

Plant Gene Register

An Endomembrane-Localized Small Heat-Shock Protein from *Arabidopsis thaliana*¹

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Nearly all eukaryotes respond to high-temperature stress by producing HSPs, which are required for survival at high temperatures (Parsell and Lindquist, 1993). Of the major gene families of HSPs, the 16- to 30-kD smHSPs are the most numerous and abundant in higher plants. Upon heat stress, plants synthesize up to 30 related smHSPs that are localized in the cytoplasm, plastids, and ER (Vierling, 1991; Helm et al., 1993). The abundance, intracellular distribution, and sequence conservation across divergent plant genera of the smHSPs suggest that they play an important role in plant adaptations to high-temperature stress (Vierling, 1991). Recent *in vitro* studies suggest that, like the other major families of HSPs, the smHSPs may function as molecular chaperones (reviewed by Parsell and Lindquist, 1993).

We previously characterized two homologous cDNAs from *Pisum sativum* and *Glycine max*, designated PsHSP22.7 and GmHSP22.0, respectively, whose encoded proteins possessed putative N-terminal signal peptides. *In vitro* and *in vivo* studies of PsHSP22.7 indicated that the encoded signal peptide is functional, and that the protein is an ER resident protein in heat-stressed peas (Helm et al., 1993). Previous observations documenting co-sedimentation of smHSPs with endomembrane fractions from heat-stressed maize and barley (Cooper and Ho, 1987; Sticher et al., 1990) suggest that ER-localized smHSPs may accumulate in all higher plants, and that these smHSPs might have important functions in protecting the endomembrane system from heat-induced damage.

As part of our studies of ER-localized smHSPs, we cloned a cDNA homologous to PsHSP22.7 from *Arabidopsis thaliana* (Table I). The AtHSP22.0 cDNA shares 60.8 and 57.6% homology with PsHSP22.7 and GmHSP22.0, respectively. The cDNA encodes a 22.0-kD polypeptide, designated AtHSP22.0. A carboxyl-terminal shock domain was

Table I. Characteristics of a cDNA encoding an endomembrane-localized smHSP from *A. thaliana*

Organism:

Arabidopsis thaliana (L.), ecotype Columbia, Brassicaceae.

Identity of Encoded Protein:

smHSP, as determined by sequence comparisons of cDNA to other plant smHSP cDNA sequences.

Techniques:

cDNA library prepared from *Arabidopsis* plants heat shocked for 90 min at 37°C (Helm and Vierling, 1989). The AtHSP22.0 was isolated by screening the library with PsHSP22.7 cDNA at reduced stringency. Both strands of the AtHSP22.0 cDNA were sequenced by dideoxy sequencing using Sequenase (United States Biochemical).

Characteristics of cDNA:

The total length is 831 bp, including 60 and 183 bp of 5' and 3' untranslated regions, respectively. Tandem start codons are located from 61 to 66, and the stop codon for this reading frame is at 646 to 648, with the longest open reading frame being 585 bp in length. A polyadenylation signal is located at 793 to 799, and the poly(A) tail extends from 813 to the 3' end of the cDNA.

Gene Copy Number:

Southern analysis of *Arabidopsis* genomic DNA using the AtHSP22.0 cDNA as a probe indicated that there is only a single gene belonging to this smHSP gene subfamily in *Arabidopsis*.

Features of the Deduced Amino Acid Sequence:

The longest open reading frame encodes a protein of 195 amino acids, with a predicted mass of 22.0 kD. A characteristic signal peptide for insertion into the ER is located from residues 1 to 24, and there is a putative C-terminal tetrapeptide for retention in the ER. Residues 100 to 155 cover a conserved region characteristic of all eukaryotic smHSPs and the α crystallin lens proteins (Vierling, 1991; Parsell and Lindquist, 1993), and thus identify this polypeptide as a product of a gene belonging to the superfamily of eukaryotic smHSPs. The processed form of AtHSP22.0 is most similar to the ER-localized smHSPs, being 84% similar (69.2% identical) and 87.1% similar (68.4% identical) to the mature forms of PsHSP22.7 and GmHSP22.0, respectively.

¹ This research was supported by U.S. Department of Agriculture Competitive Grants Office grants No. 88-37264-3914 and 90-3728 (to E.V.) and U.S. Department of Agriculture Competitive Grants Office grant No. 93-37100-9266 (to K.W.H.).

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Abbreviations: HSP, heat-shock protein; smHSP, small heat-shock protein.

identified, which classified the AtHSP22.0 gene in the smHSP gene superfamily (Vierling, 1991). Analysis of the predicted protein sequence showed that AtHSP22.0 has a putative signal peptide. When AtHSP22.0 mRNA was translated in vitro in the presence of ER-derived canine pancreatic microsomes, the encoded protein was co-translationally inserted into the microsomes and the major translation product was processed from 22 to 20 kD. This is in close agreement with the predicted masses of the precursor and processed (Von Heijne, 1983) forms of AtHSP22.0. Furthermore, AtHSP22.0 has the carboxyl-terminal tetrapeptide SKEL, which is similar to known ER retention signals (Vitale et al., 1993), indicating that AtHSP22.0 is a resident ER protein in heat-stressed *Arabidopsis* tissues. Northern analysis using the AtHSP22.0 cDNA as a probe on blots of RNA from 22°C control and 38°C heat-stressed plants indicated that AtHSP22.0 mRNA is undetectable under normal conditions and accumulates to high levels in response to heat stress.

Like PsHSP22.7 and GmHSP22.0, AtHSP22.0 is most similar to the class I cytoplasmic smHSPs, with the processed form of AtHSP22.0 being 64.5% similar to AtHSP17.6 (Helm and Vierling, 1989), a class I smHSP from *Arabidopsis*.

To our knowledge, this is the first report of an endomembrane-localized smHSP in a nonleguminous dicotyledon, and suggests that these smHSPs, like the other three classes of plant smHSPs, are widely distributed among higher plants. Experiments to test the importance of these smHSPs

for thermotolerance and to determine their function will focus on AtHSP22.0, the single ER-localized smHSP in *Arabidopsis*.

Received July 11, 1994; accepted July 15, 1994.

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The GenBank accession number for the sequence reported in this article is U11501.

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