant and/or herbicide tolerant crops have been commercialized. Genetically engineered plants and animals capable of producing biotechnology drugs more cheaply than current methods (called pharming) are also being developed and in 2009 the FDA approved the sale of the pharmaceutical protein antithrombin produced in the milk of genetically engineered goats.

Definition

Genetic engineering alters the genetic makeup of an organism using techniques that introduce heritable material prepared outside the organism either directly into the host or into a cell that is then fused or hybridized with the host. This involves using recombinant nucleic acid (DNA or RNA) techniques to form new combinations of heritable genetic material followed by the incorporation of that material either indirectly through a vector

system or directly through micro-injection, macro-injection and micro-encapsulation techniques. Genetic engineering does not include traditional animal and plant breeding, in vitro fertilisation, induction of polyploidy, mutagenesis and cell fusion techniques that do not use recombinant nucleic acids or a genetically modified organism in the process. Cloning and stem cell research, although not considered genetic engineering, are closely related and genetic engineering can be used within them. Synthetic biology is an emerging discipline that takes genetic engineering a step further by introducing artificially synthesized genetic material from raw materials into an organism.

If genetic material from another species is added to the host, the resulting organism is called transgenic. If genetic material from the same species or a species that can naturally breed with the host is used the resulting organism is called cisgenic. Genetic engineering can also be used to remove genetic material from the target organism, creating a knock out organism. In Europe genetic modification is synonymous with genetic engineering while within the United States of America it can also refer to conventional breeding methods.

History

Humans have altered the genomes of species for thousands of years through artificial selection and more recently mutagenesis. Genetic engineering as the direct manipulation of DNA by humans outside breeding and mutations has only existed since the 1970s. The term "genetic engineering" was first coined by Jack Williamson in his science fiction novel *Dragon's Island*, published in 1951, one year before DNA's role in heredity was confirmed by Alfred Hershey and Martha Chase, and two years before James Watson and Francis Crick showed that the DNA molecule has a double-helix structure.

In 1972 Paul Berg created the first recombinant DNA molecules by combined DNA from the monkey virus SV40 with that of the lambda virus. In 1973 Herbert Boyer and Stanley Cohen created the first transgenic organism by inserting antibiotic resistance genes into the plasmid of an *E. coli* bacterium. A year later Rudolf Jaenisch created a transgenic mouse by introducing foreign DNA into its embryo, making it the world’s first transgenic animal. In 1976 Genentech, the first genetic engineering company was founded by Herbert Boyer and Robert Swanson and a year later and the company produced a human protein (somatostatin) in *E.coli*. Genentech announced the production of genetically engineered human insulin in 1978. In 1980, the U.S. Supreme Court in the Diamond v. Chakrabarty case ruled that genetically altered life could be patented. The insulin produced by bacteria, branded humulin, was approved for release by the Food and Drug Administration in 1982.

The first field trials of genetically engineered plants occurred in France and the USA in 1986, tobacco plants were engineered to be resistant to herbicides. The People’s Republic of China was the first country to commercialize transgenic plants, introducing a virus-resistant tobacco in 1992. In 1994 Calgene attained approval to commercially release the Flavr Savr tomato, a tomato engineered to have a longer shelf life. In 1994, the European Union approved tobacco engineered to be resistant to the herbicide bromoxynil, making it

the first genetically engineered crop commercialized in Europe. In 1995, Bt Potato was approved safe by the Environmental Protection Agency, making it the first pesticide producing crop to be approved in the USA. In 2009 11 transgenic crops were grown commercially in 25 countries, the largest of which by area grown were the USA, Brazil, Argentina, India, Canada, China, Paraguay and South Africa.

In 2010, scientists at the J. Craig Venter Institute, announced that they had created the first synthetic bacterial genome, and added it to a cell containing no DNA. The resulting bacterium, named Synthia, was the world's first synthetic life form.

