Isolation of cDNAs from *Brassica napus* encoding the biotin-binding and transcarboxylase domains of acetyl-CoA carboxylase: assignment of the domain structure in a full-length *Arabidopsis thaliana* genomic clone

Kieran M. ELBOROUGH,* Russel SWINHOE, Robert WINZ, Johan T. M. KROON, Lee FARNSWORTH, Tony FAWCETT, José M. MARTINEZ-RIVAS and Antoni R. SLABAS

Lipid Molecular Biology Group, Biological Sciences Department, University of Durham, South Road, Durham DH1 3LE, U.K.

One independent and two overlapping rape cDNA clones have been isolated from a rape embryo library. We have shown that they encode a 2.3 kb and a 2.5 kb stretch of the full-length acetyl-CoA carboxylase (ACCase) cDNA, corresponding to the biotin-binding and transcarboxylase domains respectively. Using the cDNA in Northern-blot analysis we have shown that the mRNA for ACCase has a higher level of expression in rape seed than in rape leaf and has a full length of 7.5 kb. The level of expression during rape embryogenesis was compared with both oil deposition and expression of two fatty acid synthetase components enoyl-(acyl-carrier-protein) reductase and 3-oxoacyl-(acyl-

carrier-protein) reductase. Levels of ACCase mRNA were shown to peak at 29 days after anthesis during embryonic development, similarly to enoyl-(acyl-carrier-protein) reductase and 3-oxoacyl-(acyl-carrier-protein) reductase mRNA. In addition, a full-length genomic clone (19 kb) of *Arabidopsis* ACCase has been isolated and partially sequenced. Analysis of the clone has allowed the first plant ACCase activity domains (biotin carboxylase–biotin binding–transcarboxylase) to be ordered and assigned. Southern-blot analysis using the *Arabidopsis* clone indicates that ACCase is a single-copy gene in *Arabidopsis* but is encoded by a small gene family in rape.

INTRODUCTION

In our laboratory we have focused on the enzymology and regulation of lipid synthesis in plants, particularly with respect to the deposition of seed storage lipids [1]. A key component in such synthesis is the enzyme acetyl-CoA carboxylase (ACCase) (EC 6.4.1.2). ACCase catalyses the ATP-dependent carboxylation of acetyl-CoA to form malonyl-CoA, pivotal in supplying the substrate for both fatty acid [2] and flavonoid biosynthesis [3]. From an industrial viewpoint the ACCase gene/protein is also a prime target for herbicide action in dicotyledonous plants [4–6]. The nuclear-encoded protein (type I) from a variety of plant tissues has been extensively studied and there is a consensus that its subunit molecular mass is more than 220 kDa [7–14]. Recently it has become apparent that there may also be a chloroplast-encoded (type II) ACCase [15] which resembles the prokaryotic form having three separate polypeptides. The 220 kDa type-I enzyme has been purified from rape seed [13] but no DNA sequence data have been presented to date. Turnham and Northcote [16] demonstrated that, during rape embryogenesis, ACCase protein activity is induced before the deposition of storage lipid. They showed that its activity, unlike other fatty acid synthetase components, was rapidly switched off after attainment of full lipid deposition. Although this study was both comprehensive and reproducible, no analysis of rape ACCase expression, at the mRNA level, has been presented to date. Northern-blot analysis has indicated that, in the monocots maize [7] and wheat [11], the higher-plant mRNA (like animal ACCase) is exceptionally long (7.5–8 kb), impeding direct isolation of a full-length cDNA. In animals [17,18] cDNA sequences have been deduced using sets of overlapping clones but no full-length cDNA has yet been reported. A recent report by Roessler and

Ohlrogge [19] described the isolation and sequencing of a full-length algal ACCase genomic clone.

To obtain further insight into ACCase from dicotyledonous plants we attempted to isolate a cDNA for rape ACCase by heterologous probing with a wheat ACCase cDNA clone. The wheat clones identity was verified by direct amino acid sequencing [11].

