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Heat stress proteins and transcription factors

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I. Historical perspective

Two discoveries triggered the rapid growth of interest in molecular biological studies in the area of the heat stress response: (i) the finding by F. Ritossa [1] in 1962 of a new pattern of gene activity in polytene chromosomes of *Drosophila* salivary glands, and (ii) the first description of heat stress-inducible proteins (HSPs) by A. Tissieres and his group in 1974 [2]. A number of important books and reviews can be consulted to reconstruct the remarkable development of the field in the following 20 years (see refs 3–57).

In view of the complexity of the hs response, with major features conserved between bacteria, plants, insects and vertebrates, and the central role of members of the HSP families in a constantly increasing number of cellular activities, it is worth recalling the historical roots of experimental work in this field going back to the middle of the last century [37]:

In 1864, Julius Sachs [58] reported on an extended series of experiments defining the upper temperature limits of plant growth using a specially designed heat stress chamber for whole plants. The broad interest of plant physiologists in this topic has continued up to the present time and has provided insights into developmental, hormonal, circadian and seasonal influences on the intrinsic and inducible heat resistance of plants [59–65].

Another important root of hs research goes back to the first publication in 1866 by the German physician W. Busch [66] on the spontaneous regression of a skin tumour after local infection with *Streptococcus erysipelatis*. Following this, W. B. Coley [67] reported in 1893 on 47 cases of treatment of malignant surface tumours by *Streptococcus* infections or by injection of bacterial extracts (Coley's toxin). The curative effect is evidently due to hyperthermic damage to the tumour tissue and a local stimulation of the immune system. Hyperthermic treatment of cancer as well as investigations on the basis of cell death under heat stress conditions and survival of tumour cells due to induced thermotolerance became a major part of research in this field and was particularly stimulated after the discovery of induced HSP synthesis [68–72].

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Heat stress-induced developmental defects were first described by F. M. Alsop in 1919 [73]. But it was Richard Goldschmidt who elaborated the basis for a developmental genetics in his report in 1935 [74] on the hs-induction of phenocopies of *Drosophila* developmental mutants. This enormous work was based on the analysis of about 500,000 individuals. His experimental techniques were later extended by N. Petersen and H. K. Mitchell [75] to *Drosophila*, and J. German [76], summarizing numerous observations in vertebrates, put forward a hypothesis of embryonic stress resulting in formation of abnormal organ anlagen.

The brief outline of the early experimental results may help to understand the remarkable velocity and broad scope of scientific development initiated by the discovery of hs-inducible genes and the corresponding proteins [1, 2]. HSPs and the transcription factors regulating their expression (HSFs) will be the focus of this review.

II. Heat stress proteins

Survey of heat proteins as part of interacting chaperone systems

The first description of heat stress-induced proteins (HSP) in *Drosophila* [2] initiated a remarkable era of research on similar proteins in all types of organisms and on their function in stress tolerance. This led to the characterization of a rapidly increasing number of isoforms as members of, at present, 11 HSP families, structurally and functionally conserved between prokaryotes and eukaryotes. With the ongoing analysis of the whole system, more and more data on important minor HSP families emerged, and their number will certainly continue to increase. It became apparent that in eukaryotic cells the endomembrane systems [endoplasmic reticulum (ER), mitochondria, chloroplasts] harbour their own sets of proteins related to the HSP families. Due to the additional compartment in plants (chloroplasts), the complexity of the HSP families is particularly high in this group of organisms [20, 51, 77].

There is no doubt that the dramatic increase in our knowledge in this field is intimately connected with the intriguing observation that members of the HSP families act as key mediators of protein folding and protein topogenesis (molecular chaperones). The term was originally coined by Laskey et al. [78] for the role of an acidic nuclear protein (nucleoplasmin) in

nucleosome assembly. The highly suggestive concept, further developed by R. J. Ellis [13–15], has been extended to include RNA or RNPs as chaperones, e.g. of spliceosome assembly, or nuclear proteins (HMG1/2, HMG17, NAP-1, GAGA factor, SWI/SWF complex) involved in chromatin assembly and remodelling [26, 79–81]. Within the framework of this review, we will concentrate on the molecular chaperones sensu stricto, i.e. on those belonging to one of the HSP families.

Before giving a brief description and general functional characterization of the individual families, three levels of complexity are worth consideration: (i) The HSP100, HSP90, HSP70 and HSP60 systems are composed of different types of subunits with distinct functions for the whole system (chaperone machines; see details given below). (ii) Proteins of the HSP100, HSP60 and HSP20 families form large multimeric structures of 200–900 kDa. (iii) In many instances several chaperone systems interact either simultaneously or sequentially in a given situation of protein folding or organellar protein import. Three examples may serve to illustrate this type of cooperation between different proteins of the HSP families.

New synthesis of proteins. Hartl and coworkers demonstrated a sequential interaction of the HSP70 and the HSP60 systems during protein folding in *E. coli* [82–85] and provided arguments that similar arrangements may also exist in eukaryotes [86, 86a]. During ongoing protein synthesis, the newly forming polypeptides make initial contacts at the ribosomal exit tunnel with general chaperones represented by the trigger factor in *Escherichia coli* [87, 87a] and the nascent polypeptide-associated complex in mammalian cells respectively (see review by Rassow and Pfanner [44]). The subsequent steps of protein folding are evidently connected with the HSP70/HSP40 system, and the final stages of folding and assembly may require HSP60 (GroEL, TCP-1) complexes. Recently, this intricate processing pathway was enriched by yet another chaperone (HSP90). Using recombinant HSP70, HSP40 and HSP90, Freeman and Morimoto [88] demonstrated that refolding of denatured β-galactosidase is brought about by the HSP70/HSP40 complex; but HSP90 is important to stabilize the substrate in a folding-competent state.

Protein import into organelles. Basically similar, but even more complex, are the processing lines built out of four different chaperone systems which are required for protein import into organelles, e.g. into yeast mitochondria (see reviews in refs 11, 22, 50, 52). The precursor proteins in the cytosol are maintained in an import-competent, partially unfolded state by binding to the cytosolic HSP/C70 system [89]. The multisubunit import apparatus forms a tunnel through the two mitochondrial membranes for the entry of the precursor protein [34, 43]. There is evidence that the intramitochondrial HSP70 system may be involved as a force-generating system pulling the protein into the inner space. Generation of the biologically active conformation of the imported protein in the matrix needs sequential interaction with the HSP70 and HSP60 systems and evidently also the help of proteins belonging to the immunophilin family [90–92].

Activity cycle of hormone receptors. The inactive, cytosolic form of the steroid hormone receptors in vertebrates is part of a multichaperone complex (foldosome) containing two molecules of HSP90, two proteins of the immunophilin family (HSP56 and CYP40) and a small acidic protein, p23 [6, 93–95]. After hormone binding this complex undergoes extensive conformational changes liberating the receptor protein together with its hormone ligand which enters into the nucleus and binds to the promoters of hormone-responsive genes. In addition to the components given above, assembly, and probably also the ligand-triggered transformation within the 'foldosome', needs transient interaction with the HSP70/HSP40 system and two additional proteins (p60/STI1 and p48/Hip, see refs 96–99). With respect to the role of several HSPs for maintenance of the inactive cytosolic hormone receptor complex, it is an intriguing observation that hs may cause nuclear import of the unliganded receptor [100], but the same time reduce its activity as a transcriptional activator [101]. Meanwhile, similar multichaperone complexes were also identified in an in vitro assembly system for the Fes Tyr-kinase, the human HSF1 and for the AH receptor [101a].

The HSP100 family

The contours of this new family of stress proteins became apparent when the bacterial large subunits of the ClpP protease system and the yeast HSP104 were sequenced and functionally analysed [102–105]. The HSP100 proteins are characterized by two conserved boxes each of about 200 amino acid residues harbouring an adenosine triphosphate (ATP) binding site. The size of the proteins varies between 78 and 100 kDa according to the size of nonconserved spacers between the two boxes and additional C- and N-terminal sequences. In yeast and plants, there is evidence for the existence of cytosolic as well as organellar forms of the HSP100 proteins [103, 106–110].

