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The Role of Heat-Shock Proteins in Thermotolerance [and Discussion]

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The role of heat-shock proteins in thermotolerance

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SUMMARY

The role of heat-shock proteins (hsps) in thermotolerance was examined in the budding yeast *Saccharomyces cerevisiae* and in the fruit fly *Drosophila melanogaster*. In yeast cells, the major protein responsible for thermotolerance is hsp 100. In cells carrying mutations in the hsp 100 gene, *HSP 104*, growth is normal at both high and low temperatures, but the ability of cells to survive extreme temperatures is severely impaired. The loss of thermotolerance is apparently due to the absence of the hsp 104 protein itself because, with the exception of the hsp 104 protein, no differences in protein profiles were observed between mutant and wild-type cells. Aggregates found in mutant cells at high temperatures suggest that the cause of death may be the accumulation of denatured proteins. No differences in the rates of protein degradation were observed between mutant and wild-type cells. This, and genetic analysis of cells carrying multiple hsp 70 and hsp 104 mutations, suggests that the primary function of hsp 104 is to rescue proteins from denaturation rather than to degrade them once they have been denatured. *Drosophila* cells do not produce a protein in the hsp 100 class in response to high temperatures. In this organism, hsp 70 appears to be the primary protein involved in thermotolerance. Thus, the relative importance of different hsps in thermotolerance changes from organism to organism.

1. INTRODUCTION

When organisms are grown at normal temperatures and shifted suddenly to severe temperatures they die rapidly. However, if they are given a brief pretreatment at a more moderate temperature, which induces hsps, and are then shifted to severe temperatures, they die much more slowly. The effects of pretreatments are very dramatic. Differences in survival between naive and conditioned organisms are often in the range of 100- to 1000-fold. Conditioning heat treatments also provide tolerance to many other types of stress, such as exposure to ethanol and sodium arsenite. Conversely, exposure to moderate concentrations of ethanol and sodium arsenite induce tolerance both to higher concentrations of ethanol and sodium arsenite, as well as to high temperatures. As these other stresses also induce the synthesis of hsps, it has long been postulated that hsps are responsible for induced tolerance (see Lindquist 1986; Nover 1991). Until recently, however, this hypothesis remained controversial because tolerance can sometimes be induced in the absence of hsp synthesis (Carper *et al.* 1987). Genetic analysis of hsps has now proven their importance in thermotolerance. There are probably two explanations for earlier contradictory results. First, in some cases hsps are already present at normal temperatures and apparently need only be activated by the conditioning treatments. Second, hsps are almost certainly not the only protective agents involved in tolerance.

We have studied the role of two hsps in thermotolerance, hsp 100 and hsp 70. Surprisingly, the relative importance of the two proteins varies from organism to organism. In yeast cells, hsp 100 is the primary protein responsible for thermotolerance, whereas in *Drosophila*, hsp 70 is of prime importance.

2. THE ROLE OF HSP 100 IN YEAST

(*a*) *Phenotype of hsp 100 mutations*

Mutations in the hsp 100 gene of *Saccharomyces cerevisiae*, *HSP 104*, have no effect on growth at either low (25°C) or high (37°C) temperatures (Sanchez & Lindquist 1990). They do however, severely affect tolerance to extreme temperatures. When mutant and wild-type cells are shifted directly from 25°C to 50°C, they die very rapidly. (Less than 0.1% of the cells remain viable after 10 min.) With a 30 min conditioning treatment at 37°C, both mutant and wild-type cells acquire thermotolerance. In the first few minutes of exposure to 50°C, survival in the conditioned cells is 1000 times greater than in unconditioned cells. However, in the mutant this acquired tolerance is very transient. Seven minutes after the shift to 50°C, when the conditioned cells begin to die, mutant cells die 100 times more rapidly than wild-type cells. The hsp 104 protein plays a vital role in thermotolerance in log phase cells growing on a variety of different carbon sources, in stationary phase cells, and in spores. It is also essential for tolerance to high concentrations of

