Chapter-12

RECENT DEVELOPMENTS IN GENETICS
Gene Transfer and Its Applications and Biosafety of GMOs

Definition of Biotechnology
According to the United States Congress’s Office of Technology Assessment, biotechnology can be defined as any technique that uses living organisms to make or modify a product to improve plants or animals or to develop microorganisms for specific uses. The document focuses on the development and application of modern biotechnology based on new enabling techniques for recombinant DNA technology, often referred to as genetic engineering.

According to British Biotechnologist, biotechnology can be defined as application of biological organisms, system or processes to manufacturing and service industries.

According to Japanese Biotechnologists, biotechnology can be defined as a technology using biological phenomena for copying and manufacturing various kinds of useful substances.

Biotechnology is used in several different ways in aquaculture. Researchers use transgenic to introduce desirable genetic traits into the fish, thereby creating hardier stock. Transgenic involves the transfer of genes from one species into another species, in this case, fish.

Definition of Genetic Engineering:
Genetic engineering is the alteration of genetic material by direct intervention in genetic processes with the purpose of producing new substances or improving functions of existing organisms. It is a very young, exciting, and controversial branch of the biological sciences. On the one hand, it offers the possibility of cures for diseases and countless material improvements to daily life.

Definition of Recombinant DNA:
Recombinant DNA refers to a collection of techniques for creating and analyzing DNA molecules that contain DNA from two unrelated organisms. One of the DNA molecules is typically a bacterial or viral DNA that is capable of accepting another DNA molecule; this is called a vector DNA. The other DNA molecule is from an organism of interest, which could be anything from a bacterium to a whale, or a human. Combining these two DNA molecules allows for the replication of many copies of a specific DNA. These copies of DNA can be studied in detail, used to produce valuable proteins, or used for gene therapy or other applications.

Or

Recombinant DNA is a form of artificial DNA which is engineered through the combination or insertion of one or more DNA strands, thereby combining DNA sequences which would not normally occur together. In terms of genetic modification, recombinant DNA is produced through the addition of relevant DNA into an existing organismal genome, such as the plasmid of bacteria, to code for or alter different traits for a specific purpose, such as immunity. It differs from genetic recombination, in that it does not occur through processes within the cell or ribosome, but is
exclusively engineered. GloFish are a type of zebrafish with recombinant DNA. Genes for fluorescent proteins have been inserted into their genome to produce their fluorescent colors.

The **GloFish** is a trademarked brand of genetically modified (GM) fluorescent zebrafish with bright red, green, and orange fluorescent color. Although not originally developed for the ornamental fish trade, it is the first genetically modified animal to become publicly available as a pet.

**Definition of Recombinant DNA technology:**
Techniques, usually associated with genetic engineering, in which strands of DNA from different sources are spliced together to form DNA for a new life form. Gene splicing is another name for this process. This technique was first engineered by Stanley Norman Cohen and Herbert Boyer in 1973.

**Definition of Transgene:**
A transgene is a gene or genetic material which has been transferred by any of a number of genetic engineering techniques from one organism to another. In its most precise usage, the term transgene describes a segment of DNA containing a gene sequence which has been isolated from one organism and is introduced into a different organism. This non-native segment of DNA may retain the ability to produce RNA or protein in the transgenic organism or it may alter the normal function of the transgenic organism's genetic code.

**Definition of Transgenic:**
Relating to, or being an organism whose genome has been altered by the transfer of a gene or genes from another species or breed: *transgenic mice; transgenic plants.*

**Definition of In vitro:**
It refers to the technique of performing a given experiment in a test tube, or, generally, in a controlled environment outside a living organism. *In vitro* fertilization is a well-known example of this. Many experiments in cellular biology are conducted outside of organisms or cells; because the test conditions may not correspond to the conditions inside of the organism, this may result in inaccurate results. Consequently, such experimental results are often annotated with *in vitro*, in contradistinction with *in vivo*.

**Definition of In vivo:**
It means that which takes place inside an organism. In science, *in vivo* refers to experimentation done in or on the living tissue of a whole, living organism as opposed to a partial or dead one. Animal testing and clinical trials are forms of *in vivo* research.

