DISCUSSION
DISCUSSION

Rice is one of the most important cereal crop plant, the world over. The growth requirements of this plant are different than other economically important cereal plants. The climatic requirements of this crop ranges from sub-tropical to tropical. Plants are often submerged in water, therefore, it is thought that they might have evolved a different adaptability in terms of coping up with various environmental stresses including heat shock response. This expectation was reasonable since, during bright sun, the temperature of water would rise to a considerable extent, and possibly would remain so for longer period of time. Depending on the changes in weather conditions, temperature would fluctuate up and down frequently.

The synthesis of proteins has been used, as an indicator of heat shock response in this study, and the incorporation of $^{35}$S-methionine as a criterion for the proteins being synthesized. The incorporation of methionine, of course, has a limitation in the sense it will monitor the synthesis of only those proteins which contain methionine residues. It is known from the literature that heat shock proteins contain many methionine residues and only very few hsps such as hsp 26 of yeast are low in methionine content (Bossier et al., 1989). Since most of the proteins do contain methionine amino acid, this label has been used extensively to monitor protein synthesis. The results of this study indicate that the high temperature inhibits the quantum of proteins synthesized. The maximum
protein synthesis is observed at 30°C and both low and high temperature reduce the protein synthesis considerably. Therefore as indicated earlier 30°C has been taken as a normal growth temperature for rice plant.

For standardizing the various optimal conditions for incorporation of 35S-methionine into the newly synthesized proteins a detailed temporal kinetics was worked out. It is reasonable to assume on the basis of transcriptional activation of the heat shock genes, before hsps are synthesized, certain time has to elapse to allow the synthesis mRNA. Therefore, the optimal kinetic parameters were worked out. This showed that a 30 minutes to 1 hr preincubation followed by incubation in the presence of radiolabel produced the optimal heat shock response.

**Temperature and Pattern of Proteins synthesized**

Four temperature regimes (30, 35, 40, and 45°C) were used to study the protein synthesis. The overall protein synthesis declines in absolute terms at temperature above 30°C. Similar results have also been obtained with variety of other plants and tissues (Kishore and Upadhyaya, 1988; Kimpel and Key, 1985). Low temperature also affects the protein synthesis. The proteins synthesized were initially analyzed on single dimensional SDS-PAGE. The pattern of proteins synthesis is not same at 35, 40 and 45°C, and in all these cases the pattern is entirely different than that obtained at normal temperature (30°C). The analysis of pattern especially obtained at 40°C reveals that 15 proteins, called the hsps are synthesized. One can
arbitrarily classify these proteins on the basis of their molecular weight into
three categories. (i) high molecular weight consisting of hsps (60 kDa and
above) (ii) intermediate molecular weight range comprising of proteins
having molecular weight of 30 to 60 kDa, and (iii) a category of low molecular
weight class containing the proteins below 30 kDa. As it has been seen
earlier and will be discussed later that since plants synthesize a battery of
low molecular weight proteins, some authors have sub-classified the low
molecular weight class into three families of hsps, namely 15-18 kDa, 21-24
kDa and 26-28 kDa (Nagao and Key, 1989).

To resolve wide spectrum of hsps ranging from molecular weight of 15
to 84 kDa the 10-20% gradient SDS-PAGE gel was used. In this kind of gel
no proteins could be resolved below 14 kDa and it was difficult to visualize
any protein above 90 kDa. An apparent conclusion from the pattern of
proteins synthesized at heat shock temperature is that of many of the
proteins which were synthesized at 30°C shows either complete or parial
inhibition of their synthesis. Out of 12-15 hsps which can be resolved on
SDS-PAGE, 84, 81, 70 and 60 kDa belong to the high molecular weight class,
4 hsps, 50, 43, 41 and 35 kDa belong to the intermediate molecular weight
class, and 4 hsps namely 25, 21.5, 18, and 15 kDa belong to the low
molecular weight class. The general pattern of proteins observed in rice
shows that 84, 81, 70 and 60 kDa are prominent hsps in the high molecular
weight range. The low molecular weight class in rice contains several hsps.
Thus the roots of rice seedlings synthesize hsps ranging in molecular weight

