REVIEW ARTICLE
Stress response of yeast

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INTRODUCTION

All living cells display a rapid molecular response to adverse environmental conditions, a phenomenon commonly designated as the heat shock response. Since other kinds of stress have similar effects, this process can be considered as a general cellular response to metabolic disturbances. The most striking feature of the heat shock response is the induced synthesis of a set of proteins conserved during evolution, the heat shock proteins (hsps).

The heat shock response was first reported as a dramatic change in gene activity induced by a brief heat treatment of *Drosophila hydei* larvae (seen as changes in 'puffing patterns' of salivery gland polytene chromosomes; Ritossa, 1964). Until 1980 most attention was focused on the heat shock response in *Drosophila*, where, indeed, heat shock proteins were first discovered (Tissières et al., 1974). Similar findings with other eukaryotes and prokaryotes soon suggested that the response represents an evolutionarily conserved genetic system which might be beneficial for the living cell.

In yeast, in particular in the main species of investigations, *Saccharomyces cerevisiae*, a sudden temperature change generates considerable, temporary, alterations in the pattern of protein biosynthesis (Miller et al., 1979). Moreover, when yeast cells were shifted to a higher temperature, growth was transiently arrested at the G_1 phase of the cell division cycle (Johnston and Singer, 1980). Changes in the rate of protein synthesis were monitored by amino acid pulse-labelling, followed by two-dimensional gel analysis. These initial studies revealed a 10-fold or greater induction or repression of most proteins (Miller et al., 1979). Subsequent analysis demonstrated that, upon a shift of *S. cerevisiae* from 23 °C to 37 °C, out of 500 proteins examined more than 80 were transiently induced (20 of which could be classified as major heat shock proteins) and with more than 300 proteins the synthesis was reduced (Miller et al., 1982). The response to heat shock was temporary, with recovery occurring within a few hours after stress exposure.

The finding that the heat shock response is inhibited in mutants defective in RNA synthesis or processing/transport indicated that transcription plays a predominant role (Miller et al., 1979; McAlister and Finkelstein, 1980). Indeed, changes in the patterns of protein synthesis *in vivo* were found to parallel changes in the levels of translatable mRNAs (McAlister and Finkelstein, 1980). A major class of protein whose synthesis is strongly reduced are ribosomal proteins, which displayed a co-ordinated transient decrease upon shifting yeast cells from 23 °C to 36 °C (Gorenstein and Warner, 1979; Kim and Warner, 1983). The decrease in mRNA levels corresponding to repressed protein synthesis appeared to be much faster than could be explained merely by transcriptional arrest (McAlister and Finkelstein, 1980). In agreement with this finding, for ribosomal protein mRNAs a (heat-induced) temporarily enhanced decay rate was demonstrated (Herruer et al., 1985).

Although the early data suggested the universal nature of the heat shock response, differences were shown to exist in the way *Drosophila* and yeast achieve the fast changes in protein biosynthesis (Lindquist, 1981). In *Drosophila* a sudden heat shock induces a translational control mechanism, both specifically repressing the translation of pre-existing mRNAs and inducing the synthesis of mRNAs encoding hsps. In yeast such translational control of mRNAs does not occur.

The spectrum of hsps synthesized in yeast upon a stress challenge is similar to that produced in other cells. Several families can be distinguished which are designated, according to their average apparent molecular mass, hsp100 (in yeast hsp104), hsp90 (in yeast hsp83), hsp70, hsp60 (the chaperonin or groEL-family) and small-size hsps (in yeast hsp26 and hsp12); see Table 1. Several proteins homologous to hsps are synthesized constitutively, reflecting the important cellular functions performed by these proteins under normal circumstances. In addition, the rate of synthesis of several other proteins, e.g. ubiquitin, some glycolytic enzymes and a plasma membrane protein, is strongly

Table 1 Stress proteins of yeast

Designation	Cellular localization	Function
Hsp150	(Secretory)	Unknown
Hsp104	Nucle(ol)us	Stress tolerance
Hsp83	Cytosol/nucleus	Chaperone
Hsp70		
ssa1	Cytosol	Chaperone
ssa2	Cytosol	Chaperone
ssa3	Cytosol	Chaperone
ssa4	Cytosol	Chaperone
ssb1	Unknown	Unknown
ssb2	Unknown	Unknown
ssc1	Mitochondria	Chaperone
ssd1 (kar2)	Endoplasmic reticulum	Chaperone
Hsp60	Mitochondria	Chaperone
Hsp30	Plasma membrane	Unknown
Hsp26	Cytosol/nucleus	Unknown
Hsp12	Cytosol?	Unknown
Ubiquitin	Cytosol	Protein degradation
Enzymes		
Enolase	Cytosol	Glycolysis
Glyceraldehyde 3-phosphate dehydrogenase	Cytosol	Glycolysis
Phosphoglycerate kinase	Cytosol	Glycolysis
Catalase	Cytosol	Antioxidative defense

Abbreviations used: hsp, heat shock protein; GRE, glucocorticoid response element; HSG, heat shock granule; HSF, heat shock transcription factor; HSE, heat shock responsive element; URS, upstream repression site.

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induced upon exposing yeast cells to stress. These proteins, therefore, should also be considered as heat shock proteins.

The functional significance of the heat shock response is evident from the magnitude and the speed of the process. The ability of a cell to shift rapidly to heat shock protein synthesis suggests that it is pivotal for survival ('emergency response'; Lindquist and Craig, 1988). A shift from 23°C to 30 °C did not result in any major alterations in the pattern of protein synthesis (McAlister et al., 1979), but a shift to 36 °C (mild heat shock) does. Yet, the elevated temperature of 36 °C is within the normal growth range of yeast. Therefore, it has been assumed from the initial studies that the response might play a major role in the protection of cells from thermal injury. Indeed, a rapid shift in cultivation temperature of *S. cerevisiae* from 23 °C to 36 °C leads to protection from death due to a subsequent extreme (52 °C) heat treatment. It is a common feature of the response in all living organisms that it is evoked below lethal temperatures and, therefore, may provide the cell with the ability to withstand even higher, otherwise lethal, temperatures. The acquisition of stress tolerance, thus, is an important aspect of the stress response. A correlation between the cellular levels of hsps, transiently induced in yeast by a mild heat shock, and the level of acquired thermoresistance has been described as early as 1980 (McAlister and Finkelstein, 1980). However, a convincing body of data provide evidence that increased hsp levels are not sufficient for stress tolerance attainment (see below).

This Review deals with the cellular functions of hsps and other stress-induced proteins in yeast. In addition, stress-induced changes in yeast gene expression are discussed with particular emphasis on the specific *trans*-acting transcription factor HSF, its interaction with the corresponding *cis*-acting nucleotide element, HSE, and the regulation of transcription of some *HSP* genes. Also some stress-affected cellular processes are briefly discussed. Finally, data concerning the acquisition by yeast of tolerance against various kinds of environmental stress are reviewed. The concluding remarks address putative signals that trigger the stress response in yeast.

CELLULAR FUNCTIONS OF HEAT SHOCK (-INDUCED) PROTEINS

Heat shock proteins have been implicated in all major growth-related processes such as cell division, DNA synthesis, transcription, translation, protein folding and transport, membrane function; see Table 1 for a summary. The various members of the hsp subfamilies identified in yeast are discussed below.

Hsp70, mediator of protein folding

HSP70 proteins belong to the most highly conserved proteins in the cell. A 40–60 % identity exists between hsp70 proteins found in higher eukaryotes and the *E. coli* hsp70 dnaK; among eukaryotes this percentage is even higher (reviewed by Lindquist, 1986; Lindquist and Craig, 1988). The genes encoding hsp70 in *S. cerevisiae* constitute a multigene family consisting of eight members which show amino acid sequence identities ranging from 50 to 97 % (Ingolia et al., 1982; Craig et al., 1990). They are subdivided into four subfamilies, *SSA*, *SSB*, *SSC* and *SSD* ('Stress Seventy'). The *SSA* subfamily is indispensable for growth and encodes the ssa1, ssa2, ssa3, and ssa4 proteins, which are localized in the cytoplasm (Slater and Craig, 1989; Boorstein and Craig, 1990). Mutations in *SSB1* and *SSB2* result in a cold-sensitive phenotype and the cellular localization of the corresponding proteins is unknown (Craig et al., 1990). The protein encoded by *SSC1* is a mitochondrial protein (Craig et al., 1987, 1989), whereas the *SSD1* (better known as *KAR2*) gene product occurs in the endoplasmic reticulum (Normington et al., 1989; Rose et al., 1989). Both genes are essential for growth. Expression of the family members is regulated differently upon changes in growth conditions. For instance, ssa4 is a characteristic heat shock protein displaying very low basal levels of expression and a strong induction upon heat treatment. In contrast, ssa1 and ssa2 show a rather high constitutive expression. This aspect will be discussed below, in the section on stress-induced changes in yeast gene expression. Also in other eukaryotic cells multiple genes coding for similar sets of related hsp70 proteins are present.

