Heat Shock Response

Plant tissues generally respond rapidly to sudden rise in temperature by curtailing or abolishing normal protein synthesis and producing new polypeptides known as heat shock proteins. When cells are exposed to temperature 10-15°C above their normal growth temperature, they respond by changing both their transcriptional and translational patterns of gene expression (Key et al., 1985). This phenomenon known as heat shock response (hsr) was originally discovered in Drosophila (Ritossa, 1962). A shift of a Drosophila fly from its normal growth temperature at 25°C to 37°C elicits rapid induction of puffs at new locations on the polytene chromosomes and regression of preexisting ones. These observations were interpreted as a temperature induced regulation of gene transcription.

The hs response of soybean has become a paradigm of the environmental stress response in plants because of many of the original investigations of the molecular aspects of hs and related stresses have been performed with this organism as for example the synthesis of hsp (Key et al.,1981) the analysis of mRNAs (Schoffl and Key,1982) and hs genes (Schoffl and Key, 1983) the development of thermotolerance (Lin et al.,1984) and the relation of the hs response with other stresses (Czarnecka et al.,1984), aspects of expression of the heat shock genes relative to the inductive temperature regimes, experiments on transgenic expression of hs genes.
Heat shock response involves a transient, complex reprogramming of cellular activities evidently needed to protect essential structures and functions against damage during the stress period and to provide optimum conditions for the recovery (Scharf et al., 1990).

**Heat Shock Proteins**

The biosynthesis of proteins is a very sensitive metabolic process. Changes in temperature can affect both the amounts and types of polypeptides produced. The heat shock proteins can be seen to fall into two main categories: high molecular weight (over 60 kDa) and low molecular weight (14-30 kDa) (Table 1). The hsp's in higher plants were initially discovered in tobacco and soybean cells (Barnett et al., 1980; Key et al., 1981). There are however, several characteristics unique to plant hsp's. First LMW hsp's of about 15-20 kDa are extremely abundant (Schoffl et al., 1986). Second, some of the hsp's ranging in size from 21 to 30 kDa are translocated into chloroplasts (Kloppstech et al., 1985; Vierling et al., 1986). Third and the last, pollen grains do not show synthesis of typical hsp's (Cooper et al. 1984, Xiao and Mascarehans. 1985). This distribution is typical and characteristic of that seen in most higher plants. The HMW hsp's are known to have close homologies with those produced in other eukaryotes, and certain of them, e.g. hsp 70 are even related to bacterial hsp (Craig et al., 1982). It is the low molecular weight group which is the special feature of higher plants. Mansfield and Key (1987) surveyed low molecular weight hsp's
Table 1. **Heat Shock Proteins of Eukaryotes (For reference see the text).**

<table>
<thead>
<tr>
<th>Class</th>
<th>Species</th>
<th>Size (kDa)</th>
<th>Covalent modifications</th>
<th>Intracellular location</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsp 95</td>
<td>Plants</td>
<td>95</td>
<td>Phosphorylation</td>
<td></td>
<td>Proteins induced by glucose</td>
</tr>
<tr>
<td>hsp 84</td>
<td>plants</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsp 70</td>
<td>all</td>
<td>68-74</td>
<td>Phosphorylation</td>
<td>cytoplasm, cytoskeleton migration to nucleus after heat shock</td>
<td>Mainly constitutive cell proteins E.Coli equivalent is the dnaK protein</td>
</tr>
<tr>
<td>hsp 60</td>
<td>Plants</td>
<td>60</td>
<td></td>
<td>mitochondria</td>
<td>encoded by mt DNA</td>
</tr>
<tr>
<td>Low Mol.</td>
<td>Plants</td>
<td>15-27</td>
<td>Aggregate</td>
<td></td>
<td>hsp21 and hsp22</td>
</tr>
<tr>
<td>Wt. Hsps</td>
<td></td>
<td></td>
<td></td>
<td>after heat shock to give cytoplasmic granules</td>
<td>transported to chloroplasts</td>
</tr>
<tr>
<td>hsp 84</td>
<td>yeast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsp 70</td>
<td>yeast</td>
<td></td>
<td></td>
<td></td>
<td>Facilitates translocation into mitochondria and microsomes.</td>
</tr>
</tbody>
</table>

Contd.
<table>
<thead>
<tr>
<th>Class</th>
<th>Species</th>
<th>Size (kDa)</th>
<th>Covalent modifications</th>
<th>Intracellular location</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsp 26</td>
<td>yeast</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsp 95</td>
<td>vertebrates</td>
<td>92-105</td>
<td>Phosphorylation</td>
<td></td>
<td>component</td>
</tr>
<tr>
<td></td>
<td>normal cell</td>
<td></td>
<td></td>
<td></td>
<td>identical with membrane</td>
</tr>
<tr>
<td>hsp 84</td>
<td>vertebrates</td>
<td>83-90</td>
<td>Methylation and ADPribisylation</td>
<td>cytosol</td>
<td>normal cell component</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>associated with steroid receptors</td>
</tr>
<tr>
<td>small hsp</td>
<td>vertebrates</td>
<td>23-30</td>
<td>Glycosylation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hsp 84</td>
<td>Drosophila 84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low mol.</td>
<td>Drosophila 22</td>
<td></td>
<td></td>
<td>nuclear matrix</td>
<td></td>
</tr>
<tr>
<td>wt. hsps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

23
26
28
in a number of plant species, finding that dicot plants produce a higher proportion of these proteins than do monocots. Elevated temperatures (35°-40°C) induce the synthesis of a set of 10 hsps in the roots of maize seedlings. The size distribution of maize hsp is similar but not identical to that of soybean (Key et al., 1981). One of the maize hsp has a molecular weight of 70 kDa.

One striking feature of soybean hsps and for plants in general is the relatively complex constellation of LMW hsps that is induced by elevated temperature. The complexity of LMW group of hsps has been observed for a number of plant species. For example in maize system (Baszczynski and Walden, 1983; Cooper and Ho, 1983; Cooper et al., 1984). A comparative study using two-dimensional PAGE analysis of the total number of LMW proteins has identified, namely polypeptides induced by hs from non-detectable levels and polypeptides that were present at normal temperature but which increased after hs (Mansfield and Key, 1987). In soybean, 27 hsps in the 15 to 25 kDa range were detected, with 6 being enhanced and 21 being induced by heat treatment. Other plant species examined include pea, sunflower, wheat, rice, maize, millet and Panicum sp. In each of the plant species examined, the LMW hsps resolved into a diverse array of 12 to greater than 20 polypeptides upon electrophoresis. Nover and Scharf (1984) identified 18 basic proteins induced by heat shock in tomato suspension cultures of which 9 were less than 30 kDa MW. Green plants have additional organelle, the chloroplast, within which hsps have been identified following
heat treatment. These hsps have been shown to be coded by nuclear genes which are translated into cytoplasm and then transported into chloroplasts (Kloppstech et al., 1985; Vierling et al., 1986). These additional genes however cannot account entirely for the additional complexity of LMW hsp protein genes found in plants.