The aims of the present study were twofold: (i) to isolate rape ACCase cDNAs, and (ii) clone the ACCase gene from *Arabidopsis*, which has a smaller genome than rape. Any cDNA would permit us to look at the expression of ACCase (by Northern blots), generate antibodies to the overexpressed protein and construct ACCase antisense constructs with the cDNA to study down-regulation. A genomic clone would provide the means to assign the plant ACCase domain structure and in turn allow the use of PCR to synthesize probes for further rape cDNA cloning. This paper describes (i) the successful cloning and sequencing of three partial-length cDNA clones of rape ACCase, (ii) expression studies on the gene in relation to both oil deposition and expression of other fatty acid synthetase components and (iii) the isolation, characterization and domain assignment of a full-length *Arabidopsis* genomic clone.

MATERIALS AND METHODS

cDNA library construction

cDNA library from poly(A)⁺ RNA

The cDNA library used was generated using poly(A)⁺ mRNA isolated by the method of Logemann et al. [20] from mid-stage developing Jet neuf rape embryos (harvested at approximately 35 days after anthesis). First-strand synthesis was carried out using

Abbreviations used: ACCase, acetyl-CoA carboxylase; poly(A)⁺, polyadenylated; 1 × SSC, 0.15 M NaCl plus 0.015 M sodium citrate; ACP, acyl-carrier protein.

* To whom correspondence should be addressed.

The DNA sequences presented in this paper have been submitted to the EMBL/Genbank data libraries under accession numbers X77374–X77382.

```

      | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80
1  ARGNSLIYH SITKKGPLHG TQINDQYKPL GYLDQRQLAA RRSNTTYCYD FPLAFETALE QFGHYNNREL RNHARVLLSV
81 LKSLYSPISE GTSLMPVERS PGLNEFGWVA WSELMSTPEF PMGRKLLIVA NDVTFKAGSF GPREDAFFLA VTELACPKKL
161 PLIYLAPNSG ARLGVAEEIK ACFKVGWSDS VSPENGFQYI YLSPEDHARI GSSVIAHEIK LPSGETRWVI DTIVGKEDGI
241 GVENLTGSGP IAGAYSRAYN ETFTLTFVSG RTVGIGAYLA PLGMRCIQR L DQPIILTGF S TLNKL LGREV YSSHMLGGP
321 KIMGTNGVVH LTVSDDLEGV SAILDWLSYI PAYVGGPLPV LAPLDPPDR T VEYVPENSCD PRAAIAGVND NTGKWLGGIF
401 DKNSFIETLE GWARTVVTGR AKLG GIPGV VAVETQTMQ IIPADPGQLD SHERVVPQAG QVWFPSAGK TAQALMDFTR
481 KSFHCLSLRT GEGFQVGREI FSKEYFRQVA TIVENLRTYR QPVFVYIPKM GELRGGAMVV VDSQINSYV EMYAETARG
561 NVLEPEGTIE IKFRTKEMLE CMGRLDPKLI DLKARLQDPN QSEAYTNIEL LQQQIKAREK LLLPVYIQIA TKFAELHOTS
641 MRMTAKGVIK MCVEWIGSR S FFKYKLNRR I AENSLVKNR EASGDLSYK SAMGLIQDWF SKSDIPKGKE EAWTDDQVFF
721 TWKDNVSNYE LNLSELRPQK LLNPTCRDWK FRRIYRRCHK DLPIF 765

```

Figure 1 Rape ACCase transcarboxylase domain sequence

Derived amino acid sequence from rape cDNA encoding the transcarboxylase domain of ACCase. The amino acid sequence is translated from the first open reading frame shown pictorially. The full vertical lines represent stop codons and the half vertical lines ATG sequences.

poly(dT) oligo primers according to the manufacturers' instructions (Amersham International). The cDNAs generated were cloned into the *EcoRI*–*XhoI* sites of λ ZAPII as recommended by the manufacturers (Stratagene). The host bacterium used was XL-1 Blue (Stratagene).

Random-primed library

Poly(A)⁺ mRNA (5 μ g) from 35-day-old (post-anthesis) Jet neuf rape embryo was used for the construction of a random-primed cDNA library. The double-stranded cDNA was prepared using a 1 in 10 dilution of pd(N)6 primers (0.74 μ g/ μ l) according to the instructions provided with the TimeSaver cDNA synthesis kit (Pharmacia). The library was prepared in λ ZapII and packaged with Gigapack II Gold packaging extract (Stratagene). The host *Escherichia coli* strain used was XL-1 Blue (Stratagene).

Genomic library

The *Arabidopsis thaliana* library used (a gift from Dr. John Cowl, John Innes Institute, Norwich, U.K.) was made from leaf total DNA in λ FIXII and the host *E. coli* strain used was KW251.

