BIOGENETIC LAW

The biogenetic law is a theory of development and evolution proposed by Ernst Haeckel in Germany in the 1860s. It is one of several recapitulation theories, which posit that the stages of development for an animal embryo are the same as other animals' adult stages or forms. Commonly stated as ontogeny recapitulates phylogeny, the biogenetic law theorizes that the stages an animal embryo undergoes during development are a chronological replay of that species' past evolutionary forms. The biogenetic law states that each embryo's developmental stage represents an adult form of an evolutionary ancestor. According to the law, by studying the stages of embryological development, one is, in effect, studying the history and diversification of life on Earth. The biogenetic law implied that researchers could study evolutionary relationships between taxa by comparing the developmental stages of embryos for organisms from those taxa. Furthermore, the evidence from embryology supported the theory that all of species on Earth share a common ancestor.

Ernst Haeckel studied animals and evolution in Germany from 1834 to 1919. He proposed the biogenetic law while working at the University of Jena in Jena, Germany, in his 1866 book Generelle Morphologie der Organismen [General Morphology of the Organisms]. The publication unifies theories Haeckel proposed during his work throughout the 1850s and 1860s. Haeckel cited Johann Wolfgang von Goethe from Germany, Jean Baptiste Lamarck from France, and Charles Darwin from England as his main influences for creating the biogenetic law.

Haeckel proposed the biogenetic law after reading Charles Darwin's theories in The Origin of Species. Haeckel championed Darwin's theory of evolution in Germany and praised him for using information from embryology to help form his theory of evolution. Darwin argued that one could explain facts about embryology, such as the early similarity between embryos of different species, by looking at them in terms of evolution by natural selection. The fact that the more general characters of a taxonomic group tend to be present earlier in the embryo, while specialized and variable characters tend to manifest later in the embryo, indicated that these specialized features are the most recent changes to the ancestral form. Darwin proposed that the embryos of currently living species would look similar to the embryos of their ancestors and that embryos of different taxonomic groups look similar to each other because they share a common ancestor. Haeckel interpreted the data differently than Darwin, and he purported instead that **the embryonic stages of extant species represent adult forms of their previous ancestors.**

Although Haeckel cited Darwin as he proposed the biogenetic law, the two disagreed about embryology and evolution.

1. Haeckel interpreted the process of evolution as progressive, following a specified path from lower to higher animals. Darwin, however, argued that evolution wasn't progressive.
2. Darwin also argued that embryos diverged more from one another as development progressed, rather than passing through linear stages of evolutionary ancestry.
3. Because Haeckel argued that evolution was progressive, he also endorsed Jean Baptiste Lamarck's theory of acquired characters. Lamarck theorized that organisms could acquire or alter their characters by use and disuse of their anatomical parts, and that parents could pass on these acquired or altered characters to their offspring. Lamarck's theory competed with Darwin's of natural selection as the mechanism for evolution, but Haeckel incorporated both theories into the biogenetic law.

Haeckel proposed the biogenetic law so that researchers could use the stages of embryological development to help construct evolutionary (phylogenetic) trees. Haeckel claimed that phylogenesis, or the process by which groups of organisms diversify from one another, influenced the development

(ontogeny) of embryos. He theorized that the stages in an organism's ontogeny reflected the successive changes in form, from generation to generation, of that organism's evolutionary ancestors. Many scientists saw Haeckel's work as a breakthrough in recapitulation theory because he offered a physical mechanism of development that other biologists had not proposed.

Assumptions

According to Haeckel, the biogenetic law depends on three assumptions.

1. Law of correspondence:

This states that each stage of development in higher animals, such as humans, corresponds to adult stages of lower animals, such as fish. For instance, gill slits in early human embryos correspond to the gill slits in adult fish.

2. Constraints in early development:

The second assumption of the biogenetic law was that phylogenesis must occur by the addition of new characters to the end of the normal developmental process. Haeckel said that the early stages of different species' embryos look similar to each other because of developmental constraints present early in development. These constraints disappear towards the end of development, which allow for the addition of new characters and for subsequent evolution.

3. Principle of truncation:

The third assumption was the principle of truncation. Haeckel argued that if new characters were continuously added to the end of normal ontogeny, the length of embryonic development would eventually become longer than gestation periods of organisms in extant species. As a result, he theorized that early stages of development must be faster in higher organisms than in lower ones.

Haeckel also used the concept of truncation to explain inconsistencies between the stages of animals from different taxa. For instance, pigs and humans may look similar to each other as early embryos, but as ontogeny progresses, the embryos start to look different from one another.

