or possibly two recent occasions in addition to the original one proposed. This might mean that one or two of the *mecA* polymorphisms shown in Fig. 1A could have arisen in the putative donor species before horizontal transfer to *S. aureus*. Any such de novo progenitor could then have given rise independently to one of the *mecA*-based groups shown in Fig. 4.

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27. We acknowledge the technical assistance of D. Kumar, E. Ha, A. Garcia, T. Baradet, W. S. Perry, and C. S. Rampersad. We thank K. Dyke, H. Westh, D. Dubin, T. J. Foster, and H. Pomeroy for providing MRSA isolates. Supported by National Institutes of Health grant AI22159 (to R.P.N.) and the Medical Research Program of the Department of Veterans Affairs (to R.D.A. and J.N.M.).

26 June 1992; accepted 5 October 1992

Regulation of Heat Shock Factor Trimer Formation: Role of a Conserved Leucine Zipper

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The human and *Drosophila* heat shock transcription factors (HSFs) are multi-zipper proteins with high-affinity binding to DNA that is regulated by heat shock–induced trimerization. Formation of HSF trimers is dependent on hydrophobic heptad repeats located in the amino-terminal region of the protein. Two subregions at the carboxyl-terminal end of human HSF1 were identified that maintain the monomeric form of the protein under normal conditions. One of these contains a leucine zipper motif that is conserved between vertebrate and insect HSFs. These results suggest that the carboxyl-terminal zipper may suppress formation of trimers by the amino-terminal HSF zipper elements by means of intramolecular coiled-coil interactions that are sensitive to heat shock.

Organisms respond to mild heat stress and to a variety of chemical inducers by rapidly increasing the transcription and translation of heat shock protein genes (*1*). The synthesis of heat shock proteins leads to an increased concentration of molecular chaperones, which are thought to maintain the native state and folding of cellular proteins under conditions of physiological stress (*2*). In eukaryotes, a preexisting transcriptional activator, HSF (*3*), mediates activation of heat shock genes by binding to conserved, upstream response elements [heat shock elements (HSEs)] (*4*). HSF binds to the HSE with high affinity as a trimer of identical subunits (*5*, *6*).

The synthesis of HSF protein is not regulated by heat shock, but the high-affinity binding of HSF to DNA is dependent on heat shock. The heat shock–inducible binding of HSF to the HSE in *Drosophila*, vertebrates, and plants but not in yeasts *Saccharomyces cerevisiae* and *Kluyveromyces lactis* (*3*, *7–9*) requires a transition of the HSF protein (*10*). Gel-filtration chromatography, sedimentation analysis, and chemical cross-linking of the two forms of *Drosophila* HSF indicate that this transition is a conversion from monomer to trimer (*11*); studies of the human HSF1 protein have found a similar change (*12*). In *S. cerevisiae*, the absence of control over HSF binding to DNA is reflected by the constitutive formation of HSF trimers, which can occupy chromosomal HSEs in vivo under normal as well as under heat shock conditions (*13*). Control of *S. cerevisiae* HSF activity is exercised at the level of transcriptional activation, which is correlated with increased phosphorylation at a number of serine and threonine residues (*14*). Increased phosphorylation after heat stress has also been observed for human HSF (*12*, *15*), but the mechanism by which phosphorylation may activate HSF is unknown (*9*, *14*).

Our efforts to understand the stress signal transduction pathway have focused on the mechanism by which heat shock leads to the aggregation and high-affinity binding of HSF protein. The *Drosophila* and human HSF proteins synthesized in *Escherichia coli* form trimers, hexamers, and higher oligomers at nonshock temperatures, suggesting that the HSF polypeptide has an intrinsic ability to form aggregates (*12*, *16*). The formation of trimers of HSF is dependent on several arrays of evolutionarily conserved, hydrophobic heptad repeats (zipper motifs) located next to the DNA-binding domain at the NH_2-terminal end of the protein (*5*, *16*). Thus, the stability of the HSF monomer under normal conditions could be dependent on a mechanism that suppresses the aggregation of the NH_2-terminal zipper elements.

A comparison of the predicted sequences of HSF proteins cloned from *S. cerevisiae*, *Drosophila*, and a human source provides insight into the mechanism for trimer suppression. Whereas HSF proteins of all three species contain conserved sequences in the NH_2-terminal DNA-binding domain and the adjacent zipper motifs, only *Drosophila* and human HSF proteins have an additional hydrophobic heptad repeat in the COOH-terminal region (*16*, *17–19*). Because this fourth zipper is absent from the constitutively trimeric yeast HSF, we suggested that it could be involved in the suppression of the aggregation of the metazoan HSFs under normal conditions (*17*).