Proteins of the HSP100 family have chaperone activity and in some cases were shown to replace the HSP70/HSP40 system [110–112]. However, experiments by Parsell et al. [113] demonstrate that there may be an interesting peculiarity. Unlike other chaperones, the yeast HSP104 is able to resolubilize heat-inactivated luciferase, but it has no influence on heat denaturation. These results are in contrast to those of Wickner et al. [112] using the same reporter system (luciferase) and the bacterial ClpA protein as chaperone. The latter is normally part of a high molecular weight protease machine. The 84-kDa ClpA protein forms a hexamer with 12

molecules of ATP bound to it. This complex represents the substrate recognition and unfolding part of the protease machine together with a 12-mer of the small proteolytic subunit (ClpP). Evidently, the ClpA/X hexamers can function as chaperones independently of the ClpP subunit [112, 114]. This is also true for the hs-inducible ClpB form of *E. coli* and for the eukaryotic homologues where a protease subunit of the ClpP type was never found [109, 115].

Extensive genetic studies in yeast demonstrated (i) that HSP104 is an essential component for recovery after different stress treatments (hs, ethanol, arsenite but not heavy metals), (ii) that it is crucial for the high intrinsic heat resistance of spores and stationary phase cells, and (iii) that it can be replaced in this function by the *Arabidopsis* HSP101 [109, 115, 116]. Particularly interesting and intriguing is the role of HSP104 in creating the active yeast psi factor, evidently a prion-type self-modifying particle with a function as omnipotent nonsense suppressor protein [117].

The HSP90 system

This family of heat stress-inducible proteins was established by sequence comparison of representatives from *E. coli* (HtpG), *Drosophila* (HSP83), yeast (HSP90) and of the mammalian glucose-regulated protein (GRP94) localized in the ER [118–122]. Proteins of the HSP90 family bind ATP and have autophosphorylating activity [123]. They interact with the actin and tubulin cytoskeletal systems in a Ca^{2+} calmodulin-dependent process, and they were shown to have chaperone activity in vitro [124].

Despite its high abundance in the cytosol of all organisms, the function of these types of proteins remained unclear for several years. An early observation of a role of HSP90 and a 50 kDa phosphoprotein p50 for the $pp60^{v\text{-}src}$ retroviral tyrosine kinase maturation pathway [125] was much later confirmed by reconstitution in vitro [126] and functional testing in yeast [98, 99]. Meanwhile, it is evident that the HSP90/p50 system is essential also for the function of other receptor tyrosine kinases [101a, 127, 128], of Ser-protein kinases Raf and CKII [129, 129a, 130], of signal transduction pathways dependent on trimeric G-proteins [131] and of the hepatitis B virus reverse transcriptase complex [132]. Finally, a new type of HSP90 was identified as part of a complex with the retinoblastoma (Rb) tumor suppressor protein [132a], and the activity cycle and stability of the cyclin D kinase (Cdk4) is controlled by the HSP90/p50 system. In fact, the p50 (Cdc37) may represent a type of substrate targeting subunit of the HSP90 system [127, 132b].

Other examples of characteristic HSP90-containing complexes are the inactive cytosolic forms of steroid hormone receptors (HR). Initially detected in mammalian cells [133, 134], such complexes were also found in insects (*Drosophila*, ecdysteroid receptor) and water moulds (antheridiol receptor [135]). In vertebrates, the inactive HR is bound to two molecules of HSP90 and one molecule of HSP56. Generation of this hormone competent state and its activation needs ATP and interaction with the HSP70 chaperone system [98, 136]. This interesting interaction with the HSP90 and the HSP70 systems is evidently also important for the activity control and targeting of the protein kinases mentioned above [101a, 127, 129a, 132b].

The HSP70/DnaK chaperone system

Early reports on the HSP70 family and the chaperone-like functions of its members were based on five types of evidence: (i) The sequence homology between the *E. coli* DnaK and the *Drosophila* HSP70 was established by Bardwell and Craig [136a]. (ii) Haas and Wabl [137] reported on an immunoglobulin H-chain binding protein (BiP) in the rER which was later identified as GRP78 by Munro and Pelham [138]. (iii) The clathrin-uncoating ATPase was characterized as a member of the HSP70 family [139–141]. (iv) The universal function as ATP-binding proteins was used by Welch and Feramisco [142] to affinity-purify members of the HSP70 family, and Pelham and his group [143, 144] proposed an ATP-dependent function of HSP70 as a protein shuttle between cytoplasm and nucleus. (v) Finally, and most important, the availability of HSP70-defective yeast mutants led to the first experimental evidence for a role in intracellular protein translocation [145, 146].

The number of processes involving participation of the HSP70 system is constantly increasing. Most important in the framework of this review are protein folding and topogenesis as well as the autoregulatory role of chaperones for stress gene transcription in prokaryotes and eukaryotes (see table 2). Other cellular activities influenced by members of the HSP70 family are protein degradation, reorganization of cytoskeletal systems (tubulin, intermediate filaments), translation initiation, nuclear protein import and export, ribosome assembly, protection of nucleolar structure and functions under stress, interaction with DNA-topoisomerase I and DNA synthesis (for references see 38, 39, 147).

The HSP70 chaperone system is composed of three proteins: HSP70/DnaK plus HSP40/DnaJ plus HSP35/GrpE. The HSP70-type proteins contain two well-defined parts, an N-terminal ATP-binding domain and a C-terminal domain interacting with a broad range of partially folded or denatured protein substrates. Unfolded proteins stimulate the ATPase activity of HSP/C70 [148]. As evident from the more detailed characterization of the DnaK cycle of *E. coli*, substrate interaction needs cooperation of an activated DnaK/ADP complex with DnaJ. After release of the

protein substrate, regeneration of the DnaK/ATP complex requires GrpE as a nucleotide exchange factor [85, 149–151a]. Though comparable details are lacking for eukaryotic systems, the large number of HSP/C70 isoforms characterized in different cellular compartments is increasingly complemented by HSP40/DnaJ-type proteins. Moreover, the first GrpE homologue from yeast was recently described. Functional analysis of the appropriate mutants indicates a close cooperation of all three components of the HSP70 system, at least for protein import into yeast mitochondria [152–155]. Thus, we can assume that the tripartite composition of the HSP70 chaperone system is also valid for eukaryotic systems.

Interestingly, the invariant C-terminal tetrapeptide -EEVD of all eukaryotic cytosolic members of the HSP70 family is particularly important for the overall-function of the system [156]. It is not essential for efficient interaction with unfolded substrates, but deletion or mutation of the C-terminus causes marked conformational changes which render the protein more vulnerable to trypsin cleavage, enhances ATPase activity and impairs the ability to refold denatured luciferase. It is intriguing to recall that all HSP90 proteins have the same conserved -EEVD terminus.

The DnaJ-type proteins (HSP40) are evidently the primary substrate-interaction subunits of the HSP70 machinery [83, 84, 149, 155, 157]. Members of the HSP40 family contain an N-terminal 70-amino acid motif, the so-called J-domain [12], which interacts with HSP70/DnaK. The elucidation of the solution structure of the J-domain of the *E. coli* DnaJ by nuclear magnetic resonance (NMR) techniques revealed a four-helical bundle with two long antiparallel helices (H2 and H3) in the centre. A surface loop between the two helices interacts with DnaK [158]. C-terminal to the J-domain is a conserved Cys-rich zinc-finger domain which is essential for the recognition of denatured protein substrates [159].

Recently, a new cytosolic co-chaperone of the yeast HSP70 system was identified by two-hybrid screening [96, 160]. The 41-kDA protein (Hip) is not homologous to GrpE but may associate with the cytosolic HSP70/HSP40 complexes, making them independent of a GrpE-type factor. Hip is part of the chaperone complexes with newly formed proteins and increases the efficiency of the HSP70/HSP40 complex in protein folding in vitro. A Hip-type protein is evidently also another subunit of the inactive steroid receptor complex.

The total complexity of a eukaryotic HSP70 family can be given in yeast because sequence information of the total genome is now available. Originally, eight isoforms were characterized by the pioneering work of E. Craig and co-workers [161–164, 170], i.e. the four hs-inducible proteins, SSA1–4; the two cold stress-inducible proteins, SSB1 and 2 (all six are cytosolic proteins); the mitochondrial SSC1 and the ER-localized KAR2. The list has been enlarged by three new isoforms with very low levels of expression. SSC2 and SSH1 are mitochondrial proteins, SSH1 probably being involved in DNA replication, and SSI1 represents a second ER-bound isoform (E. Craig, personal communication). In addition, a new type of HSP70 subfamily with two representatives (SSE1 and 2) was identified [165]. Members of this peculiar subfamily from mammalian cells are HSP110 [166], described many years ago by Subjeck et al. [167] as a major hs-induced nucleolar protein, an ER-bound protein GRP170 [168], the HSP70RY protein [169] and possibly also the yeast protein LHS1 in the lumen of the ER [171].