Sequence similarity between hsp70 proteins extends over the entire protein, but particularly conserved regions are present in the N-terminal part. In this region of the proteins an ATP-binding site is present displaying a weak ATPase activity (Chappell et al., 1987). A crystallographic study of a bovine hsp70 revealed a surprising similarity in tertiary structure and topology between this ATPase domain and the ATP-binding core of hexokinase (Flaherty et al., 1990). This shared property of hsp70 proteins is consistent with the current view that hsps70, in general, play a part in protein–protein interactions. More precisely, hsps70 may act by binding to certain polypeptide chains, hence modifying or maintaining their conformation or interaction with other proteins. According to this general model, release from such substrates depends on ATP (Rothman, 1989; Craig and Gross, 1991). The model of an 'ATP-driven detergent' action of hsp70 was proposed by Lewis and Pelham (1985). Their basic idea was that hsp70 binds to denatured, aggregated proteins and aids their solubilization, using the energy of ATP hydrolysis for release, with simultaneous (re)folding of the proteins. [See Gething and Sambrook (1992) for a recent review on all aspects of protein folding in the cell.]

All available data fit with such a chaperone function of hsp70. The first hsp70 whose cellular function was established is the mammalian uncoating enzyme, involved in ATP-dependent release of clathrin from coated vesicles (Rothman, 1989). Furthermore, the *E. coli* hsp70, encoded by the *dnaK* gene, was originally identified as a factor implicated in bacteriophage lambda DNA replication, being involved in the ATP-dependent disassembly of the primary nucleoprotein complex formed at the origin of replication (reviewed by Georgopoulos et al., 1990; Ang et al., 1991). However, dnaK plays also an important part in the normal growth of *E. coli*. Very recently, by *in vitro* reconstitution experiments, dnaK, dnaJ and groEL (the latter two also being stress proteins) were demonstrated to serve as protein-folding chaperones in a strictly sequential fashion (Langer et al., 1992). In HeLa cells, hsps70 have been implicated in the normal nuclear transport of proteins (Shi and Thomas, 1992).

Also in yeast, hsps70 were found to fulfil a major function under normal conditions. Evidence was obtained that hsps70 facilitate translocation of polypeptides across the endoplasmic reticulum and mitochondrial membranes (Deshaies et al., 1988; Chirico et al., 1988). Yeast cells depleted of the hsp70 proteins encoded by *SSA1* and *SSA2* appeared to accumulate in the cytosol precursor forms of proteins that are normally destined for import into the endoplasmic reticulum and mitochondria, indicating that hsps70 are involved in post-translational import pathways.

The protein encoded by *SSD1* (identical to *KAR2*) is an homologue of mammalian BiP (immunoglobulin heavy chain binding protein) which is most likely identical to grp78 (glucose-regulated protein), initially identified as a protein induced upon

starvation for glucose (Shiu et al., 1977). *KAR2* was identified in a yeast mutant blocked in nuclear fusion after mating of haploid cells to form diploids (Normington et al., 1989; Rose et al., 1989). The function of BiP is uncertain but it appears to restrict transport of malfolded or aberrantly glycosylated secretory proteins from the endoplasmic reticulum to the Golgi body (reviewed by Deshaies et al., 1988; Pelham, 1989).

It is very likely that hsp70 proteins induced upon stress exposure perform functions similar to those under normal growth conditions. During stress, the cellular concentration of potential substrates, e.g. denatured proteins, is likely to increase, thus depleting the free pool of hsp70 and generating the need for an increase in the level of these proteins. Hsp70, therefore, has been considered as the cellular thermometer (DiDomenico et al., 1982; Craig and Gross, 1991). The intriguing possibility that hsp70 may also directly interact with the heat shock factor, thereby modulating its transcription activating potency, will be discussed below, in the section on the heat shock transcription factor HSF.

Hsp60, a chaperonin

Hsp60 proteins fulfil cellular functions that, presumably, are similar to those of hsp70. Hsp60 in yeast has been identified as a mitochondrial protein showing homology to the *E. coli* groEL protein (Cheng et al., 1989). *groEL* encodes a protein involved in bacteriophage head assembly (Sternberg, 1973; Georgopoulos and Hohn, 1978; reviewed by Zeilstra-Ryall et al., 1991). The yeast homologue was isolated as a nuclear mutation (mif4) preventing assembly of F_1-ATPase, cytochrome b_2 and the Rienke FeS protein of complex III (Cheng et al., 1989). The *HSP60* gene was found to be able to rescue the defect (reviewed by Pfanner et al., 1990). In mutants of *S. cerevisiae* defective in the constitutive expression of hsp60, incompletely processed proteins imported into the mitochondrial matrix appeared to accumulate (Cheng et al., 1989). Hsp60, therefore, most likely belongs to the proteins that facilitate post-translational assembly of polypeptides, commonly called molecular chaperones or 'chaperonins' (Ellis, 1987; Ellis and Vies, 1991). Proteins imported into the mitochondria do not fold spontaneously but need hsp60 function for proper folding (Ostermann et al., 1989; Neupert et al., 1990; Koll et al., 1992). Hsp60, consistently, acts in conjunction with ATP. Recently, another yeast protein, scj1, has been identified which is homologous to bacterial dnaJ (Blumenberg, 1991; Antencio and Yaffe, 1992), and may aid together with hsp70 in translocating proteins into the mitochondria.

Hsp83, cytoplasmic anchoring protein

Yeast hsp83 belongs to the family of hsp90 proteins. Also this family of heat shock genes encodes chaperone-like proteins. In eukaryotes, hsp90 is abundantly present in the cytoplasm; a small fraction translocates to the nucleus upon heat shock (reviewed by Lindquist and Craig, 1988; Schlessinger, 1990). Hsps90 have been found to interact with a variety of cellular proteins, including glucocorticoid receptors, several kinases and the cytoskeleton proteins actin and tubulin.

In particular, complex formation between hsp90 and the steroid receptors has been studied in detail (reviewed by Hunt, 1989). The receptor proteins are kept in the cytoplasm in an inactive conformation through their interaction with hsp90. Hsps90, therefore, serve as cytoplasmic anchoring proteins. Upon hormone binding to the receptor, hsp90 is released and the hormone–receptor complex moves into the nucleus and acts as a transcription factor through specific responsive nucleotide elements, GREs.

The structure of hsps90 is highly conserved from bacteria to man and shows among eukaryotes at least 50 % sequence identity. Two notable regions with a very high content of negative charges are present in all eukaryotic hsp90 proteins. In addition, the C-terminal sequence of the protein, which in itself is rather divergent, uniformly ends with an EEVD motif of unknown function.

S. cerevisiae contains two genes encoding hsp90: *HSP83* and *HSC83* (Borkovich, 1989). *HSC83* ('heat shock cognate') is a constitutively expressed gene and is only weakly induced upon stress exposure. *HSP83* is expressed at a much lower basal level and is strongly activated upon heat treatment. Notably, expression of this gene is also induced when cells enter the stationary phase (Kurtz and Lindquist, 1984) or sporulate (Kurtz et al., 1986).

Though yeast probably does not naturally respond to steroid hormones, the glucocorticoid receptor from mammalian cells can act in yeast (Metzger et al., 1988; Schena and Yamamoto, 1988). Recently yeast strains were designed in which hsp90 expression can be regulated (Picard et al., 1991). At low levels of hsp90, aporeceptors appeared to be mostly hsp90-free. Yet, they failed to enhance transcription from a GRE-containing promoter. On hormone addition the receptors were activated, but with a markedly reduced efficiency as compared with cells possessing normal hsp90 levels. Therefore, hsp90 seems to facilitate the response of the receptor to the hormone signal. Apparently hsp90 acts in the signal transduction pathway for steroid receptors.

Hsp104, protector of nucleoli?

Much less is known with regard to the cellular function of heat shock proteins with a molecular mass greater than 100 kDa. Hsp110 in mouse has a nuclear localization, and is predominantly present in the nucleoli (Subjek, 1983). It was found to be associated with the fibrillar component of nucleoli, which, most likely, is the site of rDNA. This finding suggests that it may bind to rRNA. It has been proposed, therefore, that upon heat shock hsp110 is induced to protect ribosome formation, a process very sensitive to heat stress (Nover et al., 1986; see below, in the section on processes affected by stress).

