GENETIC ENGINEERING: PROBLEM, PERSPECTIVE AND PROPOSITION

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A major breakthrough in nucleic acid research has not been sudden but rather slow. After the discovery of the nucleic acid, it took a long time to realise its importance in cellular function. The involvement of DNA and RNA in heredity and the establishment of double helical structure of DNA in 1950s and the proposition of central dogma *i.e.* the information flows from DNA to RNA to protein along with decoding of information in DNA accelerated the progress in nucleic acid research tremendously in the recent past. Thus molecular biology has now entered into an unprecedented activity and excitement and a revolution in biology has really occurred. Two related technologies, gene cloning and rapid nucleic acid sequencing have opened up new vistas in research in biology. The current technology of this revolution in biology has its origin in bacterial genetics and enzymology. The genetic engineering technology derives from and depends upon the elegant manipulation of bacterial genetics.

Current trends of research in the field of Gene regulation and cloning

The most unexpected revelation with eukaryotic gene is that the gene is often not collinear with its product *i.e.*, protein. Instead, genes contain intervening sequences (introns) which interrupt the continuity of the genetic information. What has emerged is that a gene transcribing an mRNA is much larger than is required for the nucleotide sequence in mRNA. The mRNA coding for protein is again much larger than is required for the amino acid sequence in protein. Thus much more information in the gene is necessary coding for the final product of protein. The β-haemoglobin gene in the mouse, for example, contains two intervening sequences of 116 and 646 base pairs. The coding sequence is 432 base pairs. There are several similar examples. Thus transcription of these genes entails only exon sequences with intron sequences eliminated from the final transcript. So, similar to compaction of genes *i.e.*, use of a single gene to code for two different proteins in certain bacteriophages and prokaryotes, this split gene concept emerges from the studies with eukaryotes and animal viruses. Thus altogether new enzymatic reaction, splicing, is required for the expression of genes. Thus splicing activity may have a ribosome-like structure involving a complex of structural proteins and RNAs with catalytic and specificity functions.

Another point that emerges is that the split message might help to produce variants of a single protein by different splicing of the interrupted RNA. It might be specifically applicable in the case of production of immunoglobulins. A relation between exons and protein functional units appears to have been established in lysozyme and haemoglobin. There are two important differences between the genetic signals necessary for gene expression in prokaryotes and eukaryotes (i) initiation signal for transcription and (ii) mRNA sequences at the 5' end necessary for translation into protein by ribosomes. A few base (3–12 bases) sequences known as Shine-Dalgarno sequences (SD) occur at the 5' end of prokaryotic mRNA, are complementary to 3' end of 16s ribosomal RNA and this complementarity appears to play a role in stabilising initiation complex between mRNA and ribosomes. mRNAs lacking this SD sequences are not efficiently translated by the *E. coli* cells. Thus manipulation by inserting right sequences upstream for a promoter for the proper expression of the desired gene is becoming a focal point in the case of an expression of eukaryotic genes in bacteria. Similarly if the developmental his-

tory of a gene is important, it may account for the relative inactivity of a mammalian gene (say for example globin gene) cloned in a bacteria and then transferred to mammalian cells[1].

For sometime it has been known that genomes are not static but that there is considerable scope for the movement of blocks of genetic information. Within the past decade or so, however, it has become increasingly apparent that there are various illegitimate recombinational processes which can join together DNA segments having little or no homology. Such processes play a significant role in the organization of genetic information and the regulation of this expression. This type of recombination is affected by transposable genetic elements, structurally and genetically discrete segments of DNA that have the ability to move around among the chromosomes. Although transposable elements have been studied largely in bacteria they were originally discovered in plants and are now known to exist in animals as well. Similarly the mechanism of transposable antibiotic resistance genes in bacteria may be analogous to the movement of transposable elements in yeast and in Drosophila, to the integration of RNA tumour viruses and to the mechanism of expression of immunoglobulin genes[2].

The most precise of the mutagenic strategies involves the use of synthetic oligonucleotide to direct the mutation. Not only in the case of mutation, but also the application of oligonucleotides of defined sequence has been found to be fruitfully utilized to optimise the expression of certain cloned gene if joined upstream of a promoter of a gene in a bacterium.