The HSP60/GroEL chaperone system

The bacterial components of this system were originally described by Georgopoulos et al. [172] and Hendrix [173] as part of a multisubunit complex (GroEL/GroES) with heptameric symmetry, ATPase activity and a catalytic function in phage head assembly. Independently of this, Barraclough and Ellis [174] identified a protein in plant chloroplasts (p60) associated with unassembled large subunits of ribulose-biphosphate carboxylase. In both cases, situations typical for chaperone activity were described without using the term. Finally, the identity of HSP60 with GroEL and the chloroplast p60 was demonstrated by sequencing [175, 176], and their function in protein folding and assembly was established [175, 177, 178].

The prototype of the HSP60 system is the *E. coli* GroEL/GroES complex. Fourteen subunits of GroEL (56 kDa) form a hollow-core structure of two heptameric rings. Seven subunits of the 10-kDa GroES attach to one site (trans) of the complex, leaving the other site (cis) open for entrance of the unfolded protein into the central cavity [84, 180]. To this asymmetric ternary complex (bullet state), a second GroES heptamer has to bind to the cis site with transient formation of the symmetrical football state [179]. ATP-dependent protein folding proceeds in the enlarged GroEL cavity sealed by the GroES heptamer. About 100 ATP molecules are probably needed for folding of a monomeric protein of 50 kDa [180–182].

Data pertaining to the crystal structure of the GroES heptamer [183] allow a better understanding of its role in the HSP60 machinery. The GroES monomers are built for a β-barrel with an attached mobile loop in the N-terminal part which is essential for interaction with the GroEL subunits. The heptamer forms a domelike flexible structure with a central 30 Å orifice on top. The highly dynamic structure has lead to speculation that the GroES heptamer might directly participate in the ATP-dependent protein-folding cycle.

GroEL/GroES-type systems were also described and functionally analysed for mitochondria and chloroplasts [175, 176, 184–187]. The chloroplast HSP60 complex is

a hetero-oligomer built of up to three forms of an α subunit and one form of β subunit [175, 188].
The prokaryotic type of HSP60 systems in the organelles of eukaryotic cells is complemented by a cytosolic counterpart. The so-called TCP-1 complex contains several structurally related subunits of 52–65 kDa. The hetro-oligomeric ringlike structure of about 970 kDa exhibits in vitro protein-folding activity which needs Mg-ATP [189]. Six different TCP subunits were identified from mouse cells [190], and disruption of genes coding for a given TCP1 subunit in yeast resulted in defective subunits which could not be rescued by another subunit [191]. Thus, the striking complexity of the TCP1 complex is evidently essential for its proper function. Characterization of mutants in yeast and studies in mammalian systems indicate that the assembly and dynamic changes of the actin and tubulin systems are critically dependent on the TCP system [189, 192–195a]. Recently, Gao et al. [195b] provided evidence for a small co-chaperone of 13 kDa modulating ATPase activity of the cytoplasmic TCP-1 complex. It is not structurally related to GroES.

The HSP20 family

Among the conserved HSP families, this is the least well understood (see the summaries in refs 8, 53 and 54). The complexity is variable, with a single representative in yeast (HSP26), 3–4 in mammals and about 20 in plants. In the latter, some of the members of the HSP20 family become mass proteins under heat stress conditions [196, 196a]. In addition to the abundant cytosolic members, plants are the only systems where representatives of this family were also characterized for all other chaperone-containing compartments, i.e. ER, chloroplasts and mitochondria [197–202a]. Sequence conservation is generally low and restricted to a C-terminal region of about 80 amino acid residues, the so-called α,B-crystallin domain, which indicates an evolutionary link between the HSP20 family and the numerous forms of vertebrate eye lens crystallins [8, 203]. In fact, α,B-crystallin is a common protein in many non-lens tissues of mammals, with enhanced expression under hs conditions [204]. Comparison of 85 HSP20 sequences by Caspers et al. [8] indicated that the α,B-crystallin domain may be composed of two hydrophobic β-sheet regions connected by a hydrophilic α-helical part.
All members of the HSP20 family, including the α,B-crystallins, form oligomers of 200–700 kDa [197, 203, 205–207] which exhibit ATP-*in*dependent chaperone activity [207–210]. They were found to prevent stress-induced protein aggregation and to improve the regeneration of proteins denatured by thermal stress. Jinn et al. [211, 212] described a remarkable stabilization of soluble soybean proteins against heat denaturation by the cytosolic complex of class I members of the HSP20 family. In addition, the mammalian HSP25 monomer has been shown to inhibit actin polymerization in vitro [210, 213–215], and it is tempting to speculate that actin may also be one of the cellular targets for HSP20 chaperone activities in vivo. A striking new aspect of HSP20 activity is the observation of Mehlen et al. [215a] that overexpression of human HSP27, α,B-crystallin or *Drosophila* HSP27 can protect mammalian cells against cell death caused by oxidative stress after treatment with tumour necrosis factor.
The stress-dependent aggregation of the 500 kDa oligomers to larger 40 nm complexes visible as electron dense material in the cytoplasm (heat stress granules, HSG) is probably a peculiarity of plants. Due to their extraordinary stability, these HSG can be purified as the main source of the cytosolic low molecular weight (lmw) HSPs of plants. They evidently represent RNP material with nontranslated housekeeping mRNA protected from degradation by an excess of lmw HSPs [196a, 215b].

Stress proteins as components of proteolytic systems

Accumulation of defective proteins in aggregated form is a consequence of many stress conditions [216, 217]. Alternatively, experimental creation of abnormal proteins was repeatedly found to trigger the hs response [218, 219 and refs in table 2]. Examples are the micro-injection of denatured proteins into *Xenopus* oocytes [218, 365, 366] or the synthesis or abnormal proteins in *E. coli* by incorporation of amino acid analogues in the presence of puromycin or by streptomycin-induced translational errors [373, 375].
As summarized in table 2, there is an intriguing connection between the stress-induced imbalance of protein homeostasis and its subsequent restoration by overproduction of chaperones and components of the proteolytic pathway. The efficient removal of irreversibly damaged proteins evidently needs these newly formed proteins and ATP (for summaries see refs 28, 220, 221).
1. Among the chaperone systems, HSP70/DnaK plays a key role. *E. coli* strains with mutations in the *dnaK*, *dnaJ* or *grpE* genes are defective in the energy-dependent degradation of protein fragments which are found associated with DnaK, GrpE and the Lon protease [222]. In mammalian cells, a specific isoform of the HSP70 family was characterized as a peptide-binding protein induced 20-fold under conditions of increased protein turnover [223, 224].
2. Tagging of proteins by ubiquitin is a prerequisite for proteolytic degradation in eukaryotes [9, 220]. In most organisms, the polyubiquitin gene is a general stress-inducible gene (see summary by Nover [38]; and refs 225a and 226) and, at least in yeast, this also extends to genes encoding enzymes of the ubiquitin conjugation pathway [221].

3. Ubiquitin-tagged proteins are mainly degraded in an ATP-dependent process by the high molecular weight 26S proteasome [9, 28, 221, 227]. The 20S core particle of the proteasome is an abundant multicatalytic protease built of two rings with 14 subunits each. It is combined at the entry site with a 19S ATPase particle required for unfolding of the proteins to be degraded in the inner cavity. Probably connected with the 19S complex is a 40- to 50-kDa recognition protein for the ubiquitin tag [228]. Interestingly, overproduction of a member of the yeast HSP70 family (SSB1) may suppress a defect in a proteasome subunit [229] and, vice versa, degradation of long-lived proteins is impaired in yeast strains with a mutant form of DnaJ [230].
4. Another type of stress-inducible multisubunit ATP-dependent protease, the ClpA/B:ClpP complex, has only been found in prokaryotes [102]. In eukaryotes only the chaperone part of the complex (ClpA/B = HSP100) has been identified so far (see 'The HSP100 family' above).
5. Two additional proteases, originally identified as hs-inducible proteins in *E. coli* were recently also found in eukaryotes. These are (i) the Lon protease [220, 231, 232] and its homologues in mammalian and yeast mitochondria [233, 234] and (ii) the membrane-bound, Zn-dependent FtsH protease, which in *E. coli* is involved in the turnover of the hs-specific sigma 32 factor [235, 236]. Its eukaryotic homologues are evidently subunits of a high molecular weight protease complex found in the inner membrane of yeast mitochondria [225] and plant chloroplasts [237].

