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Multi-functional acetyl-CoA carboxylase from *Brassica napus* is encoded by a multi-gene family: Indication for plastidic localization of at least one isoform

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ABSTRACT **Three genes coding for different multi-functional acetyl-CoA carboxylase (ACCase; EC 6.4.1.2) isoenzymes from *Brassica napus* were isolated and divided into two major classes according to structural features in their 5′ regions: class I comprises two genes with an additional coding exon of approximately 300 bp at the 5′ end, and class II is represented by one gene carrying an intron of 586 bp in its 5′ untranslated region. Fusion of the peptide sequence encoded by the additional first exon of a class I ACCase gene to the jellyfish *Aequorea victoria* green fluorescent protein (GFP) and transient expression in tobacco protoplasts targeted GFP to the chloroplasts. In contrast to the deduced primary structure of the biotin carboxylase domain encoded by the class I gene, the corresponding amino acid sequence of the class II ACCase shows higher identity with that of the *Arabidopsis* ACCase, both lacking a transit peptide. The *Arabidopsis* ACCase has been proposed to be a cytosolic isoenzyme. These observations indicate that the two classes of ACCase genes encode plastidic and cytosolic isoforms of multi-functional, eukaryotic type, respectively, and that *B. napus* contains at least one multi-functional ACCase besides the multi-subunit, prokaryotic type located in plastids. Southern blot analysis of genomic DNA from *B. napus*, *Brassica rapa*, and *Brassica oleracea*, the ancestors of amphidiploid rapeseed, using a fragment of a multi-functional ACCase gene as a probe revealed that ACCase is encoded by a multi-gene family of at least five members.**

Acetyl-CoA carboxylase (ACCase; EC 6.4.1.2) catalyzes the formation of malonyl-CoA from acetyl-CoA and HCO_3^- in an ATP-dependent two-step mechanism (1). This reaction represents the first committed step in fatty acid biosynthesis. In animals, ACCase is the key enzyme for the regulation of this pathway (2). In plants, ACCase also plays a regulatory role in fatty acid biosynthesis, which occurs in plastids (3–6). In the cytosol of plant cells, ACCase provides malonyl-CoA for fatty acid elongation (7), flavonoid formation (8), polyketide synthesis (9), and several other metabolic pathways (10). For both compartments different isoforms of ACCase have been discussed (11, 12). Two distinct forms of ACCase, a dissociable, multi-subunit and a multi-functional ACCase, have been identified in plants. As in prokaryotes (13), the multi-subunit, also named prokaryotic-type ACCase, consists of three dissociable components comprising a biotin carboxyl carrier protein subunit, a biotin carboxylase subunit, and a carboxyltransferase complex made up of nonidentical α and β subunits (11, 14, 15). In all plants examined to date, acetyl-CoA carboxylases with multi-functional subunits, each of more than 200 kDa, have been identified (16). Each subunit contains a biotinylated domain, a biotin carboxylase, and a carboxyltransferase domain on a single, large protein. The native form is thought to be a dimer of multi-functional subunits (17, 18). In yeast and animals, where only multi-functional (also named eukaryotic-type ACCase) isoforms are found, active enzymes are composed of four and multiple subunits, respectively (2, 19). It is presently believed that in dicotyledonous plants the multi-subunit type is located in plastids and the multi-functional form resides in the cytosol, whereas in *Gramineae* the multi-subunit form seems to be absent and isozymes of the multi-functional type are found in both compartments (11, 12).

To gain insight into the complex mechanisms by which plant cells produce malonyl-CoA in different tissues, at different times, and in different compartments of the cell (20), it is a prerequisite to isolate and characterize the corresponding ACCase genes. Sequence data from all subunits of the prokaryotic-type, plastidic ACCase—biotin carboxylase (BC; ref. 21), biotin carboxyl carrier protein (BCCP; refs. 22 and 23), α-carboxyltransferase (CT) (24), and β-CT (23, 25)—are available from different plants as well as their biochemical characterizations (23, 26, 27). Full-length cDNA sequences encoding multi-functional ACCases from alfalfa (28), wheat (29), *Arabidopsis* (30), and maize (31) have been published. ACCase genes from *Arabidopsis* (20), *B. napus* (32), and wheat (33) have been completely sequenced. Here we report on the existence and characterization of different genes encoding isoenzymes of eukaryotic-type ACCase in *B. napus*. At least one of these genes seems to code for a multi-functional ACCase targeted to plastids.