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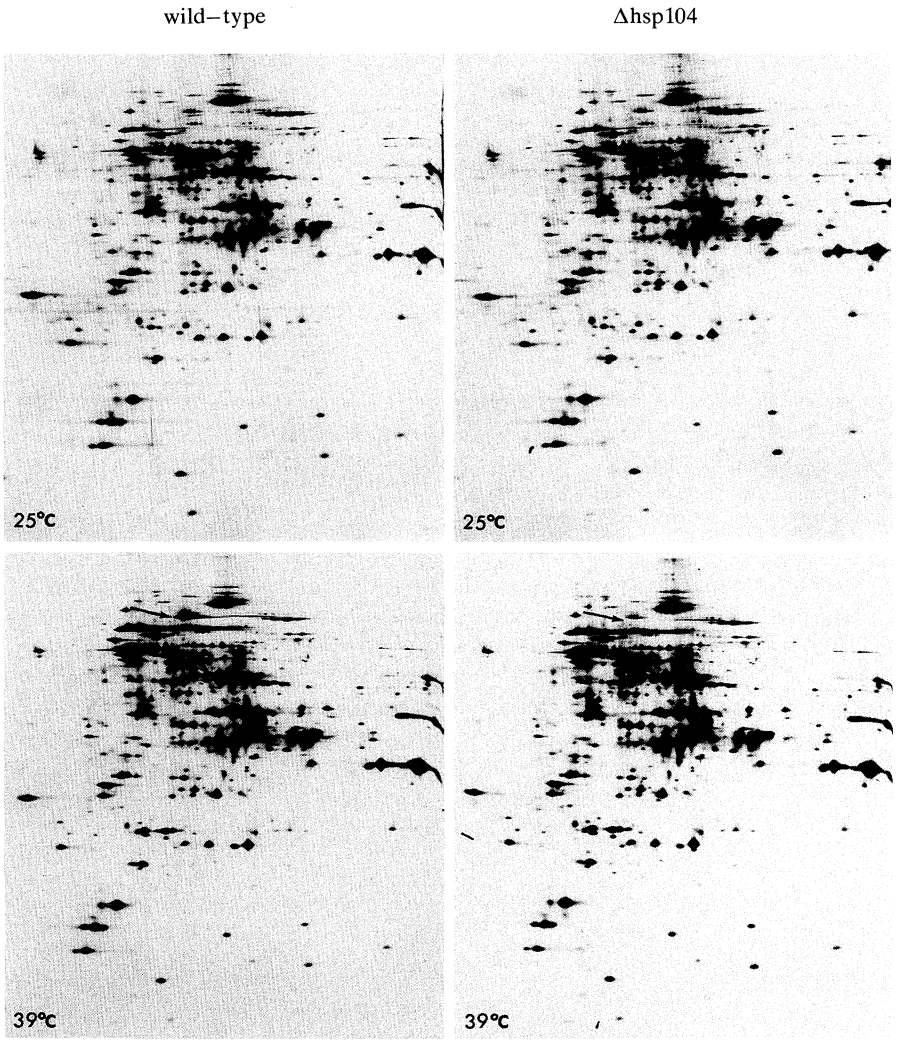

Figure 1. Analysis of proteins in wild-type and *hsp 104* mutant yeast cells. Proteins were extracted from log phase cells (9×10^6 per millilitre) which had been grown in rich medium at 25°C and exposed to 39°C for 60 min. Total cellular proteins from 7.5×10^6 cells were separated by two-dimensional gel electrophoresis (Sanchez *et al.* 1990; Daufeldt & Harrison 1984) and were visualized by silver staining. The position of hsp 104 is marked with an arrow on the heat-shocked samples.

ethanol, indicating it has broadly protective functions (Sanchez *et al.* 1990).

Since some hsp mutations have profound effects on the synthesis of other proteins (Craig & Jacobsen 1984; Finley *et al.* 1987; Werner *et al.* 1987), the question arises: is the loss of tolerance in *hsp 104* mutant cells due to an effect of hsp 104 on the expression of other proteins or due to the absence of hsp 104 itself? To investigate this question, we used two-dimensional gel electrophoresis to compare protein profiles from mutant and wild-type cells at normal temperatures and after a conditioning pretreatment at 37°C. Except for the presence or absence of hsp 104 itself, the distribution of proteins in the two strains is virtually identical (figure 1). Although we cannot exclude the possibility that a minor protein not readily detected on these gels is affected by the absence of hsp 104 and is of critical importance in thermotolerance, these data suggest that hsp 104 itself is responsible for the changes in thermotolerance.

(*b*) *Conservation of the hsp 100 family*

The hsp 100 family has been highly conserved in evolution. Heat-inducible proteins of ~100 kDa that react with antibodies raised against yeast hsp 104 peptides are observed in organisms as diverse as mammals (*Homo sapiens* and *Cricetulus griseus*), fission yeast (*Schizosaccharomyces pombe*), and bacteria (*Escherichia coli*) (Parsell *et al.* 1991). A search of the Genebank database with the hsp 104 protein sequence revealed the existence of several cloned genes encoding proteins that share between 28 and 40% amino acid identity with the yeast protein (Gottesman *et al.* 1990; Parsell *et al.* 1991) (figure 2). None of these were previously known to be heat-inducible. Recent experiments demonstrate that some are and some are not (Kitagawa *et al.* 1991; Parsell *et al.* 1991; Squires *et al.* 1991). Thus, like other hsp families, the hsp 100 family contains both heat-inducible and constitutive members. *E. coli* contains at least two proteins related

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Hsp104  MNDQTQFTERALTILTLAQKLASDHQHPQLQPIHILAAFIETPEDGSVPYLQNLIEKGRYDYDLFKKVVNRNLVRIPQQQPAPAEITPSYALGKVLQDAA
Clp B    MRLDRL.NKFQLA.AD.QS..LG.DNQFIE.L.LMS.LLNQEGGSVS.L.TSAGINA----GQLRTDI.QA.N.L..VEGTGGDVQ..QD.VR..NLCD
Clp A        MLNQELELS.NM.FAR.RE.R.EFMTVE.L.L.LLSN.SAREALEACSVDLVALRQELEAFIEQTTPVLPASEEERDTQPTLSFQRVLQRAVFHV

Hsp104  KIQKQQKDSFIAQD-HILFALFNDSSIQQIFK------------------EAQVDIEAIKQQALELRGNTRIDSRGADTNTPLEYLSKYAIDMTEQARQG
Clp B   NVAQKRG.N..SSELFV.A..ESRGTVAD.L.------------------A.GATTAN.T.AIEQM..GESVNDQ..EDQ--RQA.K..T..L..R.E..
Clp A   QSSGRNEVTGANVLVA.FSEQESQAAYLLRKHEVSRLDVVNFISHGTRKD.PTQSSDPGS.PNS.EQAGGE-----------.RMENFTTNLNQL..V.

