Mol Gen Genet (1991) 227:369–376
002689259100185Z

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Cytoplasmic male sterility in sunflower is correlated with the co-transcription of a new open reading frame with the *atpA* gene

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Received September 10, 1990 / February 6, 1991

Summary. The organization and expression of the mitochondrial (mt) genome of fertile, male-sterile and restored lines of *Helianthus annuus* and of *H. petiolaris* were compared to identify alterations which might lead to cytoplasmic male sterility (CMS). The mtDNAs of fertile and male-sterile lines differ by an 11 kb inversion and a 5 kb insertion. The rearrangements seem to be the result of recombination events within an inverted repeat of 261 bp. Detectable alterations in the transcript pattern of the rearranged mtDNA regions are restricted to the *atpA* locus. The male-sterile line *CMSBaso* shows three additional transcripts of the *atpA* locus of about 2500, 1200 and 250 nucleotides which are not detectable in *Baso*. However, the coding sequences of the *atpA* gene are entirely identical in the fertile line *Baso* and the male-sterile line *CMSBaso*. But a new open reading frame (*orfH522*) of 522 nucleotides is co-transcribed with the *atpA* gene as an additional larger transcript of about 2500 nucleotides in *CMSBaso*. *orfH522* is also included in a second additional transcript of about 1200 nucleotides. The predicted translation product of *orfH522* might play a role in CMS in sunflower.

Key words: Cytoplasmic male sterility – Sunflower – *atpA* – *orfH522* – Co-transcription

Introduction

Cytoplasmic male sterility (CMS) is a maternally inherited trait in which higher plants fail to produce functional pollen but maintain female fertility. It is thought that CMS arises from an incompatibility between nucleus and cytoplasm (Hanson and Conde 1985). In several species a correlation between CMS and rearrangements in the mitochondrial DNA (mtDNA) has been observed whereas the chloroplast DNA (cpDNA) was collinear in male-fertile and male-sterile lines (Leaver and Gray 1982; Clark et al. 1985). Rearrangements in the mtDNA lead to CMS-specific alterations in transcription and/or translation in mitochondria of maize (Dewey et al. 1986, 1988; Wise et al. 1987), *Petunia* (Rasmussen and Hanson 1989; Young and Hanson 1987; Nivison and Hanson 1989), radish (Makaroff et al. 1989), sorghum (Bailey-Serres et al. 1986) and sunflower (Siculella and Palmer 1988).

CMS in sunflower (*CMS89*), originating from an interspecific cross of *Helianthus petiolaris Nutt.* with *H. annuus L.*, was first described by Leclercq (1969). As in all other species so far investigated, CMS in sunflower is correlated with rearrangements in the mtDNA (Leroy et al. 1985; Crouzillat et al. 1987; Siculella and Palmer 1988). Rearrangements in the isonuclear lines *HA89* and *CMS89* are restricted to a mtDNA region of about 17 kb, whereas the remaining 94% of the mtDNAs are collinear (Siculella and Palmer 1988).

To investigate the molecular origin of CMS in *H. annuus* in more detail we examined the organization and the transcription of the mtDNA of isonuclear fertile and male-sterile lines, of corresponding fertility-restored lines and of *H. petiolaris*. Despite the altered transcriptional pattern of the *atpA* gene in *Baso* and *CMSBaso*, the *atpA* coding region is entirely identical in both lines, but due to rearrangements in the mtDNA a new open reading frame, *orfH522*, is co-transcribed with the *atpA* gene in male-sterile lines. The possible role of the transcription of *orfH522* in CMS in sunflower will be discussed.

Materials and methods

Plant material. The lines *Baso*, *HA89*, *CMSBaso*, *CMS89*, *RHB4* and *RHB5* of *H. annuus L.* were provided by Prof. W. Friedt (Institut für Pflanzenzüchtung, Justus-Liebig Universität, 6300 Giessen, FRG) and *H. petiolaris Nutt.* (INRA code: 199) by Dr. Serieys (INRA breeding station, Chemin de Mezouls, 34130 Mauguio, France). The genetic constitution of the lines investigated is illustrated in Table 1. *CMSBaso*, which was derived

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Table 1. Genetic constitution of the *Helianthus* lines investigated

Line	Fertility	Cytoplasm	Nuclear DNA
HA89	Fertile	*H. annuus*	*HA89*
Baso	Fertile	*H. annuus*	*Baso*
CMS89	Male sterile	*H. petiolaris*[a]	*HA89*
CMSBaso	Male sterile	*H. petiolaris*[a]	*Baso*
RHB4	Restored	*H. petiolaris*[a]	*Baso* × sf29/82GG
RHB5	Restored	*H. petiolaris*[a]	*Baso* × sf34/84GG
H. petiolaris	Fertile	*H. petiolaris*	*H. petiolaris*

[a] Cytoplasm originating from an interspecific cross of *H. petiolaris* and *H. annuus* differ in mitochondrial DNA organization from the cytoplasm of *H. petiolaris*

from *CMS89*, has the cytoplasm of *CMS89* but has a different nucleus. The fertility-restored lines *RHB4* (hybrid 138/83/GG) and *RHB5* (hybrid 160/83/GG) were derived from crosses of *CMSBaso* with two different restorer lines.

Callus was made from hypocotyl fragments of 3-week-old green plants of *Baso*, *CMSBaso* and *H. petiolaris*. Callus was grown on a modified Murashige and Skoog medium (1962) containing 1 g sucrose, 0.8 mg 6-benzylaminopurine, 1 mg 1-naphthaleneacetic acid and 0.8 g Difco bacto agar per l and transferred every 3 weeks.

