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The New Genetics: Applications and Implications For Our Future

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Introduction

In 1932, Aldous Huxley authored his famous look into the future entitled *Brave New World*. In this book Huxley predicted many things about the future of mankind and these predictions included the coming of man-made man: the test tube baby. Not only did he predict the coming of the test tube baby but he also predicted that it would take man 600 years to develop the technoloty necessary to create the test tube baby.

In Huxley's 1958 sequel entitled *Brave New World Revisited*, he reflected on his previous predictions. In the Foreword to *Revisited*, Huxley apologized to his readers for his failure to predict one event—perhaps the single greatest event—which had occurred during the preceding 26 years: the advent of atomic energy. However, he went on to make the following prophetic statement:

> The coming of atomic energy marks a great revolution in human history, but not (unless we blow ourselves to bits and so put an end to history) the greatest revolution. The revolutionary revolution will come not in the external world but in the souls and flesh of human beings.

Huxley went on to tell his readers to expect this revolutionary revolution in the world of biology. He also hinted at the rapidity with which this revolutionary revolution would come when he changed his original prediction regarding the timing of the test tube baby from 600 years to 100 years. Science and technology had progressed so far in one-quarter of a century that he was forced to modify his original prediction by five centuries!

If we accept the proposition that, included within Huxley's definition of "test tube baby" is any child which is the result of conception achieved under artificial circumstances in a laboratory environment, then we know that, had Huxley been correct in his 1932 prediction, he would have predicted the test tube baby not 600 years in the future, not 100 years in the future, but 40 years in the future! The first documented and substantiated reports of successful in-vitro fertilization were published in 1972—40 years after the publication of *Brave New World*.

As we approach the end of the 20th century, and gaze ahead into the 21st century, Huxley's prediction of a revolutionary revolution in the biological sciences and particularly in genetic engineering seems to be moving quickly and steadily toward reality. Some experts have observed and calculated that the information base in genetics alone is doubling every 18 months. This creates quite a dilemma because, although it is essential for us to be aware of these advances, it would be a full-time job to keep abreast by reading the scientific periodicals avalable to us. The purpose of this report is to give the reader a quick overview of a few of the exciting new advances in genetics.

Genetic Engineering

Genetic engineering refers to the area of genetics which deals with the shuffling of genetic material within and among organisms. This transfer of genetic material has

even been accomplished from animals to plants, an example of which is the human gene for Human Chorionic Gonadotropin (HCG) being transferred to and expressed in petunias. Now for the first time, we have plants which can produce HCG and can have positive pregnancy test results!

The concept of genetic engineering works because of one single fact: DNA is DNA is DNA. The DNA molecule which is contained in every cell of every organism is the same structure and has the same component parts. The DNA in human cells is the same as the DNA in bacterial cells which is the same as the DNA in grass and trees. Because DNA is DNA, parts of the DNA molecule from one organism can be spliced into the DNA molecule of another.

Recombinant DNA Technology

Recombinant DNA Technology refers specifically to the laboratory manipulation which makes genetic engineering possible. In order for the technology to work, it must be possible to cut DNA apart and paste it back together again. At the molecular level, however, this "cutting" cannot be accomplished with scissors or scalpel under a microscope but must be accomplished with enzymes in the test tube. The enzymes which cut the DNA are called endonucleases because they are able to cut the DNA within (endo) the molecule.

Enzymes are also used to "paste" DNA together. These enzymes are called ligases because they are responsible for the tying together or ligation of DNA parts.

