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Sequence and expression of a fused mitochondrial gene, associated with *Petunia* cytoplasmic male sterility, compared with normal mitochondrial genes in fertile and sterile plants

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The mitochondrial genome of plants specifies the trait known as cytoplasmic male sterility (CMS). By comparing recombinant mitochondrial genomes present in *Petunia* CMS and fertile somatic hybrids, a mitochondrial DNA (mtDNA) region associated with the sterility phenotype was identified. This CMS-associated mtDNA region carries a gene fusion (termed *Pcf*) which contains coding region homologous to an ATP synthase proteolipid gene (*atp*9), a cytochrome oxidase subunit II gene (*cox*II), and an unidentified reading frame, designated *urf* S. The 5′ flanking sequences are identical to those of a normal *atp*9 gene in the CMS mtDNA; the source of the 3′ flanking sequences has not been identified. S1 nuclease protection experiments have identified three *Pcf* gene transcripts, whose termini map to the same 5′ locations as three transcripts of two normal *atp*9 genes in CMS and fertile plants. In a fertile *Petunia* line, an additional transcribed *atp*9 gene with a divergent 5′ flanking region was identified. Although transcript abundance of homologous *atp*9 genes did not vary significantly between leaves and anthers in CMS and fertile lines, *Pcf* gene transcripts were four to five times higher in anthers than in leaves of the CMS plants.

1. Mitochondrial genomes specify cytoplasmic male sterility (CMS)

In a small number of the plant species that exhibit cytoplasmically inherited male sterility, the mitochondrial genome rather than the chloroplast genome has been conclusively identified as the coding location of this trait. Somatic cell fusion was required to discover which organelle's DNA was associated with cytoplasmic male sterility, because the organelle genomes are inherited together through the maternal line in most plants.

Parental chloroplast genomes usually segregate and mitochondrial genomes recombine after protoplast fusion (reviewed in Hanson 1984; Hanson & Conde 1985; Hanson *et al.* 1985). Somatic hybrid plants regenerated after fusion of protoplasts from CMS and normal fertile plants may contain one or the other parental chloroplast genome and recombinant mitochondrial genomes. In *Nicotiana*, *Petunia*, and *Brassica* somatic hybrids, chloroplast genomes have been found to segregate independently of the CMS trait (Belliard *et al.* 1978; Pelletier *et al.* 1983; Clark *et al.* 1985). For example, in *Petunia*, fertile somatic hybrids were observed to carry the chloroplast genome from the CMS parent and CMS somatic hybrids carried the chloroplast genome from the fertile parent (Clark *et al.* 1985). These somatic hybridization experiments, along with studies of mitochondrial DNA (mtDNA) and proteins

of maize CMS-T plants (Dewey *et al.* (1985) and other authors this symposium), have resulted in general acceptance of the plant mitochondrial genome as the source of the CMS phenotype.

2. Identification of a *Petunia* CMS-associated mtDNA region

The recombinant mitochondrial genomes present in somatic hybrid plants also provide a means to identify DNA regions that segregate with the CMS trait. Sufficient polymorphisms between the CMS and fertile parent's mitochondrial DNAs, as well as a number of CMS and fertile somatic hybrids, are required for use of this strategy. By examining a collection of CMS and fertile somatic hybrid *Petunia* plants produced by Shamay Izhar's laboratory (Izhar *et al.* 1983), two *Bgl*I mtDNA fragments were found to segregate with the CMS trait (Boeshore *et al.* 1985). This analysis was not simple, owing to the large size (at least 350 kilobases (kb)) of the *Petunia* mitochondrial genome, and required the use of a dozen restriction enzymes to produce enough parental-specific DNA bands whose presence or absence could be catalogued in the CMS and fertile plants.

For finer analysis of the two CMS-associated fragments, an mtDNA clone carrying both *Bgl*I fragments were isolated (Boeshore *et al.* 1985). Smaller fragments from this clone were used as probes to delineate the region that was consistently CMS-associated. From the fertile parent's mitochondrial DNA, a λ clone was obtained which was homologous to a portion of the CMS-associated DNA, but diverged in the vicinity of the *Bgl*I site that had originally distinguished the CMS and fertile plants' mtDNA (figure 1). Hybridization analysis of CMS and fertile somatic hybrids' mtDNA revealed that a particular DNA arrangement was present in stable CMS hybrids but not in fertile somatic hybrids (Boeshore *et al.* 1985). None of the probes identified a sequence that was unique to the CMS and not present in the fertile plants' mitochondrial DNA. Instead, restriction mapping could distinguish how certain sequences were arranged in the CMS compared with the fertile genome.

3. Structure of the CMS-associated mtDNA region and related regions from the fertile line's mtDNA

Sequencing of the *Petunia* CMS-associated region (Young & Hanson 1987) has confirmed the unusual mitochondrial DNA arrangement originally detected by restriction mapping. The CMS-associated region carries a fused gene consisting of incomplete coding regions of *atp*9 (the ATP synthase proteolipid subunit gene), *cox*II (cytochrome oxidase subunit II), along with an unidentified reading frame (termed *urf*S). The *cox*II portion of the gene is fused in-frame to the preceding *atp*9 sequences. This gene has been designated *Pcf* (*Petunia* CMS-associated fused).

The homologous mtDNA clone selected from the fertile parent was sequenced near the point of divergence of this clone and the clone carrying the *Pcf* gene. Sequence analysis revealed a normal *atp*9 gene on the fertile mtDNA clone (Young *et al.* 1986), with a coding region of 231 base pairs (bp). The sequence of this *atp*9 gene from −600 to +104 is nearly identical to the *Pcf* gene sequence from −600 to +104 (Young & Hanson 1987). At +105, the *Pcf* gene sequence

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diverges from *atp*9. Homology to the maize *cox*II gene begins at +117. Thus the *Pcf* gene carries less than half of the normal *atp*9 coding region, owing to divergence in the middle and 3′ portion of the coding region. In contrast, the 5′ flanking region of the *atp*9 and *Pcf* genes are indistinguishable by restriction mapping for at least 8 kb (figure 1).