The pattern of HMW hsps from a different plant species show much less variation than the LMW hsps when analysed by two-dimensional PAGE. The distribution of hsps of 68, 70, 83 and 92 kDa are remarkably similar for soybean, pea, millet, corn, and cotton (Burke et al., 1985); besides, 100 and 120 kDa proteins in tobacco (Meyer and Chartier, 1983), and 110 kDa protein in soybean are also induced. In plants, the actual temperature at which hs proteins are maximally induced is correlated with the normal growing temperature. Pea, a cold season species, maximally induces hsps around 37°C, while millet a warm season grass, maximally induces hsps around 45°C. For maize, soybean and pigeon pea, the optimal temperature is 40°C (Review Kishore and Upadhaya, 1988). In general, the synthesis of hsps is first detectable at temperatures at about 5°C above the normal growing temperature. Synthesis of hs proteins as a percentage of total protein synthesis increases strongly as the temperature of the hs approaches the optimal temperature. Above this optimum, total protein synthesis drops off precipitously, but the majority of proteins synthesised at these higher temperatures are hsps. The hsps are known to be induced by chemical agents (Table 2).
<table>
<thead>
<tr>
<th>Inducer</th>
<th>Organism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenate</td>
<td><em>Drosophila</em></td>
<td>Tanguay and Vinent (1987)</td>
</tr>
<tr>
<td></td>
<td>Soybean</td>
<td>Kimpel and Key (1985)</td>
</tr>
<tr>
<td></td>
<td>mammalian cells</td>
<td>Li, 1983</td>
</tr>
<tr>
<td>Anoxia</td>
<td><em>Drosophila</em></td>
<td>Kimpel and Key (1985)</td>
</tr>
<tr>
<td>Dinitrophenol</td>
<td><em>Drosophila</em></td>
<td>Ashburner and Bonner, 1979</td>
</tr>
<tr>
<td>1979</td>
<td>Soybean</td>
<td>Czarnecka et al., 1984</td>
</tr>
<tr>
<td></td>
<td><em>Pigeon pea</em></td>
<td>Kishore, R. Ph.D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>thesis, 1989</td>
</tr>
<tr>
<td>Heavy metals</td>
<td>Soybean</td>
<td>Czarnecka et al., 1984</td>
</tr>
<tr>
<td></td>
<td>Petunia</td>
<td>Winter et al., 1988.</td>
</tr>
</tbody>
</table>
Time Course:

For plants growing in a natural environment, gradual temperature increases during the course of the day are likely to be at least as common as sudden increase of the heat "shock type" though the latter may occur due to, for example, alternations of suns and clouds. Gradual temperature enhancement have been shown to induce hsp's synthesis in a number of species including soybean (Key et al., 1985). Moreover, during prolonged high temperature treatment there are alterations in the pattern of proteins being synthesised. Neechi et al. (1987) designated two classes of hsp's - "early" and "late" observed in five cereal species. Early hsp's were synthesised within 4 hr of a temperature rise (from 20-40°C) and late hsp's appeared only after 7 hr or more at 40°C. Similarly Cooper and Ho (1983) observed progressive changes in the pattern of hsp's over a 10 hr exposure of maize root tissue at 40°C.

Temperature Threshold

In almost all eukaryotic tissues, the synthesis of hsp's is induced rapidly when the temperature is raised above the requisite threshold. This threshold varies from species to species; for example, in maize it is approximately 35°C (Cooper and Ho, 1983) while in Sorghum it is between 35 and 37.5°C (Key et al., 1985). It is absolute temperature, rather than the magnitude of the temperature increase which appears to be significant in inducing the heat shock response (Ougham and Howarth, 1982) and this probably reflects
the optimum temperature range for the species concerned. Above the threshold, the exact nature of the response depends both on temperature and the duration of the heat treatment. For example, in millet seedlings grown at 35°C, hsps are first detectable at 40°C. At 45°C, the hsp synthesis is maximal and normal protein synthesis is greatly curtailed. At 50°C, there is a small amount of residual hsp synthesis but normal proteins are no longer made. A 55°C treatment is sufficient to abolish protein synthesis completely. For temperate plants lower temperatures are sufficient both to induce maximal hsp synthesis and to abolish normal protein synthesis.

**Pattern Changes:**

Cells of callus derived from seed embryos of Oryza sativa exposed to heat shock depressed normal protein synthesis, while synthesis of specific proteins was enhanced. Depending on whether the temperature increase was rapid or gradual, differences were observed both in amino acid incorporation and hsps produced. In addition to the hsps found in other organisms, a heat shock protein appeared to be induced at a very high temperature (45°C), after progressive temperature increase, the molecular weight of which was 33 kDa (Fourre and Lhoest, 1989). Central to the changing gene expression is the massive synthesis of heat shock proteins which belong to five families of conserved proteins (Nover et al., 1990).

The pattern of protein synthesis in roots of 3 days old seedlings is rapidly and dramatically altered when incubation temperature is raised from
25° to 40°C. One-dimensional SDS-PAGE reveals that although the synthesis of the proteins observed at 25°C continues at 40°C, a new set of hsps is induced within 20 minutes of the temperature transition. The hsps have molecular weights of 87, 85, 79, 78, 77, 72, 70, 27, 22 and 18 kDa. The ten hsps are visible on autoradiograph but not on stained gels, suggesting that proteins do not accumulate to any great extent. The induction of hsps is transitory.

In soybean, the diversity of LMW proteins can be attributed at least in part to their derivation from multigene families (Czarnecka et al., 1985; Key et al., 1985; Nagao et al., 1985; Schoffl and Key, 1982; Schoffl and Key, 1983). These proteins may also be related between different species as cDNA clones for LMW hsps of soybean hybridize with poly(A) RNA from pea, sunflower, millet and maize (Key et al., 1983).

