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Plant Gene Register

Nucleotide Sequence of an Iron Superoxide Dismutase Complementary DNA from Soybean[1]

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We have reported the isolation of a cDNA (2) for which the corresponding mRNA, called SAM46, accumulates in cultured soybean cells during cytokinin or auxin starvation (see Table I). This mRNA, which decreases rapidly in abundance following treatment of cytokinin-starved cells with 5 mM zeatin or following treatment of auxin-starved cells with 10 mM α-naphthaleneacetic acid, is detectable in expanded primary leaves but not in unexpanded primary leaves of soybean seedlings. The 5′ end of the SAM46 cDNA was shown to be 46% identical at the amino acid level to the 5′ end of the *Escherichia coli* iron superoxide dismutase gene (*sodB*) (1, 2). Furthermore, expression of the SAM46 cDNA in *E. coli* cells resulted in measurable FeSOD[3] activity (2).

The nucleotide sequence of the SAM46 cDNA and the deduced amino acid sequence of the SAM46 protein product are shown in Figure 1. The site of translation initiation is presumed to be the ATG at position 24 to 26 of the sequence because: (a) the SAM46 cDNA is a full-length cDNA, or very close to it, suggesting that translation does not begin upstream of position 24 to 26, and (b) the N-terminus of the mature soybean FeSOD is predicted, based on N-terminal amino acid sequences of other known FeSODs, to be the lysine encoded by the AAG codon at position 99 to 101 of the sequence (4), suggesting that translation does not begin downstream of position 24 to 26. The overall sequence identity between the SAM46 product and the FeSOD of *E. coli* is 41%, with most of the sequence divergence occurring at the carboxy-terminus (1). In addition, the SAM46 product is 59 and 58% identical to the products of the FeSOD cDNAs from *Nicotiana plumbaginifolia* and *Arabidopsis thaliana*, respectively (7). Again, the majority of the sequence divergence is at the carboxy-terminus.

We are currently studying several aspects of soybean FeSOD gene expression. We are interested, for example, in fully characterizing the induction of the soybean FeSOD gene in response to cytokinin or auxin starvation to determine whether this gene responds generally to stress or specifically

Table I. *Characteristics of the FeSOD cDNA from Glycine max*

Organism:
Glycine max cv Mandarin (soybean).
Location on Chromosomes:
Unknown; gene copy number reconstruction experiments suggest between 1 and 5 copies per haploid genome (D. N. Crowell, R. M. Amasino, unpublished observation).
Gene, Function, Pathway:
Soybean FeSOD gene; FeSOD (EC 1.15.1.1) catalyzes dismutation of superoxide to molecular oxygen and hydrogen peroxide (5).
Techniques:
cDNA library in Bluescript SKII+ (Stratagene, La Jolla, CA) (2); restriction fragment subcloning; dideoxynucleotide sequencing (6) of plasmid DNA (both strands).
Method of Identification:
Sequence comparison to GenEMBL database (2, 3); sequence identity to *E. coli sodB* gene (1, 2); detection of FeSOD activity in *E. coli* cells expressing SAM46 (2).
Expression Characteristics:
mRNA of approximately 1 kilobase detected by RNA blot analysis of polyadenylate-enriched RNA and total RNA (2; D. N. Crowell, R. M. Amasino, unpublished observation).
Regulation:
FeSOD mRNA accumulation induced in cultured soybean cells (*G. max* cv Mandarin) during cytokinin or auxin starvation; FeSOD mRNA accumulation induced in soybean leaves (*G. max* cv Elgin) during leaf expansion (2).
Codon Usage:
Codons not present in FeSOD open reading frame: GCG (A), AGG (R), ACG (T), TGT (C), TCG (S), CGA (R), CGC (R); amino acids with a high bias to one codon: R (AGA), I (ATT), L (CTT).
(G + C) Content:
41%.
Structural Features of Protein Product:
Coding region codes for a polypeptide of 248 amino acids with a predicted M_r of 27,824 D; first 25 amino acids are predicted to be leader sequence for chloroplast import (4, 5); predicted M_r of mature polypeptide, beginning with Lys^{26}, is 25,285 D; plant FeSODs are generally dimers consisting of two equally sized subunits and one to two atoms of iron per dimer (5).
Antibodies:
Antibodies have not been prepared by this laboratory.
Subcellular Location of Protein Product:
Chloroplast (5).
EMBL Accession No.:
M64267

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[3] Abbreviation: FeSOD, iron superoxide dismutase.