To test this hypothesis, we changed two hydrophobic residues in the fourth zipper

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motif of human HSF1, the major HSF species in human tissue culture cells (*19*), to a charged amino acid (Met^{391} to lysine) and a helix-destabilizing residue (Leu^{395} to proline) to form the mutant HSF1: M391K,L395P (Fig. 1). Wild-type and mutant HSF1 (HSF1:M391K,L395P) genes were fused to the cytomegalovirus (CMV) promoter (*20*), and the constructs were transiently expressed in human embryonic

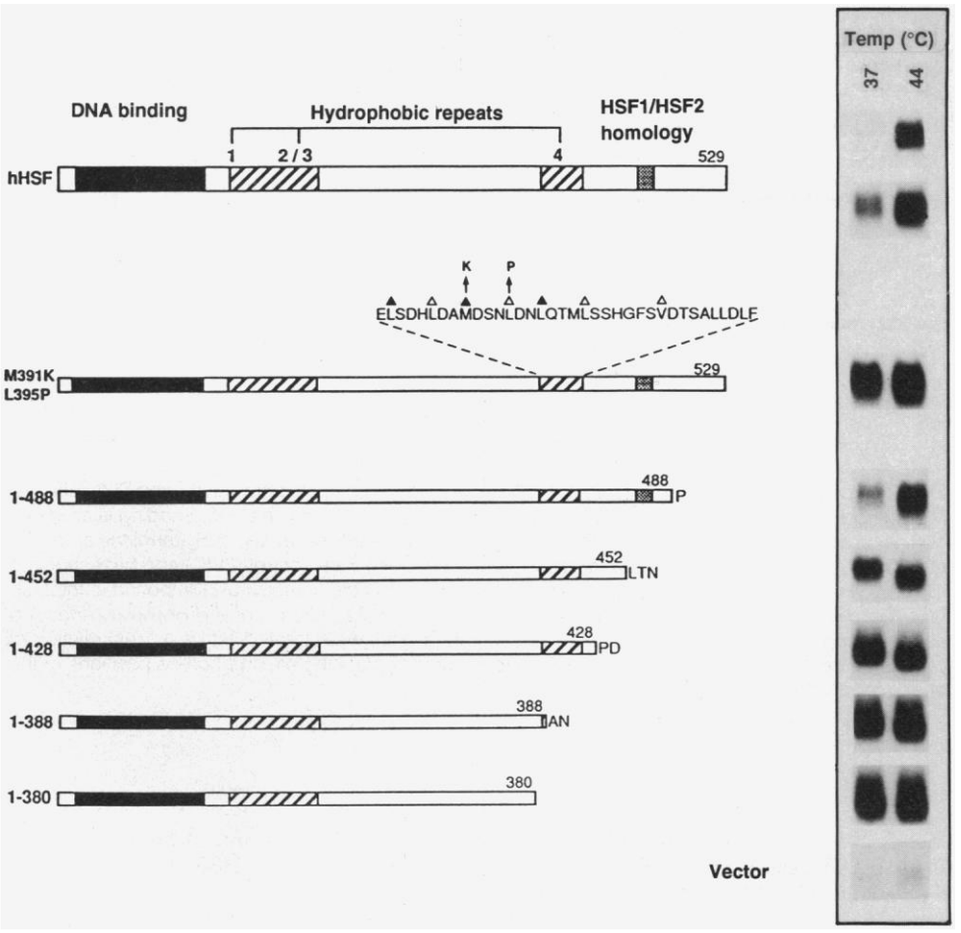

Fig. 1. Activity of wild-type and mutant human HSF1 proteins transiently expressed in 293 cells. Map of wild-type and mutant human *HSF1* (hHSF) ORFs is at left. Numbers on the right indicate the end point of the truncated fragments; amino acids in the fourth hydrophobic repeat and appended by cloning at the COOH-terminal and are represented by the single-letter code (*30*). The DNA binding region and hydrophobic repeats delineated by conserved sequence blocks are indicated by the filled and diagonally striped sections, respectively. The shaded section represents the 12-residue element conserved among vertebrate HSFs. The open and filled triangles denote hydrophobic residues at positions (a) and (d) of the heptad repeat. The gel mobility–shift analysis on extracts from unshocked (37°C) and heat-shocked (44°C) transfected cells shown at right was carried out as described (*17*) with a ^{32}P-labeled consensus HSE containing three [nGAAn] modules. Only the complex of HSF bound to the HSE is shown.