A yeast gene, *HSP104*, belonging to this family has been isolated (Sanchez and Lindquist, 1990) and sequenced (Parsell et al., 1991). Hsp104 protein is not detectable at normal growth on fermentable carbon sources, is constitutively synthesized in respiring cells (Sanchez et al., 1992), and is strongly induced following a heat shock. Expression of this protein is also activated when cells enter the stationary phase or are induced to sporulate. Like in other eukaryotes, it may be a nucleolar protein to which an important function in the acquisition of stress tolerance has been assigned (see below). Two putative sites showing homology with a nucleotide consensus binding site have been identified in hsp104. By site-directed mutagenesis these sites were shown to be essential for the stress-protective function of this protein (Parsell et al., 1991). Hsp104 displays a striking similarity to the highly conserved ClpA/ClpB protein family, first identified in *E. coli* and supposed to possess ATP-dependent protease activity (Parsell et al., 1991).

Small hsps: hsp26 and hsp12

Yeast cells contain two small hsps: hsp26 and hsp12. The small hsps form a very diverse group which, nevertheless, display

conserved structural elements (reviewed by Lindquist and Craig, 1988) and share the ability to form highly polymeric structures referred to as heat shock granules, HSGs (reviewed by Tuite et al., 1990). They show a notable and significant sequence similarity to the eye lens protein α-crystallin, in particular with respect to a highly conserved hydrophobic domain located at the C-terminus of these proteins (Tuite et al., 1990).

Hsp26 from yeast (Petko and Lindquist, 1986; Kurtz et al., 1986; Bossier et al., 1989) also is able to self-aggregate. The HSGs have a native molecular mass of 550 kDa, and contain about 20 copies of the protein (Tuite et al., 1990). Hsp26 aggregates probably accumulate in the perinuclear region of the cell (Nover et al., 1983; Leicht et al., 1986).

A universal property of the small hsps may also be their developmental regulation. Indeed, both yeast genes belonging to this group, *HSP26* and *HSP12* (Praekelt and Meacock, 1990) show, apart from a very strong stress-induction, also a dramatically increased expression following transition of cells to the stationary phase and upon induction of sporulation. The cellular role of these proteins, however, has yet to be elucidated.

Ubiquitin, mediator of proteolysis

Polyubiquitin, a protein encoded by the *UBI4* gene in yeast, is generally considered as a heat shock protein since it displays a strongly enhanced rate of synthesis under stress conditions (Finley et al., 1987). Selective, nonlysosomal proteolysis is mediated by the post-translational ubiquitination pathway (reviewed by Finley and Varshavsky, 1985; Hershko, 1988; Jentsch et al., 1990). Ubiquitin is a protein consisting of 76 amino acids which is found in eukaryotes either free or covalently bound to various other proteins. Examples of ubiquitinated proteins in mammalian cells are histones H2A and H2B, denatured globin and the platelet-derived growth factor receptor.

Attachment of ubiquitin to proteins occurs through its C-terminal glycine residue, in a series of steps: binding of ubiquitin to enzyme E1, transfer of activated ubiquitin to several conjugating enzymes E2 and, finally, joining of ubiquitin to specific proteins, sometimes through E3. Conjugation of ubiquitin to proteins can trigger their degradation. In yeast, *RAD6* has been identified as a gene encoding an E2-like enzyme, amongst others, involved in ubiquitination of histone H2B (Jentsch et al., 1987; Sung et al., 1988). A second ubiquitin carrier protein, showing sequence homology with rad6, has been identified as cdc34, a protein required for G_1 to S phase transition (Goebl et al., 1988).

Yeast ubiquitin differs in only three amino acids from mammalian ubiquitin (Finley and Varshavsky, 1985) and is encoded by a multigene family: *UBI1*, *UBI2*, *UBI3* and *UBI4*. *UBI1*, *2* and *3* encode hybrid proteins (Finley et al., 1989; Redman and Rechsteiner, 1989; Müller-Taubenberg et al., 1989; Özkaynak et al., 1987). Strikingly, in yeast, *UBI3* is fused to the gene for the small ribosomal subunit protein S37 (Finley et al., 1989), while the tail in the *UBI1*-hybrid gene encodes a so-far-unidentified protein of the large ribosomal subunit. It has been postulated that the ubiquitin moiety of the fusion protein stabilizes these ribosomal proteins during their synthesis and transfers them to the site of preribosome assembly in the nucleolus.

UBI4 (consisting of five head-to-tail arranged ubiquitin-encoding repeats) codes for a polyubiquitin precursor protein. Deletion of *UBI4* gives rise to mutants that are viable at vegetative growth conditions (Finley et al., 1987). These cells contain normal concentrations of free ubiquitin. However, these mutants were found to be very sensitive to high temperatures and other kinds of stress, such as starvation or the addition of amino acid analogues. A single ubiquitin unit put under control of the *UBI4* promoter can complement for the defect. These results indicate that *UBI4* provides ubiquitin monomers after processing of the precursor. In addition, these data suggest that ubiquitin is an essential component of the stress response system. Indeed *UBI4* (but not *UBI1*, *2* or *3*) was found to be a heat shock gene showing a rapid induction upon a temperature shift (Finley et al., 1987). Most likely, stress leads to a sudden increase in the level of damaged or denatured proteins which are very toxic for the cell. As a consequence of this effect there may be a need for excess ubiquitin against the depletion by the formation of ubiquitin–protein conjugates. Indeed, increased *UBI4* transcription was found to be triggered by the synthesis of abnormally high levels of aberrant polypeptides (Grant et al., 1989). At present it is unknown whether such ubiquitinated proteins are all degraded or are restored to their native conformation.

Other proteins involved in yeast stress response

Apart from the classical hsps discussed above, in yeast, as in other cells, several proteins have been shown or suggested to play a part in the stress response. Some of them exhibit significantly increased levels of expression following a stress treatment.

In the previous section the importance of proteolytic breakdown for cell survival has been indicated. It is not surprising, therefore, that a major protease in yeast, proteinase YscE, also appears to be involved in the stress response (Heinemeyer et al., 1991). YscE is an RNA-associated protein complex, composed of various subunits which mediate the non-lysosomal pathway of degradation of ubiquitinated proteins ('proteasome'). The gene *PRE1*, encoding a 22 kDa subunit of YscE has been isolated (Heinemeyer et al., 1991) and strains carrying a mutation in *PRE1* display enhanced sensitivity to stress. In these mutant cells protein degradation is decreased and ubiquitin–protein conjugates accumulated. It has not been reported whether expression of this gene may be induced upon stress.

Related to the occurrence of oxidative stress in yeast is the finding that catalase T is a protein whose rate of synthesis is controlled by heat shock (Belazzi et al., 1991). Catalase T is a cytosolic enzyme, encoded by the *CTT1* gene, which may contribute to protect cells against the adverse effects of heat shock. *CTT1* belongs to the genes that, in addition, are under negative cyclic AMP control (see below in this section).

In addition, several enzymes of the glycolytic pathway are induced upon heat treatment of yeast cells. One of the (three) genes encoding glyceraldehyde-3-phosphate dehydrogenase (hsp35) is induced following a temperature shock (Lindquist and Craig, 1988). Under normal conditions this enzyme is already abundantly present in yeast cells. The energy stress imposed onto cells by a heat shock may cause the observed drop in ATP levels (Findley et al., 1983). A further increased enzyme synthesis at high temperature, therefore, is beneficial, because it enables the cell to increase the rate of glycolysis, thereby restoring the intracellular level of ATP. Consistent with this assumption is the finding that two other glycolytic enzymes are also induced: enolase (eno, hsp48; Iida and Yahara, 1985) and phosphoglycerate kinase (pgk; Piper et al., 1986). An alternative explanation for the heat-induced increase of these glycolytic enzymes is that stress may damage membrane structures, resulting in a disruption of the normal coupling between electron transport and oxidative phosphorylation (Patriarca and Maresca, 1990). Notably, heat shock does not induce the gene for pyruvate kinase (pyk) which catalyses the second ATP-generating step in glycolysis (Piper et al., 1988).

An additional consequence of a stress challenge on yeast cells

is the transient dissipation of the electrochemical pH gradient across the plasma membrane, leading to a decrease in the internal pH of the cell (Weitzel et al., 1987; Coote et al., 1991). The stress-induced intracellular acidification may play a (direct or indirect) role in triggering the stress response (Coote et al., 1991; see the final section). Under normal growth conditions the pH gradient is sustained by the action of an ATP-driven proton pump, the plasma membrane ATPase. Plasma membrane ATPase is an abundant trans-membrane protein showing a highly conserved structure among eukaryotes (Aaronson et al., 1988). Maintenance of the proton gradient over the plasma membrane is essential for control of intracellular pH and nutrient uptake. It is relevant, therefore, that the *PMA* gene encoding this protein displays a sustained expression upon heat shock (Panaretou and Piper, 1990). Analysis of membranes from stressed yeast cells revealed a 30 kDa heat shock protein that may be related to plasma membrane ATPase function (Piper et al., 1990; Panaretou and Piper, 1992). Recently another stress-induced gene, *TIPI*, was identified encoding a protein presumably located at the outside of the plasma membrane (Kondo and Inouye, 1991). This gene was isolated as a cold-shock inducible gene but it displays also a heat-stimulated transcription activation.