Probe preparation and labelling

cDNA probes for screening the rape libraries were generated by the appropriate restriction endonuclease digestions of plasmid DNA. The DNA fragment required was separated from vector DNA by agarose electrophoresis and isolated using the Gene-Clean II kit (Bio 101).

The probes (200–300 ng) were radiolabelled with [α -³²P]dCTP using the Megaprime kit as recommended by the manufacturers (Amersham International) to a level of 5×10^9 c.p.m./ μ g. Unincorporated label was removed using Biospin chromatography columns (Bio-Rad).

Library screening

For both the rape random-primed library and poly(dT)-primed

cDNA library 150000 plaque-forming units were screened as previously described [11]. The filters were hybridized at 65 °C and washed four times with $1 \times$ SSC/0.1 % SDS for 30 min at 65 °C. Plasmid rescue of cDNA clones was carried out as described in the Stratagene protocol for 'in vivo excision of pSK from λ ZAPII'.

For the *Arabidopsis* genomic library 2×10^4 plaque-forming units were screened essentially as above.

Preparation of genomic DNA from positive plaques

A single positive plaque from the tertiary screen was incubated with 500 μ l of fresh KW251 cells at 37 °C for 20 min. Prewarmed Luria–Bertani medium (50 ml at 37 °C) and 500 μ l of 1 M MgSO₄ were added. The culture was incubated with mild shaking at 37 °C for 5–7 h (until lysis occurred), and 250 μ l of chloroform was added to the culture for a further 15 min at 37 °C. DNA was isolated using the Magic Lambda prep (Promega) procedure as described by the manufacturers.

Subcloning was carried out by the method of Sambrook et al. [21].

Sequencing of DNA clones

Sequencing was carried out using an Applied Biosystems Inc. 373A DNA sequencer (Durham University sequencing service; Ms. J. Bartley). Both forward and reverse primers (–21m13 and M13RP1) were used initially for all clones. Nested deletions were generated as recommended by Pharmacia (d.s. Nested Deletion Kit) and sequenced using a combination of forward and reverse primers and synthesized oligonucleotide primers. Primers were made using a 381A DNA synthesizer (Applied Biosystems Inc.). Computer analysis of DNA sequences was carried out using the SEQNET package from the SERC facility at Daresbury and DNA Strider [22].

Northern-blot analysis

Poly(A)⁺ mRNA was prepared from either 5 g of young leaf or

5 g of embryos harvested 15, 22, 29, 36, 42 and 49 days after flowering using the recommended procedure (Pharmacia mRNA purification kit). Then 1–5 μ g was loaded on to a 1% formamide/formaldehyde-agarose gel for electrophoresis. The Northern-blot procedure was carried out as described previously [11].

Lipid analysis

Preparation of lipid from seed was carried out by Dr. W. W. Christie (SCRI, Dundee, U.K.) as described previously [23].

Fatty acid isopropyl esters were analysed on a Carlo Erba model 4130 capillary gas chromatograph (Erba Scienca, Swindon, Wilts., U.K.), equipped with a split/splitless injector maintained at 260 °C. A fused silica capillary column (25 m $\times$ 0.22 mm internal diameter, film thickness 0.2 mM) coated with Carbowax 20M (Chromopack U.K. Ltd., London, U.K.) was used. The oven temperature was programmed for 3 min isothermally at 175 °C, then to 205 °C at a rate of 4 °C/min and held at the final temperature for 20 min. Hydrogen was the carrier gas. Fatty acids were identified by reference to standards and were quantified by electronic integration.

Southern-blot analysis

Total DNA isolated from rape and *Arabidopsis* leaves (10 μ g and 2 μ g per digestion respectively) was digested with *Eco*RI, *Hind*III and *Bam*HI separately for 8 h. The DNA was separated

by agarose electrophoresis, blotted and hybridized to radio-labelled probe as described by Sambrook et al. [21].

