

mally prevent "tumor" cell outgrowth. Other homeostatic mechanisms that participate in the control of angiogenesis might also be affected by a deficient immune system. The development of KS would thus depend on the balance and strength of any combination of these mechanisms. According to this model, and consistent with previous suggestions, the KS lesion may develop and "spread" without implying a metastatic process (26). This is also consistent with observations that AIDS-KS is usually a polycentric disease in which the lesions are composed of various cells with a normal diploid chromosomal pattern and lacking obvious, biological tumor properties. Further studies of AIDS-KS cells in the growth systems should lead to a better understanding of the pathophysiology of KS and to the development of treatments that do not depend on cytotoxic chemotherapy.

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and PAL-E (Sambio bv, 5400 Am. Uden, Holland); fibronectin (Hybritech, San Diego); vimentin, desmin, cytokeratin (Boehringer Mannheim, Indianapolis); keratin (AE1 and AE-3; Hybritech); and fast red (Vector Laboratories, Inc., Burlington) for use as a substrate.

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Heat Shock Is Lethal to Fibroblasts Microinjected with Antibodies Against hsp70

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Synthesis of a small group of highly conserved proteins in response to elevated temperature and other agents that induce stress is a universal feature of prokaryotic and eukaryotic cells. Although correlative evidence suggests that these proteins play a role in enhancing survival during and after stress, there is no direct evidence to support this in mammalian cells. To assess the role of the most highly conserved heat shock protein (hsp) family during heat shock, affinity-purified monoclonal antibodies to hsp70 were introduced into fibroblasts by needle microinjection. In addition to impairing the heat-induced translocation of hsp70 proteins into the nucleus after mild heat shock treatment, injected cells were unable to survive a brief incubation at 45°C. Cells injected with control antibodies survived a similar heat shock. These results indicate that functional hsp70 is required for survival of these cells during and after thermal stress.

IN ALL ORGANISMS AND CULTURED cells examined, a small group of evolutionarily conserved proteins is synthesized in response to heat and a variety of other stress-inducing agents. Most of these agents promote protein denaturation (1, 2). Of the several size classes of stress or heat shock proteins (hsps), those referred to as the hsp70 family are the most conserved and the best characterized (1, 2). In most mammalian cells there are two prominent forms of hsp70, an abundant constitutive member, hsp73, and a highly stress-inducible member, hsp72 (3). In cells grown at 37°C the majority of ~70,000 kD (70K) hsps are found in the cytoplasm. In response to heat shock, these proteins are rapidly sequestered in the nucleus with high levels accumulating in that region of the nucleolus involved in the assembly of small ribonucleoproteins and preribosomes (4-6). During recovery from heat shock, the 70K hsps accumulate in the cytoplasm where a portion colocalizes with ribosomes and polysomes (7).

One major line of evidence supports the idea that hsps, and in particular the 70K

hsps, are important in protecting or facilitating the recovery of cells from the adverse effects of heat shock. Cells given a mild heat shock treatment sufficient to elevate the expression of the heat shock proteins (or alternatively to "activate" preexisting hsps) exhibit significantly higher survival rates to a second, and what would otherwise be lethal, heat shock challenge (8). This phenomenon, referred to as acquired thermotolerance, can be elicited by pretreatment with agents other than heat, all of which have in common the ability to induce stress protein synthesis (9, 10). A number of studies have shown a strong correlation between the expression and decay of thermotolerance and the induction, accumulation, and degradation of hsps (10-12). Moreover, an inability to produce functional hsps precludes the acquisition of

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Fig. 1. Specificity of affinity-purified hsp70 antibodies determined by immunoblot analysis. Affinity purification of a mixture of four hsp70 monoclonal antibodies was as described in (27). Total cell lysates from rat fibroblasts incubated at 37°C (lane 1) or 43°C for 2 hours (lane 2), HeLa cells incubated at 37°C (lane 3) or 43°C for 2 hours (lane 4), or purified HeLa 72K and 73K hsps (lane 5) were resolved by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose. After incubation with hsp70 antibodies (1:300 dilution), the primary antibodies were visualized by incubation with horseradish peroxidase-conjugated goat antibodies against mouse IgG, followed by incubation with 4-chloro-1-naphthol/peroxide for color development. The band with an M_r of approximately 55K (lane 5) represents a degradation product of the 70K hsps. M represents the molecular weight marker lane.