**Genetically Modified Organism (GMO):**
A GMO is an organism whose genetic material has been altered using the genetic engineering techniques generally known as recombinant DNA technology. With recombinant DNA technology, DNA molecules from different sources are combined *in vitro* into one molecule to create a new
gene. This modified DNA is then transferred into an organism causing the expression of modified or novel traits. The product is also known as a Genetically Engineered Organism or GEO.

**Uses of GMOs:**

Examples of GMOs are highly diverse, and include transgenic (genetically modified by recombinant DNA methods) animals such as mice, fish, transgenic plants, or various microbes, such as fungi and bacteria. The generation and use of GMOs has many reasons, chief among them are their use in research that addresses fundamental or applied questions in biology or medicine, for the production of pharmaceuticals and industrial enzymes, and for direct, and often controversial, applications aimed at improving human health (e.g., gene therapy) or agriculture (e.g., golden rice). The term "genetically modified organism" does not always imply, but can include, targeted insertions of genes from one into another species. For example, a gene from a jellyfish, encoding a fluorescent protein called GFP, can be physically linked and thus co-expressed with mammalian genes to identify the location of the protein encoded by the GFP-tagged gene in the mammalian cell. These and other methods are useful and indispensable tools for biologists in many areas of research, including those that study the mechanisms of human and other diseases or fundamental biological processes in eukaryotic or prokaryotic cells.

**Living Modified Organisms (LMOs):**

"Living modified organisms" are any living organisms that possess a new combination of genetic material obtained through the use of modern biotechnology; they are a subset of genetically modified organisms (GMOs). Genetically modified seeds, cuttings and tissue cultures are living parts of plants and therefore LMOs.

**Gene Transfer:**

Incorporation of new DNA into an organism's cells, usually with the help of a micro organism that serves as a vector.

---

**Application of gene transfer technology for genetic improvement of fish**

The demand for fish is, traditionally met from the natural population of fish in the world. However, the level of the total worldwide annual harvest of fish has plateaued at the maximal potential level of between 100 and 150 million metric tons per year. Moreover, the worldwide decline of the natural stocks of some commercially valuable fish species and the accumulation of chemical pollutants in aquatic environments has provided a strong impetus for aquaculture. Therefore, many countries have turned to aquaculture to increase fish production over the last several decades. The outcome of these efforts has been a yield of 30 million metric tons per year that clearly proves that aquaculture has great potential to significantly increase the world production of fish and to resolve the pressing problem of meeting world demand. Success in the production of fish by aquaculture depends on several factors, such as a good genetic background of the broodstock, efficient prevention and detection of disease, understanding of the optimal physiological, environmental and nutritional conditions for growth and development, control of the reproductive cycle of the fish species, a supply of good quality water, and the application of innovative management skills. The
newly developed technologies in molecular biology and biotechnology have provided the means, such as transgenic fish technology, to improve these factors. The application of transgenic fish technology can improve a number of factors, such as growth performance, disease resistance and adaptation to inappropriate environmental conditions. A number of investigators have already reported production of transgenic fish superior to their non-transgenic counterparts. For instance, Chen et al. demonstrated that tilapia expressing extra copies of trout growth hormon, gene grow much faster than their nontransgenic counterparts which do not posses extra copies of the growth hormone gene.

**Principle of gene transfer**
Transgenics are organisms into which heterologous DNA (transgene) has been artificially introduced and the transgene stably integrated into their genomes. Although transgenics have been produced since the early 1980s, the production of transgenic fish was accomplished only a decade ago, in the early 1990s. The main principle of gene transfer is to facilitate the transfer of heterologous DNA into the nucleus of a target cell where integration into the host genome takes place. This can be accomplished using a number of techniques, such as microinjection that allows delivery of the transgene directly into the nucleus, and electroporation that facilitates the formation of temporary pores on the surface of the target cells through which the transgene is introduced into the cytoplasm where it is then delivered to the nucleus by the cellular machinery.