All these novel findings in the field of molecular biology revealed that the organisms developed, through the ages, certain ways to survive and at present the whole technology is at the hands of scientists and technologists for the benefit of mankind. This technology is now named as genetic engineering or recombinant DNA technology. This in essence entails isolation of a particular gene, hooking of this gene to a specific vector (plasmids or viral DNA) by the enzymes and then to transform the cell (either bacteria or animal or plant cells) so that the particular gene which was not present there might be established. Subsequent trimming and splicing of that gene in the cell is necessary for proper and sustained expression of that gene in the environment where it is finally put to. There is tremendous outcome of this technology in different fields of applied implications, such as (i) drugs or chemicals (ii) crop improvements (iii) energy or fuel (iv) pollution.

I. WHAT HAS ALREADY BEEN ACHIEVED IN THE FIELD OF GENETIC ENGINEERING

There are several ways by which DNA molecule can be cut and spliced or otherwise altered. The purpose of this article is not to go into details of these methodologies. The engineered DNA thus obtained must be integrated into a system of production. It then becomes a component of an industrial or otherwise useful process. The process that is central to the economic success of biotechnology has been around for centuries. It is fermentation, essentially the process used to make wine and beer. It can also produce organic chemicals including drugs using microorganism or their enzymes. Civilization actually started with the process of fermentation. Genetic engineering is not in itself an industry but a technology used at the laboratory level. It allows the worker to alter the hereditary apparatus of a living cell so that the cell can produce more of different chemicals or perform completely new functions. With this objective in mind the technology of Genetic engineering has already been applied to solve certain problems related to (i) drugs or chemicals (ii) crop improvements (agriculture) (iii) energy or fuel and (iv) pollution.

i) *Drugs or chemicals*

Genetic engineering technology introduced a revolutionary way of manufacturing biologically functional proteins such as interferons, antibodies, blood-clotting factors, insulin, growth hormones and host of other pharmocologically important compounds by fermentation. Since production of these compounds is stringently regulated in the animal cells, these

are available only in very small amounts from those tissues. Recently, insulin, interferon and somatostatin production from bacteria has given a lead in manufacturing animal polypeptide drugs through fermentation processes.

Another potential area in this is the production of vaccines. A number of human diseases such as hepatitis, measles, poliomyelitis, influenza, Burkitt lymphoma are caused by viruses. Specific antibodies are now being raised by hybridoma technique which is also included under Recombinant technology.

Efficient production of antibiotics and also the development of newer hybrid antibiotics by means of genetic engineering has already been attempted at with promising results. Human leukocyte interferon cloned in bacteria, and the testing of product out of it is in progress. A potent hybrid interferon is produced. Interferon A and D, which have 165 and 166 amino acids respectively and differ in 29 amino acids were halved into four fragments and then spliced in different combinations. Some of the hybrid AD displayed 1000 times more activity than either of its molecular precursors. Insulin produced by bacteria is also now being tested and the results are now in progress.

ii) *Crop improvements (agriculture)*

Plant cell culture and the production of whole plant from a single cell or fused cells have revolutionized the production of flowers of choice. In some of the crop plants attempts are now being made to produce hybrid of suitable variety. However, the cloning of plant genes in bacteria is still limited. Some of the chloroplastic genes RuBP-carboxylase were cloned in bacteria and thus expression was studied successfully. Genes for plant storage proteins were also successfully cloned in bacteria. Nif gene is now being tried to be transferred from bacteria to plant cell. In fact, genetic engineering technology has opened up a new vista in the field of crop improvements and in the near future, vigorous attempts will be made with plants. The possible areas of research which could be undertaken in agriculture during next twenty years will now be discussed.

iii) *Energy or fuel*

It is now advocated that basic fuel and energy carrier of the future should be hydrogen. Hydrogen can be produced by splitting of H_2O molecules (biophotolysis). Helobacterium is a great discovery which also can produce a proton gradient across the membane with the help of bacteriorhodopsin in the presence of light. Experiments are underway to mimick this process with an artificial membrane plus bacteriorhodopsin.

Petrol pump plant is another concept which entails to raise certain Euphorbia plants rich in hydrocarbons and the latex from these plants might be directly processed for oil production.

iv) *Pollution*

Pollution can be controlled in two ways: (a) by enhancing the growth and activity of microbes already present and (b) by adding new microbes to pollution sites. During the last several decades, a large number of synthetic chemicals have been released into the environment in massive amounts. Many such chemicals are halogenated aromatic compounds. Attempts have already been made to select certain micro-organisms which can degrade them efficiently. Some of the plasmids are now available endowed with the character of degrading many of the chlorinated compounds. The interactions of plasmids in the degradation of various compounds have resulted in isolating pure cultures capable of utilising such compounds rapidly as a sole source of carbon. Thus genetically engineered micro-organisms and plants might be useful in the total degradation and removal of various toxic chemicals from the environment[3,4].