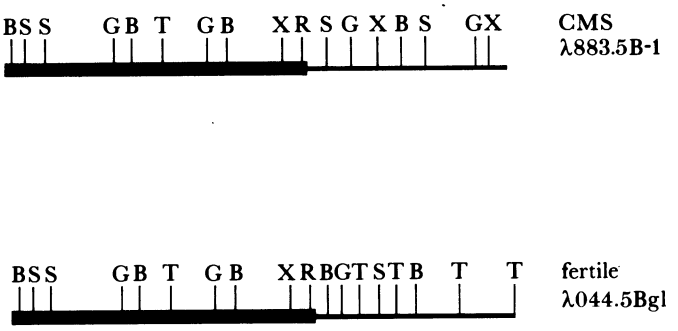

FIGURE 1. Restriction map of mtDNA regions in CMS and fertile lines. Clone λ883.5B-1 carries the *Bgl*I fragments found to be CMS-associated. The clone λ044.5Bgl from the fertile line diverges near an *Eco*R1 (R) site present at the end of the homologous region (heavy bar). The map was derived from Boeshore *et al.* (1985). The *Eco*R1 site shown is the only one which has been mapped; additional *Eco*R1 sites may exist elsewhere on the clones. Clones were completely mapped with *Bam*HI (B), *Bgl*I (G), *Sal*I (S), *Sst*I (S), and *Xho*I (X).

4. COMPARISON OF *PETUNIA atp*9 GENES AND THE *Pcf* GENE

The presence of an abnormal *atp*9 gene in the CMS line's mitochondrial DNA makes it important to inquire into *atp*9-homologous sequences in the CMS line. Two additional *atp*9-homologous regions have been detected in the CMS line's mtDNA (Rothenberg & Hanson 1987*a*). One of these regions has been sequenced and thereby found to carry a normal *atp*9 gene, which is absolutely identical to the *Pcf* gene from −568 to +104 (Rothenberg & Hanson 1988). This normal gene (now termed 88*atp*9-1) from the CMS line diverges slightly from the normal *atp*9 gene in the fertile line, but the predicted amino acid sequences are identical.

Further characterization of *atp*9 genes in the fertile parent's mitochondrial genome have revealed two hybridizing regions in addition to the 04*atp*9-1 gene previously described by Young *et al.* (1986). One of these additional homologous regions has been sequenced, revealing yet another *atp*9 gene (now termed 04*atp*9-2) carrying a normal coding region in the fertile mtDNA (Rothenberg & Hanson 1987*b*). All of the *Petunia atp*9 coding regions predict the same amino acid sequence; however, the second *atp*9 gene in the fertile genome diverges from 04*atp*9-1 at −162 and +252. The third *atp*9-homologous region in the fertile mtDNA and the second region in the CMS line still await cloning and analysis. The sequence homologies and divergence of all of the sequenced *Petunia atp*9 genes and the *Pcf* gene are summarized in figure 2. Table 1 compares two *Petunia atp*9 genes to tobacco (Bland *et al.* 1986) and maize (Dewey *et al.* 1985) *atp*9 genes.

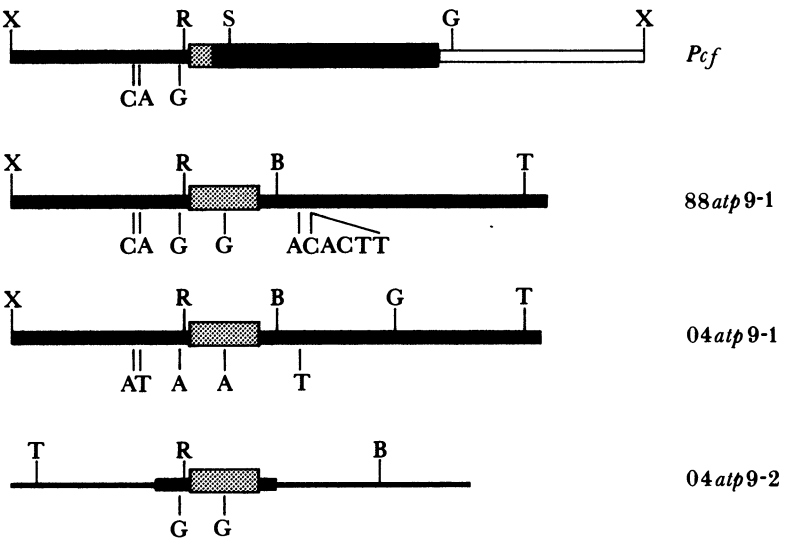

Figure 2. Restriction maps and sequence comparisons of *Pcf* and three *Petunia atp*9 genes. Letters above the bars are symbols for restriction sites (see legend to figure 1). Below the bars are shown the nucleotides that differ between the four genes in homologous regions. Heavy bar indicates homology between all four genes. The thin lines and open bar indicate non-homologous portions of *Pcf* and 04*atp*9-2, stippled area, *atp*9 coding region sequence; large black bar adjacent to *atp*9 sequences in *Pcf*, *cox*II and *urf*S portion of coding region.

