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Announcements

Conference Report

Genetic Engineering in Eukaryotes

From July 26–August 6, a NATO Advanced Study Institute conference was held at Washington State University at Pullman, USA under the title "Genetic Engineering in Eukaryotes". The meeting, organized by P. Lurquin, brought together experts working in the field of plant, yeast and animal genetic engineering, which allowed a useful exchange of information obtained from various organisms.

In the plant field, many reports were concerned with the "natural genetic engineers" *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes,* which transfer DNA to plant cells, thereby causing the Crown Gall and Hairy Root system, respectively. Although the analysis of the Hairy Root phenomenon was started only recently, it is rapidly catching up with the analysis of Crown Gall, as evidenced by reports from Gordon and Huffman (Seattle) on Rh T-DNA structure and homology with plant DNA; Tepfer (INRA, Versailles), with a remarkable account on regeneration of different species from Rh roots; and by the Schell group (Cologne, Brussels, Ghent) on transcription of Rh T-DNA and homology with Ti plasmids. Crown Gall reports included complementation studies with avirulent bacteria mutated in regions outside the T-DNA (Iyer and Nester), and the description of a chromosomal transposon insertion mutant which is both unable to bind to plant cells and avirulent, thereby confirming that binding is an essential step in transformation (Nester). From the Schell group an octopine-producing but otherwise normal potato plant was reported, which was obtained from tumours induced by a transposon insertion mutant (Wöstemeyer). Sciaky (Brookhaven) reported the occurrence of a natural *Agrobacterium* mutant with a chromosomally derived, transposon-like sequence in the same region of the T-DNA as the artificial mutant used by Wöstemeyer. Apparently, *Agrobacterium* has not only invented genetic engineering of eukaryotes before man did, but it also seems to be capable of doing its own transposon insertion mutagenesis!

Reports on two other possible plant vector systems concerned Cauliflower Mosaic Virus (CaMV) and gemini viruses.

Shillito (FMI, Basel) reported results on transfer and phenotypical expression in plant cells of selectable marker genes incorporated in CaMV-derived vectors. These results, although promising, still need a detailed analysis of the supposedly transferred DNA.

The single stranded gemini viruses discussed by Coutts (Imperial College, London) might become important as vectors for monocots, either as a system in itself, or in combination with the Ti plasmid from *Agrobacterium.* Another possible alternative for introducing DNA in plant cells, and especially monocot cells (which are insensitive to *Agrobacterium*), is liposome-mediated DNA transfer. The Lurquin-Kleinhofs group (Pullman) presented recent progress in their approach to develop such a system, which will use plant-adapted bacterial genes packaged in liposomes to complement plant nitrate reductase mutants. The isolation of the relevant genes and their modification for expression in plants is well under way. Highly efficient transfer of viral RNA packed into

liposomes (Lurquin and Rollo, Pavia) show the potentialities of liposomes to carry nucleic acids into plant cells.

Reviews and recent progress in the field of somatic plant cell hybridization and its more sophisticated variant, cytoplasmic genome transfer, was reported by Bourgin (INRA, Versailles) and Máliga (St. Louis). A whole range of new combinations of nuclear and cytoplasmic genomes can be expected in the near future, although stability and viability of these constructions might pose some problems. In this respect, the change in mitochondrial DNA restriction patterns observed upon transfer of mitochondria into heterologous cells represents a challenging problem for the future. Cocking (Nottingham) stressed the importance of a better understanding of the cytological processes involved in fusion.

The subject of yeast and animal cell transformation by various methods was treated by several speakers. Their reports showed which possibilities now exist and which problems remain. A few of these problems as discussed at the meeting are: can the site of integration of the incoming DNA be controlled (by forcing homologous recombination)? The answer for yeast seems to be yes (Williamson, ARCO, Dublin, California; Case, Athens, Georgia) whereas the situation for animal cells is more complex (Howard, NIH, Bethesda). Also, can the fate of the DNA (i.e. stable integration into the chromosomes, transient existence outside the chromosomes or independent, stable replication) be controlled? Much can be learned here from experiments with yeast currently going on with self-replicating centromere-containing vectors (Williamson). Further questions were: how can the efficiency of transformation be enhanced? How can the controlled expression of a normally processed messenger be obtained, especially in heterologous systems? What selectable markers should be used (a problem specifically addressed by Garapin, Pasteur, Paris).

Since the final state of both the DNA of interest and the carrier DNA when inserted in the chromosomes is hardly ever precisely known, this aspect of stable transformation should be kept in mind when transformants are analyzed.

An important new aspect of genetic engineering (Williamson) is the deletion, rather than the addition, of DNA sequences by homologous recombination, as shown for yeast.

McBride (NIH, Bethesda) discussed the special advantages of chromosome mediated gene transfer in animals as opposed to DNA-mediated gene transfer. Chromosome-mediated gene transfer, a field practically unexplored by workers in the plant field, can effectively bridge the gap between the cytological level and the DNA sequence level and allows an intermediate level of gene mapping.

A more exotic animal DNA transfer system is found in the infection of insects by baculoviruses (Miller, Moscow, Idaho). Analysis of this system is progressing rapidly, but transfer of foreign DNA with this potential vector has yet to be carried out. It could become of interest in the study of insect genes.

A most intriguing result was reported by Soriano (CNRS, Orléans) who presented data on the injection into rats of liposomes loaded with DNA coding for insulin. The liposomes were shown to be transported to the liver. There the insulin genes could be detected by hybridization and a specific transient increase in insulin production could be measured.

A final analysis of this novel type of transformation awaits transcription studies to show that the DNA was actually transferred into the liver cells and transcribed as the results suggest.

The discussions were completed by two round-table meetings where especially the practical aspects of DNA transformation were discussed. These included: development of new transformation methods like DEAE-mediated DNA transfer (developed by Sompayac and Danna), variability of the calcium phosphate precipitation technique, relevance of the use of homologous DNA as a carrier, possible existence of "hot spots" of integration, use of histones and of anti-lysosomal factors to protect DNA, the use and possible selection of competent cells, influence of methylation on the expression of the DNA to be transferred, and the effect of the physical state of such DNA. The discussion of these various technical aspects showed that although many cell types have now been demonstrated to be able to take up and express foreign DNA, much is left to be found out on how these processess actually work and, as a consequence, many improvements are still possible with respect to a better control of these events. With continuing technical improvement, it will become clear for which purposes genetic engineering can and cannot be used; and what it can and cannot tell us about the structure of chromosomes, gene regulation, the importance of certain DNA sequences, and the possibilities of stably changing highly complex natural living systems.

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