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Genetics Today and Tomorrow

A K SHARMA
President, Indian National Science Academy, New Delhi 110 002

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Advances in the science of genetics, the only unifying discipline in biology, have been spectacular in the last three decades. The genes have been chemically resolved, isolated, synthesized and subjected to manipulation through techniques of genetic engineering.

The scope of Genetics covers a wide spectrum, ranging from microbes to man, and the advances in each level of hierarchy have been phenomenal. In the microbial system, the prokaryotes, the gene-bearing structure is a long-chain DNA molecule named genophore. The study of gene-expression in a bacterial or a viral system involves merely the direct analysis of gene products at the level of the gene itself. The entire structure is extremely simple, being unicellular, and all activities are just confined within the small unit.

In higher organisms — plants, animals and man, the genes in the DNA molecule are organized into complex structures the chromosomes, which in addition to DNA, contain basic and non-basic proteins. The genes in chromosomes are *split* consisting of essential and non-essential sequences. Over and above, is the fact, that a heavy percentage of DNA, in the higher organism is composed of repeated sequences, the functions of which are still obscure. Such sequences as noted different centres of research including that of our own, may represent 2/3rd to 3/4th of the total amount of DNA in the organism. Their function is still an enigma in biology.

In higher organisms, the body being multicellular and highly differentiated, each and every character is the ultimate result of a series of chemical reactions initiated, originally at the gene level. As such, several intermediate steps intervene between the ultimate effect, which is analysable, and the levels of genes where the action is initially triggered. This poses tremendous complexity in the analysis of gene-expression in eukaryota as compared to that of prokaryotes.

Genetic Manipulation

Genetics has come to the forefront of scientific disciplines due to tremendous potential of the methodology developed for genetic manipulation. Leaving aside hybridization and recombination, tinkering with the gene substance owes its initiation to the discovery in 1927 by Muller demonstrating that irradiation can induce gene alterations in biological systems. Mullers' discovery led to the establishment of the science of Radiation-Genetics and later, Physical and Chemical mutagenesis — no doubt operating in a non-directed fashion.

Genetic Manipulation techniques in recent years have undergone much refinements through the use of enzymes needed for cutting and joining the DNA segments respectively at specific loci. These principally involve, introduction after isolation of DNA into the recipient bacterium, cutting the specific sequences of donor DNA and self-replicating plasmid molecule through restriction enzymes, joining the donor sequences in plasmids through ligases, followed by maintenance of the plasmid in the bacteria for cloning or propagation. The same process can be repeated with the incorporated gene in the plasmid which can be further taken off from the plasmid and introduced into the genophore of the bacterium. In suitable medium, the bacterium can propagate and the genes incorporated in it either in the plasmid or ultimately in the genophore, can find expression in the synthesis of coded enzymes.

Impact in Microbial System

The impact of recombinant DNA technique varies on microbes, plants and animals. In microbes, the organisms *per se* are deeply involved in the methodology, serving not only as vectors but also as the substrate for cloning and synthesis of the products. The genetic architecture of the organism as a whole, undergoes alteration. Recombinant DNA technique coupled with the fusion of bacterial cells are extensively made use of in conferring the property of production of antibiotics from one bacterium to another or the production of hybrid antibiotics. Similarly, the capability of nitrogen-fixation of *Klebsiella* has been transferred to other bacteria which otherwise possess no such property. The hydrocarbon-degrading capacity of the microbial genes has been cloned in a single bacterium through successive cutting and joining by specific enzymes.

Besides, in view of the simplicity of the organism, its capacity to act as vector, absorb foreign gene in its architecture, and to act as a cloning and expressing medium, the microbes offer enormous possibilities for genetic manipulation, which, with further refinements, could be carried out with predictable accuracy. The impact of these researches on degradation of pollutants and purification of environment is enormous.

Impact in Animals and Man

In animals, in general, and man, in particular, there have been remarkable advances from a fundamental standpoint. A large number of human genes have been mapped and located in specific chromosomes. Elegant techniques, have been devised, including extraction and molecular hybridization leading not merely to identification of loci but their functional significance as well.

It is now possible to make a precise diagnosis of all the inherited conditions of haemoglobin through DNA analysis. Prenatal diagnosis of genetic disorders with the aid of DNA probes has emerged as a very powerful tool in Human Genetics. With the availability of more genes for cloning, it would be possible to utilize them as probes for the diagnosis of diseases with unidentified defective structural genes. The faetal sex determination is at present a simple technique utilizing identification through specific probes of "Y-chromosomes". Nearly 30 distinct types of neoplasia have been identified with 22 specific chromosomal defects. Advances in different aspects of human genetics *per se* have led to a better understanding of the fundamental genetic structure, the gene functions, and subsequently the method for the detection of disorders.

