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Originals

Variation in mitochondrial translation products in fertile and cytoplasmic male-sterile sugar beets

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Summary. Intact and functional mitochondria were isolated from sugar beet plants (*Beta vulgaris* L.) containing normal fertile (F) or cytoplasmic male-sterile (S_1–S_4) cytoplasms. Incorporation of ^{35}S-methionine by mitochondria isolated from both roots and leaves showed approximately 20 major and ten minor translation products. Comparison of the polypeptide synthesis patterns produced by leaf mitochondria from fertile plants of three different species within the genus *Beta* revealed several taxonomically related differences. Contrary to this, the patterns of polypeptides synthesized by mitochondria from roots and leaves of sugar beet plants containing the F and S_1–S_4 cytoplasms were very similar; in the S_1 and S_2 cytoplasms no qualitative, and only a few quantitative, differences from the F cytoplasm were observed. Thus, in these cases, cytoplasmic male sterility in sugar beet is not correlated with the constitutive expression of variant polypeptides. In the S_3 cytoplasm, however, an additional 6 kDa polypeptide was synthesized and in the S_4 cytoplasm an additional 10 kDa polypeptide was observed when compared with the F cytoplasm. The expression of cytoplasmic male sterility in sugar beet may be associated with these variant polypeptides. The mitochondrial polypeptides synthesized were identical in plants with different nuclear backgrounds but with identical S_1 cytoplasms. Mitochondria from plants with variants of the S_4 cytoplasm in the same nuclear genotype also showed identical patterns of polypeptide synthesis, including the synthesis of the 10 kDa S_4-specific polypeptide. Pulse-chase experiments did not affect the synthesis of this polypeptide.

Key words: *Beta vulgaris* – Cytoplasmic male sterility – ^{35}S-methionine incorporation – Mitochondria – Sugar beet

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Introduction

Cytoplasmic male sterility (cms) is widely distributed among different plant taxa and is commonly found in plant populations (Edwardson 1970). Much circumstantial evidence implicates the mitochondrial rather than the chloroplast genome as the cytoplasmic genetic determinant of cms in higher plants (Lonsdale 1987). All cms cytoplasms studied so far are associated with a particular and unique set of nuclear restorer alleles that render male-sterile plants fully fertile. This indicates that the exact molecular mechanism by which male sterility is produced may differ among different cytotypes. It is as yet unknown to what extent the molecular mechanisms of different cms systems really differ. However, there seems to be fundamental molecular similarities between most naturally occurring cms systems. Highly rearranged mitochondrial genomes are associated with the occurrence of cms in both maize (Borck and Walbot 1982; Leaver et al. 1988; Fauron and Havlik 1989), radish (Makaroff and Palmer 1987), and sugar beet (Brears and Lonsdale 1988; Halldén et al. 1990), and may possibly constitute the molecular basis common to all spontaneously occurring cms systems.

A number of accessions belonging to different species from the section *Beta* have been classified by restriction analysis of mtDNA into five major cytoplasmic types, namely, one fertile (F) and four cms types (S_1–S_4, Halldén et al. 1990). The restriction patterns of the mtDNA from the four types of cms accessions were clearly different from each other and from all studied fertile accessions of the genus *Beta*. Restriction mapping of the S_1 mitochondrial genome revealed a multicircular organization, where homologous recombination between pairs of repeated sequences produced a range of different subgenomic circles (Lonsdale et al. 1988). The large dif-

ferences observed in mitochondrial restriction patterns between the F and the S_1–S_4 types are due to such structural rearrangements rather than to the introgression of foreign cytoplasms (Halldén et al. 1990).

In the present study we have isolated intact, functional mitochondria from roots and leaves of fertile and male-sterile plants from the five major cytoplasmic types mentioned above. Their protein patterns and their protein synthesis products have been compared to determine whether any association exists between the mitochondrial proteins synthesized and the expression of cms.

Materials and methods

Plant material

The materials used in this study are listed in Table 1. The male-sterile lines GWI-12-CMS (1), (2), (3) and (7) were obtained from Dr. R. K. Oldemeyer, Mono-Hy Sugar Beet Seed Inc., USA. All other materials were obtained from Hilleshög AB, Sweden. The variety Primahill and the breeding populations LNSZ 1, C 5489, C 37150, C 7051, C 8640 and C 8684 have been described and classified earlier (Halldén et al. 1988, 1990).

