Biotechnology and its applications

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What is Biotechnology ?

It is broadly defined as using living organisms for human benefit to make a product or solve a problem.

Biotechnology does not mean hunting and gathering animals and plants for food ; however, domesticating animals such as sheep and cattle for use as livestock is a classical example of Biotechnology.

Extended shelf-life tomato (Flavr Savr Tomato)

Herbicide resistant soybean (Roundup Ready Soybean)

History of Biotechnology

Humans have been using organisms for their benefits in many processes for several thousand years. Historical accounts have shown that the Chinese, Greeks, Romans , Babylonions and Egyptians among many others have been involved in biotechnology since nearly 2000 B.C.

Our early ancestors also took advantage of microorganisms and used fermentation to make bread, cheese, yogurt and alcoholic beverages such as beer and wine.

In 1920's *Clostridium acetobutylicum* was used by **Chaim Weizmann** for converting starch into butanol and acetone; the leter was an essential component of explosives during World War I . This raised hopes for commercial production of useful chemicals through biological processes, and may be considered as the first rediscovery of biotechnology in 20th century.

Similarly during World War II (in 1940's) the production of Penicillin (as an antibiotic discovered by **Alexander Fleming** in 1929) on a large scale from cultures of *Pencillium notatum*, marked second rediscovery of biotechnology. This was the beginning of an era of antibiotic research.

The third rediscovery of biotechnology is its recent reincarnation in the form of **Recombinant –DNA technology.** Since the 1960s, rapid development of our understanding of genetics and molecular biology has led to exciting new innovations and applications in biotechnology. As we have begun to unravel the secrets of DNA structure and functions, new technologies have led to **gene cloning,** the ability to identify and reproduce a gene of interest, and **genetic engineering,** manipulating the DNA of an organism.

Biotechnology :
A Science of Many Disciplines :

In a biotechnology tree, roots are made up of basic sciences like microbiology, genetics, biochemistry, physics, statistics, immunology, molecular and cell biology, chemical engineering, computer science and human, animal and plant physiology. The central focus or "trunk" for most biotechnological applications is genetic engineering. At the top of the tree, applications of genetic engineering can be put to work to create a product or process to help humans or our living environment.