Several lines of evidence suggest that cyclic AMP metabolism is intimately involved in the stress response. Upon heat treatment, intracellular cyclic AMP levels increase (Boutelet et al., 1985; Canonis et al., 1986; reviewed by Piper et al., 1990). Increase in cyclic AMP is correlated with a stimulation of the plasma membrane ATPase discussed above (Goffeau and Slayman, 1981; Serrano, 1983). On the other hand, a decline in cyclic AMP (and probably a consequent decrease in cyclic AMP-dependent protein phosphorylation) was found to trigger the synthesis of several heat shock proteins (Iida and Yahara, 1984; Shin et al., 1987). As a consequence of the decrease in cyclic AMP when cells enter the stationary phase, for instance, the *SSA1* (Brazzell and Ingolia, 1984), *HSP26* (Tuite et al., 1990), *HSP12* (Praekelt and Meacock, 1990) and *UBI4* (Finley et al., 1987; Tanaka et al., 1987) genes show enhanced expression. These results indicate that the pertinent *HSP* genes are under transcriptional control of two opposed signals (see below). An additional noteworthy fact is the isolation of cyclic AMP-defective mutants that show remarkable increases in stress tolerance as compared with normal cells (Iida, 1988).

Another process closely related to the stress response in yeast cells is the synthesis of trehalose. Yeast cells exponentially growing on glucose contain little trehalose (Thevelein, 1984). In spores, however, this disaccharide is accumulated, which strongly suggested its role as an energy source. Strikingly however, an enormous accumulation of trehalose (up to 100-fold) also occurs in response to a heat shock from 27 °C to 40 °C (Hottiger et al., 1987), whereas a decrease occurred when cells were shifted back to the lower growth temperature. The biosynthesis of trehalose involves two steps (reviewed by Thevelein, 1984); UDP-glucose and glucose 6-phosphate form trehalose 6-phosphate, a reaction catalysed by trehalose-6-phosphate synthase. Phosphate is then cleaved off by trehalose-phosphatase. Degradation of trehalose is mediated by trehalase, which occurs in two forms: a regulatory and a non-regulatory enzyme. The activity of neutral trehalase is thought to be regulated by cyclic AMP-dependent protein phosphorylation. In agreement with the observed dramatic accumulation of trehalose upon stress exposure, the activity of trehalose-6-phosphate synthase was found to increase 6-fold under these conditions (Hottiger et al., 1989). Surprisingly, however, also the activity of neutral trehalase is increased by a factor of 3. Obviously, turnover of trehalose is a fast process at shift-conditions. For this reason a protective role of trehalose for proteins and membranes and, thus, in maintaining the structural integrity of the cell, has been suggested (Hottiger et al., 1989; Wiemken, 1990).

It is likely that future investigations will uncover other stress-induced genes, in particular when other stress agents than heat are studied. For instance, recently metallothioneins, involved in heavy metal homeostasis and detoxification (Hamer, 1986) have been implicated in the heat shock response (Silar et al., 1991; Yang et al., 1991), and very recent evidence indicates the occurrence in yeasts of a heat-induced secretory protein (hsp150) with a so-far-unidentified function (Russo et al., 1992).

STRESS-INDUCED CHANGES IN YEAST GENE EXPRESSION

Heat shock transcription factor, HSF

In prokaryotes, the heat shock response is mediated by a specific heat-induced σ-factor, σ^{32}, that binds to core RNA polymerase and directs it to heat shock promoters (Grossman et al., 1984; Landick et al., 1984). Regulation of the level of σ^{32} is accomplished by several control mechanisms. The σ^{32} gene, *rpoH*, is constitutively transcribed by the normal (σ^{70}-containing) RNA polymerase, but the protein is extremely unstable. Upon heat shock, transcription of *rpoH* is induced (Tilly et al., 1989), the translation efficiency of the σ^{32} mRNA is increased and the protein is stabilized (Straus et al., 1987). The activity of σ^{32} may be negatively modulated by interaction with dnaK (Liberek et al., 1992), whereas, in addition, dnaJ and grpE were shown to be physically associated with this heat shock transcription factor (Gamer et al., 1992).

In eukaryotes, transcription of heat shock genes is regulated through the action of the heat shock transcription factor, HSF, which interacts with its cognate nucleotide element, the heat shock responsive element HSE (reviewed by Sorger, 1991). In higher eukaryotes, HSF binds to HSEs only after heat induction (Sorger et al., 1987), whereas, in contrast, yeast HSF was found to interact with HSEs irrespective of the transcriptional state of the *HSP* genes (Jakobsen and Pelham, 1988; Gross et al., 1990). HSF binds to the DNA as a trimer (Perisic et al., 1989; Sorger and Nelson, 1989) and, in addition, the binding of trimers to adjacent sites is highly co-operative, thus preferentially forming large complexes.

The gene encoding HSF has been isolated from the yeasts *S. cerevisiae* (Wiederrecht et al., 1988; Sorger and Pelham, 1988) and *Kluyveromyces lactis* (Jakobsen and Pelham, 1991) as well as from *Drosophila* (Clos et al., 1990), tomato (Scharf et al., 1990) and human (Schuetz et al., 1991; Rabindran et al., 1991). Tomato cells contain at least two HSFs. Human cells also contain two HSF genes but the relative levels of HSF1 and HSF2 are not yet known.

In contrast with the high conservation of the HSF-responsive element, HSFs from different organisms show little sequence similarity. The HSFs from the distantly related *S. cerevisiae* and *K. lactis*, for instance, share only 18 % amino acid identity, the similarity being mainly confined to the DNA-binding domain and the trimerization domain (see below).

HSF from *S. cerevisiae* is a protein consisting of 833 amino acids which is encoded by a single, essential gene (Sorger and Pelham, 1988; Wiederrecht et al., 1988; reviewed by Sorger, 1991). The DNA-binding region is located within residues 167–284 (Wiederrecht et al., 1988) and a further domain, encompassing residues 327–424, involved in trimerization, is required for high-affinity association with DNA (see Figure 1). The DNA-binding domain does not show a DNA-binding motif similar to other eukaryotic transcription factors; only a short match to the putative DNA-recognition helix of bacterial σ-

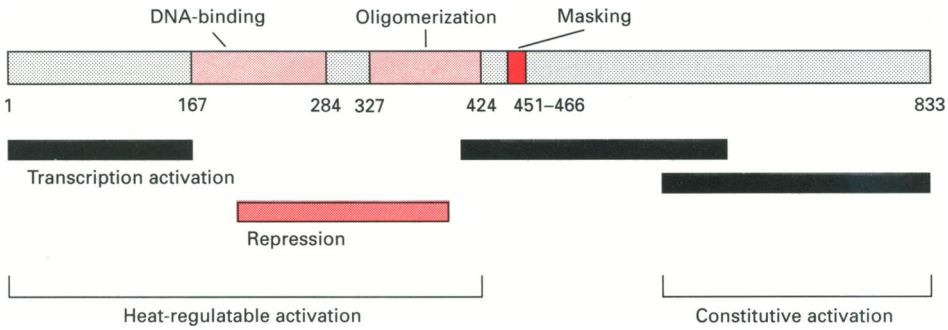

Figure 1 Domain structure of the heat shock factor HSF

The black bars indicate domains that can confer transcription activation to an heterologous, inactive DNA-binding domain (Sorger, 1990; Nieto-Sotelo et al., 1990).

factors can be distinguished (Clos et al., 1990). The trimerization domain, on the other hand, has been suggested to form a three-stranded α-helix coiled coil (Sorger and Nelson, 1989) on the basis of a leucine/isoleucine repeat structure.

Apparently, in higher eukaryotes activation of HSF to a potent transcription factor requires first induction of DNA-binding and subsequently activation, e.g. by phosphorylation (Larson et al., 1988). In yeast, however, unactivated HSF is already bound to DNA under non-shock conditions and, therefore, only the conversion of HSF into a form capable of efficiently stimulating transcription is required upon shock.

In order to unravel the mechanism by which transcription activation by HSF occurs, it should be recognized that HSF not only plays a part in heat shock-induced transcription but also in basal-level, constitutive ('sustained') transcription (Sorger, 1990). For instance, overexpression of HSF in yeast at normal growth conditions significantly increases the levels of a major hsp70 (Sorger and Pelham, 1988). In addition, as will be discussed below, 50–80 % of the basal expression of the hsp70 gene *SSA1* is mediated through HSEs.

