RECOMBINANT DNA TECHNOLOGY: APPLICATIONS IN THE FIELD OF BIOTECHNOLOGY AND CRIME SCIENCES

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ABSTRACT

There are four important applications of rDNA in the areas of human diseases prophylaxis, therapy, diagnosis and discovery. Areas of prophylaxis include vaccines and coagulation. It is now possible, through rDNA technology, to produce an effective and safer production of both live and killed vaccines with increase response and high specificity. Recombinant DNA technology approach is the identification of that protein component of virus or microbial pathogen which itself can elicit the production of antibodies having capacity to neutralize infectivity, potentially protecting the host against the pathogen. Such proteins are useful for identification of the gene coding the protein. We will discuss here the major application of recombinant DNA in field of medicine and forensic sciences.

Keywords: recombinant DNA, Tissue plasminogen activator, chemotherapy, Hybridization Probing

INTRODUCTION

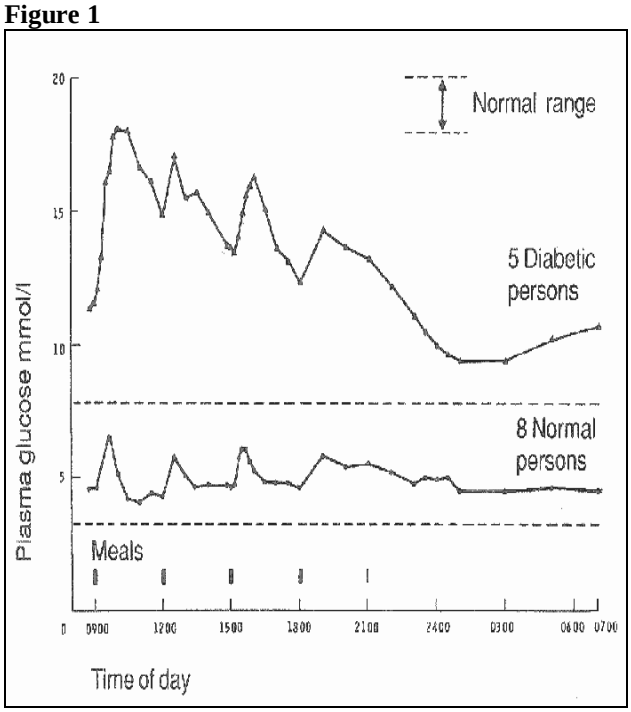
The advances in recombinant DNA technology have occurred in parallel with the development of genetic processes and biological variations. The development of new technologies have resulted into production of large amount of biochemically defined proteins of medical significance and created an enormous potential for pharmaceutical industries. The biochemically derived therapeutics is large extra cellular proteins for use in either chronic replacement therapies or for the treatment of life threatening indications[1, 2].

Applications of rDNA In Medicine: Some recombinant DNA products being used in human therapy: Using procedures like this, many human genes have been cloned in E. coli or in yeast. This has made it possible — for the first time — to produce unlimited amounts of human proteins in vitro. Cultured cells (E. coli, yeast, mammalian cells) transformed with the human gene are being used to manufacture**.**

- Insulin for diabetics
- Factor VIII for males suffering from hemophilia A
- Factor IX for hemophilia B
- Human growth hormone (GH)
- Erythropoietin (EPO) for treating anemia
- Three types of interferons
- Several interleukins
- Granulocyte-macrophage colony-stimulating factor (GM-CSF) for stimulating the bone marrow after a bone marrow transplant
- Granulocyte colony-stimulating factor (G-CSF) for stimulating neutrophil production, e.g., after chemotherapy and for mobilizing hematopoietic stem cells from the bone marrow into the blood.
- Tissue plasminogen activator (TPA) for dissolving blood clots
- Adenosine deaminase (ADA) for treating some forms of severe combined immunodeficiency (SCID)
- Angiostatin and endostatin for trials as anti-cancer drugs
- Parathyroid hormone
- Leptin
- Hepatitis B surface antigen (HBsAg) to vaccinate against the hepatitis B virus

Recombinant DNA Technology in the Synthesis of Human Insulin: The Nature and Purpose of Synthesizing Human Insulin: Since Banting and Best discovered the hormone, insulin in 1921 diabetic patients, whose elevated sugar levels (fig. 1) are due to impaired insulin production, have been treated with insulin derived from the pancreas glands of abattoir animals. The hormone, produced and secreted by the beta cells of the pancreas' islets of Langerhans, regulates the use and storage of food, particularly carbohydrates[3, 4].