RESULTS

Isolation of a partial rape ACCase cDNA encoding the transcarboxylase domain

Although ACCase has been purified from rape embryo, the amounts obtained were not amenable to protein sequencing. To study ACCase at the sequence level, we needed to isolate its cDNA. A rape-embryo-derived poly(dT)-primed λ ZapII library was screened with a partial wheat ACCase cDNA previously isolated in this laboratory. A hybridizing cDNA of 2.5 kb was taken through three rounds of screening and plasmid rescue (pRS1). The clone was fully sequenced in both directions by a combination of nested deletions and dye primer sequencing. The cDNA sequence has been submitted to EMBL (accession no. X77382). The predicted amino acid sequence from the largest

Figure 2 Comparison of rape transcarboxylase domain with known ACCase sequences

Dot matrix (DNA Strider, Stringency 9 Window 21) of derived rape ACCase amino acid sequence (transcarboxylase domain) against yeast (a), rat (b) and algal (*Cyclotella*) (c) ACCase.

Figure 3 Northern-blot analysis of rape ACCase

(a) Oil content as total fatty acid (mg/seed) in relation to the stage of rape embryogenesis. Details of the method of analysis are presented in the Materials and methods section. The three Northern blots shown, related to the different stages of embryogenesis, are all derived from the same blot after successive stripping. The probes used were as indicated in the text, and the amount of poly(A)⁺ RNA was 5 μ g for each state. Hybridization and washing conditions were as described in the Materials and methods section. Exposure was for 7 days. (b) The probe used in the Northern blot shown was the rape transcarboxylase domain cDNA derived from an embryo library. Poly(A)⁺ RNA (1 μ g) from embryos 29 days after anthesis or young leaf was used for the blot. Hybridization and washing conditions were as described in the Materials and methods section. Exposure was for 7 days. Molecular-mass markers were viewed by ethidium bromide/u.v.

Figure 4 Assignment of domain order to higher-plant ACCase

(a) Schematic diagram showing the yeast ACCase domain orders relative to the sequenced regions (hatched boxes) of the *Arabidopsis* genomic clone. The areas of sequenced genomic clone are labelled A–F for easy identification in the text. (b) (i) The translated open reading frame (ORF) from area Aii is shown in direct comparison with a region from the biotin carboxylase domain of yeast. Boxed regions represent amino acid identity. (ii) The translated open reading frame corresponding to the biotin-binding site in area C is shown in direct comparison with the biotin-binding site of yeast. Boxed regions represent amino acid identity. (iii) DNA sequence comparison by dot matrix (DNA Strider, Stringency 15 Window 23) of the rape transcarboxylase domain of ACCase and areas E/F from the *Arabidopsis* genomic clone. Amino acid sequence dot matrix comparison of the rape transcarboxylase domain with that of rat ACCase can be seen in Figure 2.

open reading frame is shown in Figure 1. Dot matrix analysis of the cDNA with previously described ACCase sequences showed it to be a partial clone of ACCase corresponding to the transcarboxylase domain (Figure 2). The predicted amino acid sequence of the rape clone showed sequence identity/similarity levels of approximately 44/61% with the yeast [24], rat [17], algae [19] and the wheat ACCase clone which had been previously authenticated by peptide sequencing [11]. As the mRNA contains a poly(A) tail and was obtained from the poly(A) fraction, it is probable that ACCase cDNA isolated was nuclear encoded.

Northern-blot analysis of rape embryogenesis

Oil production occurs at different rates during rape embryogenesis, therefore it was important to study the corresponding levels of ACCase. Although ACCase activity has been studied in relation to embryogenesis [16], no information on mRNA levels has been reported. The expression of ACCase during rape embryonic development was examined by Northern blotting using the 2.5 kb rape cDNA clone as a probe. The blots contained 5 µg of rape poly(A)⁺ mRNA prepared from a set of staged embryos taken from *Brassica napus* Jet Neuf at 15, 22, 29, 25, 42 and 49 days after anthesis. Embryos taken from the same seed set

were also analysed for oil content to monitor development. The oil-content data are presented (expressed as mg of fatty acid/seed) graphically in Figure 3(a). The Northern blot was hybridized separately to three successive probes and stripped after each in preparation for the next probe. The three probes used were embryo-derived cDNAs for enoyl-[acyl-carrier-protein (ACP)] reductase (1.15 kb), 3-oxoacyl-(acyl-carrier-protein) reductase (β -keto reductase) (1.185 kb) and ACCase (2.5 kb). All three cDNAs were highly expressed in seed material with maximum expression being co-incidental at 29 days after flowering (Figure 3a). However it appears in this experiment that the initial onset of mRNA production occurs in the order enoyl reductase, β -keto reductase and ACCase. The profile of expression of all three genes during embryogenesis was reproducible in individually probed blots with peak expression occurring at 29 days (results not shown). The enoyl-(ACP) reductase has been shown previously to exhibit temporal- and tissue-specific expression [25]. The sizes of the hybridizing bands were 1.65, 1.7 and 7.5 kb respectively as determined by size markers run on the same agarose gel as used for the blot. The level of ACCase mRNA was lower than that of enoyl-(ACP) reductase and β -keto reductase. This may be in part due to the successive stripping of the blot and degradation of the large 7.5 kb message during handling.