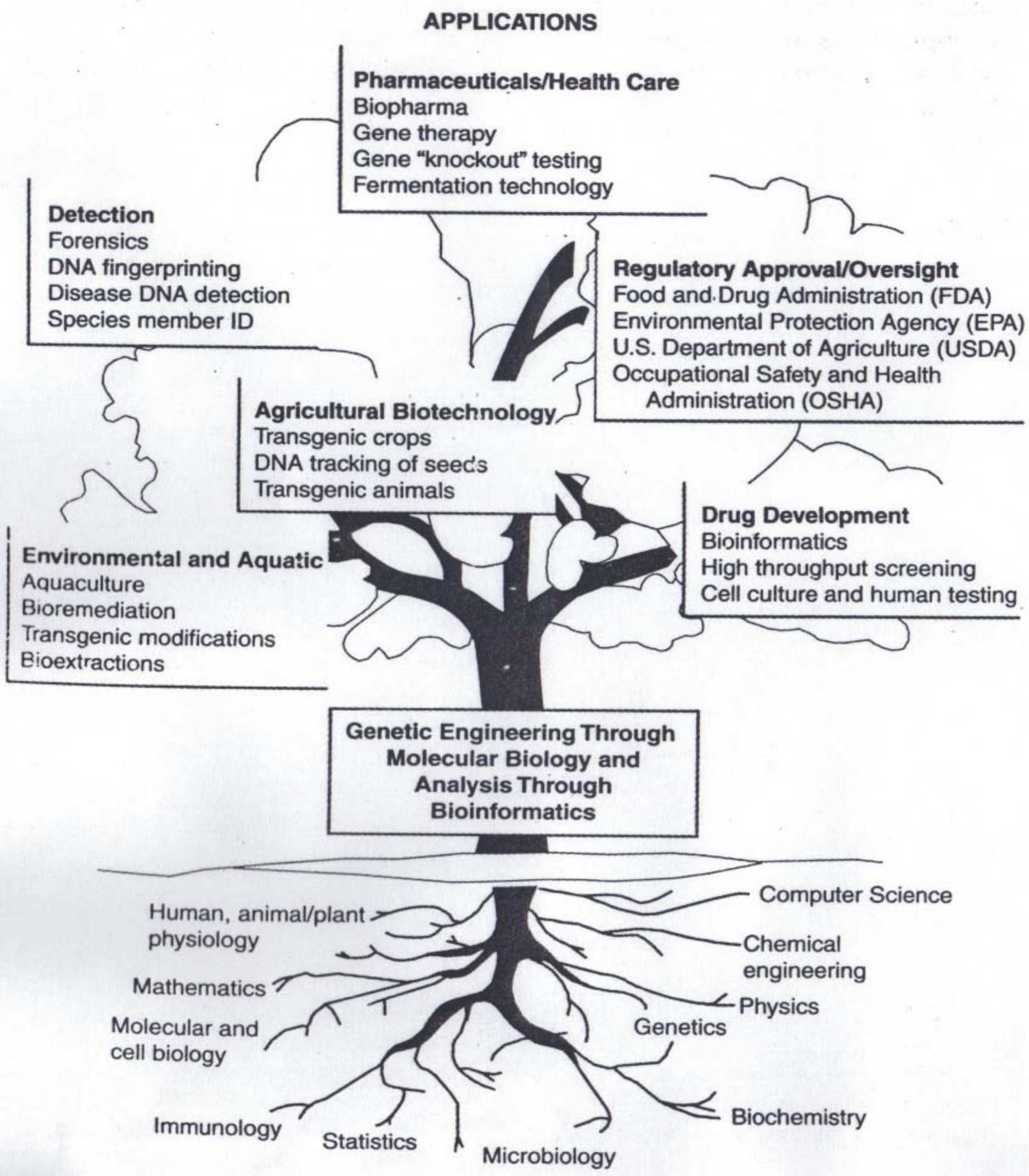


Figure 1.3 The Biotechnology Tree: Different Disciplines Contribute to Biotechnology
The basic sciences are the foundation or "roots" of all aspects of biotechnology. The central focus or "trunk" for most biotechnological applications is genetic engineering. Branches of the tree represent different organisms, technologies, and applications that "stem" from genetic engineering and bioinformatics, central aspects of most biotechnological approaches. Regulation of biotechnology occurs through governmental agencies like the FDA, USDA, EPA, and OSHA, whose roles and responsibilities will be defined in Chapter 12.

Types of Biotechnology :

- Nanobiotechnology
- Microbial biotechnology
- Agricultural biotechnology
- Animal biotechnology
- Plant biotechnology
- Forensic biotechnology
- Medical biotechnology
- Environmental biotechnology
- Bioremediation

Nanobiotechnology :

It is an entirely new field that is rapidly emerging as a major research area. One promising application of Nanobiotechnology has been the development of small particles that can be used to deliver drugs to cells

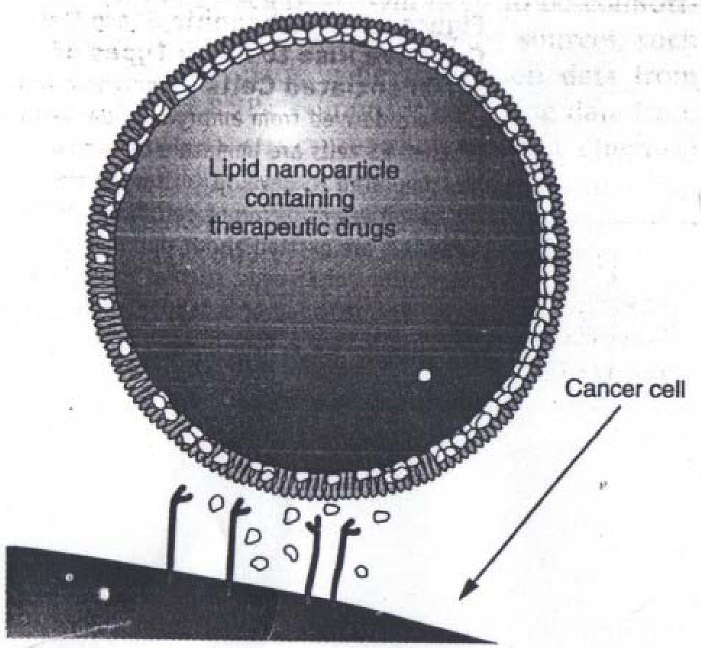


Figure 1.13 Nanobiotechnology in Action Nanoparticles containing chemotherapy agents can be specifically directed to target cancer cells by coating them with tumor specific proteins that bind to the target cells. In this way, chemotherapy agents that cannot pass through the cell membrane can be released specifically inside these target cancer cells.

Microbial Biotechnology :

Use of Yeast for making beer and wine is one of the oldest applications of biotechnology.

By manipulating microorganisms such as bacteria and yeast, microbial biotechnology has created better enzymes and organisms for making many foods , simplifying manufacturing and production processes and making decontamination processes for industrial waste product removal more efficient.

Hydrocarbon degrading bacterial strain *Pseudomonas putida* was developed by *Ananda Chakraborthy*

Sewage treatment

Organism	Substrate	Product/Application
Methanogenic bacteria	Acetate, Methanol, Format and H_2+CO_2	Methane
Pseudomonas putida	Octane, Xylene, Meta Xylene, Camphor	Biodegradation of hydrocarbons or petroleum wastes.

Microbes in Biotechnology

- Production of Organic acids.
- Production of Enzymes.
- Production of Antibiotics.
- Sewage Treatment.
- Paper Industry.
- Biomineralization.
- Biofertilizers.
- Bioinsecticides.
- Application of Genetically engineered bacteria.

