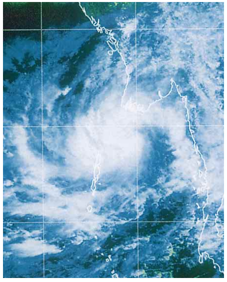

CHAPTER XIV

VIGNETTES OF BIOLOGY

THE EARLY DAYS

India has built a vibrant tradition in research and teaching in various areas of modern biology. In the early days of the twentieth century, physicist Jagadis Chandra Bose initiated experiments in Kolkata on the electrophysiology of plants. One of the earliest schools in biochemistry (first in India and second biochemistry department in the world), was established at the Indian Institute of Science, Bangalore, over 80 years ago and it continues to be one of the top research institutions in the world. Biological research in the early decades (1920-1955) was strong in the areas of enzymology, vitamins and cofactors, nutrition and organic chemistry of natural products. Some of the well-known names are: J.C. Bose in plant physiology; P.S. Sarma, who led a dynamic team in enzymology and intermediary metabolism; H.B. Cama in nutritional biochemistry; Birbal Sahni in palaeobotany; B.C. Guha in vitamins and cofactors; C.S. Vaidyanathan in plant enzymes; A.R. Gopal-Iyengar in radiation biology; B.P. Pal in agriculture; P. Maheshwari in plant embryology; Shambhu Nath De in microbiology (he was the first to describe the endotoxin of *Vibrio cholerae)*; N.N. Dasgupta in electron microscopy; and R.N. Chopra in pharmacology.

Each of them built an active school and research group so that when India became independent in 1947 and began rapidly developing its education and research institutions, it had a good number of well-trained researchers capable of creating centres of excellence. It was around this time too that notable advances were being made in the world in the areas of genetics, microbiology and biophysics. The new field of molecular biology was emerging. Thanks to the academic and research background that it already had instituted, it became possible for India to spawn research teams that could participate and contribute in these newly emerging areas of biology.

The field of human genetics is vibrant in India, and owes its strength historically to the efforts of J.B.S. Haldane who put together a team of doctors and statisticians in analyzing the prevalence of genetic disorders, unusual traits and related features in India -- *a living genetics laboratory*, as he called it. One of his students, P.M. Khan, became well-known for his comprehensive and analytical research into the biology of colorectal cancer. A welcome consequence of Haldane's efforts was to bring together genetics and statistics and establish a research group in anthropology and human genetics at the Indian Statistical Institute, Kolkata.

THE MODERN ERA

A similar and equally fruitful effort has been the establishment of biology divisions at the Atomic Energy Establishment in Mumbai, and its sibling, the Tata Institute of Fundamental Research (TIFR) at Mumbai about 40 years ago. This group pioneered research into the genetics of the fruitfly, *Drosophila melanogaster*. While classical cytogenetics was an active and useful field practised by several groups at Kolkata, Varanasi, Hyderabad, and Chennai, the group at TIFR helped usher in the fields of molecular biology, electrophysiology and mutation analysis into genetics. On a similar line were the efforts of medical researchers in bringing together the fields of nutrition, biochemistry and physiology.

Around the same time G.N. Ramachandran, himself a student of C.V. Raman, ushered in the field of molecular biophysics. This area attracted not only physical chemists, but physicists, mathematicians and computer scientists as well. To a major extent, the notable presence of Indian researchers currently in the area of bioinformatics on the one hand and in X-ray crystallography on the other, is an outcome of the 'Ramachandran effect'. In this sense, Ramachandran has been to biophysics what Haldane has been to genetics in India (see also chapter on Biophysics and Structural Biology).