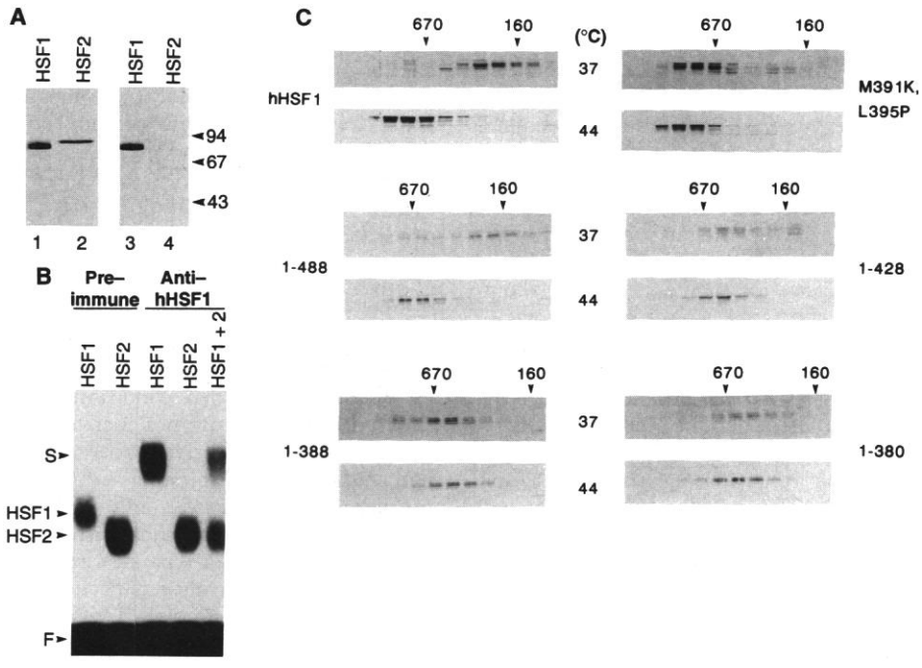

Fig. 2. Specificity of HSF1 antibodies and sizing of mutant hHSF1 proteins. (**A**) HSF1 and HSF2 cDNAs were cloned into pBluescript (Stratagene), transcribed in vitro with the mCAP RNA capping kit (Stratagene), translated in a rabbit reticulocyte lysate (Promega) according to the manufacturer's instructions with [^{35}S]methionine, and transferred to nitrocellulose. Film was exposed to lanes 1 and 2 after the protein immunoblot was probed with antibodies to HSF1 (anti-hHSF1) (lanes 3 and 4). Molecular size markers are indicated at right (in kilodaltons). We prepared polyclonal antibodies by injecting rabbits intradermally with 0.5 mg of HSF1 protein (purified from *E. coli* to 90% homogeneity), followed by another injection (0.5 mg) after 2 weeks. (**B**) HSF1 and HSF2 translated separately or together were reacted with preimmune or anti-HSF1 sera [1:50 in phosphate-buffered saline (PBS)] for 10 min before mobility-shift analysis. The positions of free DNA (F), the two HSF-HSE complexes, and the supershifted complex (S) are indicated. (**C**) Gel-filtration chromatography of extracts from nonshocked and heat-shocked 293 cells. Whole cell extracts (of 25 μl; 30 to 40 μg) were fractionated on a Superose 6 PG 3.2/30 column (Pharmacia) equilibrated in 20 mM tris (pH 7.9), 1.5 mM $MgCl_2$, 0.2 M KCl, 5% glycerol, and 1 mM dithiothreitol. Fractions (50 μl) were collected and precipitated with 10% trichloroacetic acid in the presence of 0.5 μg of bovine serum albumin. Pellets were washed in acetone and analyzed by SDS–gel electrophoresis and protein immunoblotting. Membranes were incubated with a 1:2000 dilution of anti-HSF1 antiserum diluted in PBS containing 0.05% Tween 20, washed, incubated with protein A–alkaline phosphatase conjugate (1:5000, Zymed, South San Francisco, California), and visualized with alkaline phosphatase substrate kit II (Vector Laboratories, Burlingame, California) according to the manufacturer's instructions. Arrows indicate mobility of molecular mass markers (thyroglobulin, 670 kD, and γ-globulin, 160 kD) in parallel chromatograms.

