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Characterization of *Vicia faba* mitochondrial genomes with normal *coxII* and *coxII-orf 192* chimeric genes

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Summary. Southern hybridization analysis of mitochondrial genomes from different lines and cultivars of *Vicia faba*, with respect to variability of the *coxII* gene sequence, revealed two predominant types of mitochondrial genomes. The type I mitochondrial genome contained the *coxII* gene sequence in a 6.5-kb *Bam*HI fragment. Type II had two copies of the *coxII* sequence: the first in a 6.5-kb and the second in a 2.6-kb *Bam*HI fragment. The second copy was represented by a *coxII-orf192* chimeric gene. We found several pure lines with type I and type II mitochondrial genomes. Each type of genome was stably inherited. No chimeric gene was found in mitochondria of the male-sterile line cms447. Nucleotide sequences of *Vicia faba* mitochondrial DNA (mtDNA) containing normal and chimeric *coxII* genes are presented. The sequence of the normal *coxII* gene was compared to the *coxII* gene from mitochondria of *Pisum sativum*. The similarity of nucleotide sequences and of predicted amino acid sequences between these two genes was more than 98%. A very high similarity between transcription initiation and termination signals was also observed. The sequence of the chimeric gene was characterized at the 5′ end by the almost complete sequence of the normal *coxII* gene, up to the fifth nucleotide before the termination codon. The 3′ end of the chimeric gene was represented by the 3′ part of an orf previously called *orf128+*. The full size of this orf was 576 nucleotides, and the full size of the predicted polypeptide was 192 amino acid residues. Therefore, this orf can be finally called *orf192*. Northern hybridization analysis showed that *orf192* was actively transcribed into a 1.4-kb transcript. The chimeric gene was also transcribed into a minor transcript of about 3 kb. Comparative analysis of the normal *coxII* gene and *orf192* supported the suggestion that the chimeric gene resulted from nonhomologous recombination.

Key words: *Vicia faba* – Cytochrome oxidase subunit II – Nucleotide sequence – Chimeric gene – Transcription

Introduction

Recombination in mitochondrial genomes of higher plants causes heterogeneity and divergence of these genetic systems. The extent of recombination processes varies species-specifically and is dependent on the state of plant cells: under native conditions plant mitochondrial genomes are more stable than in callus or suspension cultures (Leaver et al. 1988; Lonsdale 1989). Chimeric gene formation can be considered as one of the consequences of rearrangements in higher plant mitochondrial genomes. In many known cases chimeric genes contained a part of the *coxII* gene. Such genes were described, e.g., in mitochondrial genomes of *Zea mays* (Dewey et al. 1985; Levings and Dewey 1988) and *Petunia* (Young and Hanson 1987; Hanson et al. 1988; Pruit and Hanson 1989). Participation of the *coxII* gene in mitochondrial genome rearrangements was also confirmed by the existence of *coxII* gene fragments in the mitochondrial genome of wheat (Bonen et al. 1984). In order to reveal possible rearrangements in *Vicia faba* mitochondrial genomes involving the *coxII* gene, we performed Southern hybridization of a ^{32}P-labelled probe of the *Zea mays coxII* gene with mtDNA isolated from different lines and cultivars of *Vicia faba*. Many lines and cultivars were found with only one copy of the *coxII* gene in a 6.5-kb *Bam*HI fragment of their mitochondrial genomes. However, some lines and cultivars possessed an additional

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2.6-kb *Bam*HI fragment hybridizing with the *coxII* gene probe (Negruk and Kaushik 1988). Both fragments were cloned and mapped.

In this paper, we report the results of a detailed analysis of normal and chimeric *coxII* gene copies as regards their distribution among individual plants of line K, which contains only the normal *coxII* gene copy, and line F, which contains both copies. Additionally, we analyze: (1) individual plants of cultivar "Dornburger Ackerbohne," which is the ancestor of lines K and F; (2) individual plants of the cultivar "Black Russians," representing a natural isolate cultivated in central Russia; and (3) individual plants of line cms447. The 2.6-kb *Bam*HI fragment and about 2 kb of the 6.5-kb *Bam*HI fragment with the normal *coxII* gene were sequenced. It was found that the small *Bam*HI fragment contained a chimeric gene whose 5′ end was represented by an almost complete sequence of the *coxII* gene. Transcription of *coxII*, chimeric, and *coxIII* genes was also investigated.