Process

Isolating the Gene

Elements of genetic engineering

First, the gene to be inserted into the genetically modified organism must be chosen and isolated. Presently, most genes transferred into plants provide protection against insects or tolerance to herbicides. In animals the majority of genes used are growth hormone genes. Once chosen the genes must be isolated. This typically involves multiplying the gene using polymerase chain reaction (PCR). If the chosen gene or the donor organism's genome has been well studied it may be present in a genetic library. If the DNA sequence is known, but no copies of the gene are available, it can be artificially synthesized. Once isolated, the gene is inserted into a bacterial plasmid.

Constructs

The gene to be inserted into the genetically modified organism must be combined with other genetic elements in order for it to work properly. The gene can also be modified at this stage for better expression or effectiveness. As well as the gene to be inserted most constructs contain a promoter and terminator region as well as a selectable marker gene. The promoter region initiates transcription of the gene and can be used to control the location and level of gene expression, while the terminator region ends transcription. The selectable marker, which in most cases confers antibiotic resistance to the organism it is expressed in, is needed to determine which cells are transformed with the new gene. The constructs are made using recombinant DNA techniques, such as restriction digests, ligations and molecular cloning.

Gene Targeting

The most common form of genetic engineering involves inserting new genetic material randomly within the host genome. Other techniques allow new genetic material to be inserted at a specific location in the host genome or generate mutations at desired genomic loci capable of knocking out endogenous genes. The technique of gene targeting uses homologous recombination to target desired changes to a specific endogenous gene. This tends to occur at a relatively low frequency in plants and animals and generally requires the use of selectable markers. The frequency of gene targeting can be greatly enhanced with the use of engineered nucleases such as zinc finger nucleases, engineered homing endonucleases, or nucleases created from TAL effectors. In addition to enhancing gene targeting, engineered nucleases can also be used to introduce mutations at endogenous genes that generate a gene knockout.

Transformation

A. tumefaciens attaching itself to a carrot cell

About 1% of bacteria are naturally able to take up foreign DNA but it can also be induced in other bacteria. Stressing the bacteria for example, with a heat shock or an electric shock, can make the cell membrane permeable to DNA that may then incorporate into their genome or exist as extrachromosomal DNA. DNA is generally inserted into animal cells using microinjection, where it can be injected through the cells nuclear envelope directly into the nucleus or through the use of viral vectors. In plants the DNA is generally inserted using *Agrobacterium*-mediated recombination or biolistics.

In *Agrobacterium*-mediated recombination the plasmid construct must also contain T-DNA. *Agrobacterium* naturally inserts DNA from a tumor inducing plasmid into any susceptible plant's genome it infects, causing crown gall disease. The T-DNA region of this plasmid is responsible for insertion of the DNA. The genes to be inserted are cloned into a binary vector, which contains T-DNA and can be grown in both *E. Coli* and *Agrobacterium*. Once the binary vector is constructed the plasmid is transformed into *Agrobacterium* containing no plasmids and plant cells are infected. The *Agrobacterium* will then naturally insert the genetic material into the plant cells.

In biolistics particles of gold or tungsten are coated with DNA and then shot into young plant cells or plant embryos. Some genetic material will enter the cells and transform them. This method can be used on plants that are not susceptible to *Agrobacterium* infection and also allows transformation of plant plastids. Another transformation method for plant and animal cells is electroporation. Electroporation involves subjecting the plant or animal cell to an electric shock, which can make the cell membrane permeable to plasmid DNA. In some cases the electroporated cells will incorporate the DNA into their genome. Due to the damage caused to the cells and DNA the transformation efficiency of biolistics and electroporation is lower than agrobacterial mediated transformation and microinjection.

Selection

Not all the organism's cells will be transformed with the new genetic material; in most cases a selectable marker is used to differentiate transformed from untransformed cells. If a cell has been successfully transformed with the DNA it will also contain the marker gene. By growing the cells in the presence of an antibiotic or chemical that selects or marks the cells expressing that gene it is possible to separate the transgenic events from the non-transgenic. Another method of screening involves using a DNA probe that will only stick to the inserted gene. A number of strategies have been developed that can remove the selectable marker from the mature transgenic plant.

