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Communication

Isolation and Characterization of a cDNA Clone Encoding the Major Hsp70 of the Pea Chloroplastic Stroma[1]

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ABSTRACT

The 70-kD heat shock proteins (hsp70s) are a group of ubiquitous, highly conserved molecular chaperones that have been implicated in a variety of processes, ranging from DNA replication to protein folding and transport. To learn more about the evolution and possible functions of higher plant chloroplastic hsp70s, we isolated a cDNA clone encoding the major stromal hsp70 of pea chloroplasts, which we term CSS1 (Chloroplastic Stress Seventy). This cDNA clone encodes a 75,490-D protein that is very closely related to an hsp70 from the cyanobacterium, *Synechocystis*. CSS1 is nuclear encoded and synthesized as a higher molecular mass precursor with a chloroplastic transit peptide approximately 65 amino acids long. CSS1 mRNA was detected in RNA samples from leaves and roots of pea (*Pisum sativum*) plants grown at 18°C but increased 9- and 6-fold, respectively, after brief exposure of the plants to elevated temperature. We discuss the possible role(s) of CSS1 in chloroplastic protein transport and other processes.

Members of the hsp70[3] family are extremely highly conserved across a wide range of organisms. These proteins, which have been found in every organism so far studied, are present under normal growth conditions as well as being stress inducible. Hsp70s have been identified in a variety of cell compartments, including the cytosol, ER, mitochondria, and chloroplasts (1, 7, 20, 21, 23, 25, 28). In yeast, hsp70s located in the cytosol, ER, and mitochondria have been shown to be essential for cell viability under normal conditions (7). Thus, although hsp70s were originally identified on the basis of their stress inducibility, it is becoming increasingly clear that constitutively expressed hsp70s perform critical roles in normal metabolism (8).

Of particular interest are the recently described roles of hsp70s in the transport of proteins across membranes. Genetic and biochemical studies in yeast have shown that members of the cytosolic Ssa subfamily of hsp70s facilitate protein transport to both the ER and mitochondria (7). These hsp70s are believed to prevent precursors from assuming a transport-incompetent conformation in the cytosol. Furthermore, essential hsp70s located within the ER and mitochondria have been shown to be required for protein transport (17, 26). In the case of mitochondrial protein transport, partially translocated precursors interact with the matrix-localized hsp70, Ssc1p, which is thought to modulate precursor folding during and immediately after transport (17, 24).

Because chloroplasts are a major site of protein transport in plant cells, we undertook an earlier study of hsp70s in this organelle (20). Of the three hsp70 homologs identified in pea chloroplasts, one from the stroma (which we term CSS1 for Chloroplastic Stress Seventy) seemed a good candidate for possible involvement in chloroplastic protein transport because of its resemblance to the yeast mitochondrial hsp70, Ssc1p. To obtain more information about CSS1 and its possible function(s), we isolated a cDNA clone encoding this stromal protein. The inferred amino acid sequence of CSS1 and in vitro uptake experiments indicate that CSS1, like other nuclear-encoded chloroplastic proteins, is synthesized as a higher molecular mass precursor with an amino-terminal transit peptide. Levels of CSS1 mRNA, present in leaves and roots, increased 9- and 6-fold, respectively, after brief exposure of plants to elevated temperature. CSS1 resembles hsp70s from prokaryotes and semi-autonomous organelles and is most similar to DnaK from *Synechocystis*. We discuss the probable evolution of this protein from an hsp70 present in the prokaryotic progenitor of higher plant chloroplasts and possible function(s) of CSS1.

MATERIALS AND METHODS

Screening of cDNA Libraries

Two cDNA libraries, constructed in λgt11 and λgt10 using pea (*Pisum sativum*) leaf mRNA, were obtained from Dr. S. Gantt (13). Partial clones encoding CSS1 were identified in the λgt11 library by screening with antibodies specific for CSS1 (see below) as described in ref. 22. The 829-bp insert of λtg11/1 was radiolabeled and used to screen the λtg10 library by hybridization as described in ref. 22.