If embryos pass through the linear stages of their evolutionary ancestors, as Haeckel claimed, then the two embryos should go through the same stages until the pig reaches full development and the human continues through the subsequent stages of its evolutionary ancestry. However, in many cases, scientists found no such progressions. Haeckel hypothesized that truncation of ontogeny caused these inconsistencies. This principle of truncation influenced scientists in the US such as Alpheus Hyatt, Alpheus Packard, and Edward Drinker Cope.

Haeckel supported his biogenetic law with his drawings of embryos during different stages of development. In 1874, his work Anthropogenie included drawings of embryonic fish, salamanders, tortoises, chicks, pigs, cows, rabbits, and humans at different stages of development placed next to one another for comparison. Haeckel's drawings made the embryos of the different groups look almost identical in their earliest stages of development. He argued that they only become recognizable as species later in their respective developments. These similarities, according to Haeckel, demonstrated the linear progression from what he called lower forms to higher forms of animals, and he concluded that the stages recapitulated the evolutionary history of the organisms' ancestors.

Wilhelm His, professor of anatomy at the University of Basel in Basel, Switzerland, and at the University of Leipzig in Leipzig, Germany, opposed Haeckel's biogenetic law. He argued that embryologists shouldn't aim to construct phylogenetic trees and argued that embryologists should

instead aim to explain development. He agreed with Haeckel that one should use causal theories to explain development, but he argued that Haeckel's theory was flawed in positing the stages of development as representations of adult ancestors. He argued the Haeckel's biogenetic law overemphasized evolution as the cause of development and exaggerated the similarities between embryos of different species. He said that there were obvious differences between the early stages of embryos of different species, and that those differences, not the similarities, were important to explain development.

In the decades after Haeckel's publication of the biogenetic law, other biologists struggled to recreate Haeckel's results. Franz Keibel, a student of Wihelm His and a professor of anatomy at the University of Strasbourg in Alsace, France, tried to recreate Haeckel's drawings from his own specimens and concluded that Haeckel had exaggerated the similarity between embryos in his drawings. Keibel therefore rejected the biogenetic law and labeled it an exaggeration of the truth. In 1897, Keibel published this conclusion in the first volume of Normentafeln zur Entwicklungsgeschichte der Wirbelthiere (Standard Panels to the Developmental History of the Verterbra).

Furthermore, many scientists adopted a competing theory in the beginning of the twentieth century. In 1828, Karl Ernst von Baer at University of Königsberg in Königsberg, Prussia, had proposed what researchers later called von Baer's laws of embryology. Von Baer formulated these laws to discredit conception of recapitulation theory published in 1811 by Johann Friedrich Meckel. In his laws, von Baer stated that the more general characters of a taxonomic group appear earlier in an animal embryo than the specialized characters do. He argued that rather than animals passing through successive stages of other adult animals, they diverge from one another as development progresses. Therefore, he concluded, the stages embryos pass through during ontogeny never represent adult forms of other animals; they only represent embryonic stages of other animals. This conception was part of Darwin's 1859 account of ontogeny in The Origin of Species. Although von Baer's theory was overshadowed by recapitulation theory for most of the nineteenth century, scientists in the twentieth century began to adopt von Baer's view as the more accurate representation of development.

Haeckel's biogenetic law was further discredited by the results of experimental embryologists in the early twentieth century. Researchers abandoned Haeckel's theory when they couldn't confirm his observations. Embryologists showed that cases of recapitulation were less prevalent than were the inconsistencies between the developmental stages of normal organisms from different species.

Rejections:

As per Haeckel, all animals start their embryonic development from zygote, suggesting that all life evolved from a single cell. He believed that paleogenetic characters are ancestral traits that are retained in the embryos and coenogenetic characters are secondary, adaptive and non-ancestral that appear later in the development. However, Ernst Haeckel’s theory was criticised on three counts as follows:

1) Embryos of higher animals resemble only the embryos of lower animals and not the adults,

2) Ontogeny only shows affinity and not evolution, and

3) Retaining ancestral embryonic stage has no selection value and therefore would be wasteful and time consuming.

Modern gene theory explains the sequence of events and the subsequent end product of ontogeny in the right perspective and, therefore, Haeckel’s original theory has now been rejected.

