A cDNA Clone Encoding a Spinach 70-Kilodalton Heat-Shock Cognate

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The genes for the Hsp70s of plants, in addition to being under developmental control, are activated by a variety of diverse stresses in addition to that of a heat shock (Neumann et al., 1989). For example, wounding, water deficits, ABA, arsenite, or cold can induce the synthesis of the Hsp70s (Heikkila et al., 1984; Lin et al., 1984; Neven et al., 1992). Some members of this gene family are expressed under normal growth conditions as well as being stress inducible. Hsp70s have been identified in a number of cell compartments, including the cytosol, ER, mitochondria, and chloroplasts. The induction by various apparently unrelated stresses and chemical agents suggests that Hsp70 could play a general role in stress adaptation (Vierling, 1991).

Members of the 70-kD heat-shock gene family are highly conserved across a wide range of organisms. As such, the Hsp70s have been implicated in a variety of essential cellular processes, ranging from DNA replication to protein folding and transport. In an effort to learn more about the evolution and possible functions of higher plant Hsp70s, we isolated a cDNA clone encoding a putative cytosolic member (Hscl) of this family of proteins. This clone was obtained from a cDNA library prepared from poly(A)+ RNA isolated from cold-acclimated spinach leaf tissue. The library was screened by in situ plaque hybridization using a 597-bp hybridization probe prepared from total cDNA by PCR using primers to highly conserved regions in the N-terminal ATPase domain (Flaherty et al., 1990). The 5' and 3' oligonucleotides were GGT ATT GA(CT) CTC GGT ACC AC(CT) TAC (CG24) and TCC ACC ACC AAG GTC GAA GAC (CG14), respectively.

The characteristic features of the cDNA clone are described in Table I. Double-stranded dideoxy sequencing resulted in a 2220-nucleotide sequence with a 3' poly(A) tail. It contains an open reading frame of 1941 bp. It can encode a polypeptide of 647 amino acids with a predicted molecular mass of 70,876 D. The 647 amino acid residues exhibit a 97.8% identity with SCE70 of spinach (Ko et al., 1992) and a 94.3% identity with the tomato HSC-2 Hsp70 (Lin et al., 1991). SCE70 and Hscl may represent sequences for the same gene, but the sequence for Hscl conforms more closely to that of typical Hsp70 gene sequences. The spinach Hscl exhibits the most similarity with the ssa1-4 family of Hsp70s in yeast (Craig et al., 1993).

Hsc70s and Hsp70s in plants and other systems are known to bind to ATP and to use the energy of ATP hydrolysis for their functions. The ATP-binding domain of the bovine heat-shock cognate protein has been characterized by x-ray crys.
tallography (Flaherty et al., 1990). A calmodulin-binding motif KRAVRLRTACERAKRTLSSS has been noted for mouse, rat, and human Hsp70s (Stevenson and Calderwood, 1990). The Hsc1 protein contains a related sequence at positions 263 to 283.

Received November 23, 1993; accepted December 20, 1993.
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The GenBank accession number for the sequence reported in this article is L26243.

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