Isolation of mtDNA. Etiolated 8-day-old seedlings (20 g) were homogenized in 200 ml ice-cold extraction buffer (50 mM TRIS-HCl pH 7,4, 0.3 M mannitol, 1 mM EGTA, 1 mM $MgCl_2$, 0.5% polyclar AT, 0.1% BSA, 0.1% cysteine, 0.15%, β-mercaptoethanol) for 2×4 s with a Waring blender at low speed and passed through four layers of muslin cloth. After centrifugation at $3000 \times g$ at 4° C for 5 min, the pellet was discarded and the supernatant again centrifuged at $15000 \times g$ at 4° C for 15 min. The crude mitochondrial pellet was resuspended in 2 ml DNAse buffer (50 mM TRIS-HCl pH 7.4, 0.3 M mannitol, 10 mM $MgCl_2$, 0.05% BSA, 50 µg/ml DNAse I) incubated at 4° C for 2 h and then lysed in DNAse buffer with 1% SDS, 50 mM EDTA and 20 µg/ml proteinase K for 30 min at 65° C. CsCl density gradient centrifugation of the crude lysate with ethidium bromide results in two distinct DNA bands. The upper band consists of mtDNA whereas the lower, much weaker band is made up of traces of nuclear DNA. After butanol extraction of ethidium bromide and phenol extraction the mtDNA was used for cloning.

Molecular cloning of the mtDNA. MtDNA of the lines *Baso* and *CMSBaso* was digested with *Sal*I or *Asp*718 and cloned in pUC18 according to Maniatis et al. (1982). Colony hybridization, plasmid isolation, electrophoresis and Southern blotting were done as described (Maniatis et al. 1982). Clones containing the *atpA* gene or the *cob* gene were identified with gene probes from *Oenothera* (Schuster and Brennicke 1986, 1985).

DNA sequencing. The *atpA* gene and parts of the rearranged mtDNA region of *Baso* and *CMSBaso* were subcloned in pUC18. Regions of interest were sequenced using the dideoxy chain termination method of Sanger et al. (1977). Sequences of both strands were determined with overlaps at restriction sites. Computer analyses were performed using the "Kröger menü" (Kröger and Kröger-Block 1984).

RNA isolation, electrophoresis, transfer and hybridization. Total RNA of 8-day-old etiolated seedlings was purified by the CTAB procedure (Taylor and Powell 1982). Total RNA of callus was isolated using the guanidinium chloride extraction method described by Herdenberger et al. (1990) and separated from polysaccharides by a modified CTAB procedure (Rogers and Bendich 1988). MtRNA of etiolated seedlings was obtained from mitochondria isolated as described above. After digestion of extramitochondrial DNA, the mitochondria were further purified from the 21%/45% interphase of a discontinous Percoll density gradient (13.5%/21%/45% Percoll in 10 mM tricine-KOH pH 7.2, 0.3 M mannitol, 10 mM EDTA). MtRNA was extracted using the method of Stern and Newton (1986). Formaldehyde gel electrophoresis was done according to a modified method of Maniatis et al. (1982). Heat-denatured RNA with 0.5 µg ethidium bromide in each sample was fractionated in 1.2% agarose gels containing 0.7 M formaldehyde. The RNA was transferred to nitrocellulose by capillary blot with $20 \times$ SSPE (0.2 M NaH_2PO_4, pH 7.4, 3 M NaCl, 20 mM EDTA) overnight and the nitrocellulose was baked at 80° C for 2 h. The gene probes were labelled by random priming using α-[^{32}P]dATP and hybridized to the nitrocellulose blots as described by Maniatis et al. (1982). Nitrocellulose filters were washed for 1 h in several changes of $1 \times$SSC, 0.5% SDS at 65° C. RNA sizes were estimated using the RNA ladder (0.24–9.5 kb) from Bethesda Research Laboratories as marker.

Results

Organization of the mtDNA and of the atpA *locus of sunflower*

The mtDNA organization of fertile (*HA89*, *Baso*), male-sterile (*CMS89*, *CMSBaso*) and restored lines (*RHB4*, *RHB5*) of *H. annuus* and of *H. petiolaris* were compared by restriction fragment length polymorphism (RFLP) and Southern blot analysis to identify differences which might be correlated with CMS. The mtDNA arrangement of all lines of *H. annuus* investigated depends only on the cytoplasm and is independent of the nuclear background. The fertile lines *HA89* and *Baso*, carrying the cytoplasm of *H. annuus*, show identical mtDNA organizations. Also, the lines of *H. annuus* containing the cytoplasm of *H. petiolaris*[a] (*CMS89*, *CMSBaso*, *RHB4* and *RHB5*) seem to be identical in mtDNA organization. Fertility-restoration does not change the mtDNA arrangement.

Few differences in restriction fragment pattern can be detected between fertile and male-sterile lines. South-