STEM CELLS

Stem cells are defined as cells that have clonogenic and self-renewing capabilities and differentiate into multiple cell lineages. Stem cells are found in all of us, from the early stages of human development to the end of life. Stem cells are basic cells of all multicellular organisms having the potency to differentiate into wide range of adult cells. Self-renewal and totipotency are characteristic of stem cell. Though totipotency is shown by very early embryonic stem cells, the adult stem cells possess multipotency and differential plasticity which can be exploited for future generation of therapeutic options. All stem cells may prove useful for medical research, but each of the different types has both promise and limitations. For decades, researchers have been studying the biology of stem cells to figure out how development works and to find new ways of treating health problems. The scientific researchers and medical doctors of today hope to make the legendary concept of regeneration into reality by developing therapies to restore lost, damaged, or aging cells and tissues in the human body. This research has opened new horizons for stem cell research. Stem cell research holds tremendous promise for the development of novel therapies for many serious diseases and injuries. While stem cell-based treatments have been established as a clinical standard of care for some conditions, such as hematopoietic stem cell transplants for leukaemia and epithelial stem cell-based treatments for burns and corneal disorders, the scope of potential stem cell-based therapies has expanded in recent years due to advances in stem cell research. It is impossible to project when actual treatments or cures might emerge from such research, but the paths this research might take and potential applications have been much discussed. Stem cells can now be grown and transformed into specialized cells with characteristics consistent with cells of various tissues such as muscles or nerves through cell culture. Highly plastic adult stem cells from a variety of sources, including umbilical cord blood and bone marrow, are routinely used in medical therapies.

Historical perspective

The history of stem cell research had a benign, embryonic beginning in the mid 1800's with the discovery that some cells could generate other cells. In the early 1900's the first real stem cells were discovered when it was found that some cells generate blood cells. The term "stem cell" was proposed for scientific use by the Russian histologist Alexander Maksimov in 1908. Bone marrow transplant between two siblings successfully treated SCID in 1968. Haemopoietic stem cells were discovered in human cord blood in 1978.

Scientists at Newcastle University in England created the first ever artificial liver cells using umbilical cord blood stem cells in October 2006 . It is suggested that these stem cells have the ability to differentiate into more cell types than adult stem cells, opening up greater possibilities for cell-based therapies. Then, in early 2007, researchers led by Dr. Anthony Atala claimed that a new type of stem cell had been isolated in amniotic fluid. This finding is particularly important because these stem cells could prove to be a viable alternative to the controversial use of embryonic stem cells.

Stem cell

A stem cell is a non-specialized, generic cell which can make exact copies of itself indefinitely and can differentiate and produce specialized cells for the various tissues of the body. Stem cells are cells found in most, if not all, multi-cellular organisms. They are characterized by self-renewal and potency i.e. the ability to renew themselves through mitotic cell division and differentiating into a diverse range of specialized cell types. They are vital to the development, growth, maintenance, and repair of our brains, bones, muscles, nerves, blood, skin, and other organs. Laboratory studies of stem cells enable scientists to learn about the cells' essential properties and what makes them different from specialized

cell types. Scientists are already using stem cells in the laboratory to screen new drugs and to develop model systems to study normal growth and identify the causes of birth defects. Research on stem cells continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. Stem cell research is one of the most fascinating areas of contemporary biology, but, as with many expanding fields of scientific inquiry, research on stem cells raises scientific questions as rapidly as it generates new discoveries. Over the past year, adult stem cells have been used either exclusively or in combination with other treatments to achieve significant "healthcare benefits" for sufferers of the every tissue of human body.

Classification of stem cells on the basis of potency

Stem cells can be classified by the extent to which they can differentiate into different cell types. These four main classifications are totipotent, pluripotent, multipotent, or unipotent.

1. **Totipotent:** The ability to differentiate into all possible cell types. Examples are the zygote formed at egg fertilization and the first few cells that result from the division of the zygote.
2. **Pluripotent**: The ability to differentiate into almost all cell types. Examples include embryonic stem cells and cells that are derived from the mesoderm, endoderm, and ectoderm germ layers that are formed in the beginning stages of embryonic stem cell differentiation.
3. **Multipotent:** The ability to differentiate into a closely related family of cells. Examples include hematopoietic (adult) stem cells that can become red and white blood cells or platelets.
4. **Oligopotent:** The ability to differentiate into a few cells. Examples include (adult) lymphoid or myeloid stem cells.
5. **Unipotent:** The ability to only produce cells of their own type, but have the property of self-renewal required to be labelled a stem cell. Examples include (adult) muscle stem cells.