Beginnings of New Biology

Biological research activities in India can broadly be classified under two heads-- the classicial and the modern. Classical biology largely refers to areas where tissues, organs, whole plants, and organisms are studied. It was pursued by compartmentalized, strictly discipline-bound scientists in botany, zoology, microbiology and so on. Modern biology is largely based on molecular and cellular techniques that cut across disciplinary boundaries and utilizes common tools and techniques, be it for the study of microorganisms, plants or animals. Classical biology or systems biology was already thriving in India around the 1950s, and was done effectively in a few universities and the national agricultural research institutes. Scholars trained in botany, zoology and agriculture were the major players here. Indeed some trail-blazing discoveries and developments were made in universities. It is not commonly known that the areas of protoplast fusion, anther culture and nucellus culture, as well as micropropagation using tissue culture of plants got its earliest start in India, notably in the Department of Botany at the University of Delhi (DU). Already by the late 1950s, micropropagation of a variety of plants was regularly achieved in the laboratories there. At about the same time, plant geneticists realized the importance of hybrid seeds, mutants, selection breeding and the like. The Indian Council of Agricultural Research, which predates India's Independence , had established excellent field stations and research outlets at various places such as New Delhi and Coimbatore. Thus when the Mexican dwarf varieties of wheat were introduced to the world by Norman Borlaug, one of the earliest countries to take advantage of this was India. Based on a systems approach, involving hybrid seed selection and breeding, irrigation, use of appropriate fertilizers, post-harvest technologies and distribution of the produce, led to what is now recognized as the Green Revolution.

Modern biology as understood today had its origins in India around the 1970s. The country was fortunate in already having a cadre of scientists trained abroad in these areas. Several of them were front-ranking players in the development of many methods and in the significant discoveries made in enzymology, biopolymer structure and conformation, reverse transcription, molecular virology, ribosome structure, transcription and translation, cell culture techniques, biomolecular spectroscopy, computer methods and so on. It was roughly about the same time that the Department of Science and Technology (DST) initiated a facilitating mechanism called the National Biotechnology Board (NBTB). This Board played a proactive role in promoting specific methods and technologies, curricular programmes, research laboratories and funding research projects of individual scientists. Within a short time, NBTB matured into a full-fledged department of the government, called the Department of Biotechnology (DBT).

Biotechnology has earlier been used, in the classical sense, in India in improving livestock health and wealth. Selection breeding of chosen breeds of milch cattle, sheep, horses and other animals has been effective.

Plant Molecular Biology: Some Facets

The beginnings of plant molecular biology in India have been traced in a recent review article in *Current Science* (vol.80,2001). Only a few aspects are covered here.

Groups of scientists in Kolkata, Kalyani, New Delhi, Chennai and Bangalore have been pursuing plant biochemistry, physiology and molecular biology. The work of biochemists at IISc in the cloning of rice histone genes and their transcription was a turning point for molecular biology in India. Several groups began research in the border-line areas of hormone- and phytochrome- mediated stimulation of RNA synthesis and molecular biology of stress. The research on photosynthetic genes and their regulation has been carried out on *Vigna*, rice and *Arabidopsis*. The involvement of C^{++} and protein phosphorylation has been demonstrated in regulation of plastid gene expression. New stress proteins that are critical for overcoming stress to high temperature, salinity and drought have been discovered in rice. Interestingly, one heat shock protein (hsp) which is 110 kd is homologous with a protein of yeast, indicating the unusual conservation of the machinery to overcome stress. A number of Ca^{++} activated protein kinases have been identified. It is observed that the influx of Ca^{++} is regulated by phytochromes. Genes like nitrate reductase and PsaF are regulated by phytochrome and this involves phosphoinositide cycle. Besides its role in photosynthesis, light is indispensable for growth, elongation, maturation, flowering, reproduction and other morphogenetic phenomena. Scientists at JNU and ICGEB have been studying the preception of light signalling and gene expression, specially with reference to the photosensor, phytochrome. Several photomorphogenic mutants of *Arabidopsis* have been isolated and characterized at DU. One of these mutants, JK 224 (redesignated later as nph 1), has paved the way for molecular cloning of the hitherto unknown blue light receptor, phototropin, that regulates phototropic response in plants. Recently, genes for NPH1 and phytochromes have also been cloned and sequenced from rice and wheat, respectively. Another class of mutants display constitutive photomorphogenesis, including depression of gene expression, even in total darkness and flower precociously.