Peptidyl-prolyl cis/trans isomerases (PPIase)

In contrast to the different types of chaperones briefly discussed in the preceding sections, this rapidly growing protein family comprises enzymes which increase the rate of slow steps of protein folding. PPIases were first described in 1984 by Fischer et al. [238]. Simultaneously, Handschumacher et al. [239] reported on the first binding protein of the immunosuppressive drug cyclosporin A. The identity of both types of proteins was later shown by Fischer et al. [240].
Meanwhile, PPIases were found to be a new family of abundant, ubiquitous, heterogeneously sized proteins with representatives in all compartments of eukaryotic cells where protein synthesis proceeds (for summaries see refs 49, 241–243 and 249). Currently PPIases are divided into four subfamilies: (i) cyclosporin A-binding proteins (cyclophilins, CYP), (ii) FK506- or rapamycin-binding proteins (FKBP), (iii) parvulins and (iv) the trigger factor (TF) detected so far only in *E. coli* [87].
Though individual and multiple disruptions of the more than 10 PPIase-coding genes in yeast [241, 242] gave no hint of a significantly impaired viability, this may simply indicate the need for a more detailed investigation of the effects. At present, there are several examples of defined functions of these proteins in different organisms:
1. Expression of some CYPs and FKBPs is induced by heat or protein stress [244–246], including HSP56 (FKBP56), which is found in steroid receptor complexes. In yeast, knock-out of two inducible forms, the cytosolic CYP1 and the ER-bound CYP2, resulted in cells with markedly decreased heat resistance [246].
2. Two members of the family (CYP40, FKBP56) are intimately connected with steroid hormone receptor complexes, taking part or facilitating the rapid changes in activity state and intracellular localization of these transcription factors [247, 248]. In addition, Tai et al. [248a] observed a potentiation of progesterone receptor-mediated transcription by FK506 in a heterologous expression system (yeast), possibly due to the inhibition of the Ca^{2+}-dependent calcineurin phosphatase.
3. Evidently, CYP20 plays an important role in mitochondrial protein import in yeast and *Neurospora crassa*. Inhibition by cyclosporin A causes a delay of intramitochondrial protein folding and extended binding of imported proteins to the HSP70 and HSP60 chaperone systems [90, 91].
4. In *Drosophila*, the best characterized cyclophilin homologue, NinaA, is required for the proper folding and transport of rhodopsin from the endoplasmic reticulum to the plasma membrane [408].
5. PPIase complexes with cyclosporin A and FK506, respectively, effectively inhibit a mammalian serine/threonine protein phosphatase (calcineurin). It is very likely that the immunosuppressive effect of both types of drug is mediated by this interaction with calcineurin and that PPIase activity is not required. Evidently, other signal transduction pathways involving protein phosphorlyation/dephosphorylation may be affected as well [196, 242].
6. Recently, the trigger factor of *E. coli*, positioned at the ribosomal exit tunnel of the nascent polypeptide chain, was identified as a very potent PPIase [87, 87a] probably representing the first folding catalyst with access to the newly forming polypeptide chain. It is unclear whether the 'nascent polypeptide-associated complex' of eukaryotes has similar activities [44].
7. CYP-type proteins were reported to be involved in the intracellular replication of the parasitic protozoa of *Leishmania major* in macrophages [250].

III. Heat stress transcription factors (HSF)

Basic structure of HSFs

After the initial characterization of the promoter recognition site of eukaryotic hs genes by H. R. B. Pelham and M. Bienz [251, 252; see summaries by Nover in refs 36 and 38] attempts were made to investigate the properties of the corresponding binding protein (heat stress

transcription factor, HSF). The initial experiments of Bonner [253] and Craine and Kornberg [254] defined cytoplasmic factors of *Drosophila* cells capable of activating hs genes in vitro. Following this, Parker and Topol [255, 256] and Wu [257–259] used footprint and exonuclease protection assays to demonstrate the specific binding of crude and partially purified HSF fractions from *Drosophila* cells to the hsp70 and hsp83 genes [260]. The ultimate breakthrough came with the cloning of the yeast HSF1 gene [261, 262], followed by the characterization of the homologous genes/cDNAs from *Drosophila* [263], tomato [264, 265], *Xenopus* [359], mammals [266–268], chicken [269] and two other yeasts [270, 271].

A surprising peculiarity of the plant (tomato) system was the finding of three HSF clones with different structural and functional characteristics. Moreover, two of them are themselves hs-inducible proteins [46, 264, 265, 272]. Meanwhile, similar results were reported for other plants (see summary by Nover [41]), such as maize [273], soybean [274] and *Arabidopsis* [275 and E. Czarnecka-Verner, unpublished]. In verterbrates at least three different types of HSFs were found which can be discriminated by their response to hs and developmental signals, respectively, as well as by their tissue-specific expression [269, 276].

All heat stress transcription factors from eukaryotes have a number of common features, summarized in fig. 1. The DNA-binding domain (DBD) close to the N-terminus is flanked by a region with heptad hydrophobic repeats (HR-A/B) and a cluster of basic amino acid residues essential for nuclear import (NLS). Finally, the C-terminal part contains modules for the activator function and its regulation (AD), in some cases including another heptad hydrophobic repeat region (HR-C).

The DNA-binding domain

In common with the -AGAAnnTTCT- recognition site characteristic of all eukaryotic hs promoters, the most conserved part of HSFs is the DNA-binding domain (DBD) of about 100 amino acid residues. Central to it is a helix-turn-helix motif (HTH) evidently involved in specific DNA contacts. The secondary structure elements and the resulting three-dimensional structure were elaborated by X-ray diffraction and multidimensional NMR techniques, respectively [277–280]. The results reported for the DNA-binding domain of yeast, *Drosophila* and tomato HSFs are very similar [279] despite a structural peculiarity of the plant HSFs which lack a 10-amino acid residue unstructured loop between β-strands 3 and 4 (fig. 2). The whole tightly packed globular structure is formed by a three-helical bundle on one side and a four-stranded antiparallel β-sheet on the other. It is stabilized by interactions of the bulky hydrophobic side chains of numerous aromatic and large aliphatic amino acid residues.

The core of the DBD is tightly packed and well defined. This contrasts with the conserved 20-amino acid residue C-terminal to the fourth β-strand which end with a cluster of three to four basic amino acid residues (K/R1). Because elements of secondary structure could not be detected by NMR techniques [279, 280], Flick et al. [281] discussed a role for this region as a flexible linker between the DNA-recognition and the HR-A/B domains. This may be important for the correct positioning of the HTH motif on the DNA. However, our investigations with K/R1 mutant forms of tomato HSFsA1 and A2 indicate that this unstructured region is essential for high affinity DNA binding [410]. It is tempting to speculate that, similar to the winged helix recognition of DNA by the forkhead type of transcription factors [282], this C-terminal part of the DBD makes direct contacts with the DNA, complementing those involving the HTH motif.

Heptad hydrophobic repeats

Heptad repeat patterns (HR) of large hydrophobic amino acid residues (L, I, V, M, F, Q) are frequently found in transcription factors, but also in other proteins as domains mediating protein-protein interactions. The original idea for a three-stranded coiled-coil interaction of α-helices containing large hydrophobic amino acid residues in a heptad repeat pattern was put forward by F. H. C. Crick [283]. Later on S. L. McKnight and coworkers [282a] defined a similar repeat structure for DNA-binding proteins as a leucine zipper.

The crystal structure analysis of a 33-residue synthetic peptide derived from the oligomerization domain of the yeast GCN4 transcription factor showed a triple-stranded coiled coil. Interestingly, two helices were in parallel orientation, the third one antiparallel; but this may be a peculiarity of the artificial protein fragment [284]. Though comparable data are lacking, this is evidently also valid for HSF, as indicated by data from circular dichroism (CD) spectroscopy and chemical cross-linking of the oligomerization domain derived from the yeast HSF [284a, 285].