TABLE 1. COMPARISON OF *PETUNIA atp*9 GENES WITH THOSE OF TOBACCO AND MAIZE

(Numbers indicate percentage homology. The tobacco–*Petunia* and *Petunia*–*Petunia* flanking-region comparisons include only 196 bases of the 5′ transcribed regions because the reported tobacco sequence ends at this point. The 3′ transcribed regions were compared up to the 3′ transcript termini of 04*atp*9-1 and 88*atp*9-1.)

	flanking region homologies			coding region homologies		
	04*atp*9-1	04*atp*9-2	88*atp*9-1	04*atp*9-1	04*atp*9-2	88*atp*9-1
tobacco	91	58	91	100	> 99	> 99
maize	< 20	< 20	< 20	91	91	91
04*atp*9-1	—	61	> 99	—	> 99	> 99
04*atp*9-2	61	—	61	> 99	—	100
88*atp*9-1	> 99	61	—	> 99	100	—

5. COMPARISON OF THE *Pcf* GENE WITH THE MAIZE *cox*II GENE

The *cox*II-homologous sequences present on the *Pcf* gene do not resemble a normal *cox*II gene (Young & Hanson 1987). The *cox*II-homologous portion of the *Pcf* gene is composed of 159 codons, compared with the 260–274 codons of the maize gene. With respect to the maize *cox*II gene's coding region (Fox & Leaver 1981), *Pcf* carries three major deletions at the 5′ and 3′ end of the first exon and at the 3′ end of the second exon. However, the intron present in the maize and two other monocotyledon's *cox*II genes is absent from the *Pcf* gene. Whereas other dicotyledons, such as pea and *Oenothera*, do not contain introns in the reported *cox*II sequences (Moon *et al.* 1985; Hiesel & Brennicke 1983), preliminary sequence data of *Petunia cox*II genes indicates that an intron is present (K. Pruitt & M. Hanson, unpublished results). For this reason, the maize gene is compared to *Pcf* in figure 3.

In addition to the three major coding region deletions and absence of the intron, the *Pcf* gene diverges from the second maize exon by a six-codon deletion and a ten-codon sequence

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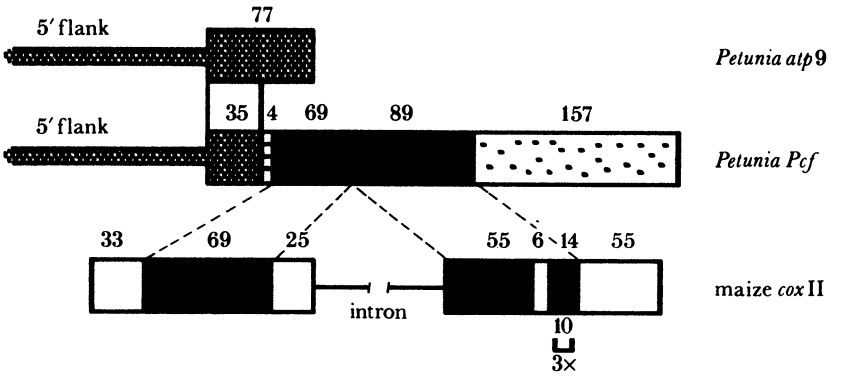

FIGURE 3. Diagram indicating homology of *Pcf* to *Petunia atp*9 and maize *cox*II. The number of codons in each segment is indicated above the bars. (Reprinted from Young & Hanson 1987; copyright Cell Press.)

tandemly repeated three times (figure 3). None of these abnormalities with respect to the maize gene has been found in the wheat, rice, pea or *Oenothera coxII* genes which have been sequenced (Bonen *et al.* 1984; Kao *et al.* 1984; Moon *et al.* 1985; Hiesel & Brennicke 1985). However, in wheat a 193 bp segment of the first *cox*II exon is repeated elsewhere in the mitochondrial genome (Bonen *et al.* 1984).

6. THE UNIDENTIFIED PORTION OF THE *Pcf* GENE

The remaining 157 codons of the *Pcf* gene consist of an unidentified reading frame, designated *urf*S. No significant sequence homologies have been found in the existing nucleic acid and protein databases. Whether the presence of *urf*S is due to the fusion of yet another gene's coding region to *atp*9 and *cox*II sequences awaits sequencing of *urf*S-homologous regions not located in the *Pcf* gene. A clone of the fertile line's mtDNA selected by hybridization with a probe encompassing *urf*S and the 3′ flanking region of *Pcf* is being subjected to mapping and sequence analysis (J. Rasmussen & M. Hanson, unpublished work).

7. TRANSCRIPTION OF THE *Pcf* GENE

Because of the presence of *atp*9 and *cox*II sequences in the CMS line's mtDNA, a *Pcf* gene probe needed to be selected carefully for S1 protection analyses of transcription. Owing to the deletion in the *cox*II-homologous portion of *Pcf* as well as fusion with *atp*9 sequences, suitably stringent conditions for annealing could be chosen so that a probe end-labelled in the abnormal *cox*II region could not be protected by RNA from a normal *cox*II gene. Likewise, to provide an *atp*9-specific probe, a fragment could be end-labelled at a site not present in either the *Pcf* gene or the uncharacterized *atp*9-homologous region of the CMS mtDNA. End-labelled sites and probes could also be chosen to distinguish the three *atp*9-homologous regions in the fertile genome.