Regeneration

As often only a single cell is transformed with genetic material the organism must be regrown from that single cell. As bacteria consist of a single cell and reproduce clonally regeneration is not necessary. In plants this is accomplished through the use of tissue culture. Each plant species has different requirements for successful regeneration through tissue culture. If successful an adult plant is produced that contains the transgene in every cell. In animals it is necessary to ensure that the inserted DNA is present in the embryonic stem cells. When the offspring is produced they can be screened for the presence of the gene. All offspring from the first generation will be heterozygous for the inserted gene and must be mated together to produce a homozygous animal.

Confirmation

Further tests using PCR, Southern Blots and Bioassays are needed to confirm that the gene is expressed and functions correctly. The organism's offspring are also tested to ensure that the trait can be inherited and that it follows a Mendelian inheritance pattern.

Applications

Genetic engineering has applications in medicine, research, industry and agriculture and can be used on a wide range of plants, animals and micro organism.

Medicine

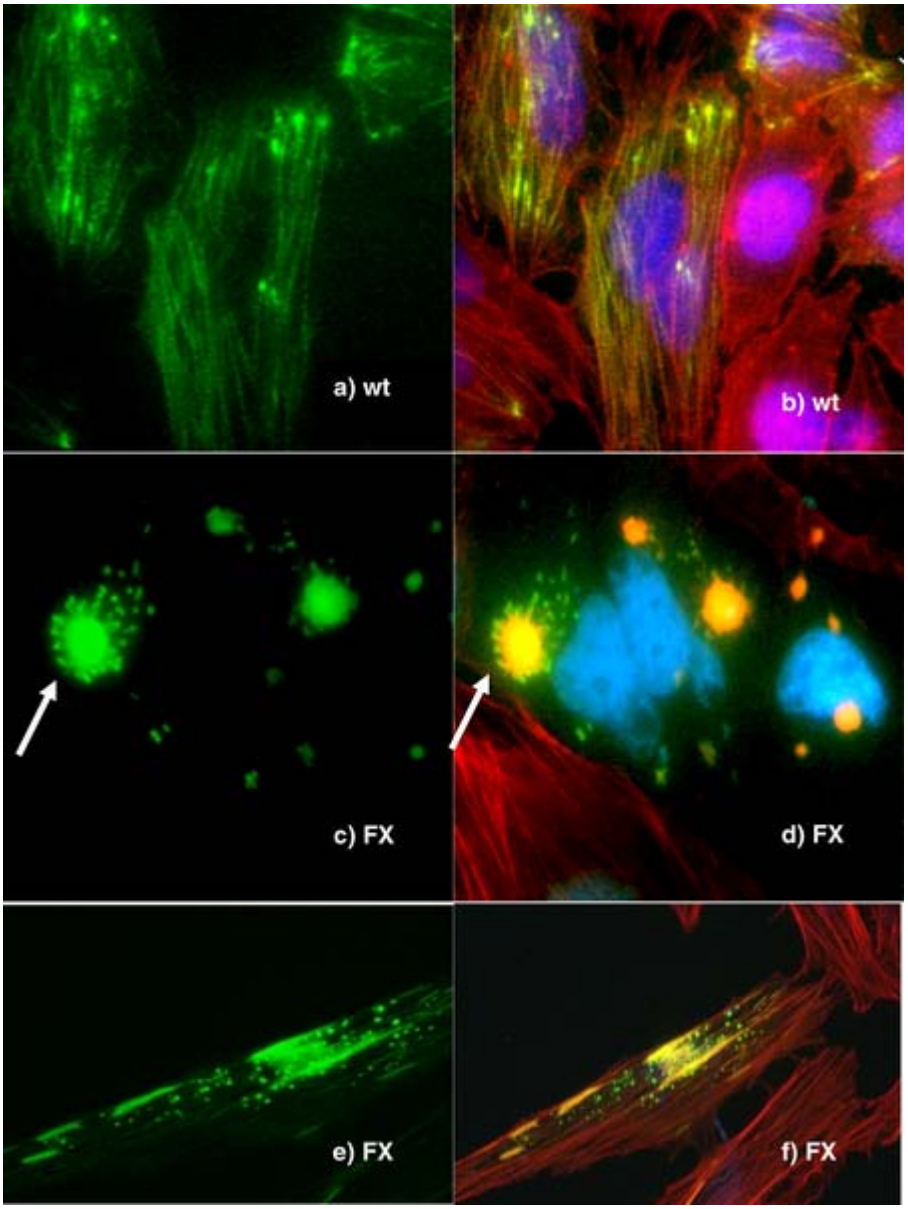
In medicine genetic engineering has been used to mass-produce insulin, human growth hormones, follistim (for treating infertility), human albumin, monoclonal antibodies, antihemophilic factors, vaccines and many other drugs. Vaccination generally involves injecting weak live, killed or inactivated forms of viruses or their toxins into the person being immunized. Genetically engineered viruses are being developed that can still confer immunity, but lack the infectious sequences. Mouse hybridomas, cells fused together to create monoclonal antibodies, have been humanised through genetic engineering to create human monoclonal antibodies.