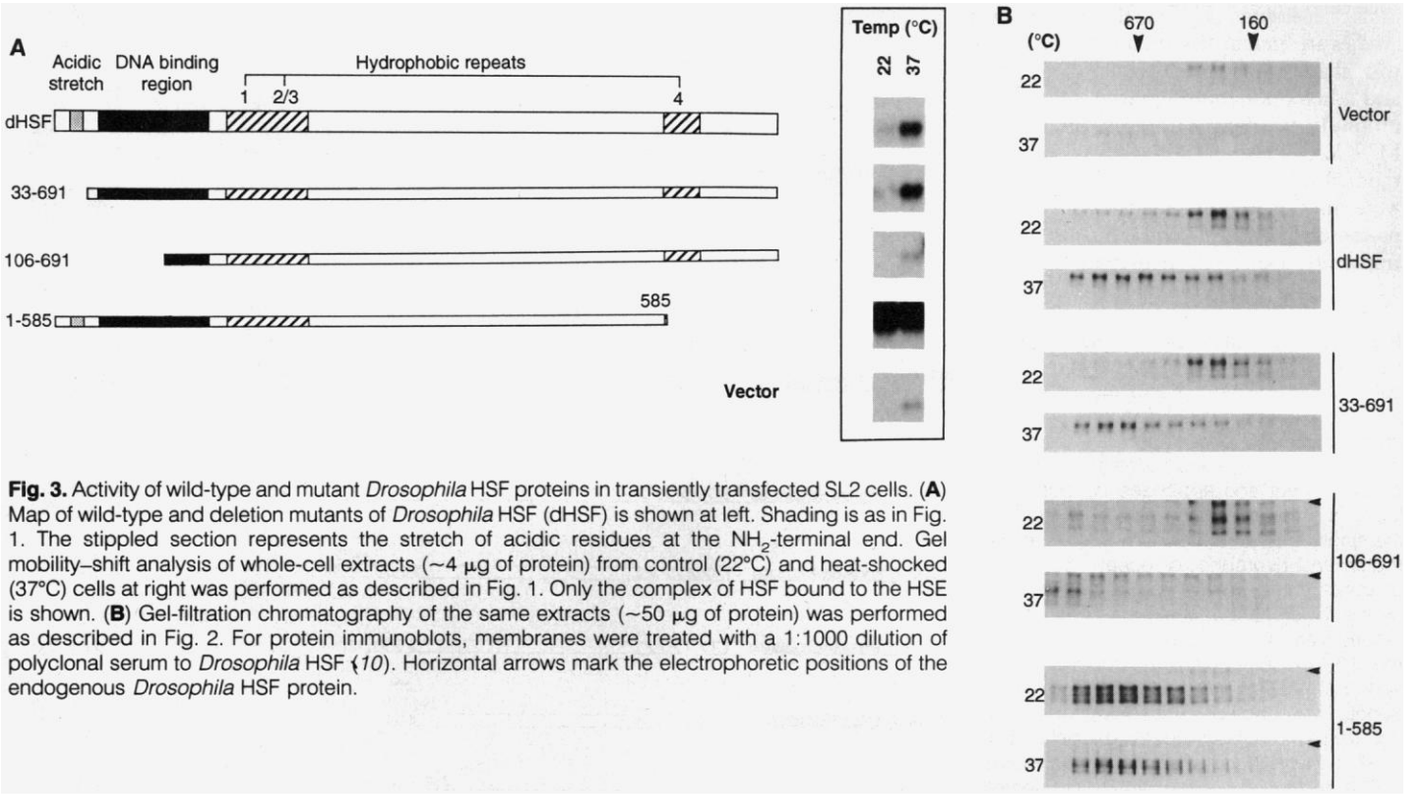

Fig. 3. Activity of wild-type and mutant *Drosophila* HSF proteins in transiently transfected SL2 cells. (**A**) Map of wild-type and deletion mutants of *Drosophila* HSF (dHSF) is shown at left. Shading is as in Fig. 1. The stippled section represents the stretch of acidic residues at the NH_2-terminal end. Gel mobility–shift analysis of whole-cell extracts (~4 μg of protein) from control (22°C) and heat-shocked (37°C) cells at right was performed as described in Fig. 1. Only the complex of HSF bound to the HSE is shown. (**B**) Gel-filtration chromatography of the same extracts (~50 μg of protein) was performed as described in Fig. 2. For protein immunoblots, membranes were treated with a 1:1000 dilution of polyclonal serum to *Drosophila* HSF (*10*). Horizontal arrows mark the electrophoretic positions of the endogenous *Drosophila* HSF protein.

