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Protoplast Mediated Transfer and Molecular Analysis of Cytoplasmic Genomes

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C. Abstract

The following is a short summary of our progress towards identifying the DNA which specifies CMS and how it acts to produce pollen abortion.

1. Organelle genomes in somatic hybrid plants.

The somatic hybrid plants their progeny and the parental lines of petunia used in this project were described by Izhar et al (1983) and Clark et al (1985). Part of the work was supported by a previous BARD project (1-135-79) given to S. Izhar and M.R. Hanson.

The organelle genomes present in Petunia lines derived following somatic hybridization of cytoplasmic male sterile (CMS) and fertile genotypes were evaluated. The chloroplast genome was observed to segregate independently of the CMS trait, eliminating chloroplast DNA as a coding location for sterility (Clark et al., 1985).

Previous work had revealed that mitochondrial DNA (mtDNA) restriction fragments characteristic of both parental lines were present in somatic hybrids (Boeshore et al., 1983). By characterizing a cosmid clone of DNA from a somatic hybrid, Rothenbert et al. (1985) demonstrated that recombination at the molecular level had occurred: DNAs from both parental mitochondrial genomes were present on the same somatic hybrid mtDNA molecule.

A somatic hybrid plant was identified in which mtDNA recombination had occurred between parental genes for the ATPase protolipid subunit (atp9). Both the somatic hybrid recombinant mtDNA and the parental mtDNAs involved in the recombination event were cloned and restriction-mapped. In order to determine whether the parental mitochondrial DNAs normally recombine at the multiple atp9 genes each carries, appropriate hybridization experiments were performed. These experiments indicate that recombination at parental atp9 loci does not routinely occur in parental lines. Thus, recombination can occur in homologous mtDNA regions following protoplast fusion even at regions where parental lines do not normally recombine (Rothenberg and Hanson, 1987).

2. Identification of mtDNA associated with the CMS trait

By comparing mitochondrial genomes from somatic hybrid plants synthesized in S. Izhar's laboratory (Izhar et al., 1983), restriction fragments which segregate with the CMS trait were identified (Boeshore et al., 1985). The mtDNA region correlated with CMS was sequenced (Young and Hanson, 1987). This region carried an abnormal gene consisting of sequences derived from an atp9 gene, a cytochrome oxidase subunit II gene (coxII), and an unidentified gene (now named urf5). A normal atp9 gene from the fertile mtDNA was sequenced for comparative purposes (Young et al., 1986). Analysis of the sequence reveals that the polypeptide specified by the CMS-associated gene could not perform the normal functions of the ATPase protolipid or subunit II of cytochrome oxidase. This information provides testable models for the mechanism of CMS.

The normal atp9 gene and the abnormal fused gene (termed Pcf) share the same 5' flanking region. The transcript termini from the 2 genes were mapped. Both genes have transcript termini mapping to -121, -266, and -522 before the coding region. Transcripts of the atp9 gene were found to be present in approximately the same amount in leaves and anthers, while transcript of the Pcf gene were approximately 5 times more abundant in anthers than in leaves. Thus, the gene associated with CMS is expressed at a higher level in the tissue where the phenotype occurs than in vegetative tissue (Young and Hanson, 1987).

In a study of the inheritance pattern using progeny of unstable sterile somatic hybrids we discovered that in some cases the whole populations consisted of fertile plants. By using mtDNA probe consisting of the fragments that was found to be correlated with CMS it has been found that these progeny lost that fragments and are thus true revertants (Clark and Izhar 1987). This is perhaps the best evidence available to indicate that the Pcf is the gene that induce CMS in petunia.

3. Identification of regulatory sequences in plant mitochondrial DNA

The sequence found at 5' transcript termini of the atp9 gene and the Pcf gene were compared to sequences at transcript termini of other plant mitochondrial genes. Two of the 3 Petunia termini mapped to sequences which were observed to be homologous to sequences in other plant mtDNAs. The sequence at the longest transcript's terminus is homologous to the known yeast mitochondrial transcription initiation signal (Young et al., 1986).

4. Technical development

During the course of this work, effective methods for isolating mitochondria and mtDNA from Petunia were developed (Hanson et al., 1986). Polypeptides synthesized by isolated mitochondria can be observed following in organello protein synthesis. This work is forming the basis of further experiments to study the protein product of the CMS-associated gene.

5. Extra particles in the cytoplasm which can casue CMS

In an attempt to detect viruses, viroids and dsRNA in CMS petunia lines we found no evidence for such particles in the cytoplasm of anther and leaf tissues of such plants (Evenor and Izhar, 1987).

6. Ultrastructural studies of CMS

Observations of anther tissues of fertile and CMS lines using EM showed that the first sign of breakdown in the CMS tissue is deformation of the mitochondrial membrane (Evenor and Izhar, will be reported in the first annual report of the next BARD project).

D. Body of report

E. Description of cooperation

There were several visits of S. Izhar in Hanson's lab. during which the results obtained and the work plan were discussed. In addition these meetings produced the continuation of the BARD project. S. Izhar continued to supply somatic hybrids for molecular analysis and gene action work in Hanson's lab. Mitochondrial DNA probes were supplied by Hanson's lab. to Izhar's lab. for further analysis of segregants of somatic hybrids and for the expression of the Pcf gene in E. coli (Not reported in the present report. Report will be submitted in the next annual report).

Ofra Soferman from Izhar's lab. visited Hanson's lab. for 3 months and Dr. E.M. Clark who completed her Ph.D. in Hanson's lab. spend one year at a post doc. in Izhar's lab. on the project and awarded BARD fellowship for additional 18 months to work in Dr. Y. Gafni's lab. in Bet Dagan.

The cooperation between the two labs produced several joint publications and the project is conducted with full coordination between the two P.I.s.

F. Benefit to agriculture

In this project the transfer of mitochondrial gene which code for CMS has been shown in petunia. This gene was characterized and much of the molecular biology work was carried out and reported. A basis for the biochemistry and physiology of CMS is now available.

This information will be important in future studies and induction of CMS in other crop species. The present study also showed that protoplast fusion can induce recombination of the DNA of the parental lines in mitochondria of the somatic hybrids. This indicate that accumulation of mitochondrial genes should be possible.

So far the petunia CMS system described by us is the most advanced system to solving and describing the phenomenon of CMS in higher plants.

G. List of publications supported by BARD grant

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