Two studies have described the functional domains in yeast HSF (Sorger, 1990; Nieto-Sotelo et al., 1990). In one study, truncated HSF proteins were introduced into cells in the absence of wild-type factor and it was found that the heat shock-induced, characteristically transient, activity of HSF and its sustained activity are mediated by physically separable transcription-activating domains (Sorger, 1990; see Figure 1). The N-terminal region of the protein, residues 1–424, mediates the transient increase of HSF activity, required for growth at elevated temperatures. Analysis of lexA–HSF fusion proteins did not reveal a particular subregion of this domain capable of conferring the transcription activating function. Rather, integrity of the entire domain seems to be essential for proper function (Sorger, 1990). A C-terminal region consisting of amino acids 584–783 is essential for sustained increase. This activating region was unmasked by deletion of N-terminal residues, resulting in a 40-fold increase of HSF activity in the absence of a heat shock (Sorger, 1990). Both activities, transient as well as sustained, are associated with a rise in the extent of phosphorylation of HSF. Up to ten Ser/Thr sites are phosphorylated at 20 °C and an additional five at 39 °C (Sorger, 1990). It is doubtful, however, whether phosphorylation plays a major functional role in transcription activation. Recently evidence was obtained that phosphorylation of *trans*-activators may occur as a consequence of the formation of a transcription initiation complex (due to exposure of the target sites) rather than as a prerequisite for this formation (Sadowski et al., 1991).

In the second study (Nieto-Sotelo et al., 1990) residues 1–63 were demonstrated to be necessary for growth under heat shock conditions. Between amino acids 208 and 648 a transcription activation domain was found to be present. Consistently, fusion of this domain to the DNA-binding domain of yAP1 generated a hybrid temperature-regulatable transcription factor. A constitutive activation domain was shown to occur between residues 410 and 648. Notably, a third element could be distinguished, between residues 208 and 394, which is responsible for repression of the transcription-activating domain under non-shock conditions (Nieto-Sotelo et al., 1990). This region encompasses part of the DNA-binding domain and the Leu repeat.

HSF from *S. cerevisiae* can functionally substitute for HSF from *K. lactis* (Jakobsen and Pelham, 1991). The repressable activation domain of both proteins shows little sequence similarity. On the other hand, the region involved in masking the activity at low temperatures contains, apart from the evolutionarily conserved DNA-binding and oligomerization domains, an additional sequence similarity: a short conserved element, RXLLKNR, located near the activator region (Jakobsen and Pelham, 1991). Very recently, by domain-swapping, deletion, and mutagenesis experiments, the importance of the central evolutionarily conserved domain in keeping HSF unactivated under non-shock conditions was confirmed (Bonner et al., 1992): a hybrid HSF–VP16 factor appeared to be as temperature-regulatable as HSF itself. The putative masking element (Jakobson and Pelham, 1991) contributed to the regulation mechanism. Adjacent to this element a run of serine residues is present. Replacing these serines by alanine or aspartic acid, however, had no effect on regulation *in vivo*. The pertinent conserved element may either bind to the structural core of the protein or to another protein, thus keeping the activator in an unactivated conformation under non-shock conditions. An appealing model is that hsp70 may serve as a repressor. Upon heat shock the demand for hsps70 strongly increases as a consequence of the elevated level of 'thermally-damaged' proteins (see above). This may lead to the dissociation of the hsp70–HSF complex, thereby derepressing its transcription-activating activity (see Figure 2). Increased synthesis of hsp70 may result in re-association of the protein with HSF, which would explain the transient nature of the response (Morimoto et al., 1990; Sorger, 1991). As mentioned before, hsp70 has as such been proposed to serve as the cellular thermometer (Craig and Gross, 1991). However, direct evidence to support this model is still missing.

Within the context of transcription activation of heat-induced genes, three unexpected recent findings deserve some comment.

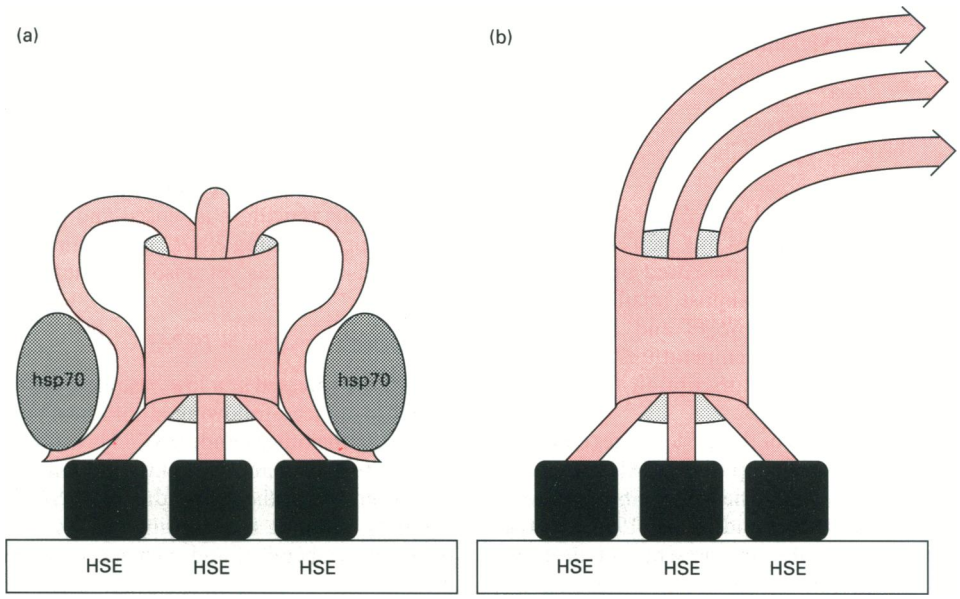

Figure 2 Model for the activation of the heat shock factor HSF

Under non-stress conditions, HSFs, bound to the HSEs as a trimer, are kept in an unactivated conformation through the interaction with hsps70 (**a**). Upon stress exposure hsp70 is released from the complex, thus enabling the factor to change into an activating-potent form (**b**).

First it was shown that HSF activates transcription of the yeast metallothionein gene (Silar et al., 1991; Yang et al., 1991). This gene, *CUP1*, is essential to prevent copper toxicity (Hamer, 1986). Metal (Cu or Ag)-induced transcription activation of *CUP1* is regulated by a specific *trans*-acting factor, ACE1, which binds to *cis*-acting metal-responsive elements in the promoter of *CUP1* (Furst et all., 1988; Furst and Hamer, 1989). Mutant strains have been isolated which are able to suppress the requirement for ACE1 in the activation of *CUP1*. Surprisingly, this suppressor gene appeared to encode a mutant form of HSF. A single amino acid substitution in the DNA-binding domain of HSF was found to strongly enhance transcription of *CUP1*, while it reduced *SSA3* gene expression. In the promoter of *CUP1* GAA repeats occur which, however, are spaced by three and four nucleotides. The results suggest HSF to be involved in transcriptional regulation of genes other than *HSP* genes. Secondly, a protein containing an amino acid sequence extensively similar to the DNA-binding domain of HSF has been identified as a suppressor of flocculation (*SFL1*; Fujita et al., 1989). This finding may reflect the presence in yeast cells of a family of (evolutionarily) related protein factors. Finally, evidence has been obtained for an HSF-independent mechanism of heat shock-induced transcription activation. In *S. cerevisiae* a set of genes has been identified which display high levels of expression after treatment with DNA-damaging compounds or heat. For one of those so-called DNA-damage response (*DDR*) genes, *DDR A2*, heat induction leads to a 20-fold increase in transcription (McClanahan and McEntee, 1986). The upstream region of the *DDR A2* gene harbours an element required for this stress regulation (Kobayashi and McEntee, 1990). Deletion of this element (−202 to −165) abolished heat-induced transcription and, in addition, this element can confer heat shock-induction onto a reporter gene. An element homologous to this region occurs in the promoter of *UBI4*, which also belongs to the *DDR* family. No sequence similarity with the HSE exists and it does not bind HSF. These data suggest the striking possibility that, in addition to HSF, at least one other factor can mediate heat-induced transcription activation.