For identification of full-length CSS1 clones, partially purified λgt10 clones were rescreened by hybridization with a radiolabeled synthetic oligonucleotide encoding a totally conserved region near the amino terminus of all hsp70s. The 20-base synthetic oligonucleotide included 2-fold degeneracy at

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[3] Abbreviations: hsp70, 70-kD heat shock protein; SSC, standard sodium citrate; TKM, 10 mM Tris-acetate (pH 7.6) 30 mM potassium acetate, 3 mM magnesium acetate, 1.0 mM DTT; IB, 50 mM Hepes-KOH (pH 7.6), 330 mM sorbitol.

three positions, and the neutral base deoxyinosine (I) was incorporated at four positions:

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5'- GGI ATC GAC CTI GGI ACI AC -3'
      T   T T
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The oligonucleotide was radiolabeled using T4 polynucleotide kinase and γ-[^{32}P]ATP as described previously (22).

Partially purified clones were plated in *Escherichia coli* Y1090 cells. Nitrocellulose filter replicas of the resulting plaques were made and pretreated as described before (22). After pretreatment, filters were washed for several hours at room temperature in prehybridization solution (6× SSC [0.9 M NaCl, 90 mM sodium citrate], 1× Denhardt's solution [22], 0.05% [w/v] sodium PPi, 50 μg/mL of yeast tRNA, 0.5% [w/v] SDS, 20% [v/v] formamide). Filters were then transferred to hybridization solution (prehybridization solution minus SDS) containing radiolabeled oligonucleotide probe and incubated overnight at room temperature. After hybridization, filters were washed three times with 150 mL of 6× SSC plus 0.5% (w/v) sodium Pi, air dried, and allowed to expose x-ray film.

Preparation of CSS1-Specific Antibodies

Antibodies that reacted specifically with a 75-kD hsp70 from spinach chloroplasts and with pea CSS1 were prepared and kindly provided by L. Bovet (Laboratorie de Physiologie végétale, Université de Neuchâtel, Switzerland). Spinach (*Spinacia oleracea*) chloroplastic soluble proteins were separated by one-dimensional SDS-PAGE. The protein band corresponding to the 75-kD chloroplastic hsp70 was excised, and the protein was eluted and used to immunize rabbits.

Antibodies specific for CSS1 were purified from the crude antiserum using partially purified CSS1 as an affinity matrix. Pea chloroplastic soluble proteins were prepared as described previously (20), except the isolation buffer was TKM plus 330 mM sorbitol, 0.1% (w/v) BSA and the chloroplast lysis buffer was TKM. A soluble protein subfraction enriched in CSS1 was prepared by ATP-agarose chromatography, using a modification of the procedure described in ref. 30. ATP-agarose (4 mL, Sigma No. A 2767) was hydrated and washed in TKM. Pea chloroplastic soluble proteins were added, and the mixture was incubated 1 h with constant mixing. The ATP-agarose beads were sedimented and washed sequentially with 2 × 8 mL of TKM, 3 × 8 mL of TKM plus 0.5 M NaCl, and 2 × 8 mL of TKM. Finally, ATP-binding proteins were eluted from the ATP-agarose beads with 3 × 4 mL of TKM plus 5 mM Mg-ATP. The three Mg-ATP washes were pooled and analyzed by SDS-PAGE and immunoblotting.

Several ATP-binding proteins were present in the pooled Mg-ATP washes. Of these, two bands corresponded to the two stroma hsp70 homologs previously identified (20). Only the protein band corresponding to CSS1 was detected by the crude antiserum described above (not shown).

ATP-binding proteins were immobilized by repeated passage through a piece of wetted Immobilon-P membrane (Millipore). The immobilized proteins were then used as an affinity matrix for the purification of CSS1-specific antibodies, using a scaled-up version of the procedure described in ref. 15. Affinity-purified antibodies appeared monospecific for CSS1 when used to probe immunoblots (not shown).

Binding and Import of in Vitro Synthesized Protein by Isolated Chloroplasts

For in vitro transcription, the cDNA insert of λgt10/9 was subcloned into the vector pSP65. Plasmid DNA was linearized and transcribed by incubation for 1 h at 40°C in a reaction mixture containing 40 mM Tris-HCl (pH 7.9), 6 mM $MgCl_2$, 2 mM spermidine, 0.5 mM each of ATP, CTP, and UTP, 0.125 mM GTP, 0.6 mM diguanosine triphosphate (GpppG), 10 mM DTT, 50 μg/mL of BSA, 40 units of RNasin, and 20 units of SP6 RNA polymerase in a final volume of 50 μL. mRNA produced in vitro was translated in the presence of [^{3}H]leucine using micrococcal nuclease-treated rabbit reticulocyte lysate (Promega) according to the manufacturer's instructions.