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from 15-84 kDa when they are grown at 10°C above their normal growth temperature. The general pattern of hsp5 in rice is comparable with that of the other plant species (Sachs and Ho, 1986). Though the size distribution of hsp5s in rice is not identical with that of soybean and maize (Key, 1981; Cooper and Ho, 1983) and pigeon pea (Kishore and Upadhyaya, 1988). The 70 kDa protein which appears in both plants and animals (Burdon, 1986) is also present in rice. Another 60 kDa hsp also appears to be most prominent one in the case of rice plant. The most important hsp5s with respect to plant species are those belonging to the low molecular weight class (Sachs and Ho, 1986). In fact all plant species studied so far synthesize low molecular weight hsp5s namely 15, 18, 20, 22, 24, and 27 kDa (Mansfield and Key, 1987; Vierling et al., 1989). Presence of these low molecular weight hsp5s possibly distinguish the heat shock response from animal to plant systems. In mammalian system and in Drosophila, a limited number of hsp5s are observed, whereas in plants a large number of hsp5s belonging to this class are synthesized. Some of the plant hsp5s of this class, after their synthesis, are transported into chloroplasts especially those ranging in molecular weight from 21-27 kDa (Vierling et al., 1986; 1988; 1989). Fourre and Lhoest, 1989 studied the hsr in rice calli derived from seed embryos. They observed a differential pattern of synthesis of hsp5s when temperature is raised abruptly or gradually. They found only few prominent hsp5s including hsp 70, hsp 89 and some in the low molecular weight range. They could also detect a 33 kDa hsp when the temperature was gradually raised to 45°C. In addition, they observed that hsp 89 and hsp 70 are modified by methylation.
A comparison of pattern of proteins observed in rice roots in the present study with the pattern observed in rice calli [Fourre and Lhoest, 1989] appears to be different. The differences in the pattern of protein synthesis observed between roots (this study) and callus might reflect the differences in the tissue or the stage of cellular differentiation.

The results of kinetic studies indicate that rice plant responds very rapidly in terms of onset of synthesis of certain hsps. Some of the hsps start appearing as early as 30 minutes to an hr after the temperature is raised to 40°C. A similar rapid response has been observed in a variety of other plant species, such as maize (Cooper and Ho; 1983); barley, rye and triticale (Necchi et al., 1987); pigeon pea (Kishore and Upadhyaya, 1988). The rapidity of the response indicates (i) since the synthesis of hsps involves the transcriptional of the hsp genes, the transcription activation of these genes must be a very rapid process, and (ii) the molecules possibly trans-acting signals involved in the regulatory cascade might already be present to perceive the signals transduced by high temperature.

The synthesis of these hsps indicates that their synthesis is transient. Since hsps can not be visualised either by Commassie blue or very sensitive silver staining which indicates that hsps do not accumulate and their synthesis is transient. Moreover a prolonged heat shock treatment leads to the inhibition of many of the hsps. Besides eukaryotes, even the production of hsps in E. coli is transient. The decrease in the synthesis of hsps appears to occur as a result of enhanced accumulation of a particular hsp, for
example, dnaK protein during heat shock (Craig, 1985; Neidhart et al., 1984). In soybean seedlings, hs mRNA accumulates maximally within few hr of heat shock and then the level declines gradually (Key et al., 1987a). These results indicate the possibility of existence of a system of auto-regulation and the system perhaps becomes operational in terms of protein degradation after few hr. The system of auto-regulation appears to introduce another level of complexity in regulation of heat shock response. First the system perceives the signal in bringing about the transcriptional activation of the hs genes, then after the synthesis of hs mRNAs and hsps, the degradative pathway takes over resulting in gradual depletion of both hs mRNAs and hsps. The prolonged heat shock somehow turns on the perceptibility of the system to temperature signals.