At Jamia Millia, the codA gene from Arthobacter globiformis has been used for transforming *Brassica juncea* cv Pusa jaikisan to enable the latter to synthesize glycinebetaine to enhance its salt and drought tolerance property. Improvement in nutritive value of crop plants, especially amino acid composition, has been a major long-term goal of plant breeding programmes. Towards this end, the cloning of seed albumin gene was initally reported from *Amaranthus hypochondriacus* at JNU. The Am A1 protein is non-allergenic in nature, rich in all essential amino acids and corresponds well with the WHO standards for optimum human nutrition. To improve the nutritional value of potato, the AmA1 coding sequence was successfully introduced and expressed in a tuber-specific and constitutive manner. A striking increase in growth and production of tubers was seen in transgenic populations and also of total protein content with a marked increase in most essential amino acids. This is the first report of a seed albumin gene with a well-balanced amino acid composition being used as a donor protein to develop transgenic plants.Of special interest is the work transferring oxalate decarboxylase gene from a fungus to tomato to improve the nutritional quality.

Engineering Bt genes in rice has been reported from Bose Institute, Kolkata and in brinjal, tomato, cabbage and rice from IARI. Plant geneticists of DU have used both conventional breeding and biotechnological approaches for crop improvement. In mustard the technology of producing hybrid seeds through the use of transgenics containing barnase and bastar genes has been developed. Barnase lines have been developed and are being diversified in the appropriate cultivars. Restorers

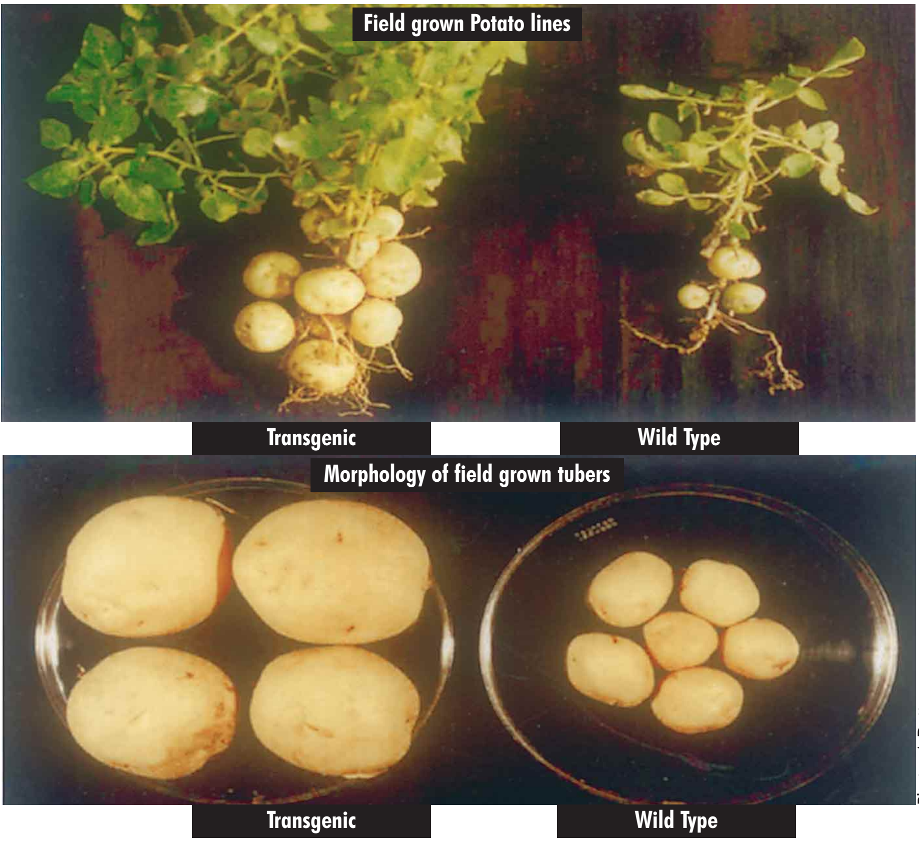

Photo: A.Datta

Designer potato for better yield and nutrition.