Use of these gene-specific probes in S1 protection experiments has resulted in the mapping of the 5′ transcript termini of the *Pcf* gene, the CMS lines' 88*atp*9-1 gene, and the 04*atp*9-1 and 04*atp*9-2 genes from the fertile parent (Young *et al.* 1986; Young & Hanson 1987; Rothenberg & Hanson 1987*b*, 1988). Three transcript termini (at −522, −266, and −121 have been identified in three of the genes: *Pcf*, 88*atp*9-1, and 04*atp*9-1 (figures 4 and 5). The mapping

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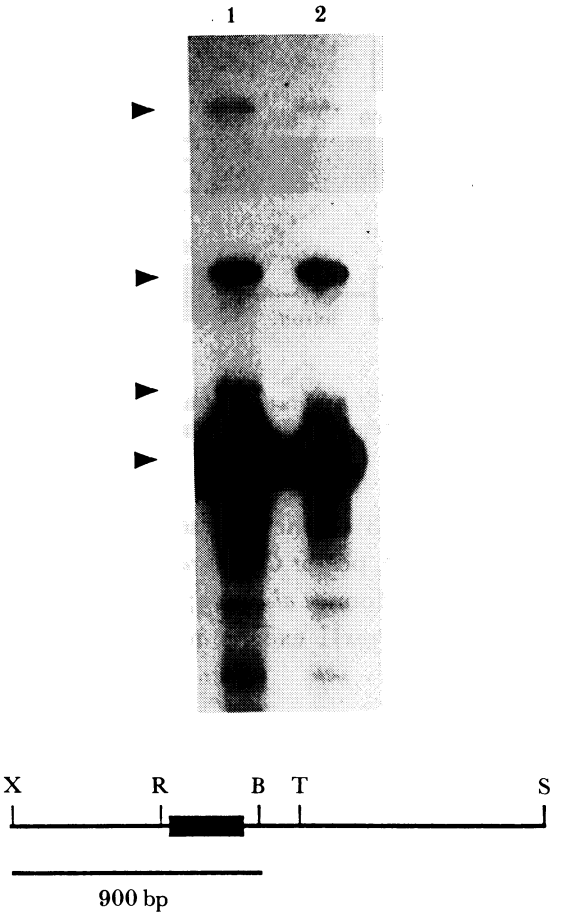

FIGURE 4. Determination of the 5′ ends of the 88*atp*9-1 transcripts by S1 nuclease mapping. A 900 bp *Xho*I–*Bam*HI fragment, ^{32}P-labelled at the *Bam*HI site, was annealed to RNA from the fertile line (1) and RNA from the CMS line (2) at 50 °C and digested with S1 nuclease. The probe was protected by 3688 RNA (2) at −121, −266, and −522 (arrows). An additional protected fragment (−164) is observed when 3704 RNA is annealed to the 3688 probe. Nucleotide polymorphisms between the CMS and fertile lines' *atp*9 genes in this region permit some clipping by S1 nuclease.

of these transcripts to identical locations was not surprising because their 5′ transcribed sequences are nearly identical. The 04*atp*9-2 gene also has a transcript terminus at −121; however, this gene diverges from the other three at −162, and three additional transcript termini have been mapped to −363, −301, and −252. The most abundant transcript from all four genes is the shortest transcript, which maps to −121.

The 3′ transcript termini of the 04*atp*9-1 and 88*atp*9-1 genes have been mapped to a single site, 200 bases past the stop codon (Rothenberg & Hanson 1987*b*, 1988). The 04*atp*9-2 gene has a transcript terminus 229 bases downstream of the stop codon. One or more additional termini for transcripts of this gene may exist further downstream, but we cannot be sure because the probe used was protected for its entire length (Rothenberg & Hanson 1987*b*). A 3′ transcript terminus has been detected approximately 1 kb downstream of the stop codon terminating *urf*S(Young & Hanson 1987).

At the 5′ transcript termini of the *Pcf* gene and the three *atp*9 genes are found short sequences which may be involved in the regulation of transcription (Young *et al.* 1986; Rothenberg &

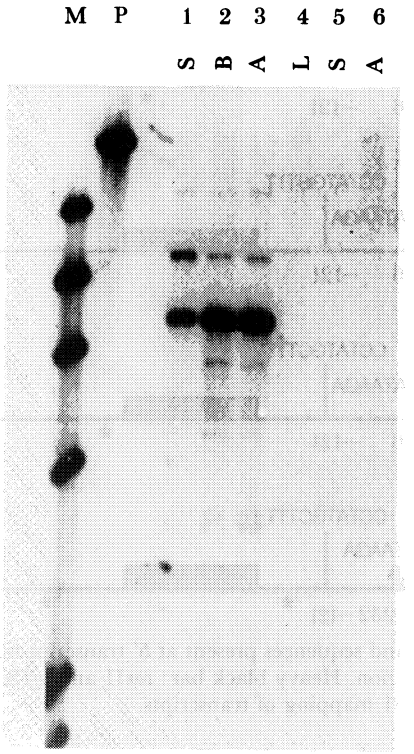

Figure 5. S1 nuclease protection of *Pcf*-specific probe by total RNA from CMS and fertile lines. Lanes 1–3: *Xho*I–*Sal*I probe (see figure 2) protected with equal amounts of RNA from suspension cultures (S), anthers in young buds (B), and mature anthers (A) of CMS line. Lanes 4–6: same probe, not protected by RNA from leaves (L), suspension cultures (S) and anthers (A) of fertile line. M: *Alu*I-digested pBR322 molecular mass marker; P, full-length probe. (Reprinted from Young & Hanson 1987; copyright Cell Press.)

Hanson 1987*b*). All four genes have identical sequences at the terminus of the most abundant transcript. In the divergent 04*atp*9-2 gene, the sequence at terminus −365 is homologous to the −121 terminus of all four genes, and the sequence at −301 is homologous to the −266 termini of the three other genes. Only one 5′ transcript terminus in 04*atp*9-2 (at −252) does not map to a sequence with significant homology to plant or yeast transcript termini. However, near this site are two short inverted repeats that may serve as transcription-regulatory signals. The location and relations of the transcript termini of these four *Petunia* genes are clarified in figure 6.