**Translational Inhibitors of synthesis of hsps**

In the present study chloramphenicol inhibits the synthesis of several proteins namely 60, 50, 21.5, and 18 kDa in rice, possibly indicating their synthesis by organellar protein synthesizing machinery. Since the hsps have been studied from root tissues they should either be associated with mitochondria or to some extent to protoplastids. Another possibility exists that they may not be from rice tissue but originate from bacteria closely associated with roots, even though precautions were taken to avoid bacterial contamination. In fact, Sinibaldi and Turpen, (1985) observed a 60 kDa polypeptide in maize seedlings which appears to be synthesized by mitochondria. Similar sized hsp has also been seen in both coleoptiles and
roots of certain cereals analyzed by Necchi et al., 1987. Subsequently certain doubts were raised whether this band was in fact of organellar origin or a consequence of bacterial contamination. Nieto-Setello et al. (1988) examined closely the possibility of hsp synthesis by mitochondria and chloroplasts and conclude that prominent band observed in 60 kDa range present in certain studies may be of bacterial origin and no in organello hsps are synthesized.

The presence of low molecular weight hsps in mitochondria isolated from pigeon pea heat shock tissue indicated the possibility that nuclear coded, cytosol translated proteins might be transported to mitochondria (Kishore, 1989). Such an association of hsps with mitochondria has been shown in the case of soybean at elevated temperature (Chou et al., 1989). Certain nuclear coded hsps belonging to low molecular weight class have also been shown to be transported into chloroplasts. Major chloroplasts associated hsps have been identified in soybean, *Glycine max* (Vierling et al., 1986); pea, *Pisum sativum* [Kloppstech et al., 1986; Vierling et al., 1985]; maize, *Zea mays* (Vierling et al., 1986), bean, *Phaseolus vulgaris* (Suss and Yordunov, 1986), petunia, *Petunia hybrida* (Vierling, 1986), tobacco, *Nicotiana tobaccum* (Kanabus et al., 1984) and *Chlamydomonas sp* (Kloppstech et al., 1985). The hsp 22 and hsp 29 after their transport into chloroplasts are associated with granna lamellae of *Chlamydomonas*. It is also demonstrated that heat shock inactivates the PS II (Photosystem II) reaction centre in a light dependent process which induces aggregation of the thylakoid proteins as well, and induction of nuclear coded hsps during
heat shock drastically accelerates the light dependent PS II and thylakoid protein aggregation (Schuster et al., 1988). On the basis of these, Schuster et al. (1988) have proposed the hypothesis that chloroplast-associated hsp s play a protective role against induced damage to the photosynthetic apparatus during heat shock. On the other hand Vierling et al. (1989) have suggested that these proteins have more general protective role.

The chloroplast hsps have been shown to be synthesized as precursor polypeptides which are processed to their mature molecular weight upon import to chloroplast (Vierling et al., 1988). The chloroplast-localized hsps show certain degree of conservation in carboxy-terminal domain from three divergent plant species, Arabidopsis, petunia, and maize (Vierling et al., 1989). DNA sequence analysis of chloroplast hsp cDNA from pea and soybean has shown that the carboxy-terminal regions of hsp 21 are homologous to similar hsps in the wide range of plant species (Vierling et al., 1988). It has further been shown that the antibodies raised against carboxy-terminal region of these hsps from pea recognize hsp precursor proteins from three divergent plant species, Arabidopsis, petunia, and maize (Vierling et al., 1989). However, antibodies raised against amino-terminal region are species-specific and primary translation products of these hsps are larger than the mature form of hsps. The precursor form contains a transit peptide when these proteins are transported into chloroplasts, which is removed during import into chloroplasts (Vierling et al., 1986; Vierling et al., 1988).
There is no other report confirming the observations of Krishnasamy et al. (1988) that certain hsps are synthesized within the chloroplasts.