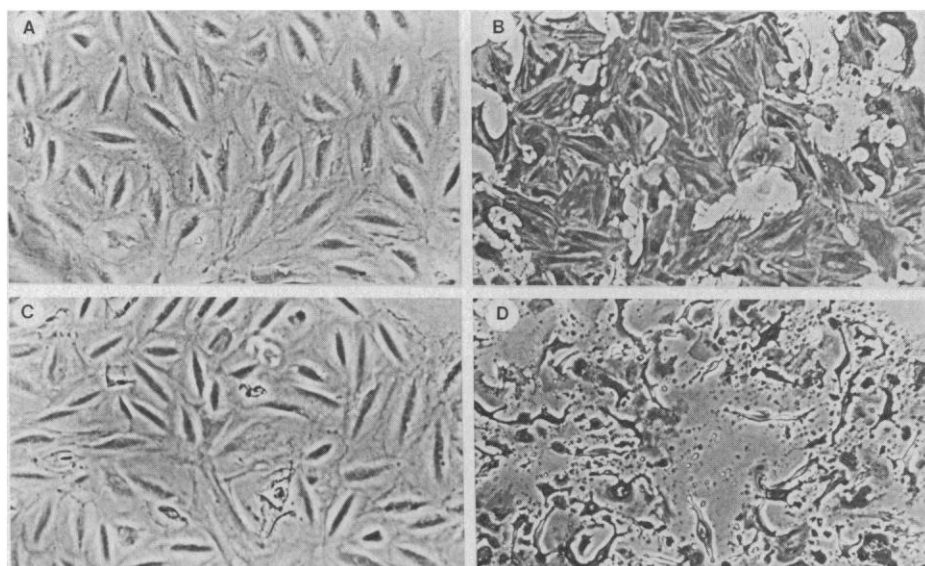
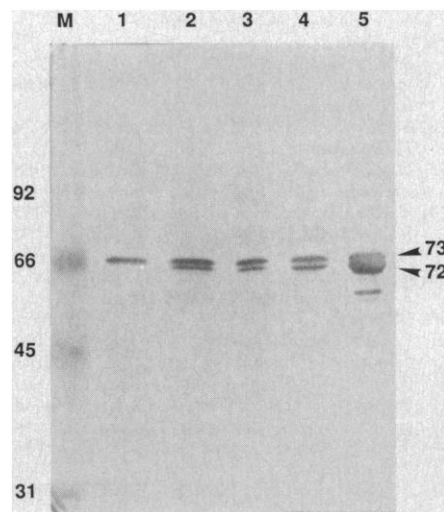


Fig. 2. Heat shock treatment is lethal to fibroblasts microinjected with hsp70 antibodies. Fields of REF-52 (rat embryo fibroblast) cells were injected with control goat antibodies against chicken IgG or hsp70 antibodies at a concentration of 5 mg/ml. After incubation at 37°C for 2 hours, phase contrast micrographs were taken of cells injected with (A) control or (C) hsp70 antibodies. The same cells were heat shocked at 45°C for 30 min, returned to 37°C, and further incubated for 24 hours. The cells were then stained for 10 min in 0.2% Trypan blue (w/v in phosphate-buffered saline), and fixed and stained with either goat antibodies against rabbit IgG (for the nonspecific control and antibodies to Fos and Ras) or goat antibodies against mouse IgG (for the antibodies to hsp70, actin, tubulin, and the C subunit of cAMP-dependent protein kinase) (19, 27). Goat antibodies against IgGs were conjugated with horseradish peroxidase to identify injected cells. (B) Cells injected with the nonspecific control antibodies, and (D) cells injected with hsp70 antibodies 24 hours after the heat shock treatment.

thermotolerance (13). The bacterial counterpart of hsp70 (dna K) is essential for bacteriophage λ replication, but this role may be distinct from that involved with growth at elevated temperature (1). Genetic analyses of yeast and bacteria indicate that the expression of the 70K hsps are needed for the survival of these organisms at elevated temperatures (14, 15). Although yeast strains containing mutations in two of the several genes encoding 70K hsps cannot form colonies at an elevated temperature (37°C), they are able to acquire thermotolerance. This indicates that in yeast, two of the hsp70

gene products are not needed to survive brief exposure to high temperatures (1, 14). Recent studies with yeast have also shown that a subset of the 70K hsps is involved in the translocation of proteins across different cellular membranes, perhaps by providing an "unfoldase" activity (16).

To determine if the 70K hsps are involved in the survival of mammalian cells subjected to thermal stress, we undertook a study in which a mixture of affinity-purified monoclonal antibodies specific for the 70K hsps were introduced into fibroblast cells by needle microinjection. Antibodies directed

against actin (17), tubulin (18), Fos (19), Ras (19), hsp28 (19), and the catalytic subunit of yeast adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase (19) were also tested under the same conditions for their effects on cell survival, and are collectively referred to as control antibodies. To demonstrate the specificity of antibodies against hsp70, immunoblot analysis was performed with total cell lysates and purified 70K hsps. The antibodies recognize only the constitutive 73K and inducible 72K hsps (Fig. 1). Rodent cells synthesized only hsp73 when grown at 37°C (Fig. 1, lane 1), whereas HeLa cells and other cell lines of primate origin synthesized both hsp73 and hsp72 at 37°C (Fig. 1, lane 3) (20). Both cell types produced hsp73 and hsp72 after heat shock (Fig. 1, lanes 2 and 4). Purified 72K and 73K hsps from HeLa cells (21) served as a positive control (lane 5).

The effects of microinjecting the control or hsp70 antibodies on cell viability before and after heat shock treatment (45°C, 30 min) are shown in Fig. 2. Pilot experiments with a Trypan blue exclusion assay indicated that approximately 90% of uninjected cells survived this heat shock treatment (22). No effect on morphology was observed after injection with control or hsp70 antibodies when cells were maintained at 37°C (Fig. 2, A and C). After heat shock, cells injected with control antibodies were viable as assessed by Trypan blue exclusion, and were morphologically similar to uninjected cells (Fig. 2B). In contrast, cells injected with hsp70 antibodies were unable to survive this treatment (Fig. 2D). Because little morphological detail remained in any of the cells injected with hsp70 antibodies after this severe heat shock treatment, cells were injected with control and hsp70 antibodies and heat shocked at 45°C, but for only 20 min. After a 24-hour recovery period at 37°C, the cells were fixed and stained for immunoglobulins (Igs).

The majority of cells injected with control antibodies (against cAMP-dependent protein kinase, Fos, tubulin, Ras, actin, hsp28, or nonspecific antigens) were not compromised in their ability to survive this treatment (see Fig. 3 for examples). In contrast, the majority of cells injected with hsp70 antibodies died and lifted from the substratum. The morphology of cells that remained attached to culture dishes after hsp70 antibody injection and heat shock was very distinct from cells injected with control antibodies. Cells injected with antibody to hsp70 showed an apparent loss of membrane integrity, which was confirmed in parallel experiments by their inability to exclude Trypan blue dye (22).

We next examined whether this effect on

cell viability was dependent on both elevated temperature and the presence of functional hsp70 antibody. Injection of control or hsp70 antibodies into confluent cells had no

apparent effect on morphology or survival when the cells were maintained at 37°C for as long as 36 hours. In addition, introduction of heat-denatured hsp70 antibody did

not affect cell survival after heat shock. Similarly, with increasing dilutions of injected hsp70 antibodies, there was a corresponding decrease in the observed heat-dependent cell killing (22). These studies indicate that cell killing requires temperature elevation in conjunction with a high intracellular concentration of functional antibody to hsp70.

We next examined whether the heat-dependent translocation of hsp70 from the cytoplasm could be prevented by microinjection of hsp70 antibodies. Rat fibroblasts were injected with control or hsp70 antibodies, heat shocked, and the distribution of the 70K hsps determined by indirect immunofluorescence. In cells injected with control antibodies, a typical nuclear and nucleolar distribution of 70K hsps, similar to that of the surrounding uninjected cells, was observed after heat shock treatment (Fig. 4, C and D). In contrast, cells microinjected with hsp70 antibodies exhibited little or no nuclear or nucleolar staining for hsp70 (Fig. 4, A and B).

We have repeated these experiments in several mammalian cell lines and have consistently observed that injection of hsp70 antibodies greatly increases the lethality of a brief but severe heat shock treatment. We do, however, find subtle differences depending on the cell line examined. HeLa cells microinjected with hsp70 antibodies show higher levels of survival than similarly injected rodent fibroblasts after heat shock treatment. This may be due to the fact that HeLa cells, and other cells of primate origin, synthesize both hsp73 and hsp72 when grown at 37°C, whereas rodent cells synthesize only hsp73 (20). We also find that rendering rodent cells thermotolerant by a prior exposure to mild heat shock enables a higher percentage of cells injected with hsp70 antibodies to survive a subsequent, and more severe, heat shock treatment.

The basis for the lethal effect of heat in cells injected with hsp70 antibodies is presumably the chelation of hsp70 proteins, resulting in their inactivation and inability to localize appropriately within cells after heat stress. Alternatively, it is possible that the formation of antibody-antigen complexes within cells as a consequence of antibody injection might affect the ability of cells to survive heat shock. However, since seven other control antibodies, including one directed against the 28K heat shock proteins, did not decrease the ability of cells to survive stress, this possibility is considered unlikely. The ability to block a specific biochemical function, in the absence of detectable non-specific effects, has previously been demonstrated for microinjection of several other antibodies (23–25).

From studies of yeast and bacteria, and a

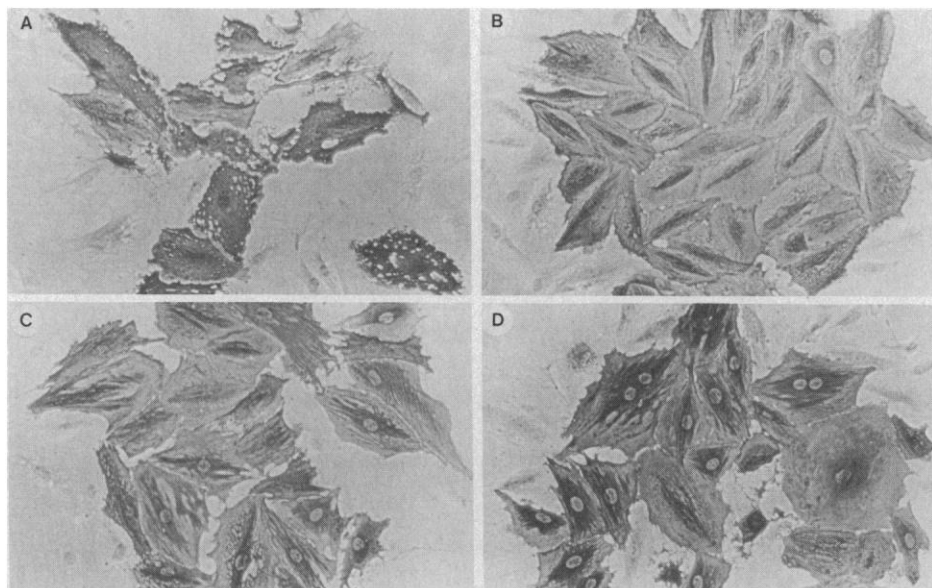


Fig. 3. Heat shock of cells injected with control and hsp70 antibodies at sublethal levels. Fields of approximately 35 REF-52 cells each, on the same culture dish, were microinjected with affinity-purified antibodies directed against (A) hsp70; (B) the catalytic subunit of yeast cAMP-dependent protein kinase; (C) Fos; and (D) tubulin. After 2 hours at 37°C, the cells were heat-shocked for 20 min at 45°C, and allowed to recover for 24 hours at 37°C. Cells were then fixed and stained for the presence of injected antibodies following the protocol in Fig. 2.

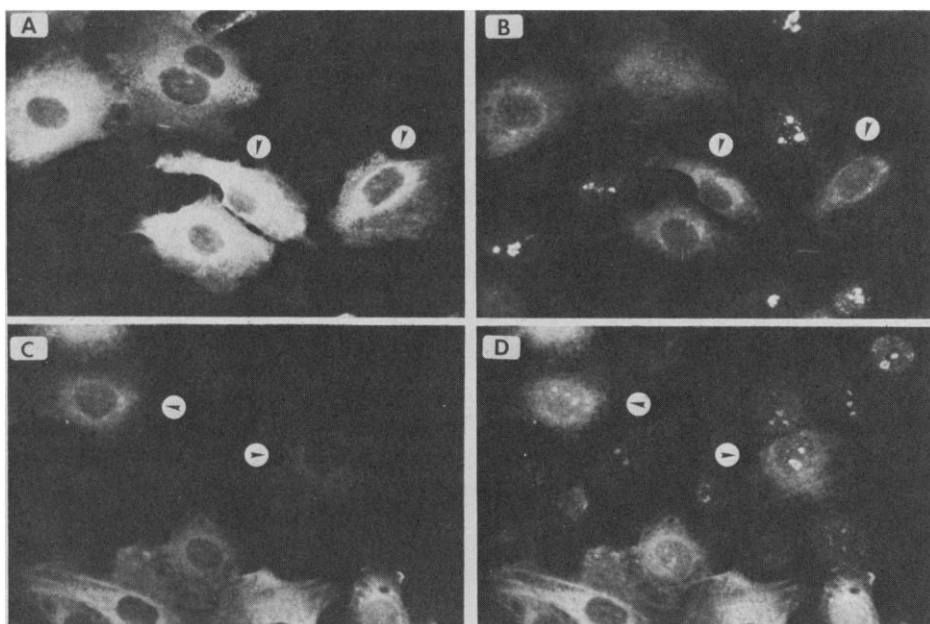


Fig. 4. Microinjection of hsp70 antibodies prevents the nuclear and nucleolar accumulation of 70K hsps after heat shock. Rat fibroblast cells, growing on glass cover slips, were injected with either control or hsp70 antibodies and heat shocked at 43°C for 2 hours. The cells were fixed and stained with either rhodamine-conjugated goat antibody against rabbit IgG or rhodamine-conjugated goat antibody against mouse IgG to identify the injected cells. The distribution of the hsp70 proteins was then determined by incubation of the cells with a rabbit polyclonal antibody specific for the 70K hsps, followed by incubation with fluorescein-conjugated goat antibodies against rabbit IgG. The cells were then examined by fluorescence microscopy with rhodamine and fluorescein filters. (A) Representative field of cells stained for the injected hsp70 antibodies and (B) the same field of cells stained for the distribution of the hsp70 proteins. (C) Field of cells injected with and stained for the control goat antibodies against chicken IgG and (D) the same field of cells stained for the distribution of 70K hsps. Arrows indicate representative cells.

limited amount of work in eukaryotes, it appears that the heat shock proteins, and in particular the hsp70 members, serve functions during normal cell growth and in cells subjected to stress. Our results indicate that functional 70K hsps are required for mammalian cells to survive conditions of brief, but severe, heat shock treatment. It has been suggested that, because of their relatively high abundance in normal and stressed cells, their intracellular localization, and their ability to bind and perhaps hydrolyze adenosine triphosphate (ATP) the 70K hsps might serve a general role in stabilizing proteins against denaturation or in promoting the renaturation of proteins in cells that have been exposed to protein denaturing (stress) agents (6, 7, 21). This role could be fulfilled by an unfoldase activity similar to that proposed for a constitutively produced member of the hsp70 family in the transport of different protein species across intracellular membranes during normal cell growth (16). These experiments, as well as those presented in (26), provide strong evidence that the

activity of hsp70 is required for the survival of cells during and after thermal stress.

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Parallel Integration of Vision Modules

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Computer algorithms have been developed for several early vision processes, such as edge detection, stereopsis, motion, texture, and color, that give separate cues to the distance from the viewer of three-dimensional surfaces, their shape, and their material properties. Not surprisingly, biological vision systems still greatly outperform computer vision programs. One of the keys to the reliability, flexibility, and robustness of biological vision systems is their ability to integrate several visual cues. A computational technique for integrating different visual cues has now been developed and implemented with encouraging results on a parallel supercomputer.

ALTHOUGH IT IS REASONABLE THAT combining the evidence provided by multiple visual cues—for example, edge detection, stereo, and color—should provide a more reliable map of the objects in a visual scene than any single cue alone, it is not obvious how to accomplish this integration. One of the most important constraints for recovering surface properties from each of the individual cues is that the physical processes underlying image formation, such as depth, orientation, and reflectance of the surfaces, change slowly in space (adjacent points on a surface are not at random depths, for instance). Standard regularization (1–3), on which many examples of the early vision algorithms are based, captures those smoothness properties well. The physical properties of surfaces, however, are smooth almost everywhere, but not at discontinuities. Reliable detection of discontinuities of the physical properties of surfaces is critical for a vision system, since discontinuities are often the most important locations in a scene: depth discontinuities, for example, normally correspond to the boundaries of an object. Thus, the output of each vision module has to be smoothed and interpolated (that is, “filled-in”), since it is noisy and often sparse; at the same time discontinuities must be detected.

Discontinuities can also be used effectively to fuse information between different visual cues (4–7) and the image data [see also (8–10)]. For instance, a depth discontinuity usually produces a sharp change of brightness in the image (usually called a brightness edge); and a motion boundary often corresponds to a depth discontinuity (and a brightness edge) in the image. The idea is thus to couple different cues—stereo, motion, texture, color, and motion—to the

image data (in particular, to the sharp changes of brightness in the image) through the discontinuities in the physical properties of the surfaces (see Fig. 1) [for early work in this direction, see (11)]. The final goal of this approach is to use information from several cues simultaneously to refine the initial estimation of surface discontinuities. In this report we will describe a first step in this direction that combines brightness edges with discontinuities in each of the modules separately.

How can this be done? We have chosen to use the machinery of Markov random fields (MRFs), initially suggested for image processing by Geman and Geman (12) [for alternative approaches see (13–16)]. Consider the prototypical problem of approximating a surface (f) given sparse and noisy data (depth data), on a regular two-dimensional lattice of sites (Fig. 2). We first define the prior probability of the class of surfaces in which we are interested. The probability of a certain depth at any given site in the lattice depends only upon neighboring sites (the Markov property). Because of the Clifford-Hammersley theorem, the prior probability has the Gibbs form:

$$P(f) = \frac{1}{Z} e^{-U(f)/T} \quad (1)$$

where Z is a normalization constant, T is a

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