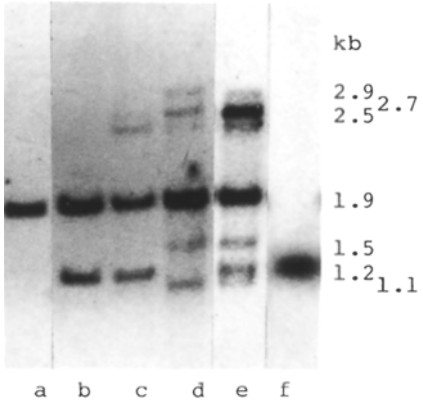

Fig. 1. Autoradiograph of hybridization of ^{32}P-labelled *coxII* gene probe with mtDNA preparations from individual 6-day-old etiolated seedlings of *Vicia faba* cv "Black Russians," digested with *Eco*RI restrictase and electrophoretically separated on a 1% agarose gel. The numbers on the *right side* of electropherogram f show the fragment sizes in kb

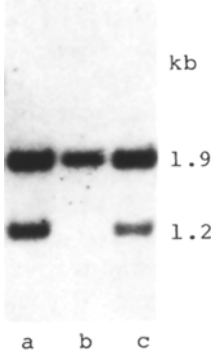

Fig. 2. Autoradiograph of hybridization of ^{32}P-labelled *coxII* gene probe with mtDNA preparations from 6-day-old etiolated seedlings of *Vicia faba: a* line F, *b* line K, and *c* cv "Black Russians," digested with *Eco*RI restrictase and electrophoretically separated on a 1% agarose gel. The numbers on the *right side* of electropherogram c show the fragment sizes in kb

Materials and methods

We have used broad bean seeds of cv "Black Russians" (All-Union Institute of Selection and Seed Production of Vegetable Crops, Moscow District, USSR), cv "Dornburger Ackerbohne," lines K and F, which differ from the standard karyotype of *Vicia faba* by homozygous translocations between chromosomes I and VI (K) and II and III (F), respectively (Institute for Genetics and Crop Plant Research, Gatersleben, Germany). Seeds of line cms447 were kindly supplied by Prof. D. Bond. Preparations of mitochondria and mitochondrial DNA were made according to Synenki et al. (1978). For Southern hybridization with mtDNA from individual plants, crude mtDNA preparations were obtained by a simplified procedure without DNase treatment. Restriction enzyme digestions, electrophoresis, labelling of DNA probes, Southern hybridization, construction of mtDNA libraries, colony hybridization, cloning of restriction fragments into pBR329, and DNA sequencing, using the standard dideoxy chain termination method (Sanger et al. 1977), were carried out according to Maniatis et al. (1982). Preparation of *Exo*III deletion clones was done according to Henikoff (1984). mtRNA isolation and Northern hybridization were performed as described by Schuster and Sisco (1986). The cloned *Zea mays coxII* gene and *Oenothera coxIII* gene sequences, kindly supplied by C. S. Levings and A. Brennicke, were used as probes.

Results and discussion

Intraspecific heterogeneity of the Vicia faba mitochondrial genome

Among 11 lines of *Vicia faba* established 30 years ago in the laboratory of Prof. R. Rieger (Schubert et al. 1982), two groups differing in the structure of their mitochondrial genomes were revealed. In lines of the first group (A, B, C, G, H, I, K), mitochondrial genomes contained only one major *Eco*RI fragment of 1.9 kb hybridizing with a ^{32}P-labelled *coxII* gene probe. In lines of the second group (D, E, F, N), mitochondrial genomes possessed, in addition to the 1.9-kb fragment, a 1.2-kb *Eco*RI fragment (Negruk and Kaushik 1988). On the basis of these differences, the ancestral cultivar "Dornburger Ackerbohne" was expected to represent a mixed population of plants with the two types of mitochondrial genomes. Southern hybridization analysis of ^{32}P-labelled *coxII* probes with *Eco*RI digests of mtDNA, isolated from 20 individual seedlings of the original cultivar, showed that 15 plants contained a mitochondrial genome of type I, and 5 plants that of type II. This confirmed our expectation.

Southern hybridization of ^{32}P-labelled *coxII* probes was also performed with *Eco*RI digests of 48 individuals of cv "Black Russians." In this cultivar mitochondrial genomes proved to be more heterogeneous, although the

majority of plants possessed mitochondrial genomes of type I or II (Fig. 1a, b). Overexposed autoradiographs revealed, for both types of mitochondrial genomes, three additional very weak signals in fragments of 2.5, 2.7, and 2.9 kb. However, for some plants these signals were stronger and could be seen without overexposition (Fig. 1c). Some plants contained mtDNA with a strong signal at 1.9 kb plus five additional weak signals in 2.9-, 2.7-, 2.5-, 1.5-, and 1.1-kb *Eco*RI fragments (Fig. 1d). In addition, we found two unique plants. The first showed seven signals: two strong ones in fragments of 1.9 and 2.7 kb, and five weaker ones in fragments of 2.9, 2.5, 1.5, 1.2, and 1.1 kb (Fig. 1e). The second plant contained a strong signal at 1.2 kb and three very weak signals in fragments of 2.9., 2.7, and 2.5 kb (Fig. 1f).