**Analysis of hsps by 2D-gel electrophoresis**

The analysis of proteins by SDS-PAGE has certain limitations. Certain protein bands might possibly represent several different proteins of similar molecular weight. Apparently the resolving power of the SDS-PAGE is limited, as we have seen only 15 hsps could be resolved on the basis of molecular weight. The separation of proteins by 2-dimensional gel electrophoresis depend not only on the molecular weight but also on their overall charge. And these charges apparently depend on the amino acid composition of polypeptides, presence or absence of detergents and pH of the solution. Nonequilibrium pH gradient gel electrophoresis (NEpHGE) is an alternate procedure for the first dimensional separation which unlike isoelectric focussing, resolves both basic as well as acidic proteins. The method is applied to the resolution of total cellular proteins with ampholytes having the pH range from pH 3.5-10. Second dimensional separation as already indicated depends on the molecular weight. The versatility of the system can easily be assessed by the power of its resolution, since over 100 polypeptides can easily be seen in a good fluorograph, whereas on SDS-PAGE only a limited number of proteins can be visualized. The results presented in the this study indicate that on SDS-PAGE only 15 hsps can be seen. However, on a typical 2-D gel approximately 44 hsps can be seen in rice. Out of these 44 hsps 11 belong to the high, 18 belong to the
intermediate and 15 belong to the low molecular weight class. It is very apparent from the results that maximum number of proteins are seen in the acidic range.

In tomato cell suspension cultures approximately 48 hsps are synthesized (Nover and Scharf, 1984). One of the striking feature of heat shock response in plants is the synthesis of multitude of low molecular weight hsps. In rice approximately 15 hsps (LMW) are synthesized. Mansfield and Key, 1987 have analysed the synthesis of low molecular weight hsps in various species. A representative picture is given in the (Table 15). It should be emphasized that in 2-D gel fluorograms, certain number of spots could be slightly variable. The number of low molecular weight hsps reported by Mansfield and Key, 1987 for rice is in good agreement with the results of the present study. The diversity of low molecular weight hsps in plants could be attributed at least in part to variation in the members of the multigene family (Schoffl and Key, 1985; Nagao et al., 1985).

As it has been discussed earlier that a multiplicity of low molecular weight hsps appears to be unique to the plant cells. Though some of the similar hsps have been observed in *Drosophila* and yeast. The amino acid composition of three different, 18 kDa soybean hsps show over 90% homology, but when they are compared with 20 and 22 kDa hsps of *Drosophila*, the overall homology is less than 40%. However, hydropathy plots indicate four major regions of strong similarity (Kimpel and Key, 1984). In the most hydrophobic domain, there is a striking conservation of
Table 15. *Low molecular weight hsp synthesis by various plant species*