MATERIALS AND METHODS

Isolation and Sequence Analysis of Genomic Clones. Isolation of clones from a genomic DNA library of *B. napus* cultivar Akela and the complete sequence of an ACCase gene represented by the overlapping clones BnACCaseg8 and BnACCaseg3 were described previously (32). Two additional clones, BnACCaseg10 and BnACCaseg1, representing distinct ACCase genes from rapeseed were partially subcloned and sequenced on both strands according to standard procedures. This includes the 4.3-kb *Xba*I fragment of the 5′ part of clone BnACCaseg10. From clone BnACCaseg1, a 8.6-kb and a 1.7-kb *Xba*I fragment as well as two 1.35-kb and 1.5-kb *Eco*RI fragments were subcloned. A 7.35-kb region of BnACCaseg1 was sequenced including the *Sac*I restriction site of the 5′ part

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Abbreviations: ACCase, acetyl-CoA carboxylase; BC, biotin carboxylase; GFP, green fluorescent protein; RACE, rapid amplification of cDNA ends.

Data deposition: The sequences reported in this paper have been deposited in the European Molecular Biology Laboratory (EMBL) database [accession nos. Y10301 (BnACCaseg1) and Y10302 (BnACCaseg10)].

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up to the third *Eco*RI site. The sequenced regions of the clones representing the three ACCase genes are indicated in Fig. 1.

Synthesis of ACCase-Specific cDNA Fragments. Analysis of cDNA ends were performed with 5′ and 3′ RACE kits from GIBCO/BRL. First-strand cDNA was synthesized from 1 μg total RNA using avian myeloblastosis virus reverse transcriptase. Similarly, first-strand cDNA was used to amplify six internal cDNA fragments by PCR (34) carrying altogether 16 exon/intron border sequences of the ACCase gene.

Construction of the Green Fluorescent Protein (GFP) Fusion Protein and Protoplast Transfection Protocol. The additional 5′ exon of the class I.1 ACCase gene (BnACCaseg8) was amplified by PCR (34) and cloned into the GFP expression vector pCK GFP S65C (35). The GFP fusion protein was transiently expressed in tobacco protoplasts transfected according to the protocol of Negrutiu *et al.* (36).

Microscopic Analysis. Transient expression studies of a mutated version of jellyfish *Aequorea victoria* GFP (S65C) (35) and the GFP S65C fusion with the putative transit peptide of the class I.1 ACCase in transformed tobacco protoplasts were performed using an Aristoplan fluorescence microscope (Leitz). Pictures depicting protoplasts 4 days after transfection were documented either in bright field or under blue light excitation with filter block I3 (blue light exciter BP, 450–490 nm; beamsplitter RKP, 510 nm; emitter LP, 520 nm) from Leitz on Kodak Ektachrome 320T and Kodak Ektachrome Panther P1600X films. For the elimination of chlorophyll autofluorescence in tobacco SR1 mesophyll protoplasts the filter set 41014 (exciter HQ 450/50; beamsplitter Q 480 LP; emitter HQ 510/50) (Chroma Technology, Brattleboro, VT) was used.

Southern Blot Analysis. Genomic DNA from *B. napus* cultivars Akela and Drakkar, as well as from *B. rapa* and *B. oleracea*, was isolated from leaves of seedlings (37). The DNA (20 μg) was treated with *Eco*RI, separated on 0.8% or 1% agarose gels, and transferred to Hybond-N nylon membranes (Amersham) (38). The blot was hybridized to a ^{32}P-labeled probe in hybridization buffer containing 3× SSC (0.15 M NaCl/15 mM sodium citrate, pH 7.4), 0.02% (wt/vol) Ficoll, 0.02% (wt/vol) polyvinylpyrrolidone 25 (PVP), and 0.1% (wt/vol) SDS at 64°C for at least 6 hr. After hybridization the blot was washed twice in 2× SSC + 0.1% (wt/vol) SDS and once in 0.2× SSC + 0.1% (wt/vol) SDS at hybridization temperature for 20 min each and finally exposed to film.