Classification of stem cells on the basis of their sources

The easiest way to categorize stem cells is by dividing them into two types: Early or embryonic and mature or adult. Early stem cells, often called embryonic stem cells, are found in the inner cell mass of a blastocyst after approximately five days of development. Mature stem cells are found in specific mature body tissues as well as the umbilical cord and placenta after birth.

1. **Embryonic stem cells:** Embryonic stem cells are self-replicating pluripotent cells that are potentially immortal. They are derived from embryos at a developmental stage before the time of implantation would normally occur in the uterus. The embryos from which human embryonic stem cells are derived are typically four or five days old and are a hollow microscopic ball of cells called the blastocyst.
2. **Adult stem cells:** Adult stem cells are undifferentiated totipotent or multipotent cells, found throughout the body after embryonic development that multiply by cell division to replenish dying cells and regenerate damaged tissues. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are found. Unlike embryonic stem cells, which are defined by their origin (the inner cell mass of the blastocyst), the origin of adult stem cells in some mature tissues is still under investigation.
3. **Pluripotent stem cells I:** Recently, a third type of stem cell, with properties similar to embryonic stem cells, has emerged. Scientists have engineered these induced pluripotent stem cellsi (iPS cells) by manipulating the expression of certain genes - 'reprogramming' somatic cells back to a pluripotent state.

Stem cell culture

Growing cells in the laboratory is known as cell culture. Human embryonic stem cells (hESCs) are generated by transferring cells from a pre-implantation stage embryo into a plastic laboratory culture dish that contains a nutrient broth known as culture medium. The cells divide and spread over the surface of the dish. However, if the plated cells survive, divide and multiply enough to crowd the dish, they are removed gently and plated into several fresh culture dishes. The process of replating or sub culturing the cells is repeated many times and for many months. Each cycle of subculturing the cells is referred to as a passage. Once the cell line is established, the original cells yield millions of embryonic stem cells. Embryonic stem cells that have proliferated in cell culture for six or more months without differentiating, are pluripotent, and appear genetically normal are referred to as an embryonic stem cell line. At any stage in the process, batches of cells can be frozen and shipped to other laboratories for further culture and experimentation.

Stem cell lines

A stem cell line is a family of constantly dividing cells, the product of a single parent group of stem cells. They are obtained from human or animal tissues and can replicate for long periods of time in vitro ("within glass"; or, commonly, "in the lab", in an artificial environment). They are frequently used for research relating to embryonic stem cells or cloning entire organism. Once stem cells have been allowed to divide and propagate in a controlled culture, the collection of healthy, dividing, and undifferentiated cells is called a stem cell line.

Applications of stem cells

The goal of any stem cell therapy is to repair a damaged tissue that can't heal itself. Ongoing research on stem cell therapies gives hope to patients who would normally not receive treatment to cure their disease but just to alleviate the symptoms of their chronic illness. Stem cell therapies involve more than simply transplanting cells into the body and directing them to grow new, healthy tissue. It may also be possible to coax stem cells already in the body to work overtime and produce new tissue.

Possible treatments by stem cells

A number of stem cell therapeutics exist, but most are at experimental stages and/or costly, with the notable exception of bone marrow transplantation. Medical researchers anticipate that adult and embryonic stem cells will soon be able to treat cancer, Type 1 diabetes mellitus, Parkinson's disease, Huntington's disease, Celiac Disease, cardiac failure, muscle damage and neurological disorders, and many others. They have suggested that before stem cell therapeutics can be applied in the clinical setting, more research is necessary to understand stem cell behavior upon transplantation as well as the mechanisms of stem cell interaction with the diseased/injured microenvironment.

Bone marrow transplants (BMT) are a well known clinical application of stem cell transplantation. BMT can repopulate the marrow and restore all the different cell types of the blood after high doses of chemotherapy and/or radiotherapy, our main defense used to eliminate endogenous cancer cells. The isolation of additional stem and progenitors cells is now being developed for many other clinical applications.

Skin replacement

The knowledge of stem cells has made it possible for scientists to grow skin from a patient’s plucked hair. Skin (keratinocyte) stem cells reside in the hair follicle and can be removed when a hair is plucked. These cells can be cultured to form an epidermal equivalent of the patients own skin and provides tissue for an autologous graft, bypassing the problem of rejection.