<table>
<thead>
<tr>
<th>Species</th>
<th>No of low mol. wt. hsp</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean</td>
<td>21</td>
<td>Mansfield and Key 1987</td>
</tr>
<tr>
<td>Pea</td>
<td>13</td>
<td>&quot;</td>
</tr>
<tr>
<td>Sunflower</td>
<td>19</td>
<td>&quot;</td>
</tr>
<tr>
<td>Wheat</td>
<td>11</td>
<td>&quot;</td>
</tr>
<tr>
<td>Maize</td>
<td>23</td>
<td>&quot;</td>
</tr>
<tr>
<td>Millet</td>
<td>16</td>
<td>&quot;</td>
</tr>
<tr>
<td>Pennisetum americanum</td>
<td>21</td>
<td>&quot;</td>
</tr>
<tr>
<td>Rice</td>
<td>13</td>
<td>&quot;</td>
</tr>
<tr>
<td>Pigein pea</td>
<td>19</td>
<td>Kishore, R, 1989</td>
</tr>
<tr>
<td>Rice</td>
<td>15</td>
<td>Present study</td>
</tr>
</tbody>
</table>
sequence gly-val-leu-thr. This hydrophobic tetrapeptide is also found in hsps 26 of yeast (Bossier et al., 1989). It has earlier been observed that small hsps and -crystallins, the water soluble structural component of the vertebrate eye lens shows a strong homology (Ignolia and Craig, 1982; de Jonet et al., 1988). -crystallins which are also low molecular weight proteins form large aggregates in the cells of lens. Perhaps the low molecular weight hsps also form large aggregates that perform a structural role in stabilizing various cellular components. In plants and Drosophila, there is evidence for such aggregation of hsps. In Drosophila a non-nuclear structure comprising heat shock polypeptides assembles and copurify with nuclear fractions during heat shock. In tomato cell culture over 80% hsps become associated with cytoplasmic heat shock grnules (Nover and Scharf, 1984). Thus, at least one function of low molecular weight hsps could be structural. These large aggregates in the cell may serve as transient cellular matrices in which various cell organelles and components are held and thereby stabilized. Such matrices would be maintained only during heat shock; once cell temperature is dropped back to normal these aggregates dissociate, allowing rapid restoration of normal cellular functions.

It has been observed in an earlier study (Necchi et al., 1987) that individual hsps associated with specific organelles during heat shock. The hsp 70 of Drosophila concentrate in nucleus during heat shock and upon recovery from heat shock, this protein leaves nucleus during the second heat shock (Velasquez and Lindquist, 1984). The association of hsps with
different cellular components suggests a possible role of hsps in the altered membrane functions that usually occurs at elevated temperature.

**PROTEINS SYNTHESIZED IN RESPONSE TO ARSANATE, DINITROPHENOL AND SALICYLATE**

The treatment of root tissues of rice with arsanate, salicylate and 2,4-dinitrophenol at 30°C results in the synthesis of several new polypeptides as compared with the proteins synthesised at 30°C. Out of the new polypeptides synthesised in all the three treatments, some of them appear to be similar to the hsps while others appear different. There are reports in literature that these chemicals mimic the effect of heat shock and bring about the heat shock response both in animals as well as in plants (Ashburner and Bonner, 1979; Czarnecka et al., 1984).

The tight coupling of electron transport and phosphorylation is disrupted by 2,4-dinitrophenol (DNP). DNP carries protons across the inner mitochondrial membrane. In the presence of DNP (uncoupler of oxidative phosphorylation) electron transport from NADH to O₂ proceeds normally, but ATP is not formed by the mitochondrial ATPase because the proton motive force across the inner mitochondrial membrane is dissipated. The loss of respiratory control leads to increased oxygen consumption and oxidation of NADH. When oxidative phosphorylation is uncoupled, the oxidation reactions of the respiratory electron transport chain continue at their maximum rate, releasing energy in the form of heat rather than in the
form of ATP. The basic question is whether these endogenous heat signals generated by short circuiting the mitochondrial proton pathway, can mimic the effect of exogenous temperature signals in bringing about the heat shock response.

Salicylate and DNP have a very similar chemical structure as shown in (Fig 20). However, salicylates are not known to induce any endogenous temperature signals. Arsanate closely resembles the Pi in structure and reactivity. In the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase, arsenate can replace phosphate in attacking the energy rich thioester intermediates. The glycolysis proceeds in the presence of arsanate but that ATP normally formed in the conversion of 1,3-bisphosphoglycerate into 3-phosphoglycerate is lost. Thus, arsanate uncouples oxidation and phosphorylation by forming a highly labile acylarsanate.

