Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Expression of a Drosophila Heat Shock Protein in Mammalian Cells: Transient Association with Nucleoli after Heat Shock

H. Pelham, M. Lewis and Susan Lindquist

Phil. Trans. R. Soc. Lond. B 1984 **307**, 301-307
doi: 10.1098/rstb.1984.0131

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1984 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

Phil. Trans. R. Soc. Lond. B **307**, 301–307 (1984) [301]
Printed in Great Britain

Expression of a *Drosophila* heat shock protein in mammalian cells: transient association with nucleoli after heat shock

H. Pelham[1], M. Lewis[1] and Susan Lindquist[2]
[1]*M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.*
[2]*Department of Biology, University of Chicago, Chicago, Illinois* 60637, *U.S.A.*

[Plate 1]

We transformed mouse L cells with a cloned *Drosophila hsp70* gene and obtained cells with either heat-inducible or constitutively expressed copies of the gene. The distribution of hsp70 in these cells was examined by indirect immunofluorescence with monoclonal antibodies specific to the *Drosophila* protein. In constitutive cells, hsp70 was present in both cytoplasm and nucleus. After heat shock, the nuclear hsp70 was transiently concentrated in nucleoli, from which it had previously been excluded; the cytoplasmic hsp70 moved to a perinuclear location, a result consistent with it being associated with intermediate filaments of the cytoskeleton. The nucleolar migration took several hours and was partly inhibited by actinomycin D, but was independent of protein synthesis; it may reflect binding to newly-synthesized rRNA. *Drosophila hsp70* was also expressed from replicating plasmids in monkey COS cells, and was found to be concentrated in nuclei even at low temperature. Migration to nucleoli occurred after heat shock. These results indicate that a single protein can have multiple interactions with cellular components, and form the basis for future studies of these interactions by *in vitro* mutagenesis and expression of the *hsp70* gene.

Introduction

Cells from all organisms respond to hyperthermia and a variety of other stresses by synthesizing a small number of proteins, the so-called heat-shock proteins or hsps (for review see Schlesinger *et al.* 1982). These proteins have a protective role, enabling the cell to survive in circumstances that would otherwise be lethal, but the precise functions that they perform remain unclear.

The most abundant and highly conserved heat-shock protein is a 70 kDa protein called hsp70. Genes coding for this protein have been isolated from a number of organisms; sequence studies show greater than 70% homology at the amino acid level between the *Drosophila*, *Xenopus* and yeast genes, and up to 50% homology between these and the corresponding gene from *Escherichia coli*, *dna*K (M. Bienz, personal communication; Ingolia *et al.* 1982; Bardwell & Craig 1984). In addition, it has become clear that unstressed cells from all organisms contain substantial levels of the same or related proteins: the *dna*K product is the seventh most abundant protein in unstressed *E. coli*, and in *Drosophila* and yeast cells genes that are about 75% homologous to hsp70 are expressed constitutively at high levels (Ingolia *et al.* 1982; Craig *et al.* 1983). Genetic analysis shows that the *dna*K gene is essential for viability, and it is likely that hsp70-like proteins perform an essential and similar function in all cells.

In attempts to define this function, the intracellular location of hsp70 has been studied by cell fractionation and autoradiographic techniques (see, for example, Arrigo *et al.* 1980; Velazquez *et al.* 1980). The *Drosophila* protein was shown to migrate into the nucleus in response

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

to stress, and has been found associated with hnRNPs (Kloetzel & Bautz 1983) and chromatin (Velazquez *et al.* 1980). Mammalian hsp70 is also found in association with the cytoskeleton (Wang *et al.* 1980), a cell surface glycoprotein (Hughes & August 1982), chromatin and the nuclear matrix (Pouchelet *et al.* 1983). Such studies are hampered, however, by the presence of multiple related proteins, and by possible artefactual binding of the abundant hsp70 to cellular structures during cell fractionation.