Figure 5 Rape ACCase biotin-binding domain sequence

(a) (i) Derived amino acid sequence from the rape cDNA encoding the ACCase biotin-binding domain. The actual biotin-binding site is shown underlined. (ii) Direct comparison of the biotin-binding site with that of the corresponding sequence of yeast ACCase. The boxed regions represent amino acid sequence identity. (b) Dot matrix comparison (DNA Strider, Stringency 9 Window 21) of derived rape ACCase amino acid sequence (biotin-binding domain) against yeast ACCase.

Embryo V leaf ACCase-expression studies

As a large part of the mature embryo consists of oil, it might be expected that ACCase would be expressed at higher levels in embryo than leaf. A Northern-blot comparison of ACCase expression in embryo 29 days after anthesis and young leaf, using the embryo-derived 2.5 kb cDNA as a probe, is shown in Figure 3(b). The 7.5 kb band that hybridizes was approximately five times more abundant in seed than in leaf, as might be expected for ACCase. The size of the full-length mRNA (7.5 kb) was consistent with the known size of the full-length mRNA for both maize and wheat ACCase [7,11].

Isolation of the *Arabidopsis* ACCase genomic clone and further rape cDNA cloning

The average insert size of our rape poly(dT)-primed cDNA library, described above, was approximately 2–2.5 kb. Therefore it was unlikely that the library would contain much more 5' cDNA. To obtain more 5' sequence a random-primed library from rape embryo mRNA was constructed. Having made a suitable library, there were two strategies available for cloning more 5' cDNA: (i) screen using the 5' region of our cDNA; (ii) screen using 5' probes from a genomic clone. We chose the second option. The strategy was to clone the ACCase genomic gene, identify the open reading frames by sequence comparison

and generate specific probes by the use of PCR. As *Arabidopsis* is related to rape and has a smaller genome, we chose to obtain the genomic clone from *Arabidopsis*. Previous data from this laboratory have shown that *Arabidopsis* DNA sequences display a high degree of identity to those of rape (not shown). We used the rape 2.5 kb cDNA clone to screen a λ FixII *Arabidopsis* genomic library and obtained one positive recombinant clone. The genomic DNA was excised from the λ clone by a partial *Sal*I digest and subcloned into pSK⁺. The genomic clone (pKLS2) was extensively subcloned through a combination of *Eco*RI/*Sal*I/*Xba*I/*Hind*III digests, and partially sequenced by both dye primer and dye terminator chemistry. We found that intron–exon boundaries could not be allocated without cDNA data. We therefore opted to sequence only enough of the genomic clone to allow generation of open-reading-frame probes for cDNA screening. The full-sequence data obtained are shown schematically in Figure 4(a) (hatched areas A, Aii, B, C, D, E and F) and have been deposited with the EMBL database (accession nos. X77375–X77381).

To map the ACCase activity domain order within the genomic clone, the open-reading-frame sequences from the different sequenced areas were compared with the first two domains of the full-length yeast cDNA (Figure 4bi and ii) and the rape transcarboxylase domain (Figure 4biii). Similarity was sufficient to allow us to assign the same order of domains to the *Arabidopsis*

Figure 6 ACCase southern-blot analysis of rape and *Arabidopsis* genomic DNA

Restriction-endonuclease-digested DNA was hybridized to the *Arabidopsis* ACCase genomic clone by Southern blot. Hybridization and washing conditions were as described in the Materials and methods section. The blot shown was exposed for 5 days; further exposure provided no extra information. Both λ HindIII and Φ X174 HaeIII DNA markers (indicated on the left-hand side) were run on the same 1% gel and viewed by ethidium bromide staining under u.v.

gene as that of yeast ACCase shown in Figure 4(a), i.e. biotin carboxylase–biotin binding–transcarboxylase.