As discussed earlier, since DNP generates endogenous temperature signals, it was conjectured that these signals can be perceived by the heat shock induction cascade in bringing about the transcriptional activation of certain heat shock genes. Since a similar kind of response is also induced by salicylate and arsanate it is not certain whether the above postulate may hold true. The other possibility is that DNP and salicylate have a similar benzene ring in their structure, which somehow, might be responsible for the inducibility of certain hsp's. Arsenate on the other hand has an entirely different chemical structure as compared with salicylate and DNP but acts as an uncoupler of oxidative phosphorylation. Considering the dissimilarities in
Fig. 20  Structural formulae of DNP and Salicylate.
their chemical structures and in their biological functions, it is very difficult to make a unifying hypothesis for these compounds in bringing about the induction of certain hsps.

**Analysis of 2D-gels of DNP, Salicylate and Arsenate treatment**

In general, studies indicate that majority of the hsps are synthesized in response to dinitriphenol, salicylate and arsenate. As discussed earlier a total of 44 hsps are synthesized at 40°C. Out of these, it appears that 2 hsps are not synthesized in the DNP treatment, one hsp is not synthesized in the salicylate treatment and 15 hsps are not synthesized in the arsenate treatment. About 9 hsps are found to be strongly induced in the salicylate treatment, approximately 7 hsps are found to be strongly induced in the DNP treatment and none of the hsp is found to be strongly induced in arsenate treatment.

It is apparent from the results of the chemical treatments that certain polypeptides are specifically induced by these chemical treatments. The chemical treatments induce the synthesis of several sets of polypeptides (i) unique to the particular chemical (ii) polypeptides whose synthesis is interdependent on heat shock as well as on the three chemicals discussed, (iii) there is a certain degree of commonality of polypeptides induced by any two of the three chemicals taken together, and (iv) the polypeptides which are not induced by heat shock signals but are induced by all three chemical treatments.
The results indicate that the synthesis of all the hspS is not brought about by the chemical treatments. This suggests the possibility that hsp genes may fall into two categories: (i) which respond to both the chemicals and heat shock and (ii) which exclusively respond heat shock. It still remains to be determined whether the heat shock element responsible for transcriptional activation of the heat shock genes also mediate the inducibility of these genes by these chemicals. It is also possible that these genes might have another regulatory element, in addition to HSE specific for chemicals in their 5'-upstream regulatory regions. Alternatively it can be argued that regulation of these genes might be exercised at the level of HSE binding proteins.

**Possible Mechanism of Activation of hsp genes**

Heat shock results in dramatic increase in the transcription of the hsp genes. A considerable progress has been made towards understanding molecular basis of the transcription of these genes. A number of hsp genes including those of plants have been cloned and sequenced. A comparison of 5'-upstream sequences of hsp genes of all the organisms studied so far reveals a short highly conserved sequence termed heat shock element (Pelham, 1985). The heat shock element has been shown to be required for thermal inducibility (Slatter et al., Craig, 1981). The heat shock element (HSE) sequence containing 10 symmetrical bases, CT- GAA--TTC-AG, represents the binding site for heat shock transcription factor (HSTF)
(Pelham, 1985). This motif has been found in the hsp genes of various other organisms e.g. yeast, slime mold, amphibians, mammals and plants (Lindquist, 1986). The functional relevance of HSEs in plants is indicated by faithful transcriptional regulation of the hsp genes in the heterologous plant cells (Baumann et al., 1987; Spena and Schell, 1987). It has been shown that Drosophila promoter functions well and confers thermoinducibility on a reporter gene (Spena et al., 1985; Spena and Schell, 1987). Recently it has been shown that 2-5 copies of soybean HSE consensus elements in the form of synthetic nucleotides are sufficient to direct the heat shock inducible gene expression from an otherwise silent promoter core element (Schoff et al., 1989).