To circumvent some of these problems, Velazquez & Lindquist (1984) isolated monoclonal antibodies which react only with the heat-inducible *Drosophila* hsp70. By indirect immunofluorescence they were able to confirm the nuclear location of the protein, and clearly show shuttling into and out of the nuclei of salivary gland cells during stress and recovery. We have used these same monoclonal antibodies to study the behaviour of *Drosophila* hsp70 expressed from a cloned gene in mouse L cells and monkey COS cells. The results suggest multiple interactions for hsp70 including a transient association with nucleoli in stressed cells.

Materials and methods

Cells and transfections

Mouse Ltk$^-$ cells and monkey COS-7 cells were maintained in DMEM with 10% foetal or newborn calf serum. L cells were transfected with plasmid DNA in the presence of calcium phosphate and tk$^+$ colonies selected in HAT medium as described by McKnight *et al.* (1981), except that 20 μg of plasmid DNA was used per 75 cm^2 flask, with no carrier DNA. COS cells were transfected as previously described (Pelham 1982), except that after removal of the DNA they were incubated in medium containing 100 μg/ml chloroquine for three hours; this increased the transfection efficiency about five-fold. The cells were fixed and stained 36–60 h after transfection. S1 mapping of the 5′ ends of hsp70 transcripts has been described (Pelham 1982; Pelham & Lewis 1983).

The plasmids used are shown schematically in figure 1. pHT1 is described elsewhere (Pelham 1982). pAH3 is derived from it; a *Sma*I fragment containing almost all of the thymidine kinase gene was removed, and the *hsp70* promoter and flanking sequences were replaced by a 280 b.p. fragment containing the adenovirus major late promoter and cap site (see Pelham & Lewis 1983). Details of this construction will be reported elsewhere.

Staining of cells

Cells were trypsinized and transferred to slides at least 24 hours before fixing. For heat-shocking, the slides were immersed in pre-warmed medium in 50 ml plastic screw-capped tubes, and the tubes placed in a waterbath. The cells were rinsed very briefly in phosphate-buffered saline, fixed in 4% paraformaldehyde containing 1% triton X-100, and stained with a mixture of three different monoclonal rat anti-*Drosophila* hsp70 antibodies followed by fluorescein-labelled goat anti-rat Fab_2 as described by Velazquez & Lindquist (1984). The samples were mounted in glycerol containing 1,4-diazabicyclo[2,2,2]octane (DABCO) as anti-fade (Johnson *et al.* 1982) and photographed on Kodak Tri-X film using a Zeiss Standard microscope fitted with epifluorescent illumination.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Results

L *cell lines expressing Drosophila hsp70*

Ltk$^-$ cells were transfected with plasmid pHT1 (figure 1) which contains the *Drosophila hsp70* gene and the herpesvirus thymidine kinase gene, by using the calcium phosphate coprecipitation procedure. Tk$^+$ cells were selected in HAT medium and a pool of approximately 100 clones was grown up and transferred to normal medium. S1 analysis of RNA extracted from these cells showed that synthesis of *Drosophila hsp70* mRNA could be induced by heat shock.

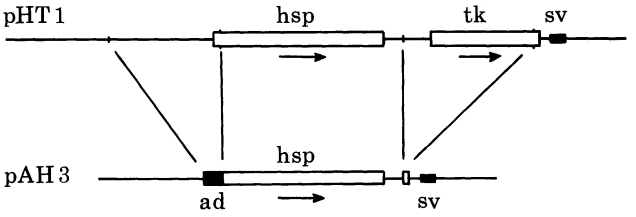


Figure 1. Schematic diagram of plasmids used in this work. The plasmids are shown arbitrarily linearized. The hsp70 (hsp) and thymidine kinase (tk) genes are indicated, together with the SV40 origin (sv). pAH3 was derived from pHT1 by deletion of the tk gene and replacement of the hsp70 promoter region with the adenovirus major late promoter (ad).

To investigate the production of hsp70 itself, the cells were stained with a mixture of monoclonal antibodies specific for *Drosophila* hsp70. Control experiments showed that untransfected Ltk$^-$ cellsdid not stain at all, with or without heat shock. About 30% of the tk$^+$ cells stained after heat shock, whereas in the absence of prior heat shock only a very few cells stained. These rare cells presumably contain an *hsp70* gene whose promoter region became rearranged during integration into the chromosome, so that it is expressed constitutively. To be able to study these in more detail, subclones from the original population were screened until a line was identified in which most of the cells could be stained withour prior stress. S1 mapping of RNA from this line showed that transcripts containing an intact coding region were indeed present constitutively (data not shown).