**Intracellular Localization of Hsps:**

Hsps have been shown to have specific subcellular localizations. For example, Vierling et al. (1986) showed transport of certain hsps into chloroplasts of soybean, pea and maize and included one major polypeptide in LMW range (21-27 kDa) in each case. Lin et al. (1984), Neuman et al. (1984) and Cooper and Ho (1987) showed localization of hsp to other subcellular compartments including mitochondria, plasma membrane and ribosomes. Certain plant species have been shown to concentrate a large proportion of LMW hsps into cytoplasmic aggregates known as "heat shock
granules" (Neuman et al., 1984; Nover, 1984). These are believed to protect mRNA and other cell components. The likelihood that their role is primarily structural is enhanced by the considerable sequence homology, between some of the LMW hsp and the bovine protein α-crystallins, which is a major structural component of the eye lens. Crystallins, the structural proteins of the eye lens, ensure the transparency and integrity of the lens throughout life. Recent sequence comparisons have shown that evolution has recruited crystallins among already existing hsps and stress inducible enzymes. The crystallins are water soluble, structural proteins that occur in high concentrations in the cytoplasm of lens fibre cells. The lack of protein synthesis in the differentiated lens fibre cells, combined with the necessity to maintain transparency by avoiding structural disintegration, demands that crystallins must be long living, stable and resistant to the deleterious influences of, for instance, radiant light, radicals and heat. α-crystallins are related to the small hsps. α-crystallins occur as large aggregates, composed of two types of related subunits, -A and -B. Surprisingly, these subunits are homologous with the small (15-30 kDa) hsp most notably in their C-terminal halves. They apparently originated from small hsp family by the classical mechanisms of gene duplication and subsequent gene divergence, allowing adaptation to novel functions. Interestingly, the promoter region of the gene for -B, but not -A, contains a perfect heat shock consensus element (HSE), although gene seems not to be induced by heat shock of lenses in organ culture. Perhaps -crystallins functions as a constitutive stress protein, conferring protection against the multitude of endogenous and exogenous
insults to which the lens is exposed. There, thus appears to be a general connection between the crystallins and stress- or development-dependent expression (de jong et al., 1989).

It has only recently been recognised that representatives of several major classes of hsps are localized to both chloroplasts and mitochondria. Yeast mitochondria have been reported to contain protein homologous to hsp 70 and Gro EL an hsp in E.coli (McMullin and Hallberg, 1988). In addition to LMW hsps identified here, chloroplasts also contain a Gro EL homology (Hemmingson et al.1988). In chloroplasts this protein is constitutive and participates in the assembly of rubisco holoenzyme. The discovery of hsps localized to chloroplasts and mitochondria extend the range of processes in which hsps function in eukaryotic cells.

It is known in Drosophila that some hsps localize specifically within the nucleus (Velasquez et al., 1980; Arrigo et al., 1980; Arrigo and Ahmad-Zadeh, 1981; Velazquez et al., 1983) and there is a suggestive evidence that hsps also associate with other organelles fractions during hs including the cytockeleton in animal cells (Schlesinger et al., 1982b) and mitochondria and ribosomes in soybean (Lin et al., 1984). The mechanism of association of hsps with the plant organelle fraction is not known, but the association is hs dependent; hs proteins made during arsenate treatment are found throughout the cytoplasm, associate with all organelles during hs. The 15-18 kDa hsps, the most abundant hs proteins in plants, associate with all the organelles examined in soybean, ribosomes, nuclei, mitochondria, and
plasma membrane fractions. Two hsp20s, 20 and 24 kDa, appear to specifically localize in the mitochondrial fraction but they do not "chase" significantly during a subsequent 30°C incubation (Key et al., 1982; Lin et al., 1984). This may indicate specific transport of these proteins into the mitochondria. At least one hsp which is post translationally transported into isolated chloroplasts. Fractionation of hsp20s from ribosomal pellet will enable to develop monoclonal antibodies to enable to more precisely define and quantitate the localization of these proteins during heat shock.

**Organelle Encoded Hsp:**

A temperature shift from 25°C to 41°C initiates the synthesis of a specific set of proteins in maize, including a peptide of 60 kDa heat shock proteins. Using an in vitro mitochondrial protein synthesising system, the evidence has been provided for this 60 kDa hsp being encoded within the organelle. Further support for this heat induced protein being encoded within mitochondria is that its synthesis is inhibited in whole seedlings by chloramphenicol. Additionally, a heat induced peptide of 62 kDa size can be detected in isolated mitochondria of a dicot plant, Brassica campestris. The function of the heat shock proteins encoded within the mitochondria are unknown. Chemicals which interfere with oxidative phosphorylation can induce the same set of stress proteins as heat shock (Ashburner and Bonner, 1979). In the inbred lines of maize, the hsp20s analysed by Sinibaldi and Turpen (1985) an abundant polypeptide of 60 kDa is synthesised by mitochondria. Similar sized hsp has been seen in both coleoptiles and roots.
of cereal species analysed by Necchi et al. (1987). Subsequently certain doubts were raised whether 60 kDa hsp observed by Sinibaldi and Turpen (1985) and 50 kDa by Necchi et al., (1987) in fact are of mitochondrial origin or this band is a consequence of bacterial contamination.

The chloroplasts have been shown to be synthesised as precursor polypeptides which are processed to their mature molecular weight upon import into chloroplasts (Vierling et al., 1988). The chloroplasts localized hsps show certain degree of conservation in carboxy-terminal domain from three different plant species. Arabidopsis, petunia, and maize (Vierling et al., 1989). The precursor form contains a transit peptide when these proteins are transported into chloroplasts (Vierling et al., 1986; 1988). However, there is no additional confirmation of the observation of Krishnasamy et al. (1988) that certain hsps are synthesized within the chloroplasts.

Recently Neto-Sotolo and Ho (1988) have examined the proteins synthesised by isolated mitochondria, chloroplast and plastids from maize tissues in order to confirm whether any of the hsps are encoded by organellar genome. They show that the prominent 62 kDa hsp band present in certain studies may be of bacterial origin and no organallar hsps are synthesized. However, they do not mention any thing concerning the 60 kDa and 59 kDa hsp bands observed (Sinibaldi and Turpen, 1985; Necchi et al., 1987).
DNA sequence analysis of chloroplasts hsp s cDNAs from pea and soybean has shown that the C-terminal halves of these proteins are homologous to low molecular weight hsp s from a wide range of eukaryotes (Vierling et al., 1988). They used a pea cDNA to construct fusion proteins containing either the C-terminal heat shock domain or N-terminal domain of the chloroplasts hsp s. The fusion proteins were overexpressed in E. coli and used to produce chloroplasts hsp specific polyclonal antibodies. The C-terminal antibodies recognised chloroplasts hsp precursors proteins from pea and three divergent plant species- Arabidopsis, Petunia and maize. The N-terminal antibodies recognised effectively only the precursor. The chloroplasts hsp s from dicotyledonous and monocotyledonous plants have conserved carboxy domain.

It has been shown that in addition to hsp s typical of eukaryotes, higher plants and algae also synthesise nuclear coded hsp s which localized to chloroplasts (Kloppstech et al., 1985; Schuster et al., 1987; Vierling, 1987; 1988). Major chloroplast hsp s have been identified in soybean (Vierling et al., 1986), pea (Kloppstech et al., 1985; Vierling et al., 1986), maize and (1987); petunia (Vierling, 1987) and Chlamydomonas (Kloppstech et al., 1985). They range in size from 21-24 kDa in different species. For pea chloroplasts hsp 21 and soybean chloroplast hsp 22, cDNAs have been isolated and shown to be 79% identical in the protein coding sequence.
Pea chloroplast hsp 21 mRNA is undetectable in control leaf tissue but increase to 0.75% of the total poly(A) RNA during 2 hr of hs treatment at 39° C (Vierling et al., 1988). Induction of chloroplast hsp mRNA is therefore the most dramatic example of environmental regulation of a chloroplast gene known to date. To investigate further the relationship between chloroplasts hsps of various plant species and to examine chloroplasts hsp accumulation and stability during stress, monospecific polyclonal antibodies against pea chloroplast hsp 21 have been prepared. The production and characterization of these antibodies and their use in demonstrating antigenic similarities among major low MW hsps of pea, Arabidopsis, petunia and maize have been shown.