Genetic engineering is used to create animal models of human diseases. Genetically modified mice are the most common genetically engineered animal model. They have been used to study and model cancer (the oncomouse), obesity, heart disease, diabetes, arthritis, substance abuse, anxiety, aging and Parkinson disease. Potential cures can be tested against these mouse models. Also genetically modified pigs have been bred with the aim of increasing the success of pig to human organ transplantation.

Gene therapy is the genetic engineering of humans by replacing defective human genes with functional copies. This can occur in somatic tissue or germline tissue. If the gene is inserted into the germline tissue it can be passed down to that person's descendants. Gene therapy has been used to treat patients suffering from immune deficiencies (notably Severe combined immunodeficiency) and trials have been carried out on other genetic disorders. The success of gene therapy so far has been limited and a patient (Jesse Gelsinger) has died during a clinical trial testing a new treatment. There are also ethical concerns should the technology be used not just for treatment, but for enhancement, modification or alteration of a human beings' appearance, adaptability, intelligence, character or behavior. The distinction between cure and enhancement can also be difficult to establish. Transhumanists consider the enhancement of humans desirable.

Research

Knockout mice



Human cells in which some proteins are fused with green fluorescent protein to allow them to be visualised

Genetic engineering is an important tool for natural scientists. Genes and other genetic information from a wide range of organisms are transformed into bacteria for storage and modification, creating genetically modified bacteria in the process. Bacteria are cheap, easy to grow, clonal, multiply quickly, relatively easy to transform and can be stored at -80°C almost indefinitely. Once a gene is isolated it can be stored inside the bacteria providing an unlimited supply for research.

Organisms are genetically engineered to discover the functions of certain genes. This could be the effect on the phenotype of the organism, where the gene is expressed or

what other genes it interacts with. These experiments generally involve loss of function, gain of function, tracking and expression.

- **Loss of function experiments**, such as in a gene knockout experiment, in which an organism is engineered to lack the activity of one or more genes. A knockout experiment involves the creation and manipulation of a DNA construct *in vitro*, which, in a simple knockout, consists of a copy of the desired gene, which has been altered such that it is non-functional. Embryonic stem cells incorporate the altered gene, which replaces the already present functional copy. These stem cells are injected into blastocysts, which are implanted into surrogate mothers. This allows the experimenter to analyze the defects caused by this mutation and thereby determine the role of particular genes. It is used especially frequently in developmental biology. Another method, useful in organisms such as Drosophila (fruit fly), is to induce mutations in a large population and then screen the progeny for the desired mutation. A similar process can be used in both plants and prokaryotes.
- **Gain of function experiments**, the logical counterpart of knockouts. These are sometimes performed in conjunction with knockout experiments to more finely establish the function of the desired gene. The process is much the same as that in knockout engineering, except that the construct is designed to increase the function of the gene, usually by providing extra copies of the gene or inducing synthesis of the protein more frequently.
- **Tracking experiments**, which seek to gain information about the localization and interaction of the desired protein. One way to do this is to replace the wild-type gene with a 'fusion' gene, which is a juxtaposition of the wild-type gene with a reporting element such as green fluorescent protein (GFP) that will allow easy visualization of the products of the genetic modification. While this is a useful technique, the manipulation can destroy the function of the gene, creating secondary effects and possibly calling into question the results of the experiment. More sophisticated techniques are now in development that can track protein products without mitigating their function, such as the addition of small sequences that will serve as binding motifs to monoclonal antibodies.
- **Expression studies** aim to discover where and when specific proteins are produced. In these experiments, the DNA sequence before the DNA that codes for a protein, known as a gene's promoter, is reintroduced into an organism with the protein coding region replaced by a reporter gene such as GFP or an enzyme that catalyzes the production of a dye. Thus the time and place where a particular protein is produced can be observed. Expression studies can be taken a step further by altering the promoter to find which pieces are crucial for the proper expression of the gene and are actually bound by transcription factor proteins; this process is known as promoter bashing.