It was noticeable from the initial experiments that the inducible cell lines varied considerably in their morphology and the time course and extent of their response to stress. We therefore continued to study these as a pool, so as to get an impression of the average behaviour of the cells. To avoid significant changes in the population, we froze aliquots of the cells at an early stage, and used them within two weeks of thawing.

Intracellular distribution of hsp70 during recovery from heat shock

Figure 2*a* and *b*, plate 1, shows the typical pattern of staining in L cells 6 h after a severe but non-lethal heat shock (45 min at 44 °C). The most brightly-stained regions of the cell are the nucleoli; the rest of the nucleus is quite dark, but there is also staining of the cytoplasm, particularly in the perinuclear area. This pattern was the same at three hours and six hours after the heat shock, but by 21 h it had changed (figure 2*c*, plate 1). The nucleoli became free of hsp70, while the rest of the nucleus stained strongly, and the cytoplasm showed an even network of staining. The time course of this change in pattern depended on the severity of the initial stress: the nucleolar staining was visible three hours after a 30 min exposure to 43°, but had vanished after 6 h. The staining pattern 20–24 h after heat shock was generally

Downloaded from rstb.royalsocietypublishing.org

indistinguishable from that obtained with unstressed constitutive cells, although the ratio of staining of nucleus and cytoplasm was somewhat variable. There did seem to be some shift of hsp70 from nucleus to cytoplasm during recovery, but unlike *Drosophila* salivary gland cells (Velazquez & Lindquist 1984), the nuclei of L cells never stained less strongly than the cytoplasm. The nuclear staining also often appeared patchy, suggesting that the protein was not freely soluble, but bound to some macromolecular structure. This patchiness, and the ability to see nucleoli as dark areas (figure 2*f*, plate 1), indicates that the nuclear staining in unstressed cells is specific, and not just an artefact due to overlying cytoplasm.

It was difficult to judge the immediate effects of heat shock on hsp70 distribution in these experiments, because the protein was synthesized continuously during the experiment, and indeed only became detectable some hours after the initial stress. To overcome this problem we examined the behaviour of pre-existing protein in constitutive cells subjected to heat shock (using both the pooled population and the constitutive subline). Figure 2*d* and *f*, plate 1, show examples of such cells stained before and immediately after a 45 min heat shock. The nucleoli are still unstained immediately after the heat shock, but some movement of the cytoplasmic hsp70 to a perinuclear location is apparent.

Figure 2*e*, plate 1 shows a constitutive cell that was heat shocked in the presence of 0.3 mM cycloheximide and allowed to recover for 3 h in the presence of the drug. Under these conditions no new hsp70 can be made, as shown by control experiments with inducible cells; nevertheless, during the 3 h after the heat shock, hsp70 in the nucleus migratres to the nucleoli and is concentrated there. This accumulation is reduced if 5 μg/ml actinomycin D is present throughout the experiment (a level sufficient to block *de novo* induction of hsp70) (figure 2*h*, plate 1), but close inspection of the cells revealed that even without new RNA synthesis the previously unstained nucleoli become stained.

Without heat shock, exposure to cycloheximide or actinomycin D for up to 4 h had no obvious effect on the hsp70 distribution (data not shown). The failure to chase the protein out of the nucleus with actinomycin D suggests that it does not just bind to short-lived hnRNA (though such binding has been reported), but must also have an affinity for some stable nuclear component such as chromatin or the matrix.

The cytoplasmic staining was quite similar to published patterns of intermediate filaments in transformed mammalian cells and *Drosophila* cells (Hynes & Desfree 1978; Falkner *et al.* 1981) which may indicate that at least some of the hsp70 is associated with the cytoskeleton. It has been reported that the distribution of vimentin (a component of intermediate filaments) is rapidly altered to a perinuclear location after heat shock of *Drosophila* or mammalian cells (Falkner *et al.* 1981). Our observations are entirely consistent with this, assuming that hsp70 remains associated with the cytoskeleton (figure 2, plate 1). Indeed, one of the more obvious effects of heat shock on L cells is their tendency to round up, which is presumably associated with cytoskeletal changes.