With respect to the oligomerization behaviour, we can probably discriminate between two basic forms of HR regions: (i) those forming triple-stranded coiled coils and (ii) those forming double-stranded zipper-type structures. Frequently, the specificity of interactions is determined by charged residues found in defined positions forming interhelical salt bridges [286–289].

Heptad hydrophobic repeats (HR-A/B) connected by a variable linker region to the DNA-binding domain are characteristic of all HSFs (fig. 1). In addition, many HSFs, including plant HSFs types A1 and A2, contain a C-terminal HR-C motif whose function will be discussed below. The HR-A/B regions are of two types [41]: Type 1, observed for plant HSFsB1 and all non-plant HSFs, represents a continuous heptad pattern of

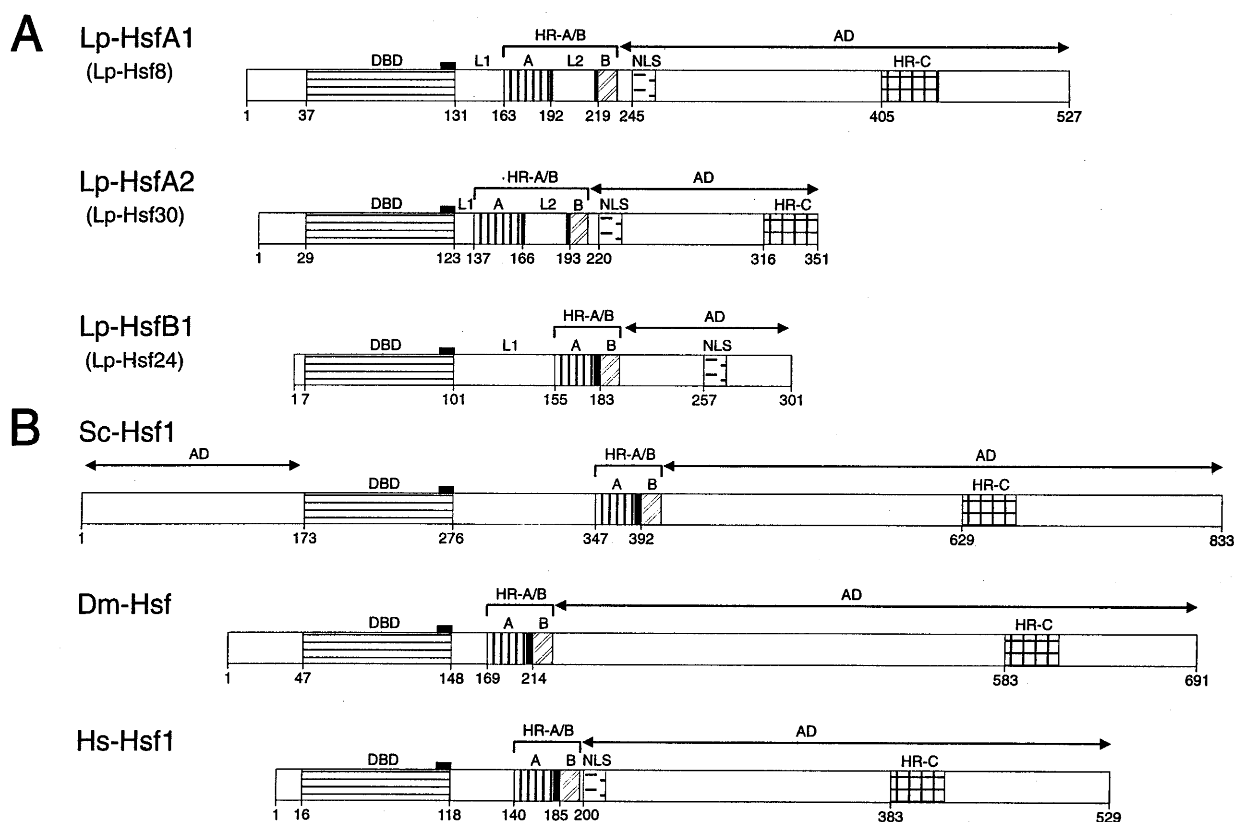
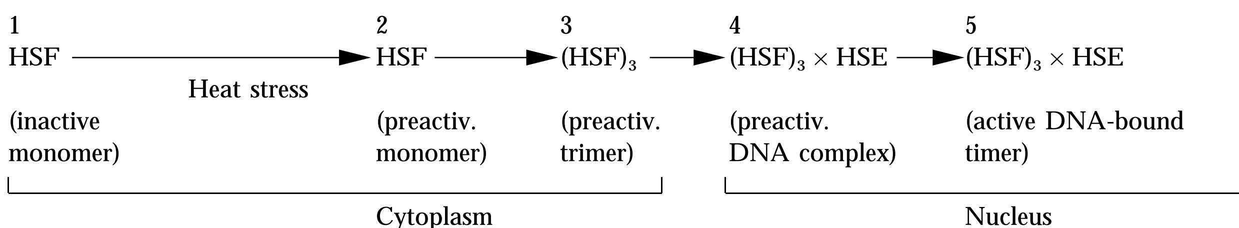


Figure 1. Basic structures of HSFs (from Nover et al. [41], with permission). Structures are exemplified in *A* by the three HSF types from wild tomato (*Lycopersicon peruvianum*, Lp) and in *B* by HSFs from baker's yeast (*Saccharomyces cerevisiae*, Sc), fruit fly (*Drosophila melanogaster*, Dm) and humans (*Homo sapiens*, Hs), respectively. DBD = DNA-binding domain; HR-A/B, HR-C = heptad hydrophobic repeats; AD = activation domain; NLS = nuclear localization signal; L1, L2 = linker sequences mentioned in the text; bar at the C-terminus of the DBD marks the position of the K/R1 motif (see text).

large hydrophobic amino acid residues. Type 2 seems to be unique to plant HSFsA1 and A2. The heptad pattern is interrupted by insertion of 21 amino acid residues and may give rise to two overlapping HR motifs. Unfortunately, the three-dimensional structure of this important part of the HSFs is unkown. Thus, the definition of the two parts (A vs B) is based on formal arguments only until more structural information is available. Interestingly, not only the heptad positions, but a number of other amino acid residues are also highly conserved, or invariant in the HR-A/B region. Among them are acidic and basic residues which might contribute to the specificity of interactions mediated by this domain (see references given above).

The activation/deactivation cycle for HSFs evidently involves positive and negative modules of the HSF itself as well as cofactors, e.g. of the chaperone families (for details see fig. 3). Though oligomerization is usually an integral part of stress activation [290–295] it is not a prerequisite for nuclear import, nor for DNA binding or function as transcription factor. HSFs with point mutations or deletions of the HR-A/B domain havebeen repeatedly reported to be active, though unregulated [294, 296, 409]. The existence of a DNA-bound but transcriptionally inactive state (form 4) of HSF represents the normal situation in unstressed yeast [261, 262, 295a]. This state can also be generated by treatment of mammalian cells with salicylate, indomethacin or other inflammatory drugs sensitizing cells to respond to a lower temperature threshold for full HSF activation [297–299]. The multistep pathway of HSF activation involving oligomerization, nuclear transport and DNA binding may be summarized as follows:



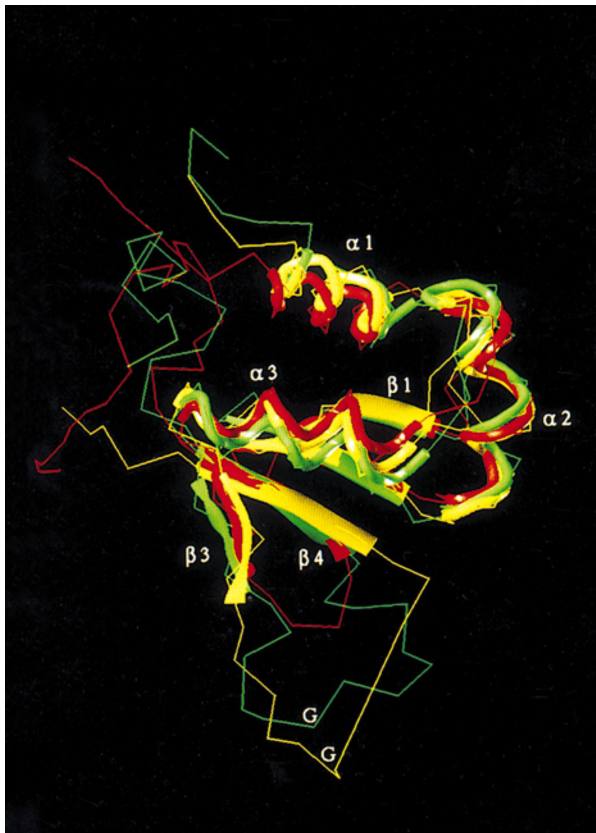


Figure 2. Superposition of the three-dimensional structures of the DNA-binding domains of HSFs from yeast (yellow), *Drosophila* (green) and tomato (red). The figure was created by J. Schultheiss (Frankfurt) by superposition of MOLSCRIPT files containing the structural information from Harrison et al. [278], Schultheiss et al. [279] and Vuister et al. [280]. The sequence of secondary structure elements is $\alpha1$, $\beta1$, $\beta2$, $\alpha2$, $\alpha3$, $\beta3$ (L), $\beta4$. The very close similarity of all three structures is evident from the position of the α-helical parts ($\alpha1$, $\alpha2$, $\alpha3$), the turn region between $\alpha2$ and $\alpha3$ as part of the HTH motif and the antiparallel β-sheet formed by β-strands $\beta1$, 2 and 4. In all three HSFs $\beta3$ is tilted with respect to the plane of the β-sheet. The remarkable difference between plant (red) and nonplant (yellow, green) HSFs is an unstructured loop (L) with 11–12 additional residues between $\beta3$ and $\beta4$ containing a conserved glycine residue in the nonplant HSFs.

The actual form of wild-type HSF imported into the nucleus is unclear. Immunofluorescence data so far have only demonstrated enhanced or exclusive nuclear localization after stress [292, 300, 410]. Cell fractionation using extracts from HeLa cells or transfected *Xenopus* oocytes demonstrated that the nuclear form of human HSF1 is trimeric, whereas the cytoplasmic form is mainly monomeric [290, 294, 295]. Our characterization of the oligomeric state of tomato HSFs in their native surrounding (cell cultures) or after expression in tobacco protoplasts did not give any evidence for monomeric states or for pronounced changes in the oligomeric state induced by shift from control to hs conditions (Scharf et al., unpublished). Thus, the simplified scheme given above reflects essential aspects of the multistep activation process of the *Drosophila* HSF and vertebrate HSF1, but must be modified for other systems including HSFs 2 and 3 of vertebrates.

Nuclear localization signal (NLS)

The nuclear import of proteins is dependent on the presence of NLS motifs formed by clusters of basic amino acids (arginine, lysine residues, K/R clusters). NLS motifs interact with cytoplasmic receptors, initiating the assembly of a multiprotein transport-competent complex which is able to pass through the nuclear pore [301, 302].
Two K/R clusters with potential function as bipartite NLS motifs [303] are found in the C-terminal flanking regions of the DNA-binding domain (K/R1) and the HR-A/B domain (K/R2), respectively (fig. 1). In contrast to earlier findings on the human HSFs1 and 2 [295, 300], only K/R2 is reponsible for nuclear import, at least for tomato HSFsA1 and A2 [410]. Although nuclear localization requires the K/R2 signal, the transport process is regulated by other parts of the protein. Common to both human and plant HSFs is the observation that the C-terminal HR-C region is somehow involved in cytoplasmic retention, e.g. by intramolecular or intermolecular shielding of the NLS motif. Truncated HSFs generated by HR-C deletions show constitutive nuclear localization together with a more or less unregulated activity [267, 269, 291, 410].

The C-terminal activator domain (AD)

Generally, this region of the HSF shows a very low degree of sequence conservation combined with a remarkable multiplicity of synergistic and partly redundant elements involved in the activator function and its regulation. Though details are far from clear, the following results may be relevant to elaborate a more general concept of HSF regulation and to understand the differences between HSF types and organisms respectively.
1. Disregarding some peculiarities of the stress-inducible HSF forms in plants, the general basis of transient activation of hs genes is the stress-dependent release of HSF from the inactive state and the restoration of this state with ongoing HSP accumulation. Multiple positive and negative regulatory modules in the C-terminal domain contribute to the function of HSFs as stress-regulated activator proteins.
2. The detection of a C-terminal heptad pattern of large hydrophobic amino acid residues (HR-C, fig. 1) suggested the model of an inactive monomeric form maintained by intramolecular interactions between the HR-A/B and the HR-C regions [267, 269, 291]. Though direct proof, e.g. by a two-hybrid assay, is lacking, there is some indirect evidence in support of this concept. Thus, deletion of the HR-C region of plant, *Drosophila* or chicken HSF creates a more active but unregulated phenotype [269, 291; Lyck et al., cf. ref. 410]. Furthermore, Baler et al. [290] demonstrated extensive confor-

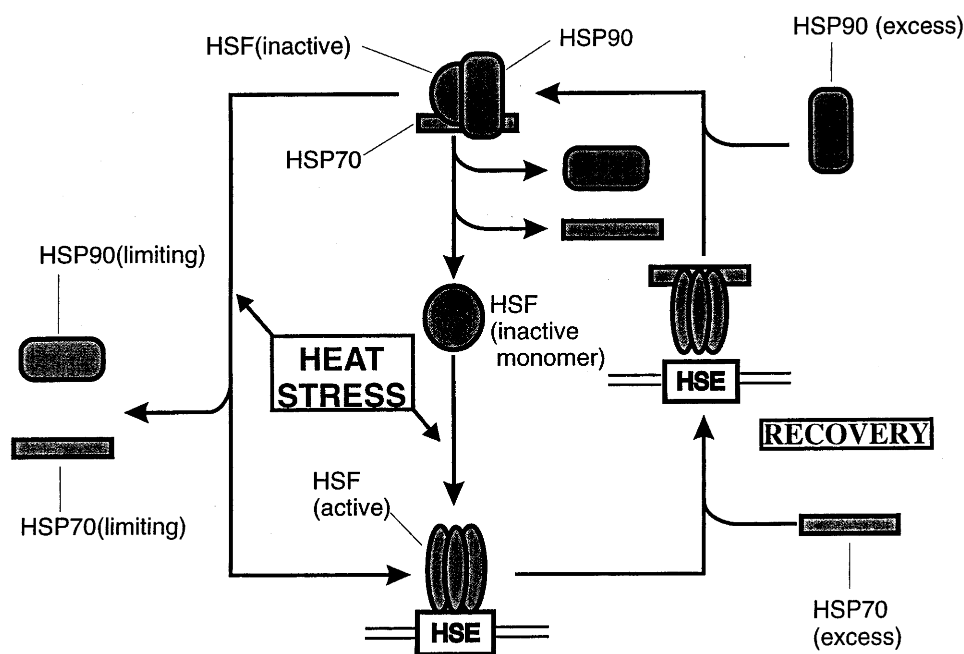


Figure 3. The HSF cycle (from Mosser et al. [316a], modified by C. Kirchner).
After heat stress, creating a situation with chaperone limitation (left), the inactive HSF monomer (centre) undergoes conformational changes with subsequent trimerization, nuclear import and DNA binding. Under conditions of chaperone excess (right) the trimer is removed from the DNA and converted to the inactive monomer by transient interaction with chaperones, e.g. of the HSP70- and HSP90-type.