Whether these putative regulatory sequences are signals for transcription initiation or processing is not known. The −266 consensus sequence has been found in a number of plant mitochondrial genes (Hiesel & Brennicke 1985; Schuster *et al.* 1986). At the time of writing, the *Pcf* gene and the two *Petunia atp*9 genes are the only plant mitochondrial genes that have transcripts mapping to the sequence ATATAGTA, which is nearly identical to the known yeast mitochondrial promoter sequence ATATAAGTA (Biswas & Getz 1986). Successful capping of primary transcripts from plant mitochondria and the development of a transcription system *in vitro* is likely to shed light on these putative regulatory sequences.

Another unique aspect of *Petunia atp*9 is the finding of two different transcribed genes with normal coding regions in the same mitochondrial genome. Pseudogenes with coding region

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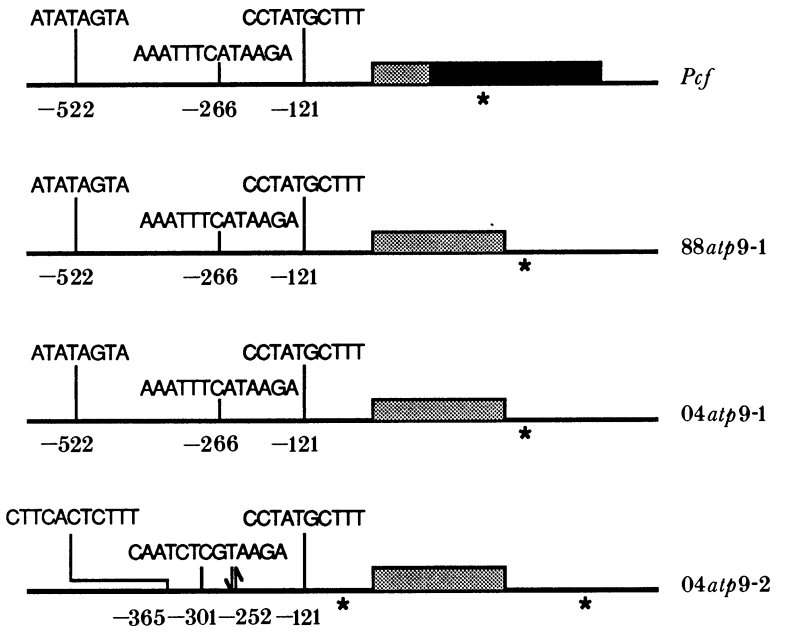

Figure 6 Diagram illustrating locations and sequences present at 5′ transcript termini of *Pcf* and three *Petunia atp*9 genes. Stippled area: *atp*9 coding region. Heavy black bar: *cox*II and *urf*S coding regions. Asterisks indicate location of probe end-labelling for S1 mapping of transcripts.

abnormalities have been found in several plant mitochondrial genomes (Bonen *et al.* 1984; Schuster & Brennicke 1986). Although two or more copies of normal mitochondrial genes have been found in other plant mitochondrial DNAs, in these cases the multiple copies were identical (Isaac *et al.* 1985; Falconet *et al.* 1985), present within repeated sequences where recombination is thought to occur (Palmer & Shields 1984; Lonsdale *et al.* 1984). Hybridization data indicate that the sequenced *Petunia atp*9 genes in the fertile genome do not recombine at detectable levels (Rothenberg & Hanson 1987*a*).

8. Transcript abundance of *atp*9 and *Pcf* in vegetative and reproductive tissues

The relative levels of transcripts of the *Pcf* gene is different tissues of the plant are of interest because of the tissue-specificity of abnormal development. CMS plants of many species grow apparently normally in comparison to their fertile relatives; usually only the development of male reproductive tissue is impaired.

In order to compare transcript abundance of the *Pcf* gene in leaves and anthers, comparative S1 protection experiments were performed with total RNA obtained from leaves and anthers (Young & Hanson 1987). Comparable measures of transcript abundance for the CMS line's 88*atp*9-1 gene and the normal fertile line's 04*atp*9-1 gene were also undertaken, because these genes and *Pcf* share putative 5′ regulatory sequences. Although suspension-culture mitochondrial RNA has also been isolated to analyse transcripts of the *Pcf* gene, in tissue comparisons total RNA was used rather than mitochondrial RNA because of the difficulty in obtaining sufficient quantities of *Petunia* anthers for isolation of mitochondria.

In *Petunia* CMS anthers, the amount of the shortest *Pcf* transcript (−121 terminus) is four

to five times greater in steady state than in leaves (Young & Hanson 1987) (figure 5). The amounts of the −121 88*atp*9–1 and 04*atp*9–1 transcripts do not vary significantly between leaf and anther tissues.

To establish whether the increase in the abundance of the *Pcf* gene transcript has a causal relation to the specificity of cell death in the anther will require additional experiments, including studies of anthers at different stages of development. The abundance of the *Pcf* transcript in the tapetal layer of the anther will be of particular interest, because the first cytological abnormalities in CMS anthers are evident in this tissue, which contains only a small fraction of the cells in the anther (Bino 1985*a*, *b*). Furthermore, the amount of the polypeptide gene product(s) of the *Pcf* gene must be examined carefully, because mitochondrial gene expression can be under translational control (Fox *et al.*, this symposium).

9. Future directions

The identification of a mitochondrial gene that segregates with the CMS phenotype in the dicotyledon *Petunia* opens new routes to understanding the molecular basis of this aberration in pollen development. Further studies of the fused genes found in CMS lines of two monocotyledonous species (Dewey *et al.* 1986; Bailey-Serres *et al.* 1986) may reveal whether abnormal intragenomic recombination events are a common theme in the generation of CMS. A key question for all the CMS systems under study is whether the presence of a fused gene's product actively works to disrupt microspores, or whether the absence of a critical gene product inhibits normal processes. In *Petunia*, sequence data concerning DNA regions homologous to *urf*S and *Pcf* flanking regions will be necessary to understand how this gene arose. Information concerning the predicted *Pcf* polypeptide product will be essential to determine why mtDNA-specified male sterility occurs.