RESULTS

Isolation and Characterization of ACCase-Specific Genomic DNA Clones. Screening of a genomic library from *B. napus* with an ACCase-specific cDNA fragment generated by PCR resulted in the isolation of 11 clones (32). Restriction mapping revealed that these clones formed three distinct groups, each representing a different gene. Representatives of each gene group fall in two major classes (class I and class II), as shown in Fig. 1. The cDNA ends corresponding to the class I.1 gene represented by the two overlapping clones BnACCaseg3 and 8 were determined by 5′ and 3′ RACE. BnACCaseg8 contains the complete coding sequence of that gene, and BnACCaseg3 carries its promoter region. To identify exon/intron borders within the structural gene, six internal cDNA fragments were obtained in addition to the cDNA ends, altogether excluding 18 of the 31 intron sequences of this gene (Fig. 1). The remaining exon/intron borders were determined by identification of consensus splice sites (39) and by comparisons of the deduced amino acid sequence with that of ACCases from other organisms. The deduced cDNA has an open reading frame of 6,912 bp and codes for 2,304 amino acids (32). Partial sequence informations of clones representing the two other genes (BnACCaseg1 and 10) include the 5′ parts of the coding regions and at least 3 kb upstream of the proposed translation start sites. The 5′ end of the corresponding cDNA of BnACCaseg1 was identified by 5′ RACE, and the putative transcription start of BnACCaseg10 by sequence comparison with the gene in BnACCaseg8.

The three ACCase genes from *B. napus* can be divided into two major classes: class I, containing two genes (represented by clones BnACCaseg8 und 10) with an additional coding exon of approximately 300 bp at the 5′ end, and class II with one gene (BnACCaseg1) carrying an intron of 586 bp in its 5′ untranslated region. The 5′ coding exon is also missing in ACCase sequences from *Arabidopsis* (20, 30), alfalfa (28), and wheat (29, 33). Analysis of the deduced amino acid sequence

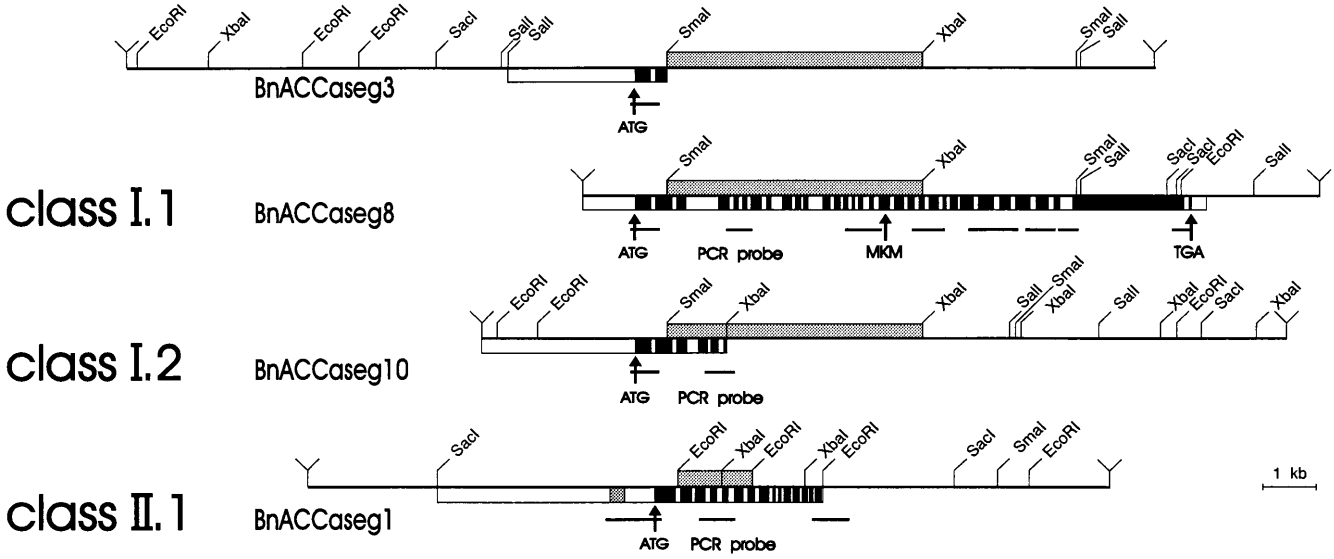

FIG. 1. Restriction map of genomic clones of ACCase genes from *B. napus*. The organization of different ACCase gene classes is shown. Clones BnACCaseg3 and 8 are overlapping and represent the complete class I.1 gene (promoter and coding sequence). Restriction fragments hybridizing with the PCR probe used for gene isolation are indicated as gray bars. Regions analyzed by sequencing are shown as white bars interrupted by black boxes describing exons of the coding region or by a gray box pointing to an intron in the 5′ untranslated region (BnACCaseg1). Exons that are confirmed by sequencing of cDNA fragments generated by reverse-transcriptase–PCR are underlined. Positions of the identified start ATG and stop TGA codons as well as the MKM biotin binding motif are indicated. The "Y" at the ends of each clone symbolizes *Xba*I, *Sac*I, *Not*I, and *Sal*I restriction sites.