Figure 1



Fluctuations in Diabetic Person's Blood Glucose Levels, Compared with Healthy Individuals: Although bovine and porcine insulin are similar to human insulin, their composition is slightly different. Consequently, a number of patients' immune systems produce antibodies against it, neutralizing its actions and resulting in inflammatory responses at injection sites. Added to these adverse effects of bovine and porcine insulin, were fears of long term complications ensuing from the regular injection of a foreign substance, as well as a projected decline in the production of animal derived insulin[5]. These factors led researchers to consider synthesizing *Humulin* by inserting the insulin gene into a suitable vector, the E. coli bacterial cell, to produce insulin that is chemically identical to its naturally produced counterpart. This has been achieved using Recombinant DNA technology. This method (fig. 2) is a more reliable and sustainable method than extracting and purifying the abattoir by-product.

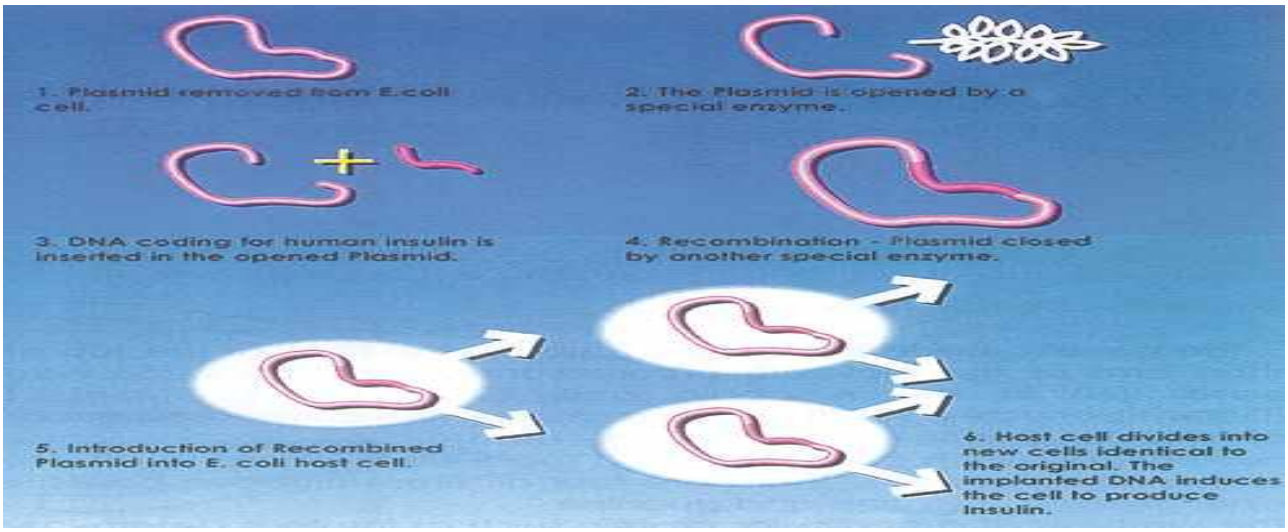


Figure 2 An overview of the recombination process.

Understanding the genetics involved.

The Structure of Insulin: Chemically, insulin is a small, simple protein. It consists of 51 amino acid, 30 of which constitute one polypeptide chain, and 21 of which comprise a second chain. The two chains (fig. 3) are linked by a disulfide bond.

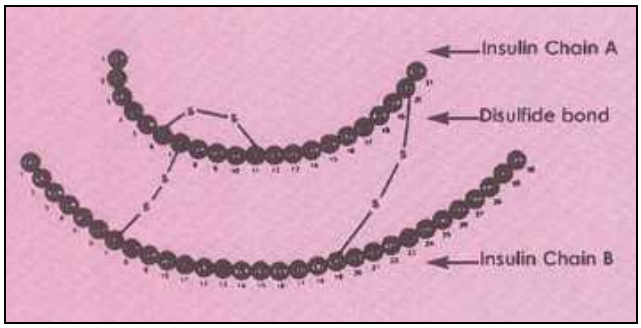


Figure 3

Inside the Double Helix: The genetic code for insulin is found in the DNA at the top of the short arm of the eleventh chromosome. It contains 153 nitrogen bases (63 in the A chain and 90 in the B chain).DNA Deoxyribonucleic Acid), which makes up the chromosome, consists of two long intertwined helices, constructed from a chain of nucleotides, each composed of a sugar deoxyribose, a phosphate and nitrogen base. There are four different nitrogen bases, adenine, thymine, cytosine and guanine the synthesis of a particular protein such as insulin is determined by the sequence in which these bases are repeated[6, 7] (fig. 4).

