

**Plant Gene Register**

# A cDNA Clone Encoding *Chlamydomonas reinhardtii* Preferredoxin

Mariana Stein, Jean-Pierre Jacquot,\* and Myroslawa Miginiac-Maslow

Physiologie Végétale Moléculaire, Bâtiment 430, UA 1128 Centre National de la Recherche Scientifique, Université de Paris-Sud, 91405 Orsay Cedex, France

Fd's are ubiquitous low mol wt proteins containing iron sulfur center(s) involved in numerous electron transfer reactions. In plants, one of the major functions of Fd is to provide the reducing power for chloroplastic NADP photoreduction. In addition, Fd is also needed for nitrite, ammonia, and sulfite assimilation as well as for pseudocyclic electron flow and fatty acid metabolism and for the light regulation of chloroplastic enzymes (Orme-Johnson, 1973). This protein is encoded by nuclear genes and is, therefore, produced as a precursor that is subsequently cleaved inside the chloroplast (Smeekens et al., 1985). We have previously purified *Chlamydomonas reinhardtii* Fd and determined its primary structure by direct amino acid sequencing (Schmitter et al., 1988). Based on this sequence and using the polymerase chain reaction, we have isolated a nucleotidic sequence coding for the mature portion of *C. reinhardtii* Fd and shown that the deduced amino acid sequence was identical with only one substitution Thr<sup>7</sup> to Ser (Table I). In addition, we demonstrated that *Escherichia coli* cells were able to direct the synthesis of *C. reinhardtii* Fd polypeptide and to reassemble it together with an iron-sulfur center (Rogers et al., 1992).

In this paper we report the sequence of a cDNA encoding *C. reinhardtii* preferredoxin. A  $\lambda$ gt11 cDNA was sequenced (527 bp) and found to encode a 126-amino acid precursor with a molecular mass of 13,250 D, compared to the mature form, which contains 94 amino acids and has a molecular mass of 9,908 D. The deduced amino acid sequence was completely homologous to the sequence reported by Schmitter et al. (1988). In addition, the sequence revealed the structure of the 32-amino acid transit peptide (molecular mass 3342 D), which is as follows: MAMAMRSTFAARVGAKPA-VRGARPASRMSCMA.

Several lines of evidence indicate that the first ATG is the initiation codon. First, it is preceded by TCA and AAA, triplets theoretically coding for Ser and Lys but rarely if ever used for nuclear genes in *C. reinhardtii*. Second, the flanking sequence surrounding the initiation codon (AAAAATGGC) fits well the eukaryotic translation initiation consensus (Wedel et al., 1992). Finally, the beginning of the transit peptide (MAMAM) is highly similar to the MAQM sequence reported by Wedel et al. (1992) and to the MAMAT of Hoffmann et al. (1988), and the dipeptide MA is overwhelm-

**Table I.** Characteristics of a cDNA clone coding for *Chlamydomonas reinhardtii* preferredoxin

Organism:	<i>Chlamydomonas reinhardtii</i> .
Function:	Encodes the precursor for Fd, a protein essential for chloroplastic NADP photoreduction.
Clone Type, Designation:	cDNA, Fd 9.
Source:	cDNA library ( $\lambda$ gt11), a gift from Michel Clermont-Goldschmidt.
Method of Identification:	Amino acid sequencing, polymerase chain reaction cloning, screening of the $\lambda$ gt11 library with labeled polymerase chain reaction fragment.
Sequencing Strategy:	Plasmid sequencing in Bluescript SK+.
Features of cDNA Structure:	Sixty-five percent homology to <i>Silene pratensis</i> cDNA (relates to the 288 nucleotides coding for the mature protein).
Structural Features of the Deduced Protein:	Identical with the direct amino acid sequencing of <i>C. reinhardtii</i> Fd. About 70% homology to higher plant Fd's.
Expression	Recombinant ferredoxin can be expressed in <i>E. coli</i> reassociated with its iron-sulfur center (Rogers et al., 1992).
Antibodies:	None available.
Subcellular Localization:	Chloroplast.

ingly found to start chloroplastic transit peptides (von Heijne et al., 1989).

The putative transit peptide displays a very high content of Ala, Met, and Arg. Nine amino acid residues are absent, most notably the acidic residues Asp and Glu, as well as the hydrophobic residues Ile, Leu, Trp, and Tyr. Hydroxylated residues are present, but their content is not higher than in mature Fd. Overall, the peptide is very positively charged; no clear homology to other Fd transit peptides and no obvious secondary structure can be deduced as observed by Pilon et al. (1992). From the NH<sub>2</sub>-terminal sequence of mature Fd, we can deduce that the processing of *C. reinhardtii* preferredoxin occurs between Ala and Tyr residues, Ala being often found next to the processing site of chloroplastic precursor proteins (Keegstra et al., 1989).

\* Corresponding author; fax 33-1-69-85-37-15.

Received January 4, 1993; accepted February 11, 1993.

Copyright Clearance Center: 0032-0889/93/102/1349/02.

The GenBank accession number for the sequence reported in this article is L10349.

#### LITERATURE CITED

- Hoffmann NE, Pichersky E, Malik VS, Ko K, Cashmore AR** (1988) Isolation and sequence of a tomato cDNA clone encoding subunit II of the photosystem I reaction center. *Plant Mol Biol* **10**: 435–445
- Keegstra K, Olsen LJ, Theg SM** (1989) Chloroplastic precursors and their transport across the envelope membranes. *Annu Rev Plant Physiol Plant Mol Biol* **40**: 471–501
- Orme-Johnson WH** (1973) Ironsulfur proteins—structure and functions. *Annu Rev Biochem* **42**: 159–204
- Pilon M, Rietveld AG, Weisbeek PJ, de Kruijff BB** (1992) Secondary structure and folding of a functional chloroplast precursor protein. *J Biol Chem* **267**: 19907–19913
- Rogers JW, Hodges M, Decottignies P, Schmitter JM, Gadal P, Jacquot JP** (1992) Isolation of a cDNA fragment coding for *Chlamydomonas reinhardtii* ferredoxin and expression of the recombinant protein in *Escherichia coli*. *FEBS Lett* **310**: 240–245
- Schmitter JM, Jacquot JP, de Lamotte-Guéry F, Beauvallet C, Dutka S, Gadal P, Decottignies P** (1988) Purification, properties and complete amino acid sequence of the ferredoxin from a green alga, *Chlamydomonas reinhardtii*. *Eur J Biochem* **172**: 405–412
- Smeekens S, van Binsbergen J, Weisbeek P** (1985) The plant ferredoxin precursor: nucleotide sequence of a full length cDNA clone. *Nucleic Acids Res* **13**: 3179–3194
- von Heijne G, Steppuhn J, Herrmann RG** (1989) Domain structure of mitochondrial and chloroplast targeting peptides. *Eur J Biochem* **180**: 535–545
- Wedel N, Klein R, Ljungberg U, Andersson B, Herrmann RG** (1992) The single copy gene *psbS* codes for a phylogenetically intriguing 22 kDa polypeptide of photosystem II. *FEBS Lett* **314**: 60–66