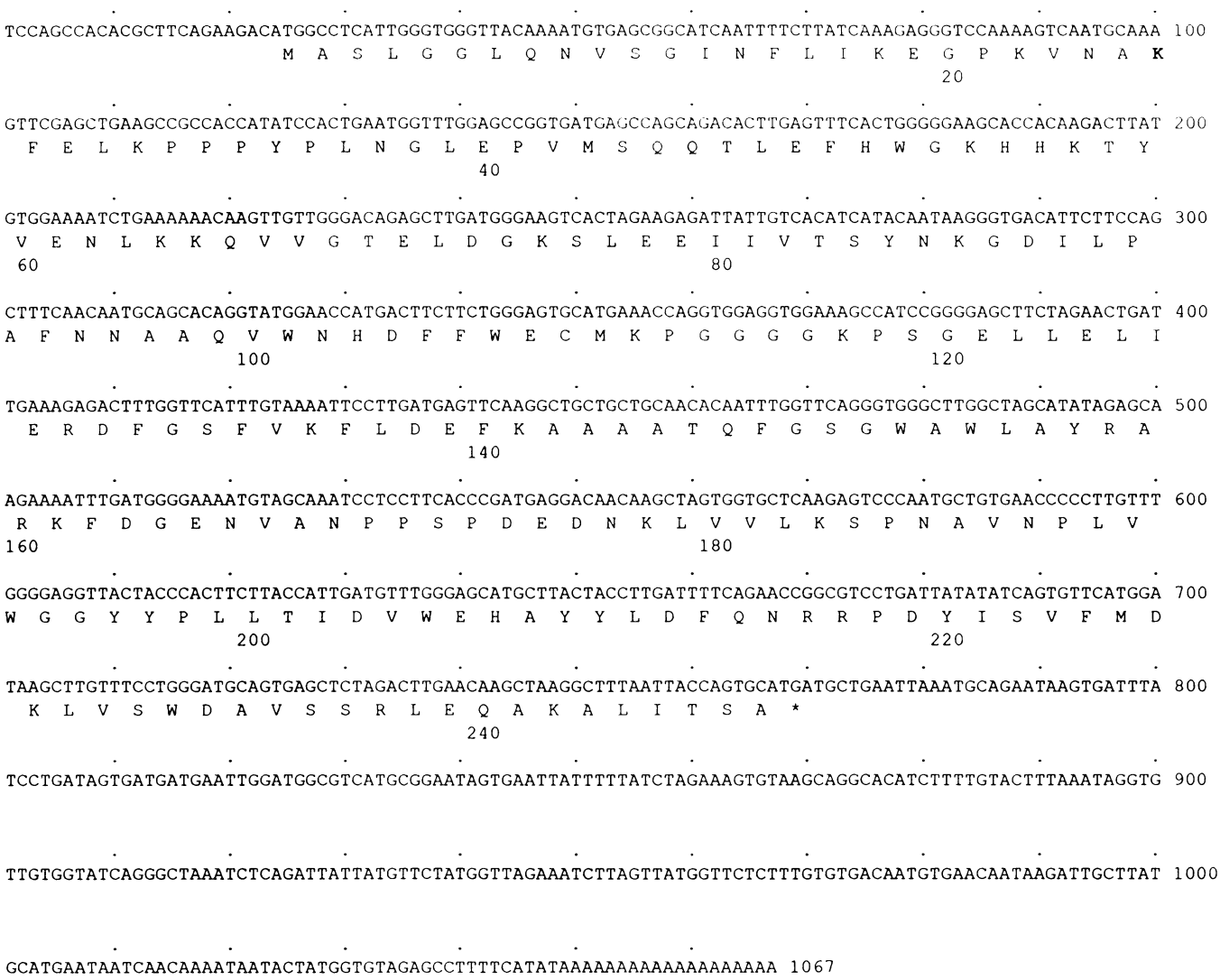

Figure 1. Nucleotide sequence of the SAM46 cDNA and deduced amino acid sequence of the SAM46 protein product The amino acid assumed to be the N-terminus of the mature soybean FeSOD (Lys^{26}) is in bold face. This assumption is based on N-terminal amino acid sequences of other known FeSODs (4).

to phytohormones. We are also interested in studying FeSOD gene induction during leaf expansion. In particular, we would like to determine whether a causal relationship or a coincidental relationship exists between leaf expansion and FeSOD gene induction. Finally, we are beginning studies of the import of the SAM46 protein product into chloroplasts and its assembly with iron into a functional enzyme.

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