It has been shown that three divergent plant species Arabidopsis, petunia and maize, all contain a LMW hsp which is antigenically related to the chloroplast localized 21 kDa hsp of pea. It is concluded that these hsps are also chloroplasts protein on the basis of (i) their antigenic similarity to pea hsp 21 (ii) evidence that hsps are synthesised as precursor proteins of high MW than the form that accumulate in vivo and (iii) the demonstration that the hsps which accumulate in Arabidopsis, and maize correspond in MW to major hsps imported into chloroplasts in vitro. The LMW chloroplasts hsps have now been identified in seven plant species, Arabidopsis, maize, pea, soybean, Phaseolus, and Chlamydomonas (Kloppstech et al., 1985).
The different plant species show an interesting pattern of cross reactivity with C-terminal and N-terminal antisera. The C-terminal fusion protein included the entire region of hsp 21, referred to as "heat shock domain", which is homologous to LMW hsps of plants and other eukaryotes (Vierling et al., 1988). The C-terminal antiserum reacts strongly with in vitro translation products of all four species. The N-terminal antiserum reacts strongly only with the translation products of pea in which the amount of hsp precipitated appears identical to that obtained with the C-terminal antiserum. Failure of N-terminal antiserum to precipitate the heterologous hsps suggests that N-terminal domain is not highly conserved as the C-terminal heat shock domain.

**Heat shock protein gene structure**

**Heat Shock Element (HSE):**

The first heat shock promoter to be studied in detail was that of the Drosophila hsp 70 gene. Multiple copies of this gene exist in Drosophila, and sequence analysis identified 355 nucleotides of conserved sequence 5' to the transcription unit of two hsp 70 genes (Karch et al., 1981). Identification of sequences necessary for heat induction was initially accomplished by introduction of Drosophila genes into cells of other organisms (Corces et al., 1981; Pelham and Bienz, 1982). The heat inducibility of the hsp 70 gene in heterologous systems demonstrated that the recognition signal is similar in widely diverged species. Comparison of the upstream regions of different hs
genes suggested that the key features of the HSE is a consensus sequence with dyad symmetry CTnGAAAnnTTCnAG (Table 3) (Pelham, 1982). Further comparision of hs gene promoter reveals that the minimum consensus element is the dyad C-GAA--TfC-G (Pelham, 1985; Bienz, 1985). Although Drosophila HSEs function to permit heat inducible expression in heterologous systems, the temperature optimum for expression corresponds to that of the recipients cells (Corces et al., 1981; McMahon et al., 1984). When the Drosophila hsp promoter was fused to a marker gene and introduced back into Drosophila cells, a single HSE was not sufficient for activity (Dudler and Travers, 1984; Mansfield, 1986; Amin et al., 1985; Simon et al., 1985). Additional 5'sequences including a second HSE with only 6/8 match to the consensus are required. Drosophila seems to require two HSEs, whose locations are somewhat flexible, and this requirement versus a single HSE in animals appears to be a genuine species difference (Pelham, 1987). Sequence comparisions of plant hs genes has identified similarities to the HSE in all hs genes that have been sequenced thus far. Almost all plant hs genes sequenced to date contain multiple HSEs in the 5' flanking region of the gene. The hs genes of soybean consist of several families of genes for which the largest family (class I) consists of 13 15-18 kDa proteins. One feature of this most abundant class is the tandem overlapping HSEs proximal to the TATA motif. The distance between the 5'end of the TATA motif to the 5'end of the HSE in this family of soybean genes is 31 nucleotides.
Table 3. **Comparision of soybean and Drosophila Sequences-Potential Proximal HS Promoter or Regulatory Elements (HSEs).**

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Source</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTnGAAnnTTnCnAG or CnnGAAnnTTCnnG</td>
<td>Drosophila</td>
<td>Pelham, 1985.</td>
</tr>
<tr>
<td>(-57) CTggt tgTgAG (-44)</td>
<td>Drosophila hsp 27</td>
<td>Ingolia and Craig, 1981.</td>
</tr>
<tr>
<td>(-61) CTcGAAttTCgGC (-48)</td>
<td>Drosophila hsp 70</td>
<td>Pelham, 1982.</td>
</tr>
<tr>
<td>(-60) CTcGAaTaTaAG (-47)</td>
<td>Soybean hs 6871</td>
<td>Schoffl et al. 1984.</td>
</tr>
<tr>
<td>(-62) CTgGAACaTaCaAG (-49)</td>
<td>Soybean Gm hsp 17-5-E</td>
<td>Czarnecka et al. 1985.</td>
</tr>
<tr>
<td>(-62) CTgGAACgTacaCg (-49)</td>
<td>Soybean Gm hsp 17-5-M</td>
<td>Nagao et al 1985.</td>
</tr>
<tr>
<td>(-63) CCCGAATCTTCtgg (-50)</td>
<td>maize hsp 70</td>
<td>Rochester et al. 1986.</td>
</tr>
</tbody>
</table>
The promoter structure of the 26 kD general stress proteins gene Gm26-A, appears complex with one major and two minor start sites for transcription. Each start site is associated with a TATA motif 27-30 bp upstream and sequences with low homology to HSE. Each start site appears to be the activity of a separate subpromoter and shows a distinctive induction pattern of expression to a variety of stresses (Czarnecka et al., 1987). The soybean hsp 70 gene (Roberts and Key, 1989) and the Arabidopsis hsp 70-2 gene (Wu et al., 1988) also have overlapping proximal HSEs.

The extent of conservation of nucleotides within the HSE is different. Nover (1987) has compiled the percent usages of each nucleotide from 69 HSEs as follows. 5'C (80) T (52), A (45), G (91), A (90), A (88)nn, T (86), T (98), C(91), T (42), A (73), G (75) 3'. Similar comparisons for the plant HSEs using only the proximal HSEs reveal percentages of 5'-C (89), T (85), g (58), G (100), A (100), A (92), a/c (38), T (85), T (62), C (100), a (58), A (69), G (85), 3'. One interesting aspect that is revealed when the tandem overlapping HSEs of the class I 15-18kDa hs genes are aligned is a second dyad consensus of inverted nature to the HSE, TTCnnGAA, created by the overlap. It is intriguing that each of the class I genes sequenced to date has 100% identity to this dyad. Recent experiments with heat shock transcription factor from Hela cells extracts show that such overlapping sites compete for factor more efficiently than do single HSE (Pelham, 1987).
Heat Shock Protein Structure

**High molecular weight heat shock proteins:**

The most abundant hsps induced in many organisms, but not in plants, has a molecular weight between 68 and 74 kDa. This major protein family is referred as hsp 70, reflecting a molecular weight in Drosophila where initial characterization was done. This heat inducible protein has been highly conserved throughout evolution. A polyclonal antibody prepared against purified chicken hsp 70 cross reacted with a protein of approximately 70 kDa from yeast, slime mold, maize seedlings, worms, frogs, flies, rodents and humans [Kelley and Schlesinger, 1982]. The bacterium, E.coli has a simple hsp 70 related gene dna K, with 48% identity to the Drosophila hsp 70 gene (Bardwell and Craig, 1984). The eukaryotes have evolved families of related genes with a complexity and number of genes composing the families differing among species (Craig, 1985; Lindquist, 1987).