mational changes of human HSF1 during hs activation. But in the present state of our knowledge, it is essential to note that many effects could also be explained by intermolecular interactions, e.g. with chaperones or with other not yet identified coregulators (see below).
3. Activity tests with C-terminal deletion forms of the tomato HSFs [272] led us to identification of short peptide motifs with a central tryptophan residue (Trp elements) as essential elements for the activator function. They are part of more extended C-terminal regions with a high density of aromatic (**A**) and bulky hydrophobic (**H**) as well as acidic (**A**) amino acid residues (see also review by Scharf et al. [46]). Similar **AHA** regions are common C-terminal markers of yeast and vertebrate HSFs as well (see table 1). Repeatedly, synergistic subdomains were defined by testing fusion constructs with heterologous DNA-binding domans. In two remarkable cases, the activator function could be reduced to short peptide motifs with a characteristic and indispensible pattern of aromatic and large hydrophobic amino acid residues (underlined residues of AHA modules). For the human HSF1, the sequence -FSVDTSALLDLF- fits this description [304], and for the tomato HSFA2 it is -VADDIWEELLS- [272 and Treuter and Nover, unpublished].
Though these core modules are integral parts of a much more complex activator domain, their composition and function are reminiscent of similar **AHA** modules in the centre of the activator domains of other transcription factors (see table 1 and refs 305 and 306). With the exception of Sp1, these motifs are negatively charged. However, the significance of the acidic amino acid residues is not clear [307]. The aromatic hydrophobic side chains are essential for protein contacts to other components of the basal transcription apparatus (see references given in table 3). Xiao et al. [308] argue that similar motifs close to the C-terminal part (CTD) of the largest subunit of RNAP II may compete for these binding sites in the preactivated state. Thus, release of the CTD by competition with activator proteins may help in assembly and/or activation of the transcription complex [309]. CTD phosphorylation is an important step for the transition from the initiation state to elongation by the RNAP II holoenzyme complex [310, 311].
4. A central aspect of the hs response is the maintenance of the inactive state of HSF under nonstress conditions. Different regions of the C-terminal domain have been identified as negative regulatory elements. Clearly, deletion of the HR-A/B region [271, 409] or HR-B plus flanking regions [304, 312] creates active but unregulated HSF forms. On the other hand, deletions or mutations of C-terminal regions that involve the potential HR-C domain may have similar effects [269, 291, 410]. It seems likely that both parts of the molecule are involved in intramolecular interactions to stabilize the inactive monomer [291].
Evidently, HSF phosphorylation/dephosphorylation plays an important role in this context. Hoj and Jakobsen [313] reported on the significance of a Ser-rich heptapeptide motif in the C-terminal activator domain

Table 1. AHA modules involved in the activator function and protein contacts of transcription factors. (For reviews see Triezenburg [306]; Tjian and Maniatis [305].)

Transcr. factor[a] (Source)	Class[b]	Function	Test system[a]	AHA modules[c]	Remarks[d]	References
HSV VP16 (M)	—	Viral activator protein	M, Y	438-ALDDFDLDM- 470-MADFEFEQMF-	Bipartite activator region; interacts with TBP and TFIIB	308, 389–391
RelA (M)	—	p65 subunit of NF-κB	M, Y	442-LQFDDEDLGALL- 535-SIADMDFSALL-	Additive effect of several AHA moduls required	392
Sp1B (M)	ZnF	GC box-binding activator	M, Y, D	454-QVSWQTLQLQNLQV-	Not acidic; interaction with $TAF_{II}110$ and $TAF_{II}40$	367
p53 (H)	—	Mammalian tumor suppressor protein	M	14-LFQETFSDLWKLLPE-	Interacts with TBP, $TAF_{II}40$, $TAF_{II}60$, HSP70 and viral oncogenes, (Ad5 E1B)	393–395
c-Jun (H)	bZip	Part of Fos/Jun complex, binding to AP1 sites	M	107-QEGFAEGFVRAL-	Similar module (HOB2) found in c-Fos	396
E2A (M)	HLH	E-box binding protein	M, Y	9-PVGTDKELSDLLDFSMMFP-	HLH proteins involved in muscle and B-cell development	397
C/EBPα (M)	bZip	CCAAT-binding protein	M	63-IDISAYIDPNDEFLADLF-		398, 399
NRF1 (M)		Nuclear factor controlling respiratory genes	M, Y	358-QNW- 451-LVQIPVSMYQTVV		412
hRXRα (M)	ZnF	Human retinoid X receptor	M, Y	447-IDTFLMEMLEAPHQMT*	Interacts with TBP, TAF110 and the RXR repressor, similar C-terminal motifs found in other RXR, RAR and TR	413–415
GCN4 (Y)	bZip	Activator of genes for aa synthesis	Y	89-LDDAVVESFFSS6M 108-FEYENLEDNSKEWTSLFD-	Additive effects between both AHA modules	400, 411
GAL4 (Y)	ZnF	Activator of gal regulon	Y, P	861-MDDVYNYLFDDEDT*	Interacts with the GAL80 repressor, TBP and two coactivating proteins	307, 308, 401
Heat stress transcription factors (HSF)						
Hs-HSF1 (H)	HTH	Human	M	401-MLSSHGFSVDTSALLDFSP-		304
Lp-HSFA1, Lp-HSFA2(P)	HTH	Tomato	P, Y	HSFA1: 447-GADIDWQSGLL- 466-VGDPFWEKFLQ- HSFA2: 292-VADDIWEELLS- 332-VKTPEWGEELQ-	Additive effects of both modules	272 and unpubl. results
Kl-HSF1 (Y)	HTH	Yeast	Y	582-FFQDLQNNIDKQEESIQE IQDWITKLNPGPGEDGNTPIF-	Similar motif found in Sc-HSF1 (aa 629–666)	296
Dm-HSF	HTH	*Drosophila*	D, P		C-terminal activator (aa 629–691) contains an extended AHA motif	402

[a]Organisms are abbreviated as follows: D = *Drosophila*; H = human; M = mammals; P = plants; Y = yeast.
[b]Classification of transcription factors (TF) according to their DNA-binding motifs are indicated with the following abbreviations: bZip = basic region/leucine-zipper; HTH, HLH = helix-turn-helix and helix-loop-helix motifs; ZnF = zinc finger (for reviews see refs 403, 404).
[c]The sequence of the N-terminal or C-terminal AHA motifs are enriched in amino acid residues with aromatic (F = phenylalanine; Y = tyrosine; W = tryptophan), large hydrophobic (I = isoleucine; L = leucine; M = methionine; V = valine) and acidic (D = aspartic acid; E = glutamic acid) side chains. The number indicates the position of the first amino acid residue in the whole protein. Residues underlined were shown to be particularly important for function, e.g. by analysis of mutant proteins.
[d]Protein contacts which may be essential for the activator function involve several components of the basal transcription complex, e.g. TBP (TATA-binding protein), TFIIB (transcription initiation factor II B) and TBP-associated proteins (TAFs).

of the *Kluyveromyces* HSF. Phosphorylation in this region observed under hs conditions is evidently a prerequisite for inactivation of the HSF in this yeast. Though many HSFs were shown to be phosphoproteins and to change the phosphorylation state under stress conditions [261, 262, 314, 315], this is the first example of a possible function attributed to this dynamic modification. Recently, the role of phosphorylation in the

reversion of HSF activity was also confirmed for human HSF1 [297, 299, 316].

5. The intensive characterization of different sequence motifs of HSF which might explain the stress-regulated phenotype is parallelled by investigations of specific chaperones as putative coregulators in the restoration or maintenance of the inactive state. The *intra*molecular interactions discussed for the C-terminal domain are complemented by *inter*molecular interactions. There is genetic and biochemical evidence that stress proteins of the HSP70 family are key components in this respect, probably acting by sensing the accumulation of denatured proteins in the stressed cells (table 2). The model given in figure 3 summarizes our present concept elaborated in various detail for yeast, vertebrate and plant systems. Probably, the main function of chaperones is to restore or maintain the inactive state of HSFs. From functional tests with plant HSFs (Kirchner and Scharf, in preparation), it is very likely that besides the HSP70 chaperone machinery, HSP90 is also involved. Thus, the system of coregulators required for control of HSF activity is evidently very similar to that connected with steroid hormone receptors in vertebrates. Whether this similarity also includes mechanistic aspects remains to be elaborated.

IV. Developmental control of hs gene expression in plants

After more than 20 years of investigations of hs-induced proteins, our knowledge is far from complete. In particular, some of the 11 major HSP families are barely or not all characterized in plants. As mentioned above, the remarkable complexity of the HSP pattern in plants results from two peculiarities: (i) in addition to the mitochondria and ER, the chloroplasts harbour their own set of chaperones; (ii) in contrast to most other organisms, the HSP20 family usually comprises more than 20 different proteins (see 'The HSP20 family' above). Considering this multiplicity of genes, it is not surprising that genotype and tissue-specific differences in expression patterns after induction by hs or other stressors occur [317–321]. The situation simply reflects the central role of chaperones for protein homeostasis and the evolution of multivalent promoters controlling HSP expression under different stress and developmental conditions. Though hs-induced gene activation and subsequent HSP synthesis are part of the general hs response in practically all tissues, there are specific observations on peculiarities in developing pollen [322–324], germinating seedlings [317, 325] and during somatic embryogenesis [320, 326]. In the latter case, HSP synthesis is evidently controlled primarily at the translational level.