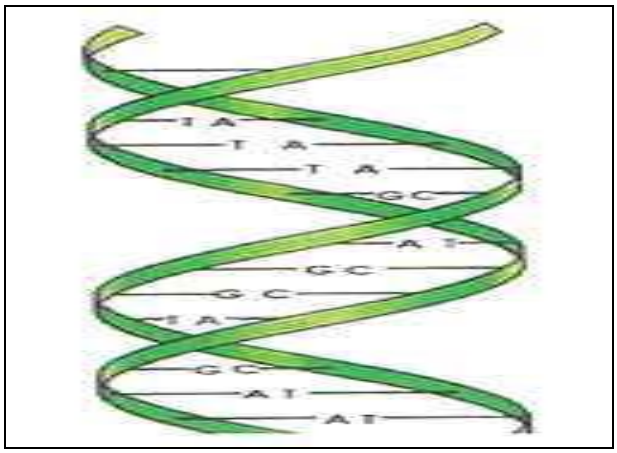


Figure 4

DNA Strand with the Specific Nucleotide Sequence for Insulin Chain B: Insulin synthesis from the genetic code. The double strand of the eleventh chromosome of DNA divides in two; exposing unpaired nitrogen bases which are specific to insulin production (fig. 5).

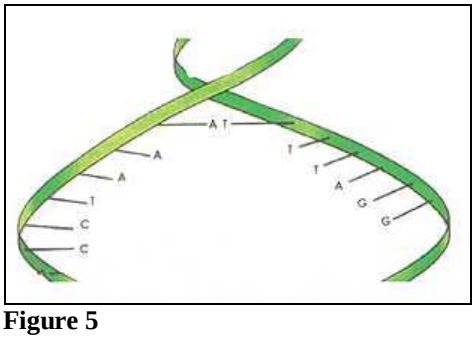


Figure 5

Unraveling strand of the DNA of chromosome 11, with the exposed nucleotides coding for the B chain of Insulin: Using one of the exposed DNA strands (fig.6) as a template, messenger RNA forms in the process of transcription (fig. 7).

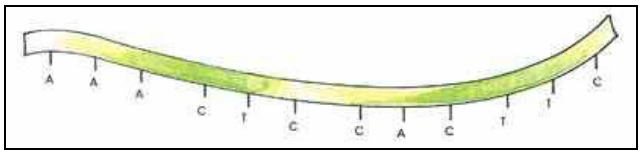


Figure 6 single strand of DNA coding for Insulin chain B.

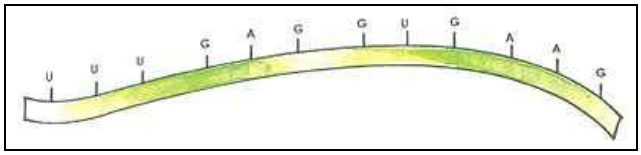


Figure 7

The (m) RNA Strand: The role of the mRNA strand, on which the nitrogen base thymine is replaced by uracil, is to carry genetic information, such as that pertaining to insulin, from the nucleus into the cytoplasm, where it attaches to a ribosome (fig. 8).

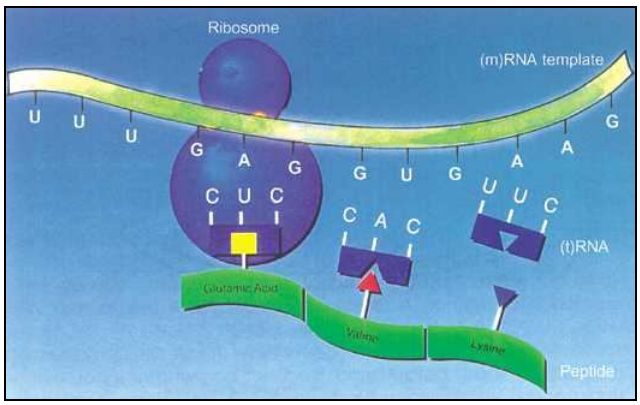


Figure 8

Process of translation at the Ribosome *the nitrogen bases on the mRNA are grouped* into threes, known as codons. Transfer RNA (tRNA) molecules, three unpaired nitrogen bases bound to a specific amino acid, collectively known as an anti-codon (fig.9) pair with complementary bases (the codons) on the mRNA.