- ***AmA1 protein is non-allergenic***
- ***Expression of AmA1 leads to:***

    More than two-fold increase in tuber number,
    3-3.5 fold increase in tuber yield in terms of fresh weight, and
    35-45% increase in total protein content.

with the barstar gene are also available for these lines. Mustard lines with zero erusic acid and zero glucosinolate have been developed and are being tested in multilocation trials.

In cotton, pure lines have been developed for regeneration *in vitro* and used for developing insect resistant transgenics.

Molecular Biology and Medical Research

Advent of new biology has opened up unprecedented opportunities for biomedical research. These techniques have been utilized to identify, clone and sequence genes responsible for pathogenesis of a host of viruses, bacteria and parasites. On the basis of these studies antigens could be identified, monoclonal or polyclonal antibodies produced to purity for developing specific diagnostic tests and useful immunoprophylactic and immunotherapeutic vaccines. This has also helped in unravelling the molecular mechanisms responsible for cell multiplication, differentiation and death, thus providing valuable insights into the fields of developmental biology and oncology. An interesting outcome of these developments was not only to enhance the capabilities of medical researchers but the evolution of a whole generation of biomedical scientists in universities and research institutions.

Contemporary Centres of Biological Research Activity

Amongst the internationally known players in contemporary biology are -- the Indian Institute of Science, Bangalore; Tata Institute of Fundamental Research, Mumbai; the National Centre for Biological Sciences, Bangalore; Jawaharlal Nehru University, New Delhi; Madurai Kamaraj University, Madurai; Pune University, Pune; University of Hyderabad, Hyderabad; MS University, Vadodra; Banaras Hindu University, Varanasi; and the Bose Institute, Kolkata. Equally active are the centrally funded national laboratories such as the Centre for Cellular and Molecular Biology (CCMB) and Centre for DNA Fingerprinting and Diagnostics (CDFD), both in Hyderabad; Indian Institute of Chemical Biology, Kolkata; National Institute of Immunology, New Delhi; Centre for Biochemical Technology, Delhi; Central Drug Research Institute, Lucknow; Institute of Microbial Technology, Chandigarh; and the UNDP-aided International Centre for Genetic Engineering and Biotechnology, New Delhi (which has its twin at Trieste, Italy). Significant agro-biotech research goes on in the ICAR laboratories and state agricultural universities such as the ones at Hyderabad and Pant

Nagar.

Some of the more notable contemporary contributions in the area of modern biology and biotechnology in India are: (I) macromolecular conformation -- both theory and experiments, (ii) protein structure and function, (iii) designer peptides, (iv) glycobiology, (v) procaryotic transcription and drug action, (vi) enzymological approaches to drug design, (vii) vaccines and immunology, (viii) DNA typing and lineage analysis, (ix) transgenic plants, (x) expression vector systems for protein production, (xi) genomics, (xii) functional enzymology, and (xiii) molecular virology.

Several excellent hospitals and medical centres across the country are engaged in research and development activities in medical sciences. Some of these are the All India Institute of Medical Sciences, New Delhi; Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow; Christian Medical College, Vellore; Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram; Postgraduate Institute of Medical Sciences, Chandigarh; the Cancer Research Institute, Mumbai, L.V.Prasad Eye Institute; Hyderabad; Sankara Nethralaya, Chennai; Jawaharlal Nehru Institute of Postgraduate Medical Research, Pondicherry; and Manipal Academy of Higher Education, Manipal. Mention must be made of their work in the areas of neurobiology, cataract, corneal and retinal research, cancer epidemiology and surveys, cardiomyopathy, leprosy, and medical genetics. (see chapter on Medical Sciences).