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References

Bailey-Serres, J., Hanson, D. K., Fox, T. D. & Leaver, C. J. 1986 Mitochondrial genome rearrangement leads to extension and relocation of the cytochrome *c* oxidase gene in sorghum. *Cell* **47**, 567–576.

Belliard, G., Pelletier, G., Vedel, F. & Quetier, F. 1978 Morphological characteristics and chloroplast DNA distribution in different cytoplasmic parasexual hybrids of *Nicotiana tabacum*. *Molec. gen. Genet.* **165**, 231–237.

Bino, R. J. 1985*a* Ultrastructural aspects of cytoplasmic male sterility in *Petunia hybrida*. *Protoplasma* **127**, 230–240.

Bino, R. J. 1985*b* Histological aspects of microsporogenesis in fertile, cytoplasmic male sterile and restored fertile *Petunia hybrida*. *Theor. appl. Genet.* **69**, 423–428.

Biswas, T. & Getz, G. S. 1986 Nucleotides flanking the promoter sequence influence the transcription of the yeast mitochondrial gene coding for ATPase subunit 9. *Proc. natn. Acad. Sci. U.S.A.* **83**, 270–274.

Bland, M. M., Levings, C. S. III & Matzinger, D. F. 1986 The tobacco mitochondrial ATPase subunit 9 gene is closely linked to an open reading frame for a ribosomal protein. *Molec. gen. Genet.* **204**, 8–16.

Boeshore, M. L., Hanson, M. R. & Izhar, S. 1985 A variant mitochondrial DNA arrangement specific to *Petunia* stable sterile somatic hybrids. *Pl. molec. Biol.* **4**, 125–132.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Bonen, L., Boer, P. H. & Gray, M. W. 1984 The wheat cytochrome oxidase subunit II gene has an intron insert and three radical amino acid changes relative to maize. *EMBO J.* **3**, 2531–2536.

Clark, E. M., Izhar, S. & Hanson, M. R. 1985 Independent segregation of the plastid genome and cytoplasmic male sterility in *Petunia* somatic hybrids. *Molec. gen. Genet.* **199**, 440–445.

Dewey, R. E., Levings C. S. III & Timothy, D. H. 1986 Novel recombinations in the maize mitochondrial genome produce a unique transcriptional unit in the Texas male-sterile cytoplasm. *Cell* **44**, 439–449.

Dewey, R. E., Schuster, A. M., Levings, C. S. III & Timothy, D. H. 1985 Nucleotide sequence of F_0-ATPase proteolipid (subunit 9) gene of maize mitochondria. *Proc. natn. Acad. Sci. U.S.A.* **82**, 1015–1019.

Falconet, D., Delorme, S., Lejeune, B., Sevignac, M., Delcher, C., Bazetoux, S. & Quetier, F. 1985 Wheat mitochondrial 26S ribosomal RNA gene has no intron and is present in multiple copies arising by recombination. *Curr. Genet.* **9**, 169–174.

Fox, T. & Leaver, C. J. 1981 The *Zea mays* mitochondrial gene coding cytochrome oxidase subunit II has an intervening sequence and does not contain TGA codons. *Cell* **26**, 315–323.

Hanson, M. R. 1984 Cell culture and recombinant DNA methods for understanding and improving salt tolerance of plants. In *Salinity tolerance in plants: strategies for crop improvement* (ed. R. D. Staples), pp. 335–359. New York: John Wiley.

Hanson, M. R. & Conde, M. F. 1985 Functioning and variation of cytoplasmic genomes: lessons from cytoplasmic-nuclear interactions affecting male fertility in plants. *Int. Rev. Cytol.* **94**, 213–267.

Hanson, M. R., Rothenberg, M., Boeshore, M. L. & Nivison, H. T. 1985 Organelle segregation and recombination following protoplast fusion: analysis of sterile cytoplasms. In *Biotechnology in plant sciences: relevance to agriculture in the eighties* (ed. M. Zaitlin, P. Day & A. Hollaender), pp. 129–144. New York: Academic Press.

Hiesel, R. & Brennicke, A. 1983 Cytochrome oxidase subunit II gene in mitochondria of *Oenothera* has no intron. *EMBO J.* **2**, 2173–2178.

Hiesel, R. & Brennicke, A. 1985 Overlapping reading frames in *Oenothera* mitochondria. *FEBS Lett.* **193**, 164–168.

Isaac, P. G., Brennicke, A., Dunbar, S. M. & Leaver, C. J. 1985 The mitochondrial genome of fertile maize (*Zea mays* L.) contains two copies of the gene encoding the α-subunit of the F_1-ATPase. *Curr. Genet.* **10**, 321–328.

Izhar, S., Schlicter, M. & Swartzberg, D. 1983 Sorting out of cytoplasmic elements in somatic hybrids of *Petunia* and the prevalence of the heteroplasmon through several meiotic cycles. *Molec. gen. Genet.* **190**, 468–474.

Kao, T.-H., Moone, E. & Wu, R. 1984 Cytochrome oxidase subunit II gene of rice has an insertion sequence within the intron. *Nucl. Acids Res.* **12**, 7305–7315.

Lonsdale, D. M., Hodge, T. P. & Fauron, C. M.-R. 1984 The physical map and organization of the mitochondrial genome from the fertile cytoplasm of maize. *Nucl. Acids. Res.* **12**, 9249–9261.

Moon, E., Kao, T.-H. & Wu, R. 1985 Pea cytochrome oxidase subunit II has no intron and generates two mRNA transcripts with different 5′ termini. *Nucl. Acids Res.* **13**, 3195–3212.