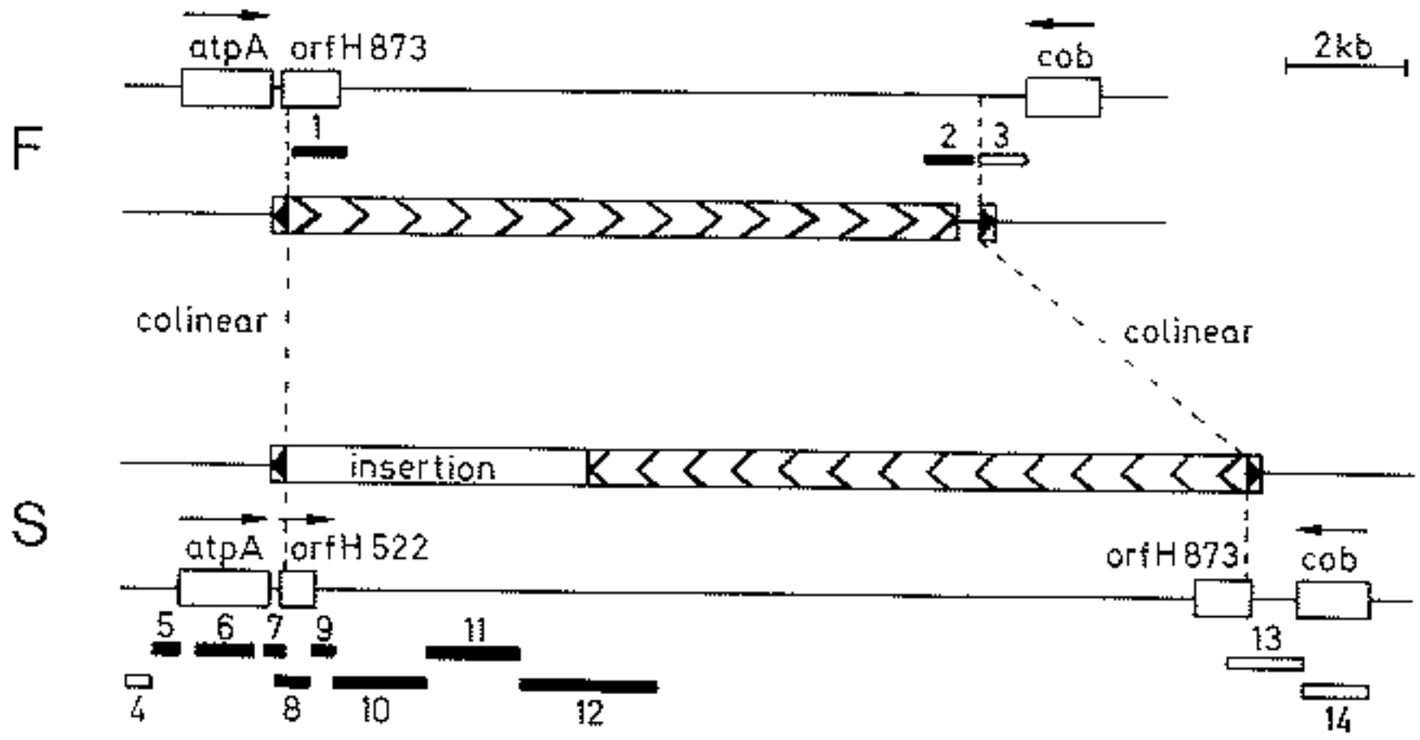

Fig. 1. Comparison of the rearranged mitochondrial (mt) DNA region of sunflower. The mtDNA of the fertile line *Baso* (F) and the male-sterile line *CMSBaso* (S) differ in an 11 kb inversion (*arrowheads*) and a 5 kb insertion. The rearranged mtDNA region is flanked by an inverted repeat (*solid arrows*) of 261 nucleotides. The *small arrows* above the *atpA* gene, the *cob* gene and *orfH522* (*open boxes*) indicate their direction of transcription. There are no transcripts of *orfH873* detectable. Below the mtDNA the origin of the gene probes 1–14 used for Northern hybridization to mtRNA and to total RNA (*solid boxes*) or to total RNA (*open boxes*) of etiolated seedlings is indicated

ern blot analysis with different heterologous mitochondrial gene probes only show differences in the signal pattern of male-sterile and fertile lines when probed with *atpA* (Schuster and Brennicke 1986) or *cob* (Schuster and Brennicke 1985) gene sequences (unpublished results).

The mtDNA organization of the lines investigated seems to be identical in restriction fragment pattern and Southern blot analysis to those lines described by Leroy et al. (1985), Crouzillat et al. (1987), and Siculella and Palmer (1988). According to Siculella and Palmer (1988), rearrangements in the mtDNA of the sunflower lines *HA89* and *CMS89* are restricted to a region of about 17 kb the ends of which are defined by the *atpA* and *cob* genes. We cloned the mtDNA of etiolated seedlings of *Baso* and *CMSBaso* using the restriction enzymes *Sal*I and *Asp*718 and compared the structures of the rearranged mtDNA region, including the *atpA* and the *cob* gene sequences, by gene walking and restriction mapping (Fig. 1).

The mtDNA of *Baso* and *CMSBaso* differ in an 11 kb inversion and a 5 kb insertion/deletion. Southern hybridization experiments using mtDNA fragments from the 5 kb insertion/deletion indicate that this sequence is absent from the mtDNA of fertile lines of *H. annuus* and from *H. petiolaris* (data not shown). Weak hybridization signals which are detectable in all lines might be due to short repeated sequences. The 5 kb region is an insertion of unknown origin because it is absent in the mtDNA of both parental lines. Also, the mtDNA organization of *H. petiolaris* and of the fertile lines of *H. annuus* seems to be collinear for at least 7 kb in the 5′ and the 3′ flanking regions of the *atpA* gene (data not shown).

The upstream region of the *atpA* gene is collinear for at least 7 kb in all lines investigated of *H. annuus* and in *H. petiolaris* (data not shown). Up to at least 900 bp of the 5′ flanking sequences and the entire coding sequences of the *atpA* gene are identical in the fertile line *Baso* and the male-sterile line *CMSBaso* (data not shown). The *atpA* gene includes 1530 nucleotides encoding a polypeptide with a molecular mass predicted from the DNA sequence of about 55474 Da (Köhler et al. 1990; EMBL accession number X53537 and X55963).

The homology between the *atpA* loci of *Baso* and *CMSBaso* stops 194 nucleotides downstream of the *atpA* stop codon. The sequence homology ends with a region that is homologous to the first 30 nucleotides of *orfB* of *Oenothera* (Hiesel et al. 1987; Fig. 2). In *CMSBaso* the homology to *orfB* is extended to 57 bp.

Different open reading frames start with the region of homology to *orfB* in *Baso* and *CMSBaso*. In *Baso*, an open reading frame of 873 nucleotides (*orfH873* in Fig. 1, nucleotides 1698–2570 in Fig. 2) begins downstream of the *atpA* gene. In *CMSBaso*, *orfH873* is located 659 nucleotides downstream of the *cob* gene. Hybridization to total RNA or mtRNA of etiolated seedlings, using a probe containing nucleotides 1794–2197 of the open reading frame (Fig. 1, probe no. 1), failed to detect any transcript corresponding to *orfH873*. Due to rearrangements downstream of the *atpA* gene a new open reading frame of 522 nucleotides (*orfH522*, Figs. 1, 2) is present in the male-sterile line *CMSBaso*. The first 57 bp of *orfH522* are homologous to *orfB* of *Oenothera*. All following sequences of *orfH522* show no homology to sequences in the EMBO data library, however, 11 nucleotides downstream of *orfH522* a sequence of 45 nucleotides is homologous to the beginning of the *atp9* gene of sunflower (Recipon 1990).