Hsp104  KLDPVIGREEEIRSTIRVLARRIKSNPCLIGEPGIGKTAIIEGVAQRIIDDDVPIILQGAKLFSLDLAALTAGAKYKGDFEERFKGVLKEIEESKTLIVL
Clp B   ........D....R..Q..Q..T.N..V......V.....V..L.....NGE..EGLK.RRVLA..MG..V.....R.E....L....NDLAKQEGNVI.
Clp A   GI..L....K.LERA.Q..C..R.N..L.V..S.V.....A..L.W..VQG...EVMADCTIY...IGS.L..T..R....K...AL..QL.-QD.NSI.

Hsp104  FIDEIHMLMGNGKD-------DAANILKPALSRGQLKVIGATTNNEYRSIVEKDGAFERRFQKIEVAEPSVRQTVAILRGLQPKYEIHHGVRILDSALVT
Clp B   ....L.TMV.A..ADGA---M..G.M.....A..E.HCV....LD...QYI...A.L......VF......ED.I......KER..L..H.Q.T.P.I.A
Clp A   ......TII.A.AASGGQ--V....LI..L..S.KIR...S..YQ.FSN.F...R.LA......DIT...IEE..Q.IN..K....G..D..YTAK.VRA

Hsp104  AAQLAKRYLPYRRLPDSALDLVDISCAGVAVARDSKPEELDSKEQSIAIDSSRDKSSRERVECRLHTKRKLKLARQKEASLQEELEPLRQRYNEEKHGHE
Clp B   ..T.SH..IAD.Q...K.I..I.EAASSIRMQI........RLDRR.-.QLKLEQQALMK-.SDEAS.KR.DMLNEELSDKERQYSE.EEEWKA..ASLS
Clp A   .VE..VK.IND.H...K.I.VI.EAG.RAR.MPV..RKKT------------------------------------------------------------

Hsp104  ELTQAKKKLDELENKALVAERRYDTRTAADLRYFAIPDIKKQIEKLEDQVAEEERRAGANSMIQNVVDSDTISETAARLTGIPVKKLSESENEKLIHMER
Clp B   GTQTI.AE.EQAKIAIEQ.R.VG.LARMSE.Q.GK..ELE..L.AATQLEGKTM.------LLR.K.TDAE.A.VL..W.....SRMM...R...LR..Q
Clp A   ------------------------------------------------------------.NVAD.ESVV..IAR..E.SV.Q.DRDT.KNLGD

Hsp104  DLSSEVVGQMDAIKAVSNAVRLSRSGLANPRQP-ASFLFLGLSGSGKTELAKKVAGFLFNDEDMMIRVDCSELSEKYAVSKLLGTTAGYVGYDEGGFLTN
Clp B   E.HHR.I..NE.VD.....I.R..A...D.NR.IG......PT.V.....C.AL.N.M.DSDEA.V.I.M..FM..HS..R.V.APP.....E...Y..E
Clp A   R.KML.F..DK..E.LTE.IKMA.A..GHEHK.VG....AGPT.V....VTVQLSKA.---GIELL.F.M..YM.RHT..RVI.APP....F.Q..L..D

Hsp104  QLQYKPYSVLLFDEVEKAHPDVLTVMLQMLDDGRITSGQGKTIDCSNCIVIMTSNLGAEFINS---------QQGSKIQESTKNLVMGAVRQHFRPEFLN
Clp B   AVRRR....I.L..........FNIL..V.....L.D...R.V.FR.TV........SDL.QE---------RF.ELDYAHM.E..L.V.SHN.....I.
Clp A   AVIKH.HA...L..I.......FNIL..VM.N.TL.DNN.RKA.FR.VVLV..T.A.VRETERKSIGLIHQDNSTDA---------.EEIKKI.T...R.

Hsp104  RISSIVIFNKLSRKAIHKIVDIRLKEIEERFEQNDKHYKLNLTQEAKDFLAKYGYSDDMGARPLNRLIQNEILNKLALRILKNEIKDKETVNVVLKKGKS
Clp B   ..DEV.V.HP.GEQH.AS.AQ.Q..RLYK.L.ER--G.EIHISD..LKL.SEN..DPVY.....K.A..QQ.E.P..QQ..SG.LVPGKVIRLEVNEDRI
Clp A   .LDN.IW.DH..TDV..QV..KFIV.LQVQLD.KG--VS.EVS...RNW..EK..DRA.....MA.V..DNLKKP..NEL.FGSLV.GGQ.T.A.D.E.N

Hsp104  RDENVPEEAEECLEVLPNHEATIGADTLGDDDNEDSMEIDDDLD908
Clp B   VAVQ
Clp A   ELTYGFQS.QKHKAEAAH
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Figure 2. Alignment of the yeast hsp 104 protein with the *E. coli* ClpA and ClpB proteins. Amino acids are designated by the single letter code. Positions where the ClpA and ClpB proteins are identical to the yeast protein are marked by a dot. Dashes indicate introduced gaps.

to hsp 100, ClpA and ClpB (Gottesman *et al.* 1990). Mutations in *clpB*, the gene which is heat-inducible and which has a higher level of homology with yeast hsp 104, cause *E. coli* cells to die more rapidly when exposed to 50°C. Thus it is likely that the heat-inducible members of the hsp 100 family will serve an important role in thermotolerance in many organisms.