Apart from Primahill, which is a triploid variety, all of the male-sterile materials in Table 1 are diploid. The GWI-12-materials have all been backcrossed to the same nuclear non-maintainer genotype. The accessions C 7051, C 8640 and C 8684 were all represented by completely sterile plants.

Table 1. Origin and categorization of the investigated sugar beet material

Fertile/sterile cytoplasmic major types	Fertile/sterile phenotypic expression	Material	Origin of cytoplasm
F	f	LNSZ 1	*Beta vulgaris*
F	f	C 5489	*B. maritima* from Portugal
F	f	C 37150	*B. procumbens*
S_1	s	Primahill	*B. maritima* from Turkey
S_2	s	C 7051	*B. maritima* from Morocco
S_3	s	C 8640	*B. cicla* from Yugoslavia
S_4	s	GWI-12-CMS (7)	*B. sp.* from Manchuria; PI 141919
S_1	s	Primahill	*B. maritima* from Turkey
	s	C 8684	*B. maritima* from Turkey
	s	GWI-12-CMS (1)	*B. sp.* from India; PI 120704
S_4	s	GWI-12-CMS (2)	*B. sp.* from Turkey; PI 164747
	s	GWI-12-CMS (3)	*B. sp.* from Pakistan; PI 177272
	s	GWI-12-CMS (7)	*B. sp.* from Manchuria; PI 141919

Isolation of mitochondria

The methods used for the isolation and purification of mitochondria from leaves and roots are described in Lind et al. (1991). Determination of protein concentrations, enzyme activities, latencies, and respiration rates were according to Lind et al. (1991).

The purified root and leaf mitochondria had approximately 90%, or better, intact outer membranes, as measured by the latency of cytochrome c oxidase activity. The inner membranes, as judged by the latency of malate dehydrogenase activity, were also highly intact ($\geq$93%). Since structural integrity does not necessarily imply functional integrity, the respiratory properties of the mitochondria were measured with succinate (root mitochondria) and malate (leaf mitochondria) as substrates. Root mitochondria state-3 respiration rates of 300–350 nmol (mg protein)$^{-1}$ min^{-1}, and respiratory control (RC) ratios and ADP/O ratios of 3.0–3.8 and 1.4–1.6, respectively, were routinely observed. Leaf mitochondria showed state-3 respiration rates in the range of 210–260 nmol (mg protein)$^{-1}$ min^{-1} and RC and ADP/O ratios of 3.2–3.9 and 1.9–2.2, respectively. Thus, both root and leaf mitochondria were fully functional, and the high rates of respiration indicate a low degree of contamination. No consistent differences in enzyme activities or respiratory properties were observed between the different fertile and male-sterile lines.

Protein synthesis

Protein synthesis of the intact and purified mitochondria were according to Lind et al. (1991). Incorporation of [^{35}S]methionine (30 µCi per sample, >800 Ci/mmol, Amersham) was assayed with 20 mM succinate / 2 mM ADP and 25 mM malate / 2 mM ADP as the energy-generating systems. Inhibitors were regularly added to the incubation medium to check for contaminating cytosolic (100 µM cycloheximide) or plastidic (400–800 µM erythromycin) translational activity according to Lind et al. (1991). The level of bacterial contamination was monitored by plating samples of mitochondrial suspensions on blood agar plates and counting the number of colonies that developed within 48 h.

For pulse-chase experiments, mitochondria were labelled as above for 30–60 min and then final concentrations of 1 mM unlabelled methionine and 100 µg/ml chloramphenicol were added. After a further 30, 60 and 90 min, samples were taken out and processed as described below. The incorporation of [^{35}S]-methionine into polypeptides was measured as the amount of trichloroacetic acid-precipitable material, according to Mans and Novelli (1961).