Investigations of developmental control of hs gene expression in plants have concentrated on lmw HSPs (HSP20 family). Three major parts of development are summarized in table 3: early meiotic stages of pollen formation, fruit ripening and somatic embryogenesis. In most cases, only one or a few representatives of the whole set of hs-induced HSP20 isoforms are observed in a particular developmental context. The same is true for the few examples, where other chaperones, e.g. of the HSP70 family, were included. A particularly convincing documentation of the variability of hsc70 gene expression in tomato tissues was presented by Duck et al. [327] using in situ hybridization data. In addition, Wang and Lin [328] reported on the complete replacement of a seed-specific HSC70 in germinating mung bean by a new vegetative isoform, and storage protein synthesis in ripening seeds is intimately connected with increased levels of GRP78 (BiP) in the rER [329–331]. Denecke et al. [330] characterized six different cDNA clones for BiP isoforms in tobacco. But expression patterns at the protein level and possible functional differences remain to be investigated. Also, it is not surprising that light-induced synthesis of small stress proteins has repeatedly been observed. They are part of developing chloroplasts [333] of mitochondria [334] or belong to the cytosolic members of the HSP20 family [335]. In this context, it is also worth mentioning that synthesis of hs-regulated mRNAs is under circadian control in pea seedlings with a maximum at the end of the night and early in the morning [336]. Finally, there are several reports indicating developmental as well as light- and stress-dependent control of synthesis of other chaperones. Examples are the chloroplast Cpn60 [337], HSP90 [338], HSP104 [106, 339] and PPIases [340].

Table 2. Autoregulation of the hs response: Evidence for the role of denatured or malformed proteins in hs signal transduction.

1.	Heat and chemical stressors cause protein denaturation/aggregation; newly synthesized proteins are particularly affected [216, 217, 377, 361–364].
2a.	Injection of denatured proteins into *Xenopus* oocytes leads to HSF activation [218, 365, 366].
2b.	Synthesis of recombinant or mutant/defective proteins triggers HSP synthesis [368–375].
3.	Unfolded abnormal or denatured proteins interact with the HSP70/DnaK system [83, 84, 151, 155, 157, 376–378].
4.	Genetic and biochemical evidence that the level of free HSP70 controls the extent of hs response [10, 161, 316, 345, 379–381].
5.	ATP depletion of cells results in increased levels of denatured proteins and hs induction [216, 382].
6.	HSFs interact with HSP70 [380, 384, 406]; large complexes of both proteins are found in cytoplasm of non-stressed NIH-3T3 cells [383].
7.	HSF activity in non-stressed tobacco protoplasts is repressed by co-expression of HSF with HSP70 and HSP90 (C. Kirchner and K. D. Scharf, unpublished). Overexpression of mammalian HSFs in mammalian cells and *Xenopus* oocytes results in unregulated activity [290, 292], whereas overexpression of HSC/P70 in mammalian and *Drosophila* cells was found to accelerate the deactivation of HSF [316, 345, 380, 384].
8.	Regulation of the *E. coli* sigma 32 stability and activity cycle by the DnaK/DnaJ/GrpE system [7, 149, 157, 385–388, 407].

Table 3. Developmental expression of plant heat stress genes and proteins.
(See summaries by Nover, 1991 [38]; Winter and Sinibaldi, 1991 [360]; Waters et al., 1996 [54], na, not analysed)

	Organism; developmental process	Proteins[a]	hs mRNAs	Remarks	References
A.	*Pollen development*				
	1. Lily, maize	na	HSP18 (class II)	Detected as meiosis-related multi-gene family (cDNA); expressed during meiotic prophase, but not in mature protein	323, 345–348
	2. Tobacco, tomato	HSP17	na	Expression of HSP17 during meiotic stages of pollen development	341
B.	*Flower development and seed ripening*				
	3. Pea, ripening pods, seed germination	lmw HSPs class I & II and HSP71.2	HSPs 70, 20, 17	Coordinate expressions of class I & II mRNA and proteins during mid-maturation of seeds; decay during germination	342, 343
	4. Tomato; fruit ripening, leaf senescence	na	pTOM56 = HSP17	mRNA levels increasing in response to ripening or senescence; not induced by ethylene	349, 350
	5. Tomato; fruit	na	HSC70	Detection by in situ hybridization, highest levels in tapetum cells (ovary), seed integument and developing embryo	327
	6. Tobacco, tomato, pea, bean, maize; seed development and germination	HSP17 (class I)	na	HSP17 accumulates in ripening seeds; degraded during germination; pattern of isoforms different from hs-induced pattern	341
	7. Wheat; germinating seeds	HSPs 94, 70, 60 40, 17, 14	HSPs 70, 60, 40, 17, 14	In vitro translation of mRNAs from wheat germs; except for HSP70, levels of hs mRNAs decrease upon germination	351
	8. Sunflower, seed ripening	HSP17.6	HSP17.6 (classes I & II)	In seedlings also induced by heat, osmotic stress or ABA	352, 353
C.	*Somatic embryogenesis*				
	9. *Arabidopsis*; flower development	na	na	HSP18 promoter × gus construct expressed in sepals, filaments, styles	354
	10. *Arabidopsis*; seed ripening	HSPs17.4 and 17.6	na	Class I HSP17.4 is strongly expressed in devel. seeds, but only traces of HSP17.6; rapidly degraded during germination; only 10% of normal HSP17.4 level observed in ABA-response mutants *(abi 3*-1, *abi 5-1)*	344
	11. Rice, barley, maize, tobacco	GRP78 (BiP)		The ER-localized member of the HSP70 family is essential for storage protein synthesis; six different BiP clones identified in tobacco; BiP level in germinating barley aleurone cells enhanced by GA3 treatment	329–332, 355
	12. Maize, mature seeds	na	Class I lmw HSPs	Identification of clones by sequencing of cDNAs from mature endosperm	323a
	13. Sorghum bicolor	na	lmw HSPs	In vitro translation of mRNAs from dry seeds	356
	14. Alfalfa; somatic embryogenesis	na	HSP18 (class I)	Highest levels detected in early developmental stages	357
	15. Tobacco, induced embryogenesis from	na	HSP17 (class I)	Detected during starvation-induced embryogenesis; already present in pollen	358
	16. Carrot, somatic embryogenesis	na	HSP17 (class I and II)	hs-induced synthesis of HSPs regulated at translational level, based on preformed mRNA	326, 405

The most concise sets of data on developmental expression of HSP20 proteins in plants were provided by zur Nieden et al. [341] and the group of E. Vierling [342–344]. Comparing the lmw HSP (class I) levels in ripening and germinating seeds of tobacco, tomato, pea, bean and maize, our group [341] reported on the accumulation of HSP17 (class I) in mid to late phases of seed ripening and their degradation during germination. The pattern of lmw HSPs is different from the hs-induced pattern but shows great variability between the plant species investigated. The same is true for the tissue-specific and intracellular distribution of the HSP17 isoforms. Surprisingly, a considerable amount of the protein is found in the nuclei in different tissues of embryos from maize, soybean and tomato, as well as in the storage protein compartments (protein bodies) of soybean and tomato cotyledons [341]. De Rocher and Vierling [342, 343] investigated seed maturation in pea where only cytosolic class I and class II lmw HSPs accumulate simultaneously with the presence of the corresponding mRNAs. No ER- or chloroplast-specific proteins are formed under these conditions. The lmw HSPs are only found in the developing embryo but not in the surrounding somatic tissue. The same is true for a single isoform of the HSP70 family (HSP71.2) whose expression in nonembryonic tissue is only observed after hs-induction [343].

With regard to the specific patterns of HSPs expressed within a particular developmental context (table 3), it is very unlikely that the normal stress-responsive signal transduction pathway is operative. The only direct evidence for a long-discussed alternative stems from experiments of Wehmeyer et al. [344] using abscissic acid (ABA) response mutants of *Arabidopsis*. In this case a single lmw HSP (HSP17.4, class I) is the dominant HSP of developing seeds. The fairly high levels found in wild-type *Arabidopsis* seeds are reduced to one-tenth in two ABA-insenstive mutants (*abi-3-1*, *abi 5-1*). The *abi3* locus probably codes for a transcription factor which affects the accumulation of storage proteins. Unfortunately, nothing is known about the nature of the *abi5* gene product and about the mechanism whereby *abi3* and *abi5* gene products may influence expression of lmw HSPs.

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