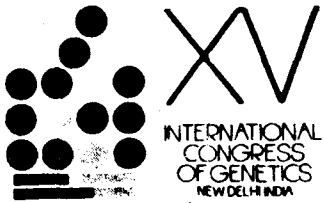
XV INTERNATIONAL CONGRESS OF GENETICS

PLENARY SYMPOSIA AND SYMPOSIA SESSIONS

Summaries of Contributions



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SUMMARIES OF CONTRIBUTIONS

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SYMPOSIA SESSIONS

*Symposia Session.

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Plenary Session I

Genetic Engineering

GENETIC ENGINEERING IN NEUROPEPTIDE AND NEURORECEPTOR RESEARCH

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A new approach to the understanding of neural and hormonal peptides as well as neurotransmitter receptors has recently been provided by the application of recombinant DNA techniques. The primary structures of three opioid peptide precursors, i.e., the corticotropin-β-lipotropin precursor, preproenkephalin A and preproenkephalin B, have been elucidated by determining the nucleotide sequences of cloned DNAs complementary to their mRNAs. All of the endogenous opioid peptides thus far identified are derived from these three precursors. In addition, some novel neural and hormonal peptides have been discovered from the cDNA sequences. The three precursors each contain multiple repeated peptide units, thus representing multi-hormone precursors. The genes encoding the respective precursors have been isolated and characterized. The striking similarities found among the structural organizations of these multi-hormone precursors as well as their genes suggest that these genes may be evolutionarily related. The structures of the corticotropin-releasing factor precursor and its gene are also discussed.

The primary structures of all subunits of the fish electric organ acetylcholine receptor (with a subunit structure of $\alpha_2\beta\gamma\delta$) have been deduced from the nucleotide sequences of cloned cDNAs encoding the respective subunit precursors. The four subunits exhibit marked amino acid sequence homology and are similar in hydrophilicity profile and predicted secondary structure, thus being most probably oriented in a pseudosymmetric fashion across the membrane. The transmembrane topology of the subunit polypeptides and the locations of functionally important regions, such as the acetylcholine binding site and the transmembrane segments which may be involved in the ionic channel, have been proposed. The amino acid sequence homology among the acetylcholine receptor subunits suggests that the genes encoding them have been generated from a single common ancestor by gene duplications.

Other Speakers

STUDIES WITH HUMAN ONCOGENES

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INTERFERON GENES

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STRUCTURE AND EXPRESSION OF H_2 GENES

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Plenary Session II

Biotechnology and Its Varied Applications

SYNTHETIC VACCINES

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Advances in fractionation technology have improved the safety and efficacy of several classical vaccines. Nevertheless, in a number of cases this conventional approach is difficult and even hopeless because: (a) the source of the natural antigens can be dangerous (hepatitis B HBs antigen) and/or impossible to prepare in sufficient amounts (Malaria sporozoite vaccine); (b) certain natural structures, even when easily available, can cross-react with host tissues (for instance streptococcal M protein and cardiac antigen). Therefore, artificial preparations are being intensively studied as substitutes for native antigenic determinants using modern technologies such as recombinant DNA, monoclonal antibodies, computer-graphics programme, synthesis of peptides or carbohydrates, induction of anti-anti-idiotypic antibodies.

Highly purified or even synthetic antigens of the future are likely to be weak protective immunogens and will therefore need external adjuvants. A new class of agents have been intensively studied in recent years. Indeed, since 1975, the active subunit of FCA (Freund's complete adjuvant), Nac-Mur-L-Ala-D-isoGln called muramyl dipeptide or MDP, and several hundred analogs have been synthesized. One of these derivatives, murabutide, is presently evaluated in clinical trials. This adjuvant is active even in an aqueous medium and, contrarily to alum, can be lyophilized, and enhances slightly the level of specific IgE.

Previous experiments using (poly-Tyr-poly-Glu)-(poly-D-L-Ala)-poly-L-Lys, (T, G-A--L) or a synthetic fragment of the coat protein of the coliphage MS2 have demonstrated that MDP, particularly after coupling, was a powerful adjuvant for synthetic antigens even after administration in saline. More recently, antibodies capable of protecting against the toxicity of the diphtheria toxin have been obtained after injection of a totally synthetic conjugate made of MDP and synthetic diphtheria peptides coupled to the A--L chain.