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BnACCaseg 8   MEMRALVSCSAAGNGASDRFRLSNVSPWITSARGASGSDSPATVKLGSSSMIRAFKGVSIYKNKTRRNVLSQRNKQFRPMAYLGRKDLSSPDPTSFCDND 100
BnACCaseg 10  MEMRALVSCSAAGNGASDRFRLSNVSPWITSARGASGSDSPATVKLGSSSMIRAFKGVSIYKNKTRRNVLSQRNKQFRPMAYLGRKDLSSPDPTSFCDND 100
BnACCaseg 1   ..........................................................................................................
Arabidopsis   ..........................................................................................................

BnACCaseg 8   ISEPQGTGSINGNDHSAV.......RVSQVDEFCKAHGGKRPIHRILVATNGMAAVKFIRSVRAWSYQTFGSEKSISLVAMATPEDMRINAEHIRIADQF 193
BnACCaseg 10  ISEPQGTGSINGNDHSAV.......RVSQVDEFCKAHGGKRPIHSILVATNGMAAVKLIRSVRAWSYQTFGSEKSISLVAMATPEDMRINAEHIRIADQF 193
BnACCaseg 1   .....MAGSVNGVQ....TPGRNHVSVSEVDDFCIALGGKRPIHSILIANNGMAAVKFIRSVRTWAYETFGTERALLLVGMATPEDMRINAEHIRIADQF  91
Arabidopsis   .....MAGSVNGNL.HSAVGPGINYETVSQVDEFCKALRGKRPIHSILIANNGMAAVKFIRSVRTWAYETFGTEKALLLVGMATPEDMRINAEHIRIADQF  94

BnACCaseg 8   MQVPGGTNNNNYANVHLIVEMAEATGVDAVWPGWGHASENPELPDALKAKGVIFLGPTAASMLALGDKIGSSLIAQAADVPTLPWSGSHVKIPPGSSLVT 293
BnACCaseg 10  MQVPGGTNNNNYANVHLIVEMAQATGVDAVWPGWGHASENPELPDALKAKGVIFLGPTAASMLALGDKIGSSLIAQAADVPTLPWSGSHVKIPPGSSMVT 293
BnACCaseg 1   VEVPGGTNNNNYANVQLIVEMAETTRVDAVWPGWGHASENPELPDALKAKGIIFLGPPAASMAALGDKIGSSLIAQAADVPTLPWSGSHVKIPPDSSLVT 191
Arabidopsis   VEVPGGTNNNNYANVQLIVEMAETTRVDAVWPGWGHASENPELPDALDAKGIIFLGPPASSMAALGDKIGSSLIAQAADVPTLPWSGSHVKIPPYSNLVT 194
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FIG. 2. Comparison between deduced N-terminal amino acid sequences of ACCase genes from *B. napus* [BnACCaseg8 (32); BnACCaseg1, accession no. Y10301; BnACCaseg10, accession no. Y10302] and *Arabidopsis* (20). The first 100 residues deduced from BnACCaseg8 and 10 are encoded by an additional 5′ exon. Amino acids that are not identical as compared with that encoded by BnACCaseg8 are indicated in reverse print.

of the additional class I coding exon revealed characteristics of a transit peptide (ref. 40; Fig. 2). There were a high amount of serine and threonine residues, very low amounts of acidic amino acids, and a positively charged domain in the center of the sequence between the positions of amino acid 53 and 86 (Fig. 2). To examine the function of the putative transit peptide, the corresponding DNA sequence was fused to that of a mutated version of the jellyfish *Aequorea victoria* GFP (S65C) (35). Upon transient expression in tobacco protoplasts the GFP fusion protein was localized in chloroplasts (Fig. 3 *a* and *b*). Green fluorescence in plastids was especially visible in small protoplasts with only a few chloroplasts (Fig. 3*b*). In larger protoplasts with many chloroplasts, the green fluorescent color was often covered by the red autofluorescence of chlorophyll. However, by using a special filter set, the red autofluorescence was suppressed, and therefore, the intensity of green fluorescence was enhanced (not shown). By transient expression of GFP without the proposed transit peptide, green fluorescence was only observed in the cytoplasm (Fig. 3*c*).

