DEVELOPMENTS IN RECOMBINANT DNA TECHNIQUES

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ABSTRACT

Since the day Oswald Avery and his group in 1943 proved that DNA is the genetic material a new era started in the field of biological sciences. The research efforts that have gone in and the developments that have been made since then in the field of molecular genetics have been colossal and mind-boggling.

The monumental research in the basic science of life processes within the cell led to the first successful cloning of a foreign gene in Escherichia coli by Boyer, Cohen and Chang in 1973. This was a turning point. Since, then several thousands of genes from any conceivable source, from virus to man, have been successfully cloned and expressed.

Genetic engineering became a technological reality when a human gene coding for a 14 amino acid peptide neurotransmitter, somatostatin was cloned in E.coli and expressed, in 1976, when the first genetic engineering company, Genentech was formed in the USA and when the recombinant DNA-produced insulin, Humulin went to market in 1982. Now, several value-added products such as hormones, life saving drugs, vaccines, enzymes and other proteins are produced by recombinant DNA technology. Cloning, expression and secretion of human and other mammalian gene products in yeast, Saccharomyces cerevisiae have become a routine practice. Several human growth factors, alpha-interferon, beta-endorphin, somatostatin, prochymosin, urinary plasminogen activator, alpha-galactosidase, pancreatic phospholipase A2, interleukin-3 and HIV1 and HIV2 proteases are some of them.

Development of an experimental technique in mid-1980s by Kary Mullis revolutionized molecular genetics. This was polymerase chain reaction technique (PCR) which made possible a whole new approach to the study and analysis of genes. PCR enabled the researchers to produce enormous numbers of copies of any specific DNA fragment without resorting to cloning and amplification. E.coli DNA polymerase which was used earlier for this purpose, which is heat-sensitive and had to be added repeatedly, was soon

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replaced by Taq polymerase. Taq polymerase derived from a bacterium, Thermus aquaticus which was isolated from a hot spring (75°C) has a temperature optimum of 72°C and is reasonably stable even at 94°C. Single addition of this enzyme is sufficient for the complete set of amplification cycles which enabled the automation of the PCR process. Several companies are now manufacturing thermal cyclers which have become a boon to genetic engineers.

Another recent development in the areas of molecular genetics is protein engineering which has opened up new vistas. This promises unlimited potential to provide tremendous advances in science, medicine and industry. Protein engineering basically involves creation of mutant proteins with desired properties. The necessary steps involved are identification of the native protein of interest, cloning and expressing it and characterization of the protein by a variety of analytical methods to understand the functional domain of the molecule. Once the active sites are identified, the protein engineer makes alterations in these sites and studies the new properties. The desired property may be improvement in stability, catalytic activity, receptor binding, specificity, pharmaco kinetic properties or immunogenicity or a combination of one or more of these. Basic knowledge of in vivo synthesis of protein in prokaryotes and eukaryotes, and its primary, secondary, tertiary and quarternary (if any) structures is essential for a protein engineer.

Oligonucleotide directed site-specific mutagenic techniques are used to bring about specific mutations in the desired site of the target DNA. Single stranded DNA phage, M13 was used earlier for this purpose. But with the advent of the efficient PCR technique the work has become easy. Oligonucleotide (generally called oligos) fragments complementary to the target DNA but having the required base changes can be synthesized easily and used for this purpose. Detailed techniques with examples will be described.