kidney 293 cells by DNA transfection (*21*). Human 293 cells have a low endogenous amount of HSF-binding activity, which is shown by the gel mobility–shift assay of extracts prepared from cells transfected with the expression vector alone (Fig. 1; vector). Extracts prepared from cells expressing the wild-type human HSF1 protein showed a range of basal HSF-binding activity under normal conditions; two separate experiments that display the lower and upper limits of basal activity are shown (Fig. 1). Despite the variation in basal activity, all experiments performed with the full-length *HSF1* gene showed a significant increase of binding activity (about tenfold) after heat shock. These observations indicated that the wild-type human HSF1 protein was regulated in 293 cells in a manner similar to the endogenous HSF proteins (*22*). When the binding activity of HSF1:M391K,L395P was analyzed, the amount of the binding activity in unshocked cells was substantially increased (about sevenfold) over the activity of the wild-type HSF1 protein (Fig. 1). In addition, a small increase of HSF1:M391K,L395P binding activity (about 1.5-fold) was reproducibly observed after heat shock. In these and all subsequent transfection experiments, protein immunoblot analyses indicated that equivalent amounts of HSF1 proteins were expressed in cells with and without the heat shock treatment (*12*). Thus, the two amino acid changes introduced in the fourth zipper motif caused significant, although incomplete, derepression of HSF1-binding activity in unshocked cells.

To determine whether other regions of HSF were involved in suppressing the DNA-binding activity under normal conditions, we constructed a set of nested 3′ deletions of the human *HSF1* open reading frame (ORF) (Fig. 1) and compared the activity of the mutant proteins with the activity of full-length HSF1 (*20*). Like the full-length human HSF1, HSF1:1–488 showed little DNA-binding activity in unshocked cells and increased DNA-binding activity after heat shock. In contrast, all other deletion mutants tested: [1–452, 1–428, 1–388, and 1–380 (Fig. 1) and 1–353, 1–339, and 1–283 (*12*)] showed high amounts of binding to DNA when expressed under normal conditions, and the binding activity did not increase further after heat shock. The constitutively high amount of DNA-binding activity observed with HSF1:1–452 and HSF1:1–428, both of which retain the fourth zipper motif, indicates that a separate region in the COOH-terminus must participate in stabilizing the HSF monomer (*23*).

The observed increases in DNA-binding activity of the double point mutant (HSF1:M391K,L395P) and the deletion mutants (HSF1:1–452, 1–428, 1–388, and 1–380) under nonshock conditions suggested that these mutant proteins should be constitutively aggregated as a trimer. To test this prediction, we fractionated transfected cell extracts by gel-filtration chromatography and analyzed the column fractions by protein immunoblot analysis. Because human cells have two species of HSF (HSF1 and HSF2) that share sequence similarity in the DNA-binding domain and leucine zipper motifs, we first determined the specificity of the antibodies to HSF1. Antibody staining of HSF1 but not HSF2 protein was shown by protein immunoblots (Fig. 2A). Antibodies to HSF1 also reacted specifically by supershifting the protein-DNA complex containing HSF1 but not the complex containing HSF2 in a gel mobility–shift assay (Fig. 2B). An identical result was obtained when both HSF1 and HSF2 were cotranslated and treated with antibody in the same reaction, indicating that HSF1 and HSF2 proteins do not form mixed oligomers.

The wild-type HSF1 monomer extracted from unshocked 293 cells chromatographed with a relative molecular mass of ~200 kD, whereas the HSF1 trimer extracted from heat-shocked cells chromatographed as a broad peak at ~1000 kD (Fig. 2C) (*24*). In contrast, the majority of HSF1:M391K,L395P protein isolated from unshocked 293 cells chromatographed at ~1000 kD; no significant increase in size was observed when the transfected cells were subjected to heat stress. These observations suggest that the COOH-terminal zipper motif is involved in maintaining the monomeric state of the HSF1. Gel-filtration chromatography and protein immuno-

blot analysis of the inducible mutant HSF1:1–488 demonstrated a heat shock–dependent transition in the size of the native protein that was similar to the transition of the full-length HSF1, whereas the constitutive mutants HSF1:1–428, 1–388, and 1–380 formed large species under normal conditions, presumably trimers, and did not exhibit a significant increase in size after heat shock (Fig. 2C). The protein immunoblot analysis also confirmed that the amount of mutant HSF1 proteins expressed in unshocked and heat-shocked 293 cells was approximately equivalent.