Altogether, among 48 plants analyzed we found 31 with a mitochondrial genome of type I, 12 with a mitochondrial genome of type II, 3 with a mitochondrial genome shown on Fig. 1d, and 1 each with a mitochondrial genome shown in Fig. 1e, f. The plants with mitochondrial genomes shown in Fig. 1d–f are rare exceptions, as confirmed by hybridization of ^{32}P-labelled *coxII* gene probes with *Eco*RI digests of pooled mtDNA, isolated from several dozen etiolated seedlings of lines F, K, and from cv. "Black Russians," respectively (Fig. 2). Thus, both cultivars "Dornburger Ackerbohne" and "Black Russians" were heterogenous for their mitochondrial genomes. In both cases, approx. 70% of plants contained a mitochondrial genome of type I and ca. 25%, a mitochondrial genome of type II. In order to study the mode of inheritance of both types of mitochondrial genomes, the distribution of 1.9- and 1.2-kb *Eco*RI fragments, among progenies of lines K and F and among individual F_1 hybrid plants obtained by reciprocal crosses between lines K and F, was analyzed. The hybrid nature of progenies of crosses was ascertained by analysis of individual karyotypes. Southern hybridization experiments showed that all 22 plants of line F and all 12 plants of line K analyzed contained mitochondrial genomes of type II and type I, respectively. A total of 13 hybrid seedlings with line K as a cytoplasm donor contained a mitochondrial genome of type I, and 19 hybrid seedlings with line F as a cytoplasm donor contained a mitochondrial genome of type II. Thus, both types of mitochondrial genomes were stably inherited. This led to the conclusion that the 1.2-kb *Eco*RI fragment is the result of an irreversible recombination.

Sequence of the Vicia faba coxII gene and comparison with the Pisum sativum coxII sequence

pBR329 DNA-cloned 6.5-, and 2.6-kb *Bam*HI fragments of *Vicia faba* mtDNA, including two different copies of the *coxII* gene, were obtained and mapped (Zeinalov et al. 1990). Nucleotide sequences of the complete 2.6-kb *Bam*HI fragment and of about 2 kb of the larger *Bam*HI fragment containing the *coxII* gene were determined. It was found that the 6.5-kb *Bam*HI fragment possessed a normal *coxII* gene very similar to the *Pisum sativum coxII* gene (Moon et al. 1985) (Fig. 3). No introns were present in either gene. The predicted translation product from the *Vicia faba coxII* gene was one amino acid residue longer than that of *Pisum sativum*. It contained an additional threonine residue coded by ACG in position 1732–1734 (Fig. 3). The same additional codon was found at corresponding positions in *coxII* genes of rice and *Zea mays* (Moon et al. 1985). Thus, the full length of the predicted polypeptide was 259 amino acid residues. The similarity of predicted amino acid sequences between broad beans and pea was more than 98%. In addition to the insertion of an ACG codon in position 1732–1734, we found seven single nucleotide substitutions in the coding part of the gene. Three of these, at positions 1311, 1443, and 1659, did not cause substitutions of amino acid residues (Fig. 3). In the other four cases substitutions of nucleotides caused substitutions of amino acid residues. The total similarity of nucleotides between *Vicia faba* and *Pisum sativum coxII* genes was also more than 98%.

Nucleotide sequences upstream and downstream from the *coxII* coding region were also very similar between *Vicia faba* and *Pisum sativum*. Among 630 nucleotides upstream from the initiation codon, only eight nucleotide substitutions occurred, and among 203 nucleotides downstream from the termination codon, eight nucleotide substitutions were found between the two genes (Fig. 3). Moon et al. (1985) determined positions of transcription initiation and termination sites in *P. sativum coxII* gene. These sites were perfectly identical to corresponding regions of the *V. faba coxII* gene (Fig. 3). For this reason we suggest that *V. faba coxII* transcripts are initiated and terminated at the same sites.

Within the *V. faba* gene sequence, three pairs of short direct repeates were detected (Fig. 3). Two of them, each 11 nucleotides long, were localized several hundred nucleotides upstream from the coding region. Of the third pair of direct repeats, each 10 nucleotides long, the first repeat occupies position 681–690, and the second position 1232–1241 (within the coding region).

Common upstream and downstream sequences

There are also some other interesting sites upstream and downstream from the coding region of the *V. faba coxII* gene. A possible hairpin structure was observed at position 674–710, not far from the possible transcription initiation site (Fig. 3, 4a). The same structure was found in the *coxII* gene of *P. sativum*. When we compared sequences located upstream from initiation codons of the *V. faba coxII* gene and the mt-plasmid 2 (Wahleithner and Wolstenholme 1987), which corresponds to the mini-