Industrial

By engineering genes into bacterial plasmids it is possible to create a biological factory that can produce proteins and enzymes. Some genes do not work well in bacteria, so

yeast, a eukaryote, can also be used. Bacteria and yeast factories have been used to produce medicines such as insulin, human growth hormone, and vaccines, supplements such as tryptophan, aid in the production of food (chymosin in cheese making) and fuels. Other applications involving genetically engineered bacteria being investigated involve making the bacteria perform tasks outside their natural cycle, such as cleaning up oil spills, carbon and other toxic waste.

Agriculture

Bt-toxins present in peanut leaves (bottom image) protect it from extensive damage caused by European corn borer larvae (top image).

One of the best-known and controversial applications of genetic engineering is the creation of genetically modified food. There are three generations of genetically modified crops. First generation crops have been commercialized and most provide protection from insects and/or resistance to herbicides. There are also fungal and virus resistant crops

developed or in development. They have been developed to make the insect and weed management of crops easier and can indirectly increase crop yield.

The second generation of genetically modified crops being developed aim to directly improve yield by improving salt, cold or drought tolerance and to increase the nutritional value of the crops. The third generation consists of pharmaceutical crops, crops that contain edible vaccines and other drugs. Some agriculturally important animals have been genetically modified with growth hormones to increase their size while others have been engineered to express drugs and other proteins in their milk.

The genetic engineering of agricultural crops can increase the growth rates and resistance to different diseases caused by pathogens and parasites. This is beneficial as it can greatly increase the production of food sources with the usage of fewer resources that would be required to host the world's growing populations. These modified crops would also reduce the usage of chemicals, such as fertilizers and pesticides, and therefore decrease the severity and frequency of the damages produced by these chemical pollution.

Ethical and safety concerns have been raised around the use of genetically modified food. A major safety concern relates to the human health implications of eating genetically modified food, in particular whether toxic or allergic reactions could occur. Gene flow into related non-transgenic crops, off target effects on beneficial organisms and the impact on biodiversity are important environmental issues. Ethical concerns involve religious issues, corporate control of the food supply, intellectual property rights and the level of labeling needed on genetically modified products.

Other uses

In materials science, a genetically modified virus has been used to construct a more environmentally friendly lithium-ion battery. Some bacteria have been genetically engineered to create black and white photographs while others have potential to be used as sensors by expressing a fluorescent protein under certain environmental conditions. Genetic engineering is also being used to create BioArt and novelty items such as blue roses, and glowing fish.

Opposition and criticism

A 2010 study of Canola found transgenes in 80% of wild (uncultivated or "feral") varieties in North Dakota, meaning 80% of the plants which had established themselves in the area were genetically engineered varieties. The researchers stated that "we found the highest densities of [such transgene-containing] plants near agricultural fields and along major freeways, but we were also finding plants in the middle of nowhere" adding that "over time,..the build-up of different types of herbicide resistance in feral [natural] canola and closely related weeds, like field mustard, could make it more difficult to manage these plants using herbicides."