A comparison of N-terminal amino acid sequences of the three different ACCases from *B. napus* and the ACCase from *Arabidopsis* (20) is shown in Fig. 2. The two class I genes were very similar to each other with respect to their coding regions. The deduced amino acid sequence of the partially sequenced clone BnACCaseg10 had 98% identical residues compared with that of BnACCaseg8. But the promoter regions of the two class I genes were substantially different (not shown). The enzyme encoded by the *B. napus* class II.1 gene (represented by clone BnACCaseg1) showed a higher identity to the ACCase from *Arabidopsis* (94% identical amino acids) than to the

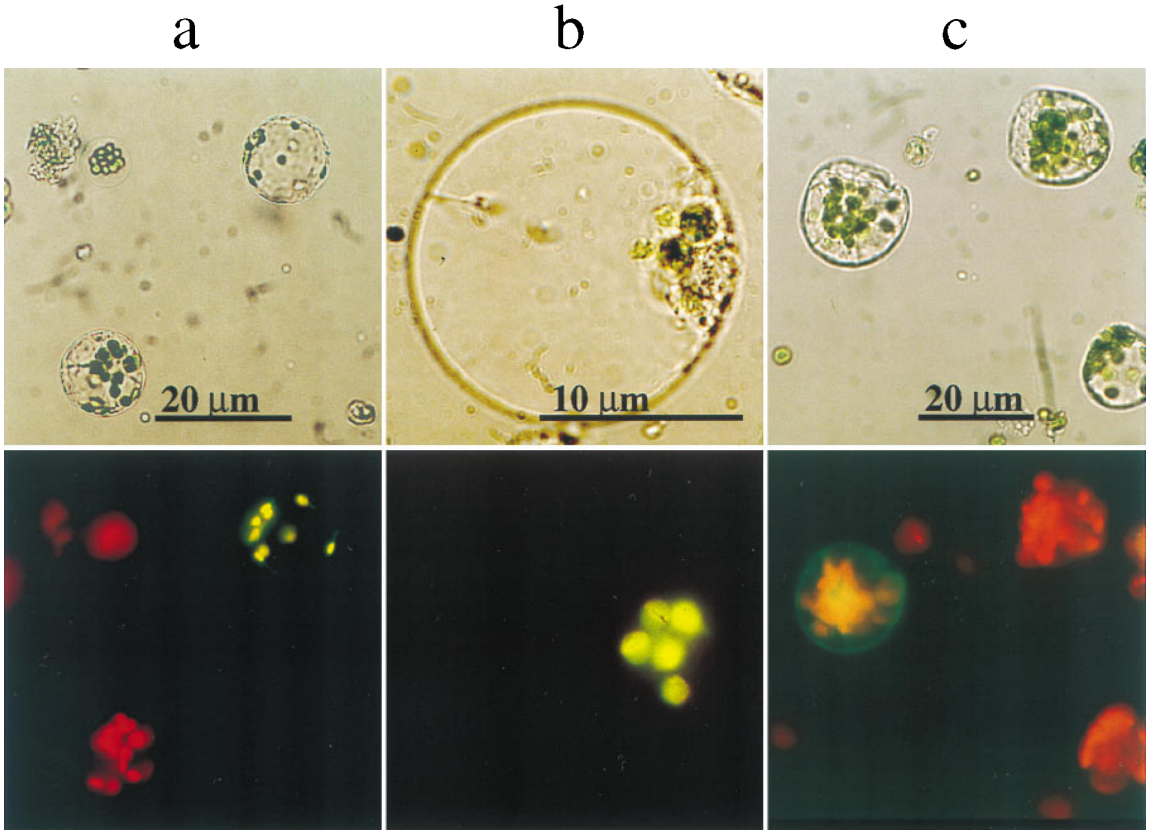

FIG. 3. Subcellular localization of green fluorescent protein (GFP S65C) with (*a* and *b*) or without (*c*) fusion to the peptide sequence encoded by the additional 5′ exon of the ACCase class I.1 gene in tobacco protoplasts. The additional 5′ exon was amplified by PCR and cloned into the GFP expression vector pCK GFP S65C (35). The GFP as well as the GFP fusion protein was transiently expressed in tobacco protoplasts transfected according to the protocol of Negrutiu *et al.* (36). Pictures depicting protoplasts 4 days upon transfection were documented either in bright field (*Upper*) or under blue light excitation (*Lower*) with filter block I3 (blue light exciter BP, 450–490 nm; beamsplitter RKP, 510 nm; emiter LP, 520 nm) from Leitz on Kodak Ektachrome 320T and Kodak Ektachrome Panther P1600X films.