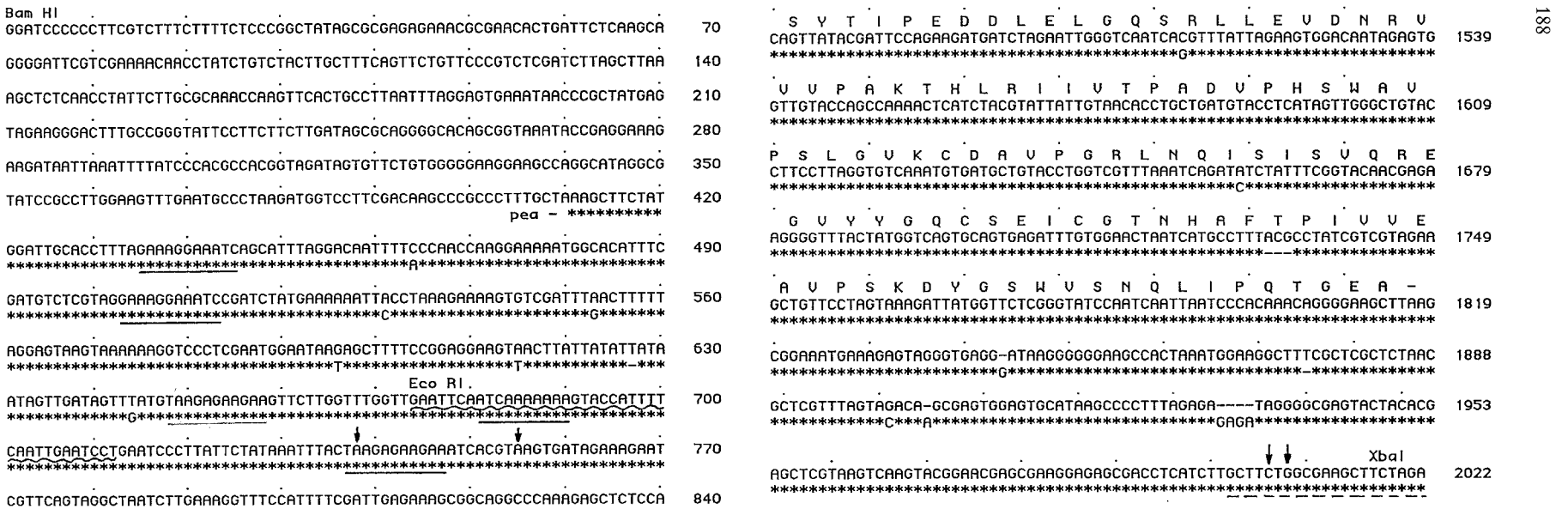

Fig. 3. The nucleotide sequence of the 2,022-bp fragment of broad mtDNA that contains the *coxII* gene. The *Bam*HI and *Xba*I sites at the ends of this sequence are identified. The direction of transcription is indicated by *horizontal arrow* and the predicted amino acid sequence is shown. The nucleotide sequence of the *coxII* gene of *Pisum sativum* is aligned beneath the *Vicia faba* gene: an *asterisk* indicates a nucleotide that is conserved relative to the broad bean sequence; a dash indicates a nucleotide that is absent. *Vertical arrows* show positions of transcription initiation and termination sites determined for *P. sativum* by S1 mapping (Moon et al. 1985). Three pairs of short direct repeats found in this sequence are *underlined*. Two segments of the *V. faba* sequence that can fold into a hairpin structure near transcription initiation (Fig. 4a) and termination (Fig. 4b) sites are tagged by a *wavy underline* and a *dashed underline*, respectively

circular plasmid CCC1A (Negruk et al. 1985), we found a perfect decanucleotide sequence homology, starting from the tenth nucleotide upstream from initiation codons of the *coxII* gene and the orf of mt-plasmid 2 (Fig. 5a). Additionally, we found regions of high sequence homology immediately upstream from these homologous sequences (Fig. 5b). These regions were arranged in opposite orientation.

After analysis of the *P. sativum coxII* gene sequence near transcription termination sites, a possible hairpin structure was found at position 967–988 (Moon et al. 1985) (Fig. 4b). In *V. faba* it corresponded to position 2001–2022. As seen from Figs. 3 and 4b, several nucleotides more were needed to overlap the corresponding *P. sativum* hairpin structure completely. Nevertheless, this structure of the *V. faba coxII* gene must be similar to that of *P. sativum*, since neighbouring parts of these two genes were perfectly homologous in sequence.

The coxII-orf192 chimeric gene

The complete sequence analysis of the 2.6-kb *Bam*HI fragment showed that 1,038 nucleotides upstream from the initiation codon were perfectly homologous to the corresponding region of the normal *coxII* gene (Fig. 6). Furthermore, the coding region of the *coxII* gene copy of the 2.6-kb *Bam*HI fragment, excluding the last five nucleotides before the termination codon, was also similar to that of the normal *coxII* gene. However, downstream from position 1810 we found a different sequence. Therefore, the *coxII* region was not terminated at the position of the normal *coxII* gene. Instead, we observed continuation of an open reading frame that was terminated by a TAG codon 306 nucleotides downstream from position 1810 (Fig. 6). Analyzing the open reading frame, we predict a chimeric polypeptide consisting of 359 amino acid residues.

The next question was: what is the origin of the 3′ end of the chimeric gene? To answer this question, we hybridized a ^{32}P-labelled, cloned 3′ end sequence of the chimeric gene from nucleotides 1916 to 2538 (Fig. 6; clone 563) and the 2.6-kb *Bam*HI fragment with *Bam*HI digests of *V. faba* mtDNA. We found that clone 563 hybridized not only with the 2.6-kb *Bam*HI fragment, but also with a 7.8-kb *Bam*HI fragment (Fig. 7). Additional experiments demonstrated that the 7.8-kb fragment also contained a *coxIII* gene. Physical mapping of the 7.8-kb *Bam*HI fragment showed the sequence in the chimeric gene 3′ end to be located near the *coxIII* gene sequence (Fig. 8).