II. WHAT ARE THE IMPORTANT EVENTS WHICH ARE LIKELY TO OCCUR AT THE END OF THIS CENTURY

Of special note was the general feeling of optimism and enthusiasm not only the intriguing scientific problems themselves, but also the emerging application of new microbiology of producing usable energy for the future as well as production of life-saving drugs and vaccines and agricultural outputs.

a) *Genetic engineering for curing and correcting human diseases*

One goal of recombinant DNA technology is the cure of human genetic diseases. As a step toward the goal hereditary defect is corrected by microinjecting a single gene into a mammalian cell. More than 2000 human genetic diseases have been described, diseases caused by the inheritance of a defective gene coding for a defective protein. Some of these diseases can be treated but none can be cured. A cure might be effected either by correcting the mistake in DNA structure that is responsible for the disease or by transfering a normal, functional gene into the defective cells. Therefore, one of the exciting applications might be gene therapy in human patients. This gene therapy will entail not only correction of defective genes but also correcting the controlling genes involved in the regulation of certain gene product say for example, the globin genes. Sickle cell anemia and thalassemia might be corrected in the near future by applying recombinant DNA technology.

For the cure of these diseases, many drugs which are not available even in minimal quantities will be made available in plenty and cheaper when their production is stipulated through fermentation process by selecting suitable bacteria containing particularly the specific genes for the end products, one is looking for. For example steroid hormones (Cortisone; testosterone, estradiol) will be obtained through fermentation by genetically engineered strain. Many of the enzymes *i.e.*, α-amylase, asparaginase, protease, ethanol dehydrogenase, glucose isomerase, pepsin, renin will also obtained similarly. Besides, insulin, interferon, somatostatin some of the peptide hormones, endorphins enkephalens, glucagon, vasopressin, human growth hormones; Vaccines for leukemia, hepatitis, influenza, some type of cancers, malaria, leprosy, diarrhoae, cholera, filaria will be obtained through rDNA technique including hybridoma.

b) *Recombinant DNA for plant genetic improvement*

There appear to be many potential applications of rDNA technology for the development of genetically superior crop plants. There is as usual a certain lag in utilising genetic engineering technology with the plant cell. Though, plant cell culture has attained its height in raising plants from a single cell, there are certain lacunae in getting suitable vectors as well as well studied marker for using in this technology.

The prospect of transforming nonlegumes into nitrogen fixers *via* recombinant DNA technology has been receiving a great deal of attention If feasible, this transtormation would obviate much of the necessity for chemical fertilizers, whose synthesis is linked to petroleum prices. Similar approaches and greater or lesser degrees of difficulty attend the genetic engineering of the other traits, such as disease resistance, stress resistance and toxin tolerance etc., in plants and it is presumed that substantial contributions will be made in this direction during the next 20 years or so.

The construction of an osmotic tolerance plasmid, the manipulation and cloning of genes against environmental stresses including salt, sugar and other end product tolerance including toxic products will give a new dimension[5].

Recombinant DNA technology holds the promise of enabling the creation of entirely new crops, whose yields potential, insect and disease resistance far surpass those of existent cultivars[6]

Barriers to application of Recombinant DNA techniques in plants

i) The problem of eliciting the expression of cloned genes inserted into plant cells: Although the plant cell is titipotent, the regulation and expression of plant genes during ontogeny is complex. Synthesis or non-synthesis of the metabolic products of different biochemical pathways is highly dependent upon the plant organ, the stage of development, the environment and even the specific genotype or species. A number of crop plants are tetraploid or hexaploid. Thus, for a transplanted gene to be expressed, it should exhibit Mandelian dominance.

ii) Adequate genetic markers are not available to plant genetic engineers: There exists in plants, multiple alternative pathways for the synthesis and catabolism of metabolites which may be

genetically activated as necessary to maintain plant viability.

iii) The problem of regeneration: Cereals and other monocots have proven especially difficult to regenerate from protoplasts. Thus biotechnology for regeneration should be perfected.

iv) Plasmid vectors for plant genes should be developed.