An imperfect inverted repeat of 25 nucleotides, which could form a stem-loop structure, is present downstream of the *atpA* gene of the fertile line *Baso*, but is missing in the male-sterile line. The *cob* gene and its 5′ flanking region are collinear for at least 6 kb in *Baso* and *CMSBaso*. The homology ends 688 bp downstream of the *cob* gene with a region that represents an inversion of the 3′ region of the *atpA* locus. This inverted repeat (IR) of 261 bp flanks the rearranged mtDNA region in both lines (Fig. 1; nucleotides 1467–1727 in Fig. 2). The

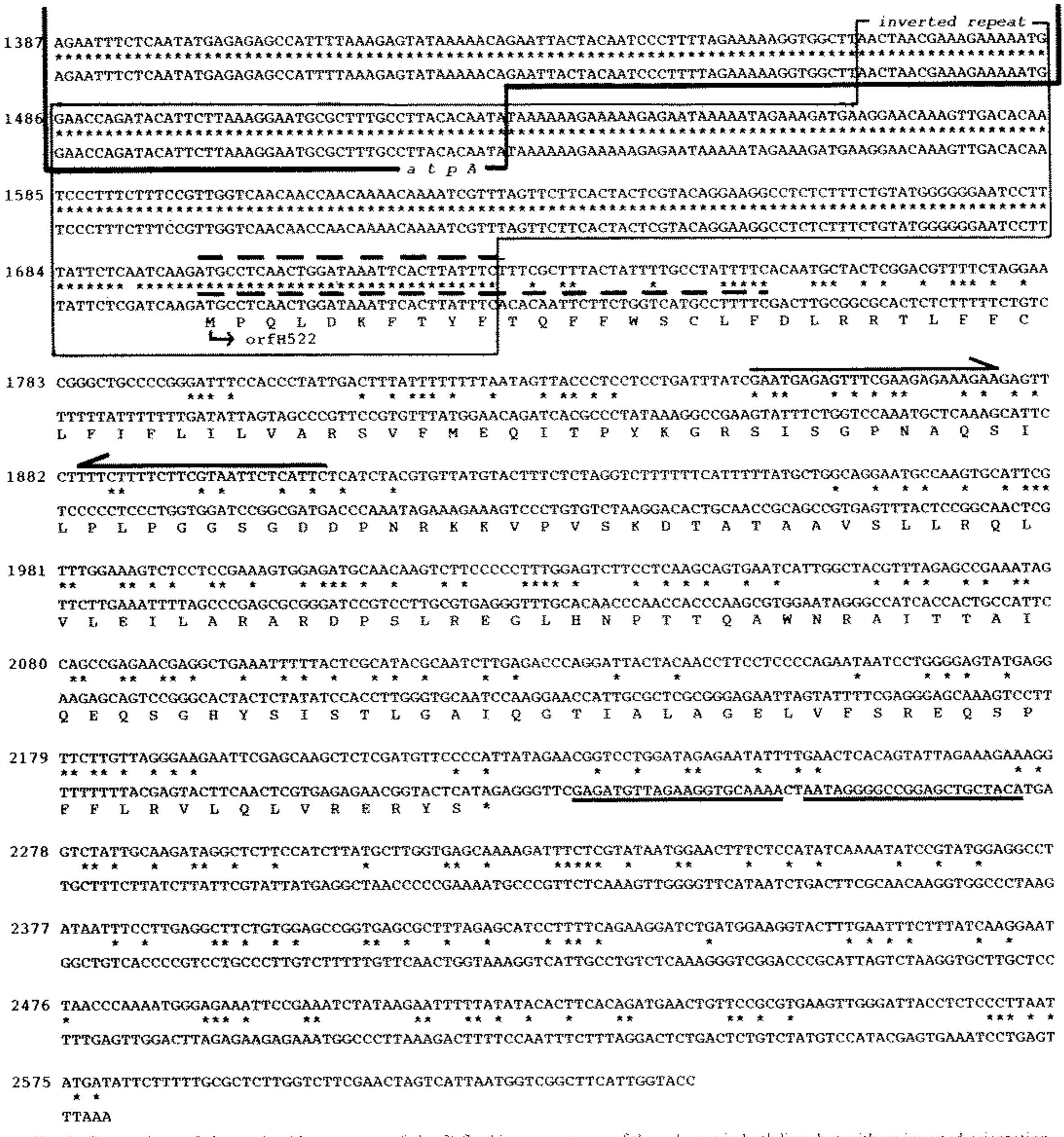

Fig. 2. Comparison of the nucleotide sequence of the 3′ flanking sequences of the *atpA* locus. Alignment of the 3′ flanking sequences of the *atpA* locus of the fertile line *Baso* (top line) and the male-sterile line *CMSBaso* (bottom line). The numbering of the nucleotides starts from the predicted translation start of the *atpA* gene. Sequence homologies between the two lines are indicated by *asterisks*. The *bold framed* region at the beginning represents the end of the coding sequences of the *atpA* gene. The *light framed box* marks a sequence of 261 nucleotides that is also present downstream of the *cob* gene in both lines but with an inverted orientation (inverted repeat, IR). Regions of homology to the *orfB* from *Oenothera* or the *atp9* gene of sunflower are marked with *dotted lines* above or with *solid lines* beneath the sequences. An incomplete IR of 25 nucleotides located downstream of the *atpA* gene of the fertile line is *overlined* with *horizontal arrows*. The amino acid sequence of an open reading frame of 522 nucleotides (*orfH522*), which is only present in the male-sterile line, is indicated below the coding region

IR contains the last 64 nucleotides of the *atpA* coding region and its downstream sequences. The last 30 nucleotides of the IR are homologous to *orfB* of *Oenothera*. The IR is also found upstream of the *coxIII* gene in the hybrid seed line *H. annuus Gloriasol* (Quagliariello et al. 1990).