The DNA sequence identity of the eight genes for hsp 70 family of yeast ranges from 96% to about 50% (Craig, 1985) and these sequence homologies have led to grouping of these genes into four subfamilies SSA1-SSA4; SSB1 to SSB2; SSC1; SSD1. SSA1 and SSA2 are constitutively expressed (Werner-Washburne et al., 1987) and strains carrying mutations in these genes are temperature sensitive for growth. The deletion of SSA1 and SSA2 results in the constitutive synthesis of other hsps (Craig and Jacobson, 1984). The SSA3 and SSA4 genes are strictly heat inducible. Limited data are available from hsp 70 genes from plants. Two hsp 70 genes have been isolated and sequenced from maize; one contains the entire coding region predicting a
646 amino acid protein, and the other contain only part of the coding sequence and 270 bp of 5'-upstream sequence (Rochester, et al., 1986). Both maize genes are induced thermally and each contains an intron in the same position at position 71. Sequence of the first exon of two maize genes show 90% amino acid sequence homology. Maize hsp 70 has 68% identity with Drosophila hsp 70 (Ingolia et al., 1980) and 75% identity with Xenopus (Bienz, 1984) and human hsp 70 (Hunt and Morimoto, 1985). The maize hsp 70 has 70% identity with the E.coli dnaK protein, which is similar to the homology between Drosophila and E. coli protein.

The hsp 70 related family from Arabidopsis consists of at least 12 polypeptides, most of which are constitutively expressed (Wu et al., 1988). Three hsp 70 genes have been isolated from Arabidopsis, but only one of these genes hsp 70-1 is expressed at significant levels. HSP 70-1 is expressed at control temperatures and is induced approximately 4 or 5 fold after heat shock. Partial sequence data is available for each of the Arabidopsis hsp 70 genes, and deduced amino acid sequences indicates the presence of an intron in the same position as in the two maize hsp 70 genes (Rochester et al., 1986), a petunia hsp 70 gene (Winter et al., 1987) the Drosophila HS cognate, hsc-1 gene (Ingolia and Craig, 1982) and a rat HSC 70 gene (Sorger and Pelham, 1987). Three cloned genes from Arabidopsis are member of a small family and are closely related to the hsp 70 genes found in other species (Wu et al., 1988). HSP70-1 is 89% identical to hsp 70-2 and 83%,
79%, and 72% identical to maize hsp 70, the Drosophila HSP-70 cognate and Drosophila hsp 70 respectively (Wu et al., 1988).

The petunia genome encodes a hsp 70 multigene family for which one genomic clone sequence has been determined (Winter et al., 1987). This gene is expressed constitutively; however, hs. arsenate and heavy metals significantly enhance expression.

The complete cDNA and corresponding genomic sequence for a soybean hsp 70 gene has been determined (Roberts and Key, 1989). The predicted amino acid sequence for the soybean hsp 70 polypeptide contains 645 amino acids. The gene is expressed at very low levels at control temperatures, and is thermally inducible to much higher levels.

A second prominent HMW hsp produced by all eukaryotes is in the range of 83 to 93 kDa. This protein class is referred to as hsp 83. Evidence indicates, however, that hsp 83 has been used to isolate the corresponding hsp 83 related genes from soybean (Roberts and Key, 1985) and maize (Sinibaldi et al., 1985) using a Drosophila hsp83 gene fragment as probe.

**Low Molecular Weight Heat Shock Proteins:**

(I) 15-18-kDa Family:

Characterization of soybean HS cDNA and genomic clones has demonstrated that soybean LMW hsp genes represent several multigene families with domains of homology with evolutionary distant organisms.
including Drosophila, Xenopus (Nagao et al., 1986). The area of highest conservation resides in the carboxylic portions. Based upon hybrid-select translation and DNA sequence analysis, the largest soybean family (class I) consists of 13 proteins. DNA sequences representative genome clones of this family have been worked out (Scholl et al., 1984; Nagao et al., 1985). Based on the genes sequenced thus far, molecular weight range of this family is 17.3 and 18.5 kDa (Key et al., 1987b). Four class I soybean hs proteins genes of 17.3 to 18.5 kDa show greater than 90% amino acid homology with approximately two-thirds of the nucleotide changes being silent substitutions (Nagao et al., 1985). Comparison of a 18.5 kDa hs protein sequence, Gm hsp 18.5-v, with the four 17 kDa sequences showed 96% amino acid identity when compared individually to each of the four 17 kDa proteins.

(ii) 21 to 24 kDa Family:

An additional family of soybean hsp genes encoding 21-24 kDa proteins is represented by cDNA clones and genomic clones. Amino acid sequence alignment shows that homology variations between clones within this size class may be as much as the variation between these and the 17kDa protein (Scholl et al., 1984). This region of maximum conservation is located towards the carboxyl terminal of the protein. The lower homology in the amino terminal portion of the protein may represent the functional divergence for such phenomenon as specific localization. For example, Lin et al., (1984) demonstrate the specific localization of 21 to 24 kDa hsp's to a
mitochondrial enriched fraction during hs. When the mitochondrial fraction was incubated with protease, the 21 to 24 kDa hsps were resistant to digestion, suggesting that these proteins were protected within the membrane or transported into the mitochondria. Second, these proteins do not delocalize from the mitochondria during control temperature chase suggesting that they are within the membrane than outside as are 15-18 kDa hsps that do delocalize during the chase period and are protease sensitive.

(iii) 26-28 kDa Family:

HS genes encoding 26 to 28 kDa proteins can be divided at least in two classes. One class represents a family of general stress proteins. This family of 4-6 genes is expressed constitutively at control temperatures, and synthesis is enhanced with hs and numerous other agents including arsenate (Kothary and Candido, 1982), heavy metals, high salt, anaerobiosis, water stress and abscissic acid treatment (Czarnecka et al., 1984). Hydropathy analysis of the deduced amino acid sequence compared to the smaller hsps indicate a high degree of relatedness within the carboxyl half of the protein. While clearly related to hsps, the lower amino acid sequence identity suggests that this protein is highly diverged and may therefore be specialised for general stress adaptation in soybean (Czarnecka et al., 1987).