corresponding region of rapeseed ACCase encoded by the clone BnACCaseg8 (89% identical amino acids) in its 5′ region. The first seven amino acids were identical in both *B. napus* class II.1 and *Arabidopsis* enzymes. In most cases where differences were detectable in the amino acid sequence between the ACCase encoded by the clone BnACCaseg1 (class II.1) and that encoded by clone BnACCaseg8 (class I.1), the corresponding amino acids of class II.1 ACCase were identical with that of the *Arabidopsis* ACCase (Fig. 2).

5′ RACE experiments to determine the 5′ ends of cDNAs coding for the class II ACCase led to the identification of three groups of cDNA fragments with different nucleotide sequences. The cDNA fragments of all three groups lacked the sequence coding for the proposed transit peptide found in clone BnACCaseg8. Nine 5′ cDNA fragments belonging to one group with 100% sequence identity to the clone BnACCaseg1 began at various positions in the 5′ region of the gene.

Hybridization Experiments. Southern blot analysis using *Eco*RI-treated genomic DNA of three *Brassica* species and the 766-bp *Eco*RI/*Xba*I DNA fragment of BnACCaseg1 (Fig. 1) as a probe revealed four (*B. rapa*) and five (*B. napus* and *B. oleracea*) cross-hybridizing bands as shown in Fig. 4. Three of the bands identified by Southern blot analyses with genomic DNA of *B. napus* are represented by the isolated genomic clones described in Fig. 1. The largest hybridizing fragment corresponds to the class I.1 gene, the approximately 10-kb fragment is correlated with the class I.2 gene, and the 1.3-kb band with the class II.1 gene. For the additional hybridizing fragments (5.3 kb and 2.7 kb) visible in all tested species, corresponding genomic ACCase clones are missing.

DISCUSSION

The results described in this paper demonstrate that in *B. napus*, multi-functional ACCase is expressed by a multi-gene family. Characterization of the three identified ACCase genes from *B. napus* by sequencing allows for a classification into two classes: one class with an additional 5′ exon in the coding region of two ACCase genes and another class represented by one gene that lacks the 5′ coding exon and contains an intron in the 5′ untranslated region. The 100-aa sequence encoded by the additional first exon of the class I.1 gene is composed of a large number of serine and threonine residues, very low amounts of acidic amino acids, and a positively charged domain in the center of the sequence, altogether representing features of transit peptides (40). This putative transit peptide clearly targets GFP fused to it to chloroplasts of transformed tobacco protoplasts. These results strongly suggest that the additional N-terminal amino acid sequence acts as a transit peptide, which may also target class I ACCases to plastids. The occurrence of a multi-functional ACCase isoform in plastids was thus far demonstrated only for monocots (12, 17). In plastids of *B. napus*, a dicotyledonous plant, the existence of a multi-functional ACCase in addition to a multi-subunit one (23, 26) poses the question as to which isozyme catalyzes the first committed step in fatty acid biosynthesis. Therefore, experiments eliminating one or the other isoform by antisense repression or overexpression studies will show which isoform is the most important in providing malonyl-CoA for *de novo* fatty acid biosynthesis in rapeseed. Class I.1 and I.2 genes have different promoter sequences, indicating the possibility of an even more complex regulation. Southern blot analyses with the first *Xba*I/*Sma*I DNA fragment of the clone BnACCaseg8 encompassing the additional 5′ exon as a probe confirmed the existence of at least two class I ACCase isogenes (not shown).

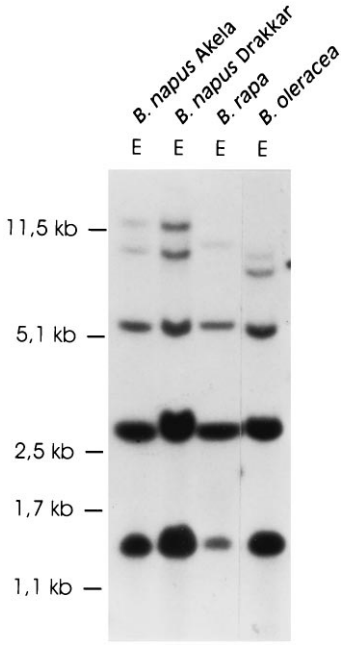

FIG. 4. Southern blot analysis with genomic DNA from *B. napus* cultivars Akela and Drakkar, *B. rapa*, and *B. oleracea*. Genomic DNA digested by *Eco*RI (E) was probed with the 766-bp *Eco*RI/*Xba*I fragment of clone BnACCaseg1 encompassing exon 2-4 of the class II.1 ACCase gene.