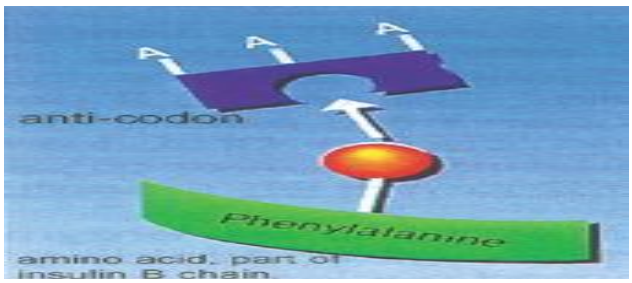


Figure 9

The reading of the mRNA by the tRNA at the ribosome is known as translation. A specific chain of amino acids is formed by the tRNA following the code determined by the mRNA. The base sequence of the mRNA has been translated into an amino acid sequence which link together to form specific proteins such as insulin[4, 8]. The Vector (Gram negative E. coli). A weakened strain of the common bacterium, Escherichia coli (E. coli) (fig. 10), an inhabitant of the human digestive tract, is the 'factory' used in the genetic engineering of insulin.

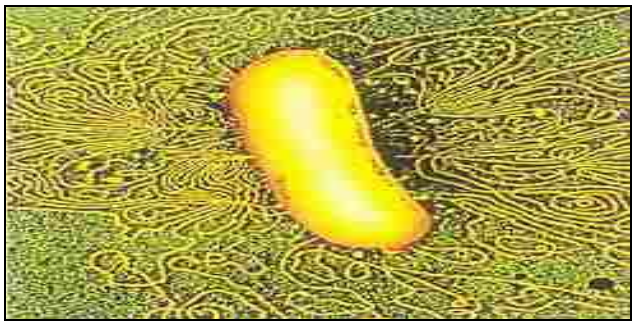


Figure 10

The insulin is introduced into an *E. coli* cell such as this.

When the bacterium reproduces, the insulin gene is replicated along with the plasmid, a circular section of DNA (fig. 11). E. coli produces enzymes that rapidly degrade foreign proteins such as insulin. By using mutant strains that lack these enzymes, the problem is avoided.

Figure 11

Electron Micrograph of the Vector's Plasmid: In E. coli, B-galactosidase is the enzyme that controls the transcription of the genes. To make the bacteria produce insulin, the insulin gene needs to be tied to this enzyme[9].

Inside the genetic engineer's toolbox: Restriction enzymes, naturally produced by bacteria, act like biological scalpels. (fig.12), only recognizing particular stretches of nucleotides, such as the one that code for insulin.

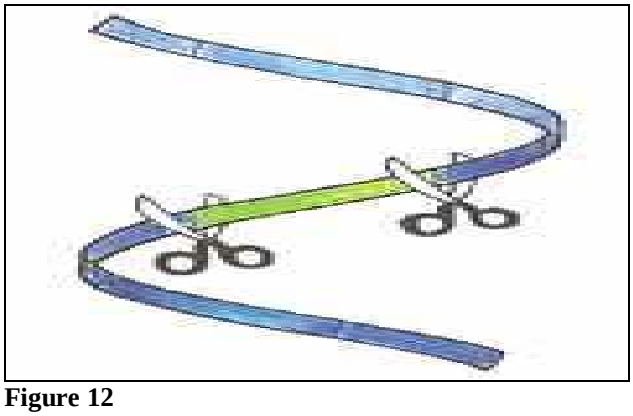


Figure 12

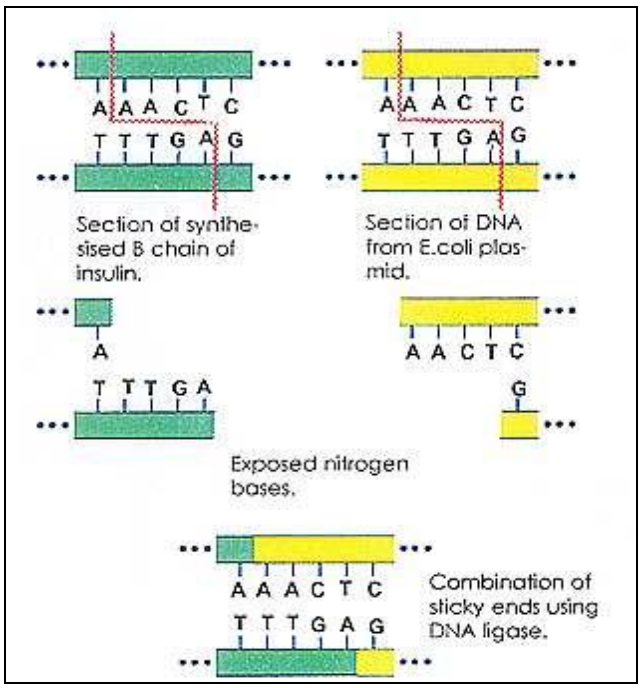
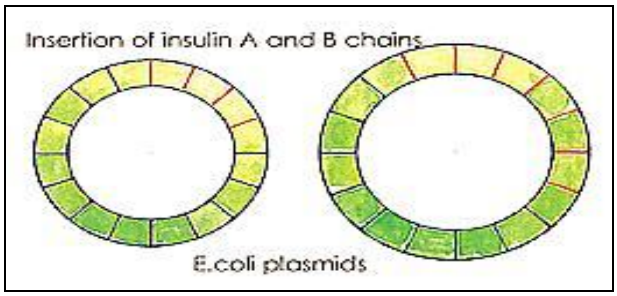