A striking feature of one subset of plants hsps is the localization in chloroplasts (Kloppstech et al., 1985; Vierling et al., 1986; Schuster et al., 1987). Schuster et al. (1988) have presented results that support a role for
the chloroplast-imported hsps in preventing damage to the photosystem II reaction centre during hs in the light; a more general protective role for these proteins, not exclusive of a role in protection of PS II against photoinhibition, has been suggested by Vierling et al., (1988).

One method that has been used extensively to illustrate relationships of hsps has been analysis of hydropathic profile (Kyte and Doolittle, 1982). The conserved hydropathy domains of hsps not only illustrate relatedness among diverse organisms but also interpreted as functional conservation through evolution (Suss and Yordanov, 1986; Nagao et al., 1985; Nagao et al., 1986).

**Expression and Regulation of Synthesis of hsps :**

The expression of hs genes has been investigated for a variety of experimental conditions (Nagao et al., 1986; Key et al., 1987a). Some LMW hsps in soybean appear to be constitutively expressed at low levels in embryos during early stages of germination.

A great deal of information has accrued on the cis-acting regulatory sequences of hs genes. While it is now clear that many more sequence elements are involved in regulating hs gene expression. The HSE defined from initial work in Drosophila (CTnGAAnnTTCnAG) has been highly conserved across widely divergent eucaryote. One HSE is necessary and sufficient for conferring heat inducibility on a hs or some chimeric gene construct, yet the level of expression is highly modulated by several other DNA sequence "elements".
The expression of both hsp 17.4 and hsp 18.2 appears to be transcriptionally induced by hs as these transcripts are undetectable in control tissues. This feature differs somewhat from that of the HSP 70-1 gene from Arabidopsis. The HSP 70-1 gene is constitutively expressed and the level of corresponding mRNA is increased only 4 or 5 fold after heat shock (Wu et al., 1988). Non coordinate expression of these hsp genes is probably due to the number and location of HSE-like sequences and the existence of the CAAT box motif.

Two different concepts for mutant selection and gene manipulation of the hs response system appear promising.

(1) The first concept is the selection of cells and plants with constitutively repressed or non-inducible hs genes. Regulatory mutants of this type may result from a deficiency in the transactive HSTF, which is required for the induction of all or most of the hs genes. Mutations in genes of other factors acting in trans, for example, enhancer sequence-binding proteins, may generate a similar phenotype. A selection of such mutants may require a prior construction of chimeric genes, containing a selectable resistance marker or producing a toxic compound fused with a hs promoter (Bonner et al., 1984). A negative effect on the transcription of hs genes may also be generated if multiple copies of HSE sequences, which compete with the natural binding site for HSTF, are introduced into cells by genetic engineering (Xiao and Lis, 1986).
Another approach to block the expression of hsps uses genetically engineered hs genes, which produce antisense hs mRNA. This strategy has been successfully used in Drosophila (McGarry and Lindquist, 1986) and it may also be applicable to plants. Because the soybean hsps are highly conserved (90%) it should be possible to repress the expression of a whole multigene family by a constitutively transcribed antisense RNA of one gene.

It has been shown that antisense RNAs transcribed from genes that are stably integrated into the genome can be used to inhibit the expression of an endogenous cellular gene. Drosophila tissue culture cells were stably transformed with a gene encoding a heat inducible RNA complementary to the message for hsp 26. These cells produced much less hsp 26 after heat shock than did the untransformed cells. The inhibition was very specific and expression of the closely related heat shock proteins hsp 22, hsp 23 and hsp 28 was unaffected. By varying the copy number of the antisense gene, the degree of inhibition was varied over a broad range. Reducing the rate of hsp 26 synthesis did not appear to affect the synthesis of any other protein during either heat shock or recovery (McGarry and Lindquist, 1986). (ii) The other concept is the generation of plants that overexpress a certain, eventually manipulated hsp. Such plants would be useful to examine the biological effects of protein dosages by genetic engineering seems to be a simple way to enhance a gene product. Manipulation or replacement of the natural hs promoter may cause a hsp to accumulate under non stress temperature conditions in the cell (Pelham, 1984). The constitutive
expression of a hs gene may answer a number of questions pertaining to stability of hs mRNA and proteins: level of constitutive versus regulated expression; fitness and thermotolerance of transgenic plants.

There is a little information on the mechanism of regulation of the response. Regulation clearly occurs at both transcription and translation levels. Results indicate that during hs normal cellular mRNAs persist in the cells but are translated very inefficiently if at all. In soybean the mRNA levels for the small subunit of rubisco and an auxin regulated gene decreases dramatically during hs. of the total poly (A)RNA pool, the concentration of many poly(A) RNAs decline after 2 hr hs. Thus there must be a selective mechanism for preferential initiation/ and or elongation of hsps on hs mRNAs. In Drosophila, cell free lysates from heat shocked cells do selectively translate hs mRNAs from a pool of total cellular mRNAs. When tissues are returned to normal temperature after hs, the normal cellular mRNAs are once again efficiently translated. In soybean seedlings under prolonged heat treatment, hs mRNAs cease to be translated after about 6 hr and translation of normal mRNA recovers. The molecular basis for such translation regulation is not known. The absence of mRNA in tissues that have not been heat shocked indicates that the induction of the hs response is obligately regulated at the level of transcription.
Transcriptional Control of Hsp Synthesis:

The messages coding for the hsps result from new transcription and preferentially translated during heat shock. That is normal RNAs are rapidly cleared from the ribosomes to make way for the translation of the hs mRNA. It has been shown for the first time that transcription of plant hs genes may occur in heterologous plant cells in a thermo-regulated fashion with faithful initiation. The conserved promoter elements, present in the 5' upstream region of soybean class I hs genes are possibly recognised by transcription. They are possibly recognised by transcription factors which regulate the hs response in soybean and in sunflower by similar mechanisms. At least two protein factors which interact by sequential binding with DNA regions covering the TATA box sequences and the upstream control element of Drosophila hs genes have already been identified. The resemblance of the respective binding sites in animals and plants suggest a common mechanism for the transcriptional activation of hs genes in nature.

The molecular mechanisms of the transcriptional regulation by a hs promoter has become one of the major targets of the research in plants, soybean genes still playing a leading role. The availability of the new gene transfer techniques also made it possible to manipulate the hs genes and to study their expression in a foreign genetic background, particularly in transgenic tobacco (Schofl and Baumann, 1985; Spena et al., 1985; Gurley et al., 1986; Rochester et al., 1986; Schofl et al., 1986; Baumann et al., 1987; Spena and Schell, 1987) revealed conserved hs promoter element HSE
which probably interact with HSTF to initiate thermo-regulated transcription in native and transgenic plants.

The same level of transcripts is generated from a soybean hs gene in transgenic tobacco plants (Schoffl et al., 1986). The functional analysis of 5' upstream sequences of this gene suggests a bipartite promoter structure. Proximal sequences containing the conserved HSE like element 5' GT-GAA-TTC-AG responsible for thermo-induction and distal enhancer like sequences for maximal transcription (Baumann et al., 1987).