Pea chloroplasts, isolated as described before (6), were resuspended at 1 mg of Chl/mL in IB. In vitro translation products were brought to 330 mM sorbitol by addition of 5× IB. For precursor binding, 20 μL of translation products plus IB were added to 50 μL of chloroplasts, and the mixture was incubated for 15 min on ice in the dark. Intact chloroplasts were recovered by centrifugation through 40% Percoll (6) and resuspended in IB or IB plus thermolysin (100 μg/mL) and 0.5 mM $CaCl_2$. Samples without thermolysin were stored on ice in the dark for 45 min. Samples containing thermolysin were incubated for 30 min on ice in the dark. Protease reactions were terminated by addition of Na_2EDTA to 5 mM, and chloroplasts were repurified as above except the Percoll contained 5 mM Na_2EDTA.

For protein uptake, 20 μL of translation products plus IB were mixed with 50 μL of chloroplasts and 1.8 μL of 100 mM Mg-ATP, and the mixture was incubated for 30 min at room temperature. Intact chloroplasts were reisolated and treated with thermolysin as described above.

Northern Blot Analysis of CSS1 mRNA Levels

Pea seedlings were grown for 14 d at 18°C under fluorescent light (12 h light/12 h dark). For heat-stress treatment, seedlings were transferred to 37°C for 1.5 h immediately before tissue harvesting. Total RNA was isolated from leaves and roots of control and heat-stressed seedlings using guanidinium thiocyanate and sedimentation through 5.7 M CsCl as described in ref. 22. Each RNA sample (15 μg) was separated by electrophoresis and transferred to a nitrocellulose membrane. A 432-bp *Pst*I/*Eco*RI fragment of the insert of λgt10/9 was radiolabeled and incubated with the membrane overnight at 42°C in hybridization solution containing 50% formamide (22). The membrane was then washed at 68°C, 2 × 5 min in 2× SSC plus 0.1% (w/v) SDS and 2 × 20 min in 0.1× SSC plus 0.1% (w/v) SDS (22). Bound probe was quantitated using a Molecular Dynamics PhosphorImager and ImageQuant software.

RESULTS

Isolation of a Full-Length λgt10 cDNA Clone for CSS1

In initial attempts to obtain a cDNA clone encoding CSS1, a λgt11 expression library, constructed using pea leaf mRNA,

was used (13). To detect clones encoding CSS1, the library was screened with antibodies directed against 75-kD soluble proteins from spinach chloroplasts. This antiserum was chosen because it contained antibodies that reacted specifically with a soluble 75-kD hsp70 present in spinach chloroplasts and with CSS1 from pea chloroplasts (3, 12). To reduce the possibility of detection of cDNA clones for other proteins, antibodies specific for CSS1 were purified from the crude antiserum using partially purified CSS1 as an affinity matrix.

Several positive clones were identified with the CSS1-specific antibodies. However, restriction digestion of the purified λ-DNA demonstrated that none of the these clones contained a cDNA insert long enough to encode a 70-kD protein. The nucleotide sequence of the cDNA insert of one of the clones (λgt11/1) was determined and found to consist of 829 bp, which encoded a polypeptide with homology to the carboxy-terminal region of hsp70s (data not shown).

To obtain a full-length clone, the cDNA insert from λgt11/1 was excised, radiolabeled, and used as a hybridization probe to screen a λgt10 cDNA library (also constructed using pea leaf mRNA). Initially, 64 positive clones were identified with the radiolabeled cDNA probe. To identify those clones likely to contain full-length inserts, positive isolates were rescreened using a 20-residue synthetic oligonucleotide probe encoding an absolutely invariant region (Gly–Ile–Asp–Leu–Gly–Thr–Thr) that is present very near the amino terminus of all hsp70s studied to date.