The potency of MDP or derivatives has been also studied in totally

different models: hormonal vaccines prepared in view of immunological castration of cattle or following the pioneering studies of Talwar of controlling fertility. Thus, male mice have been successfully immunized with a completely synthetic vaccine administered in saline containing LH-RH (luteinizing hormone-releasing hormone) directly coupled to MDP-Lys. Other studies have shown that a synthetic C-terminal fragment characteristic of the β-chain of HCG coupled to a carrier and administered with MDP can enhance the level of anti-HCG antibodies. Very recently, several promising results have been obtained with new synthetic bacterial and viral antigens. For example, synthetic vaccines have been shown to protect against a *Streptococcal* infection or *Escherichia coli* heat-stable toxin. However, most studies deal with viruses. Several sequences of the surface antigen HBs, the influenza hemagglutinin, or the foot and mouth virus coat protein have been reproduced and tested for their immunogenicity. Antibodies capable of protecting against influenza and still more against the foot and mouth disease were produced as well as antibodies binding the natural HBs. Aminoacid sequences containing major antigenic determinants of the poliovirus have also been described very recently as potential synthetic antigens. Finally, recent reports on the antigenic structure of malaria sporozoites indicate that the synthetic approach is considered also in the field of parasitology.

Synthetic vaccines are no longer considered as an unrealistic venture. But, two very important issues have still to be confronted: the choice of an adjuvant and of a suitable carrier or, better still, coupling directly antigens to adjuvants. It must be noted that such possibilities have already been proved to be effective in experimental models.

APPLICATION OF PLANT TISSUE CULTURE IN AGRICULTURE AND FORESTRY

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Recent advances in plant tissue culture have led to a rapid increase in its application to agriculture. Micropropagation, mutation, embryo culture, production of haploids and protoplasts, somatic hybridization and genetic engineering are the major developments in the past three decades, which hold promise of exciting new applications of tissue culture.

Methods have been developed for the micropropagation of a wide range of species so that natural variation can be rapidly utilized for increasing production or disease resistance. Plantlets of orchids and other ornamentals, oil palm and several fruit and vegetable species are commercially produced by tissue culture. Pioneering work has been carried out in India on

the multiplication of major forest species, such as teak, eucalyptus and bamboo. High biomass yields of fast growing eucalyptus elites hold promise of a major contribution to the production of energy and chemical feedstocks and to conservation of forests. Methods have also been developed for rapidly multiplying plantation crops such as turmeric, ginger, cardamom and work is in progress on coconut, cashew and rubber. Meristem culture is also being widely used for producing virus-free potato, banana, cassava and sugarcane. The increase in yield of mosaic virus-free sugarcane by this method was about 20 per cent compared to that of the virus-infected plants.

The discovery in India that haploids can be produced by anther culture has led to its extensive use, especially in China, for plant breeding and the production of improved varieties of cereals.

Mutation breeding by tissue culture offers economy in space, time, labour and expense and relative ease of selection compared to methods using seeds or plant segments. Mutation of sugarcane, wheat, turmeric and other species in tissue culture has yielded superior varieties. This method is potentially of value with all economic crops.

Embryo culture is of importance for hybridization if the hybrid embryo is infertile *in vivo*. Leaf mosaic virus-resistant papaya hybrids were produced by embryo culture when the hybrid embryo was found to abort on the plants.

The production of plant protoplasts, their fusion and regeneration to yield hybrids is one of the most exciting developments in tissue culture. Though protoplast regeneration has so far been obtained only with relatively few species or only a few varieties of a species, the possibility of crossing sexually incompatible species and applying recombinant DNA technology to plants has led to extensive work in this field. *In vitro* production of hybrids was achieved by intraspecific fusion with tobacco and *Petunia* and interspecific crosses of potato and tomato. The transfer of specific genes or groups of genes (such as Nif genes), plant organelles (such as chloroplasts), DNA fragments or irradiated pollen, as well as protoplast fusion are of interest for genetic engineering of plants.

Several major difficulties hamper research in tissue culture and its application. Most of the work is empirical and methods which are suitable for one species are often inapplicable to other species or even to other varieties of a species. Plating efficiency with most species is low or even zero, since single cells of most plants fail to grow. Regeneration of protoplasts is difficult with most species. Suitable genetic markers and vectors for DNA transfer are not available in most cases and selection of the required hybrids after fusion of somatic cells is difficult. Solutions to these problems will contribute not only to major applications of tissue culture to agriculture and forestry, but also to basic studies on chromosome mapping, hormone action and other aspects of plant biochemistry.

APPLICATION OF GENETICS TO MICROBIAL PRODUCTION

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Overproduction of microbial metabolite or enzyme is a necessary factor for the industrial process using microorganism. The requisites for getting a microbial metabolite efficiently in a large amount may be arranged according to the steps of the flow of a substrate into the metabolite as follows:

(1) Efficient permeation of substrate material into the microbial cell ---promotion of the permeation of the substrate.

