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Plant Gene Register

Plasmid ω-3 Fatty Acid Desaturase cDNA from *Ricinus communis*[1]

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Chloroplast membrane lipids are rich in the polyunsaturated fatty acids hexadecatrienoic acid and α-linolenic acid. Except for the introduction of the first unsaturation into 18-carbon fatty acids, these polyunsaturated fatty acids arise by sequential desaturation of lipid-linked fatty acids in the chloroplast or the ER. Genes (designated *fad3*) encoding the desaturation of linoleic (18:2) to linolenic acid (18:3) in the ER of *Brassica napus* (Arondel et al., 1992) and *Arabidopsis* (Yadav et al., 1993) have recently been cloned by genetic methods. A highly homologous gene (*fad7*) encoding the enzyme catalyzing the same desaturation step in the chloroplast has recently been isolated from *Arabidopsis* and soybean (Iba et al., 1993; Yadav et al., 1993).

In the course of our studies of fatty acid metabolism in the developing endosperm of castor (*Ricinus communis*) seeds, we isolated a cDNA (pFL1) with strong sequence similarity to these ω-3 desaturases, by screening a cDNA library at moderately low hybridization stringency with the *Brassica* probe (Table I). This 1958-bp clone encodes a 1380-bp open reading frame corresponding to a protein of 52,558 D. The cDNA exhibited a high degree of sequence identity at the nucleotide (amino acid) level with the *fad7* sequence from *Arabidopsis* (78% [73%]), the *B. napus fad3* sequence (70% [72%]), and a cyanobacterial Δ12 desaturase (Wada et al., 1990) (47% [27%]), suggesting that this castor cDNA encodes a plastid ω-3 desaturase. The amino terminus of the predicted castor protein has an extension similar to that of the fad7 protein but not found in the fad3 protein. This amino-terminal extension is rich in the hydroxy amino acids Ser and Thr (23% of the first 78 residues), a characteristic feature of the transit peptide of plastid proteins (Keegstra et al., 1989), supporting the assignment of the castor clone as a plastid desaturase. The sequence similarity between these ω-3 desaturases demonstrates the high degree of conservation between the ER and plastid forms and between the same enzymes from different species of higher plants.

Table I. *Characteristics of the pFL1 cDNA from Ricinus communis*

Organism:
Ricinus communis L. cv Baker 296.
Clone Type; Designation:
cDNA, full length; pFL1.
Gene Product:
Plastid ω-3 fatty acid desaturase.
Function:
Desaturates lipid-linked hexadeca-7,10-dienoic acid to hexadeca-7,10,13-trienoic acid and octadeca-9,12-dienoic acid to octadeca-9,12,15-trienoic acid.
Techniques:
A *Brassica napus* cDNA encoding the microsomal ω-3 fatty acid desaturase was used to probe 10^5 colonies of a pYES2.0 cDNA library from *R. communis* developing endosperm and embryo. Three positives obtained gave the same restriction pattern. Both strands of the largest clone (pFL1) were completely sequenced.
Method of Identification:
Sequence comparison to *B. napus fad3* cDNA (Arondel et al., 1992) and *Arabidopsis fad7* cDNA (Iba et al., 1993).
Features of cDNA:
The clone is 1958 nucleotides in length and consists of a 344-nucleotide 5′ untranslated region, a 1380-nucleotide open reading frame, and a 234-nucleotide 3′ untranslated region. The possibility that the large nature of the 5′ untranslated region of pFL1 is due to a cloning artifact was not eliminated. The open reading frame encodes a 460-amino acid protein with a calculated M_r of 52,558.
Antibodies:
None available.
Subcellular Location:
Not tested, predicted to be in the plastid.

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