Given the high level of conservation in the hsp 100 family, we asked whether the *E. coli* ClpA or ClpB proteins could substitute for the yeast hsp 104 protein in thermotolerance. Cells that express ClpA and ClpB in the hsp 104 mutant background were created by placing the coding sequences for these proteins under the control of the *HSP 104* promoter. The expression of ClpA and ClpB was confirmed by immunological analysis of electrophoretically separated proteins (figure 3*a*). Coomassie staining demonstrated that the proteins accumulated to a level that approached that of the hsp 104 protein in wild-type cells (data not shown). Neither protein was capable of restoring thermotolerance in the *hsp 104* deletion strain (figure 3b). As discussed below, the function of the ClpA protein in *E. coli* may be different from that of the hsp 104 protein in yeast. The ClpB protein, however, is known to play a role in survival at high temperatures. Its failure to provide thermotolerance in the hsp 104 deletion strains suggests that the hsp 100 proteins might cooperate with other proteins to provide tolerance and that the surfaces that govern these interactions have evolved to the point where the *E. coli* protein cannot function in yeast cells. This would not be surprising. Several other hsps have been shown to require specific partner proteins for function *in vivo* and *in vitro* (Lindquist & Craig 1988; Gething & Sambrook 1992; Georgopoulos 1992).

(*c*) *Possible mechanism of hsp 104 action*

Prior to our recent experiments on yeast hsp 104, the only member of the hsp 100 family that had been studied at the biochemical level was ClpA. This protein serves to regulate the activity of the dodecameric ClpP protease (Katayama *et al.* 1988). Electron micrographs of yeast cells exposed to moderately high temperatures reveal large aggregates, primarily in the nucleus, that are conspicuous in mutant cells but barely detectable in the wild-type. Based upon the fact that most other hsps function in protein folding, we suspect that these aggregates are, at least in part, composed of proteins denatured by high temperatures (Y. Sanchez & S. Lindquist, unpublished observations). This suggests that hsp 104 mutant cells are killed at high temperatures by the accumulation of proteins into insoluble aggregates, in keeping with previous hypotheses (Pelham 1986). In the light of these findings, the homology between hsp 104 and

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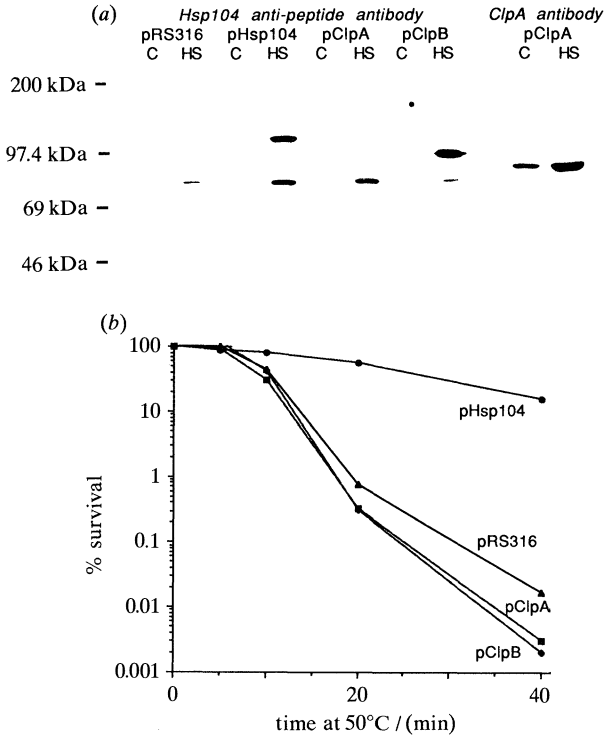

Figure 3. Neither *E. coli* ClpA nor ClpB can rescue thermotolerance in yeast cells deficient for hsp 104. A haploid yeast strain disrupted for the *HSP 104* gene was transformed with a *CEN* plasmid encoding one of the following: (i) the wild-type *HSP 104* gene (pHsp 104); (ii) the *E. coli clpA* gene under control of the *HSP 104* promoter (pClpA); (iii) the *E. coli clpB* gene under control of the *HSP 104* promoter (pClpB); and (iv) vector alone (pRS316). (*a*) Total yeast proteins (1×10^6 cells per lane) were electrophoretically separated on 7.5% polyacrylamide gels and transferred to Immobilon filters. Filters were probed with antisera raised against a peptide sequence from hsp 104 which reacts with both hsp 104 and ClpB (as well as another 71 kDa yeast protein) or antisera raised against ClpA (a gift from M. Maurizi). (*b*) The ability of ClpA and ClpB to substitute for hsp 104 in thermotolerance was assessed. Yeast cells were grown at 25°C to a density of approximately 2×10^6 cells per millilitre, given a conditioning preheat treatment at 37°C for 30 min and transferred to 50°C for various amounts of time. Cells were then diluted in ice-cold YPDA and plated on YPDA plates.