Agriculture Biotechnology:

- In "ag-biotech" we examine a range of topics from genetically engineered, pest resistant plants that do not need to be sprayed with pesticides .

- Genetic manipulation of plants have been used for over 20 years to produce genetically engineered plant with altered growth characteristics such as drought resistance, tolerance to cold temperature and greater food yields.

- Plants can be engineered to produce a wide range of pharmaceutical proteins (Molecular pharming) in a broad array of crop species and tissues.

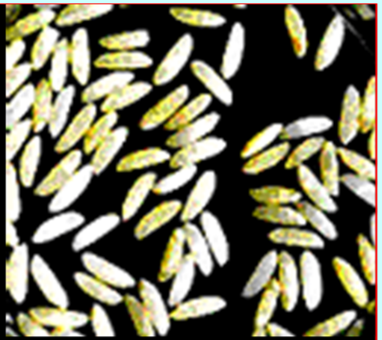
- The cost of producing plant materials with recombinant proteins is often significantly lower than producing recombinant proteins in bacteria.

- Agricultural waste and other high cellulose sources will have to become efficient sources of energy through new decomposition and fermentation methods resulting from biotechnology.

- **Biogas production** is the best example: Conversion of cellulose into methane through methanogenic bacteria

- **Introduction of Nif gene** into the plants through *Agrobacterium.*

I. Genetic Engineering



E. Examples

2. Golden Rice

- GM rice containing genes that produce beta-carotene
  - Can be converted to vitamin A
  - Vitamin A deficiency (VAD) may cause weakened immune systems, partial to total blindness, and increased chance of death
  - VAD causes 350,000 cases of blindness and has been linked to 1 million+ deaths each year
- Highly controversial

a. Proponents
  - Reduce incidence of blindness and other VAD related health disorders
  - 1/2 lb of rice/day will keep VAD symptoms away

b. Opponents
  - Nutritional deficiencies will prevent people from absorbing beta-carotene from rice
  - Concentrations of beta-carotene in rice are low, and an average woman would need to eat 16 lbs of golden rice a day to get 100% of daily requirement
  - Alternatives like leafy green vegetables or unpolished rice are better, cheaper sources of vitamin A
  - Western corporations are trying to control rice production

Courtesy International Rice Research Institute

Recombinant DNA through Genetic Engineering.

- **Steps involved in production of recombinant DNA.**
- Isolate gene
- Prepare target DNA
- Insert DNA into plasmid
- Insert plasmid back into cell
- Plasmid multiplies
- Target cells reproduce
- Cells produce proteins
- 1. Isolate Gene
- The gene for producing a protein is isolated from a cell. The gene is on the DNA in a chromosome. Special DNA cutting proteins are used to cut out certain sections of DNA. The gene can be isolated and then copied so that many genes are available to work with.

- 2. Prepare Target DNA
- In 1973, two scientists named Boyer and Cohen developed a way to put DNA from one organism into the DNA of bacteria. This process is called recombinant DNA technology. First, a circular piece of DNA called a plasmid is removed from a bacterial cell. Special proteins are used to cut the plasmid ring to open it up.

- 3. Insert DNA into Plasmid
- The host DNA that produces the wanted protein is inserted into the opened plasmid DNA ring. Then special cell proteins help close the plasmid ring.

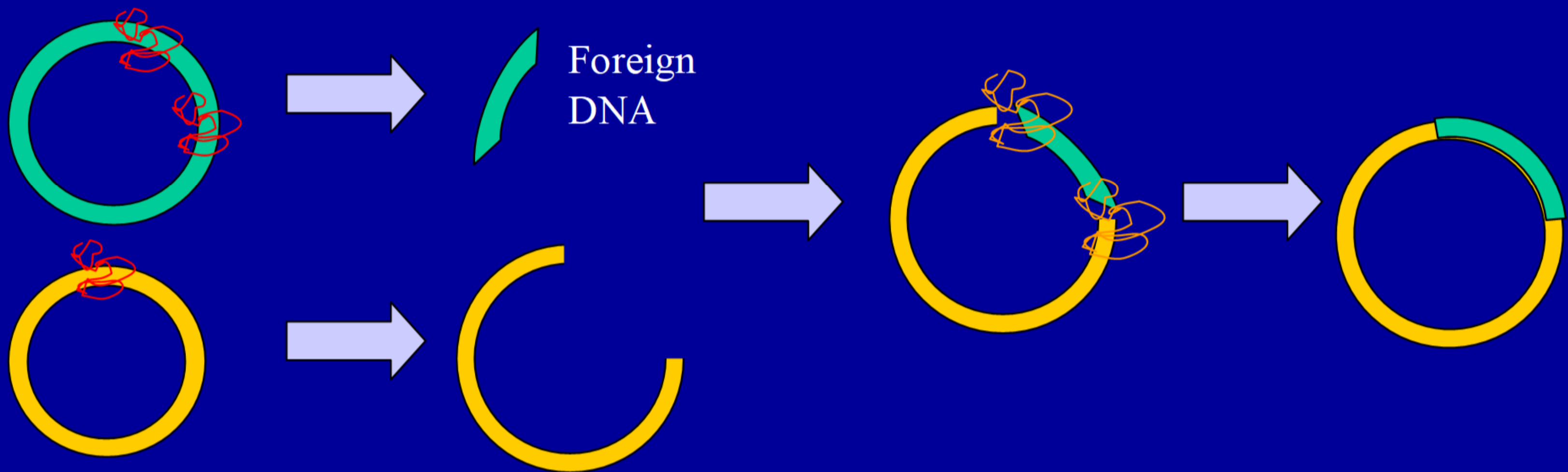
Recombinant DNA through Genetic Engineering