Expression of hsp70 in monkey COS *cells*

COS cells are SV40-transformed monkey fibroblasts that constitutively produce T antigen and will replicate to high copy number any plasmid containing an SV40 origin of replication (Gluzman 1981). We have previously shown that the *Drosophila hsp70* gene is regulated in this system (Pelham 1982). Antibody staining of COS cells tranfected with pHT1 confirmed that protein was only detectable after heat shock. Initial experiments showed that it was concentrated

Downloaded from rstb.royalsocietypublishing.org

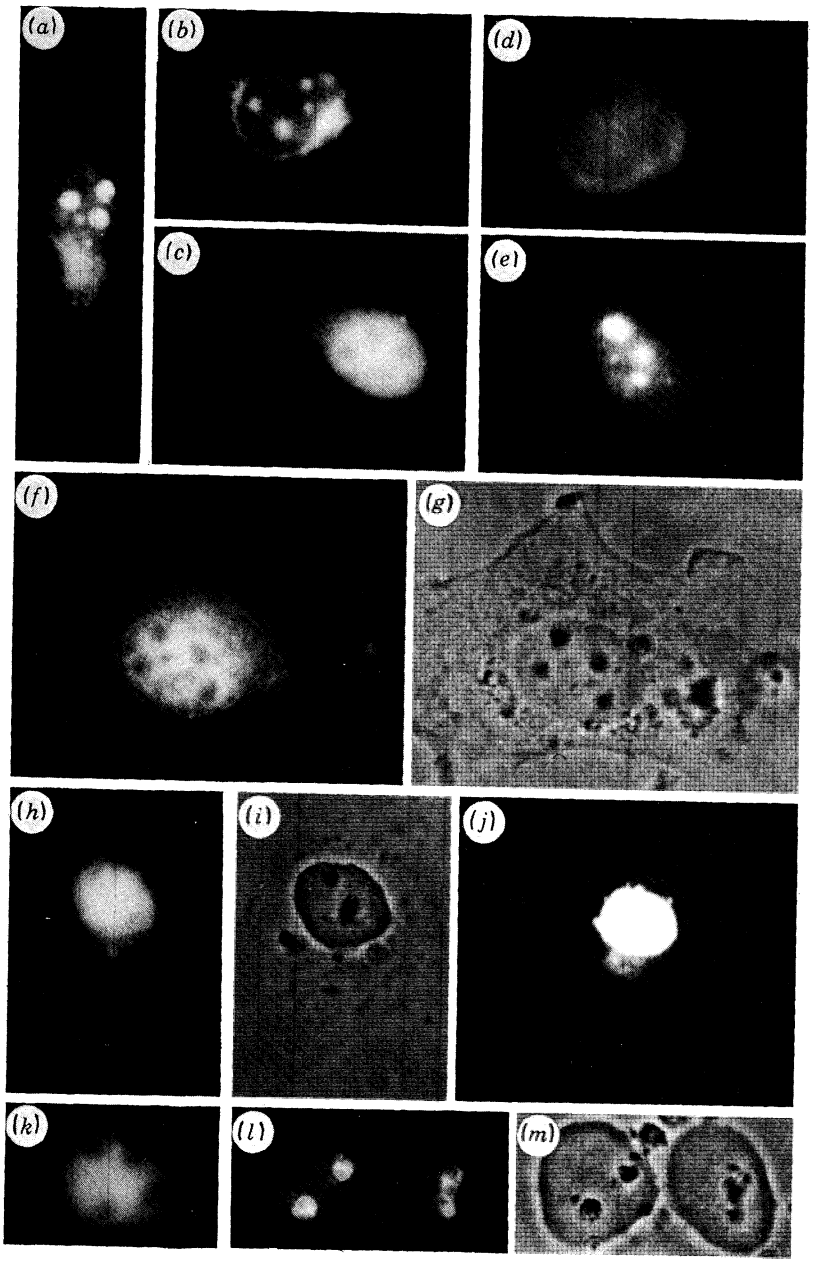


FIGURE 2. Hsp70 distribution in L cells and COS cells. Hsp70 was stained by indirect immunofluorescence; (*g*), (*i*) and (*m*) show the phase-contrast images of (*f*), (*h*) and (*l*) respectively. (*j*) is printed at a magnification of 0.6 relative to the others.