ClpA, and the known function of ClpA in regulating a protease, suggested that hsp 104 might protect cells from killing at high temperatures by stimulating a protease to dissolve protein aggregates.

To test this possibility, we employed several assays designed by others to investigate defects in protein degradation in yeast (Seufert & Jentsch 1990). Wild-type and hsp 104 mutant cells were compared with respect to their rates of turnover of naturally short-lived yeast proteins and of abnormal proteins containing the amino acid analogue canavanine (figure 4). Cells were pulse-labeled and the release of radioactive amino acids was measured during a chase period. At both 25°C and 37°C, conditioned wild-type and mutant cells were essentially identical in their rates of degradation of both normal proteins and canavanine-substituted proteins. These results contrast sharply with those obtained when known proteolytic pathway mutants are examined (Seufert & Jentsch 1990). The growth of yeast cells deficient in proteolysis is also much more sensitive to the presence of amino acid analogues than is the growth of wild-type cells. (Presumably analogues produce toxic levels of abnormal proteins in cells unable to degrade them.) In contrast to known protease mutants, the canavanine sensitivity of wild-type and hsp 104 mutant cells are indistinguishable.

Our results do not exclude the possibility that hsp 104 functions to stimulate the proteolysis of protein aggregates that accumulate at high temperatures. Indeed, in *E. coli* the general proteolytic defects of ClpA mutants are subtle, becoming apparent only when the influence of an additional protease is eliminated by mutation (Katayama *et al.* 1988). However, given the 100- to 1000-fold increase in thermotolerance seen in the presence of the hsp 104 protein, it seems unlikely that such a subtle role in protein degradation is its primary function.

An alternative hypothesis is that hsp 104 acts as a molecular chaperone to prevent the formation of protein aggregates or to promote their dissolution. Genetic analyses of the relationship between hsp 104 and hsp 70, which is known to stabilize proteins in an unfolded conformation, support this hypothesis:

1. In cells that have reduced levels of constitutive hsp 70 expression (*ssa1 ssa2* double mutants), mutations in the hsp 104 gene severely reduce growth rates at all temperatures (Sanchez *et al.* 1993). Mutations in the hsp 104 gene do not reduce rates of growth in cells carrying wild-type copies of the hsp 70 genes (Sanchez & Lindquist 1990).
2. In the hsp 104 mutant background, mutations that eliminate heat-inducible expression of hsp 70 (*ssa1 ssa3 ssa4* triple mutants) have a marked effect on thermotolerance. Specifically, they substantially reduce the residual, transient thermotolerance that is observed when hsp 104 deficient cells are conditioned by a preheat treatment and exposed to 50°C (Sanchez *et al.* 1993). In the presence of the hsp 104 protein, these hsp 70 mutations do not impair thermotolerance (Werner *et al.* 1987).
3. Over-expression of hsp 70 partially compensates for the loss of hsp 104 in thermotolerance. When hsp 70 is over-expressed in the hsp 104 deletion strain, from a high copy-number *SSA1* plasmid, it substantially increases the survival of preconditioned cells at 50°C (Sanchez *et al.* 1993).

Thus, hsp 104 plays an important role in growth in hsp 70-deficient cells and hsp 70 plays an important role in thermotolerance in hsp 104-deficient cells. These data strongly suggest that the functions of hsp 70 and hsp 104 are closely related *in vivo*. We believe that the proteins are either working on parallel pathways that partially overlap or are working on different steps in the same pathway. For example, hsp 70 might capture proteins as they are unfolded at high temperatures and pass them to hsp 100 for refolding. Such a scheme is reminiscent of the manner

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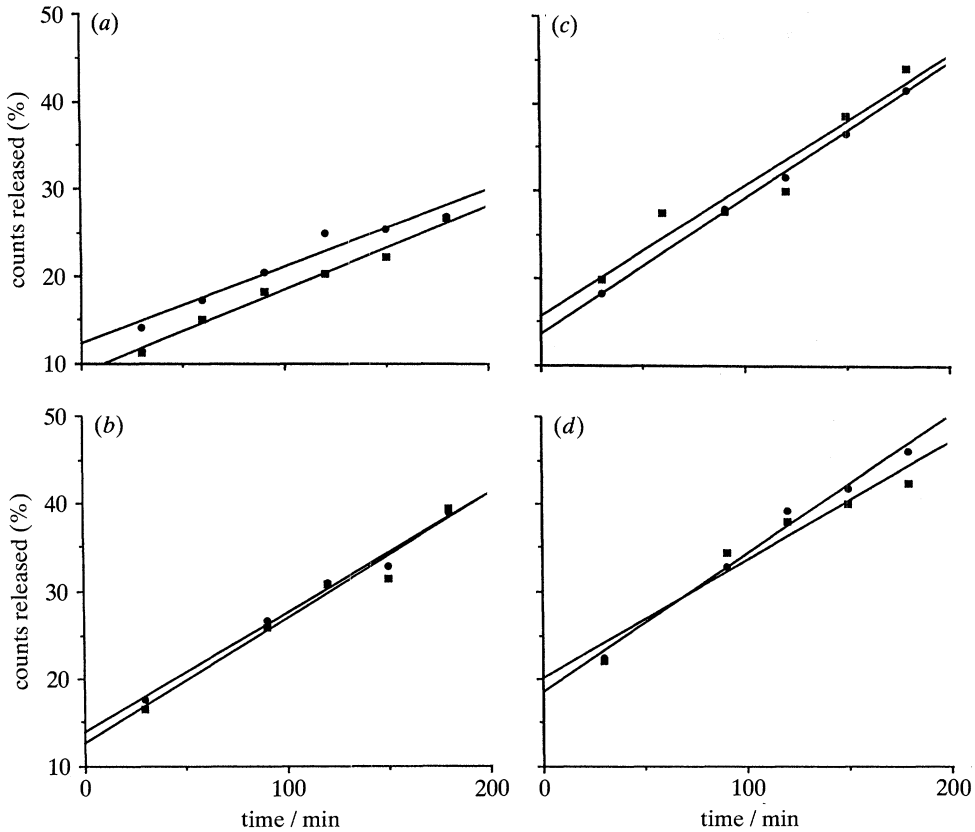