Fourteen isolates were detected in this secondary screen, and purified phage DNA from six of these was analyzed by restriction digestion. All six isolates contained inserts of approximately 2.5 kb, sufficient to encode a protein larger than 70 kD. One clone (λgt10/9) was selected for further analysis, and the nucleotide sequence of its 2474-bp cDNA insert is shown in Figure 1. The 3′ region of this cDNA was identical with the nucleotide sequence of λgt11/1, except the polyadenylation tail of λgt10/9 included 41 additional adenosine residues. Translation of the longest open reading frame (from base 46 to base 2163) gave a derived polypeptide 706 amino acids in length (Fig. 1). This inferred amino acid sequence was compared to the translations of all the sequences in the GenEMBL sequence library, using the GCG program TFASTA (10), and was found to resemble a large number of hsp70s, being most similar to the DnaK homolog from the cyanobacterium *Synechocystis*.[4]

Initiation of translation at position 46 would produce a polypeptide homologous to hsp70s but containing an amino-terminal extension of approximately 65 amino acids (based on comparison with other hsp70s). The sequence of this extension resembled the sequences of transit peptides that direct proteins to the chloroplastic stroma (14, 18, 27), being rich in the hydroxylated amino acids, serine and threonine, and devoid of acidic residues. It was not possible to determine

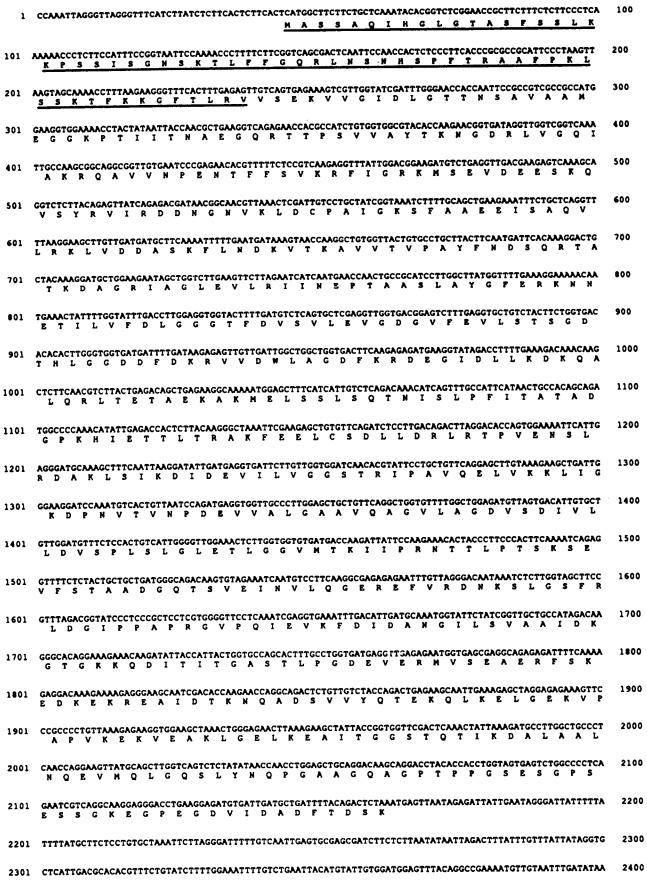

Figure 1. Nucleotide and inferred amino acid sequences of CSS1. The nucleotide sequence of the cDNA insert of λgt10/9 was completely determined in both directions using a Sequenase II kit (U.S. Biochemicals) and subclones generated using convenient restriction sites of Exonuclease III. The inferred amino acid sequence of the longest open reading frame is shown below the nucleotide sequence. The putative transit peptide is underlined. The DNA sequence will appear in the GenBank sequence library.

precisely the junction of the putative transit peptide and the mature hsp70-related protein because the amino acid sequence of the mature protein is not known. However, cleavage after the valine residue at position 65 (from the initiator methionine) seemed a likely possibility. This prediction was based on the presence of arginine at position 64, because arginine is commonly found one residue removed from the cleavage site of chloroplastic transit peptides (14). Furthermore, cleavage at this site would produce a mature protein with an amino terminus similar to other hsp70s.