Inducible L cells: (*a*), (*b*) 6 h after heat shock (45 min at 44°); (*c*) 21 h after heat shock.

Constitituve L cells; (*d*) immediately after heat shock; (*e*) 3 h after heat shock, 0.3 mM cycloheximide present throughout; (*f*), (*g*) not heat shocked; (*h*), (*i*) 3 h after heat shock, 5 μg/ml actinomycin D present throughout.

COS cells transfected with pAH3: (*j*), (*k*) not heat shocked (only the nucleus is visible in (*k*)); (*l*), (*m*) 3 h after heat shock (45 min at 44°).

(*Facing p.* 304)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

in the nucleus and remained there for at least 24 h after the heat shock. For subsequent experiments we used a plasmid that expresses *hsp70* constitutively from the adenovirus major

Figure 2*j*, plate 1, shows a typical positive cell. Most of the hsp70 is in the nucleus, but excluded from the nucleoli (see figure 2*k*, plate 1). The cytoplasm is also stained, faintly but reproducibly. The cytoplasmic staining varied from diffuse to granular or fibrillar, and as with L cells suggests some interaction of hsp70 with the cytoskeleton.

Although high levels of hsp70 are synthesized in COS cells, the nuclear distribution does not seem to depend on this. There can be great quantitative variation in the staining of different cells, but even in the faintest ones the pattern was the same. Thus even more so than the L cells, COS cells differ from *Drosophila* salivary gland cells in concentrating hsp70 in the nucleus in the absence of stress. It is of course true that the cells contain highly replicated plasmids and in many ways resemble virus-infected cells; however, as far as transcriptional induction of the hsp70 promoter is concerned, this does not amount to stress (Pelham 1982). The pattern of staining was quite stable: it was constant from 24 to 72 h after transfection, was unaffected by cycloheximide, and incubation in 5 μg/ml actinomycin D for 4 h caused at most only a slight reduction in nuclear staining (data not shown).

Upon heat shock, the cytoplasmic staining was not greatly altered, but there was a slow accumulation of hsp70 in nucleoli. Thus in both L and COS cells, *Drosophila* hsp70 appears to have an affinity for the cytoskeleton and for some stable nuclear component, and is transiently associated with nucleoli after heat shock. The differences in intensity of nuclear staining may reflect the very different morphology and growth characteristics of the cells, or perhaps the presence of replicating plasmids in the COS cells.

Discussion

Hsp70 is one of a family of proteins that have been very highly conserved throughout evolution, but we cannot be certain that the behaviour of *Drosophila* hsp70 in animal cells is the same as that of the endogenous mammalian hsp70. There are, however, several indications that the behaviour we see is typical of mammalian hsp70. For example, mammalian hsp70-like proteins are found associated both with the cytoskeleton and with the chromatin and nuclear matrix fractions of heat-shocked cells (Wang *et al.* 1980; Pouchelet *et al.* 1983). Reorganization of the vimentin cytoskeleton in response to heat shock is also a common feature of both *Drosophila* and mammalian cells (Falkner *et al.* 1981). A report from Welch *et al.* (1982) also mentions that proteins reacting with a polyclonal antiserum raised against hsp70 are present in both cytoplasm and nucleus of a variety of mammalian cells, with a moderate concentration in nuclei after heat shock. Thus although we do not see such a dramatic stress-dependent shuttling of hsp70 between nucleus and cytoplasm in mammalian cells as occurs in *Drosophila* salivary glands, this may reflect a genuine difference between the cell types rather than a failure of the heterologous assay system. We have also expressed *Drosophila hsp70* in *Xenopus* tissue culture cells, which grow at the same temperature as flies, and in these too there is residual nuclear staining even after prolonged recovery (M. Bienz, S. Lindquist & H. Pelham, unpublished results). It would be wrong to place too strong an interpretation on the quantiative aspects of our results, but it seems likely that the basic phenomena we observe are quite general.