The recombinant DNA technique has given a new dimension to pharmacology to a great extent, the best example being the production of insulin in a bacterial system. The isolation of insulin-coding gene from mammals, its incorporation into

the bacterium through a plasmid vector and ultimately the production of insulin in the bacterium serving as a factory, are established facts. Similar possibilities do exist for a wide range of interferons, the antibody proteins, which are specific for a large number of diseases, including possibly cancer. The capacity of the bacterium to accept the genes of higher organisms and act as a suitable medium of their expression are the key factors in these operations. Ultimately, it amounts to utilization of the bacterium as a production centre for a variety of drugs including insulin, interferons, human hormones and a host of pharmaceuticals. Simultaneously, the fusion of lymphocytes with antibody-producing cells—the hybridoma technology—has paved the way for the production of monoclonal antibodies.

Thus, in the mammalian system, emphasis has been laid towards three different approaches; (*i*) identification of genes in chromosomes, (*ii*) diagnosis of genetic disorders, and finally (*iii*) the production of drugs through the introduction of desired gene in the bacterium. Nowhere there has been an alteration in the genetic architecture of the individual or the introduction of foreign genes as in case of prokaryotes. excepting in melignancy.

The coming decade would undoubtedly witness much more developments in this direction utilizing bacteria as a medium for production of a variety of drugs. But the ultimate objective of curing the genetic disorder at the genetic level is yet to be attained. This dimension of research which does not contemplate the production of drug in a bacterial factory in the artificial medium but visualizes its production in the human body through the incorporated gene is yet to make a solid base. It implies, the isolation of interferon or insulin-coding gene from the donor and its introduction to the human recipient, ensuring continuous production of the drug in the human system. Such incorporation may involve the functioning of the recombinant bacterium as a factory within human system or ultimately the introduction of the gene in the human chromosome itself. With the present rate of development of technology, the recombinant bacteria serving as drug production centre in human organ instead of *in vitro* may be possible in near future, though not necessarily the introduction of foreign gene in the chromosomes of man.

Impact in the Plant System

In the plant system, there has been phenomenal advances in different facets of genetics. The identification and analysis of fine structure of chromosome, study of sequence complexity, including additional genetic elements, population analysis, improvement through breeding, mutation and chromosome substitution techniques have paid very high dividends both from fundamental and utilitarian standpoints. But recombinant DNA technique in this case, is designed to achieve the objective of restructuring plants own genetic component through introduction of gene by specific enzymes as in the case of bacteria. The execution of this task in plants is fraught with innumerable limitations as compared to that in microbes where the task is quite simple as the object is unicellular with no differentiation.

One of the advantages of the plant system is its totipotency, which theoretically enables any cell to regenerate into a whole plant in the proper medium. Significant advances have been made in cell, tissue, and organ culture which have led to production of innumerable plants of desired genetic constitution without involving the complicated process of sexual reproduction and fertilization. In fact, induction of mutation followed by the regeneration in culture has become an effective tool in securing improved variety of crops within

a short-span of time which through conventional breeding practices would have taken benerations to achieve. One can secure haploid cells in culture, induce desired mutations through a random mutagenic treatment and regenerate the selected individuals in multiples. Further, regeneration of homozygous plants through artificial induction of doubling assures the purity and stability of the individual, One can hardly doubt the phenomenal advances made and the potential of *in vitro* technique for propagation and improvements in agriculture, horticulture and forestry.

However, the other facet of genetic manipulation in which foreign genes either from different plants or from microbes, need direct introduction into the recipient chromosome through recombinant DNA technology as in bacteria suffers from serious constraints. Lately, a case of successful transfer of protein 'Phaseolin' from *Phaseolus* to sunflower, has been reported.

Most of the works so far carried out deal with the insertion of genes from higher organisms into the microbial recipient where the enzymes coded by the former for pharmaceuticals, and drugs like insulin, interferon and growth hormones are expressed. There has been only one single example of the insertion of a bacterial gene for antibiotic resistance into *Petunia* cells in culture.

The failure in direct introduction of the genes of a microbe or a plant to another plant through recombinant DNA technique can be attributed to various factors. Plant genome is exceedingly complex involving at least 5 to 6 million genes in an average crop plant. A large number of genes are located outside the chromosome such as in chloroplastids and mitochondria. Each gene has three regions—the promoter, the series of nucleotides coding for the enzyme the structural sequences and the terminators. All these sequences require identification before introduction of a foreign gene in the system.