Just as a surgeon must be able to manipulate his scalpel precisely in order to accomplish his task, so a geneticist must be able to cut DNA at specific points. Fortunately, each endonuclease recognizes and cuts only one specific sequence of nucleotide bases contained in the DNA molecules and this allows the geneticist the control necessary to accomplish his task. The "cut-points" in the DNA molecule are designated by the occurrence of palindromes. A palindrome is a sequence of letters, numbers, or nucleotides which reads the same from left to right as from right to left. Examples of palindromes include Hannah, radar, toot, and 100,001. Since DNA is a molecule which consists of a double strand of nucleotides and since these nucleotides always pair up the same way, the palindrome will "switch over" in the middle of the sequence. For instance, the GAA AAG palindrome will actually look like this on the double strand:

---- GAA TTC ----
---- CTT AAG ----

Since each endonuclease enzyme is restricted to a particular palindrome sequence, they are referred to as restriction endonucleases. The EcoR1 enzyme is the endonuclease which recognizes the nucleotide sequence used in the above example. It is interesting to note that the name EcoR1 refers to the enzymes' origin—it was isolated from the bacteria E.coli (Eco)—and the fact that it was the first restriction endonuclease so isolated (R1).

Application

The technology referred to above can be used to make multiple copies or clones of a gene. Many strains of bacteria contain circular molecules of DNA called plasmids. These circles of DNA can be isolated and manipulated in the laboratory. A DNA plasmid from E.coli can be cut open with one of the many restriction nucleases yielding an open circle. A human gene, for example, the gene for the protein insulin, can then be isolated from human white blood cells using the same enzyme. The human insulin gene will insert into the open circular plasmid from E.coli. The new larger circle of DNA is tied together with ligase and is called a recombinant plasmid because we have recombined human DNA with bacterial DNA. The recombinant plasmid can then be placed back into E.coli cells

by the process of transformation. As those cells grow in the laboratory, the human insulin gene will replicate every time the bacterial cell divides. These rapidly growing bacterial cells are actually making copies or clones of the human gene. The copies of the insulin gene can later be isolated from the recombinant plasmids by using the same restriction enzyme. When the E.coli plasmid is carrying the human gene, the plasmid is referred to as a replication vector.

If the human gene is inserted into a bacterial plasmid "downstream" from a promoter or on-off switch, the bacterial promoter can activate the human gene so it can be expressed. That means the bacterial cells which contain the recombinant plasmid can manufacture the human protein, i.e., insulin. The plasmid with the promoter used to express the gene is called an expression vector. This technology is now used by The Eli Lilly Company to produce an unlimited supply of pure insulin synthesized in the laboratory and marketed under the trade name Humulin. Previously insulin for diabetics had to be isolated from the pancreas of pigs and cows. This technology has also been applied to permit the manufacture of human interferon, growth hormone, Factor VIII, tissue plasminogen activator, tumor necroses factor, erythropoietin, and many others.

Some of these genes are too complex for E.coli to handle. For instance, the gene for Factor VIII which makes the protein necessary for the proper clotting of blood is made up of 186,000 nucleotide pairs and has 26 exons. The Factor VIII gene had to be inserted into Chinese hamster cells and was then expressed by the mammalian expression vector, in-vitro.

Another application of the technology is based upon analysis of DNA fragments. If DNA which has been isolated from an individual is mixed in a test tube with a particular restriction enzyme, the DNA will be cut into small fragments. The size of the fragments will depend upon the location of the cut-points or restriction sites on the DNA. Since the number and position of the restriction sites vary from person to person, the lengths of the fragments and the number of fragments will also vary. Once exposed to an endonuclease, an individual's DNA fragments can be separated using the process of electrophoresis. This process will result in an electrophoretic pattern which is unique to that individual.

It has been discovered that some specific electrophoretic patterns may be associated with a genetic disease. By using restriction fragment length polymorphisms (RFLPs) in some families it is possible to make presymptomatic diagnoses. For instance, Huntington's Disease (HD) is a slow, progressive, physical and mental deterioration of the central nervous system. It is caused by an autosomal dominant gene and; therefore, it is expected, by chance, to be passed from an affected parent to half of his children. Huntington's, however, may not manifest itself until the affected person is 35 years of age or older. In the past, the children of an individual with HD had to wait with great anxiety to see what would happen to them in mid-life. But today, in some families by using RFLPs, the geneticist can distinguish with great accuracy those young people who carry the HD gene. This can be done by evaluating several family members both normal and those affected with HD. If all of the normal individuals in the family have three fragments but those with HD have four, the extra fragment serves as the RFLP marker for diagnosis. RFLPs can now be used to diagnose numerous genetic conditions in certain families. These conditions include: Duchennes muscular dystrophy, polycystic kidney, cystic fibrosis, neurofibromatosis, and familial polyposis coli.