Figure 4. Rates of protein turnover are similar in wild-type (filled circles) and hsp 104 mutant cells (filled squares). Yeast cells were grown at 25°C in SD medium (substituted with required amino acids) to a density of approximately 3×10^6 cells per millilitre and were given a 30 min conditioning preheat treatment at 37°C. To assess short-lived protein turnover cells were labelled with ^{3}H-isoleucine (75 μCi ml^{-1} culture medium) for 5 min at (*a*) 25°C, or (*b*) 37°C. To measure turnover of abnormal proteins cells were pretreated with canavanine (20 μg ml^{-1} culture medium) for 90 min at (*c*) 25°C, or (*d*) 37°C prior to labeling as above. Cells were then washed twice and incubated in growth media supplemented with 100 μg ml^{-1} isoleucine and 0.5 mg ml^{-1} cycloheximide. At timepoints after initiation of the chase, aliquots of the culture were removed and analysed for radioactivity in the total culture versus the culture supernatant. Protein turnover is given as the percent radioactivity released during the chase as described (Seufert & Jentsch 1990). Viable cell counts done at timepoints during the chase indicate no loss in viability during the experiment.

in which hsp 70 and hsp 60 cooperate in normal protein folding in mitochondria (Georgopoulos 1992; Gething & Sambrook 1992). Other models are also consistent with the data, but all point to a general function for hsp 100 in protein assembly–disassembly reactions. This does not preclude a relationship between the functions of hsp 100 and ClpA. In the absence of ClpA, the ClpP protease can be activated by low concentrations of detergent (Etyan *et al.* 1989). It seems likely, therefore, that ClpA may stimulate the ClpP protease by enhancing the access of substrates, either by promoting the unfolding of these substrates or by liberating the protease from a repressed conformation.

4. THE ROLE OF HSP 70 IN *DROSOPHILA*

Unlike most other organisms we have examined, *Drosophila* does not produce a major, heat-inducible protein in the hsp 100 size class (Lindquist 1980). It does, however, produce hsp 70 in greater abundance than is observed in any of these other organisms. This is due, in large part, to the amplification of the heat-inducible hsp 70 gene of *Drosophila*. Five or six virtually identical copies are present per haploid genome (Craig & McCarthy 1980). Thus, our yeast strain, in which a high copy number hsp 70 plasmid compensates for the loss of hsp 104 in thermotolerance, mimics the hsp expression pattern of *Drosophila*. The natural

Table 1. *Hsp 70 in* Drosophila *tissue culture*

hsp 70 alteration in transformants	effect on thermotolerance
extra copies of the wild-type *hsp 70* gene	increases the rate at which thermotolerance is acquired at 36°C
addition of antisense *hsp 70* genes	decreases the rate at which thermotolerance is acquired at 36°C
frame shift mutation at codon 337	blocks the development of thermotolerance
coding sequences regulated by the metallothionein promoter	thermotolerance induced by copper in the absence of heat

absence of hsp 100 induction in *Drosophila* provides a particularly clean system in which to investigate the involvement of hsp 70 in thermotolerance.

Several different strategies were employed to investigate the role of hsp 70 in *Drosophila* tissue culture cells (summarized in table 1). First, we altered the rate at which hsp 70 accumulates by transforming the cells with additional copies of the wild-type gene or with antisense genes. Cells that over-expressed hsp 70 developed thermotolerance at increased rates; cells that under-expressed hsp 70 developed thermotolerance at reduced rates. Second, since hsp 70 functions by protein/protein interactions we asked whether mutations in the hsp 70 coding region could interfere with the normal development of thermotolerance. A frame shift mutation, which eliminates the carboxy-terminal half of the protein, does just that. Third, we asked if transferring hsp 70 coding sequences to a heterologous promotor could place thermotolerance under the control of an independent induction system. We found that thermotolerance could be induced by copper in cells transformed with a metallothionein-regulated gene. Cells carrying the vector alone showed no increases in thermotolerance. Taken together, these experiments provide compelling evidence for the role of hsp 70 in thermotolerance in *Drosophila*. The latter experiment not only demonstrates the importance of hsp 70, but also suggests that increased expression of hsp 70 alone is sufficient to provide a major increase in thermotolerance (Solomon *et al.* 1991).