The most striking effect of heat shock is the transient movement of the nuclear hsp70 into nucleoli. This process occurs during recovery; it takes some hours and is partly blocked by

Downloaded from rstb.royalsocietypublishing.org

actinomycin D. Since hsp70 appears to be capable of binding to RNA (DiDomenico *et al.* 1982), the migration could be explained by binding of the protein to either pre-existing or newly-synthesized rRNA. In fact it has been known for a long time that nucleoli are very sensitive to the kind of heat shocks used in our experiments (for review see Simard *et al.* 1974). rRNA synthesis and processing, ribosome assembly and transport from the nucleolus are specifically and severely inhibited within 10–30 min. This inhibition is accompanied by morphological changes in the nucleoli that are clearly visible in the electron microscope, but the effects are fully reversible when the cells are allowed to recover at 37°. Recovery is associated with exaggerated RNA synthesis and accumulation of RNP in the nucleoli, with reduced export of pre-synthesized rRNA. One can thus speculate that one function of hsp70 may be to bind to the excess rRNA, either to protect it from degradation until it can be assembled into ribosomes, or to prevent it from binding to other sensitive cellular structures. HnRNPs are also severely disrupted by heat shock (Mayrand & Pederson 1983); hsp70 has been reported to bind hnRNA (Kloetzel & Bautz 1983), and this association might have a similar function. When normal RNA metabolism is restored, hsp70 would return to other nuclear binding sites. Other functions for hsp70 are also possible, of course. Its binding to the cytoskeleton suggests a structural role, although since mRNA aso interacts with the cytoskeleton this too could conceivably involve RNA binding (Cervera *et al.* 1981).

It is clear that hsp70 expressed from a single defined gene can have multiple interactions with cellular components. We can now mutate this gene *in vitro* and study the effects on the behaviour of the expressed protein. In this way it may be possible to define functional domains of the protein, and correlate properties such as RNA binding with intracellular location.

References

Arrigo, A. P., Falkan, S. & Tissieres, A. 1980 Localization of the heat-shock-induced proteins in *Drosophila* melanogaster tissue culture cells. *Devl Biol.* **78**, 86–103.

Bardwell, J. C. A. & Craig, E. A. 1984 Major heat shock gene of *Drosophila* and the *E. coli* heat-inducible *dna*K gene are homologous. *Proc. Natn Acad. Sci. U.S.A.* **81**, 845–848.

Cervera, M., Dreyfuss, G. & Penman, S. 1981 Messenger RNA is translated when associated with the cytoskeletal framework in normal and VSV-infected HeLa cells. *Cell* **23**, 113–120.

Craig, E. A., Ingolia, T. D. & Manseau, L. J. 1983 Expression of *Drosophila* heat-shock cognate genes during heat shock and development. *Devl Biol.* **99**, 418–426.

DiDomenico, B. J., Bugaisky, G. E. & Lindquist, S. 1982 The heat shock response is self-regulated at both the transcriptional and post-transcriptional levels. *Cell* **31**, 593–603.

Falkner, F-G., Saumweber, H. & Biessmann, H. 1981 Two *Drosophila* melanogaster proteins related to intermediate filament proteins of vertebrate cells. *J. Cell Biol.* **91**, 175–183.

Gluzman, Y. 1981 SV40-transformed simian cells support the replication of early SV40 mutants. *Cell* **23**, 175–182.

Hughes, E. N. & August, J. T. 1982 Coprecipitation of heat shock proteins with a cell surface glycoprotein. *Proc. Natn. Acad. Sci. U.S.A.* **79**, 2305–2309.

Hynes, R. O. & Desfree, A. T. 1978 10 nm filaments in normal and transformed cells. *Cell* **13**, 151–163.