Figure 13

An Analogous Look at Restriction Enzymes: This makes it possible to sever certain nitrogen base pairs and remove the section of insulin coding DNA from one organism's chromosome so that it can manufacture insulin[5, 11] (fig. 13). DNA ligase is an enzyme which serves as a genetic glue, welding the sticky ends of exposed nucleotides together.The first step is to chemically synthesise the DNA chains that carry the specific nucleotide sequences characterizing the A and B polypeptide chains of insulin (fig. 14).

Human Insulin Structure. Amino Acid RNA to DNA Conversion: The required DNA sequence can be determined because the amino acid compositions of both chains have been charted. Sixty three nucleotides are required for synthesizing the A chain and ninety for the B chain, plus a codon at the end of each chain, signaling the termination of protein synthesis. An anti-codon, incorporating the amino acid, methionine, is then placed at the beginning of each chain which allows the removal of the insulin protein from the bacterial cell's amino acids. The synthetic A and B chain 'genes' (fig. 15) are then separately inserted into the gene for a bacterial enzyme, B-galactosidase, which is carried in the vector's plasmid. At this stage, it is crucial to ensure that the codons of the synthetic gene are compatible with those of the B-galactosidase.

Figure 15



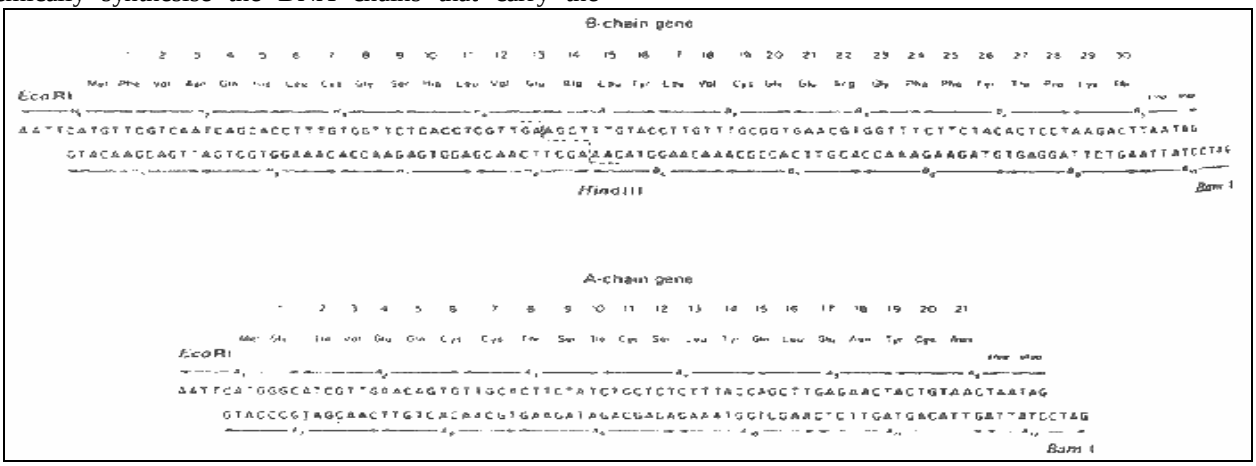


Figure 14

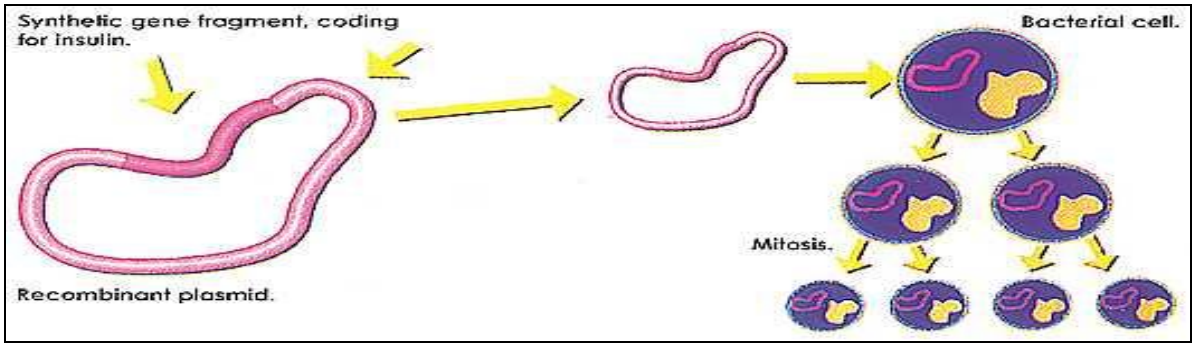


Figure 16

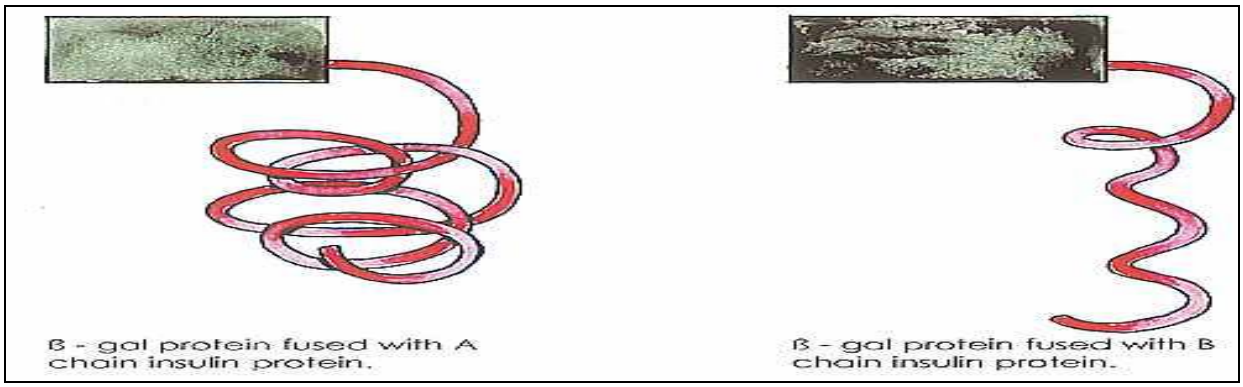


Figure 17

The recombinant plasmids were then introduced into E. coli cells. Practical use of Recombinant DNA technology in the synthesis of human insulin requires millions of copies of the bacteria whose plasmid has been combined with the insulin gene in order to yield insulin. The insulin gene is expressed as it replicates with the B-galactosidase in the cell undergoing mitosis (fig. 16).