Gel electrophoresis

After an incubation period of 30 to 90 min, the incorporation of radioactivity was terminated by the addition of 1 ml of ice-cold wash medium containing 10 mM of unlabelled methionine. The mitochondria were pelleted by centrifugation at 14,000 *g* for 5 min in an Eppendorf centrifuge and solubilized in 50 µl of Laemmli's (1970) sample buffer by incubation at 90 °C for 6 min. The polypeptides were fractionated on 15% (w/v) SDS-polyacrylamide slab gels according to Laemmli (1970), or on 12–18% (w/v) gradient SDS-polyacrylamide slab gels containing sucrose in the separation gel (Hames 1981), or on 12–22.5% (w/v) gradient SDS-polyacrylamide slab gels containing 4 M urea. The gels were fixed and either: (1) stained with Coomassie brilliant blue, de-stained, dried onto Whatman 3 MM paper and subjected to direct autoradiography using Amersham β-max film at −80 °C, or (2) impregnated with a fluor (Amplify, NEN) and fluorographed using Amersham β-max film at −80 °C. The molecular weights of the labelled polypeptides were estimated by running molecular weight markers in parallel (2.5–17 kDa, 14–94 kDa, Pharmacia).

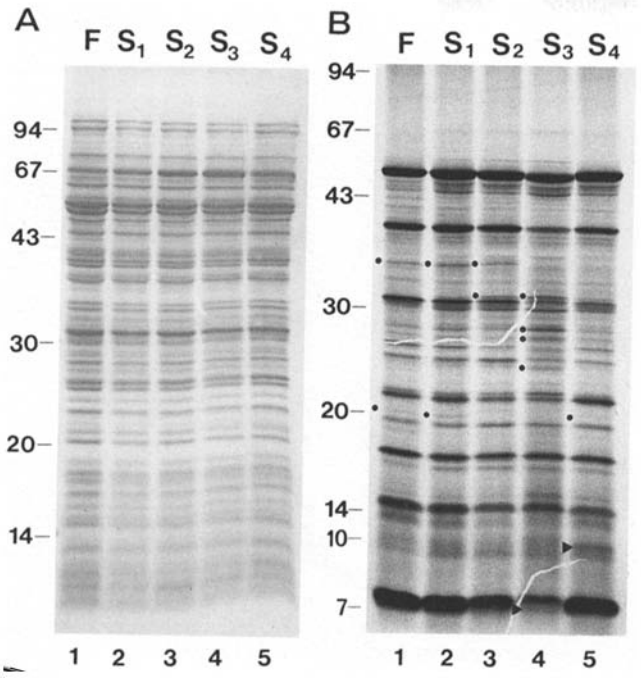

Fig. 1 A, B. Analysis of root mitochondrial polypeptides and *in organello*-protein synthesis products of the five major cytoplasmic groups. **A** Polypeptides separated on 12–18% (w/v) gradient SDS-PAGE and stained with Coomassie brilliant blue. *Lane 1*, F cytoplasm (LNSZ 1); *lane 2*, S_1 cytoplasm (Primahill); *lane 3*, S_2 cytoplasm (C 7051); *lane 4*, S_3 cytoplasm (C 8640); *lane 5*, S_4 cytoplasm [GWI-12-CMS (7)]. **B** Direct autoradiography of the gel in **A** showing *in organello*-protein synthesis products. Each lane contained 2×10^5 cpm. Quantitative differences are marked by *dots*

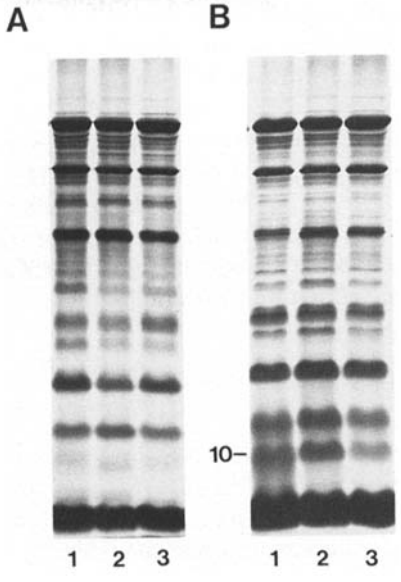

Fig. 2 A, B. Direct autoradiography of *in organello*-labelled polypeptides separated on 15% (w/v) SDS-PAGE. **A** Variation in the protein synthesis of root mitochondria from the S_1 cytoplasm in three different nuclear backgrounds. *Lane 1*, Primahill; *lane 2*, C 8684; *lane 3*, GWI-12-CMS (1). **B** Variation in the protein synthesis of root mitochondria from different S_4 cytoplasms in an isogenic nuclear background (GWI-12). *Lane 1*, GWI-12-CMS (7); *lane 2*, GWI-12-CMS (2); *lane 3*, GWI-12-CMS (3). Each lane contained 2×10^5 cpm

Results

Protein patterns and protein synthesis products of isolated root mitochondria

Five major types of fertile and male-sterile cytoplasms are known to be present in beets, the F and the S_1–S_4 types (Halldén et al. 1990). Root mitochondria were isolated from plants with these five different types of cytoplasm. The mitochondrial preparations showed identical polypeptide profiles after Coomassie blue staining of SDS-polyacrylamide gels (Fig. 1 A), indicating: (1) little differential contamination of the mitochondrial preparations with other subcellular components, and (2) that the different male-sterile types are very closely related to the male-fertile type.