Comparative computer analysis of the 2.6-kb *Bam*HI fragment sequence revealed that the 3′ end of the chimeric gene sequence downstream from the junction point overlapped with the *orf128+* sequence located downstream from the *coxIII* gene (Macfarlane et al. 1990). This allowed us to reconstruct the total orf, which was previously called *orf128+*. Figure 9 shows the reconstructed sequence starting from position 1500 of the 2023-bp fragment, sequenced by MacFarlane et al. (1990), and terminating at the end of the chimeric gene. The length of the overlapping region was 113 nucleotides. The only difference that we found in this region was C at position 1925 in our variant, and A at the same position in the variant described by Macfarlane et al. (1990). It caused the substitution of the amino acid residue asparagine for histidine. The length of the pre-

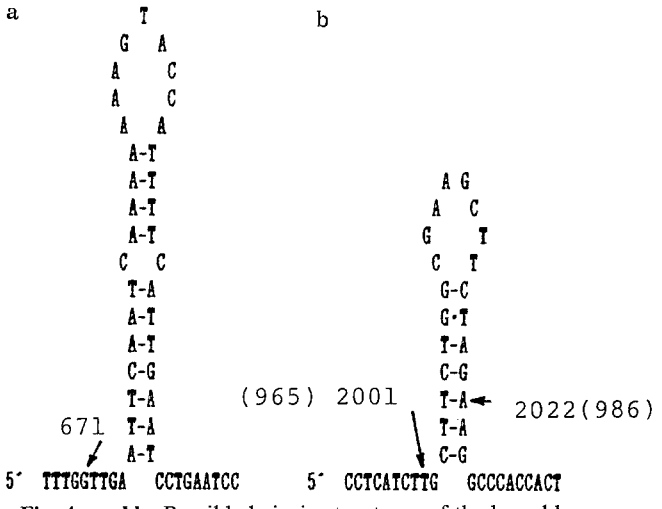

Fig. 4a and b. Possible hairpin structures of the broad bean sequence near the predicted transcription initiation site (position 676–708) (**a**), and of the pea sequence near transcription termination (position 967–988) (**b**)

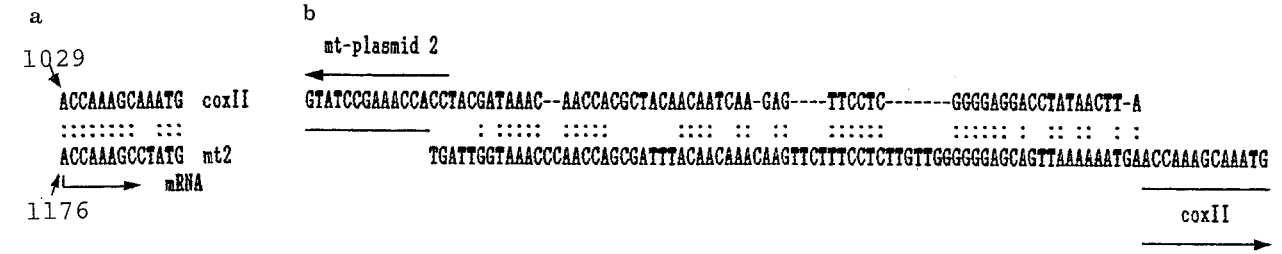

Fig. 5a and b. Homologies between sequences present upstream of the *Vicia faba coxII* gene and of the short orf in *V. faba* mt-plasmid 2 (Wahleithner and Wolstenholm 1987). Direction of transcription is indicated by *horizontal arrows*. A *dash* indicates a nucleotide that is absent. Short homologous sequences (**a**) are inserted into corresponding long homologous sequences (**b**) in opposite orientation