Protein Encoded by the cDNA Insert of λgt10/9 is Imported and Processed by Isolated Chloroplasts

To verify that the protein encoded by the cDNA insert of λgt10/9 was a chloroplastic hsp70, the protein was expressed in vitro, and its competence for uptake by isolated chloroplasts was examined. The cDNA insert was subcloned into the plasmid pSP65, allowing transcription using SP6 RNA polymerase. The resulting mRNA was translated in the pres-

[4] Domoney et al. (11) recently reported the nucleotide sequence of pCD7, a partial cDNA clone encoding a pea protein related to hsp70s. The cDNA insert of pCD7 is identical with bases 1420 through 2091 of the insert of λgt10/9. Radiolabled probes derived from this partial clone and from the extreme 3′ end of λgt10/9 both hybridized to single bands on northern blots washed at high stringency (Fig. 4, ref. 11). Therefore, it seems likely that pCD7 is a partial clone of CSS1.

ence of [^{3}H]leucine using rabbit reticulocyte lysate, giving rise to a 74-kD primary translation product (Fig. 2, lane T). Several other minor translation products of lower molecular mass were also seen and probably represented initiation at internal codons specifying methionine, because they were not imported by isolated chloroplasts (see below) and, therefore, probably did not contain the amino-terminal transit peptide.

Isolated chloroplasts were incubated with translation products under conditions that would support either binding of chloroplastic precursors to the surface of the chloroplasts or transport of the precursors across the chloroplastic envelope. After incubation, the chloroplasts were treated with the protease thermolysin to remove external proteins and were analyzed by SDS-PAGE and fluorography. As shown in Figure 2, lane B, only the major 74-kD translation product associated with chloroplasts under conditions that support precursor binding but not transport (incubation on ice, in the dark). This 74-kD protein was completely removed when the chloroplasts were treated with thermolysin (Fig. 2, lane B + P), indicating that it was bound to the outer surface of the chloroplasts (6).

When chloroplasts and translation products were mixed and incubated in the presence of 2.5 mM Mg-ATP (conditions that support precursor transport across the envelope membranes), a single radiolabeled protein band of approximately 69 kD[5] was observed (Fig. 2, lane I). This protein was not removed by treatment of the chloroplasts with thermolysin (Fig. 2, lane I + P), indicating that it was inside the chloroplastic envelope. The apparent molecular mass of the internalized protein was very close to the 68.5 kD predicted for the mature protein that would be produced by removal of the putative 65 amino acid transit peptide.

To verify that the protein encoded by λgt10/9 was CSS1 and not the other stromal hsp70 homolog previously identified in pea chloroplasts (20), the products of in vitro protein uptake were analyzed using a combination of immunoblotting and fluorography. Proteins from chloroplasts that had been allowed to import the radiolabeled translation products were separated by SDS-PAGE, transferred to an Immobilon-P membrane, and either subjected to fluorography or probed with antibodies against hsp70s. Careful realignment of the fluorographs and immunoblots indicated that the 69-kD import product coelectrophoresed with the major stromal hsp70, CSS1, and not with the other stromal hsp70 homolog previously identified (data not shown).

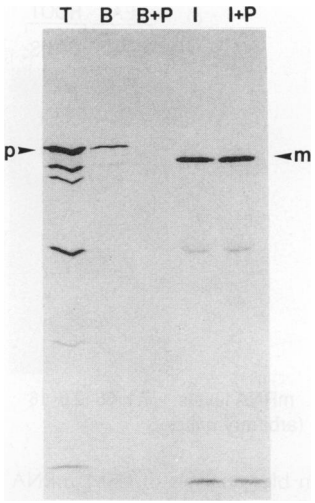

Figure 2. The protein encoded by the cDNA insert of λgt10/9 is imported by isolated chloroplasts. Fluorogram of ^{3}H-labeled proteins bound to and imported by isolated chloroplasts. Lanes: T, products of in vitro translation of the cDNA insert of λgt10/9 subcloned into pSP65; B, translation products associated with isolated chloroplasts after incubation under conditions that support precursor binding; B + P, same as B except chloroplasts were posttreated with the protease thermolysin; I, translation products associated with chloroplasts after incubation under conditions that support protein import; I + P, same as I except chloroplasts were posttreated with thermolysin. Samples were loaded on an equal Chl basis. Arrowheads indicate precursor (p) and mature (m) forms of CSS1.

CSS1 mRNA is Heat Inducible

We previously reported that the steady-state levels of chloroplastic hsp70 homologs do not change significantly after brief exposure of pea plants to elevated temperature (20). However, the techniques used did not allow us to determine the level of expression of genes encoding the chloroplastic hsp70s. In the present work, we have readdressed the question of the heat inducibility of CSS1 using a more refined assay.