Transcript analysis of the rearranged mtDNA region. Northern blots with probes covering the insertion and flanking regions of the rearranged mtDNA of *Baso* and *CMSBaso* were performed to investigate whether there are differences in transcript pattern between the fertile and the male-sterile lines which could be responsible for CMS in sunflower. The origin of the probes, which were used for hybridization to mtRNA and/or total RNA of etiolated seedlings, is illustrated in Fig. 1 below the mtDNAs.

Only the *atpA* locus and the downstream region of the *cob* gene show differences in transcript pattern between *Baso* and *CMSBaso*. Both probes covering the downstream region of the cob gene (probes 3 and 13 in Fig. 1) contain the IR of 261 bp. Cross-hybridization of the IR to the transcripts of the *atpA* gene leads to the same differences in transcript pattern as observed for the *atpA* gene (data not shown). Using as probe the *cob* gene of *Oenothera*, which maps upstream of the IR, all lines show only one transcript of about 2200 nucleotides (data not shown). Using probes covering the downstream region of the IR (probes 1 and 2 in Fig. 1) no transcripts are detectable at all.

The *atpA* locus is the only other region which shows differences in the transcript pattern between *Baso* and *CMSBaso* within the mtDNA regions investigated. In etiolated seedlings of the male-fertile line *Baso* only one transcript of the *atpA* gene of about 1700 nucleotides is detectable (Fig. 3). In contrast, the male-sterile line *CMSBaso* shows three additional transcripts of the *atpA* locus besides the transcript of about 1700 nucleotides. An additional larger transcript of about 2500 nucleotides is visible in the male-sterile line when probes nos. 5 (nucleotides −290 to 4), 6 (345–1289), 7 (1466–1754), 8 (1647–2191), or 9 (2215–2576) are used. A second additional transcript of about 1200 nucleotides hybridizes to probes covering the IR or the downstream region of the *atpA* gene of the male-sterile line (probes 7, 8 or 9). The third additional transcript of about 250 nucleotides is only detectable with a probe located downstream of *orfH522* (probe 9). The transcripts of about 3100 nucleotides and of about 500 nucleotides, which can be observed in both lines with probe no. 9, seem to be the result of cross-hybridization to the transcripts of the *atp9* gene. A region of 45 nucleotides of the probe is homologous to the *atp9* gene of sunflower.

A probe covering the 5′ flanking region of the *atpA* gene from nucleotides −290 to −700 (probe 4 in Fig. 1) or probes located downstream of nucleotide 2576 (probes 10, 11 and 12 in Fig. 1) fail to detect any transcripts of *Baso* or *CMSBaso*. Also, transcripts are not detectable with a probe of the fertile line *Baso* covering the downstream region of the *atpA* gene (probe 1 in Fig. 1, nucleotides 1794–2197 in Fig. 2)