Ingolia, T. D., Slater, M. R. & Craig, E. A. 1982 Saccharomyces cerevisiae contains a complex multigene family related to the major heat shock-inducible gene of *Drosophila*. *Molec. Cell. Biol.* **2**, 1388–1398.

Johnson, G. D., Davidson, R. S., McNamee, K. C., Russell, G., Goodwin, D. & Holborow, E. J. 1982 Fading of immunofluorescence during microscopy: a study of the phenomenon and its remedy. *J. immunol. Methods* **55**, 231–242.

Kloetzel, P. M. & Bautz, E. K. F. 1983 Heat-shock proteins are associated with hnRNA in *Drosophila* melanogaster tissue culture cells. *EMBO Jl* **2**, 705–710.

Mayrand, S. & Pederson, T. 1983 Heat shock alters nuclear ribonucleoprotein assembly in *Drosophila* cells. *Molec. Cell. Biol.* **3**, 161–171.

McKnight, S. L., Gavis, E. R., Kingsbury, R. & Axel, R. 1981 Analysis of transcriptional regulatory signals of the HSV thymidine kinase gene: identification of an upstream control region. *Cell* **25**, 385–398.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Pelham, H. R. B. 1982 A regulatory upstream promoter element in the *Drosophila hsp70* heat-shock gene. *Cell* **30**, 517–528.

Pelham, H. R. B. & Bienz, M. 1982 A synthetic heat-shock promoter element confers heat-inducibility on the herpes simplex virus thymidine kinase gene. *EMBO Jl* **1**, 1473–1477.

Pelham, H. R. B. & Lewis, M. J. 1983 Assay of natural and synthetic heat-shock promoters in monkey COS cells: requirements for regulation. In *Gene expression* (ed. D. Hamer & M. Rosenberg), U.C.L.A. symposia on molecular and cellular biology, vol. 8, pp. 75–85. New York: Alan R. Liss.

Pouchelet, M., St-Pierre, E., Bibor-Hardy, V. & Simard, R. 1983 Localization of the 70000 dalton heat-induced protein in the nuclear matrix of BHK cells. *Expl Cell Res.* **149**, 451–459.

Schlesinger, M., Ashburner, M. & Tissieres, A. (eds) 1982 *Heat shock from bacteria to man.* New York: Cold Spring Harbor Laboratory.

Simard, R., Langelier, Y., Mandeville, R., Maestracci, N. & Royal, A. 1974 Inhibitors as tools in elucidating the structure and function of the nucleus. In *The cell nucleus* (ed. H. Busch), vol. 3, pp. 447–487. New York: Academic Press.

Velazquez, J. M., DiDomenico, B. & Lindquist, S. 1980 Intracellular localization of heat shock proteins in *Drosophila*. *Cell* **20**, 679–689.