The transport rate of some substrates to cell has been found to be a limiting factor. Mutant which has the increased transport rate of a substrate or precursor into cell can be isolated. Such a mutant can be obtained by two methods: One is the selection of a rapidly growing or large colony on a plate containing the compound as a sole source of carbon; another method is the isolation of a strain sensitive to a compound which is an analog of the substrate compound and inhibitory to the growth of the microorganism.

(2) Efficient proceeding of the metabolic reactions concerned---increase in enzyme formation and enzyme activity.

An auxotrophic mutant which cannot produce a regulatory effector or co-repressor overproduces and excretes the precursor or a related metabolite of the blocked reaction when grown on a limiting supply of the required nutrient. A mutant that has lost some biosynthetic regulation can be obtained as an analog-resistant and as a prototrophic revertant from the auxotroph having a deficiency in a regulatory enzyme. The combination of auxotrophy and genetic deregulation is apparently generally useful to obtain an increased yield of a primary metabolite. Multiple markers also contribute to the yield by stabilizing the productivity against back mutation during fermentation. In the method used for obtaining a multiple regulatory mutant by sequentially adding several analog-resistance markers to a single strain, there is a possibility that cross-resistance or reversion of prior mutations cause the failure to get a multiple mutant which completely lacks individual regulatory mechanisms. This is avoided by the use of transduction technique.

Constitutive mutant and mutant resistant to catabolite repression are useful for the production of catabolic metabolite or enzyme.

Recent development of cell fusion and recombinant DNA technology has made possible genetic recombination between different species and the increasing of the biosynthesizing activity of a microorganism by increasing gene copy number or improving the microorganism in relation to the substrate and environmental conditions, such as temperature.

Immobilization of enzymes or cells increased the stability of the enzymes

concerned and made possible continuous production using the immobilized enzyme column (bioreactor).

(3) Blockage of side reaction to prevent the flow of the substrate or metabolic intermediate into the other pathway.

(4) Blockage of breakdown or metabolism of a metabolite produced.

(5) Efficient leakage of the metabolite into the environment of the cell.

The importance of excluding the permeability barrier was most clearly recognized in the case of glutamic acid production. Escape of metabolite from the cell prevents an effective feedback control of the metabolite within the cell, and allows continued synthesis of the metabolite.

Some recent examples which satisfy these requisites will be mentioned.

HYBRIDOMAS—A NEW DIMENSION FOR IMMUNODIAGNOSTICS AND IMMUNOTHERAPY

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The hybridoma technology enables the availability in large amounts of homogeneous antibodies of consistent characteristics. Clones can be selected for a high degree of specificity and other desirable traits e.g. affinity, antibody subclass, etc. These can provide valuable source material for qualitative and quantitative immunoassays. Introduction of non-isotopic methods, in place of radioisotopes, has further brought these assays within reach of laboratories with modest equipment facilities. The Non-Isotopic Immunoassays can also be carried out in field conditions. Some developments in this context will be reviewed.

Monoclonal antibodies by virtue of the advantages stated above have also found applications for a variety of therapeutic uses. The anti-zona pellucida antibodies can be used to block fertilization of the egg by sperm. A single injection confers protection for several months. The anti-LHRH monoclonals can be employed for suppression of estrus in domestic pets. The antibodies given at early stages of pregnancy have also been found effective in termination of pregnancy in rodents and in primates suggesting their possible use in the development of a non-surgical method for menstrual regulation and termination of pregnancy. Monoclonals against cell surface antigens can help in imaging of metastatis and as vehicles for delivery of drugs and toxins to cancer cells.

Plenary Session III

Genetics and Society

GENETIC ENGINEERING IN THE UNITED STATES

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Genetic engineering has become an important reality, and a continuing stream of results from pure and applied research guarantee an even greater role in the future. Advances in techniques for recombinant DNA, production of monoclonal antibodies, tissue culture and protoplast fusion are being energetically exploited. These tools for genetic engineering have been supplemented by excellent means of determining the location of genes and amino acid sequences of proteins. In addition, it is feasible to construct entirely new genes and to modify the known ones. Thus far, the principal practical results have been obtained with genetic manipulation of microorganisms, notably *Escherichia coli*. However, the genomes of other prokaryotes and many eukaryotes are now being altered using an increasing array of new vectors and techniques.

Human and other animal genes have been incorporated into plasmids of *E. coli* and expression of these genes has resulted in the production of human insulin and many other pharmaceutical products that are now undergoing clinical tests.