The new RFLP technology gives rise to many ethical questions: should the child of a parent with HD be tested for the HD gene? If so, when? Should RFLPs be used for prenatal diagnosis? Should an HD fetus be aborted knowing that person will be healthy for 35 years and knowing we may have a cure for HD by that time?

There are some RLFP differences that are so unique that no two individuals, except for identical twins, are the same. These are based upon the natural variation among

repeated DNA sequences within our non-functional (not protein producing) DNA. These fragment size differences are called VNTRs (variable number of tandem repects) and are being used in forensic science. The identification of a rapist can be confirmed by matching the DNA fragments from the suspect to the DNA fragments of the semen found at the crime site. It will be possible to identify a murderer by matching the suspect's DNA to hair, skin or blood left by the murderer at the murder site. In addition, another new procedure, polymerese chain reaction (PCR) permits very small amounts of DNA to be multiplied over a million-fold in a few hours resulting in the quantities of DNA necessary for RFLP analysis.

RFLPs are also the key to the human genome sequencing project. The goal of the project is to work out the entire human DNA sequence for all twenty-four (24) human nuclear chromosomes. The project is expected to take 10-12 years to complete at a cost of about 15 million dollars. The complete human sequence contains an estimated 3 billion nucleotide pairs.

Other discoveries and applications of the recombinant DNA technology can only be mentioned briefly: The discovery that some enzymes are RNA molecules called ribozymes instead of proteins has shattered the old "all enzymes are proteins" rule. Human mitochondrial DNA analysis has led to the realization that we all evolved from a common female ancestor now referred to as "Eve". DNA from an Egyptian mummy 2,400 years old has been isolated and cloned, as has DNA from the brain of a human found in a Florida peat bog that was 8,600 years old. Genetically engineered plants that are frost resistant have received the necessary legal approval for field testing. The T. plasmid, a tumor including plasmid from a bacterium, has been used to carry foreign genes into plants. The RFLP for muscular dystrophy has led to the discovery of the actual gene responsible for the disease and analysis of its product named dystrophin. This technology has also been instrumental in the discovery, analysis, and vaccine development for HIV, the AIDS virus.

As we look to the future, the applications of the genetic engineering technology appear to be infinite. Gene therapy via gene transplantation may soon be possible to correct some human genetic diseases. The therapy will involve obtaining bone marrow cells from the affected individual and isolating the pluripotent stem cells from the marrow. The stem cells will then be infected in a petri plate with a cancer causing virus which has been modified in two ways: the genes necessary for cancer induction have been removed and the gene missing in the affected individual has been inserted. The modified virus therefore serves as a vector to carry the "good" gene into the stem cells. Once the stem cells contain a new normal gene, they are put back into the patient. The new stem cells will give rise to new red and white blood cells which will carry the normal gene and make the missing protein. As the cells are distributed throughout the body they will also distribute the previously missing protein and, in so doing, correct the genetic defect.

Some investigators believe that placing genes into the fertilized egg might be a future possibility. It is already possible to inject the human gene for growth hormone into a fertilized mouse egg and get mice which are twice normal size. Other investigators have injected a cancer causing oncogene into mouse eggs and have produced mice which transmit the oncogene to their progeny. These mice have even been patented. As we look at the future implications of this work it is easy to speculate about using the same procedures to insert a gene into a human egg soon after conception to correct a human gene defect.

As progressive and prophetic as Aldous Huxley was, he did not have an inkling as to the tremendous and far-reaching possibilities of his revolutionary revolution. Today's scientific community has a two-fold responsibility as we look to the future. One. . . . to continue to innovate work in the laboratory and two. . . . to inform the lay public of the upcoming social implications of this work.