

FIRST INTERNATIONAL CONGRESS OF PLANT MOLECULAR BIOLOGY, HELD AT SAVANNAH, GEORGIA, USA—AN OVERVIEW

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The First International Congress of Plant Molecular Biology was organized by the International Society for Plant Molecular Biology at Savannah, Georgia, from 27 October to 3 November, 1985.

The Congress scientific programme consisted of five Plenary Sessions, 33 concurrent sessions of proffered papers and two sessions of poster presentations.

The Congress was attended by about 2000 participants from 24 countries. The distinctive features of the Congress were: (i) the very broad coverage, which included practically every area of plant molecular biology, (ii) the participation by almost every leading scientist in molecular biology area from all over the world, and (iii) the preponderance among participants of younger scientists and students from the US universities.

In the following is given a summary of the major points of interest.

At the inaugural ceremonies, the highlight of which was the announcement of the award of the University of Georgia bicentennial medallions to Dr. M. S. Swaminathan and Dr. Barbara McClintock. The keynote address titled "Plant Research and World Agriculture" was delivered by Dr. Swaminathan who emphasized on the following points:

—Increasing productivity; reducing cost of production; minimizing risks; enhancing returns; providing greater resource neutrality in the feasibility of adoption of new technologies; providing better consumer and market quality.

In his address he also laid greater emphasis on the ecological sustainability of high yield technology, which would require:

—Conservation of genetic resources; genetic evaluation and utilization; reducing risk from pests, pathogens and weeds; scientific land use and minimization of pollution hazards.

He concluded his talk with an optimistic note suggesting increasing reliance on biotechnology research in terms of practical achievements in agriculture.

Dr Ernest G. Jaworski of the Monsanto Company talked on the University and the Industry Research Partnerships in Biotechnology and emphasized that

biotechnology with all of its exciting ramifications in the fields of agriculture, nutrition, health care and many other fields has stimulated close associations between industry and universities. While industrial support of basic academic science is not especially new, the magnitude of that support has heightened interest and concerns over the relationships. A number of major corporations in the United States and in Europe have established significant programs with universities to support both specific and broad research areas.

Gene expression: Dr R. B. Goldberg of the Department of Biology, University of California at Los Angeles, presented work which showed that expression of soybean seed protein genes is developmentally regulated in tobacco plants. A Ti-plasmid-derived vector was used to transform tobacco plants with soybean lectin and Kunitz trypsin inhibitor genes. These genes are represented approximately once per tobacco genome, while Kunitz trypsin inhibitor gene is expressed in tobacco seeds and leaves, the lectin gene is only expressed in seeds. In addition, four non-seed protein genes flanking the lectin gene were also expressed normally in tobacco indicating that five closely linked soybean genes are regulated correctly during the tobacco life cycle. These studies show that soybean seed protein genes contain *cis*-acting sequences which regulate their developmental-specific expression, and that these genes retain their developmental specificities in nonleguminous plant cells.

Dr Nam-Hai Chua of the Laboratory of Plant Molecular Biology, the Rockefeller University, New York, talked of phytochrome-induction, light regulation, tissue specificity and position effects in the expression of nuclear genes for chloroplast proteins in transgenic plants. They have isolated from peas, three members (E9, 3A and 3C) of the multigene family for the small subunit (*rbcS*) of ribulose-1, 5-bisphosphate carboxylase and from wheat one gene (*whABB.7*) for the chlorophyll *a/b*-binding protein (*Cab*). All the four genes are expressed predominantly in leaves and their expression is regulated by light and/or phytochrome.

Dr R. G. Hermann of the Institute of Botany, the University of Dusseldorf, West Germany, summarized information on "Expression of the Chloroplast Genome (Plastome)". The genetic information of plastids, the plastome, is deposited in a single, highly reiterated circular DNA molecule of approximately 150 kilobase pairs. This chromosome is known to

encode part of the potent organellar translation machinery and components involved in the fundamental process of photosynthesis. The genes are found on both DNA strands and are generally not interrupted. They may overlap but are not organized typical operons, although some clustering can be observed. Their transcripts are not polyadenylated. The synthesis of transcriptionally active RNA generally involves polycistronic transcription and RNA processing. The genes for components of the ATP synthase and cytochrome b/f complex are coordinately expressed in the dark as well as in the light. However, transcription and translation of components for the photosystems I and II appear to be uncoupled (dark/light vs. light) and the individual subunits of these complexes may not appear simultaneously during membrane genesis although photosynthetic activity can be detected early. Dr Christopher J. Leaver of the Department of Botany, University of Edinburgh, talked about "Mitochondrial Genes, Mutations and Male Sterility". The mitochondrial (mt) genomes of higher plants range in size from Ca. 200–2400kb. Only a small fraction of the mtDNA has a coding function as revealed by analysis of RNA transcripts or mitochondrial translation products. Evidence from *in organello* translation experiments suggests that plant mitochondria synthesise between 20 to 30 polypeptides, the majority of which are components of multisubunit enzyme complexes of the inner membrane.