The fourth leucine zipper is conserved in the sequence of *Drosophila* HSF protein. We deleted a region of the *Drosophila* HSF ORF corresponding to the COOH-terminal 106–amino acid residues of the protein, including the fourth zipper (Fig. 3A), and expressed the mutant protein (*Drosophila* HSF:1–585) in *Drosophila* Schneider 2 (SL2) cells under the control of the metallothionein promoter (*25*). Gel mobility–shift analysis of *Drosophila* HSF:1–585 revealed a high amount of constitutive DNA-binding activity in the extracts of unshocked cells and no increase in binding activity after heat shock of the transfected cells (Fig. 3A) (*26*). Similar results were observed with COOH-terminal deletions of the *Drosophila* HSF ORF that extended to amino acid residues 473 and 361, respectively (*12*). In contrast, the full-length *Drosophila* HSF protein displayed inducible binding activity after heat shock, similar to the endogenous *Drosophila* HSF present in cells transfected with the expression vector alone.

The DNA-binding region of *Drosophila* HSF is separated from the NH_2-terminal end of the protein by a stretch of glutamic acid residues. We tested the ability of these NH_2-terminal sequences to affect the regulation of HSF activity by deleting sequences corresponding to the NH_2-terminal 33–amino acid residues of *Drosophila* HSF and analyzing the activity of the mutant protein in transfected cell extracts. As shown by the gel mobility–shift assay in Fig. 3A, *Drosophila* HSF:33–691 retained heat shock regulation of the DNA-binding activity, indicating that the stretch of acidic residues is not involved with this aspect of HSF function. Further deletion into the DNA-binding region (*Drosophila* HSF:106–391) completely abolished the ability of the mutant protein to bind to the HSE.

We determined the extent of aggregation of the truncated *Drosophila* HSF proteins by gel-filtration chromatography and protein immunoblotting of the column fractions. The *Drosophila* HSF mutant lacking zipper 4, *Drosophila* HSF:1–585, aggregated as a high molecular mass species when expressed under normal and heat shock conditions (Fig. 3B). In contrast, the endogenous *Drosophila*

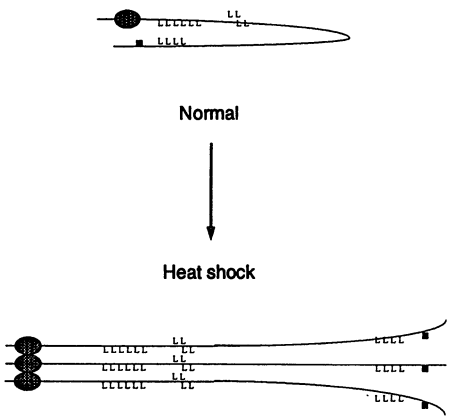

Fig. 4. Model for the repression of HSF1 trimer formation. The HSF molecule is shown as a line with the shaded oval representing the NH_2-terminal DNA binding domain. The hydrophobic heptad repeats are indicated by the L's (not necessarily leucines), and the regulatory region COOH-terminal to the fourth zipper motif is indicated by the square. The HSF monomer is proposed to be stabilized by direct interactions between the NH_2- and COOH-terminal zippers. The orientation of the COOH-terminal regulatory regions with respect to the NH_2-terminal end of the HSF monomer is arbitrary, as is the lack of interaction between COOH-terminal zippers in the HSF trimer.

HSF, the full-length *Drosophila* HSF, and *Drosophila* HSF:33–691 showed a heat shock–dependent transition from a species of relative molecular mass ~250 kD to a broad peak at ~1000 kD (*24*). *Drosophila* HSF:106–691, which lacks a large section of the DNA-binding region, retained a heat shock–inducible oligomeric transition that was roughly similar to the transition observed for the endogenous protein in the same cell extract. This result suggests that a major part of the DNA-binding domain is not involved, at least for *Drosophila* HSF, in the interactions that stabilize the HSF monomer.

Our results show that two regions of human HSF1 are necessary for the maintenance of the monomeric state under physiologically normal conditions. The first region is a leucine zipper motif that is conserved between the *Drosophila* and vertebrate factors; the second region, defined by the end points of the two deletion mutants HSF1:1–488 and 1–452, contains a 12-residue element (amino acids 463 to 474) conserved among the vertebrate HSF1 and HSF2 proteins (67% identity) (*27*). Both regions appear to be required for proper control because mutations in either lead to aggregation and high-affinity binding to DNA in the absence of a heat stress.

How these two regions act to repress trimer formation and the high-affinity DNA binding of HSF is unknown. We suggest that the COOH-terminal leucine zipper could associate with the zippers located at the NH_2-terminal region of the protein by intramolecular coiled-coil interactions (Fig. 4). Such an interaction would mask the NH_2-terminal zippers and suppress their ability to form trimers. The second control region downstream of the leucine zipper may function through a direct interaction with another part of the human HSF1 protein to stabilize the coiled-coil interactions or may be required for the proper folding of the COOH-terminal regulatory domain. The possibility that one or both regulatory regions of the protein are stably complexed with another molecule that masks the NH_2-terminal zippers cannot be excluded (*28*).