Heat shock responsive element, HSE

It has been well established that in yeast, as in other eukaryotes, a specific *cis*-acting promoter element mediates the heat shock response on transcription. This heat shock responsive element, HSE, represents the binding site for the *trans*-acting transcription factor HSF. The HSE was originally defined for *Drosophila HSP* genes as the sequence CNNGAANNTTCNNG, displaying a dyad symmetry characteristic for binding sites of multimeric DNA binding proteins (Parker and Topol, 1984). Later on, the definition has been revised on the basis of results obtained with site-directed mutagenesis (Xiao and Lis, 1988). Functionally-defined HSEs should be considered as modular elements encompassing at least three repeats of the sequence GAA in alternating orientations and separated from each other by two nucleotides. A gap of 5 bp between two modules is tolerated provided that the elements flanking the gap are direct repeats. *In vitro*, the minimal DNA sequence required for the formation of a stable complex with *Drosophila* HSF is an inverted 5 bp-repeat, GAA, irrespective of whether it has a tail-to-tail or a head-to-head arrangement. The aforementioned data have led to the model of trimerization of HSF binding to the DNA (Perisic et al., 1989; Sorger and Nelson, 1989).

Comparison of yeast HSEs indicates that they are composed of three to five appropriately positioned GAA-modules (Boorstein and Craig, 1990). On the other hand, a single HSE is sufficient to confer heat-inducible expression onto a reporter gene (Kirk and Piper, 1991), although in general multiple HSEs act co-operatively to mediate transcription activation of heat shock genes.

The conserved nature of the *cis*/*trans*-combination HSF–HSE emphasizes the general evolutionary conservation of tran-

scriptional activation mechanisms among eukaryotes (reviewed by Guarente and Bermingham-McDonogh, 1992).

Transcription regulation of HSP-genes

Transcription of *HSP70*

The yeast hsp70-encoding genes *SSA1* and *SSA2* show a significant basal level of expression (reviewed by Craig et al., 1990). Only *SSA1* gene expression is strongly increased upon a mild heat shock; *SSA2* gene expression is almost unaffected by a stress treatment. On the other hand, *SSA3* and *SSA4* gene products are barely detectable at normal temperatures but display a very strong increase in the rate of transcription following a temperature upshift (Craig et al., 1990).

The upstream DNA region of each of these *SSA* genes contains multiple sites showing a close relationship to the HSE consensus sequence, which are sufficient to confer heat-inducibility onto a reporter gene (reviewed by Boorstein and Craig, 1990). In the *SSA1* promoter a number of HSEs are present but deletion analysis indicated that only one of these ('HSE2') is most important for the heat-inducible expression (Slater and Craig, 1987). Removal of 'HSE3', located further upstream and having a perfect match with the consensus, did not significantly affect heat-inducible expression of *SSA1*. 'HSE2' can confer heat shock-induced transcription onto a reporter gene. However, the basal level of expression conferred by this element was found to be considerably decreased when 'HSE2' was flanked by its original surrounding sequences (Park and Craig, 1989). This finding suggested the involvement of a negative regulatory element which, by deletions and point mutations, indeed could be shown to overlap this HSE (Park and Craig, 1989). This URS ('upstream repression site') has some sequence homology with a URS found in other yeast gene promoters (Luche et al., 1990). Consistently, mutations in the URS resulted in elevated levels of basal expression. For basal level expression of *SSA1* the heat shock factor HSF appeared to be essential (Park and Craig, 1989).

The HSE elements in the promoter of the *SSA3* gene are also involved in enhancement of the level of expression at heat shock conditions (Boorstein and Craig, 1990). In addition this gene is transcriptionally induced under conditions of lowered intracellular cyclic AMP, such as starvation.

SSA4 is the only canonical *HSP70* gene in yeast showing an extremely low level of expression at normal growth conditions and dramatically induced levels upon heat shock (Boorstein and Craig, 1990). Again an upstream region containing an extended match to the conserved HSE is necessary and sufficient for heat-inducible regulation.

Yeast cells bearing mutations in *SSA1* and *SSA2* display a high basal level of expression of other heat shock proteins even at a normal (23 °C) growth temperature (Craig and Jacobsen, 1984). In addition, it has been established that transcription activation of *SSA4* in these *ssa1/ssa2* mutants is mediated by the HSE in its promoter (Craig and Jacobsen, 1984).

These data lend support to the hypothesis that hsp70 may act as an autoregulator: it inhibits, directly or indirectly, its own expression. As discussed above, a tentative model presumes that hsp70 exerts its negative feed-back through the heat shock factor HSF.

Very recently, three independently acting *cis*-elements in the promoter of the *KAR2* gene were identified (Mori et al., 1992). In addition to a heat shock responsive element (HSE) and a GC-rich region functionally involved in the high constitutive rate of transcription of this gene, a 22 bp sequence was discovered which mediates the response to unfolded proteins accumulating in the endoplasmic reticulum. This so called UPR-element is able to confer responsiveness to unfolded proteins onto an heterologous gene and its structure is similar to that of regulatory regions of the homologous mammalian genes (Mori et al., 1992). UPR turned out to represent a specific (*in vitro*) binding site of a protein factor. How the presence of unfolded proteins in the endoplasmic reticulum is sensed and may lead to the activation of a factor regulating *KAR2* gene transcription in the nucleus is an intriguing but as yet unsolved question.

Transcription regulation of *HSP83*

HSP83 is expressed at a low basal level at normal conditions and is highly induced following a mild heat shock (Borkovich et al., 1989). On the other hand, *HSC83* is expressed at a 10-fold higher constitutive level and is slightly induced upon a heat challenge. The promoter of the heat-inducible *HSP83* has been analysed in detail by genomic footprinting (Gross et al., 1990a,b). A combination of chemical and enzymic footprinting techniques was used to obtain a high resolution map. The *HSP83* promoter contains three HSEs that fully match the consensus sequence (Farelly and Finkelstein, 1984). Only one of these putative HSF-binding sites, the most proximal 'HSE1', appeared to be bound by a protein *in vivo*, within the major groove of the DNA (Gross et al., 1990a,b). In addition, the TATA box was found to be occupied, most likely by the basal transcription factor TFIID, and in this case binding occurs to the sugar-phosphate backbone of the DNA. Both protein-binding elements are on one side of the helix causing a marked local distortion of the DNA in the chromatin, which is in agreement with the general view that protein–protein interactions play a major part in transcription activation (Ptashne, 1988).

Binding of HSF to 'HSE1' was found to be irrespective of the transcriptional state – basal or induced – of the *HSP83* gene, which is in agreement with the finding that this HSE is also required for basal level expression of the *HSP83* gene (McDaniel et al., 1989). Moreover, mutational analysis of 'HSE1' confirmed that this element is absolutely required for both basal and induced expression (Gross et al., 1990a). On the other hand, some point mutations were preferentially found to reduce constitutive expression of *HSP83* and had no effect on the heat-inducible expression (Gross et al., 1990a). Therefore, HSEs may exist in functionally distinct subclasses. This is also emphasized by genomic footprinting showing that 'HSE2' and 'HSE3' are vacant, probably reflecting a lower affinity of HSF for these sequences.

Similar experiments performed for the *HSC83* gene revealed, in addition to a TATA box and a heat shock element ('C.HSE1') another factor-binding site 40 bp upstream of 'C.HSE1' (Gross et al., 1990a,b). Perhaps the 10-fold higher basal level of expression of *HSC83* as compared with *HSP83* is due to this (so far) unknown factor.

Transcription regulation of *HSP26*

HSP26 gene expression is strongly enhanced following a heat shock (Petko and Lindquist, 1986), during stationary phase growth (Kurtz et al., 1986) and upon induction to sporulation (Rossi and Lindquist, 1989). Several deletion and insertion mutations have been analysed (Susek and Lindquist, 1990) which demonstrated that regulation of *HSP26* gene expression primarily takes place at the transcriptional level. The promoter of *HSP26* has quite complex characteristics and, unlike the *HSP* promoters discussed above, appears to be composed of both

repressing and activating elements. In the DNA region up to −500 matches to the consensus HSE sequence are present but none of them are essential for heat shock-induced transcription activation. They might act in a co-operative fashion (Susek and Lindquist, 1990).

Evidence for the presence of a transcriptional repressing element was provided by the finding that several upstream promoter deletions rendered *HSP26* expression strongly constitutive. Moreover, an upstream DNA fragment (−501 to −332) is able to confer transcriptional repression to an heterologous gene. This finding has led to the tempting proposal that the main features of the regulation of *HSP26* expression involve repression of basal transcription activity under normal conditions and derepression at heat shock conditions. This mechanism would significantly differ from the mode of transcription regulation of other heat shock genes in yeast (Susek and Lindquist, 1990). No distinction could be made so far between *cis*-acting elements involved in heat-shock control and those implicated in developmental regulation.

Transcription regulation of *HSP12*

HSP12 shows a pattern of gene expression that is quite similar to that of *HSP26* (Praekelt and Meacock, 1990). *HSP12* is also developmentally regulated and is strongly induced upon heat shock. During exponential growth, hsp12 mRNA levels are low, but following a temperature shift the concentration increases several-hundred-fold.