All these barriers perhaps will be solved by the year 2000 A.D.

c) *Recombinant DNA for production of fuel and energy*

This is another area, though not isolated, is expected to yield certain exciting results in the coming years. Splitting of H_2O to hydrogen has been developed by plants through the ages. If the genes for this enzyme complex are cloned into a bacterium then the splitting of water can be obtained by those bacteria and the reaction will be driven by the sunlight. Nitrogenase can also act under certain conditions as hydrogenase. It is expected that this splitting of water might be achieved at the commercial level[7].

Processes for the utilization of cellulosic biomass to produce liquid fuel, have three features in common.

i) They employ some means of pretreatment to affect some initial size reduction and more often cause a dissociation of liquid and cellulose.

ii) They involve either acid or enzymic hydrolysis of the cellulose and hemicellulose to produce mono and disaccharides and

iii) They employ fermentation to produce ethanol or other chemicals.

Investigation will be enhanced to integrate chemically and enzymatically treated cellulose and hemicellulose with a fermentation process whether it be with yeast or thermophillic bacteria. High cellulose production and repression-resistant organisms by genetic engineering will be achieved. Immobilized cell technology will be used for conversion of cellulose and cell wall material directly to alcohol. Fermentation of a wide variety of compounds, such as heterocyclic compounds and all types of agricultural wastes produce acetate as a major end product. Some of the methane bacteria convert acetic acid in very high yields to methane and CO_2. Another unknown area in the series of reactions is the overall conversion of CO_2 and molecular hydrogen to methane. Similarly clostridial fermentations that produce butanol and acetone will definitely add to the list of achievements. More attention to the biocatalysts really found in nature, say, the enzymes associated with cellular bodies and membranes, will be given. Bacterial strains, will be constructed to recover the residual oil from the abandoned well.

During the past five years, 40–50 companies have been started in USA to do business by applying genetic engineering technology. These companies have raised $\$4\times10^8$ in capital to support their efforts. In addition, many of the country's largest chemical, pharmaceutical, food processing and oil companies have made substantial internal financial and personnel commitments in this area of research.

III. TO COPE WITH THE TREMENDOUS PROGRESS IN THIS FIELD OF RESEARCH WHAT CHANGES IN THE TRAINING POGRAMME IN THE UNIVERSITIES AND THE ATTITUDES IN THE INDUSTRIES SHOULD BE INTRODUCED PARTICULARLY IN INDIA

In the Universities genetic engineering training programmes should be undertaken. This programme should be designed to provide a year of intensive, hands-on-training in modern techniques of molecular biology with special reference to nucleic acid biochemistry and molecular genetics and fermentation technology. This, in my opinion, will be best achieved if the staff members of biotechnology companies as well as the Universities participate in the programme. This will be a post M.Sc., and post B.Sc., course with intensive practical work preferably in a company. The companies should come forward to support this type of course by providing the research fellowships and other costs.

Secondly, biological resource facilities *i.e.*, academic institutions and industries cooperation in centers for tissue culture, cellulose hydrolysis, computerized identification of

microbes, gene banks and assay standardization procedure should be established to develop genetic engineering technology.

Thirdly, intensive interaction between universities and industries to identify the local problems is a primary necessity to harvest the fruits of this modern technology. Agencies are to cooperate with scientific community to help define research needs ranging from fundamental to practical in various areas.

In United States such interactions between the universities and companies are forthcoming. How to achieve this in India is a matter for further discussion between the universities/centers and the interested companies. What is primarily needed is to establish a link between education and work. It is gratifying that the Department of Science and Technology, Government of India has already constituted a Biotechnology Board to promote research in this ever-opening area of research. The Council of Scientific and Industrial Research, Government of India has already decided to open up a new laboratory for genetic engineering and applied microbiological work. The University Grants Commission should come forward with the academic programme and the industries with cooperation to take advantage of this newly developed technology.

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ANNOUNCEMENT

INDIAN INSTITUTE OF CHEMICAL ENGINEERING AWARDS

Dr. B. D. Kulkarni of National Chemical Laboratory, Poona, has been awarded the Amar Dye-Chem Award for excellence in research and development. Dr. R. A. Mashelkar, Deputy Director, National Chemical Laboratory, Poona, has been awarded the Herdillia Award for excellence in basic research in Chemical Engineering.