We suggest that the COOH-terminal interactions that constrain the structure of the HSF monomer are disrupted by heat shock, thereby freeing the NH_2-terminal zippers for intermolecular interactions in the HSF trimer. Such a disruption could be caused directly by a rise in temperature (*29*) or by secondary events caused by heat stress. These events may include posttranslational modifications of HSF protein, physicochemical changes in the cellular environment, or a decrease in the free pool of molecular chaperones. Disruption of the COOH-terminal zipper interactions could also occur through the binding of chemical inducers known to activate the heat shock response.

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sedimentation on glycerol gradients. Discrepancies in the extent of oligomerization reported by our laboratory (*16*) and by Perisic and co-workers (*6*) are most likely related to the greater tendency of bacterially expressed HSF to aggregate as hexamers and higher oligomers and to the anomalous migration of HSF when analyzed by nondenaturing polyacrylamide gradient gel electrophoresis.
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18. In addition to the cloning of two human HSF cDNAs [HSF1 (*17*) and HSF2 (*19*)], several HSF cDNAs have been cloned from tomato (*7*) and mouse [K. D. Sarge, V. Zimarino, K. Holm, C. Wu, R. I. Morimoto, *Genes Dev.* **5**, 1902 (1991)]. The COOH-terminal leucine zipper is conserved in the mouse and to some extent in the tomato HSFs. The necessity for multiple HSFs is unclear because yeast and *Drosophila* HSFs are apparently single-copy genes; it may reflect a cell or tissue restriction or a functional diversity related to the varied nature of the stress signals.
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20. The cDNA for human HSF1 (including the 5′ and 3′ untranslated regions) was subcloned into pCMV5 (a derivative of pCMV3), which has a multiple cloning site downstream of the CMV promoter-enhancer. Point mutations in the leucine zipper motif were introduced by site-directed mutagenesis. For constructing the deletion mutants, we subcloned the entire cDNA into pCMV6 (derived from pCMV5), which has stop codons downstream of the multiple cloning site. COOH-terminal nested deletions were obtained with the double-stranded nested deletion kit (Pharmacia). The end points of all deletion mutants and the sequence of the point mutation were confirmed by sequencing. For transfection experiments, plasmids were prepared by alkaline lysis and purified by polyethylene glycol precipitation and ethidium bromide–CsCl gradient centrifugation.
21. Human embryonic kidney 293 cells transformed with adenovirus [F. L. Graham and J. Smiley, *J. Gen. Virol.* **36**, 59 (1977)] were grown for 18 to 24 hours in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in T-25 tissue culture flasks coated with poly-lysine (1×10^6 cells per flask). Cells were synchronized by a thymidine block (3.3 mM for 18 to 20 hours). Two hours after release from the block, cells were washed twice with opti-MEM (Gibco/BRL) and incubated with the lipofectin-DNA complex for 5 hours. We prepared the complexes by combining 10 μl of lipofectin (BRL) and 1 μg of DNA in a final volume of 1.2 ml. Transfections were ended with the addition of 1.2 ml of DMEM containing 20% FBS, and we allowed the cells to express transfected DNA for 12 to 14 hours. Before the cells were harvested, flasks were either kept at room temperature or heat-shocked at 44°C for 20 min. Cells were harvested by gentle scraping, and whole-cell extracts were prepared as described [V. Zimarino, C. Tsai, C. Wu, *Mol. Cell. Biol.* **10**, 752 (1990)].
22. In order to achieve optimal regulation of HSF1 in 293 cells, we adjusted the conditions for DNA transfection and expression. In general, the amount of protein expressed in transfected cells was limited by the use of small amounts of DNA (1 μg) and by short transfection and expression times. Longer transfection or expression times resulted in a high constitutive amount of DNA-binding activity in transfected cells that were not subjected to heat stress.
23. This finding is consistent with our observation that the double amino acid substitution in the fourth zipper alone does not lead to a completely constitutive DNA-binding activity.
24. The molecular size of HSF1 monomer and trimers are deceptively high when analyzed by gel-filtration chromatography, which only measures the Stokes radius. The positions of the globular marker proteins indicated in the figure serve as reference points and do not accurately estimate the native size of the HSF protein. The resolution of the microbore Superose 6 column (Pharmacia) was decreased because we applied a 25-μl sample volume in order to detect the expressed HSF1 protein.
25. Expression plasmids for *Drosophila* HSF were constructed by insertion of polymerase chain reaction (PCR) fragments containing the full-length or truncated ORF (the sequence 5′-AATTCAAA-3′ was added upstream of the ATG codon) into pRmHa-3 vector between the *Drosophila* metallothionein promoter-leader and the alcohol dehydrogenase gene 3′ end. We transfected CsCl-purified plasmids into SL2 cells using lipofectin according to the protocol of L. Sondergaard (personal communication). Briefly, about 3×10^6 cells in M3 medium supplemented with 10% heat-inactivated FBS were grown in T-25 flasks at 22°C for 24 hours. The medium was replaced with serum-free M3 containing 50 μl of lipofectin-DNA complex. We prepared complexes by combining 30 μl of lipofectin with 20 μl of diluted DNA (2 μg), and allowed the transfections to proceed for 24 hours. Transfections were stopped by the addition of FBS to a final concentration of 10%, and we allowed cells to recover for 24 hours before adding $CuSO_4$ to a final concentration of 0.7 mM. After 16 hours of induction, the cells were collected either directly or after 15 min of heat shock at 37°C, and whole-cell extracts were prepared as described [V. Zimarino and C. Wu, *Nature* **327**, 727 (1984)].
26. The amount of DNA-binding activity of mutant 1–585 is significantly higher than the activity of the full-length *Drosophila* HSF and is due to overexpression of the mutant protein in transfected cells. However, even when the expression was reduced, this protein was fully active under nonshock conditions.
27. The 12-residue element is not found in the sequence of *Drosophila* HSF. It is possible that another element may serve the same function.
28. The molecular chaperone Hsp70 has been suggested to act as a negative regulator of HSF trimer formation (*2, 3, 16*), and there is evidence for interactions between Hsp70 and HSF in vitro, although the biological significance of these interactions remains to be determined [K. Abravaya, M. P. Myers, S. P. Murphy, R. I. Morimoto, *Genes Dev.* **6**, 1153 (1992); R. Baler, W. J. Welch, R. Voellmy, *J. Cell Biol.* **117**, 1151 (1992); S. K. Rabindran *et al.*, in preparation]. However, the size of the HSF monomer in cell extracts as measured by gel filtration and sedimentation analysis is incompatible with a stable association between HSF and Hsp70 (*11*).
29. The induction temperature of hHSF1 is lowered to 37°C when hHSF1 is expressed in *Drosophila* cells (J. Clos, S. K. Rabindran, J. Wisniewski, C. Wu, in preparation).
30. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
31. We thank B. Howard (National Institutes of Health) for pCMV5, T. Bunch for pRmHa-3, W. Herr and L. Goldstein for gifts of expression vectors, T. J. Schuetz and R. E. Kingston for the HSF2 cDNA clone, L. Sondergaard for the SL2 cell transfection protocol with lipofectin, J. Eldridge for oligonucleotide synthesis, and C. Klee for suggestions. R.I.H. was supported by a Howard Hughes Medical Institute–NIH Research Scholarship.

