Plant Gene Register

A Wheat (Triticum aestivum) cDNA Clone Encoding a Plastid-Localized Heat-Shock Protein

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Higher plants produce both high mol wt (in the range of 70,000–110,000) and LMW (in the range of 15,000–30,000) HSPs in response to heat-stress conditions. During periods of heat stress, plant cells produce an abundance of LMW HSPs that are encoded by gene families (Vierling, 1991). The physiological function of these proteins remains obscure, but the evolutionary conservation of this response in higher plants suggests that they perform some important function in protection of plants from heat. LMW HSP genes have been isolated from a variety of crop plants such as soybean, pea, carrot, wheat (Triticum aestivum), and corn. Recently, Vierling (1991) classified these proteins into four families, two of these encode cytoplasmic proteins, one represents endomembrane proteins, and one includes the plastid-localized HSP genes.

The question of nonfortuitous association of LMW HSPs with plastids during and after heat shock has been addressed by several research groups who concurred that HSPs with mol wts ranging from 21,000 to 28,000 are specifically localized in chloroplasts during and after heat stress (for review, see Vierling, 1991). These nuclear-encoded HSPs consist of an amino-terminal transit peptide region and a carboxyl-terminal mature peptide that has significant similarity with other cytoplasmic LMW HSPs. The translation products of these mRNAs are transported to plastids where they are associated with either the soluble stroma portion or the grana region of the thylakoid membranes. Comparison of the amino acid sequences of plastid-localized HSPs from petunia, Arabidopsis, pea, soybean, and corn has led to identification of three conserved regions designated I, II, and III (Chen and Vierling, 1991). Regions I and II are also shared by cytoplasmic HSPs, but region III is exclusively present in plastid-localized HSPs.

The first monocotyledonous cDNA encoding a plastid-localized HSP26 was isolated from corn by Nieto-Sotelo et al. (1990). Using this cDNA clone as a probe, we previously isolated a cDNA clone from wheat, which encodes an HSP of mol wt of 26,600 (Tahsp26.6a) (Weng et al., 1991). We have reported 82, 50, and 61% identity between wheat HSP26 and corn, pea, and soybean plastid-localized HSPs, respectively. In this communication, we describe a cDNA that encodes a second member of the plastid-localized HSP gene family in wheat (Table I).

By using hybridization/selection and in vitro translation protocols described previously (Weng et al., 1993), we found that the HSP26.6 family in wheat consists of at least two members that differ slightly in their mol wt. Therefore, we selected a clone that was distinct from Tahsp26.6a in terms of restriction enzyme digestion patterns and insert size. Nucleotide sequence of this cDNA, Tahsp26.6b, is 1007 bp with a 5' noncoding region of 82 bp, a coding region of 711 bp, and a 3' noncoding region of 214 bp. A putative poly(A) signal (AATAAG) is present at 29 bp upstream of the 3' end of the cDNA clone (Joshi, 1987). Overall similarity between Tahsp26.6b and Tahsp26.6a, the other member of this family, indicate that the coding regions are 96% identical, whereas 5' and 3' untranslated regions of these genes show 37 and 78% similarity, respectively. Therefore, it appears that this cDNA represents another member of wheat HSP26.6 family. To date, only one member of plastid-localized HSP gene families from Arabidopsis, Petunia, soybean, pea, wheat, and corn have been characterized (Chen and Vierling, 1991; Vierling, 1991; Weng et al., 1991). This is the first example of a second member of a plastid-localized gene family that has been isolated and characterized.

The deduced amino acid sequence of HSP26.6b is 97% identical with wheat HSP26.6a protein. All three conserved regions, including the 'Met bristles' in plastid-localized HSPs are present in HSP26.6b (Chen and Vierling, 1991). The first 50 amino acids have the characteristic features of putative transit peptides as described by Nieto-Sotelo et al. (1990). We are in the process of producing rabbit polyclonal antibodies against wheat HSP26.6, and in the future we will examine the intracellular and intraplasmid distribution of HSP26 during heat stress. This may assist in understanding the function of these proteins in plastid protection. Moreover, recent reports of successful wheat transformation (Vasil et al., 1992) and the presence of a small number of highly similar members of this gene family are likely to make the antisense HSP26.6

Abbreviations: HSP, heat-shock protein; LMW, low molecular weight.

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Table 1. Characteristics of the cDNA encoding HSP26.6b from wheat

<table>
<thead>
<tr>
<th>Organism:</th>
<th><em>Triticum aestivum</em> L. var Mustang.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location:</td>
<td>Nuclear-encoded LMW HSP, posttranslational transport to plastids.</td>
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<td>Techniques:</td>
<td>A CDNA library construction in λZap II vector (Stratagene) using poly(A)+ RNA from leaves of wheat seedlings heat shocked for 2 h (Weng et al., 1993). Screening of the library with radioactive maize HSP26 cDNA probe (Nieto-Sotelo et al., 1990). Phagemid rescue and subcloning. DNA sequencing using dideoxy chain termination method.</td>
</tr>
<tr>
<td>Method of Identification:</td>
<td>DNA and protein comparison with other plastid-localized HSPs using CCG computer programs (Devereux et al., 1984).</td>
</tr>
<tr>
<td>Characteristics of the Deduced Amino Acid Sequence:</td>
<td>Open reading frame of 237 amino acids including a 40- to 50-amino acid putative transit peptide followed by two consecutive stop codons. Calculated mol wt of TaHSP26.6b is 26,587 with predicted isoelectric point of 10.13.</td>
</tr>
<tr>
<td>Expression Pattern:</td>
<td>A transcript of about 1 kb accumulates progressively in response to heat-shock conditions at 37°C when no homologous mRNA is present under nonheat stress conditions. The mRNA accumulation is extremely rapid and is detectable after about 7 min of heat shock at 37°C. Roots without functional plastids also express this gene at slightly lower level of abundance than leaves of heat-stressed plants.</td>
</tr>
<tr>
<td>Gene Copy Number:</td>
<td>Southern blot analysis and in vitro hybrid selection/translation experiments indicate presence of at least two genes in this family.</td>
</tr>
<tr>
<td>Subcellular Location of Protein:</td>
<td>Although not examined, it is expected to be in the heat-stressed plastids.</td>
</tr>
<tr>
<td>Availability of the Antibodies:</td>
<td>Work in progress in our laboratory.</td>
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</table>

mRNA experiments plausible. This may provide some insights into the physiological functions of these proteins in heat-stressed plants.

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