

## Plant Gene Register

# A Gene Encoding Acetyl-Coenzyme A Carboxylase from *Brassica napus*<sup>1</sup>

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ACCCase (EC 6.4.1.2) is one of the key regulatory enzymes in fatty acid biosynthesis catalyzing the formation of malonyl-CoA from acetyl-CoA and bicarbonate in an ATP-dependent reaction providing the substrate for fatty acid synthesis. ACCCase from plants is proposed to be a dimer consisting of identical subunits of larger than 200 kD (Egli et al., 1993; Gornicki and Haselkorn, 1993). To date the sequence of a plant ACCCase gene has not been reported. Here we describe the sequence of an ACCCase gene from rapeseed (*Brassica napus*) (Table I).

Based on regions conserved between ACCCase from chicken and *Escherichia coli* BC (Kondo et al., 1991), a specific hybridization probe was generated by PCR from cDNAs synthesized from poly(A<sup>+</sup>) RNA of immature seeds of *B. napus*. Degenerate oligonucleotide primers for PCR were deduced from amino acids 305 to 312 and 384 to 391 of chicken ACCCase and resulted in amplification of a 260-bp fragment covering the exon sequences in the region of nucleotides 4300 to 4813 of the rapeseed sequence reported here. This fragment encodes 84 amino acids showing 88.4 and 65.1% similarity to ACCCase of chicken and BC from *E. coli*, respectively, and was used as a probe for the isolation of clones from a rapeseed genomic library constructed in  $\lambda$ -FIX II (Stratagene, La Jolla, CA).

Fragments of two overlapping genomic clones were subcloned and sequenced. A comparison with protein sequences of the ACCCase from chicken, yeast, *Cyclotella cryptica*, and *E. coli* allowed us to postulate the presence and location of 31 introns. Potential promoter elements were localized at position 2283 to 2286 (CAAT box) and 2416 to 2419 (TATA box). A putative ATG start codon is located at position 2506 to 2508. The Lys residue in the common motif for biotinyla-

tion, -Met-Lys-Met- (MKM) (Samols et al., 1988), is encoded by nucleotides 7391 to 7393, and a TGA stop codon is provided by nucleotides 13,253 to 13,255. A polyadenylation signal sequence (AATAAA) is found at position 13,284 to 13,289 and, using 3' rapid amplification of cDNA ends, a site of polyadenylation was detected at position 13,405. The deduced protein consists of 2305 amino acids and has a molecular mass of 256 kD. Amino acid similarities between the ACCCase of rapeseed and chicken or yeast or *Cyclotella cryptica* or *E. coli* BC were found to be, respectively, 60, 61, 58, and 58%, whereas *E. coli* BCCP and CT did not show substantial homology. The sequence comparison revealed clearly that the different domains in ACCCase, BC, BCCP, and CT are arranged in the same order as in ACCCase from other eukaryotes: BC-BCCP-CT (Al-Feel et al., 1992; Roessler and Ohlrogge, 1993).

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The EMBL, GenBank, and DDBJ accession number for the sequence reported in this article is X77576.

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Abbreviations: ACCCase, acetyl-CoA carboxylase; BC, biotin carboxylase; BCCP, biotin carboxyl carrier protein; CT, carboxytransferase.

**Table I.** Characteristics of rapeseed ACCase gene**Organism:**

Rapeseed (*Brassica napus*).

**Genomic Organization and Gene Copy Number:**

Southern analysis indicates that rapeseed ACCase is encoded by a small gene family with four members.

**Sequence, Source, Method of Identification:**

Two overlapping genomic clones, *BnACCaseg3* and *BnACCaseg8*, were isolated from a genomic library in  $\lambda$ FIX II using as a probe a cloned PCR fragment. The PCR fragment was amplified using degenerate oligonucleotides representing conserved regions of chicken ACCase and *E. coli* BC (Kondo et al., 1991). Sequence 1 to 1,904 of the ACCase gene belongs to clone *BnACCaseg3*, carrying the promoter region and a 3' truncated coding sequence. The sequence from 1,905 to 13,757 corresponds to clone *BnACCaseg8*, which carries part of the promoter and the entire coding sequence.

**Sequencing Strategy:**

Exonuclease III deletion subcloning and complete sequencing on both strands by the dideoxy chain-termination method using universal or specific oligodeoxyribonucleotides as primers was performed.

**Confirmation:**

The proposed rapeseed ACCase sequence is based on homology to ACCase protein sequences from chicken (J03541), yeast (M92156) *C. cryptica* (L20784), and *E. coli* (BC: M79446 or M80458; BCCP: X14825;  $\alpha$ CT: M96394;  $\beta$ CT: M68934) (GenBank accession numbers in parentheses).

**Features of Gene Structure:**

A total of 13,757 nucleotides, including 2,505 bp upstream and 505 bp downstream of the coding region, was sequenced. The ACCase gene consists of an open reading frame of 10,747 bp interrupted by 31 intron sequences. One exon is remarkably long compared to the other exons covering most of the CT domain.

**Features of Protein Structure:**

The open reading frame of a deduced cDNA (6915 bp) codes a protein of 2305 amino acids. Biotinylation occurs at the Lys residue at position 807, which is embedded in the conserved MKM motif. The domains of the multifunctional rapeseed ACCase are arranged as BC-BCCP-CT.