20 July 1992; accepted 3 November 1992

Rate and Mechanism of Nonhomologous Recombination During a Single Cycle of Retroviral Replication

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Oncogenes discovered in retroviruses such as Rous sarcoma virus were generated by transduction of cellular proto-oncogenes into the viral genome. Several different kinds of junctions between the viral and proto-oncogene sequences have been found in different viruses. A system of retrovirus vectors and a protocol that mimicked this transduction during a single cycle of retrovirus replication was developed. The transduction involved the formation of a chimeric viral-cellular RNA, strand switching of the reverse transcription growing point from an infectious retrovirus to the chimeric RNA, and often a subsequent deletion during the rest of viral DNA synthesis. A short region of sequence identity was frequently used for the strand switching. The rate of this process was about 0.1 to 1 percent of the rate of homologous retroviral recombination.

Highly oncogenic retroviruses have incorporated cellular proto-oncogene sequences between their long terminal repeats (LTRs). Most hypotheses for the origin of highly oncogenic retroviruses from cellular proto-oncogenes and replication-competent retroviruses propose an initial formation of a chimeric retrovirus–proto-oncogene RNA (*1, 2*). This chimeric RNA results either from transcription of DNA after a deletion that fuses 5′ viral sequences to cellular sequences or from readthrough transcription, which is often followed by abnormal splicing. An additional recombination step is then needed to add 3′ viral sequences and to form a highly oncogenic retrovirus. The 3′ viral–proto-oncogene junctions, when compared to the parental viral and proto-onco-

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