Biotech Products through Basic Research

With regard to marketing of products, a few notable examples have come through in the last few years which have involved active academic-industry collaboration. There are two companies, both of the medium range, that have been able to produce and market effective hepatitis B vaccine, through collaborations with academic institutions. Other products hitting the market through such combinations are streptokinase, interferon, and a general-purpose salt-inducible expression vector system, which is of great use for both academic and large-scale production of proteins from recombinant DNA technology. This vector has been patented in the US and licensed to a company there for global marketing. In addition there is a whole host of diagnostic kits that have been developed in academic institutions and given to industry for marketing and sales. Amongst the more notable of these is a diagnostic kit that exploits an agglutination method using whole blood to detect the presence of the AIDS virus. Laboratories are working on the development of a cholera vaccine, leprosy vaccine, and a vaccine against the Japanese encephalitis virus. While the leprosy vaccine has already been licensed to a firm, the other two are expected to do so shortly.

Turning to industry, perhaps the most prevalent biotechnology industry in India in almost every major city is that of tissue culture and micropropagation of horticulture and plantation crops. There are several major players such as SPIC, A.V. Thomas, Godrej, but there are also many small-scale micropropagators. It is interesting to note that during the severe winter of 1994-95 in Europe, tulips, the favourite of the Dutch people, were exported in cargo loads by air from Hyderabad to Amsterdam -- a biotechnological twist to the phrase 'carrying coal to Newcastle' (see chapter on Plant Sciences).

Among the major Indian drug firms utilizing modern biology are Ranbaxy, Dr. Reddy's Laboratories, Cadila, Unichem, Cipla and so on. The notable point about these firms is that each one of them is completely home grown and now has full fledged R&D laboratories associated with it. Apart from classical methods of drug discovery, several of them have dived right into the quest for new molecules. Three such new molecules have already appeared on the drug scene and are being licensed to firms outside the country. The firm Biocon, near Bangalore, is a success story in the customproduction of enzymes and biochemicals for specific clients. Astra Zeneca has its research and development centre at Bangalore, where it concentrates on drugs for infective diseases, and screening of candidate molecules.

Today there are a couple of biotechnology suppliers, such as Bangalore Genii (started by a former academic), who supply to academic institutions their molecular biologicals, in competition with companies such as Sigma, Gibco - BRL, and Amhersham. These companies supply enzymes, oligos and probes, offer synthesis and sequencing services for both peptides and nucleic acids, and also offer minor laboratory equipment.

Research Funding and Support

The DBT not only funds extramural research projects but has also started institutions and centres of excellence in areas of modern biotechnology such as the National Institute of Immunology, National Centre for Cell Sciences, Centre for DNA Fingerprinting and Diagnostics, and the National Centre for Brain Research. It also suports university centres of excellence, somewhat in the fashion of the CNRS system in France. The DBT has come out with guidelines on recombinant DNA techniques, transgenics, and other issues (see chapter on Department of Biotechnology).

In addition to the DBT, other governmental agencies such as the Indian Council of Medical Research (ICMR), Indian Council of Agricultural Research (ICAR), Department of Science and Technology (DST), Council of Scientific and Industrial Research (CSIR), and the Bureau of Research in Nuclear Sciences (BRNS), as well as the Universitiy Grants Commission are other sources of research funding in biotechnology in the country. It has been estimated that close to 60% of all grants-in-aid offered by these national agencies goes into areas of modern biology. The governmental funding for science and technology amounts to about 1 per cent of its budget.

Professional associations in the area of modern biology have also been active for over two decades now. Notable amongst these are the Society of Biological Chemists of India (which is the oldest, over 50 years in existence), Indian Society of Cell Biology, Indian Immunological Society, Association of Indian Microbiologists, Indian Biophysics Society, and the informal and yet very effective annual meeting called the Guha Research Conference. These have led to camaraderie and cooperation between investigators within the country so that and scholars, exchange of materials and sharing of equipment has become far more common in the areas of biotechnology and modern biology than in other branches of science in India.