

Plant Gene Register

Genomic Nucleotide Sequence of a Gene Encoding a Microsomal ω -3 Fatty Acid Desaturase from *Arabidopsis thaliana*¹

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This study is a first step in the study of the genetic regulation of the desaturation of lipids with regard to membrane properties and to the synthesis of storage lipids. In higher plants, ω -3 fatty acid desaturases catalyze the desaturation of hexadecadienoic (16:2) and linoleic (18:2) acids that are esterified to a glycerolipid molecule. The features of these enzymes have been well characterized by analyzing mutants of *Arabidopsis thaliana* (Somerville and Browse, 1991). The *fad3* mutant has reduced levels of linolenic acid (18:3) and exhibits a concomitant increase in linoleic acid (18:2) in extrachloroplastic membranes and storage lipids (Lemieux et al., 1990). The *fad3* cDNA was recently cloned by chromosomal walking (Arondel et al., 1992). Except for the transit peptide, the deduced amino acid sequence of the *fad3* gene showed 70% identity to that of the *Arabidopsis fad7* gene, which encodes a chloroplast ω -3 desaturase (Iba et al., 1993). The high degree of homology between the *fad3* and the *fad7* gene products suggests that these genes are derived from a common ancestral gene. The deduced amino acid sequence of ARG1, an auxin-induced gene from mung bean (Yamamoto et al., 1992), showed 68% identity to that of the *fad3* gene.

Here we report the genomic nucleotide sequence of the *fad3* gene (Table I). The *fad3* gene has seven introns and its structure is very similar to that of the *fad7* gene (Iba et al., 1993). The intron/exon structure of both genes is highly conserved with respect to number and position. The first (624 bp), the second (547 bp), and the third (479 bp) intron of the *fad3* gene are longer than those of the *fad7* gene (324, 466, and 389 bp, respectively). The GT and AG dinucleotides that usually characterize the 5' and 3' splice sites are present at the extremities of each intron. The region upstream from the ATG initiation codon (973 bp) exhibits a high AT content

Table I. Characteristics of *Arabidopsis fad3* gene

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| Organism: | <i>Arabidopsis thaliana</i> , Columbia ecotype. |
| Mutant: | The mutant lines BL1 (Lemieux et al., 1990), G30, and 1E5 (James and Dooner, 1990) have been isolated from <i>A. thaliana</i> . |
| Chromosome Location: | Chromosome 2, 0.4 centimorgan from ASA2 (Arondel et al., 1992). |
| Source: | λ GEM11 genomic library. |
| Cloning and Sequencing Technique: | Library was probed by the <i>Arabidopsis fad3</i> cDNA clone (Iba et al., 1993). One positive clone, λ g2, was digested with several restriction endonucleases to generate overlapping subclones in pBluescript II (Stratagene). Both strands of the nucleotide sequences of these subclones were determined by the di-deoxy chain-termination method. |
| Features of Gene Structure: | A total of 4250 bp, including 973 bp upstream and 116 bp downstream of the open reading frame; open reading frame interrupted by seven AT-rich introns, all at locations equivalent to those of the <i>Arabidopsis fad7</i> gene. |
| Confirmation: | Identified by the cDNA sequence. |
| Expression Characteristics: | Northern analysis showed that the 1.3-kb transcript was expressed in both leaf and root tissues and accumulated at a very high level in leaf tissue (Yadav et al., 1993). |
| Subcellular Localization of the Protein: | ER. |

(71%). A putative TATA box is present 131 bp upstream from the initiation codon. A TCA motif (TCATCTTCTT) can be detected 448 bp upstream from the initiation codon. This motif frequently has been found to occur in the promoter and the noncoding regions of stress-inducible genes (Goldsbrough et al., 1993). A variant (75% identity) of the ABA-responsive element TACGTGGC (Mundy et al., 1990) of the *rab-16A* gene of rice and another variant (89% identity) of the elicitor-responsive element AACCAACA (Ohl et al.,

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1990) of a Phe ammonia-lyase promoter of *A. thaliana* are also found 476 and 461 bp upstream of the initiation codon, respectively.

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