A transcriptional factor has been identified that binds specifically to HSE. This protein termed HSTF, was originally purified from Drosophila cells [Parker and Topol, 1984] and was shown to be required for in vitro/in vivo transcription of Drosophila hsp 70 gene. The yeast HSE binding protein and Drosophila HSTF bind to each other's HSEs, approximately with equal affinity, as detected by using monoclonal antibodies prepared against 70 kDa HSE binding protein purified from yeast. The yeast HSTF genes has been isolated from a Saccharomyces cerevisiae expression library in gt11 (Wiederrecht et al., 1988). Sorger and Pelham, (1988) find that HSTF is essential for viability of yeast cells at temperature between 18 and 300C and is involved in transcription even in unstressed cells. It seems likely that the function of HSTF at normal temperature is to promote transcription perhaps
in combination with other factors of those hsp genes that are expressed at the basal level. The activity of synthetic promoter containing HSTF binding site increases more than two fold when cells are heat stressed. This change in activity of HSTF is accompanied multiple changes in phosphorylation states. Sorger and Pelham, (1988) proposed that expression of hsp genes in yeast is modulated by phosphorylation of DNA-bound HSTF and this leads to a more efficient interaction of the factors with other components of the transcriptional machinery. It has been shown that HSTF interacts especially to the heat shock element sequences, and this factor (HSTF) appears to have a role in transcriptional activation of hsp genes. However, as discussed earlier, the HSTF is present both in normal and heat shocked tissues, and only difference between these was phosphorylation of this factor (Sorger and Pelham, 1988). It is not clear how does heat shock induce phosphorylation in this particular protein. It is possible that alteration in the HSTF might be achieved through denaturation or damage by agents which induce hsp synthesis. A proposal has been made that intracellular accumulation of abnormal damaged proteins may trigger hsp genes transcription (Findley et al., 1984). Normally abnormal proteins are degraded within the eukaryotic cells by an ubiquitin dependent degradation system (Hershko et al., 1982). They are attached by N-terminal amino group to ubiquitin, a 76 amino acid protein through a peptide bond, and becomes substrate for proteolysis. Thus it may be that heat shock treatment results in increase in abnormal proteins requiring ubiquitination and this may lead to a transient shortage of ubiquitin (Burdon, 1986). However, there is a temperature sensitive mutant of mouse
cell line in which no ubiquitination is possible at non-permissive temperature, yet hsp60 are synthesized (Findley et al., 1984). This observation is difficult to reconcile with the preposition that transcription of hsp genes is a consequence of insufficient amount of ubiquitin.

In all the cases investigated so far, the HSTF preexists under nonstress conditions but is activated by hs or chemical stressors. The native HSTF of Drosophila and yeast are homotrimers. The activation by hs is associated with phosphorylation as shown in Drosophila, yeast and mammals (Fig. 19). Interestingly, the in vitro activation of the HSTF from HeLa cells can be brought about not only by hs but also by low pH, Nonidet or urea, and this transformation of the HSTF is inhibited by glycerol. These results suggest that changes of protein conformation is involved. South-Western screening of a tomato cDNA library in gt11 led to the identification of three potential HSTF genes (Scharf et al., 1990). They all share a putative DNA binding domain of ~90 amino acid residues which is very similar to the corresponding region of yeast HSTF.

It is tempting to speculate that the hs response initially involves activation of a constitutive factor such as HSTF8 to bind to HSE as shown by gel retardation assays. Under continued stress conditions newly formed factors, e.g. HSTF24 and HSTF30, may be required to assist or respectively replace HSTF8 in its activity to facilitate hs gene transcription. A homooligomeric form of HS TF8 may be replaced by heterooligomeric forms involving HSTF8, 24 and/or 30. Crosslinking of the DNP complexes and
Fig. 19  A generally accepted model for the transcriptional activation of the heat shock genes.
careful footprint experiments with different hs promoter will reveal the supposed fine tuning of hs transcription. It may be that changes in the composition of the 'heat stress transcriptosome' by interaction of with different HSTF components are involved in the well known differential response of individual genes with respect to the type of inducer, to the temperature threshold and the time course of expression (Scharf et al., 1990).