- **3. Insert DNA into Plasmid**
- The host DNA that produces the wanted protein is inserted into the opened plasmid DNA ring. Then special cell proteins help close the plasmid ring.
  4. Insert Plasmid back into cell
- The circular plasmid DNA that now contains the host gene is inserted back into a bacteria cell. The plasmid is a natural part of the bacteria cell. The bacteria cell now has a gene in it that is from a different organism, even from a human. This is what is called recombinant DNA technology.
  5. Plasmid multiplies
- The plasmid that was inserted into the bacteria cell can multiply to make several copies of the wanted gene. Now the gene can be turned on in the cell to make proteins.
- **6. Target Cells Reproduce**
- Many recombined plasmids are inserted into many bacteria cells. While they live, the bacteria's cell processes turn on the inserted gene and the protein is produced in the cell. When the bacterial cells reproduce by dividing, the inserted gene is also reproduced in the newly created cells.
  7. Cells Produce Proteins
- The protein that is produced can be purified and used for a medicine, industrial, agricultural, or other uses.

Modifying Genes

- Also called recombinant DNA technology, molecular cloning, and genetic engineering.

1. Restriction enzymes are used to “cut” DNA segments from one genome.
2. DNA ligases are used to “paste” them into another genome.



Potential Benefits

Humanitarian:

- Pest resistance
- Herbicide resistance
- Cold tolerance
- Drought tolerance

→ Improved farming → Cheaper food / More food → **Reducing world hunger and improving world health**

Increased nutrition → **Reducing world hunger and improving world health**

Edible vaccines → **Reducing world hunger and improving world health**

Environmental: reduced use of herbicides and chemicals in farming.

What are genetically modified foods?

- Also called genetically modified organisms (GMO).
- Involves the insertion of DNA from one organism into another OR modification of an organism's DNA in order to achieve a desired trait.

4

Arctic fish DNA

+

5

strawberry

= A strawberry resistant to frost

Examples of GMO's

- **Golden rice** – rice that contains beta-carotene (Vitamin A), which is not found in regular rice.
- **Bt corn** – corn that contains a chemical normally found in a bacterium (*Bacillus thuringiensis*) that is toxic to insects but not to humans.
- **Herbicide resistant plants.**

Animal Biotechnology:

❖Animals can be used as Bioreactors to produce important products.
Goats, cattle, sheep and chickens are being used as sources of medically valuable proteins such as antibodies – protective proteins to destroy foreign materials.

❖Production of transgenic animals : For the large scale production of human therapeutic proteins female transgenic animals can be created that express therapeutic proteins in their milk .

❖Transgenic animals contain genes from another sources.

❖Human genes for clotting of proteins can be introduced into cows for the production of these proteins in their milk.

❖Animal cloning : Dolly was the first mammal created by a cell nucleus transfer process.

❖Although animal cloning has elicited fears and concern about the potential for human cloning, scientists are generally excited about the techniques used to clone animals for a number of reasons. For instance , these techniques may lead to the cloning of animals that contain genetically engineered organs that can be transplanted into humans without any fear of tissue rejection.

