

Plant Gene Register

A Maize Acetyl-Coenzyme A Carboxylase cDNA Sequence¹

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ACCCase (EC 6.4.1.2) catalyzes synthesis of the malonyl-CoA required for subsequent synthesis of fatty acids and secondary metabolites in plants. ACCCase activity is positively correlated with rates of fatty acid synthesis in both leaves and developing oil seeds, and it is likely to play a key regulatory role in plant lipid synthesis (Post-Beittenmiller et al., 1993). In maize (*Zea mays*), most ACCCase activity is associated with a high molecular weight, dimeric, MF, plastid-localized polypeptide that is sensitive to inhibition by aryloxyphenoxypropionate and cyclohexanedione herbicides (Egli et al., 1993). Complete coding sequences for MF ACCCase polypeptides have been published for wheat (Gornicki et al., 1994) and for several dicotyledons (Roesler et al., 1994; Schulte et al., 1994; Shorosh et al., 1994). Partial maize and rice ACCCase cDNAs have also been reported (Ashton et al., 1994; T. Sasaki, unpublished data). Here we describe the complete coding sequence of a maize ACCCase (Table I).

Antiserum to the major maize ACCCase polypeptide (ACCCase I; Egli et al., 1993) was used to select potential maize ACCCase cDNA clones from an oligo(dT)-primed lgt11 expression library derived from A188 maize seedling leaf (Dr. Stephen Gantt, University of Minnesota). Plaques from 14 of 800,000 clones were strongly recognized by the antiserum. Four clones that contained 3.5- to 5.4-kb inserts were partially sequenced and found to be identical. The remaining 5' coding sequence was obtained by partial sequencing of a 15-kb genomic clone whose 3' end hybridized to an ACCCase cDNA probe (nt 3900–5932). The corresponding cDNA sequence was obtained by three successive rounds of RT-PCR, using oligonucleotide primers based on genomic apparent exon (5') and known cDNA (3') sequences. PCR products corresponded to nt 1 to 240, 217 to 610, and 537 to 2094 of the final sequence and were cloned into PCR-script (Stratagene). The original 5.4-kb cDNA clone No. 18–5 and PCR products from at least three

Table I. Nucleotide and deduced amino acid sequence of maize ACCCase

Organism:

Zea mays (inbred A188).

Gene Location:

Nuclear encoded; one of two maize cDNAs, corresponds to partial maize ACCCase cDNA pA3 (Ashton et al., 1994).

Method of Identification:

ACCCase I antiserum recognizes lgt11 β -galactosidase-ACCCase fusion proteins produced by clone 18–5 and others. Portions of clone 18–5 were used to identify a corresponding genomic clone, from which sequence RT-PCR 5' primers were designed that allowed amplification of the remaining 5' cDNA.

Confirmation:

The predicted maize ACCCase polypeptide sequence is highly identical with *Brassica* (X77576), alfalfa (L25042), and wheat (U10187) ACCCases and is 99.5% identical with the C-terminal 1307 amino acids of maize inbred B73 ACCCase (pA3; Z24449) (GenBank accession Nos. in parentheses).

Features of the cDNA Structure:

Reported cDNA sequence of 7470 nt includes a 6975-nt coding region, a 459-nt 3' untranslated region, and 36 nt of the 5' untranslated region. A single mRNA of 8.3 kb was recognized by a cDNA probe (nt 3900–5932). A 2215-nt genomic sequence corresponding to nt 1 to 931 contains four introns, at positions 240 (460 nt), 296 (480 nt), 663 (148 nt), and 872 (76 nt).

Structural Features of Proteins:

The predicted polypeptide of 2325 amino acids contains a biotinylation site at position 806, within the conserved MKM motif (Toh et al., 1993). Functional domains of the maize ACCCase polypeptide are in the order biotin carboxylase, biotin carboxyl carrier protein, carboxyltransferase.

Antibody:

Prepared to ACCCase I polypeptide (Egli et al., 1993); available.

reactions per oligonucleotide pair were sequenced in both directions by the dideoxy chain-termination method, using either Sequenase II (United States Biochemical) or ABI 373 (Applied Biosystems) protocols. No sequence differences were found in regions of clone overlaps.

The first Met codon in the cDNA (nt 37–39) was identified as the start codon based on its similarity to consensus

Abbreviations: ACCCase, acetyl-CoA carboxylase; MF, multi-functional; nt, nucleotide; RT, reverse transcription.

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initiation sequences (Lutcke et al., 1987; Kozak, 1989). An in-frame stop was found in the genomic sequence 6 nt upstream of the sequenced cDNA, and RT-PCR of this region suggested that it was also present in cDNA. The coding sequence 3' end was defined by a double stop after nt 7011. The translated coding sequence predicted a polypeptide of 2325 amino acids (257 kD), which was 79 to 81% identical with MF ACCases from alfalfa (Shorrosh et al., 1994), wheat (Gornicki et al., 1994), and a 158-amino acid predicted polypeptide of a rice expressed sequence tag (T. Sasaki, unpublished data; GenBank accession No. D39099) but only 53 to 55% identical with MF ACCases from other eukaryotes. In a PILEUP alignment of plant ACCases (Genetics Computer Group, Madison WI), Met¹ of both maize and *Brassica napus* ACCases was located about 130 amino acids upstream of the conserved sequence VDEFCKALGG, compared to only 25 amino acids upstream for other plant ACCases. The arrangement and amino acid sequence of binding sites (Shorrosh et al., 1994) for ATP (maize amino acids 318–333), biotin (amino acids 799–811; biotin at 806), acetyl-CoA (amino acids 1952–1961), and carboxybiotin (amino acids 1662–1711) were highly conserved among all MF ACCases.

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The GenBank accession number for the sequence reported in this article is U19183.

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