One of the crucial factors for a plant geneticist is the identification of a suitable vector for carrying the passenger or foreign gene into a suitable recipient system. The protoplast being naked (devoid of cell wall), and theoretically having the capacity of regeneration, is a convenient recipient medium. However, though potatoes of *alpha alpha* as well as several Solanaceous plants grow very well from the protoplast, others like corn, wheat or soybeans do not do so. The technique therefore calls for further improvements for universal applicability.

Of the vectors moreover, the only one demonstrated to be effective is the Ti plasmid associated with *Agrobacterium tumafaciens* —a bacterium responsible for crown gall in legumes. But its application restricted the specificity being mainly confined to species infected by Agrobacterium alone. Its range is to be further widened by genetic manipulation so that it can be utilized to carry genes to cereals from grasses or from bacteria too. Of the other vectors, only cauliflower mosaic virus appears promising.

The key to the success of genetic engineering in higher organisms is a precise understanding of the mechanism of control of differentiation. Each and every cell contains the same genetic complement but growth and differentiation being sequential and phasic, only certain genes are switched on at the proper time, the others being shut off during that period. Genes for photosynthesis are present in all organs but expressed only in the leaf and not in the root. An understanding of this mechanism is a prerequisite to the identification of the control elements regulating gene expression.

Even after a foreign gene is inserted in the living system through a vector, it is difficult to ensure that it would be expressed only in the organ needed and not elsewhere. The issue is further complicated by the fact that a character in a plant is not necessarily controlled by one or many gene(s) in a

single chromosome, but rather they are more likely to remain distributed in different chromosomes. The basis for their simultaneous functioning responsible for the harmonious expression of the character is not yet fully understood. The manifestation of the tremendous potential of plant genetic engineering would await an understanding of the basic factors involved in regulation of differentiation with the inherent biochemistry and physiology of regulating factors. Theoretically, there is potential of isolation and incorporation of the genes for resistance to herbicide or drought or capacity to thrive in marginal, salty or acidic soil, to crops and commercially important species. In relation to nitrogen fixation, where 17 genes are involved in bacteria, recombinant strains with the capacity for fixation or accelerated fixation either as free-living organisms or as symbionts in legumes are demonstrable. But the transfer of these genes with their individual control elements from the bacterium to the plant presents innumerable fundamental problems. The potential is unlimited but what is needed is a basic understanding of the genetic control of differentiation.

One can visualize advances in the methodology for the transfer of genes from one plant to another in view of the similarity in the nature of the genes — being split in both, and similarity of the control signals. The heavy amount of additional DNA offers enormous scope of utilization of these sequences in genetic manipulation techniques.

Future Scope

The coming decade would undoubtedly witness major advances in genetics vis-a-vis recombinant DNA technology. The identification of almost all human genetic disorders through random DNA probes is within the realm of possibility. More bacterial factories would be established for the production of insulin, interferons, hormones as well as other pharmaceuticals. One can look forward to a rapid method for environmental purification with the aid of more powerful recombinant organisms. Nitrogen-fixation through more efficient microbes harbouring the cereals would undoubtedly be achieved in near future.

The cell, tissue and organ culture including the protoplast has opened up new vistas for rapid propagation, enrichment of genetic diversity and introduction of genes in the system. Protoplast fusion would enable the regeneration of novel hybrids in plants, unimaginable in the history of conventional hybridization. The impact of these methodologies in the improvements of cereals, medicinal and commercially valuable species, can hardly be overrated.

But the ultimate objective of altering the genetic architecture of a plant for improvement in agriculture or the human organ for amelioration of diseases, through recombinant DNA technology, may not possibly be attained in near future. A prerequisite for the realization of this aim is a basic understanding of the control signals regulating differentiation in eukaryota. The sequential and phasic growth and diversification of organs, in the higher hierarchy of evolution, is one of the most challenging problems in biology. With the intensification of researches on genetics, biochemistry and physiology, one can hope to have in near future, a deep insight into the fundamentals of the genic and chromosomal control of differentiation in higher organisms. This will pave the way in later years for the introduction of genes in the genetic architecture of higher eukaryotes. If achieved, even though in distant future, it will be a remarkable feat of genetic engineering — fulfilling the very objectives of a scientist — search for truth, enrichment of qualily of life, and alleviation of human suffering.