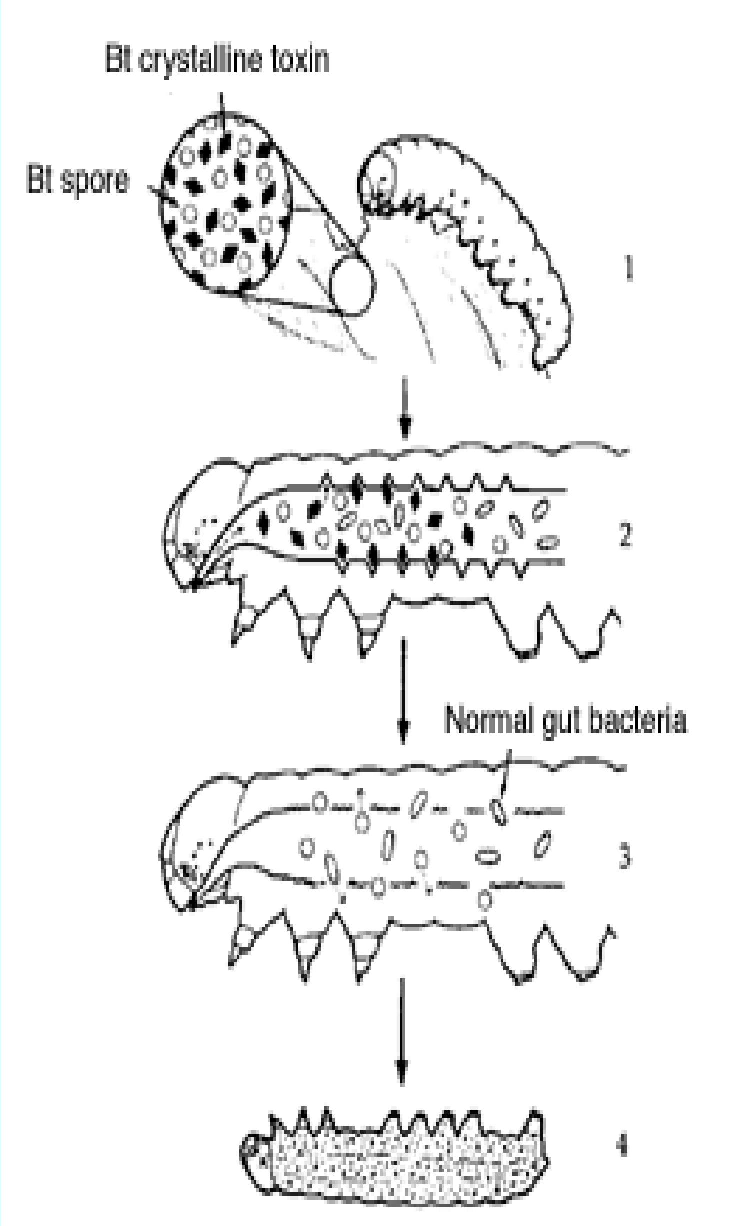
Selective breeding : **Recently scientists at Children's Hospital at Boston produced a transparent zebrafish named as Casper. Casper was created by mating a zebrafish mutant that lacked reflective pigments with a zebrafish that lacked black pigments. Scientists believe that Casper will be important for drug testing and *in vivo* study of stem cells and cancer.**

Casper has already proven to be valuable for studying how cancer cells spread : Scientists injected fluorescent tumor cells into the fish's abdominal cavity and were able to track migration of cancer cells to specific locations in the body.

Plant Biotechnology:

Production of **transgenic plants – Bt (*Bacillus thuringiensis*) cotton**

Bacillus thuringiensis **was registered as a plant pesticide in 1961, which produces a crystalline protein (cry gene) that kills harmful insects and their larvae**



Making transgenic plants

There are several methods for introducing genes into plants, including

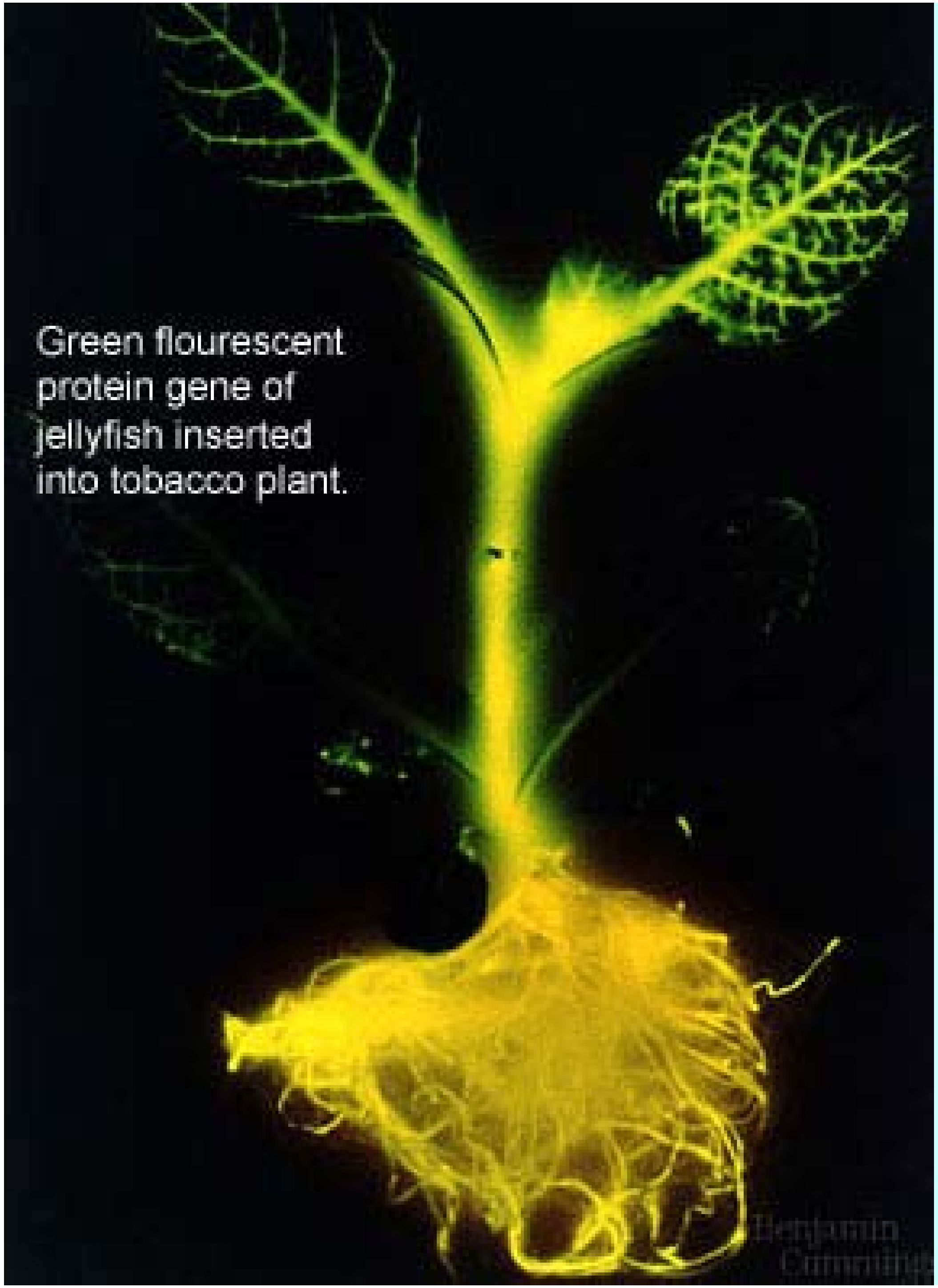
1. infecting plant cells with plasmids as vectors carrying the desired gene
2. shooting microscopic pellets containing the gene directly into the cell.

