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## Cloning and Characterization of the *Arabidopsis thaliana* Germin-like Protein (GLP1) Gene (Accession No. [AF090733](#))

Clay Carter and Robert Thornburg (\*)

Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa 50011

(\*) Corresponding author:  
FAX: 1-515-294-0453  
Email: thorn@iastate.edu

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Germin is a 130 kDa pentameric glycoprotein composed of 26 kDa subunits that was originally identified in wheat (McCubbins *et al.*, 1987). This protein is found in cereal cell walls and is an oxalate oxidase (Lane *et al.*, 1993). Oxalate oxidase catalyzes the oxidative breakdown of oxalate into carbon dioxide and hydrogen peroxide. Other plant species have been examined for germins. This search led to the discovery of numerous germin-like proteins (GLPs), which have been identified from a variety of species including both monocots and dicots. Although the actual role(s) of germin and germin-like proteins in the plant is unknown, there is evidence for several proposed functions. These include a possible function in plant responses to osmotic stress (Lane, *et al.*, 1992), the secondary hydration of wheat embryos (Lane, 1991), and the transportation of cell wall components to the cell wall during rapid growth phase of germinating wheat embryos (Lane, *et al.*, 1993). It has been hypothesized that germin produces hydrogen peroxide for cell wall crosslinking (Lane, *et al.*, 1993; Zhang, *et al.*, 1995). Hydrogen peroxide is required for the peroxidase catalyzed cross-linking of cell wall components (Varner and Linn, 1989). Thus, germin may contribute to cell wall restructuring.

Hydrogen peroxide has also been shown to play a central role in plant defenses. It has direct antimicrobial effects (Lachman, 1986) and has been proposed to function as a secondary messenger in the activation of defense gene expression. Because germin produces hydrogen peroxide, it has been suggested to play a role in plant defense mechanisms. Recently, several groups have found that both germin and oxalate oxidase activity increase during powdery mildew attack of barley (Dumas *et al.*, 1995; Zhang *et al.*, 1995; Hurkman and Tanaka, 1996). Whether this increase is induced by a specific pathogen or is a response to an altered osmotic state is unknown. Interestingly, none of the dicot GLPs have been shown to have oxalate oxidase activity; however, Wei *et al.* (1998) have isolated a powdery mildew fungus responsive non-oxalate oxidase GLP cDNA from papilla-resistant barley. It is possible that non-oxalate oxidase GLPs may utilize substrates other than oxalate to generate activated oxygen species. Because of the possibility that germin-like proteins may function in plant defenses, we have started to characterize the GLP gene family in Arabidopsis.

cDNAs encoding GLP1 have been previously isolated and characterized (Membré, *et al.*, 1997, Carter, *et al.*, in press). The GLP1 gene in Arabidopsis is the most highly expressed of the GLP genes. It is 2.5 to 14 times more highly expressed than other GLP genes (Carter *et al.*, in press). GLP1 is expressed in green shoots, etiolated seedlings, and whole seedlings.

An *Arabidopsis thaliana* genomic library was kindly provided by Dr. Daniel Voytas (Iowa State University). The library contains partially digested MboI fragments of *Landberg erecta* genomic DNA in Stratagene's lambda FIX vector. This library was screened with the *Arabidopsis thaliana* GLP1 cDNA (Accession No. [U75206](#)). Five plaques positively hybridizing to the GLP1 cDNA were purified to homogeneity. One lambda phage that cross-reacted strongly with the GLP1 cDNA was mapped with a battery of restriction enzymes. From this analysis a 1.4 kb HindIII fragment was chosen for isolation and sequencing. This fragment was subcloned into the plasmid pUC8 to form the vector pRT444.

The insert of the pRT444 vector was sequenced and found to contain the GLP1 gene. Sequencing reactions were performed using the Applied Biosystems Prism Dye-deoxy Cycle Sequencing Kit. The reactions were run on an Applied Biosystems Prism 377 DNA sequencer (Perkin-Elmer Corp.). Sequencing of the insert was initiated from known vector sequences. Based upon these sequencing runs, additional sequencing primers were designed to extend new sequence. Sequence was continued until the insert was completely sequenced. The entire sequence of the GLP1 gene was independently confirmed on the opposite strand. DNA sequences were performed in duplicate or triplicate for each run.

The GLP1 gene encodes a 208 amino acid protein with a pI of 9.3 and a MW of 21559 Da. Amino acids 1 to 18 encode a classical signal sequence resulting in extracellular targeting of the GLP protein (Carter *et al.*, in press). Amino acids 19 to 208 encode the mature protein. A single site of N-glycosylation occurs at Asn(50). Like the GLP3b gene, the GLP1 gene does not contain an intron. This is different from most GLP genes. Most GLP genes contain an intron located near amino acid 40 (Carter and Thornburg, unpublished data). The genes encoding germin-like proteins in the *Arabidopsis* GLP Subfamily 3 (GLP1 and GLP3b) lack this intron. The wheat germins also lack introns.

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**Table 1.** Characteristics of the GLP1 gene from *Arabidopsis thaliana*

Organism:

*Arabidopsis thaliana*

Gene Product:

Germin-like Protein 1 (GLP1) related to oxalate oxidase from cereals

Characteristics of the GLP1 gene:

The sequenced DNA fragment of the GLP1 gene was a 1387 bp HindIII fragment containing the entire GLP1 gene.

Promoter

96 nucleotides of the promoter are located in this HindIII fragment [1..96] TATA box [49..62] (10/13) to canonical plant TATA box (Joshi, 1987) no significant repeats, palindromes, or hairpin loops were detected.

5' untranslated region

50 nucleotides of the 5' UTR were identified [97..146]

coding region

623 nucleotides [147..770] terminates with an ochre codon (TAA) [771..773] the GLP1 gene does not contain an intron

3' untranslated region

156 nucleotides [774..930] there is no clear poly A signal the poly A addition site is at 832 of this sequence. no significant repeats, palindromes, or hairpin loops were detected within the 3' untranslated region

3' flanking region

457 nucleotides [930..1387] no significant repeats, palindromes, or hairpin loops were detected within the 3' flanking region

**Differences between Landsberg Erecta and Columbia:**

Only a single nucleotide difference was identified as position 930 of the genomic sequence where a G was identified in the genomic Landsberg clone and a T was identified at this position in the Columbia cDNA.

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