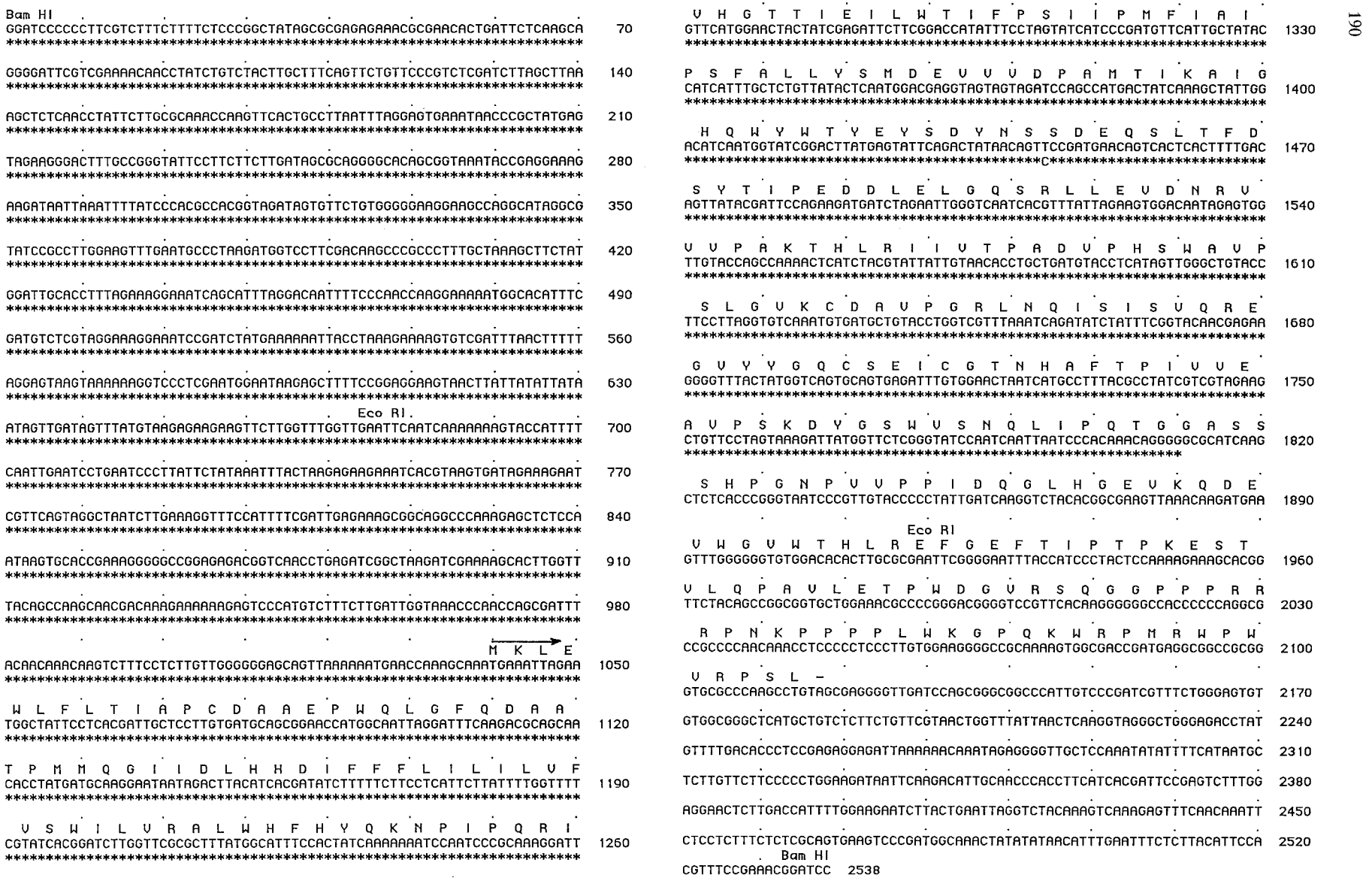

Fig. 6. The nucleotide sequence of a 2,538-bp fragment of broad bean mtDNA that contains the *coxII-orf192* chimeric gene. The *Bam*HI sites at the ends and two *Eco*RI sites inside this sequence are indentified. The direction of transcription is indicated by a *horizontal arrow* and the predicted amino acid sequence is shown. The nucleotide sequence of the normal *coxII* gene of *Vicia faba* is aligned beneath the chimeric gene: and *asterisk* indicates a nucleotide that is conserved within the chimeric gene sequence

dicted polypeptide which could be translated from the new orf was 192 amino acid residues (molecular weight: 19,506). Therefore, we called this new open reading frame *orf192*.

Using the method of Eisenberg et al. (1984) we have characterized positions and sequence of transmembrane helices of the predicted *orf192* polypeptide. We found two such helices in positions 6–28 and 41–71 amino acid residues.

Comparative analysis of sequences of the *V. faba* normal *coxII* gene and *orf192* did not reveal any sequence homology in regions that could participate in recombination. Thus, it is possible that the *coxII-orf192* chimeric gene arose as a result of nonhomologous recombination.

Transcription of the coxII-orf192 chimeric gene

The similarity of the 5′ end regulatory regions between normal and chimeric genes, and the occurrence of plants containing only the chimeric gene in their mitochondrial genomes (Fig. 1f), suggest that the chimeric gene might be transcribed. To verify this, Northern hybridization analysis of ^{32}P-labelled *coxII* gene probes with mtRNA isolated from seedlings of *Vicia faba* cv "Black Russians" was performed. We found a main signal in a 1.2-kb RNA band and two minor signals in 1.1- and 3-kb RNA bands (Fig. 10a). When we used a probe of a fragment of *orf192*, the distribution of signals was different. In this case, three major signals were found in 1.4-, 1.6-, and 3.-kb RNA bands, and two minor signals at 0.8 and 2.5 kb (Fig. 10b). It is difficult to interpret these data since, as mentioned before, cv "Black Russians" represents a heterogenous population containing only 25% of plants with the type II mitochondrial genome. Therefore, we hybridized ^{32}P-labelled probes of the *coxII* gene, the *orf192* (clone 563), and the *coxIII* gene with mtRNA isolated from seedlings of lines F (with the chimeric gene) and K (without the chimeric gene). In these experiments, the *coxII* gene probe gave the main signal in the 1.2-kb RNA band of both lines (Fig. 10c, d). mtRNA from these two lines differed in a minor signal found at 3-kb only in line F (Fig. 10c). The ^{32}P-labelled clone 563 (*orf192*) gave one main signal in the 1.4-kb RNA band of both lines (Fig. 10e, f). However, mtRNA from line F contained two additional signals in the 1.6-, and 3-kb RNA bands (Fig. 10e).