Expression of Heat Shock Genes in Transgenic Systems:

Transgenic gene expression has been essential in the characterization of hs promoters. Nover (1987) has summarized the results using hs genes and corresponding fusion constructs in heterologous and homologous transformation systems. These experiments confirm the necessity of the HSE for thermal regulation but also affirm the complexity of hs response in identifying many additional regions that affect hs gene expression. Gene transfer experiments have been performed in plants to identify sequences relevant in heat inducible expression. A 457 bp upstream sequence derived from the Drosophila hsp 70 gene was fused to the neomycin phosphotransferase gene (NPTII) and the resulting chimera was transferred in tobacco callus where an increase in NPTII activity was detected after heat treatment (Spena et al., 1985). The hs-neo-chimeric gene is heat regulated in roots, stems, and leaves, but not in pollen of regenerated tobacco plants.
(Spena and Schell, 1987). The lack of heat induction in pollen is consistent with previous reports (Cooper et al., 1984; Xiao and Mascarenhans, 1985) which indicate that pollen do not express hs genes.

A chimeric gene containing the NPTII coding region under the control of the hsp 70 promoter from Drosophila was integrated and found to be expressed in a heat regulated fashion, in tobacco tissue (Spena et al., 1985). This further supported the evolutionary conservation of heat shock response, that is Drosophila hsp70 genes can be expressed in heterologous systems.

A genomic DNA fragment containing a soybean heat shock gene hs6871 was inserted into the T-DNA region of the Agrobacterium tumifaciens pTiC58 plasmid. A strain carrying the modified Ti plasmid was used to incite tumors in sunflower hypocotyles. The expression of heat shock gene was investigated by Northern blot analysis of RNA and S1 nuclease mapping of the transcriptional start site. Heat shock induced poly(A) mRNA was detected in tumor tissues only after incubation at 40°C (heat shock) and not at the normal growth temperature (28°C). Transcripts from hs6871 are faithfully initiated in sunflower, starting at the same site in the DNA as in soybean. The low levels of transcripts initiating correctly on hs6871 in sunflower is consistent with a general tissue specific reduction in the expression of partially homologous native heat shock genes in sunflower tumor. It is not clear whether hs 6871 specific mRNAs are translated in sunflower into a functional protein. This can be tested upon availability of
antibodies or high level of transcription of the gene (Schoffl and Baumann, 1985).

Gurley et al., (1986) studied the expression of a soybean Gm hsp17.5E gene containing 3.2 kb of upstream flanking sequence in tumors incited on sunflower hypocotyles. Upon hs the steady state level of GmHsp17.5E transcripts reached a level comparable to that in soybean hypocotyles. Deletion from position -3250 to -1175 showed no effect on thermoinducible transcription at control temperature suggesting the involvement of additional temperature related regulatory sequences. Deletion of all sequences upstream of -95 resulted in low level thermal induction (2-5%). Therefore, while sequences both necessary and sufficient for thermal inducibility reside downstream of -95, sequences between -1175 to -95 are necessary for full transcription under hs conditions (Gurley et al., 1986).

The studies have been carried out to test whether the replacement of a native heat shock promoter by a viral promoter results in constitutive transcripts levels of the respective genes in transgenic plant. The 35S promoter of the cauliflower mosaic virus was linked to the protein coding region of the genomic heat shock gene hs 6831, encoding a 17.6 kDa hsp of soybean. After transformation of tobacco plants with this chimeric construction using a disarmed Agrobacterium binary vector, abundant mRNA at 25°C was equal to that generated by the native heat shock promoter at 40°C, however, it was markedly reduced by heat shock applied to the transgenic plants. These findings suggest a sufficiently high stability of hs
mRNA produced at normal growth temperature to direct constitutive expression of heat shock proteins.

Two maize hsp70 genes have been isolated and used to construct a hybrid gene which was transferred into petunia cells (Rochester et al., 1986). The expression of this gene in a generated petunia plant was thermally induced and transcript initiated correctly; however, the level of expression appeared to be significantly less than the endogenous level in maize shoots.

Expression of a chimeric gene construction (truncated 35S-promoter-chloramphenicol acetyl transferase) placing two partly overlapping soybean HSEs upstream of the reporter gene was found to be hs dependent in transgenic tobacco. The transcription was initiated correctly, but expression did not display organ specificity nor effect by light. In a different reporter gene construct, the HSEs were inserted into the pea rbc.S-3a (a light regulated gene) 5' flanking region which rendered the reporter gene light inducible and organ specific expression was observed under hs conditions (Shittmatter and Chua, 1987).

Using the technique of differential hybridization screening, they have isolated cDNAs for two low-molecular- mass heat shock proteins and their corresponding genes, hsp17.4 and hsp18.2 from Arabidopsis thaliana. These two genes encode polypeptides that are 79.2% identical to each other with respect to amino acid sequences and contain several overlapping sequences
that are similar to the consensus sequences for HSE. The 5' region of the hsp 18.2 gene was fused, in frame, to the E.coli gene B-glucuronidase (GUS) and the product has been introduced into petunia by Agrobacterium mediated transformation. It has been shown that GUS activity in transformed petunia plants is enhanced by heat shock (Takahashi and Komeda, 1989).

**Heat Shock Transcription Factor**

Heat stress treatment of cell cultures of Lycopersicon peruvianum results in activation of preformed transcription factor(s) (HSF) binding to the heat stress consensus element (HSE). Using appropriate synthetic HSE oligonucleotides, three types of clones with potential HSE binding domains were isolated from a tomato λ-gt11 expression library by DNA-ligand screening. One of the potential HSF genes is constitutively expressed, the other two are hs-induced. Sequence comparisons define a single domain of 90 amino acid residues common to all three genes and to the HSE-binding domain of the yeast HSF. The domain is flanked by proline residues and characterized by two long overlapping repeats. It has been speculated that the derived sequence is also representative for other eukaryotic HSF and that the existence of several different HSF is not unique to plants (Scharf et al., 1990).

The HSTF specifically binds to the HSE, thereby allowing induction of hs genes. This factor was originally detected in Drosophila tissue culture extracts and was partly purified on the basis of its ability to stimulate
transcription in vitro of the hsp 70 promoter (Parker and Topol. 1984). Independent studies by Wu (1985) detected HSE binding activity and nuclease protection of chromatin at heat shock but not at control temperature. Kingston et al. (1987) found a factor in nuclear extracts of human cells that binds to HSE of human hsp 70 gene. Zimarino and Wu (1987) showed that Drosophila tissue culture cells stimulated by hs contain a high level of hs activator proteins which binds specifically to the HSE. HSTF and HSAP (Heat-shock Activator Protein) studies show that HSTF is required for growth at normal temperature. Sorger and Pelham (1988) demonstrate that phosphorylation correlates with the transcriptional activation of HSTF following hs. Yeast heat shock factor is an essential DNA-binding protein that exhibit temperature dependent phosphorylation (Sorger and Pelham, 1988). Heat shock promoter contain one or more binding sites for a specific heat shock factor. It is proposed that expression of heat shock genes in yeast is modulated by phosphorylation of DNA bound HSF, and that this leads to a more efficient interaction of the factor with other components of the transcriptional machinery.