Most recently we have begun to test the role of hsp 70 in thermotolerance in whole flies. A long-term goal is to elucidate the nature of heat-induced developmental anomalies and the functions of hsps in providing protection against them (Zimmerman & Cohill 1991). We have used the flp recombinase system (Golic and Lindquist 1989) to increase the copy number of hsp 70 genes in the *Drosophila* genome. The effect of this amplification on thermotolerance was examined by incubating early embryos at 36°C prior to a severe heat shock at 41°C (Welte *et al.* 1993). When thermotolerance was assessed as the number of embryos that survive to hatching, early embryos carrying extra copies of the hsp 70 gene acquired thermotolerance more rapidly than embryos that did not. This result indicates that hsp 70 is limiting for thermotolerance in the early embryo. It also suggests that it may be possible to alter the thermotolerance of even very complex developing organisms by altering the expression of hsp 70 genes in their genome. It is notable, however, that embryos that carried the extra hsp 70 genes did show an increased rate of survival to adulthood. A trivial explanation is that the extra hsp 70 genes in these transformants were not expressed in some tissue that is required for larval or pupal development. An alternative explanation is that while hsp 70 is beneficial in some tissues, it is detrimental in others. Indeed, expression of hsp 70 in the absence of heat inhibits growth in both tissue culture cells and in whole flies (Feder *et al.* 1992). In yeast cells, overexpressing hsp 104 has no adverse affect on growth (D. Parsell & S. Lindquist, unpublished data). Restoring hsp 100 expression in *Drosophila* might provide a simpler pathway for improving thermotolerance. However, it may be that the loss of hsp 100 expression in *Drosophila* is not just an evolutionary accident, but has occurred in response a detrimental effect that hsp 100 has on some aspect of *Drosophila* physiology. We have made considerable progress in elucidating the factors that control thermotolerance and continue to explore avenues that will allow us to manipulate the thermotolerance of higher eukaryotes in a meaningful way.

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Discussion

P. Viitanen (*du Pont de Nemours, Wilmington, U.S.A.*). Has Professor Lindquist seen any evidence that granules of hsp 70 can dissociate to release soluble hsp 70?

S. Lindquist. We have tried to demonstrate this but without success. This is puzzling because if the formation of these granules is irreversible and therefore represents an inactivation mechanism, it would seem simpler just to degrade the hsp 70. The observations with early embryos suggest that it may be easier to regulate a granule assembly process in the short time of ten minutes than regulate a degradation process.

P. Viitanen. Has Professor Lindquist estimated the turnover number of the ATPase activity of hsp 104 or looked for any peculiar ion requirements or modifications?

S. Lindquist. The K_m for ATP is about millimolar and the turnover rate is about 1000 times that of hsp 70. The pH optimum is at 9.0 so we are not sure how active it is inside the cell. We have not looked for autophosphorylation yet.

R. Jaenicke (*Department of Biophysics and Physical Biochemistry, University of Regensburg, Germany*). Why do acetate and galactose increase the amount of hsp 104; is this a question of nutrient stress or of regulation?

S. Lindquist. When provided with acetate and galactose the cells grow primarily by respiration, but with glucose they ferment to produce ethanol. Once the glucose is depleted the ethanol is then respired. We have evidence that hsp 104 can protect against the toxic effects of ethanol. So it is possible that the regulation of hsp 104 expression is related to respiration in such a way that the protein is produced in respiring cells to protect against high concentrations of ethanol in the growth medium.

A. Horwich (*Department of Molecular Genetics, Yale University, U.S.A.*). Is the fertilization of the oocyte associated with the loss of thermotolerance, or do the nurse cells transfer heat shock proteins along with other cytosolic proteins?

S. Lindquist. The oocyte is exquisitely sensitive to heat; it becomes heat-tolerant only at the blastoderm stage when hsp 70 becomes inducible. The oocyte in fact contains no inducible hsp 70 at all: this protein is specifically excluded from the package of other heat shock proteins that pass from the nurse cells into the oocyte. We think the explanation may be that hsp 70 is detrimental to rapid cell division.

F.-U. Hartl (*Memorial Sloan-Kettering Cancer Center, New York, U.S.A.*). The authors have observed in the hsp 104 mutant cells the formation of protein aggregates under mild heat shock conditions. Can these aggregates be resolved by the expression of wild-type hsp 104?

S. Lindquist. We have not tested this possibility; it is an interesting question whether these aggregates can be resolved or prevented from forming by the wild-type hsp 104.

F.-U. Hartl. This is part of a more general question as to whether cells contain a machinery to resolve protein aggregates or just one to prevent their formation.

S. Lindquist. We plan to test this possibility by expressing wild-type hsp 104 from a galactose promoter in cells that have been exposed to high temperatures.

R. J. Ellis (*Department of Biological Sciences, University of Warwick*). Professor Lindquist says that *Drosophila* oocytes do not contain hsp 70; how does this fit with the idea that one function of hsp 70 is to bind to nascent polypeptides?