The Process of Mitosis: The protein which is formed consists partly of B-galactosidase, joined to either the A or B chain of insulin (fig.17). The A and B chains are then extracted from the B-galactosidase fragment and purified.

The two chains are mixed and reconnected in a reaction that forms the disulfide cross bridges, resulting in pure *Hamelin* - synthetic human insulin (fig. 18).

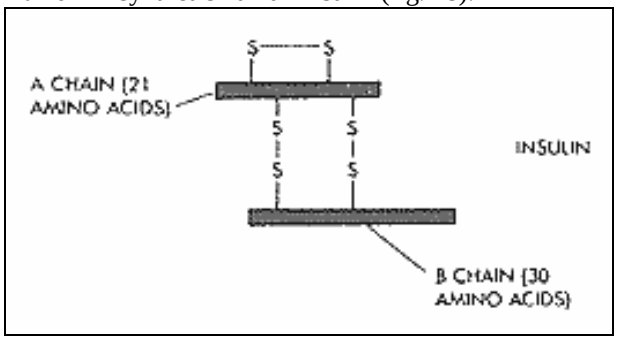


Figure 18

Biological Implications of Genetically Engineered Recombinant Human Insulin: Human insulin is the only animal protein to have been made in bacteria in such a way that its structure is absolutely identical to that of the natural molecule[13, 14]. This reduces the possibility of complications resulting from antibody production. In chemical and pharmacological studies, commercially available Recombinant DNA human insulin has proven indistinguishable from pancreatic human insulin. Initially the major difficulty encountered was the contamination of the final product by the host cells, increasing the risk of contamination in the fermentation broth. This danger was eradicated by the introduction of purification processes. When the final insulin product is subjected to a battery of tests, including the finest radio-immuno assay techniques, no impurities can be detected. The entire procedure is now performed using yeast cells as a growth medium, as they secrete an almost complete human insulin molecule with perfect three dimensional structures. This minimizes the need for complex and costly purification procedures.