The transcriptional activation requires a specific heat shock promoter element (HSE) whose sequence is similar in species as diverse as humans, Drosophila, and the yeast (Bienz and Pelham, 1987). HSEs are binding sites for a specific heat shock factor (Wu, 1985; Parker and Topol, 1984; Sorger and Pelham, 1987; Kingston et al., 1987) and there is evidence that this factor, when isolated from yeast heat shocked cells can stimulate
transcription of heat shock genes (Wu et al., 1987; Wiederrecht et al., 1987). HSF has been purified by affinity chromatography on HSE sequences (Sorger and Pelham, 1987; Wiederrecht et al., 1987; Wu et al., 1987). Because of the induction of heat shock genes is not blocked by inhibitors of protein synthesis, HSF must preexist in cells in an inactive state (Zimarino and Wu, 1987; Kingston et al., 1987; Sorger and Pelham, 1987). The crucial regulatory step appears therefore to be the conversion of HSF into an active form.

**Phosphorylation of HSTF Correlates with Transcriptional Activation:**

The activity of synthetic promoters containing HSF binding sites increases more than 200 fold when cells are heat shocked from low growth temperatures. This increase in activity is associated with extensive covalent modification of the HSF polypeptide, and the phosphatase sensitivity of HSF from heat shocked cells indicates that most, if not all, of the modification represents the addition of phosphate groups. It thus appears that the ability of HSF to promote transcription is controlled by phosphorylation.

One simple model is that phosphorylation of clusters of amino acids can create an analog of the acidic activation sequences found in other transcription factors such as GAL 4 and GCN 4 (Hope and Struhl, 1986; Ma and Ptashne, 1987), and that these acidic regions are required for interaction with a TATA binding protein or with RNA polymerase.
The ability to modulate the overall acidity of such a cluster by phosphorylation would allow the activity of HSF to be varied rapidly and continuously according to the physiological need of the cell. This hypothesis is supported by the observation that progressive increases in both HSF activity and phosphorylation occur in parallel over a range of normal and heat shock temperatures. By mutational analyses of the cloned HSF gene it should be possible to identify amino acid sequences responsible for transcriptional activation and test the prediction of such a model (Fig. 19).

Possible Roles of Hsps

The heat shock response has been intensively studied and much is known about the organisation and regulation of heat shock genes but there is still less information about the function of hsps.

One polypeptide which has been identified as a hsp, at least in animal cells (Bond and Schlesinger, 1985), is the small protein ubiquitin. So called because its almost universal occurrence in eukaryotic tissues, it functions in ATP dependent degradation of proteins. Based on the findings that ubiquitin is a hsp, Munro and Pelham (1985) have formulated a hypothesis (as yet unproven) for the induction of heat shock response by accumulation of proteins damaged by heat or other stresses. Ubiquitin has not yet been shown to be a hsp in plants.
Different groups of hsps may have different functional properties, which together contribute to the thermotolerant phenotype. Each group encompasses members of at least one multigene family encoding hsps with similar molecular masses, amino acid homology and with common biochemical properties. Thus mutational changes of a single hsp may have very little, if any, phenotypic consequences because its deficiency may be functionally compensated for by another hsp from the same family. Only mutation in the regulation have significant effect on the viability and growth of cells after heat stress as evidenced in E. coli, in which mutations block the expression of all or one group of small hsps.

On the basis of such evidence, Pelham (1986) suggested a general model that hsp 70 binds to hydrophobic regions of the proteins, either naturally exposed or accessible as a result of denaturation, that binding can be reversed with the aid of ATP hydrolysis, and that it results in prevention or disruption of inappropriate protein-protein interaction.

In experiments in which the translation products of poly(A) RNA obtained from heat shocked pea plants were transported into isolated intact chloroplasts, it was found that only chloroplasts isolated from heat shocked plants integrated the hsp 22 into the thylakoid. The ability of such chloroplasts to integrate hsps into the thylakoids required a pretreatment of minimum of 15 min at 38°C of the pea plants before isolation of chloroplasts. In other experiments it has been observed that hsp 22 and 29 are associated
with the granna lamellae fraction of Chlamydomonas thylakoids. It is demonstrated that heat shock inactivates the PSII reaction center in a light dependent process which induces aggregation of the thylakoid proteins as well. Inhibition of nuclear coded hsp's during heat shock drastically enhanced the light dependent inactivation of PSII and the thylakoid protein aggregation process. The result supports the hypothesis that chloroplasts hsp's play a protective role against light induced damage to the photosynthetic apparatus during heat shock (Schuster et al., 1988).

Newly synthesized cytosolic precursors of mitochondrial proteins have to maintain an unfolded conformation to be competent for membrane translocation. This is achieved by interaction with hsp's of relative molecular mass (70 kDa) in the cytosol and by the action of other factors which might include ATP-dependent 'unfolding enzymes.' Precursors traverse the mitochondrial membranes in an extended conformation at translocation contact sites between the outer and inner membranes. Amino-terminal presequences are cleaved by a matrix-localized, metal dependent processing enzyme. The mechanisms underlying the folding and assembly of proteins imported into proteins are of importance. The recently identified stress protein, hsp 60, is the first mitochondrial component known to be essential in this process. Hsp 60, is a nuclear coded, constitutively expressed hsp residing in the mitochondrial matrix. The consequences of loss of hsp 60 function have been analysed in a temperature sensitive lethal yeast mutant defective in the gene coding for hsp 60. Mutant cells are deficient in the
assembly of several mitochondrial proteins of the matrix, inner membrane and intermembrane space. The mitochondrial hsp-60 functions in the folding of proteins imported into mitochondria. Folding occurs at the surface of hsp-60 in an ATP-mediated reaction, followed by release of the bound polypeptides. Hsp-60 catalyzes protein folding (Ostermann et al., 1989).

Exceptions to the Heat Shock Response In higher plants, the most intensively studied exception to the standard response is that shown by pollen. Germinating maize pollen fails to synthesise any of the normal hsps in contrast to all other maize tissue (Cooper et al., 1984), though two novel polypeptides are produced in response to a heat shock. In growing pollen tubes of Tradescantia despite the fact that a prior exposure to gradually increasing temperature protects against otherwise injurious exposure to 41°C, there is no concomitant synthesis of hsps (Xiao and Mascarenhans, 1985). Since, this study also failed to demonstrate the presence of preformed hsps in pollen grains, the mechanism of induction of thermotolerance in pollen remains obscure but appears not to depend upon heat shock response.