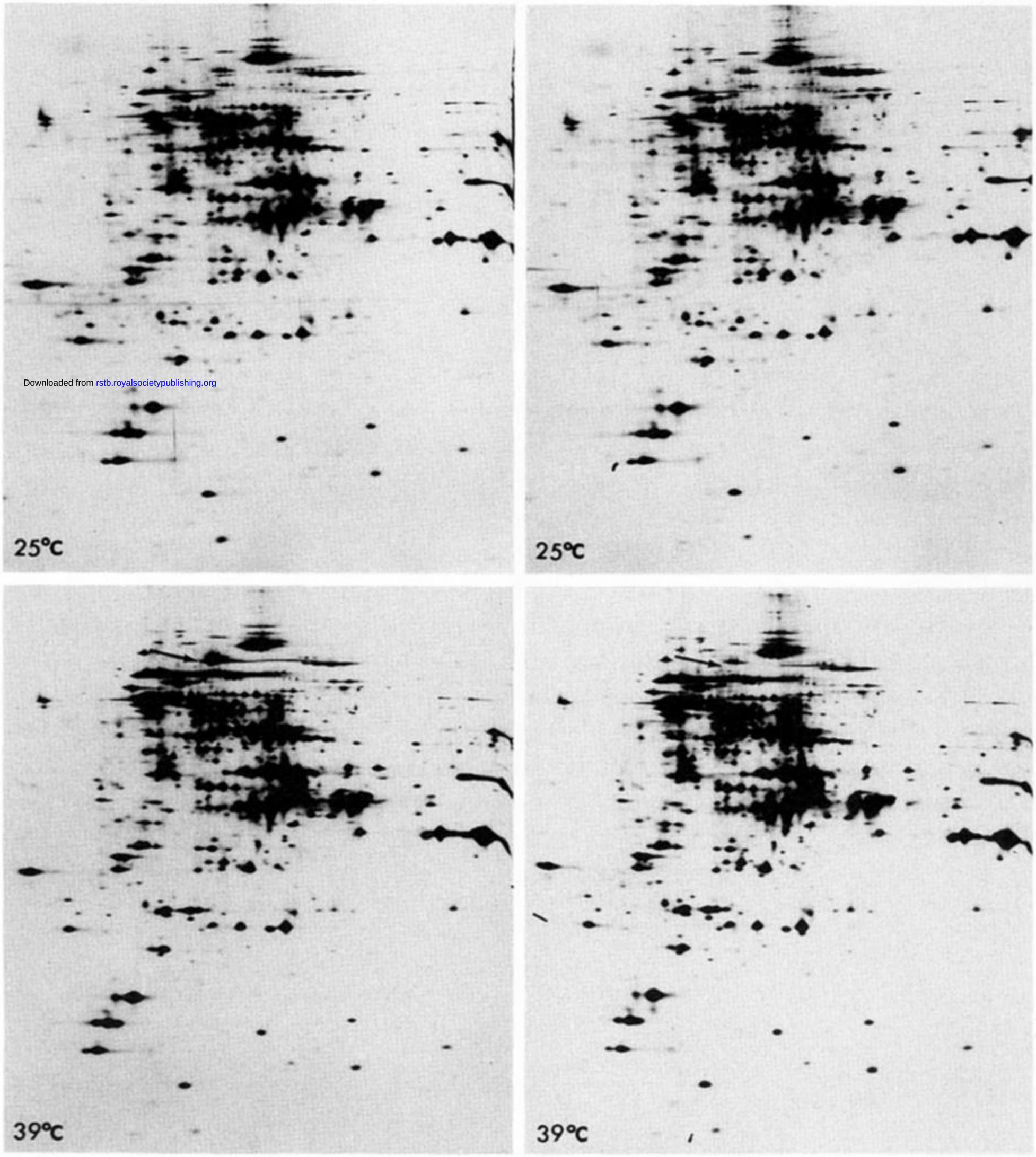
S. Lindquist. My remarks apply only to the inducible forms of hsp 70; *Drosophila* oocytes contain large amounts of constitutive hsp 70. It is becoming clear that the functions of the inducible forms of hsp 70 are not the same as those of the constitutive forms.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

W. J. Welch (*Department of Medicine and Physiology, University of California, San Francisco, U.S.A.*). In primate cells the highly inducible form of hsp 70 is also constitutively expressed.

S. Lindquist. Gloria Lee has transformed rat cells with extra copies of hsp 70 genes; these extra genes provide thermotolerance but they also slow down the growth rate. *Drosophila* seems to be a special case for two reasons: the cells do not produce hsp 104 proteins but they contain several copies of hsp 70 genes under tight regulation.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



gure 1. Analysis of proteins in wild-type and *hsp 104* mutant yeast cells. Proteins were extracted from log phase lls (9×10^6 per millilitre) which had been grown in rich medium at 25°C and exposed to 39°C for 60 min. Total llular proteins from 7.5×10^6 cells were separated by two-dimensional gel electrophoresis (Sanchez *et al.* 1990; aufeldt & Harrison 1984) and were visualized by silver staining. The position of hsp 104 is marked with an arrow n the heat-shocked samples.