In contrast to animals, there is no real distinction between somatic cells and germinal cells.

Somatic tissues of plants, e.g., root cells grown in culture, can be transformed in the laboratory with the desired gene grown into mature plants with flowers.

If all goes well, the transgene will be incorporated into the pollen and eggs and passed on to the next generation.

In this respect, it is easier to produce transgenic plants than transgenic animals.


Green flourescent
protein gene of
jellyfish inserted
into tobacco plant.
Benjamin
Cummings

Disease Resistance.

Genes that provide resistance against plant viruses have been successfully introduced into such crop plants as tobacco, tomatoes, and potatoes.

Tomato plants infected with tobacco mosaic virus (which attacks tomato plants as well as tobacco). The plants in the back row carry an introduced gene conferring resistance to the virus. The resistant plants produced three times as much fruit as the sensitive plants (front row) and the same as control plants. (Courtesy Monsanto Company.)	

Herbicide Resistance.

Questions have been raised about the safety — both to humans and to the environment — of some of the broad-leaved weed killers like 2,4-D. Alternatives are available, but they may damage the crop as well as the weeds growing in it. However, genes for resistance to some of the newer herbicides have been introduced into some crop plants and enable them to thrive even when exposed to the weed killer.

Effect of the herbicide bromoxynil on tobacco plants transformed with a bacterial gene whose product breaks down bromoxynil (top row) and control plants (bottom row). "Spray blank" plants were treated with the same spray mixture as the others except the bromoxynil was left out. (Courtesy of Calgene, Davis, CA.)

Protoplast fusion :

Callus cells have the capacity to redifferentiate into shoot and root, and a whole flowering plant can be produced.

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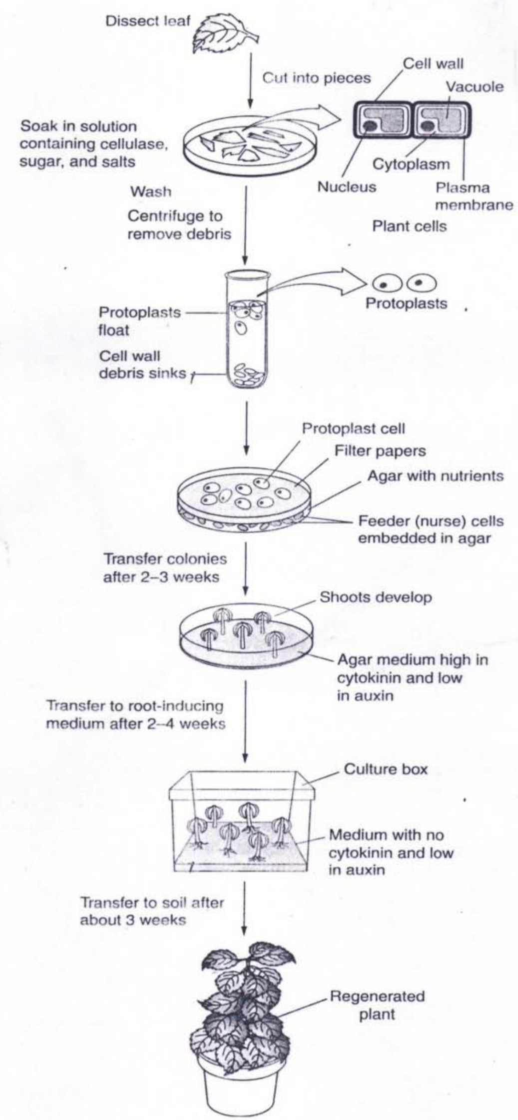