The cytoplasmic male sterile phenotype (CMS) has been shown to be associated with sequence reorganisation of the mitochondrial genome and synthesis of characteristic variant mitochondrial translation products. For example, in one line of sorghum the variant polypeptide has been identified as a higher molecular weight form of subunit I of cytochrome *c* oxidase. DNA sequence analysis indicates the presence of additional amino acids at the C-terminus which accounts for the increase in molecular weight.

Mobile genetic elements: Prof. Peter Starlinger of the Institute of Genetics, University of Cologne, said that in some respects, plant transposable elements resemble those of other organisms. Transposable element *Ac* is 4.5 Kb long, has several open reading frames, terminates in inverted repeats (1 mismatch) and creates a duplication of 8 bp at its site of integration. *Ac* differs from non-plant transposons because at its either end, duplications and inversions (DIRT) are found.

Numerous revertants of transposable element induced mutations have been isolated, cloned and

sequenced. It is shown that DNA sequence is not restored in these revertants. It is thus clear that transposable elements, while walking through the genome, leave footsteps behind and generate the DNA sequence diversity needed in evolution.

Dr Richard B. Flavell of the Plant Breeding Institute, Cambridge, discussed the role of DNA movement in the evolution of DNA sequences in plant genomes: nuclear, chloroplast and mitochondrial. Many duplicated genes have been described within species where the duplicated sequences must have moved following duplication. Also, the presence of dispersed repeats within and between species imply that new families of DNA sequences arise, move among the chromosomes and spread through populations at a high rate during evolution.

The movement and subsequent fixation of DNA sequences are not confined to nuclear chromosomes alone. Sequences in mitochondrial genomes are frequently rearranged. Chloroplast DNA sequences are found in mitochondrial genomes and the nuclei contain sequences also present in mitochondria and chloroplasts.

Dr Elliot M. Meyerowitz of the Division of Biology, California Institute of Technology, discussed the use of P-elements in *Drosophila* transformation. P-elements are a class of transposable elements found in some strains of *Drosophila melanogaster*. These elements get transposed into random locations in the *Drosophila* chromosomes even when recombinant plasmids containing these elements are injected into *Drosophila* embryos under appropriate circumstances. Since only the termini of the element are required for transposition, foreign DNA sequences inserted between the termini can be introduced to the *Drosophila* genome. This technique has allowed identification and separation of sequences that dictate the time and tissue of gene expression. Tissue- and time-specific expression, in *Drosophila*, of *E. coli* β -galactosidase, has been achieved by fusion of the bacterial gene to *Drosophila* *Sgs-3* control sequences.

Plant microbial interactions: Dr Adam Kondorosi from the Institute of Genetics, Biology Research Centre of the Hungarian Academy of Science, summarized information on molecular biology of nitrogen fixation symbiosis. In all fast-growing rhizobia, the *nod* genes are localised on large indigenous plasmids in the vicinity of genes coding for enzyme nitrogenase. In *Rhizobium meliloti*, the essential *nod* genes are arranged into two clusters: one is conserved in many *Rhizobium* species both functionally and at nucleotide

sequence level (*common nod* genes), while the other contains genes determining plant host specificity of nodulation (*hsn* genes). Directed Tn5 mutagenesis of the two clusters resulted in 5 different classes of *Nod*-mutants. In *E. coli* minicells, in *R. meliloti* and *E. coli* *in vitro* transcription/translation systems the *nod* protein-coding regions were delimited and the open reading frames were determined by nucleotide sequencing. On this basis 4 common *nod* genes and at least 3 *nod* genes in the *hsn* region were identified. Physical and genetic analyses of these genes indicate that they are subject to a specific control involving the plant host, and this may be mediated via highly conserved specific sequences found in the 5'-flanking region of most *nod* genes.

Dr Klaus Hahlbrock, Biochemistry Department, Max-Planck Institute for Plant Breeding, Koln, gave an overview of defence reactions of plants to fungal infections. Resistance of plants to potential pathogens is the result of a combination of constitutive and rapidly inducible defence mechanisms. These can formally be divided into structural barrier components and soluble materials, which include antibiotics (phytoalexins) and enzymes which are directly or indirectly involved in the growth inhibition or killing of the invading organism. Studies with rapidly inducible reactions, using both infected tissue from intact plants and plant cell cultures treated with fungal elicitors have shown that at least some of the defense reactions are induced via gene activation. Inducible genes include those encoding enzymes of phenylpropanoid pathway and groups of so-called pathogen-related proteins. All responses studied so far can be triggered not only by various types of pathogens, but also by biotic and abiotic stimuli.

Dr Ab Van Kammen from the Department of Molecular Biology, Agricultural University, Wageningen, The Netherlands, discussed relationship between plant viruses and their hosts. For a number of plant RNA viruses e.g. tobacco mosaic virus (TMV), cowpea mosaic virus (CPMV), brome mosaic virus (BMV) and alfalfa mosaic virus (AIMV), and DNA viruses e.g. cauliflower mosaic virus (CaMV) and maize streak virus (MSV), the complete nucleotide sequence of the genome has been elucidated which has provided detailed understanding of the organization of the viral genome at the molecular level. The main lines of the expression of the information encoded on the genome of these viruses is understood, the virus-specified proteins have for the greater part been identified and there is a growing understanding of the function of these proteins in virus replication.