Brain cell transplantation

Stem cells can provide dopamine - a chemical lacking in victims of Parkinson's disease. It involves loss of cells which produce the neurotransmitter dopamine. The first double-blind study of fetal cell transplants for Parkinson's disease reported survival, release of dopamine from the transplanted cells and a functional improvement of clinical symptoms. However, some patients developed side effects, which suggested that there was an over sensitization to or too much dopamine. Although the unwanted side effects were not anticipated, the success of the experiment at cellular level is significant.

Treatment for diabetes

Diabetes affects millions of people in the world and is caused by abnormal metabolism of insulin. Normally, insulin is produced and secreted by the cellular structures called the islets of langerhans in the pancreas. Recently, insulin expressing cells from mouse stem cells have been generated. In addition, the cells self-assemble to form structures, which closely resemble normal pancreatic islets and produce insulin. Future research will need to investigate how to optimize conditions for insulin production with the aim of providing a stem cell-based therapy to treat diabetes to replace the constant need for insulin injections.

Scientists and stem cell research

Scientists believe that stem-cell research could lead to cures for a myriad of diseases afflicting humans. Anti-abortion groups, some religious groups, and conservative citizens say that using cells from embryos is immoral because it destroys life. However, recent news has shown that support for stem cell research by a 2-1 margin and say that it should be funded by the federal government, despite controversy over the use of human embryos.

CLONING

A clone may be defined as:

1. A cell, group of cells, or organism that is descended from and is genetically identical to a single common ancestor, such as a bacterial colony whose members arose from a single original cell.
2. An organism descended asexually from a single ancestor, such as a plant produced by layering or a polyp produced by budding.
3. A DNA sequence, such as a gene, that is transferred from one organism to another and replicated by genetic engineering techniques.
4. Individual organisms that arise asexually from the somatic, or body, cells of the parent rather than from the specialized sexual cells.

Cloning may thus be defined as:

1. To make multiple identical copies of a DNA sequence.
2. To create or propagate an organism from a clone cell.
3. To reproduce or propagate asexually.
4. To produce a copy of one thing.

Cloning creates a genetically identical copy of an animal or plant. Transgenic animal and clone for the study of gene regulation and expression has become commonplace in the modern biological science now. The sheep Dolly was the world's most famous clone animal, but it was not the first one. Many animals - including frogs, mice, sheep and cows had been cloned before Dolly. Plants have been often cloned since ancient people. Human identical twins are also clones. Dolly was the first mammal to be

cloned from an adult cell, rather than an embryo. This was a major scientific achievement of Dolly, but also raised scientific and ethical concerns. Recent years, many various species and cells from which viable somatic cell were cloned offspring have been produced. Production of mammals by nuclear transfer has become a useful tool for propagating valuable animals and can be used as a method to produce genetically modified animals. But, the use of the technology has been limited because of the low survival rate of foetuses during the last trimester of gestation and compromised postnatal health of the offspring. There are many factors for the inefficiencies not fully understood, which may be related to many factors such as the oocyte-donor cell interaction, the stage of the donor cell cycle, inadequate placentation, inappropriate or incomplete nuclear reprogramming following nuclear transfer, and the type of donor cell used. Now, the high rate of fetal loss in the third trimester and the increased calf loss in the first month of life in clones compared with conventional pregnancies and calves are primary limitations for the widespread application of this technology.

The stage of the donor cell cycle is a major factor in the success of nuclear transfer in mammals. Quiescent donor cells arrested in the G0 or G1 stage of the cell cycle have been used to produce cattle, pigs, mice, and sheep. Methods of arresting cells in this phase of the cell cycle have been explored using reversible cycle inhibitors. However, serum starvation is often used as a donor cell treatment prior to nuclear transfer.

Gene therapy can be defined as the deliberate transfer of DNA for therapeutic purposes. Many serious diseases such as the tragic mental and physical handicaps caused by some genetic metabolic disorders may healed by gene transfer protocol. Gene transfer is one of the key factors in gene therapy, and it is one of the key purposes of the clone.

Somatic cell nuclear transfer

1. **Cell preparation and culture:**

A primary cell line is isolated from a mature animal such as a sheep. Isolated cells are washed in Dulbecco minimal essential medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 1% (v/v) penicillin/streptomycin, and the cells are seeded into six-well culture plates following typical cell culture techniques.

2. **Oocyte preparation and nuclear transfer:**

Recipient oocytes are washed and selected after they are removed from animal. Only oocytes that have a homogenous cytoplasm and at least three layers of cumulus cells are selected for in vitro maturation. Selected oocytes are incubated; oocytes are vortexed after maturation to remove expanded cumulus cells. Donor cells (one per oocyte) are microsurgically placed into the perivitelline space evacuated during enucleation, ensuring intimate contact between the donor cell and the recipient oocyte.