The occurrence of a multi-functional isoform along with a multi-subunit isoform in developing rapeseed embryos is supported by biochemical experiments carried out by Roesler *et al.* (26). In two high-erucic-acid cultivars, the BC polypeptide was present throughout embryo development. In contrast, the eukaryotic-type ACCase was only detected early in embryo development, although erucic acid is mainly formed at later developmental stages. This could mean that the detected peak expression of the multi-functional ACCase reflects the demand for malonyl-CoA for fatty acid biosynthesis in plastids rather than the demand for malonyl-CoA for elongation of fatty acids to erucic acid in the cytosol. Previous studies indicated that ACCase might be responsible for the limitation of lipid synthesis in rapeseed, because ACCase activity was reduced before accumulation of lipids was achieved (41, 42). In the early work of Turnham and Northcote in 1983 (41), expression of a 225-kDa biotin-containing protein, supposed to be a multi-functional ACCase, was observed during embryo development. Protein patterns of avidin-binding proteins and ACCase activity were investigated in seed extracts of different developmental stages. The highest ACCase activity was measured in extracts showing the occurrence of a protein band with an apparent molecular mass of 225 kDa as analyzed on a SDS polyacrylamide gel.

Until now it was believed that in most higher plants, multi-functional ACCase is exclusively localized in the cytoplasm and the multi-subunit isoform in the plastids. An exception are the *Gramineae.* So far, all analyzed members of this family have multi-functional ACCases in both compartments and lack the multi-subunit form (12). *Gramineae* are susceptible to the selective grass herbicides (graminicides) of the aryloxyphenoxypropionic acid type (fenoxaprop) and the cyclohexanedione type (sethoxydim), which inhibit ACCase activity, thus blocking fatty acid biosynthesis (43). The target of these herbicides is probably the plastidic, eukaryotic-type ACCase, whereas the prokaryotic type absent in *Gramineae* is resistant (11). However, Evenson *et al.* (44) reported that a multi-functional ACCase in *Lolium multiflorum* is resistant to the aryloxyphenoxypropionate diclofop.

The ACCase encoded by the class II.1 gene like those from alfalfa (28), *Arabidopsis* (20, 30), and wheat (29, 33) are lacking a transit peptide and probably represent cytosolic isoforms. Presumably the class II can be also divided into subclasses. 5′ RACE products obtained to define the 5′ cDNA end of the

class II.1 gene led to the identification of three different groups of class II cDNA fragments, with one corresponding to BnACCaseg1. None of the nine 5′ cDNA fragments corresponding to the class II.1 gene starts with the same nucleotide, suggesting various transcription start points. In the promoter of the class II.1 ACCase gene there is no sequence similar to a TATA box. In animal cells the absence of a TATA sequence has been observed in housekeeping genes (45, 46).

A comparison of deduced amino acid sequences covering the BC domain of the three rapeseed ACCases with those from multi-functional ACCases of other organisms as well as those of *Escherichia coli* and tobacco BC subunits from prokaryotic-type ACCases is shown as a dendrogram in Fig. 5. Striking differences are observed between the sequences of the two proposed plastidic class I ACCases encoded by BnACCaseg8 and 10, and of the class II ACCase encoded by BnACCaseg1, and of other known plant ACCases, which all lack a transit peptide. BC domains of ACCases from the dicotyledonous plants *B. napus*, *Arabidopsis thaliana*, and alfalfa show higher similarity to each other than to those of the monocotyledonous plants, wheat and maize. The cDNA of a multi-functional ACCase from maize (31) codes for a protein with an N-terminal extension of about 120 additional amino acids when compared with that from wheat (29). Possibly, the maize ACCase represents a plastidic isoform, whereas the ACCase from wheat is thought to be a cytosolic enzyme. The existence of 227-kDa ACCase proteins in extracts of maize mesophyll chloroplasts was previously demonstrated (17). The deduced sequence of the first 100 N-terminal amino acids of the maize ACCase shows no significant similarity with that of the transit peptide of the class I ACCases from *B. napus*. Generally, the primary structure of transit peptides is not very conserved (40). The dendrogram indicates that the similarity among multi-functional ACCases of dicotyledonous plants is higher than that among the proposed plastidic ACCases derived from rapeseed (dicot) and wheat (monocot). Moreover, the primary structures of BC domains from plant multi-functional ACCases are much more similar to that of animal and yeast multi-functional ACCases than to the amino acid sequence of the BC subunit from tobacco prokaryotic-type ACCase.