Sequence data from an open reading frame at the 5' end of the genomic clone (area Aii) showed marked homology (49.5/64% identity/similarity at the derived amino acid level) to the 5' region of yeast ACCase (Figure 4bi). The 3' end of the cloned genomic fragment (19 kb) was sequenced and shown to be homologous to the 3' end of the rape 2.5 kb cDNA clone isolated from the poly(dT)-primed mRNA library (Figure 4biii). As we had approximately 1.3 kb 5' to area Aii, we reasoned that it was likely that we had cloned the full-length genomic clone.

As the sequence of the *Arabidopsis* genomic was apparently similar to the rape cDNA isolated (86% identity in the exons of areas E and F), it was clear that the genomic clone could be used to isolate further rape cDNAs. We generated a specific probe via PCR of area C within the genomic clone and used it to screen the random-primed library generated from rape embryo mRNA. Two cDNA clones (pRS8 and pRS6 containing 2.0 kb and 1.1 kb cDNA respectively) were isolated and sequenced. The cDNA from each was shown to overlap. The full combined derived amino acid sequence (pRS8/6 2.38 kb cDNA size) is presented in Figure 5(ai) (EMBL accession no. X77374). The sequence analysis of the clones indicated homology to the

sequences of yeast (39/58% identity/similarity), rat (38/59% identity/similarity) and algal (34/54% identity/similarity) ACCases. Within the cDNA sequence is the highly conserved [26] biotin-binding site (Val-Met-Lys-Met), shown in Figure 5(ai) as the underlined region. Direct comparison with the yeast biotin-binding site is shown in Figure 5(aii). Interestingly, the sequence also showed identity at its 5' end to the 3' portion of the yeast biotin carboxylase domain. These data demonstrate that the domain order in rape (biotin carboxylase–biotin binding–transcarboxylase) is consistent with the domain assignment of *Arabidopsis*.

Southern-blot analysis

As it was not known how many genes for ACCase are present in rape and *Arabidopsis*, total DNA was analysed by Southern blotting. Total DNA from both rape and *Arabidopsis* was digested with three separate restriction enzymes and blotted. The *Arabidopsis* genomic clone indicated that ACCase genes would most likely be relatively large. The size dictated that it was not possible, using partial cDNAs as probes, to gain an accurate estimate of the gene copy number by Southern blot. The blot was therefore hybridized to the full *Arabidopsis* genomic clone (19 kb) which had been random-prime labelled. The sum of the *Arabidopsis* bands that hybridized to the probe was approximately 20 kb (Figure 6). As the genomic clone is approximately 19 kb, and showed a similar pattern when digested with the same enzymes (results not shown), we deduced that there is only one gene present in *Arabidopsis*. Although the rape profile is more complicated, it can be seen that it consists of a relatively small gene family (Figure 6).

DISCUSSION

In this paper we present the first report of a cDNA sequence from a dicotyledonous plant that equates to the biotin-binding and transcarboxylase domains of ACCase. Rape seed ACCase is probably nuclear encoded and the mRNA of 7.5 kb corresponds to the full-length message as deduced by Northern-blot analysis. Such analysis clearly shows that ACCase is highly regulated with respect to both tissue and temporal expression. This is consistent with earlier studies on the activity of the ACCase protein in maturing seed material.

Comparison of the expression of ACCase with both enoyl-(ACP) reductase and β -keto reductase indicates that they show similar temporal expression. The level of all three mRNAs increases continually throughout the lipid-deposition phase reaching a peak at 29 days after anthesis. This is entirely consistent with the increasing demand on the lipid-synthesizing machinery. The *Arabidopsis* gene isolated enabled us to deduce the domain order (biotin carboxylase–biotin binding–transcarboxylase) and structure of higher-plant ACCase. It has also allowed us to design probes that can eventually be used to isolate cDNAs that span the whole 7.5 kb of ACCase.

It would appear from Southern-blot analysis that ACCase in *Arabidopsis* is encoded by a single gene. Enoyl-(ACP) reductase is also encoded by a single-copy gene (unpublished work from this laboratory). This raises interesting questions over the differential control of this gene between housekeeping (leaf) and specialized (seed triacylglycerol storage) lipid function in the two tissues. It is interesting to speculate that it may consist of a multifunctional promoter and enhancer elements.