The Issue of Hypoglycaemic Complications In The Administration of Human Insulin: Since porcine insulin was phased out, and the majority of insulin dependent patients are now treated with genetically engineered recombinant human insulin, doctors and patients have become concerned about the increase in the number of hypoglycaemic episodes experienced. Although hypoglycaemia can be expected occasionally with any type of insulin, some people with diabetes claim that they are less cognisant of attacks of hypoglycaemia since switching from animal derived insulin to Recombinant DNA human insulin. In a British study, published in the 'Lancet", hypoglycaemia was induced in patients using pork or human insulin, the researchers found "no significant difference in the frequency of signs of hypoglycaemia between users of the two different types of insulin." An anecdotal report from a British patient, who had been insulin dependent for thirty years, stated that she began experiencing recurring, unheralded hypoglycaemia only after substituting Recombinant DNA human insulin for animal derived insulin. After switching back to pork insulin to ease her mind, she hadn't experienced any unannounced hypoglycaemia. Eli Lilly and Co., a manufacturer of human insulin, noted that a third of people with diabetes, who have been insulin dependent for over ten years[10, 15], "lose their hypoglycaemic warning signals, regardless of the type of insulin they are taking." Dr Simon P. Wolff of the University College of London said in an issue of *Nature*, "As far as I can make out, there's no fault (with the human insulin)." He concluded,"I do think we need to have a study to examine the possible risk."

Although the production of human insulin is unarguable welcomed by the majority of insulin dependent patients, the existence of a minority of diabetics who are unhappy with the product cannot be ignored. Although not a new drug, the insulin derived from this new method of production must continue to be studied and evaluated, to ensure that all its users have the opportunity to enjoy a complication free existence.

Application of rDNA In Forensic Sciences: The applications of molecular biology in forensics center largely on the ability of DNA analysis to identify an individual from hairs, blood strains and other items recovered from the crime scene.

In popular media, these techniques are called genetic fingerprinting, though the more accurate term for the procedures used today is DNA profiling. As well as

identification of criminals, DNA profiling can also be used to infer if two or more individuals are members of the same family. This type of study is called kinship analysis and its main day-to-day application is in paternity testing.

DNA Analysis in the Identification of Crime Suspects: It is probably impossible for a person to commit a crime without leaving behind a trace of his or her DNA. Hairs, spots of blood and even conventional fingerprints contain traces of DNA, enough to be studied by the polymerase chain reaction (PCR). The analysis does not have to be done immediately and in recent years a number of past crimes have been solved and the criminal brought to justice because of DNA testing that has been carried out on archived material[4, 15, and 16]. The basis of genetic fingerprinting and DNA profiling is that identical twins are the only individuals who have identical copies of the human genome. The human genome is more or less the same in everybody - the same genes will be in the same order with the same stretches of intergenic DNA between them. But the human genome, as well as those of other organisms, contains polymorphisms, positions where the nucleotide sequence is not the same in every member of the population. The polymorphic sites which are used as DNA markers in genome mapping includes restriction fragment length polymorphisms (RFLPs), short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs). All three can occur within genes as well as in intergenic regions, and altogether there are several million of these polymeric sites I the human genome, with SNPs being the most common.

Genetic Fingerprinting by Hybridization Probing: This is first method for using DNA analysis to identify individuals. This technique was not based on any of the types of polymorphic site, but on different kind of variation in the human genome called a hyper variable dispersed repetitive sequence. As the name indicates this is a repeated sequence that occurs at various places (dispersed) in the human genome. The key feature of these sequences is that their genomic positions are variable: they are located at different positions in the genomes of different people (fig 1). To prepare a fingerprint a sample of DNA is digested with a restriction endonuclease and the fragments separated by agarose gel electrophoresis and a Southern blot prepared. Hybridization to the blot of a labeled probe containing the repeat sequence reveals a series of bands each one representing a restriction fragment that contains the repeat (fig 2). Because the insertion sites of the repeat sequence are variable, the same procedure carried out with a DNA sample from a second person will give a different pattern of bands. These are the genetic fingerprints for those individuals.

DNA Profiling By PCR of Short Tandem Repeats: The more powerful technique of DNA profiling avoids the limitations of hybridization analysis of dispersed repeat sequences. Profiling makes use of polymorphic sequences called STRs. STR is short sequence, one to 13 nucleotides in length that is repeated several times in a tandem array. In human genome, the most common type of STR is the dinucleotide repeat [CA]n where 'n', the number of repeats, is usually between 5 and 20.(fig 3). The number of repeats in a particular STR is variable because repeats can be added or, less frequently[17], removed by errors that occur during DNA replication. In the population as a whole, there might be as many as ten different versions of a particular STR, each of the alleles characterized by a different number of repeats. In DNA profiling the alleles of a selected number of different STRs are determined. This can be achieved quickly and with very small amounts of DNA by PCRs with primers that anneal to the DNA sequences either side of a repeat. After the PCR, the products are examined by agarose gel electrophoresis with the size of the band or bands indicating the allele or alleles present in the DNA sample that has been tested (fig4). Two alleles of an STR can be present in a single DNA sample because there are two copies of each STR, one on the chromosome inherited from the mother and one on the chromosome from the father. Because PCR is used, DNA profiling is very sensitive and enables results to be obtained with hairs and other specimens that contain just trace amounts of DNA. The results are unambiguous, and a match between DNA profiles is usually accepted as evidence in a trial.