Recently, a number of laboratories have been changing the sequences of nucleotides in known genes and have obtained corresponding new proteins. In this way, one laboratory produced a human type interferon having enhanced effectiveness against viruses. Other laboratories are seeking to use genetic engineering to obtain enzymes suitable as catalysts in industrial processes. These applications require that artificial genes be produced that can code for unusually rugged proteins capable of withstanding harsh conditions of temperature and pH.

Incorporation of foreign genes into *E. coli* is only one of many ways in which genomes are being altered. For the purpose of production of pharmaceuticals and other substances, yeasts, molds and bacteria are being studied. For example, renin is being produced by expression of the renin gene by yeast.

Animal tissue cells in culture are being transformed by viruses. For this purpose, both simian virus-40 and papilloma viruses have been employed. When cultured mouse or human cells are treated with SU-40, DNA from the virus is incorporated in their chromosomes.

The most spectacular results have been obtained by using newly fertilized mouse eggs. When foreign genes are injected into the male nucleus, they are often incorporated into the mouse chromosomes. The new genes are expressed in the mouse throughout its lifespan and are inheritable.

Thousands of different, highly specific monoclonal antibodies have been produced. These are now being employed as pharmaceuticals, diagnostic aids and as useful tools in fundamental biological research.

To date genetic engineering of plants has not produced many practical applications. However, simple tissue culture of plants has led to new species with greatly altered chromosome numbers and to mutants with enhanced resistance to herbicides. The methods of tissue culture lend themselves to speedy identification of new varieties. As yet there have been limited examples of incorporation of foreign genes into plant genomes. The agent commonly employed is a plasmid from an *Agrobacterium*. However, a number of skilled researchers are seeking additional vectors to carry genetic material and some are likely to succeed. Ultimately, the successful classical methods of plant breeding will be combined with products of plant genetic engineering to create new and superior plants.

THE GENETICS REVOLUTION

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Genetics, like mathematics, has historically had an ethereal quality because the object of its concern, the gene, was an abstract concept. With the discovery of the structure of DNA, that abstraction took on some reality, but it was not until we could physically isolate and study in detail the nature of genes that genetics provided materials for practical manipulation. We are now entering an era in which genetics will revolutionize biotechnology, medical practice, and our concept of biological individuality. Furthermore, molecular genetics is providing us with a detailed understanding of gene control and is sure to provide the basis for an understanding of development. The remarkable abilities provided by genetics and the information that will flow from genetics present scientists and those interested in public policy with the duty to see that societies derive the maximal benefit and the least disruption from these powerful advances.

GENETICS AS A UNIFYING FORCE IN SCIENCE AND SOCIETY

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Human genetics—like all other sciences—does not develop in a sociologic vacuum. It is the work of social groups of human beings who are influenced by the society at large. To a biologist, one reason for excitement is the discovery that much of the conceptual framework for biological evolution could also be usefully applied in the context of social change. Thus, the first part of my paper summarizes how adaptation could evolve under natural selection before specific knowledge of biological transmission was available. As early as 10,000 to 15,000 years ago in the Stone Age, man began, first unconsciously and then consciously, to domesticate certain plants and animals and selectively breed them to fit them to their own need. All this early breeding work was in fact highly successful and it formed one of the bases for the development of civilization. Also it is interesting to note that in ancient times many cereals such as wheat, barley and oats were first cultivated and animals such as cattle, sheep, goats and pigs were first domesticated in some nuclear areas including China and India. It was only later that the change to an economy of food production spread in all directions thus reaching Europe and Africa.

With the rediscovery of Mendelism in 1900, there was an almost immediate application of Mendelian principles to plants and animals. As a result genetics gave a strong impetus to the development of modern society. During the last 30 years there has been a dramatic increase in food production or the "Green Revolution" as called by the plant geneticists. This achievement is not the result of increasing the acreage planted, but of using new varieties of plants and developing hybridizing techniques. So the second part of my paper deals with the impact of modern genetics on agriculture, medicine and society with emphasis on two achievements—molecular biology and chromosome research—which have not only altered human genetics as a pure science but also brought marked progress in its application for human welfare.

The third part of my paper points to the importance of the crucial role played by genetic knowledge in the handling and management of four big issues that particularly confront our developing countries. They are energy requirement, population pressure, food supply and environmental pollution. Examples of using genetic approaches in solving these problems in China are given in detail with special emphasis on the successful control of Chinese population through systematic education, planned parenthood, genetic counselling and prenatal diagnosis etc.