These mitochondrial preparations were allowed to synthesize polypeptides according to Lind et al. (1991). Root mitochondria from the five different fertile and cms cytoplasms showed very similar patterns of labelled polypeptides (Fig. 1B). However, the S_3 and S_4 cytoplasms showed one qualitative, or possibly strong quantitative, difference each, compared with the pattern of the fertile type of cytoplasm (Fig. 1 B, arrowheads). The S_3 cytoplasm showed either a loss or a dramatic reduction in the synthesis of a polypeptide with an apparent mobility of 7 kDa. The variant polypeptide synthesized by S_4 mitochondria had an apparent mobility of 10 kDa. In addition, only a few quantitative differences (marked by dots) were observed. In particular: (1) the S_3 cytoplasm showed some clear quantitative differences compared with the other cytoplasms, and (2) the F, S_1 and S_2 cytoplasms showed a more pronounced synthesis of a 34 kDa polypeptide than did the other cytoplasms.

The range of mitochondrial mutations which can be detected by separation of *in organello*-labelled translation products on SDS-PAGE is somewhat limited; e.g., point mutations resulting in amino-acid substitutions will not be detected in this kind of analysis. There are several sources contributing to the variation seen in these experiments, but all reported differences have been consistently observed in at least three independent experiments.

The polypeptides synthesized by root mitochondria of the S_1 cytoplasm in three different nuclear backgrounds are compared in Fig. 2A. MtDNA from the three S_1 cytoplasms showed identical restriction profiles after *Sal*I, *Bam*HI and *Eco*RI digestion (data not shown). Neither qualitative nor quantitative differences were observed for any of the synthesized polypeptides. Thus, there was no detectable effect on mitochondrial protein

synthesis of varying the nuclear background. A similar result was obtained when protein synthesis by root mitochondria from three lines with variants of the S_4 cytoplasm were studied in an isogenic nuclear background (Fig. 2B). The three cytoplasms (Table 1) are known to show small differences in their mtDNA restriction profiles (Mikami et al. 1985), but no differences in their polypeptide synthesis could be detected (Fig. 2B). In particular, the synthesis of the previously mentioned 10 kDa polypeptide was observed in all three cytoplasms.

Protein patterns and protein synthesis products of isolated leaf mitochondria

Since sugar beet mitochondria are known to synthesize organ-specific polypeptides (Lind et al. 1991), we performed the same type of analysis on leaf mitochondria. Again, the mitochondrial preparations of the F and S_1–S_4 cytoplasmic types were indistinguishable after Coomassie staining of SDS-polyacrylamide gels (Fig. 3A). This also indicates that no differential contamination occurred in leaf mitochondrial preparations. When protein patterns of root and leaf mitochondrial preparations were compared after Coomassie staining (compare Figs. 1A and 3A), four major differences were detected. With the exception of these four polypeptides, which are probably involved in glycine oxidation (Lind et al. 1991), the polypeptide patterns of root and leaf mitochondria were highly similar. Thus, little or no differential contamination was found between mitochondria isolated from different organs (root and leaf).

Leaf mitochondria from the five different lines synthesized a virtually identical spectrum of polypeptides, except for the mitochondria from the S_3 and S_4 cytoplasms, which characteristically differed from the other cytoplasms in their synthesis of polypeptides with molecular weights of 6 kDa and 10 kDa, respectively (Fig. 3B). Leaf mitochondria from the S_3 cytoplasm showed marked synthesis of a novel polypeptide of 6 kDa whereas the 7 kDa polypeptide above it was equally represented in all lines. This is in contrast to root mitochondria from the S_3 cytoplasm where the 7 kDa polypeptide showed a lowered abundance, and no synthesis of the 6 kDa polypeptide. The 10 kDa polypeptide seen in the S_4 cytoplasm is presumably the same polypeptide as was detected in root mitochondrial preparations from plants containing the S_4 cytoplasm (Figs. 1B and 2B). Thus, the 10 kDa polypeptide seems to be constitutively expressed in mitochondria isolated from the S_4 cytoplasm. Some minor quantitative variation in band intensity can also be seen in Fig. 3B. Much of this is probably within the range of the experimental variation seen in this type of experiment.