3. **Nuclear transfer unit fusion and activation:**

The donor cell and recipient cytoplasm of the nuclear transfer couplets are fused approximately 22–24 hours post maturation by a single direct electrical pulse (40 V) delivered through needle-type electrodes.

4. **Embryo transfer:**

Embryos that reaches the blastocyst stage are transferred into recipient animals approximately 7 days after synchronized estrus. One or two embryos per recipient are non-surgically introduced into the uterine horn ipsilateral to the ovary containing a palpable corpus luteum.

5. **Calving:**

Calving is done with a standing surgical route on approximately day 272 of gestation through cesarean section.

Other methods:

The Roslin Technique

The Roslin Technique is a variation of somatic cell nuclear transfer that was developed by researchers at the Roslin Institute. The researchers used this method to create Dolly. In this process, somatic cells (with nuclei intact) are allowed to grow and divide and are then deprived of nutrients to induce the cells into a suspended or dormant stage. An egg cell that has had its nucleus removed is then placed in close proximity to a somatic cell and both cells are shocked with an electrical pulse. The cells fuse and the egg is allowed to develop into an embryo. The embryo is then implanted into a surrogate.

The Honolulu Technique

The Honolulu Technique was developed at the University of Hawaii. In this method, the nucleus from a somatic cell is removed and injected into an egg that has had its nucleus removed. The egg is bathed in a chemical solution and cultured. The developing embryo is then implanted into a surrogate and allowed to develop.

Artificial Twinning

While the previously mentioned techniques involve somatic cell nuclear transfer, artificial twinning does not. Artificial twinning involves fertilization of a female gamete (egg) and separation of resulting embryonic cells in the early stages of development. Each separated cell continues to grow and can be implanted into a surrogate. These developing embryos mature, eventually forming separate individuals. All of these individuals are genetically identical, as they were originally separated from a single embryo. This process is similar to what happens in the development of natural identical twins.

IN VITRO FERTILIZATION

IVFET can be used to overcome infertility caused by numerous conditions including tubal disease, endometriosis and oligospermia. A first step in IVFET is to prepare the woman for removal of eggs (oocytes). Two methods are used to accomplish this. Sometimes oocytes can be obtained during a natural cycle of a woman by determining the time of the marked increase in the luteinizing hormone level in the blood, which precedes ovulation by about 1.5 days. Using a natural cycle, however, frequent blood samples must be analyzed to exactly pinpoint the increase in this hormone level. Only one mature egg is usually extracted by this method. Alternatively, follicular growth and maturation, which leads to ovulation, can be induced by the use of various fertility drugs such as human menopausal gonadotrophin. The subsequent development of ovarian follicles can be monitored by ultrasound and by measuring blood oestrogen levels. By this method, which is most commonly used today, more than one oocyte is stimulated to develop and can be obtained for fertilization.

Just before the timed ovulation would occur, oocytes are removed from the ovary either by laparoscopy or by needle aspiration guided by ultrasonography. The eggs, with their adherent nurse cells, are placed in a petri dish so that their state of maturation can be assessed using the state of dispersion of the attached cells as a marker. Fertilization of the mature egg is accomplished by incubation for approximately 24 hours in the petri dish with washed sperm that have been treated to ensure capacitation. Fertilization is defined by the visible presence of two pronuclei in the newly formed zygote.

The first cleavage of the zygote occurs approximately 1.5 days after insemination. A catheter is used to transfer the dividing embryo into the lumen of the uterus between the 2- and 16-cell stage. To supplement the natural luteal phase, hormones such as progesterone are sometimes administered after transfer of the embryo, (or embryos if more than one oocyte has been fertilized) to the uterus. Pregnancy is established when the developing embryo implants itself into the wall of the uterus. Implantation can be documented by a measured increase in blood levels of human chorionic gonadotrophin.

Sometimes, a greater number of mature eggs are harvested than can usefully be implanted. Increasingly, these excess eggs are fertilized and preserved by cryopreservation for subsequent use.

Sometimes the donation of spermatozoa, eggs, or in some cases fertilized zygotes, are necessary. Excess eggs collected from one female donor patient undergoing IVFET can be fertilized and implanted in a recipient uterus which has been synchronized with the donor's cycle. Artificial insemination using donor spermatozoa is a common technique. This technique can be used to improve breeds of livestock, preserve biodiversity and treat infertility.