The gene may be regulated both by heat shock and by cyclic AMP, as evidenced by analyses of mutants defective in cyclic AMP-dependent protein phosphorylation (Praekelt and Meacock, 1990). In a *cyr1-2* mutant containing low intracellular levels of cyclic AMP, high levels of hsp12 mRNA are present during exponential growth. In contrast, in a *cyr1-2,bcy1* double mutant, whose protein kinase is constitutively active, these high levels do not occur. Cyclic AMP-dependent protein kinase may regulate both positive and negative transcription factors by phosphorylation (Tanaka et al., 1988) and, thus, control the level of *HSP12* expression. Notably, in minimal medium *HSP12* displays a low level of induction upon heat shock, which may be correlated with the low levels of cyclic AMP under those conditions. Evidence is available that, similar to the *HSP26* gene, also the *HSP12* promoter is activated upon a stress challenge by a derepression mechanism (P. A. Meacock and U. M. Praekelt, unpublished results; J. Varela and W. H. Mager, unpublished results).

Transcription regulation of the phosphoglycerate kinase gene

Levels of the phosphoglycerate kinase mRNA in *S. cerevisiae* are increased about 6-fold by transcriptional activation when fermentative cultures are subjected to a heat shock (Piper et al., 1986). The promoter of the *PGK* gene was searched for the presence of HSE elements and two imperfect matches with the consensus sequence were found at −165 and −365. The element at −365 seems to be functionally involved in heat-induced transcription activation (Piper et al., 1988). This HSE is located adjacent to the major upstream activation site (UAS) of the *PGK* gene, a binding site for the abundant protein factor RAP1 at about −460 (Chambers et al., 1990). The effect of the putative HSE on phosphoglycerate kinase mRNA synthesis is only evident when the UAS is deleted (Piper et al., 1988) since in the presence of the UAS, heat shock had a relatively small effect on phosphoglycerate kinase mRNA levels. The role of the HSE may be to sustain transcription of the *PGK* gene when, following a stress challenge, transcription of non-heat shock genes is suddenly arrested. The effect of heat shock on *PGK* gene expression appeared to be dependent on the carbon source as only fermentative cultures show elevated phosphoglycerate kinase mRNA levels (Piper et al., 1988). Using glycerol as a carbon source heat shock-induction did not occur, but addition of glucose rapidly induced the ability to enhance phosphoglycerate kinase mRNA levels. These data indicate a catabolite control of the induction phenomenon.

Processes affected by stress

Heat damages a wide variety of cellular processes and cellular structures. As stated in the introduction, stress exposure dramatically changes the pattern of gene expression. Apart from the increased levels of gene expression discussed above, transcription of many genes is transiently inhibited upon a temperature shock but it is unknown how this sudden arrest of transcription is brought about. Perhaps an essential component of the transcription machinery is extremely sensitive to stress. An alternative explanation is that conformational changes in chromatin occur which cause the arrest. It is striking that the HSF-mediated transcription activation can escape the inhibition.

Studies of two nuclear processes, ribosome formation and pre-mRNA splicing, point to drastic changes in nucle(ol)ar structure. It has been observed that ribosome assembly in mammalian cells is quite sensitive to a temperature stress and that rRNA processing slows down after heat shock leading to the accumulation of precursor rRNA (Sadis et al., 1988). In yeast, a strong inhibition of the rate of synthesis of rRNA has been found upon heat treatment (Veinot-Drebst et al., 1989). In addition, splicing of mRNA precursors was demonstrated to be disrupted by a severe heat shock (reviewed by Yost et al., 1990; Yost and Lindquist, 1991). A mild heat treatment prior to a shift to severe temperatures protects splicing. During such a pre-treatment no protein synthesis is required, but protein synthesis is needed for a rapid recovery of the splicing process after a sudden severe heat shock.

Notably, in *Drosophila*, *HSP83* is the only *HSP* gene that contains an intron. Indeed, following a stress challenge, pre-mRNA for hsp83 accumulates (Yost and Lindquist, 1985). The inhibition of splicing at high temperatures may explain why intron-less *HSP* genes have evolved. Perhaps *HSP83* has escaped from this selection because of the abundance of its gene product at normal conditions (Yost et al., 1990).

For mammalian cells a role of heat shock proteins in protection and repair of ribosome assembly and spliceosome assembly has been implicated. Upon heat shock, hsp70 migrates into the nucleus in order to associate with polypeptides that form insoluble complexes at increased temperatures (Pelham, 1990). Hsp70 also moves into the nucleolus to associate with partially assembled ribosomes (Munro and Pelham, 1985). Presumably nuclear proteins become partially denatured upon a temperature shock hence exposing hydrophobic regions that tend to interact and form insoluble aggregates (Pelham, 1990). The (auto-) regulatory role of hsp70 in recovery from stress may, thus, involve the promotion of disaggregation (Pelham, 1986). During the recovery process hsp70 moves back to the cytoplasm. Consistent with this proposed function of hsp70 is the finding that certain yeast hsp70 mutants that constitutively overproduce other heat shock proteins display protection of splicing at high temperatures without the need of a pre-treatment at mildly increased temperatures (Yost and Lindquist, 1991). Also hsp104 may play a part in the recovery of RNA splicing after a severe heat shock (Yost and Lindquist, 1991).

Finally, heat shock has a profound effect on RNA metabolism due to the selective degradation of mRNAs (reviewed by Yost et al., 1990). During shock conditions some normal cellular mRNAs display a transient rapid decay. In yeast, in particular ribosomal protein mRNAs have been shown to undergo a rapid degradation upon a mild temperature shift (Herruer et al., 1988; Mitsui and Tsurugi, 1991). On the other hand, during recovery from heat shock, while the levels of normal cellular mRNAs are being restored, hsp mRNAs are selectively degraded (Yost et al., 1990). This rapid decay is probably mediated by a *cis*-acting element present in the trailer regions. An appealing alternative explanation for the observed differences in turnover-rate of hsp mRNA is that these mRNAs are intrinsically unstable but are stabilized during stress exposure.

OTHER STRESS AGENTS AND THE ACQUISITION OF STRESS TOLERANCE

Studies on the stress response of living cells have so far mainly been focused on the effects of heat treatment. In particular for yeast, however, it has been demonstrated that, apart from heat, other stress agents can induce similar responses (reviewed by Watson, 1990). Heat shock protein synthesis has been reported to occur upon exposure of yeast cells to ethanol (Plesset et al., 1982; P. Moradas Ferreira, unpublished results), high salt (Varela et al., 1992), desiccation, heavy metals, arsenite (Chang et al., 1989), hydrogen peroxide (Collinson and Dawes, 1992) and amino acid analogues. It is clear, however, that the responses to the various stress agents are not identical (see below). The sensitivity of yeast for the induction of the stress response is manifest by the recent finding that conversion of yeast cells to spheroplasts (by incubating them in the presence of lyticase) evokes the expression of *HSP70* and *HSP83* genes (Adams and Gross, 1991). Treatment of cells with thiolutin (an inhibitor of all three RNA polymerases) induced a 5-, 25- and 50-fold increased transcription of *HSP83*, *SSA4* and *HSP26*, respectively (Adams and Gross, 1991). Another drug commonly used to inhibit transcription, phenanthroline, also caused elevated mRNA levels for some hsps, but in this case probably due to stabilization of the respective mRNAs (Adams and Gross, 1991).

Pre-treatment of yeast cells at mildly elevated temperatures leads to the attainment of tolerance against a severe heat shock. Thermotolerance develops rapidly in yeast after a shift from 23 °C to 37 °C reaching a maximum at 2 h and decreasing in the next 8–24 h (McAlister and Finkelstein, 1980). Consistent with the finding that other stress agents can induce similar responses as heat, it was demonstrated that heat treatment of *S. cerevisiae* results in a marked increase in ethanol tolerance as compared to control cells (Watson and Cavicchioli, 1983). The effect of ethanol on yeast has particularly been investigated since high ethanol concentrations inhibit fermentation and growth. This effect has been associated with plasma membrane ATPase activity (Rosa and Sá-Correia, 1992). Both ethanol and thermal stress lead to a slight but significant drop of internal pH which is related to the ATPase activity (Weitzel et al., 1987; Pampulha and Loureiro-Dias, 1989). Similarly, cells pre-exposed to osmotic stress were shown to become not only osmo-tolerant but also acquired thermotolerance (Trolmo et al., 1988; Varela et al., 1992). The reverse, however, is not true. Obviously, under both stress conditions no identical sets of proteins are synthesized.

