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Plant Gene Register

A cDNA Encoding the Endoplasmic Reticulum-Luminal Heat-Shock Protein from Spinach (*Spinacia oleracea* L.)[1]

James V. Anderson, Lisa G. Neven, Qin-Bao Li, Dale W. Haskell, and Charles L. Guy*

Institute of Food and Agricultural Science, Program for Plant Molecular and Cellular Biology, Department of Environmental Horticulture, University of Florida, Gainesville, Florida 32611

HSP70s are an evolutionarily conserved family of 70-kD proteins better known as molecular chaperones (Ellis and van der Vies, 1991). Molecular chaperones assist the in vivo assembly and folding of polypeptides during normal growth without themselves becoming part of the final folded protein (Gatenby, 1992). HSP70s show differential expression to a variety of abiotic or biotic factors (for review, see Craig, 1989; Gething and Sambrook, 1992).

In eukaryotes, one of the best-characterized organellar HSP70s is that of the ER-luminal HSC70. During normal growth the ER-luminal HSC70 is required for translocation, folding, and assembly of secretory and transmembrane proteins passing through the ER secretory pathway (Vogel et al., 1990). However, the ER-luminal HSC70s will bind to misfolded, underglycosylated, and mutant polypeptides, which in turn generally causes the increased expression of the HSC70 (Hendershot et al., 1988; Fontes et al., 1991). Like other HSP70s, the ER-luminal HSC70 contains a highly conserved N-terminal ATP-binding domain and shows increased ATP hydrolysis (Flynn et al., 1989) when peptides are bound at the less-conserved C-terminal peptide-binding domain. The ER-luminal HSC70 also undergoes autophosphorylation of a Thr residue (Freiden et al., 1992), which may have a regulatory role in its function.

A cDNA library made from cold-acclimated spinach (*Spinacia oleracea* L.) leaf tissue in λZapII was screened with polyclonal antibody to spinach HSC70s (Neven et al., 1992) and with polymerase chain reaction-generated spin350 (Neven et al., 1992). This led to the isolation of a partial clone (p73) of 1387 bp, which was identified as a C-terminal portion of the ER-luminal HSC70. Clone p73 was then used to further screen a cDNA library for full-length clones of the ER-luminal HSC70. p522 was the largest clone isolated (2436 bp). Sequence homology analysis showed that the amino acid sequence from p522 has a 91.5% homology to the ER-luminal HSC70 (BLP4) of tobacco (Denecke et al., 1991). Southern blots indicated that the spinach ER-luminal HSC70 is encoded by a single gene (our unpublished data), which contrasts with that reported for tobacco (Denecke et al., 1991). The data in this report (Table I) show the characteristics of p522. As previously indicated mRNA to the spinach ER-luminal HSC70 is expressed during normal growth conditions, up-regulated during cold acclimation of leaf tissue, but is not expressed during either heat shock or water stress.

Table I. *Characteristics of a cDNA coding for the ER-luminal HSC70 of spinach*

Organism:
Spinacia oleracea L. cv Bloomsdale.
Genome Location:
Nuclear genome; chromosome location unknown.
Gene Copy Number:
Single copy.
Gene Function:
Encodes for an ER-luminal HSC70, which functions as a molecular chaperone involved in the translocation and processing of secretory proteins.
Source:
cDNA library in λZapII constructed from poly(A)$^+$ RNA from cold-acclimated spinach leaf tissue.
Sequencing Technique:
Dideoxy chain termination method using an Applied Biosystems automated sequencer.
Method of Identification:
Screening with spin350 (Neven et al., 1992) and p73. Amino acid sequence comparison with GenBank, EMBL, Protein Information Resource, and SwissProt sequence data bases using University of Wisconsin Genetics Computer Group.
Expression Characteristics:
ER-luminal HSC70 mRNA is expressed during normal growth. The mRNA levels are up-regulated during cold acclimation but are not expressed during either heat-shock or water-stress conditions.
Structural Features of the Protein:
An open reading frame of 2004 bp encodes a 668 amino acid protein that includes an N-terminal signal peptide of 28 amino acids. The mature protein is 640 amino acids with a predicted M_r of 70,700 and an isoelectric point of 4.9.
Antibodies:
Polyclonal mouse serum, hybridoma culture supernatants, and monoclonal ascites recognize native, denatured, and recombinant ER-luminal HSC70.

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* Corresponding author; fax 1–904–392–3870.

Abbreviations: HSC70, 70-kD heat-shock cognate; HSP70, 70-kD heat-shock protein.

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The GenBank accession number for the sequence reported in this article is L23551.

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