Mitochondria isolated from plants containing the S_4 cytoplasm were incubated with ^{35}S methionine for

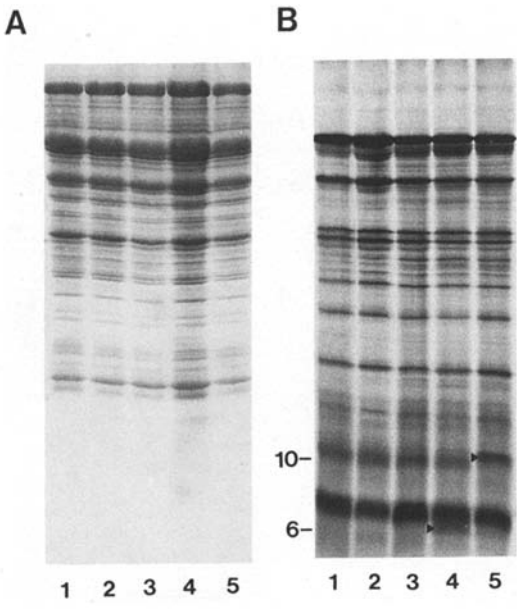

Fig. 3A, B. Analysis of leaf mitochondrial polypeptides and *in organello*-protein synthesis products of the five major cytotypes. **A** Polypeptides separated on 12–18% (w/v) gradient SDS-PAGE and stained with Coomassie brilliant blue. *Lane 1*, F cytoplasm (LNSZ 1); *lane 2*, S_1 cytoplasm (Primahill); *lane 3*, S_2 cytoplasm (C 7051); *lane 4*, S_3 cytoplasm (C 8640); *lane 5*, S_4 cytoplasm [GWI-12-CMS (7)]; **B** Direct autoradiography of a gel as in **A**. Each lane contained 50 µg mitochondrial protein and approximately 3.5×10^5 cpm

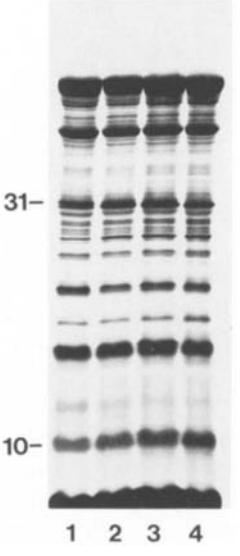

Fig. 4. Pulse-chase experiment as a control for the presence of precursor molecules or breakdown products in a preparation from S_4 cytoplasm. Direct autoradiography of *in organello*-labelled polypeptides separated by 12–18% SDS-PAGE. *Lane 1*, 60 min labelling with ^{35}S-methionine. *Lanes 2–4*, 60 min labelling with ^{35}S-methionine followed by 30, 60 and 90 min chase with unlabelled methionine. Each lane contained 5×10^5 cpm from the same mitochondrial preparation [GWI-12-CMS (7)]

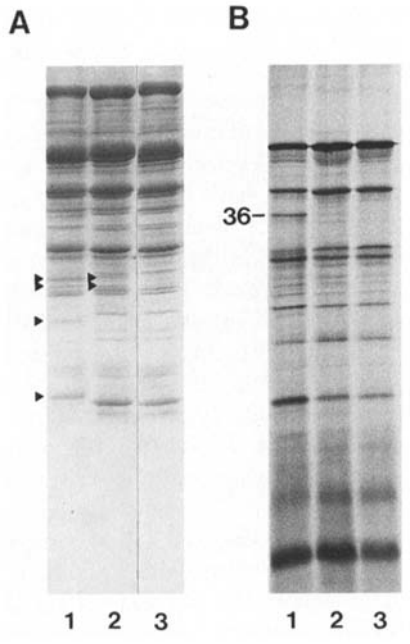

Fig. 5 A, B. Comparison of leaf mitochondrial polypeptides and *in organello*-protein synthesis products from fertile plants of three different species. **A** Polypeptides separated on 12–18% SDS-PAGE and stained with Coomassie brilliant blue. *Lane 1*, *B. procumbens* (C 37150); *lane 2*, *B. maritima* (C 5489); *Lane 3*, *B. vulgaris* (LNSZ 1). **B** Direct autoradiography of a gel as in **A**. Each lane contained 50 µg mitochondrial protein and 3.5×10^5 cpm