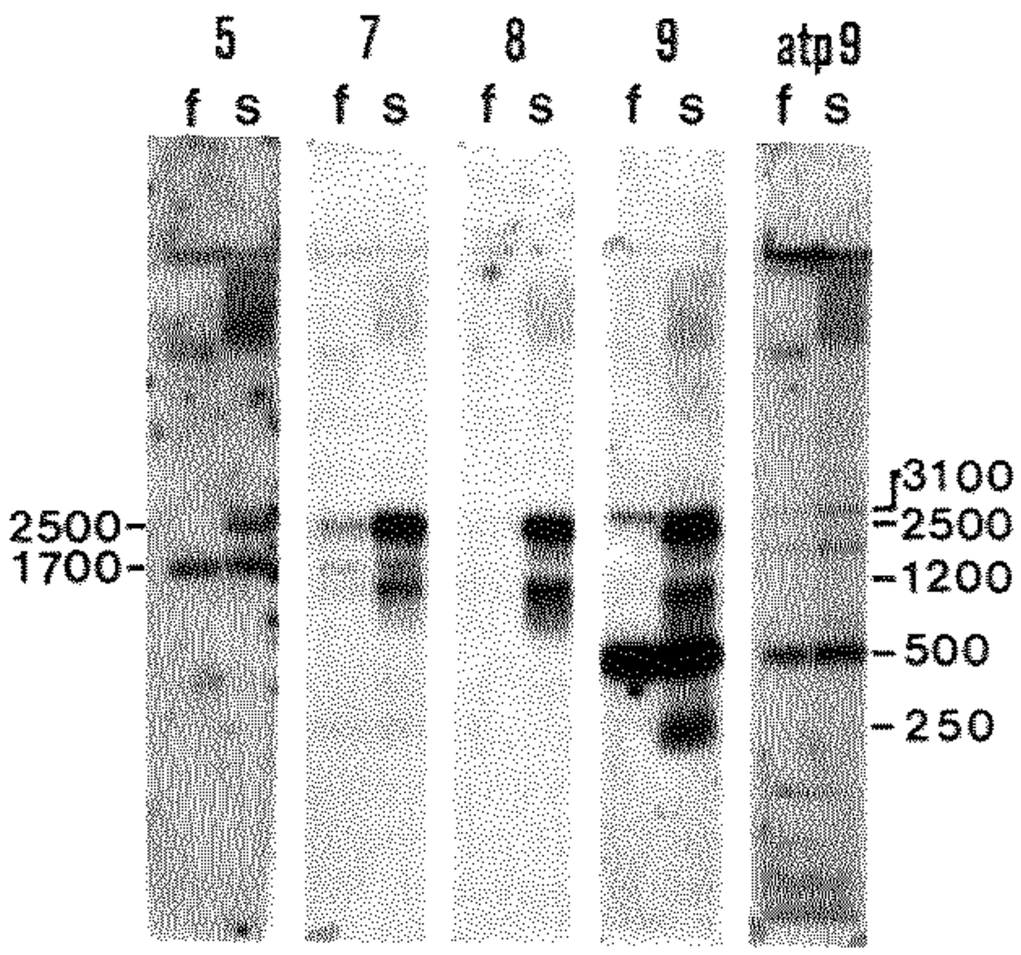

Fig. 3. Transcript analysis of the *atpA* locus. MtRNA (2 μg) from etiolated seedlings of the fertile line *Baso* (f) and the male-sterile line *CMSBaso* (s) were hybridized to probes covering different parts of the *atpA* gene and its upstream and downstream regions. The location of probes 5, 7, 8 and 9 on the mtDNA of the male-sterile line *CMSBaso* (S) is shown below the Northern blot and in Fig. 1. A probe of 2.9 kb (*atp9*) including the *atp9* gene of tobacco (Bland et al. 1986) was used to check the origin of the additional transcripts which are visible with probe 9. The bands at the top of all blots are due to contamination with mtDNA. Transcript lengths are designated in nucleotides

orfH522 *is co-transcribed in* CMSBaso

Hybridization with single-stranded DNA probes revealed that the additional transcripts of the male-sterile line *CMSBaso* are transcribed from the same DNA strand as the *atpA* gene. Transcript analyses using different probes of the *atpA* locus showed that *orfH522* is co-transcribed with the *atpA* gene in *CMSBaso* (Fig. 3). All probes, including parts of the *atpA* gene and a probe containing the last 10 bp of *orfH522* and its downstream sequences (probe 9, nucleotides 2215–2576), hybridize to the larger transcript of the *atpA* gene of about 2500 nucleotides in *CMSBaso*. In addition, the complete *orfH522* seems to be present on a transcript of about 1200 nucleotides. With probes covering the upstream or the downstream region of *orfH522* (probes 7 and 9) a transcript of about 1200 nucleotides is detectable in *CMSBaso*.

Comparison of the transcript pattern of the atpA locus of CMSBaso and its parental lines

CMS89, from which *CMSBaso* originated, arose from an interspecific cross of *H. petiolaris* with *H. annuus*. To determine whether the co-transcription of *orfH522* is specific for the male-sterile lines or whether it is a feature of the cytoplasm of *H. petiolaris*, we compared the transcript patterns in callus from *CMSBaso*, *Baso* and *H. petiolaris*, (Fig. 4). Both male-fertile parental lines, *H. petiolaris* with the cytoplasm of *H. petiolaris* and *Baso* with the cytoplasm of *H. annuus*, show only one transcript of the *atpA* gene of about 1700 nucleotides. Therefore, the co-transcription of *orfH522* seems not to be a feature of the cytoplasm of *H. petiolaris* itself. It might be specific for the cytoplasm of the male-sterile lines.