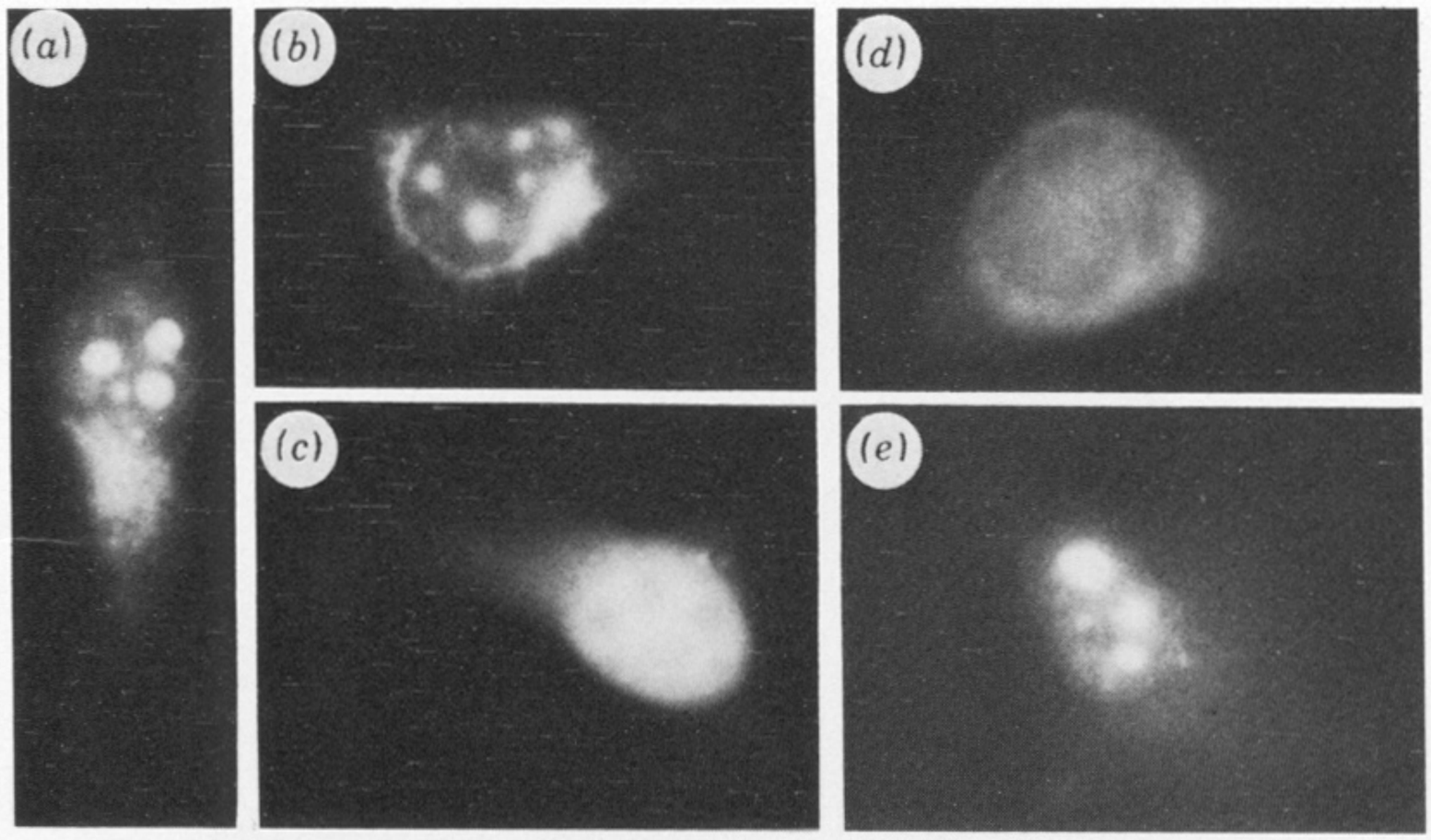
Velazquez, J. M. & Lindquist, S. 1984 *Hsp70*: nuclear concentration during environmental stress; cytoplasmic storage during recovery. *Cell* **36**, 655–662.

Wang, C., Asai, D. J. & Lazarides, E. 1980 Heat shock proteins are methylated in avian and mammalian cells. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1541–1545.

Welch, W. J., Garrels, J. I. & Feramisco, J. R. 1982 In *Heat shock from bacteria to man* (ed. M. Schlesinger, M. Ashburner & A. Tissieres), pp. 257–266. New York: Cold Spring Harbor laboratory.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



Downloaded from rstb.royalsocietypublishing.org

FIGURE 2. Hsp70 distribution in L cells and COS cells. Hsp70 was stained by indirect immunofluorescence; (*g*), (*i*) and (*m*) show the phase-contrast images of (*f*), (*h*) and (*l*) respectively. (*j*) is printed at a magnification of 0.6 relative to the others.

Inducible L cells: (*a*), (*b*) 6 h after heat shock (45 min at 44°); (*c*) 21 h after heat shock.

Constitituve L cells; (*d*) immediately after heat shock; (*e*) 3 h after heat shock, 0.3 mM cycloheximide present throughout; (*f*), (*g*) not heat shocked; (*h*), (*i*) 3 h after heat shock, 5 μg/ml actinomycin D present throughout.

COS cells transfected with pAH3: (*j*), (*k*) not heat shocked (only the nucleus is visible in (*k*)); (*l*), (*m*) 3 h after heat shock (45 min at 44°).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES