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16. From 75 heavy-atom–containing solutions, heavy-atom derivatives were screened by the sinking crystal method (*17*). Data were collected from some 18 crystals on a MSC Rigaku AFC 5R. Corrections were made for decay and absorption (*18*). A $K_2Pt(NO_2)_4$ derivative was located on the threefold symmetry axis by matching its isomorphous and anomalous difference Patterson functions with peaks from an E map, which was generated from the isomorphous differences by the direct methods program MITHRIL (*19*). This model was refined by the Fhle method (*20*) with REFINE2 (*21*). A $Bi(NO_3)_3$ derivative was located in a general position from a difference Fourier map with phases from the Pt derivative. The Bi derivative was refined by the origin-removed Patterson correlation method (*22*), and phases based on it returned the correct position of the Pt atom in a difference Fourier map. Both of these heavy atoms are located in chemically reasonable places on the protein surface: the Bi site is near the carboxyl terminus and Glu^8 side chain, and the Pt ion occupies a crevice near the side chains of Lys^4 and Glu^8.
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Antibody-Mediated Activation of *Drosophila* Heat Shock Factor in Vitro

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Eukaryotic cells respond to elevated temperatures by rapidly activating the expression of heat shock genes. Central to this activation is heat shock–inducible binding of the transcriptional activator, termed heat shock factor (HSF), to common regulatory elements, which are located upstream of all heat shock genes. The DNA binding activity of the inactive form of *Drosophila* HSF was induced in vitro by treatment with polyclonal antibodies to the purified, in vivo–activated factor. This finding, together with observations that high temperature and low pH activate HSF binding in vitro, suggests that the inactive form of HSF can directly recognize and transduce the heat shock signal without undergoing a covalent modification of protein structure.

ORGANISMS RESPOND TO AN ELEVATION in the ambient temperature by the rapid transcription and translation of RNAs encoding heat shock proteins (*1*). The transcriptional response to heat shock in eukaryotes is mediated by a positive control element, the heat shock element (HSE), which is present in multiple copies upstream of all heat shock genes (*2*). A transcriptional activator protein, termed heat shock factor (HSF), binds to HSEs (*3–8*) and activates transcription of heat shock genes in vitro in a binding site–dependent manner (*9–11*). Although the sequence of the HSE has been highly conserved in evolution, there are differences in the properties of HSF from a range of eukaryotic species (*6, 8*). Electrophoretic analyses of HSF purified from *Saccharomyces cerevisiae*, *Drosophila*, and human cells indicate that the factors have molecular sizes of 150, 110, and 83 kD, respectively (*11–13*). In addition, HSF is bound constitutively to the HSE in yeast (*6, 8, 14*), whereas HSF in *Drosophila* and vertebrates is unable to bind to the HSE unless the cells are heat shocked (*3, 5–8*). The relative increase in HSF binding is correlated with the severity of the heat shock stimulus, suggesting that the temperature-dependent induction of HSF binding activity is a critical regulatory switch in the activation of heat shock gene transcription in higher eukaryotes.

Experiments with protein synthesis inhibitors indicate that de novo protein synthesis is not required for the induction, reversal, and reinduction of HSF binding activity in *Drosophila*, *Xenopus*, and human cells (*5, 8, 15*). These studies indicate that HSF binding is likely to be regulated by a posttranslational mechanism in higher eukaryotes. Moreover, the binding activity of human HSF in HeLa cell cytoplasmic extracts is induced in vitro simply by heat treatment, suggesting that the inactive form of human HSF is able to sense the temperature elevation (*16*). Furthermore, low pH (5.8 to 6.4) activates human HSF in vitro (*17*). Using the gel mobility shift assay, we have found that heat (35°C) and low pH (KH_2PO_4 buffer, pH 6.5) also stimulate the DNA binding activity of HSF in unshocked cyto-

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plasmic extracts of a *Drosophila* cell line (SL2) (Fig. 1). The optimal temperature (35°C) for the in vitro activation of *Drosophila* HSF is close to the temperature (37°C) that elicits a maximal heat shock response in intact cells (*18*). Once heat-treated in vitro, the HSF binding activity remained stable, even at low temperatures or when returned to neutral pH (*19*).

We have now discovered that treating unshocked SL2 cytoplasmic extracts with polyclonal antiserum to the purified, in vivo–activated form of *Drosophila* HSF (anti-HSF) could also induce HSF binding activity in vitro (Fig. 1). [Antibodies have previously been shown to affect the activities of other proteins. For example, autoantibodies to the insulin receptor have been found to mimic the effect of insulin in down-regulating the receptor protein (*20*).] The increase of HSF binding activity mediated by the antiserum (about tenfold) appeared to be higher than the increase caused by heat or low pH treatment (Fig. 1) and was comparable to the increase observed when intact cells were heat-shocked (*8*). In addition to stimulating HSF binding activity, the interaction of anti-HSF with HSF slowed the migration of the HSF-HSE DNA complex in the gel mobility shift assay. This decreased migration is likely to be due to the additional binding of one or more antibodies to the complex. Other protein-DNA complexes show a similar decreased migration when bound to specific antibodies (*21, 22*).

The antibodies to HSF are specific for *Drosophila* HSF (Fig. 2). HSF purified by affinity chromatography from heat shock–induced *Drosophila* SL2 cells, *S. cerevisiae*, and human HeLa cells was incubated with either preimmune mouse serum or anti-HSF, before binding to ^{32}P-labeled HSE DNA for the gel mobility shift assay. The presence of either serum did not affect formation of complexes of HSF bound to HSE (denoted "B"). Only the *Drosophila* HSF-HSE complex revealed an interaction with anti-HSF, as evidenced by a greater retardation of gel mobility. As might be expected, no increase of binding activity was observed when *Drosophila* HSF that was activated in vivo was bound to antibody. The specificity of the HSF-HSE interaction in the gel shift assay was confirmed by the inhibition of complex formation in the presence of excess unlabeled HSE DNA. Protein gel blot assays also showed that the antibody reacted with the 110-kD *Drosophila* HSF polypeptide (*19*).

Antibodies to HSF were only able to activate in vitro the HSF prepared from *Drosophila* cells. Anti-HSF induced HSF binding activity in unshocked cytoplasmic extracts of a different *Drosophila* cell line (K_c), but did not induce the activity in cytoplasmic extracts of HeLa cells (Fig. 3a). The HeLa cell extracts did contain "activatable" HSF, because an increase in HSF bind ing activity was observed when the HeLa extracts were incubated at a higher temperature. The possibility that the inducing activity is a nonspecific substance in mouse serum was excluded by a demonstration that preimmune serum and antiserum to an unrelated *Drosophila* transcription factor, FTZ-F1 (*23*), were both unable to induce the HSF binding activity in cytoplasmic extracts of unshocked *Drosophila* cells (Fig. 3b). These results indicate that the stimulation of HSF binding in vitro is due to specific antibody recognition of *Drosophila* HSF protein. Further analyses indicated that the antibody-mediated stimulation of HSF binding was not affected by dilution (up to tenfold) of the cytoplasmic extract, nor by performing the antibody reaction at 4°C (*19*). Since the antiserum was specific for HSF protein that was activated in vivo, it seems likely that the activation in vitro is caused by an antibody-mediated stabilization of the active form of HSF. Such a stabilization would irreversibly shift the thermodynamic equilibrium between the inactive and active forms of HSF in the direction of the active species.

Our experiments showing antibody-mediated activation of HSF binding in vitro were performed with crude cytoplasmic extracts, which typically contain a basal level of HSF binding activity. We have separated this activity from the inactive form of HSF by chromatography of unshocked cytoplasmic extracts. The basal HSF binding activity was assayed by the standard gel mobility shift procedure, and the inactive form of HSF,

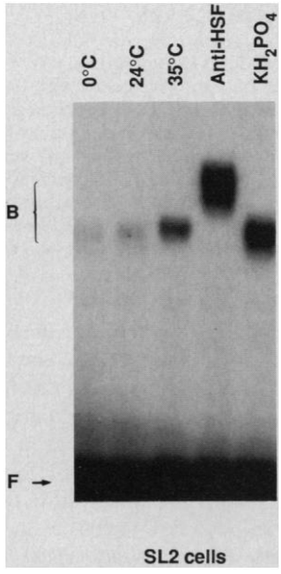

Fig. 1. Gel mobility shift analysis showing in vitro activation of *Drosophila* HSF. Cytoplasmic extracts were prepared from unshocked SL2 cells essentially as described (*27*). Samples (5 μl) of extract were incubated for 10 min at the indicated temperatures, with 50 mM KH_2PO_4 at 24°C (pH 6.5) (final volume 6 μl), or with 1:10 dilution of anti-HSF (final volume 6 μl). Samples were transferred to ice, an equal volume of Dignam's solution A [(*27*), modified to contain 150 m*M* KCl] was added, and incubated on ice for 10 min with 30 fmol of a ^{32}P-labeled consensus HSE (*8*) and 2 μg of poly(dI-dC) · poly(dI-dC). The samples were subjected to electrophoresis at room temperature on a 1% agarose gel in 0.5× tris-borate-EDTA buffer and autoradiographed (*15*). Addition of 40-fold molar excess of unlabeled HSE resulted in inhibition of binding to the labeled HSE (*19*); therefore, all the protein-DNA complexes denoted B (bound) are specific. The free DNA is denoted by F. Antibody-mediated activation of HSF was observed with final serum dilutions ranging from 1:5 to 1:100 (*19*). Anti-HSF was prepared from *Drosophila* HSF purified to 95% homogeneity (*12*). HSF (0.5 μg) was applied to nitrocellulose membrane filters (4 mm by 4 mm) (Schleicher & Schuell BA85). The square was inserted subcutaneously in a dorsolateral location in a mouse. At 3-week intervals, antigen (0.5 μg of HSF) was again applied. Dilutions up to 1:1000 of the immune serum showed positive immunohistochemical staining (Vector Laboratories, Burlingame, CA) with 5 ng of purified HSF protein dotted on nitrocellulose. Preimmune serum diluted to final concentrations ranging between 1:10 and 1:1000 tested negative for staining with HSF.

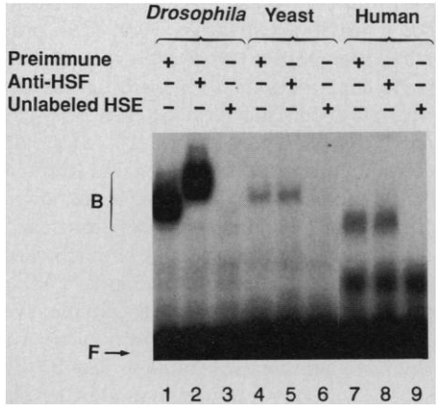

Fig. 2. Specificity of the anti-HSF serum. HSF was purified from the indicated heat-shocked cells by affinity chromatography essentially as described (*12*). (Lanes 1, 2, 4, 5, 7, and 8) HSF (2 μl) was incubated at 4°C for 20 min with 1 μl of a 1:2 dilution of preimmune serum or anti-HSF. After the addition of 1 μl of bovine serum albumin (50 mg/ml), 1 μl of ^{32}P-labeled HSE (30 fmol), and 1.5 μl of a mixture containing 1 μg *Escherichia coli* DNA and 10 μg of poly(dI-dC) · poly(dI-dC), the samples were further incubated for 10 min at 25°C. (Lanes 3, 6, and 9) HSF (2 μl) was incubated as above, except that serum was absent, and 1 μl of a 40-fold excess of unlabeled HSE DNA was included in the final incubation. The samples were subjected to electrophoresis as in Fig. 1.

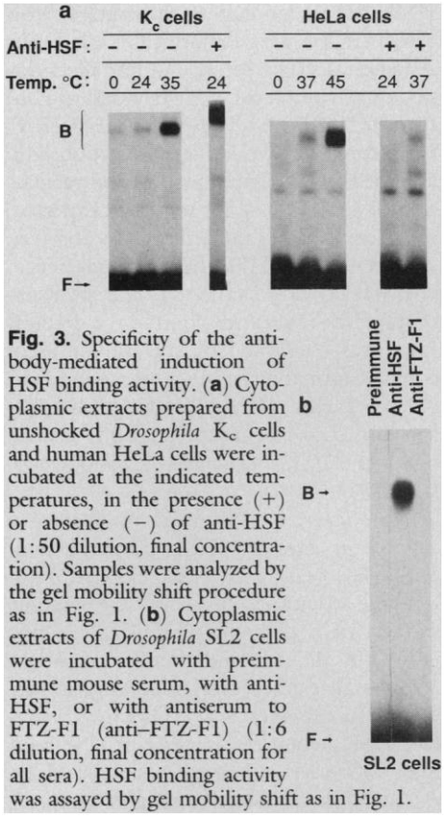

Fig. 3. Specificity of the antibody-mediated induction of HSF binding activity. (**a**) Cytoplasmic extracts prepared from unshocked *Drosophila* K_c cells and human HeLa cells were incubated at the indicated temperatures, in the presence (+) or absence (−) of anti-HSF (1:50 dilution, final concentration). Samples were analyzed by the gel mobility shift procedure as in Fig. 1. (**b**) Cytoplasmic extracts of *Drosophila* SL2 cells were incubated with preimmune mouse serum, with anti-HSF, or with antiserum to FTZ-F1 (anti–FTZ-F1) (1:6 dilution, final concentration for all sera). HSF binding activity was assayed by gel mobility shift as in Fig. 1.

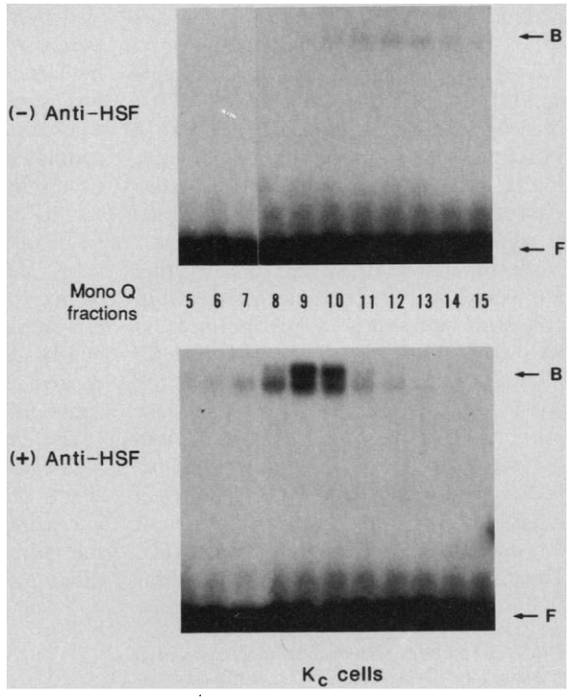

Fig. 4. Mono Q column chromatography of the basal HSF activity and the inactive form of HSF. Cytoplasmic extracts of 3×10^9 unshocked *Drosophila* K_c cells were equilibrated in chromatography buffer [CB: 20 mM tris-HCl (pH 7.9), 5% glycerol, 0.5 dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride] containing 0.1 M NaCl. A portion (0.5 ml) of the crude extract (protein, 15 mg/ml; total volume, 7 ml) was diluted with an equal volume of CB, NP-40 was added to a final concentration of 0.05%, and the sample was applied to a 0.2-ml Cibachron Blue–Sepharose (Pharmacia) column. The column was washed with 1 ml of CB (with 0.1 M NaCl), and protein was eluted sequentially with 0.26 ml of CB containing 0.5 M NaCl and 1.0 M NaCl. The 1.0 M NaCl fraction contained HSF, as assayed by antibody-mediated activation of HSF binding activity. The Cibachron Blue–1.0 M NaCl fraction (total volume 0.3 ml) was diluted to 1.0 ml with CB (with 0.1 M NaCl) and was applied to a 0.1-ml narrow bore Mono Q (Pharmacia) column. The column was washed with 0.4 ml of CB containing 0.2 M NaCl, and protein was eluted with a 0.5-ml gradient of CB containing 0.2 M to 1.0 M NaCl. Chromatography on the Cibachron Blue and Mono Q columns each typically resulted in a fivefold purification of the inactive form of HSF. (**Bottom**) Mono Q fractions (0.04 ml) were collected, and 3-μl portions were assayed by incubation for 10 min at 24°C with 27 μl of CB containing bovine serum albumin at 20 mg/ml, and 0.5 μl of anti-HSF serum (1:55 final dilution). A 2-μl mixture containing 30 fmol of ^{32}P-labeled HSE and 0.5 μg of poly(dI-dC) · poly(dI-dC) was added to each sample, and the sample was incubated for 10 min at 24°C before gel electrophoresis as in Fig. 1. (**Top**) The same Mono Q fractions were also analyzed in the absence of anti-HSF serum.

after treatment with anti-HSF, was assayed similarly. Although both activities generally cofractionated on a range of chromatographic resins, they could be separated by anion-exchange (Mono Q) chromatography (Fig. 4). The basal activity was eluted in Mono Q fractions 10 to 14, and the inactive HSF was eluted in fractions 6 to 11 (Fig. 4). Although the two activities overlapped in fractions 10 and 11, the bulk of the inactive HSF in fractions 6 to 9 displayed no detectable basal DNA binding activity. The increase in DNA binding activity of inactive HSF after activation in vitro is therefore greater than the tenfold increase previously determined for unfractionated extracts (*24*).

The lack of antibody-mediated stimulation of DNA binding activity in Mono Q fractions 12 to 14 suggests that the basal activity represents a distinct HSF subpopulation in cytoplasmic extracts that is already fully competent to bind DNA. In addition, the elution of the basal activity from the Mono Q column at higher NaCl concentrations suggests that this activity may be more negatively charged than the inactive form of HSF. From experiments involving treatment of human HSF with potato acid phosphatase, it has been suggested that the fully activated form of HSF is modified by phosphorylation (*16*). The higher retention of the basal HSF activity on the Mono Q column may reflect a similar phosphorylation. The existence of a basal activity may reflect a need for some activated HSF in the cell under normal tissue culture conditions. It is also possible that the basal activity may have been induced artificially during the preparation of the cytoplasmic extract.

Although the inactive form of HSF can be induced to bind DNA in vitro, the ability of the in vitro–activated factor to stimulate transcription of heat shock genes is unknown. It is possible that this form of HSF, although capable of binding DNA, is not fully capable of stimulating transcription because of the lack of a secondary modification. There is suggestive evidence for phosphorylation that may lead to a fully activated HSF protein in mammalian cells (*16*), and the transcriptionally active yeast HSF protein also appears to be phosphorylated (*6*).

Recent studies have revealed that the active, DNA binding form of HSF is apparently trimeric in structure (*22, 25*). The oligomeric nature of the inactive form of HSF in the cytoplasmic extract is unknown. In addition, it remains to be determined whether the antibody-mediated induction of HSF binding occurs by an alteration of HSF protein conformation or by displacement of a regulatory molecule (*26*). In any event, the ability to activate HSF binding in vitro by specific antibody recognition (and by agents that generally affect protein structure such as temperature and low pH) suggests that the inactive HSF complex in the cytosol can respond to the heat shock or stress signal and convert that signal to a change in protein structure and function directly, without the use of enzymatic intermediaries. Our results do not necessarily exclude covalent modification of HSF protein or a regulatory factor as an alternative mechanism for activating HSF binding in vivo.

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Specific Tropism of HIV-1 for Microglial Cells in Primary Human Brain Cultures

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Human immunodeficiency virus (HIV) frequently causes neurological dysfunction and is abundantly expressed in the central nervous system (CNS) of acquired immunodeficiency syndrome (AIDS) patients with HIV encephalitis or myelopathy. The virus is found mostly in cells of the monocyte-macrophage lineage within the CNS, but the possibility of infection of other glial cells has been raised. Therefore, the effects of different HIV-1 and HIV-2 strains were studied in primary cultures of adult human brain containing microglial cells, the resident CNS macrophages, and astrocytes. These cultures could be productively infected with macrophage-adapted HIV-1 isolates but not with T lymphocyte–adapted HIV-1 isolates or two HIV-2 isolates. As determined with a triple-label procedure, primary astrocytes did not express HIV *gag* antigens and remained normal throughout the 3-week course of infection. In contrast, virus replicated in neighboring microglial cells, often leading to their cell fusion and death. The death of microglial cells, which normally serve immune functions in the CNS, may be a key factor in the pathogenesis of AIDS encephalitis or myelopathy.

HUMAN IMMUNODEFICIENCY VIRUS not only infects T lymphocytes and cells of the monocyte-macrophage lineage (*1–3*) but also often spreads to the nervous system and causes a variety of neurological dysfunctions (*4*) such as acute and chronic meningitis, inflammatory and sensory neuropathies and myelopathy, as well as encephalitis (*5*). Encephalitis gives rise to a decline in memory and cognitive functions, in addition to sensory and motor disturbances, generally known as AIDS dementia. Pathologically, the CNS diseases caused by HIV-1 are characterized by the presence in several cortical and subcortical regions, as well as in the basal ganglia and spinal cord, of perivascular and infiltrating macrophages and lymphocytes, microglial nodules, and multinucleated giant cells (*5, 6*). These features are accompanied by diffuse gliosis and various degrees of demyelination in the white matter of brain or spinal cord. In postmortem samples of brains from AIDS patients, expression of HIV genes has been detected by in situ hybridization in multi- and mononucleated cells that express monocyte-macrophage lineage markers and, more rarely, in endothelial cells, astrocytes, and oligodendrocytes (*7–9*). In addition, HIV *gag* antigens have been shown by immunohistochemistry to be in individual microglial cells and microglial nodules (*7*). Moreover, macrophages infected with HIV have been identified in cultures derived from the brain of AIDS victims (*1*). These studies indicate that the principal sites of viral replication within the CNS are cells that express markers for the macrophage-monocyte lineage, including microglial cells.

An in vitro system is clearly desirable to study the mechanisms of entry, replication, and assembly of HIV in CNS cells; to examine whether certain strains of HIV have a preferential tropism for specific cell types; and to determine the fate of CNS cells infected by HIV. Although adult CNS neurons have not yet been maintained in culture, all nonneuronal cell types of the brain have been cultured successfully, including astrocytes, oligodendrocytes, and microglial cells (*10, 11*). Adult human brain tissue was resected from temporal lobe as a therapeutic measure in patients with intractable epilepsy (this form of epilepsy is resistant to drug therapy; histopathologically, tissue is normal or shows mild gliosis). Tissue was enzymatically dissociated; cells were seeded onto plastic dishes and cultured as described (legend to Fig. 1). These primary cultures contained significant numbers of microglial cells expressing monocyte-macrophage lineage markers such as LDL-R (low-density lipoprotein receptor) (*12*), Leu M3 and Leu M5 (*13*), EBM11 (*14*), and OX42 (*15*); astrocytes that express GFAP (glial fibrillary acidic protein) (*16*); and flat cells that express cell surface fibronectin (fibroblasts or meningeal cells) (*17*), as seen by fluorescence microscopy (legend to Fig. 2). The survival of microglial cells was optimized by addition of giant cell tumor (GCT) supernatant, a mixture of cytokines (*18*). Higher density cultures of purified microglial cells were obtained from a freshly resected and dissociated malignant glioma by means of a differential adhesion method (legend to Fig. 3). Cultures were infected at 14 to 18 days in vitro with HIV-1 and HIV-2 strains of different origins and cellular tro-

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