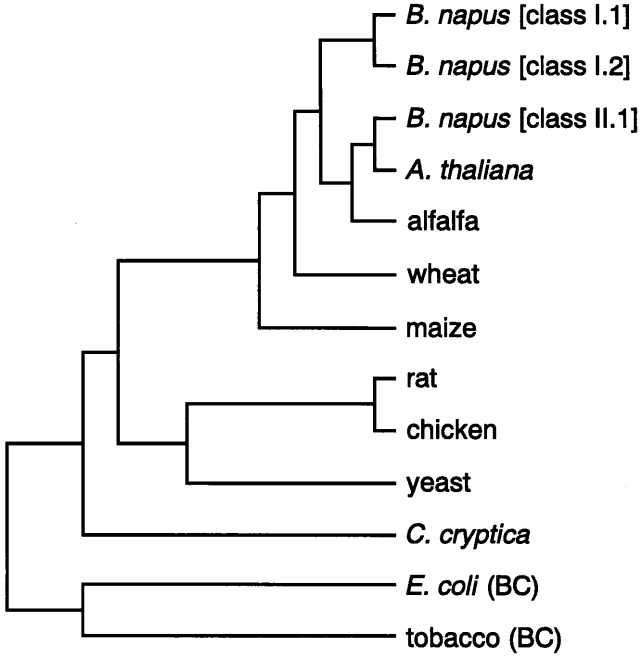

FIG. 5. Dendrogram based on a comparison of deduced amino acid sequences covering BC domains or BC subunits from ACCases of different organisms using the computer program GCG PILEUP (47). BC domain sequences were identified showing significant similarity to the primary structure of *E. coli* BC. The increase in length of the horizontal lines schematically indicates the degree of decreasing similarity. Amino acid sequences of BC domains and BC subunits of ACCases are taken from following organisms: *B. napus* (32), *Arabidopsis thaliana* (20), alfalfa (28), wheat (29), maize (31), rat (48), chicken (49), yeast (50), *Cyclotella cryptica* (51), *E. coli* BC (52), and tobacco BC (21).

Southern blot analysis revealed multiple ACCase genes in *B. napus*, three of which are represented by the isolated genomic clones. The observed 5.3-kb and 2.7-kb bands visible in all tested *Brassica* species may both represent an unidentified ACCase gene if these two bands were the result of an *Eco*RI restriction site within the cross-hybridizing part of that gene. It is not very likely that the unidentified gene(s) represented by the 5.3-kb and 2.7-kb bands correspond to the BC subunit of multi-subunit ACCase, because nucleotide sequences between prokaryotic-type (28, 52) and eukaryotic-type ACCases are not very conserved. Apart from this gene(s), the existence of at least two genes in *B. rapa* and three in *B. oleracea*, the parents of amphidiploid *B. napus*, suggests a multi-gene family of at least five gene members coding for multi-functional ACCases in rapeseed. The genomic organization of two genes seems to be very similar in the parents, so that the parental genes are only visible as three bands in *B. napus*. Provided that the gene(s) represented by the 5.3-kb and 2.7-kb bands also encodes a multi-functional ACCase there would be an even more complex eukaryotic-type ACCase gene family in *B. napus*. The difference in the pattern of cross-hybridizing bands in the DNA of parental and progeny species are probably explainable by restriction fragment-length polymorphisms between different *B. rapa* or *B. oleracea* varieties. Evidence for multiple ACCase genes in other plants have been obtained in *Arabidopsis* (20), maize (53), wheat and soybean (29), as well as alfalfa and rice (28).

The identification of several ACCase genes in *B. napus* further emphasizes the versatile functions of ACCase isoenzymes in plant cells. Future promoter analyses and overexpression studies of the class I.1 gene under the control of different promoters in transgenic rapeseed plants will lead to a better understanding of the role of eukaryotic-type ACCase for fatty acid biosynthesis in plants. Currently aspects are being investigated as to whether the multi-functional ACCase plays a crucial role in *de novo* fatty biosynthesis in plastids of *B. napus* and whether the ACCase gene might provide a powerful tool for directing the metabolic flux toward oil accumulation in seeds by genetic engineering.

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