Studying Kinship by DNA Profiling

Related Individuals Have Similar DNA Profiles: Your DNA profile like all other aspects of your genome is inherited partly from your mother and partly from your father. Relationships within a family therefore become apparent when the alleles of a particular STR are marked on the family pedigree (fig 5). In this example, we see that three of the four children have inherited the 12-repeat allele from the father. This observation in itself is not sufficient to deduce that these three children are siblings, though the statistical chance would be quite high if the 12-repeat allele was uncommon in the population as whole. To increase the degree of certainty, more STRs would have to be typed but, as with identification of individuals, the analysis need not be endless, because a comparison of nine STRs gives an acceptable probability those relationships that are observed a real.

DNA Profiling and the Remains of the Romanovs: An interesting example of the use of DNA profiling in a kinship study is provided by work carried out during 1990s on the bones of the Romanovs, the last members of the Russian ruling family. Tsar Nicholas II was deposed at the time of the Russian Revolution and he and his wife, the Tsarina Alexandra, and their five children were imprisoned. Then all seven were killed along with their doctor and various servants. Later after the fall of communism, the bodies were recovered from their roadside grave.

STR Analysis of the Romanov Bones: The bodies that were recovered were little more than a collection of bones, those of adults and children intermingled with no indications as to which belonged to the Romanovs and which to their doctor and servant. However, the only juvenile bones among the collection should have belonged to the children of the Tsar and Tsarina. This means that the bones of the Tsar and Tsarina could be identified by establishing which of the adults could be the parents of the children. DNA was extracted from the bones from each individual and five STRs typed by PCR. In fact just two of these STRs provided sufficient information for the male

and female parents of the children to be identified unambiguously (fig 6). But were these indeed the bones of the Romanovs or could they be the remains some other unfortunate group of people? To address this problem the DNA from the bones was compared with DNA samples from living relatives of the Romanovs. This work included studies of mitochondrial DNA, the small 16kb circles of DNA contained in the energy-generating mitochondria of cells. These mitochondrial DNA studies showed that the bones were indeed those of Tsar Nicholas, Tsarina, Alexandra and three of their daughters.

Missing Children: Only three children were found in the Romanovs grave. Alexei, the only boy, and one of the four girls were missing[15, 17]. During the middle decades of the twentieth century several women claimed to be a Romanov princess, because even before the bones were recovered there had been rumors that one of the girls, Anastasia, had escaped the clutches of the Bolsheviks and fled to West. Regrettably, DNA testing has shown that none of these claimants could have been a daughter of the Tsar and Tsarina.

Sex Identification by DNA Analysis: DNA analysis can also be used to identify the sex of an individual. The genetic difference between the sexes is the possession of a Y chromosome by males, so detection of DNA specific for the Y chromosome would enable males and females to be distinguished. DNA tests can also be used to identify the sex of an unborn child. Finding out if a fetus is a boy or a girl is usually delayed until the anatomical differences have developed and the sex can be identified by scanning, but under some circumstances an earlier indication of sex is desirable. An example is when pedigree of the family indicates that an unborn male might suffer from an inherited disease and the parents wish to make an early decision about whether to continue the pregnancy. A third application of DNA-based sex identification and the one that has been responsible for many of the developments in this field is in the analysis of archaeological specimens[18]. Male and female skeletons can be distinguished if key bones such as the skull or the pelvis are intact, but with fragmentary remains, or those of young children, there are not enough sex-specific anatomical differences for a confident identification to be made. If ancient DNA is preserved in the bones a DNA-based method can tell the archaeologists if they are dealing with a male or a female.

CONCLUSION

Recombinant DNA is artificially created from two or more DNA incorporated into a single molecule. Genetic engineering, recombinant DNA technology, genetic modification/manipulation and gene splicing are terms that are applied to the direct manipulation of an organism's gene. The development of these new technologies have resulted into production of large amount of biochemically defined proteins of medical significance and created an enormous potential for pharmaceutical industries. The biochemically derived therapeutics is large extra cellular proteins for use in either chronic replacement therapies or for the treatment of life threatening indications. Recombinant DNA technology has also an important role in forensic science in identification of criminals, DNA profiling to study kinship analysis and in paternity testing.

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