Application of plant molecular biology to economic problems:

Dr C. J. Arntzen, du Pont de Nemours Co., Willmington, U.S.A., discussed the importance of the tools of molecular biology for use in analysing the basis for resistance to three groups of herbicides: the triazines, the sulfonylureas, and glyphosate. In each case, single amino acid substitutions in target enzymes have been shown to confer high levels of resistance to the herbicides. These studies are providing new insights to our understanding of "small molecule-protein" interactions. The availability of genes which confer resistance to herbicides is also leading to numerous studies of gene transfer to crop species.

Dr C. A. Ryan, Washington State University, Pullman, U.S.A., discussed stress reactions of plants. Plants from *Solanaceae* and *Leguminosae* can respond to attacks of insects and microorganisms by activating genes that code for the synthesis of defense proteins, such as proteinase inhibitors, or for enzyme systems that produce complex defense chemicals, such as phytoalexins. Both classes of genes can be activated in tissues of specific plants by oligo polysaccharides that can be fragmented from either plant or fungal cell walls by hydrolytic enzymes. This suggests that similar fragments may be involved in a universal early recognition system to activate plant defense genes. Genes from two non-homologous proteinase inhibitors, called Inhibitor I and Inhibitor II, have been isolated from potato and tomato genomic libraries and characterized to further understanding the mechanism(s) of their wound-regulation.

Dr W. R. Scowcroft, CSIRO, Canberra, Australia, gave an account of the theoretical aspects of causation of somaclonal variation and its potential for crop improvement activity. Somaclonal variation is a widespread phenomenon in plants regenerated from cell and tissue culture. There is increasing application of somaclonal variation to plant breeding as well as concern about its impact on clonal uniformity during micropropagation and germplasm conservation.

Some somaclonal mutants are not associated with cytologically observable chromosome alterations and these may represent fine-structural changes. Others appear to be the result of genomic rearrangements (translocations, deficiencies) which occur at relatively high frequency.

For plant improvement, the enhanced frequency of genomic rearrangements during culture provide a new option to introgress alien genes from wild relatives

into domesticated crops. This approach along with *in vitro* selection and rapid screening using molecular probes is now being used for disease resistance breeding in wheat.

Plant transformation systems: Dr Thomas Hohn, Basel, Switzerland, evaluated cauliflower mosaic virus as a plant DNA vector. Like other plant viruses, CaMV populations have a high capability to remove inserts that are affecting viral fitness. Therefore, it should be ensured that: sites of insert must be located outside vital viral genes; total length of the hybrid DNA must not exceed the length of wild type DNA; inserts should not interfere with the reverse transcription mode of CaMV genome replication, and inserts must be adapted to the mode of CaMV transcription and translation.

Dr Ingo Potrykus, Basel, Switzerland, gave an account of direct gene transfer to plants and gene isolation from plants. He stated that incubation of protoplasts with a gene under control of plant expression signals provides an efficient, routine method for stable integration of foreign genes into plant genomes without any host range limitation. It also enables direct isolation of single copy genes from plant genomes.

Neomycinphosphotransferase (NPH(3')II) from Tn5 confers resistance to aminoglycosidic antibiotics. They have constructed a selectable hybrid gene by placing the protein coding region under the control of 5'/3' expression signals from CaMV gene VI. This construction has been used to study 'direct gene transfer', and its stable integration and expression through successive generation.

Dr Jeff Schell reported on the use of 'Ti-Plasmid

vectors to study the transfer and regulation of expression of chimaeric genes in plants'. Plant gene-vectors derived from the Ti-plasmid of *A. tumefaciens* were used to introduce a number of chimaeric genes in tobacco plants. These chimaeric genes were constructed to study the involvement of 5' upstream sequences in the regulation of gene expression in plants. It was shown that regulatory sequences derived from the light inducible gene coding for the enzyme chalcone synthase, could be used to obtain light regulated expression of a neomycine phosphotransferase reporter enzyme.

Other reported experiments addressed the problem of tissue specific expression of genes in plants. When leaf-specific genes were tagged by a DNA insertion and reintroduced in potatoes and tobacco, it was shown that the introduced genes were expressed in a tissue specific manner. Another chimaeric gene construction was used to demonstrate that regulatory sequences derived from a *Drosophila* heatshock gene could be used to obtain a heatshock dependent expression of the Tn5 Neomycine phosphotransferase in tobacco plants. It was also reported that cells of potato discs were transformed when co-cultivated with Ti plasmid containing gene for glyphosate resistance. Such cells could be regenerated into whole plants which stably expressed herbicide resistance.

Two compensations on cards for the US \$150 registration fee were a wine and cheese party and a dinner. The wine and cheese party was a disaster; it generated a stampede for food and drink, the like of which I have never seen. My sole compensation for a ruined evening was that it did not occur in India and Indians (mercifully) were not responsible for causing the stampede!