Embryo transfer is most commonly performed after 72 hours (day 3 post retrieval). 'Blastocyst transfer' is generally performed at 120 hours (day 5 post retrieval). Blastocyst transfer will be detailed below; the principal advantage of blastocyst transfer is the replacement of fewer embryos (generally 1–2), given their apparent higher implantation potential. Transfer of fewer day 3 embryos reduces the incidence of higher order and twin multiple gestations.

The objective of embryo transfer is to maximize the chance for pregnancy while limiting the number of multiple gestations. Both of these outcomes are directly correlated with the number of pre-embryos transferred. The optimal number of embryos to transfer is individualized, based on the individual's expected implantation rate per embryo. Maternal age and embryo quality are important factors determining the implantation potential for each embryo.

EMBRYO TRANSFER

'Embryo transfer' refers to the transplantation of a mammalian preimplantation embryo into the reproductive tract of a recipient female so that it may implant and continue to develop to birth. Mammalian embryos of many species can develop in vitro from fertilization to the blastocyst stage (approximately 100 cells), but at this point they must implant in the uterus in order for embryogenesis to proceed normally. For this reason, the ability to produce live young, or even mid-term fetuses, from isolated preimplantation embryos depended historically on the development of embryo transfer techniques. The first successful embryo transfer was performed in 1890 in the rabbit.

The embryo transfer process is similar to the process for a pap smear. The doctor will insert a speculum into the woman's vagina to keep the vaginal walls open. Using ultrasound for accuracy, the doctor will then pass a catheter through the cervix and into the womb. From there, the embryos are passed through the tube and into the womb. The process is usually pain free and rarely requires any sedatives. Some women may feel discomfort as a result of having the speculum inserted or from having a full bladder, which is required for ultrasound. The process is short, and the bladder can be emptied immediately after.

Fresh embryo transfer: Once eggs have been fertilized, they are cultured for 1-2 days. The best embryos are chosen to transfer directly to the woman's uterus.

Frozen embryo transfer: Any healthy embryos that were not used in the first transfer can be frozen and stored for future use. These can be thawed and transferred to the uterus.

Blastocyst embryo transfer: If many healthy embryos develop after the fertilization, it is common to wait to see if the embryos develop into blastocysts. Blastocyst embryo transfer has a higher success rate than the standard embryo transfer on day 3. However, it may pose risks later in pregnancy and should not always be recommended.

RIBOZYME TECHNOLOGY

Ribozymes (or RNA enzymes) are catalytic RNA capable of cleaving target RNA molecules in a sequence-specific manner. Ribozymes were discovered in the early 1980s in two different systems, *Tetrahymena thermophila* and ribonuclease P of *Escherichia coli*, as self-cleaving molecules, lending support to the notion that current biologic systems evolved from a so-called ‘‘RNA world’’. Subsequently, other catalytic RNA were discovered in plant viruses that could be used to cleave a target RNA in transcription. These included the hammerhead ribozyme, identified in the virusoid from lucerne transient streak virus, the hairpin ribozyme, identified from the ‘‘minus’’ strand of satellite RNA of tobacco ringspot virus, and the hepatitis delta ribozyme.

ISSUES IN RIBOZYME DESIGN AND DELIVERY

One of the central issues in ensuring the success of a ribozyme based experiment is the proper selection of the target gene and the optimal design of the ribozyme. The selected target gene is usually prominently involved in producing the phenotype of interest; however, a major issue that must be considered in the design of a ribozyme experiment includes knowledge of the half-life of the target message and protein. The more stable the target gene and protein, the more prolonged inhibition may be necessary to produce the desired suppression of expression and change in biologic phenotype. Thus, many target genes may not be susceptible to transient transfections and may require prolonged expression, which in in vitro studies entails the use of stable transformants expressing the ribozyme under antibiotic selection.

The selection of the specific sequence within the target gene is another issue that deserves attention in the design of a ribozyme. In short, this differs from target to target and must be tested with the design of each new molecule, as ribozymes have been successfully designed to target the 50 end of the mRNA, the 30 untranslated region, or regions in between. A major determinant of an active ribozyme is accessibility of the target RNA, which is greatly reduced by double-stranded regions of folded single stranded RNA and sites of interaction with RNA-binding proteins.