60 min prior to a chase period of 0–90 min. This type of pulse-chase experiment (Fig. 4), revealed no changes in the pattern of polypeptides synthesized, except for a decrease in intensity of one band at 31 kDa. In a study of sugar beet mitochondria isolated from roots and leaves of plants containing the F type of cytoplasm, Lind et al. (1991) also reported that a 31 kDa polypeptide decreased in intensity with increasing chase period. In particular, the S_4-specific 10 kDa polypeptide was as stable as the other polypeptides resolved in this gel system.

When the polypeptide patterns of the F-type (*Beta vulgaris*) mitochondrial preparation were compared with those of fertile accessions from two other species, *B. maritima* and *B. procumbens*, several differences were detected (Fig. 5A, arrows). Since most of the differences distinguished the polypeptide pattern of *B. procumbens* from those of the two other species, and since *B. procumbens* belongs to a different section of the genus *Beta* from the two other species, we assume that these differences reflect the differences expected between more-or-less distantly related species.

In organello-protein synthesis did not reveal any differences in mitochondrial protein synthesis between the *B. vulgaris* and the *B. maritima* preparations (Fig. 5B, lanes 2 and 3). The pattern of protein synthesis in mitochondria isolated from *B. procumbens* (Fig. 5B, lane 1) showed marked synthesis of a 36 kDa polypeptide. Thus, the number of different mitochondrial proteins (both nuclear- and mitochondrially-encoded) are correlated with the taxonomic distances between the three different species.

Discussion

The expression of cytoplasmic male sterility

Cms is expressed phenotypically only during later stages of anther development and microsporogenesis. The time and mode of action of the nuclear-cytoplasmic interaction resulting in degeneration of the tapetum and microspores are unknown, but three major alternatives are possible for the cytoplasmic component in this interaction: (1) One or several S-specific cytoplasmic genes are constitutively expressed and interact with nuclear maintainer/restorer genes in one or several of the tissues in anthers, causing sterility/fertility. (2) One or several mitochondrial genes in S cytoplasms are transcribed or translated in a time- and organ-specific manner in certain nuclear backgrounds, resulting in the appearance of the cms phenotype. (3) The general performance of the mitochondria during anther development (rate of replication, rate of transcription, biochemical turnover etc.) critically depends upon the structural arrangement of the mitochondrial genome (number and type of mtDNA molecules present within a given mitochondrion) and upon the distribution of mtDNA molecules over different mitochondria, resulting in the degeneration of the microspores in nuclear maintainer backgrounds.

The approach adopted in the present investigation has been to compare the mitochondrial proteins synthesized in all the different cytoplasmic types known in sugar beet. This type of experiment should ideally be done on mitochondria isolated from a number of different developmental stages and tissues. Mitochondria from the tissues of the developing flowers would be particularly interesting. It has, however, not been possible for us to isolate intact mitochondria from the flowers of sterile sugar beet plants (Lind et al. 1991). Consequently, this paper only discusses data obtained from root and leaf mitochondria.

The ideal material should also include near isogenic lines, some of which allow the sterility to be expressed, and some of which are restored to fertility. Since restoration is not required in the production of hybrid sugar beet varieties, such lines have not been developed. Consequently, lines carrying an S-type cytoplasm but with the plants restored to fertility through the action of nuclear fertility-restoring genes have not been available to us. We circumvented this problem by using inbred populations segregating for male-fertile and male-sterile phenotypes.

Male-fertile and male-sterile plants were first scored for their phenotype, and were then used for mitochondrial isolation. This paper presents data from such material which, in general, showed a highly male-sterile phenotype.

Similar patterns of polypeptides synthesized by mitochondria from the fertile and cms S_1 and S_2 cytoplasms

In organello-protein synthesis by sugar beet mitochondria isolated from either root or leaf did not differ qualitatively among the fertile and the S_1 and S_2 cms lines. Thus, the present study shows that the dramatic differences in mtDNA restriction profiles of the fertile and S_1 and S_2 male-sterile cytoplasms are not associated with any constitutively expressed qualitative differences in mitochondrial polypeptide synthesis.