Palmer, J. D. & Shields, C. R. 1984 Tripartite structure of the *Brassica campestris* mitochondrial genome. *Nature, Lond.* **307**, 434–440.

Pelletier, G., Primard, C., Vedel, F., Chetrit, P., Remy, R., Rousselle, P. & Renard, M. 1983 Intergeneric cytoplasmic hybridization in *Cruciferae* by protoplast fusion. *Molec. gen. Genet.* **191**, 244–250.

Rothenberg, M. & Hanson, M. R. 1987*a* Recombination between parental mitochondrial DNA following protoplast fusion can occur in a region which normally does not undergo intragenomic recombination in parental plants. *Curr. Genet.* **12**, 235–240.

Rothenberg, M. & Hanson, M. R. 1987*b* Differential transcript abundance of two divergent ATP synthase subunit 9 genes in the mitochondrial genome of *Petunia hybrida*. *Molec. gen. Genet.* **209**, 21–27.

Rothenberg, M. & Hanson, M. R. 1988 A functional mitochondrial ATP synthase proteolipid gene produced by recombination of parental genes in a *Petunia* somatic hybrid. *Genetics, Princeton* **118**, 155–161.

Schuster, W. & Brennicke, A. 1986 Pseudocopies of the ATPase α-subunit gene in *Oenothera* mitochondria are present on different circular molecules. *Molec. gen. Genet.* **204**, 29–35.

Schuster, W., Hiesel, R., Isaac, P. G., Leaver, C. J. & Brennicke, A. 1986 Transcript termini of messenger RNAs in higher plant mitochondria. *Nucl. Acids Res.* **14**, 5943–5954.

Young, E. G., Hanson, M. R. & Dierks, P. M. 1986 Sequence and transcription analysis of the *Petunia* mitochondrial gene for the ATP synthase proteolipid subunit. *Nucl. Acids Res.* **14**, 7995–8006.

Young, E. G. & Hanson, M. R. 1987 A fused mitochondrial gene associated with cytoplasmic male sterility is developmentally regulated. *Cell* **50**, 41–49.

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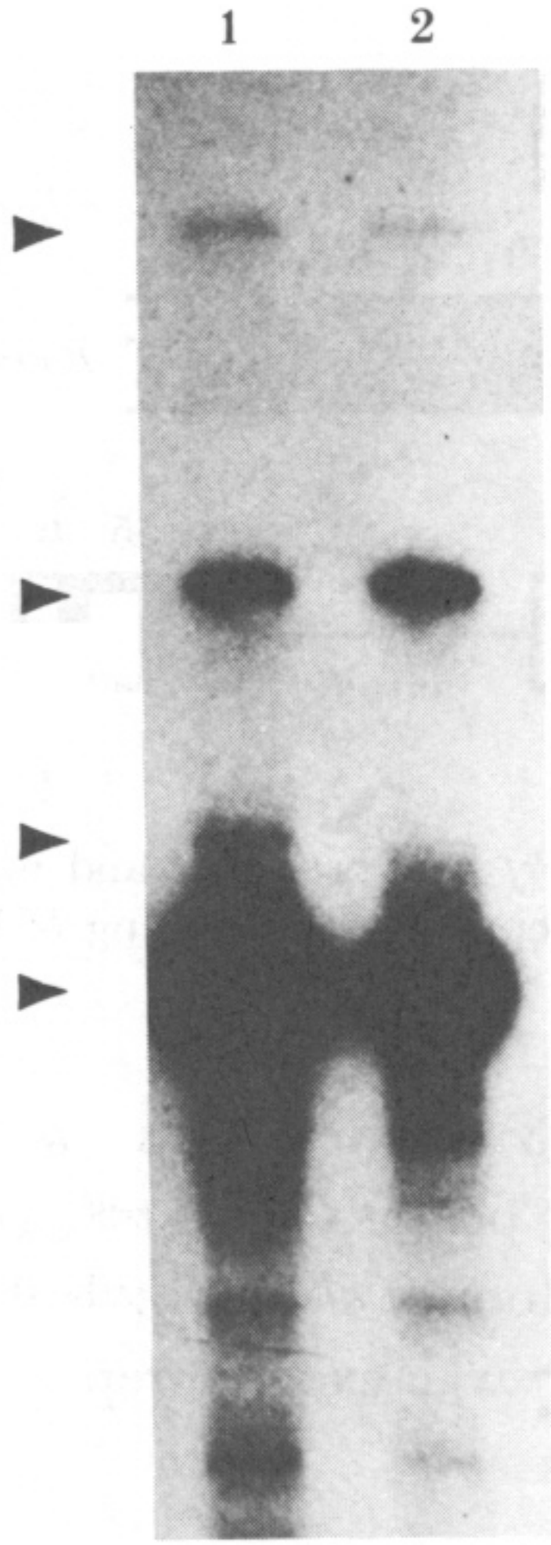