Figure 6.2 Protoplast Fusion and Regeneration of a Hybrid Plant After dissecting a plant leaf, it is possible to create protoplasts by digesting the cell wall with the enzyme cellulase. To create a hybrid plant, fuse protoplasts from different plants by culturing the fused cells in sterile media that stimulates shoots (cytokinin) and roots (auxin).

Vaccines for plants :

Researchers have recently inserted a gene from the tobacco mosaic virus (TMV) into tobacco plants. The gene produces a protein found on the surface of the virus and, like a vaccine, turns on the plant's immune system. In this case tobacco plants with this gene are immune to TMV.

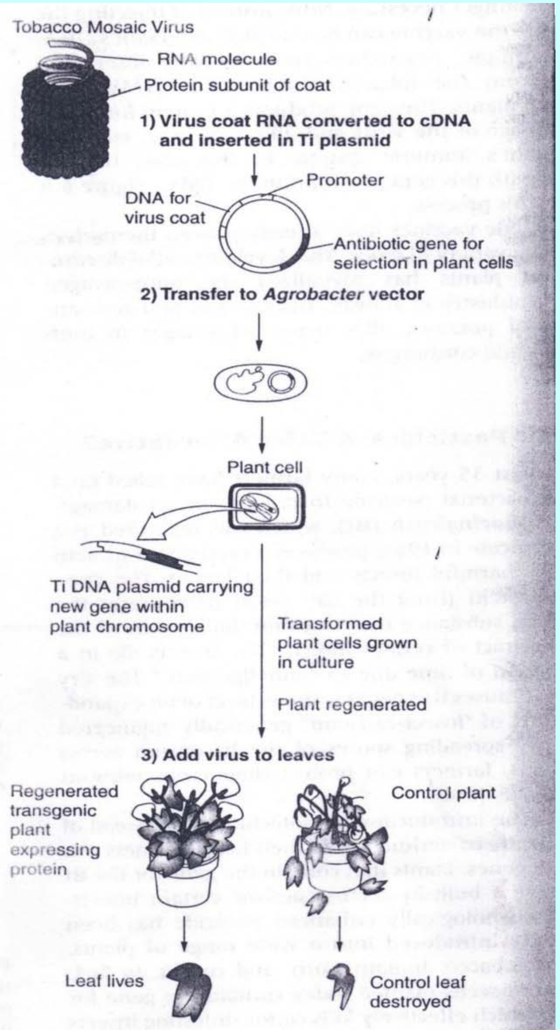

Figure 6.8 Plant Vaccines Transgenic plants expressing the TMV coat protein (CP) were produced by *Agrobacterium*-mediated gene transfer. When the generated plants expressing the viral CP were infected with TMV, they exhibited increased resistance to infection. Whereas control plants developed symptoms in 3 to 4 days, transgenic CP-expressing plants resisted infection for 30 days.

Synthetic seeds : Through tissue culture techniques embryoids are produced which are coated with the gel.

Tissue culture technique : Plantlets are produced at commercial level from the desirable plant through tissue culture technique.

Forensic biotechnology:

DNA fingerprinting : a method for detecting an organism's unique DNA pattern –is a primary tool used in forensic biotechnology.

DNA fingerprinting can be accomplished using trace amounts of tissue, hair, blood or or body fluid left behind at a crime scene.

It has many other applications , including its use in paternity case for pinpointing a child's father and for identifying human remains.

Medical biotechnology

- **Medical biotechnology is involve in the whole spectrum of human medicine**
- **Over 325 million people worldwide have been helped by biotechnology drugs and vaccines.**
- **Gene therapy : In gene therapy genetic disease condition s can be treated by inserting normal genes into a patient or replacing diseased genes with normal genes.**
- **Stem cell technologies are some of the newest , most promising aspects of medical biotechnology.**
- **Stem cells are immature cells that have the potential to develop and specialize into nerve cells, blood cells, muscle cells and virtually any other cell type in the body.**
- **Production of insulin and interferon.**

Edible vaccines

- Plant serves as a cheap and safer production system for subunit vaccines. The edible vaccines can be easily ingested by eating plants.
- Transgenic plants have been developed for expressing antigens derived from animal viruses.For examples:

Antigen	Host plant
Rabies glycoprotein	Tomato
Foot & mouth viruses	Arabidopsis
Herpes virus B surface antigen	Tobacco
Cholera toxin B subunit	Potato

Bioremediation :

- The use of biotechnology to process and degrade a variety of natural and man made substances, particularly those that contribute to environmental pollution.
- Bioremediation is being used to clean up many environmental hazards that have been caused by industrial progress.
- By Stimulating the growth of oil degrading bacteria many miles of shore line of Alaska were cleaned up nearly three time faster than the chemical cleaning.