From the initial studies (McAlister and Finkelstein, 1980), it has been suggested that heat shock proteins play an essential role in the acquisition of stress tolerance. In many aspects, indeed, the level of acquired thermal resistance shows a narrow correlation with induced cellular level of hsps. For instance, a yeast mutant, temperature-sensitive for growth at 39 °C by a defect in RNA transport from nucleus to cytoplasm and, therefore, incapable of synthesizing hsps at this temperature, failed to become resistant to a subsequent challenge at 55 °C (McAlister and Finkelstein, 1980). Since then, circumstantial evidence has become available that supports the idea that heat shock protein synthesis is a prerequisite for the development of stress-tolerance by yeast. A heat shock resistant mutant of *S. cerevisiae* (*hsr1*; Iida and Yahara, 1984) has been isolated with high constitutive levels of two hsps. Also *cyr1-2* mutants, having low intracellular cyclic AMP contents, constitutively synthesize hsps and are relatively resistant against lethal temperatures, while *bcy1*-mutants (deficient in cyclic AMP-dependent protein kinase) fail to attain thermotolerance (Shin et al., 1987). It is noteworthy, furthermore, that yeast cells in stationary phase are intrinsically more resistant to various stress agents than are exponential-phase cells (Schenberg-Frascino and Moustacchi, 1972; Parry et al., 1976; Walton et al., 1979). Under those growth conditions several hsps were shown to display enhanced rates of synthesis (see above). The heat shock response of *S. cerevisiae* cells during stationary growth differs from that occurring at exponential since the former shows long-term resistance in contrast with the transient nature of the latter (reviewed by Watson, 1990). Finally, studies performed with defective mutants of the *SSA1* and *SSA2* genes have been interpreted as evidence for a causal relationship between hsp levels and the acquisition of thermotolerance, since these double mutants are thermosensitive for growth at 37 °C (Craig and Jacobsen, 1984). On the other hand, a direct correlation between hsps and the attainment of stress-resistance so far has not been established. Rather, evidence is accumulating that hsps may not be needed for stress-tolerance acquisition but for a rapid recovery from the stress-affected situation, thus serving as components of the cellular defense mechanism.

The first argument against an obligatory role of hsps in the acquisition of stress tolerance was provided by the finding that *S. cerevisiae* cells pretreated with cycloheximide to block protein synthesis still develop thermotolerance (Hall, 1983). In addition, administration of inhibitors of both cytoplasmic and mitochondrial protein synthesis did not interfere with the heat-induced ethanol- or thermo-tolerance (Watson et al., 1984).

Analysis of *ssa1/ssa2* mutants demonstrated that their capability to attain thermotolerance is similar to that of control cells (Craig and Jacobson, 1984). These mutants appeared to be even more resistant against a short treatment at 52 °C than wild-type cells. Moreover, except for *HSP104*, analysis of disruption mutants did not reveal an important function of any hsp in the acquisition of stress-resistance. *HSP104* deletion mutants, however, do fail to acquire thermotolerance (Sanchez and Lindquist, 1990) and rescue of this capacity could be gained by transformation of the disruption strain with the wild-type gene. Very recently, a further analysis of the role of hsp104 in the acquisition of stress tolerance was reported (Sanchez et al., 1992). Respiring cells, displaying a constitutive expression of *HSP104*, were found to be basally more resistant against heat shock than cells in a fermentative culture. This selective advantage is absent in a *hsp104* deletion mutant. The attainment of tolerance against high ethanol concentrations (and to a minor extent also to arsenite) is similarly dependent upon a functional *HSP104* gene (Sanchez et al., 1992). These data strongly suggest the requirement of this heat shock protein for the acquisition of tolerance against stressful conditions.

So far, overproduction of hsps has not been found to render yeast cells thermoresistant. The *HSP83* gene, for instance, has been introduced into yeast on a multicopy plasmid. The resulting

3-fold increased induction of this protein upon heat shock did not lead to a corresponding increase in the level of thermotolerance normally attained (Finkelstein and Strausberg, 1983). Also a *HSP26* gene under control of the *GAL* promoter, allowing induction prior to the heat treatment, or the *PGK* promoter, did not alter the ability of cells to acquire tolerance (Tuite et al., 1990). Of course, this type of experiment does not exclude that hsps are involved in stress tolerance acquisition, but increased levels, obviously, are not sufficient to confer elevated levels of protection.

Yeast cells exposed to stress circumstances temporarily stop growing by an arrest at G_1 in the division cycle. Using cell cycle inhibitors it has been shown that arresting cells in G_1, S or G_2 phase of the mitotic cycle is not a stress condition that induces thermotolerance (Barnes et al., 1990). Arrested cells remained as sensitive to thermal death as growing cells, providing evidence for the conclusion that the full spectrum of hsps is not necessary for thermotolerance induction.

Convincing evidence that thermotolerance attainment does not require hsp synthesis was obtained by uncoupling both processes (Smith and Yaffe, 1991). A yeast strain bearing a mutation in the gene for the heat shock factor HSF (*hsf1-m3*) was isolated which causes a temperature-sensitive growth defect. The mutation prevents activation of HSF and therefore leads to a general block of heat shock-induced protein synthesis. However, it does not affect the acquisition of thermotolerance.

Finally, with regard to the development of stress tolerance by yeast cells, the synthesis of trehalose should be mentioned (see also above). Changes in intracellular trehalose levels have been correlated with the acquisition of tolerance, both against heat and desiccation (Hottiger et al., 1989; Wiemken, 1990). Again, however, it is unclear whether increased concentrations of trehalose are sufficient to render cells tolerant against stress conditions or just contribute to the protection of cellular structures at those adverse conditions.

WHAT IS THE TRIGGER?

The data summarized above 'stress' the pleiotropic nature of the heat shock response in yeast (and other living cells). Many cellular events reflect molecular consequences of the stress exposure and deal with protection, survival and repair. A remaining intriguing, so far unsolved question, however, is by which cellular component(s) the stress condition is sensed.

First, intracellular pH has been suggested to play an essential part in triggering the stress response, since the acquisition of thermotolerance is enhanced in cells having an acidic external environment (pH 4.0) as compared with a neutral one (Coote et al., 1991). Probably, plasma membranes become leaky for protons as a consequence of the stress challenge. At an external pH of 4.0 passive diffusion of protons may lead to internal acidification that, subsequently, may trigger the stress response. How changes in the pH_i might lead to, amongst others, stress protein synthesis is so far unknown.

A more classical view is that some proteins in the cell are very sensitive to stress-induced denaturation. (Although this may also be a pH-dependent process.) By exposure of cells to adverse circumstances, these denatured proteins might recruit hsps70 from their complex with the heat shock factor HSF. In this way hsps70 could positively regulate the expression of their own and other *HSP*-genes. Also in principle denatured nascent polypeptides may be signalled by the hsp70 'control-system'. For this reason in *E. coli* ribosomes have been implicated in sensing the stress response (van Bogelen and Neidhart, 1990). It is clear, on the other hand, that at least some *HSP* gene promoters, in addition to the HSF-mediated control, are regulated by other signals such as cyclic AMP. These data indicate the possible involvement in the stress response of protein phosphorylation. Another finding emphasizing that (de)phosphorylation may play a part, is the modification of HSF itself. Even if phosphorylation of HSF is a consequence of the formation of an active transcription initiation complex, kinases and phosphatases may play a regulatory part in the return to the normal growth conditions. Furthermore, it cannot be excluded that protein-(de)phosphorylation fulfils a key role in the expression of the response through a so far unidentified regulatory circuit.

By analysis of mutants defective in certain steps of well-known signal transduction pathways, the relevance of protein modifications will be elucidated. Moreover a comparative analysis of the responses evoked by different stress circumstances will contribute in revealing the nature of the actual trigger(s) of the process.

CONCLUDING REMARKS

In conclusion, the data reviewed in this article clearly demonstrate the pleiotropic nature of the stress response of yeast. Many questions with respect to the underlying molecular mechanisms remain to be answered. Evidently, the stress response is not limited to the action of the classical heat shock proteins and the heat-responsive transcription factor HSF, but involves many more factors and processes presently under study. These proteins and molecular events contribute to either protecting and repairing cells after exposure to stress (transient response) or their adaptation to prolonged stress (sustained response). It will be the challenge of future studies to unravel the functional links in this regulatory network, the more so since they are expected to play an important part under normal cellular growth conditions as well.

It is obvious that increased knowledge about the stress protection and adaptation mechanisms in yeast is of major both fundamental and biotechnological importance. Because of the universal nature of the stress response, further insight into the response of yeast is also relevant to improve the understanding of defense mechanisms in other cell types. For instance, a direct relationship exists between the expression of stress proteins and specific pathological conditions (Morimoto et al., 1990) and antigens from a wide variety of pathogens were identified as members of hsps (Young, 1992). Therefore, although the stress response certainly displays cell-specific features, the many shared characteristics render yeast an attractive model to investigate the response from bacteria to man.

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