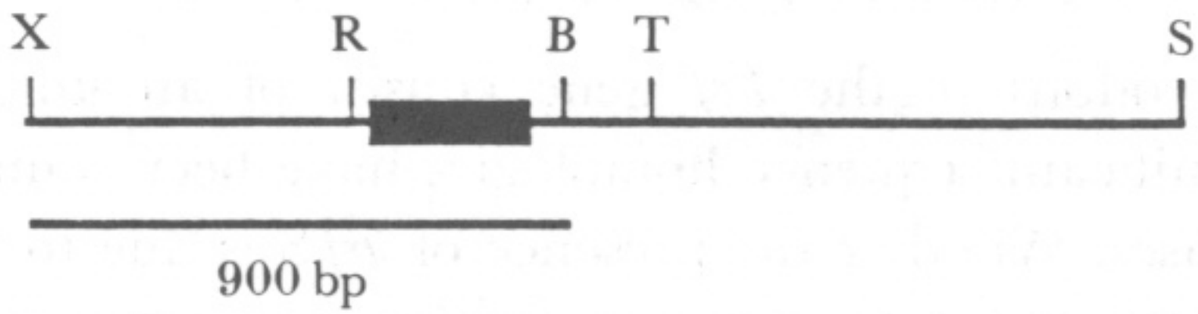

FIGURE 4. Determination of the 5′ ends of the 88*atp*9-1 transcripts by S1 nuclease mapping. A 900 bp *Xho*I–*Bam*HI fragment, ^{32}P-labelled at the *Bam*HI site, was annealed to RNA from the fertile line (1) and RNA from the CMS line (2) at 50 °C and digested with S1 nuclease. The probe was protected by 3688 RNA (2) at −121, −266, and −522 (arrows). An additional protected fragment (−164) is observed when 3704 RNA is annealed to the 3688 probe. Nucleotide polymorphisms between the CMS and fertile lines' *atp*9 genes in this region permit some clipping by S1 nuclease.

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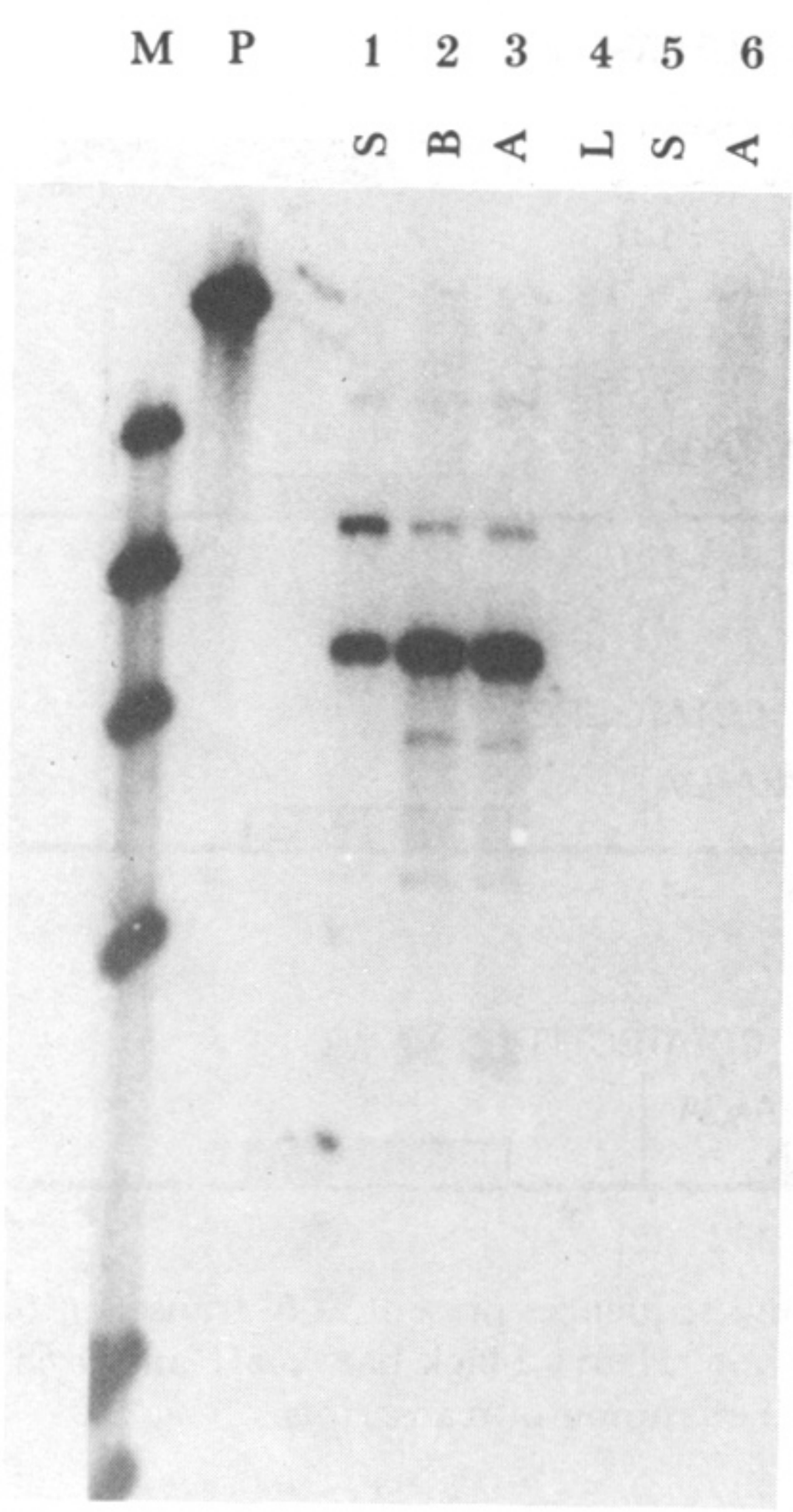

Figure 5. S1 nuclease protection of *Pcf*-specific probe by total RNA from CMS and fertile lines. Lanes 1–3: *Xho*I–*Sal*I probe (see figure 2) protected with equal amounts of RNA from suspension cultures (S), anthers in young buds (B), and mature anthers (A) of CMS line. Lanes 4–6: same probe, not protected by RNA from leaves (L), suspension cultures (S) and anthers (A) of fertile line. M: *Alu*I-digested pBR322 molecular mass marker; P, full-length probe. (Reprinted from Young & Hanson 1987; copyright Cell Press.)

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