Is the α Subunit of the Mitochondrial F_1-ATPase Involved in CMS in Sunflower?

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Summary

The mtDNA in cytoplasmic male sterile sunflower shows characteristic rearrangements in the 3'-flanking region of the *atpA* gene coding for the F_1-ATPase α subunit. It is made up by an 11 kb inversion and a 5 kb insertion unique to the PET1 type of CMS. Part of the insertion is a novel open reading frame, designated *orfH522*, which is co-transcribed with the *atpA* gene. This organisation of the mitochondrial genome is found in cytoplasmic male sterile and fertility-restored plants. A 16 kDa polypeptide appears to be correlated with the presence of the *orfH522* since it is only detectable in CMS lines and fertility-restored hybrids of sunflower. It remains to be shown whether the *orfH522* encodes for the unique 16 kDa protein.

Using a monoclonal antibody to the F_1-ATPase α subunit from maize in an ELISA system, we could determine identical amounts of the α subunit of the F_1-ATP synthase in mitochondrial extracts from cytoplasmic male sterile (CMSBaso), fertile (Baso), and fertility-restored (RB5) sunflower. Thus, the α subunit of the F_1-ATPase seems to have no impact upon CMS in sunflower, although, tissue-specific variations cannot be excluded.

Introduction

Cytoplasmic male sterility (CMS) in sunflower is correlated with alterations in the mitochondrial genome. The rearranged area in the mtDNA consists of a 11 kb inversion and a 5 kb insertion flanked by the *atpA* and the *cob* gene (2, 3; fig. 1).

Only the *atpA* gene coding for the α subunit of the mitochondrial F_1-ATP synthase shows alterations in the transcript pattern depending on nuclear background and cytoplasm (1, 2). A new open reading frame, *orfH522*, is co-transcribed with the *atpA* gene as an additional larger transcript in the male sterile lines (2). Correlated with the appearance of this co-transcript is the detection of a novel 16 kDa polypeptide in male sterile sunflower lines and fertility-restored hybrids (1). In order to determine whether this co-transcription affects the expression of the α subunit of the mitochondrial F_1-ATPase, we performed an ELISA immuno-quantification of the α subunit using monoclonal antibodies. Additionally, we raised a polyclonal antiserum to a fusion protein from an expression vector containing the *lacZ* gene and parts of the *orfH522* sequence to find out whether the *orfH522* encodes for the 16 kDa protein using the Western blot technique.

Material & Methods

The isolation procedure for mitochondria from sunflower seedlings and the *in-organello* translation technique using ^{35}S-labelled L-methionine were described elsewhere (1). The F_1-ATPase was purified from etiolated seedlings of *Helianthus annuus* L. cv. Albena using a modified $CHCl_3$ release method followed by DEAE-anion exchange chromatography. The indirect ELISA was performed as described by 4. Apart from the commercial cultivar Albena, we used the following sunflower lines or hybrids:

Line or hybrid	Male fertility	Cytoplasm	Nuclear genome
HA89	fertile	*H. annuus*	*H. annuus*/HA89
CMS89	sterile	*H. petiolaris**	*H. annuus*/HA89
Baso	fertile	*H. annuus*	*H. annuus*/Baso
CMSBaso	sterile	*H. petiolaris**	*H. annuus*/Baso
R1 (hybrid 457/84/GG)	restored	*H. petiolaris**	*H. annuus*/CMS89 x sf1673/83GG
R2 (hybrid 19/86/GG)	restored	*H. petiolaris**	*H. annuus*/CMS89 x I_{11}sf36/85
RB5 (hybrid 160/83/GG) (=Kä 1809/87)	restored	*H. petiolaris**	*H. annuus*/CMSBaso x sf34/82GG

*) The cytoplasm of this interspecific crossing differs in the organisation of its mtDNA from the wild type cytoplasm of *H. petiolaris*

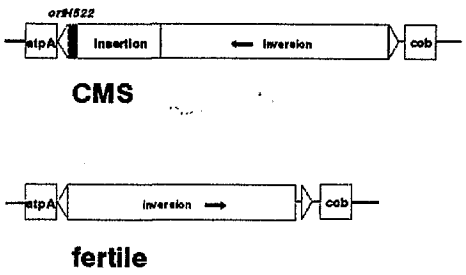

Fig. 1: Comparison of the rearranged mitochondrial (mt) DNA region of sunflower. The mtDNA of the fertile line Baso (fertile) and the cytoplasmic male sterile line CMSBaso (CMS) differ in an 11 kb inversion and a 5 kb insertion, part of which ist the novel open reading frame, *orfH522*. The rearranged mtDNA region is flanked by an inverted repeat (arrowheads) of 261 nucleotides.