Total RNA was isolated from leaves and roots of control and heat-treated pea seedlings. Levels of mRNA specifying CSS1 were then analyzed on northern blots by hybridization to a radiolabeled probe consisting of the extreme 3′ 432 bp of the cDNA insert of CSS1. The first 108 bp of this probe encoded the 36 carboxy-terminal amino acids of CSS1, and the remainder was 3′-noncoding DNA. Because the carboxy-terminal region of hsp70s is poorly conserved, the probe was expected to hybridize only to CSS1 mRNA.

As shown in Figure 3, a single RNA band was detected in samples from leaves and roots of control and heat-stressed plants. The apparent size of this RNA species was close to the 2.5 kb predicted from the cDNA insert of λgt10/9. The relative amount of CSS1 mRNA present in each sample was estimated by measuring the intensity of the detected band using a digitized image of the northern blot. CSS1 mRNA was approximately three times more abundant in leaf tissue than in root tissue. Furthermore, the level of CSS1 mRNA present in leaves and roots increased approximately 9- and 6-fold, respectively, after 1 h of exposure of the plants to elevated temperature.

Analysis of the Relatedness of CSS1 to Other hsp70s

As noted above, the inferred amino acid sequence of CSS1 was most similar to that of the DnaK homolog from *Syne-*

[5] We previously reported (20) that the molecular mass of CSS1 was 78 kD, which differs from the 69 kD reported here. As noted above, the radiolabeled protein band seen in Figure 3, lanes I and I + P, comigrated with the protein previously identified as CSS1. This difference of calculated molecular mass is probably due to a change in SDS-PAGE conditions used in the previous and present works.

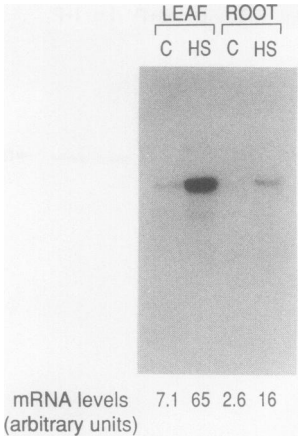

Figure 3. Northern blot analysis of CSS1 mRNA levels present in total RNA samples isolated from leaves and roots of control (C) and heat-stressed (HS) pea plants. Numbers at bottom indicate the relative amount of ^{32}P-labeled probe that hybridized to each sample (arbitrary units).

chocystis (5). CSS1 was also very similar to chloroplastic hsp70s recently identified in three algal species (21, 23, 28). To learn more about the relation of CSS1 to prokaryotic and other hsp70s, we compared the amino acid sequences of CSS1 and several other hsp70s using the GCG sequence comparison program, GAP (10). The results of these comparisons are summarized in Table I. CSS1, *Synechocystis* DnaK, and the algal chloroplastic hsp70s (represented in Table I by the red alga *Porphyra umbilicalis* [21]) were all strikingly (>80%) similar to one another. These proteins were also quite similar (>72%) to hsp70s from mitochondria and *E. coli*. It is interesting that the pea chloroplastic hsp70 was less similar to a pea cytosolic hsp70 than to prokaryotic, chloroplastic, and mitochondrial hsp70s.

DISCUSSION

We have isolated a cDNA clone encoding CSS1, the major stromal hsp70 of pea chloroplasts. CSS1 is synthesized as a 706-amino acid precursor protein, containing an amino-terminal transit peptide approximately 65 amino acids long.

CSS1 was originally identified as a chloroplastic protein. However, mRNA specifying CSS1 was detected in RNA samples from roots as well as leaves, indicating that this protein is probably also present in plastid types other than chloroplasts. The observed lower level of CSS1 expression in roots compared to leaves is similar to the pattern of expression of plastid-localized hsp21 after heat shock (4). Chen et al. (4) suggested that the lower expression in roots may be a reflection of the smaller number of plastids present in root cells than in leaf cells and that the amount of hsp21 per plastid may be similar in the two organs. The same explanation could apply to CSS1 expression.

Levels of CSS1 mRNA were higher in RNA samples isolated from leaves and roots after exposure of plants to elevated temperature. A corresponding increase in the amount of CSS1 protein following heat shock has not been observed (20). One possible explanation for this is that heat shock leads to higher CSS1 turnover and the increase of mRNA is required to maintain a constant level of CSS1 in plastids.