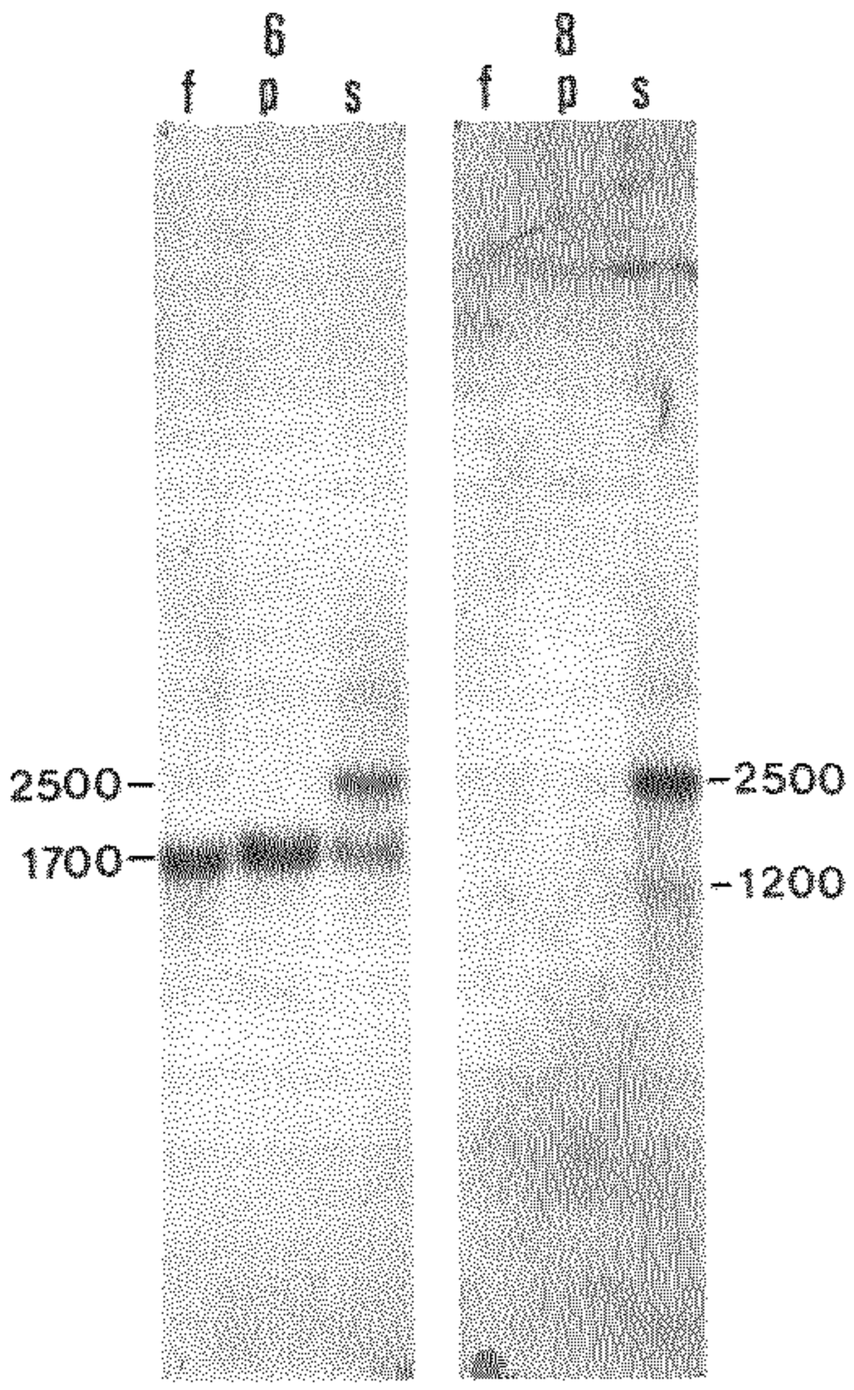

Fig. 4. Comparison of the transcript pattern of the *atpA* gene of the line *CMSBaso* and its parental lines. Total RNA of callus of *Helianthus petiolaris* (p, fertile) and of the lines *Baso* (f, fertile) and *CMSBaso* (s, male-sterile) of *H. annuus* were hybridized to probes for the *atpA* gene (6) or *orfH522* (8). The origin of probes 6 and 8 is indicated below the Northern blot and in Fig. 1

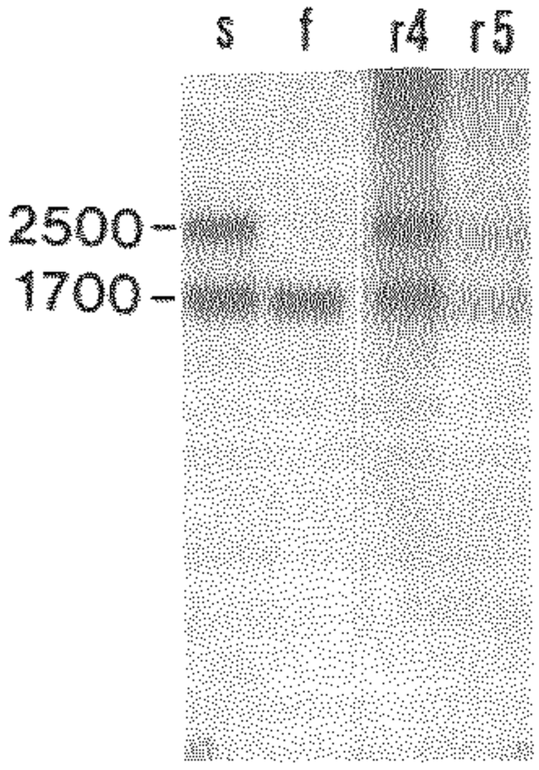

Fig. 5. Transcript pattern of the *atpA* gene of fertility-restored lines. MtRNA of etiolated seedlings of *Baso* (f), *CMSBaso* (s) and of the fertility-restored lines *RHB4* (r4) and *RHB5* (r5) were probed with a fragment internal to the *atpA* coding region (probe 6 in Figs. 1, 4). Cross-hybridization to mtDNA is the cause of the contamination in lane r4. Transcript lengths are specified in nucleotides

Transcript pattern of the atpA *gene of fertility-restored lines*

The fertility-restored lines *RHB4* and *RHB5* of *H. annuus* contain dominant nuclear genes that restore fertility of plants carrying the *H. petiolaris*[a] cytoplasm. Fertility restoration seems to have no influence on the co-transcription of *orfH522* (Fig. 5). The additional larger transcript of the *atpA* gene of *CMSBaso*, which contains *orfH522*, is also detectable in etiolated seedlings of the fertility-restored lines *RHB4* and *RHB5*.

Discussion

Our results indicate that CMS in sunflower is correlated with the co-transcription of a new open reading frame, *orfH522*, in male-sterile lines.

Rearrangements, which are possibly the result of recombination within an inverted repeat of 261 bp, lead to an 11 kb inversion and a 5 kb insertion in the mtDNA of the male-sterile line *CMSBaso* compared to the male-fertile lines *Baso* and *H. petiolaris*. Within the entire rearranged mtDNA region only the *atpA* locus shows differences in transcript pattern between *Baso* and *CMSBaso*. According to Siculella et al. (1990) the coding region of the *atpA* gene of the lines *HA89* and *CMS89* of sunflower differs in two mutations which lead to one conservative alteration in the amino acid composition. However, the coding sequences of the *atpA* gene and its 5′ flanking sequences for at least 900 bp are entirely identical in the lines *Baso* and *CMSBaso* of sunflower (data not shown). In addition, no difference is detectable in molecular weight, isoelectric point or peptide pattern of the F_1-ATPase subunit α between etiolated seedlings of fertile, male-sterile and restored lines of sunflower (Horn et al. 1991). Therefore it seems unlikely that the F_1-ATPase subunit α plays a role in CMS in sunflower. However, we cannot exclude tissue-specific alterations in the transcription or translation of the *atpA* gene caused by the additional transcripts of the male-sterile lines.

The *atpA* locus of the male-sterile line *CMSBaso* shows three additional transcripts of about 2500, 1200

and 250 nucleotides. Both male-fertile parental lines, *H. petiolaris* with the cytoplasm of *H. petiolaris* and *Baso* with the cytoplasm of *H. annuus*, show only one transcript of the *atpA* locus of about 1700 nucleotides. Therefore, the additional transcripts of *CMSBaso* seem to be a feature of the cytoplasm of *CMSBaso*. This is in accordance with the observation that the mtDNA organization of the *atpA* locus of *H. petiolaris* and *Baso* are colinear, but differ from that of *CMSBaso*.

The three additional transcripts of *CMSBaso* could be the result of three different transcription initiation processes. However, it seems more likely that the additional smaller transcripts of about 1200 and 250 nucleotides, which seem to be less abundant, are the result of extensive processing of the larger transcript of about 2500 nucleotides. The transcript of the *atpA* gene of *CMSBaso* of about 1700 nucleotides might also be a product of processing, but it could also be that incomplete termination of transcription leads to the two transcripts of the *atpA* gene of *CMSBaso* of about 1700 and 2500 nucleotides.