The *Arabidopsis* exons and the rape cDNA sequences show high similarity at the nucleotide level, as has been previously shown for many cloned genes including β -keto reductase and

enoyl-(ACP) reductase (results not shown). Comparison of the *Arabidopsis* data with those for yeast and animal ACCase also reveals an exceptionally high similarity at the amino acid level with the domain order preserved.

With the availability of cDNAs to rape ACCase, we are now able to investigate further its importance in metabolic regulation of the lipid pathway by antisense constructs. It may be that switching off ACCase synthesis will effect a cascade of metabolically related activities, as has been shown with RUBISCO [27].

We thank Karen White and Zeneca seeds for their invaluable assistance throughout. This work was supported under the AFRC LINK scheme 'Crops for Industrial Use' in association with Zeneca seeds, project no. E15. R.W. is supported by SERC grant no. GR-H06910.

REFERENCES

- Slabas, A. R. and Fawcett, T. (1992) *Plant Mol. Biol.* **19**, 169–191
- Harwood, J. L. (1988) *Annu. Rev. Plant Mol. Biol.* **39**, 101–138
- Packter, N. M. (1980) in *The Biochemistry of Plants; Lipids: Structure and Function* (Stumpf, P. K., ed.), vol. 4, pp. 535–570, Academic Press, New York
- Burton, J. D., Gronwald, J. W., Somers, D. A., Gengenbach, B. G. and Wyse, D. L. (1989) *Pestic. Biochem. Physiol.* **34**, 76–85
- Walker, K. A., Ridley, S. M., Lewis, T. and Harwood, J. L. (1990) *Phytochemistry* **29**, 3743–3747
- Nakahira, K., Hayashi, O., Uchiyama, M. and Suzuki, K. (1990) *J. Pest Sci.* **15**, 245–247
- Ashton, A. R., Jenkins, C. L. D. and Whitfield, P. (1994) *Plant Mol. Biol.* **24**, 35–50
- Charles, D. J. and Cherry, J. H. (1986) *Phytochemistry* **25**, 1067–1071
- Egin-Bühler, B. and Ebel, J. (1983) *Eur. J. Biochem.* **133**, 335–339
- Egin-Bühler, B., Loyal, R. and Ebel, J. (1980) *Arch. Biochem. Biophys.* **203**, 90–199
- Elborough, K. M., Simon, J. W., Swinhoe, R., Ashton, A. R. and Slabas, A. R. (1994) *Plant Mol. Biol.*, **24**, 21–34
- Hellyer, A., Bambridge, H. E. and Slabas, A. R. (1986) *Biochem. Soc. Trans.* **14**, 565–568
- Slabas, A. R. and Hellyer, A. (1985) *Plant Sci.* **39**, 177–182
- Slabas, A. R., Hellyer, A. and Bambridge, H. E. (1986) *Biochem. Soc. Trans.* **14**, 716
- Li, S.-J. and Cronan, J. E. (1992) *Plant Mol. Biol.* **20**, 759–761
- Turnham, E. and Northcote, D. H. (1983) *Biochem. J.* **212**, 223–229
- Lopez-Casillas, F., Bai, D.-H., Luo, X., Kong, I.-S., Hermodson, M. S. and Kim, K. H. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5784–5788
- Takai, T., Yokoyama, C., Wada, K. and Tanabe, T. (1988) *J. Biol. Chem.* **263**, 2651–2657
- Roessler, P. G. and Ohlrogge, J. B. (1993) *J. Biol. Chem.* **268**, 19254–19259
- Logemann, J., Schell, J. and Willmitzer, L. (1987) *Anal. Biochem.* **163**, 16–20
- Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Marck, C. (1988) *Nucleic Acids Res.* **16**, 1829–1836
- Wolff, R. L. and Fabien, R. J. (1989) *LeLait* **69**, 33–46
- Al-Feel, W., Chirala, S. S. and Wakil, S. J. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4534–4538
- Kater, M. M., Koningstein, G. M., Nijkamp, H. J. J. and Stuitje, A. R. (1991) *Plant Mol. Biol.* **17**, 895–909
- Bai, D. H., Moon, T. W., Lopez-Casillas, F., Andrews, P. C. and Kim, K. H. (1989) *Eur. J. Biochem.* **182**, 239–245
- Quick, W. P., Schurr, U., Fichtner, K., Schulze, E. D., Rodermeil, S. R., Bogorad, L. and Stitt, M. (1991) *Plant J.* **1**, 51–58