CSS1 was found to resemble most closely hsp70s found in cyanobacteria and the algal chloroplasts, followed by mitochondrial and *E. coli* hsp70s (Table I). The high degree of similarity of CSS1 and *Synechocystis* DnaK is probably an indication that the pea chloroplastic and cyanobacterial hsp70s are evolutionarily related. It seems likely that CSS1 evolved from an hsp70 present in the prokaryotic progenitor of higher plant chloroplasts, which, according to the endosymbiont hypothesis, was probably a cyanobacterium. Similar proposals have been made for the origins of the three recently reported algal chloroplastic hsp70s and are supported by the findings that all three of these hsp70s are encoded on the plastid genome (21, 23, 28).

Table I. *Comparisons of Seven hsp70s*

Hsp70	Percent Amino Acid Similarity (Identity)					
Synechocystis DnaK[a]	84 (71)					
Porphyra chlp.[b] hsp70[c]	81 (68)	84 (73)				
pea mito. hsp70[d]	74 (56)	73 (58)	72 (55)			
pea cytos. hsc70[e]	67 (47)	69 (48)	68 (49)	67 (50)		
E. coli DnaK[f]	74 (55)	75 (57)	72 (56)	77 (60)	67 (49)	
yeast mito. SSC1p[g]	72 (55)	71 (53)	70 (54)	75 (61)	68 (49)	75 (59)
	pea chlp. CSS1[h]	*Synecho.* DnaK	*Porphyra* chlp. hsp70	pea mito. hsp70	pea cytos. hsc70	*E. coli* DnaK

[a] Ref. 5. [b] Abbreviations: chlp., chloroplastic; mito., mitochondrial; cytos., cytosolic; *Synecho.*, *Synechocystis*. [c] Ref. 21. [d] Ref. 29. [e] E. Vierling, personal communication. [f] Ref. 2. [g] Ref. 9. [h] Present work.

Interestingly, Ko et al. (19) recently reported the isolation of a cDNA clone encoding Sce70, an hsp70 associated with the envelope of spinach chloroplasts. It is nearly identical with plant cytosolic hsp70s (19) but has much lower similarity to CSS1 (data not shown). Presumably, Sce70 is derived from a cytosolic hsp70 and was contributed to the endosymbiont from the host.

Nothing is known about the function(s) of chloroplastic hsp70s. However, the fact that CSS1 is expressed in roots as well as in leaves indicates that this protein is probably involved in processes common to different plastid types and is not restricted to functions occurring in photosynthetic tissues.

Some functional information may be inferred from studies of hsp70s similar to CSS1. Although the functions of the most closely related hsp70s, *Synechocystis* DnaK and the three algal chloroplastic hsp70s, have not yet been elucidated, *E. coli* DnaK has been extensively studied and is known to be involved in a number of diverse processes, including protein folding, protein export, and DNA replication (reviewed in ref. 8). CSS1 may act in some or all of the analogous processes in chloroplasts. For instance, CSS1 could facilitate correct folding of newly synthesized or transported proteins in the stroma. Furthermore, CSS1 might act in the transport of proteins from the stroma to other chloroplastic compartments such as the thylakoids, inner envelope membrane, and envelope intermembrane space. Such intraorganellar protein transport has been proposed for both chloroplasts and mitochondria to have evolved from prokaryotic protein export (16) and, therefore, may involve prokaryotic-like hsp70s. Additionally, CSS1 could act in chloroplastic DNA replication.

Finally, CSS1 may facilitate chloroplastic protein import. As noted earlier, CSS1 resembles the yeast mitochondrial hsp70, Ssc1p, both in its similarity to prokaryotic hsp70s and in its intraorganellar location. Ssc1p has recently been shown to be required for the import of proteins by mitochondria (17). It has been proposed that ATP-dependent interaction of precursors with Ssc1p in the matrix somehow drives protein transport and may explain the observed requirement for NTP hydrolysis in the matrix. We believe that CSS1 may be the chloroplastic analog of Ssc1p, and, therefore, we propose that these two proteins may have common functions in organellar protein import. ATP-dependent interaction of precursors with CSS1 in the stroma might explain the observed dependence of chloroplastic protein import on stromal ATP hydrolysis (18).

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