An imperfect inverted repeat of 25 nucleotides is located downstream of the *atpA* gene in the fertile line *Baso* which could form a stem-loop structure. Comparable inverted repeats are found downstream of several plant mitochondrial genes (Schuster et al. 1986). Their function is not yet clear, but they could play a role in termination, processing or transcript stability. Due to the rearrangements downstream of the *atpA* gene the inverted repeat is absent in *CMSBaso*. This could be involved in producing the differences in transcript pattern between *Baso* and *CMSBaso*.

In the male-sterile line *CMSBaso* a new open reading frame, *orfH522*, is located downstream of the *atpA* gene. *orfH522* is co-transcribed with the *atpA* gene on a polycystronic transcript of about 2500 nucleotides in the male-sterile line *CMSBaso*. In *Oenothera*, maize, and tobacco there is evidence for polycistronic mitochondrial messengers (Hiesel and Brennicke 1985; Bland et al. 1986; Wissinger et al. 1988). In addition, co-transcription of the CMS-associated *pcf* gene with the *nad3* gene and the *rps12* gene has been described for *Petunia* (Rasmussen and Hanson 1989). *orfH522* is also included in a second additional transcript of *CMSBaso* of about 1200 nucleotides. Translation from a polycystronic messenger seems therefore not to be necessary for the expression of *orfH522* as a polypeptide.

The 5′ flanking sequences of *orfH522* show homology to the 5′ flanking sequences of several mitochondrial genes as described by Pruitt and Hanson (1989) for the *coxII* gene of *Petunia*. The function of these conserved non-coding sequences in plant mitochondria is not known. In association with the rearrangements in the mtDNA they might participate in generation of the different transcripts of the *atpA* locus.

The co-transcription of *orfH522* is the only detectable difference in transcript pattern of *Baso* and *CMSBaso* within the 5 kb insertion and the flanking sequences of the rearranged mtDNA regions. We cannot exclude the possibility of low abundance transcripts which may not be detectable by Northern hybridization (Finnigan and Brown 1990).

Transcript analysis of the sunflower lines *HA89* and *CMS89* using mtRNA revealed only one major alteration in transcript pattern (Siculella and Palmer 1988). Using clones covering 82% of the CMS mitochondrial genome and heterologous clones of several mitochondrial genes (*coxI*, *coxII*, *coxIII*, *cob*, *26SRNA*, *18SRNA*, *atpA*, *atp9*, *atp6*, *rsp13*, *ndh1*) only differences in the transcript pattern of the *atpA* gene were observed. According to restriction fragment pattern, Southern analysis and to the organization of the rearranged mtDNA region, the lines *HA89* and *CMS89* described by Siculella and Palmer (1988) seem to be collinear with the lines we investigated; additionally, the transcript pattern of the *atpA* gene seems to be identical (Horn et al. 1991). In contrast to their results, we found another region downstream of the *cob* gene which shows differences in Northern hybridization between the lines *Baso* and *CMSBaso*, however, this difference is due to cross-hybridization with the *atpA* transcripts. Nevertheless, it is likely that the lines described by Siculella and Palmer (1988) and the lines we investigated are identical in their mtDNA organization and that the co-transcription of *orfH522* is the only difference in transcription between male-sterile and male-fertile lines of sunflower.

Restoration of fertility seems to have no influence on the co-transcription of *orfH522* with the *atpA* gene. Etiolated seedlings of fertility-restored lines (RHB4 and RHB5) show the same transcript pattern as *CMSBaso*. An additional transcript of about 2500 nucleotides is also detectable in fertility-restored lines which derive from crosses of *CMS89* with different restorer lines (Horn et al. 1991). However, there might be an influence of fertility restoration on the additional transcripts of about 1200 and 250 nucleotides, of the *atpA* locus of the male sterile line *CMSBaso*.

If the predicted polypeptide product of *orfH522* plays a role in CMS in sunflower, fertility restoration could work by modifying the transcription of these smaller transcripts or by post-transcriptional regulation. For the *pcf* gene product of *Petunia* (Rasmussen and Hanson 1989) a post-transcriptional regulation has been assumed, whereas fertility restoration in maize T-cytoplasm is regulated at the transcript level (Dewey et al. 1986). Because all investigations were made with etiolated seedlings or callus we cannot exclude a tissue-specific regulation of restoration. In *Petunia* the fused mitochondrial gene associated with CMS is developmentally regulated (Young and Hanson 1987). A 4- to 5-fold increase of the *pcf* transcript in comparison to other transcripts is observed in anthers.

The polypeptide product of *orfH522* has a deduced molecular weight of about 19.5 kDa or of about 14.5 kDa if the single internal start codon is used and no RNA editing is assumed (Gualberto et al. 1989; Covello and Gray 1989). In view of this molecular weight, the possible polypeptide product of *orfH522* could be identical with a 16 kDa polypeptide which is expressed in mitochondria of male-sterile and restored lines of *H. annuus* but is absent in male-fertile lines of *H. annuus* and in *H. petiolaris* (Horn et al. 1991).

CMS in sunflower could be the result of the co-transcription of *orfH522* in male-sterile lines. It remains to

be shown how the transcription of the *atpA* locus is regulated in sunflower and whether the 16 kDa polypeptide is indeed the translation product of *orfH522*.

Acknowledgments. We thank Prof. W. Friedt and Dr. Serieys for providing the seed material, Prof. A. Brennicke and Prof. C.S. Levings III for the gene probes and Dr. M. Kroeger for doing the sequence comparison with the EMBO data library. This research was supported by grants of the Deutsche Forschungsgemeinschaft to K.Z. (Schwerpunktprogramm "Genetische Mechanismen der Hybridzüchtung").

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Communicated by R.O. Herrmann