Results

The purified mitochondrial F_1-ATP synthase from sunflower consists of at least six subunits (fig. 2), of which α and β could be identified immunologically. Their molecular weight was determined as 54 and 52 kDa, respectively. The monoclonal antibody specifi-

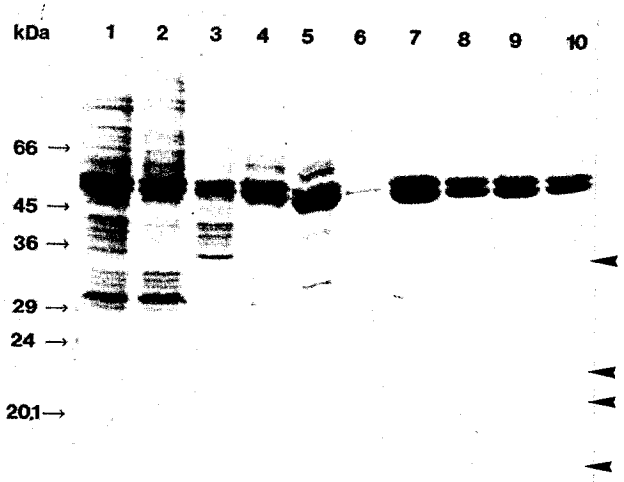

Fig. 2.: Isolation of the mtF_1-ATPase from etiolated sunflower seedlings. SDS-PAGE (15 % acrylamide) of representative fractions of the isolation protocol: (1) = mitochondria, (2) = pellet of the 1st ultracentrifugation, (3) = supernatant of the 1st ultracentrifugation, (4) = $CHCl_3$ extract, (5) = supernatant of the 2nd ultracentrifugation, (6 to 10) = fractions no. 3 to 7 of the DEAE-anion exchange column. Figures on the left indicate molecular masses in kDa. The subunits of the mtF_1-ATPase are marked with arrowheads.

cally reacted with the α subunit (fig. 3). According to our ELISA experiments, the amount of α subunit of the F_1-ATP synthase is identical in mitochondria of male sterile, male fertile, and fertility-restored sunflower seedlings (figs. 4 & 5).

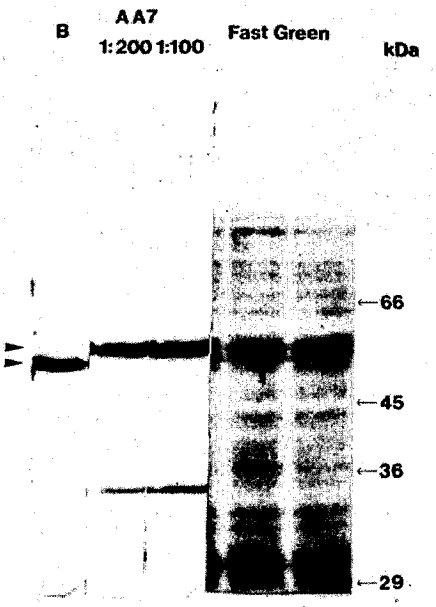

Fig. 3: Immunological identification of the α and β subunits of the mtF_1-ATPase of *Helianthus annuus* L. cv. Albena. Total mitochondrial protein was transferred from SDS-PAG (12.5 % acrylamide) to cellulose nitrate. A Western blot was performed using the polyclonal antiserum to subunits α and β of the mtF_1-ATPase of *Vicia faba* L. (= B), and the monoclonal antibody to the F_1-ATPase α subunit of maize (= AA7). The signals below the α subunit probably indicate proteolytic degradation. Proteins were stained with Fast Green, the immunoreaction was performed with alkaline phosphatase coupled to the 2nd antibody. 5-bromo-4-chloro-3-indolyl phosphate-*p*-toluidinic salt and *p*-nitroblue tetrazolium chloride served as substrates. The figures on the right indicate the molecular masses in kDa, subunits α and β of the mtF_1-ATPase are marked with arrows.

For the immunological detection of the 16 kDa protein, we raised a polyclonal antiserum to the fusion protein encoded by *lacZ* and *orfH522* in rabbit (fig. 6). This antiserum did not recognise any of the mitochondrial proteins separated by SDS polyacrylamide electrophoresis (data not shown).