HUMAN GENOME PROJECT

The Human Genome Project (HGP) was one of the great feats of exploration in history - an inward voyage of discovery rather than an outward exploration of the planet or the cosmos; an international research effort to sequence and map all of the genes - together known as the genome - of members of our species, Homo sapiens. Completed in April 2003, the HGP gave us the ability, for the first time, to read nature's complete genetic blueprint for building a human being.

The Human Genome Project (HGP) was the international, collaborative research program whose goal was the complete mapping and understanding of all the genes of human beings. All our genes together are known as our "genome." The HGP was the natural culmination of the history of genetics research. In 1911, Alfred Sturtevant, then an undergraduate researcher in the laboratory of Thomas Hunt Morgan, realized that he could - and had to, in order to manage his data - map the locations of the fruit fly (Drosophila melanogaster) genes whose mutations the Morgan laboratory was tracking over generations. This culminated in HGP later.

HGP researchers have deciphered the human genome in three major ways: determining the order, or "sequence," of all the bases in our genome's DNA; making maps that show the locations of genes for major sections of all our chromosomes; and producing what are called linkage maps, complex versions of the type originated in early Drosophila research, through which inherited traits (such as those for genetic disease) can be tracked over generations.

The HGP has revealed that there are probably about 20,500 human genes. The completed human sequence can now identify their locations. This ultimate product of the HGP has given the world a resource of detailed information about the structure, organization and function of the complete set of human genes. This information can be thought of as the basic set of inheritable "instructions" for the development and function of a human being.

The International Human Genome Sequencing Consortium published the first draft of the human genome in the journal Nature in February 2001 with the sequence of the entire genome's three billion base pairs some 90 percent complete. A startling finding of this first draft was that the number of human genes appeared to be significantly fewer than previous estimates, which ranged from 50,000 genes to as many as 140,000.The full sequence was completed and published in April 2003.

Upon publication of the majority of the genome in February 2001, Francis Collins, the director of NHGRI, noted that the genome could be thought of in terms of a book with multiple uses: "It's a history book - a narrative of the journey of our species through time. It's a shop manual, with an incredibly detailed blueprint for building every human cell. And it's a transformative textbook of medicine, with insights that will give health care providers immense new powers to treat, prevent and cure disease."

The tools created through the HGP also continue to inform efforts to characterize the entire genomes of several other organisms used extensively in biological research, such as mice, fruit flies and flatworms. These efforts support each other, because most organisms have many similar, or "homologous," genes with similar functions. Therefore, the identification of the sequence or function of a gene in a model organism, for example, the roundworm C. elegans, has the potential to explain a homologous gene in human beings, or in one of the other model organisms. These ambitious goals required and will continue to demand a variety of new technologies that have made it possible to relatively rapidly construct a first draft of the human genome and to continue to refine that draft. These techniques include:

- DNA Sequencing
- The Employment of Restriction Fragment-Length Polymorphisms (RFLP)
- Yeast Artificial Chromosomes (YAC)
- Bacterial Artificial Chromosomes (BAC)
- The Polymerase Chain Reaction (PCR)
- Electrophoresis

Of course, information is only as good as the ability to use it. Therefore, advanced methods for widely disseminating the information generated by the HGP to scientists, physicians and others, is necessary in order to ensure the most rapid application of research results for the benefit of humanity. Biomedical technology and research are particular beneficiaries of the HGP.

However, the momentous implications for individuals and society for possessing the detailed genetic information made possible by the HGP were recognized from the outset. Another major component of the HGP - and an ongoing component of NHGRI - is therefore devoted to the analysis of the ethical, legal and social implications (ELSI) of our newfound genetic knowledge, and the subsequent development of policy options for public consideration.

PROTEOMICS

Proteomics is the large-scale study of proteins. Proteins are vital parts of living organisms, with many functions. The term proteomics was coined in 1997 in analogy with genomics, the study of the genome. The word proteome is a portmanteau of protein and genome, and was coined by Marc Wilkins in 1994 while he was a PhD student at Macquarie University. Macquarie University also founded the first dedicated proteomics laboratory in 1995.

The proteome is the entire set of proteins that are produced or modified by an organism or system. Proteomics has enabled the identification of ever increasing numbers of protein. This varies with time and distinct requirements, or stresses, that a cell or organism undergoes. Proteomics is an interdisciplinary domain that has benefitted greatly from the genetic information of various genome projects, including the Human Genome Project. It covers the exploration of proteomes from the overall level of protein composition, structure, and activity. It is an important component of functional genomics.

Proteomics generally refers to the large-scale experimental analysis of proteins and proteomes but is often specifically used to refer to protein purification and mass spectrometry.