The S_1 and S_2 cms cytoplasms are, therefore, strong candidates for being cytoplasms of a type that express their sterility-inducing effects in a temporally and spatially restricted way. The mitochondrial expression associated with cms may appear in the whole flower or in one or several of the tissues of the anther, e.g., the tapetum cell layers. We (Lind et al. 1991) detected organ-specific differences in mitochondrial polypeptides synthesized by sugar beet plants. However, no unique polypeptide was found to be synthesized in the flower mitochondria isolated from fertile plants, compared to those of root and leaf mitochondria. At the same time we were unable to isolate mitochondria from flowers of sterile plants. Consequently, we do not know if there are mitochondrially encoded genes with a flower-specific expression that are associated with cms.

Due to the complexity of higher plant mitochondrial DNAs we do not know whether higher plant mitochondria are present in a homoplasmic or heteroplasmic condition. If heteroplasmy occurs, and is involved in the expression of cms, then the associated polypeptide differences may well be of a quantitative rather than a qualitative nature. Whether any of the quantitative differences between the F, S_1 and S_2 types of cytoplasm are causally related to the expression of male fertility or sterility is unknown at present. However, since the observed differences are only minor, and may well be within the range of experimental variation, we find it difficult to investigate this possibility further. Note that we do not exclude a possible involvement of heteroplasmy in the generation of the cms phenotype.

Boutry et al. (1984) have reported that mitochondria from green leaves of fertile sugar beet plants synthesize two polypeptides of 21 and 32 kDa, which are not found in the leaves of sterile plants (plant containing the S_1 cytoplasm). We cannot confirm this observation (Fig. 3 B).

Different patterns of polypeptides synthesized by mitochondria from the fertile and cms S_3 and S_4 cytoplasms

Isolated leaf mitochondria from plants containing the S_3 and S_4 cms cytoplasms synthesize unique polypeptides of molecular weights 6 kDa and 10 kDa, respectively. Similar strong qualitative differences have been observed in the T-, S- and C-type cytoplasms of maize (Forde and Leaver 1980; Forde et al. 1978), in different cms cytoplasms in sorghum (Dixon and Leaver 1982), in *Petunia* (Nivison and Hanson 1989), and in faba bean (Boutry and Briquet 1982; Horn et al. 1991). In maize, the function of a 13 kDa polypeptide strongly associated with the cms T phenotype has been thoroughly investigated in a number of studies (reviewed by Levings and Brown 1989). This polypeptide is not synthesized by N-type mitochondria, but is synthesized by T-type mitochondria from a number of non-green tissues including tassels (Newton and Walbot 1985). In *Petunia*, the S-*pcf* locus has been investigated in detail. The *pcf* gene is transcribed in a number of different organs and at particularly high levels in the anthers. Such transcripts can be found in both male-sterile and fertility-restored lines, although the abundance of the corresponding protein is reduced in restored lines (Nivison and Hanson 1989), indicating a modification of the expression of the *pcf* gene by a nuclear restorer gene.

Thus, such constitutively expressed S cytoplasmic genes may interact with nuclear genes that are strongly temporally and spatially regulated, and ultimately result in the male-sterile phenotype. The strong potential effects of anther- and, in particular, tapetum-specific nuclear gene expression have been nicely demonstrated in an artificial system by Koltunow et al. 1990. They fused the 5′ region of a tapetum-specific gene to a cytotoxic diphteria toxin A-chain gene. This resulted in the destruction of the tapetum and the production of male-sterile transgenic plants.

Root mitochondrial preparations from plants containing the S_3 and S_4 cytoplasms also differ from those of the other cytoplasms in their lack of synthesis of a 34 kDa polypeptide. This polypeptide was never present in any of the leaf mitochondrial preparations, but occurred in preparations of flower mitochondria from fertile plants (Lind et al. 1991). It is again unknown if this polypeptide is synthesized in the flowers of sterile plants. Thus, the 34 kDa polypeptide shows a complex pattern of synthesis over different organs and cytotypes, and cannot be excluded as a possible determinant of cms.

Further studies on organ-specific mitochondrial polypeptide synthesis in combination with an identification of the variant polypeptides of the S_3 and S_4 cytoplasms are probably the best alternatives for further investigations of the biochemical and physiological mechanism of cms in *Beta*.

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