**Fish species used for gene transfer studies**
To date, a range of fish species, such as Japanese medaka (*Oryzias latipes*), zebra fish (*Brachydanio rerio*), trout (*Onchorynchus mykiss*), Atlantic salmon (*Salmo salar*), common carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), tilapia (*Oreochromis*), loach (*Misgurnus anguillicaudatus*) and goldfish (*Carassius aurata*), have been used for gene transfer studies. The species of fish to be used for gene transfer studies is determined according to the purpose of the study. Fish species that are small in size, such as Japanese medaka (*Oryzias latipes*), is chosen to the test efficiency of a method or to establish a model system for basic or applied studies. However, larger species such as tilapia are chosen if the transgenic fish will be used as a bioreactor or for aquaculture purposes.
Methods used for gene transfer:
A variety of gene transfer techniques, have been used to produce transgenics. Different gene transfer techniques having various advantages and disadvantages depending on the species of animal to be used for gene transfer studies are as follows:
1. Calcium precipitation,
2. Microinjection,
3. Lipofection,
4. Retrovirus Infection,
5. Electroporation,
6. Embryonic stem cells,
7. Sperm mediated gene transfer and
8. Particle gun bombardment.

For instance, methods such as microinjection and electroporation are currently used for gene transfer in egg-laying fish, whereas others, such as retroviral vector-mediated gene transfer, are used in live-bearing fish species. These two methods currently used for gene transfer in fish are summarized below.

Microinjection
Microinjection is considered the most effective method of gene transfer in high vertebrates such as mice and sheep. In this technique, the transgene is directly microinjected into the male pronuclei of fertilized eggs. However, the pronuclei of fertilized eggs in fish studied to date are not visible, and transgenes are usually injected into the egg cytoplasm. Although this method is successful in transferring DNA into fish embryos, it is a very tedious, laborious and time-consuming procedure for animals such as fish that produce a large number of eggs (3).

Electroporation
Electroporation utilizes a series of short electric pulses to permeate the cell membrane and allow the transgenes to enter the cells. The presence of a tough chorion layer around the fish eggs reduces efficiency, but removal of the chorion layer, physically or chemically, increases the efficiency of this technique. However, removal of the chorion is not applied routinely because it is a tedious procedure and introduces additional stress on newly fertilized eggs. Electroporation has been shown to be the most effective means of gene transfer in fish since a large number of fertilized eggs can be treated in a short time by this method (9,10). There are a number of commercially available electroporators, such as Beakon 2000 (Baekon Co.) that can be effectively used for gene transfer studies in fish.

Controversy over GMOs
The use of GMOs has sparked significant controversy in many areas. Some groups or individuals see the generation and use of GMO as intolerable meddling with biological states or processes that have naturally evolved over long periods of time, while others are concerned about the limitations
of modern science to fully comprehend all of the potential negative ramifications of genetic manipulation.

While some groups advocate the complete prohibition of GMOs, others call for mandatory labeling of genetically modified food or other products. Other controversies include the definition of patent and property pertaining to products of genetic engineering and the possibility of unforeseen local and global effects as a result of transgenic organisms proliferating.

In 2004, Mendocino County, California became the first county in the United States to ban the production of GMOs. The measure passed with a 57% majority. In 2005, a standing committee of the government of Prince Edward Island in Canada began work to assess a proposal to ban the production of GMOs in the province. PEI has already banned GM potatoes, which account for most of its crop.

Currently, there is little international consensus regarding the acceptability and effective role of modified "complete" organisms such as plants or animals. A great deal of the modern research that is illuminating complex biochemical processes and disease mechanisms makes vast use of genetic engineering.

**Biosafety of GMOs**

The vast majority of work with GMOs in contained use is inherently safe. This is because most work involves the insertion of genes into micro-organisms that have been deliberately "crippled" with disabling mutations so that they will not grow outside of the controlled environment of a laboratory test tube. Safety is thus built into the experimental design. Only a small number of activities involve GMMs that are not disabled and still capable of growth outside of the laboratory. Risk assessments for all such work must be submitted to the regulatory authorities for approval before work can commence. The regulatory authorities are thus in a position to ensure that the work is carried out in facilities and equipment that contain the infectious materials during the work activities. The assignment of these containment measures tends to be done on a precautionary basis to allow some margin of safety, where there is any uncertainty over the risks. Gradually as knowledge increases some precautionary safeguards are being removed, where there is clear evidence that they are not necessary, and efforts are being focussed on ensuring the safety of those projects where there are tangible hazards. The safety record in this industry is extremely good.