Profiles of *coxIII* gene transcripts in lines F and K were similar. For both lines, a major signal was found at 1.2 kb and a minor one at 2.8 kb (Fig. 10g, h). *coxIII* gene probes were used because it was found by Macfarlane et al. (1990) and in our laboratory that *orf192* was located near the 3′ end of the *coxIII* gene. Therefore, we wanted to know whether or not chimeric gene formation interferes with transcription of the *coxIII* gene. Since this was not the case, we concluded that the chimeric gene was weakly transcribed into a transcript of about 3 kb. The origin of the signal in the 1.6-kb RNA band is not clear.

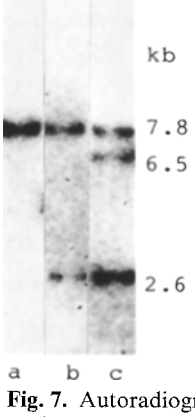

Fig. 7. Autoradiograph of hybridization of ^{32}P-labelled gene probes: *a* – *coxIII; b* – *orf192* (563 clone); *c* – chimeric *coxII-orf192*, with mtDNA preparations from 6-day-old etiolated seedlings, digested with *Bam*HI restrictase and electrophoretically separated on a 1% agarose gel. The numbers on the *right side* of the electropherogram c show the fragment sizes in kb

Mitochondria of line cms447 do not contain a coxII-orf192 chimeric gene

It has been reported that chimeric genes may be causally linked to male-sterile phenotypes and that very often

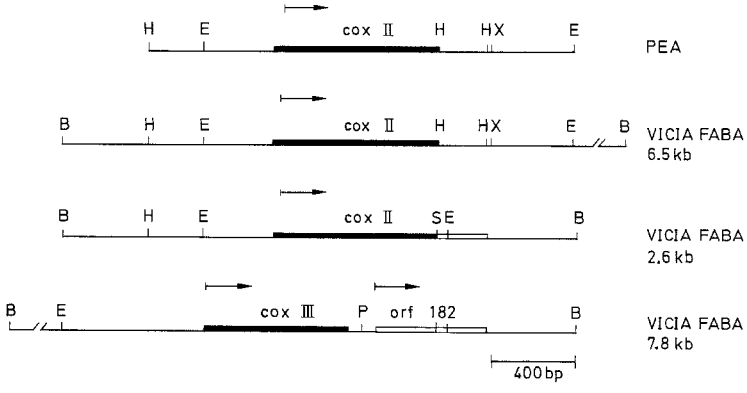

Fig. 8. Restriction maps of the 2.1-kb *Hin*dIII-*Eco*RI fragment of *P. sativum* mtDNA that contains the *coxII* gene, the 6.5-kb *Bam*HI fragment of *V. faba* mtDNA containing the normal *coxII* gene, the 2.6-kb *Bam*HI fragment of *V. faba* mtDNA that contains the chimeric *coxII-orf192* gene, and the 7.8-kb fragment of *V. faba* containing *coxIII* and *orf192* genes. The restriction endonucleases are indicated as: H-*Hin*dIII, B-*Bam*HI, E-*Eco*RI, X-*Xba*I, P-*Pst*I, S-*Sma*I. Direction of transcription is indicated by arrows

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GGTATATGAAGGAACCAAACAGTGGATTTAGGGATGAAAGCTCGAATACAAAGATAACCGGGCTTTTCCA 1570

AAGAATTACTGCAGCTTTCCCAGCTTCGTTATCCTTTGAATTACTCCTAATTTTTCTATTCCTAGTGTCA 1640

M  R  N  K  N  F  L  Y  S  F  L  L  L  V  G  V  S  Y  L  L  C  L  I  L
TGAGAAATAAAAACTTTCTATACTCATTCCTTCTCTTAGTCGGTGTCTCCTATTTACTTTGTCTCATCTT 1710

  G  E  S  E  V  F  W  A  L  L  S  K  V  G  Y  S  G  T  T  R  A  I  F
GGGTGAGAGTGAAGTTTTTTGGGCCTTGCTCTCAAAGGTGGGATACTCCGGTACGACGCGAGCCATATTT 1780

 I  P  F  L  K  L  T  G  C  S  G  R  L  A  L  V  L  F  F  A  V  K  A
ATACCATTTCTCAAATTGACAGGTTGCTCCGGAAGGCTGGCTCTTGTTCTGTTTTTCGCCGTGAAAGCGG 1850