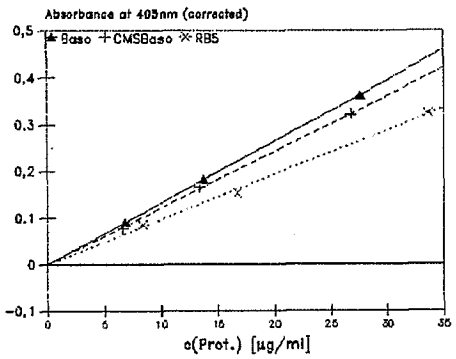

Fig. 4: Immunological quantification of the F_1-ATPase α subunit in Baso, CMSBaso, and the restored hybrid RB5 of sunflower by indirect ELISA using the monoclonal antibody AA7 (1:200). The diagram shows the dependence of light absorbance on the amount of total protein submitted to the test. Absorbance values were corrected to start the linear regression line at zero. Submitochondrial particles obtained from mitochondria of the resp. lines purified by Percoll-density gradient centrifugation served as antigen. Immuno-reaction of alkaline phosphatase coupled to the 2nd antibody with *p*-nitrophenyl phosphate.

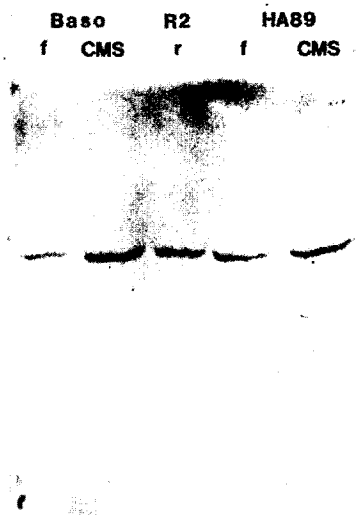

Fig. 5: Western blot to identify the α subunit of the mtF_1-ATPase in the lines Baso (f = male fertile), CMSBaso, HA89, and CMS89, and in the fertility-restored (= r) hybrid R2, using the monoclonal AA7 (1:200).

Discussion

We purified the mitochondrial F_1-ATP synthase from sunflower seedlings and were able to show that the monoclonal antibody AA7 reacted specifically to its α subunit (fig. 3). Although we found no significant differences in the amount of mitochondrial F_1-ATPase α subunit between the male fertile line Baso, the male sterile line CMSBaso, and a restored hybrid (figs. 4 & 5), tissue and/or stage specific differences cannot be ruled out. Thus, we try to isolate radioactively labelled samples of the 16 kDa protein unique to male sterile

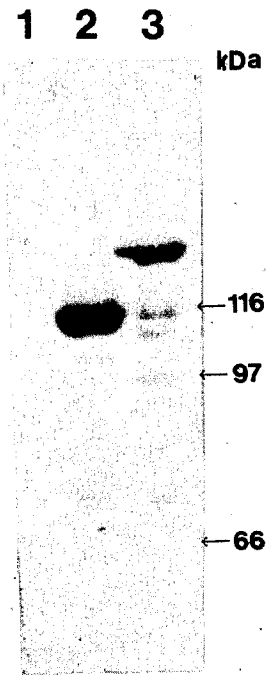

Fig. 6: Testing of the polyclonal antiserum from rabbit to the β-galactosidase-*orfH522* fusion protein encoded by a chimeric gene in the vector pEX-1. Total protein of *E. coli* was submitted to SDS-PAGE 3 h after heat induction of the lambda promoter, and transferred to cellulose nitrate, the immuno-reaction was carried out using alkaline phosphatase coupled to anti-rabbit IgG with 5-bromo-4-chloro-3-indolyl phosphate-*p*-toluidinic salt and *p*-nitroblue tetrazolium chloride as substrates. The figures on the right indicate the molecular masses in kDa, subunits α and β of the mtF_1-ATPase are marked with arrows: (1) = *E. coli* POP2136 without vector pEX-1, (2) = *E. coli* POP2136 with vector pEX-1 coding for β-galactosidase, (3) = *E. coli* POP2136 with vector pEX-1 coding for the β-galactosidase-*orfH522* fusion protein, the figures indicate the molecular masses in kDa.

and restored sunflower by isoelectric focussing in high pH ranges in order to obtain probes for amino acid micro sequencing of the 16 kDa polypeptide. This assay could prove whether the *orfH522* encodes for this protein unique for cytoplasmic male sterile and fertility-restored sunflower by synthesis of an oligonucleotide and subsequent hybridization to mtDNA. The non-detection of the 16 kDa polypeptide using the antiserum to the fusion protein by Western blotting could either be due to the very small amounts of this polypeptide or to conformational changes in the protein caused by the fusion to β-galactosidase.

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