ENVIRONMENTAL BIOTECHNOLOGY

‘Environmental Biotechnology’ involves specific applications of biotechnology to the management of environment and related socio-economic and developmental issues, keeping in view the concept of sustainable development. ‘Environmental biotechnology’ encompasses issues like:

1. Environmental monitoring;
2. Restoration of environmental quality;
3. Resource/residue/water-recovery/utilization/treatment through application of r-DNA technology;
4. Substitution of non-renewable resources by renewable ones;
5. Strain improvement for degradation of highly-toxic pollutants with the production of chemicals;
6. Global changes;
7. Biological diversity.

Biological Challenges of the 21st Century:

Numerous problems and challenges have the potential to be solved by biotechnology. For many of the greatest challenges – such as curing life – threatening human diseases – the barriers to overcoming these challenges are not insurmountable. Answer lie in our ability to better understand biological processes and design and adapt biotechnological solutions.

- Human genome project - to solve all of our medical problems
- To find out the substitute of crude oil
- To develop micro organisms for degradation of plastic
- To develop strains of microorganisms for the productions of hormones, vitamins, alkaloids, vaccines etc.,

SNP: Single Nucleotide Polymorphisms are single Nucleotide changes or mutations in DNA sequences.

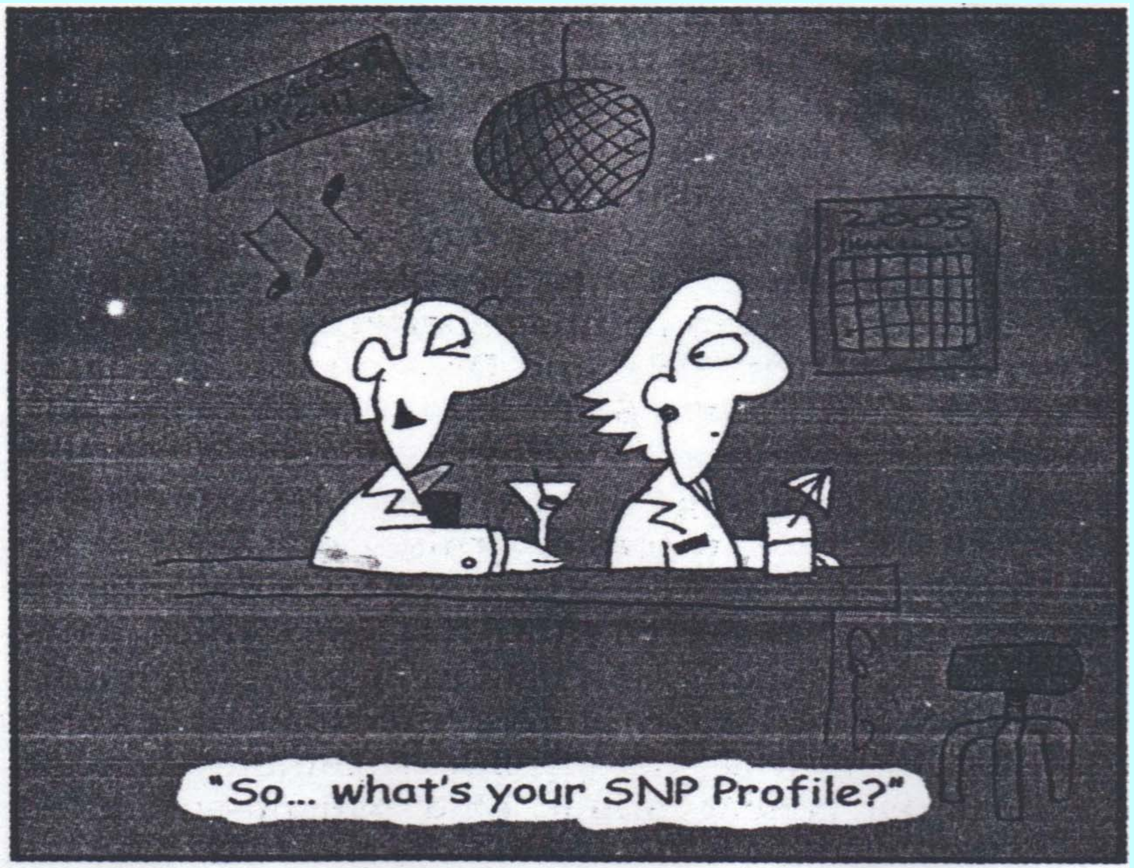

Figure 1.12 Secrets of the Human Genome In the future, we will have unprecedented knowledge of our genetic makeup including SNPs and other markers of genetic diseases. Can you think of possible ethical, legal, and social implications of such information?

Thank you!