V  N  G  T  L  F  K  D  F  F  S  C  M  E  E  A  G  P  S  S  G  A  S  S
TGAATGGAACCCTCTTCAAAGATTTTTTCTCTTGTATGGAAGAAGCTGGGCCGTCTTCGGGCGCATCAAG 1920

  S  H  P  G  N  P  V  V  P  P  I  D  Q  G  L  H  G  E  V  K  Q  D  E
CTCTCACCCGGGTAATCCCGTTGTACCCCCTATTGATCAAGGTCTACACGGCGAAGTTAAACAAGATGAA 1990

                          Eco RI
 V  W  G  V  W  T  H  L  R  E  F  G  E  F  T  I  P  T  P  K  E  S  T
GTTTGGGGGGTGTGGACACACTTGCGCGAATTCGGGGAATTTACCATCCCTACTCCAAAAGAAAGCACGG 2060

V  L  Q  P  A  V  L  E  T  P  W  D  G  V  R  S  Q  G  G  P  P  P  R  R
TTCTACAGCCGGCGGTGCTGGAAACGCCCCGGGACGGGGTCCGTTCACAAGGGGGGCCACCCCCCAGGCG 2130

  R  P  N  K  P  P  P  P  L  W  K  G  P  Q  K  W  R  P  M  R  W  P  W
CCGCCCCAACAAACCTCCCCCTCCCTTGTGGAAGGGGCCGCAAAAGTGGCGACCGATGAGGCGGCCGCGG 2200

 V  R  P  S  L  -
GTGCGCCCAAGCCTGTAGCGAGGGGTTGATCCAGCGGGCGGCCCATTGTCCCGATCGTTTCTGGGAGTGT 2270

GTGGCGGGCTCATGCTGTCTCTTCTGTTCGTAACTGGTTTATTAACTCAAGGTAGGGCTGGGAGACCTAT 2340

GTTTTGACACCCTCCGAGAGGAGATTAAAAAACAAATAGAGGGGTTGCTCCAAATATATTTTCATAATGC 2410

TCTTGTTCTTCCCCCTGGAAGATAATTCAAGACATTGCAACCCACCTTCATCACGATTCCGAGTCTTTGG 2480

AGGAACTCTTGACCATTTTGGAAGAATCTTACTGAATTAGGTCTACAAAGTCAAAGAGTTTCAACAAATT 2550

CTCCTCTTTCTCTCGCAGTGAAGTCCCGATGGCAAACTATATATAACATTTGAATTTCTCTTACATTCCA 2620

                Bam HI
CGTTTCCGAAACGGATCC  2633
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Fig. 9. Nucleotide sequence of *orf192* constructed by comparison between the 2,023-bp fragment of *V. faba* mtDNA that contains the *coxIII* gene (Macfarlane et al. 1990), and the 2,538-bp fragment of *V. faba* mtDNA that contains the *coxII-orf192* chimeric gene. The direction of transcription is indicated by a *horizontal arrow*. Overlapping sequences of these two fragments are *underlined*. The predicted amino acid sequence is shown. The nucleotide sequence is started from nucleotide 1501 of the 2,023-bp fragment

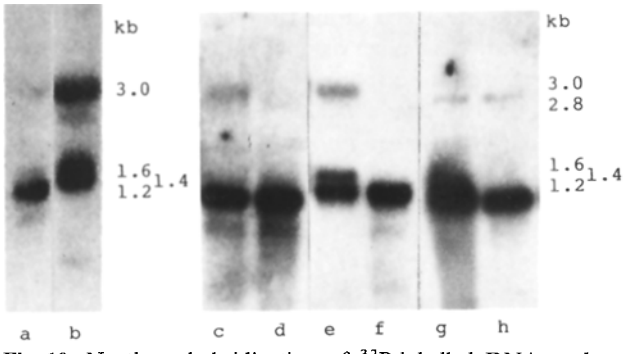

Fig. 10. Northern hybridization of ^{32}P-labelled DNA probes with mtRNA isolated from *Vicia faba:* cv "Black Russians" (*a, b*); line F (*c, e, g*); line K (*d, f, h*). The ^{32}P-labelled gene probes: *coxII* (*a, c, d*); *orf192* (*b, e, f*); *coxIII* (*g, h*). The numbers on the *right sides* of electropherograms *b* and *h show the fragment sizes in kb*

these chimeric genes contain a portion of the *coxII* gene (Dewey et al. 1985; Young and Hanson 1987; Levings and Dewey 1988; Hanson et al. 1988; Pruit and Hanson 1989). In this context, we asked the question: is the *coxII-orf192* chimeric gene present in the mitochondrial genome of *Vicia faba* cms cytoplasm? To answer this, ^{32}P-labelled *coxII* probes were hybridized with *Eco*RI digests of mtDNA from seedlings of line cms447. It was found that the mitochondrial genome of line cms447 did not contain the chimeric *coxII-orf192* gene.

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