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Concomitant Changes in Mitochondria and Intermediate Filaments During Heat Shock and Recovery of Chicken Embryo Fibroblasts

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Abstract Utilizing video-enhanced differential interference contrast microscopy of chicken embryo fibroblasts, we observed dramatic changes in the localization and morphology of mitochondria shortly after cells were subjected to a *mild* heat shock At normal temperatures mitochondria were distributed in the cell cytoplasm as elongated, tubular, and dynamic organelles but upon heat shock they moved to the perinuclear region and formed a tight ring of short swollen and—in some cases—fused vesicles Vital dye staining of mitochondria with rhodamine 123 and indirect immunofluorescence staining with antibodies against the mitochondrial-matrix protein, HSP 60, confirmed these results Using cells double labeled with antibodies to vimentin and the HSP 60 protein, we found that the changes in mitochondria were accompanied by perturbations of the intermediate filament network that we and others have reported previously for heat shocked cells Microtubules remained largely unaltered by our heat shock treatment and the redistribution of intermediate filaments and mitochondria occurred even in the presence of taxol, a microtubule stabilizing drug

The effects of heat shock on mitochondria were reversed when cells were returned to normal temperatures and their recovery to their normal state coincided with return of normal intermediate filament morphology This recovery was blocked in cells treated with actinomycin D during heat shock, a result indicating that a heat shock protein may be required for recovery These data are consistent with previously published observations that mitochondria are associated with the intermediate filament network but they extend this interaction to a cell system responding to a physiological stress normally experienced by the intact organism

Key words: vimentin filaments, lactic acid, differential interference contrast microscopy rhodamine 123, heat shock protein 60

The hallmark of the heat shock response is the rapid and selective activation of genes encoding a set of conserved proteins collectively called the heat shock or stress proteins (HSPs) This response is recognizable in every species examined—from bacteria to man [reviewed in Lindquist, 1986, Schlesinger, 1990, Nover, 1991 and references therein] A wide variety of chemical reagents and physical treatments, usually perceived as detrimental to normal cellular growth and function, can elicit the stress response and HSPs are postulated to have crucial roles in protecting the cell during stress and in restoring cellular activities affected by stress-imposed damage However, a cell's response to stress, such as a heat shock, is not limited to the induction and synthesis of heat shock proteins Virtually every major cellular activity can be affected by stress, but the extent of the changes depends on the intensity of the stress and the specialized functions of the cell type Our laboratory has identified many metabolic activities and morphological features that change when chicken embryo fibroblasts (CEF) are stressed by a mild heat shock [Kelley and Schlesinger, 1982, Collier and Schlesinger, 1986, Collier et al , 1988, Schlesinger et al , 1989, Bond and Schlesinger, 1985] Among the morphological changes detected in these avian cells is a striking reorganization of the vimentin-containing intermediate filament network, which collapses into

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a perinuclear ring soon after a mild stress. This response is a transient one and upon removal of the stress, there is a slow recovery to a normal intermediate filament morphology. In contrast, the microtubule and microfilament networks remain largely unaltered by this heat stress [Collier and Schlesinger, 1986].

To detect additional early events in a stressed cell we have used video-enhanced differential interference contrast (DIC) microscopy, a recently developed technology that allows one to observe real-time events in a living cell. There were clear changes in the cell nucleus soon after the temperature increased but a striking alteration also occurred in the cell cytoplasm as long tubular organelles migrated toward the nuclear membrane where they appeared to grow rounder and shorter. We subsequently identified these structures as mitochondria by using a specific dye and antibodies to a mitochondrial matrix protein. This stress-related translocation of mitochondria was strikingly similar to our previously reported changes in the intermediate filament network of avian cells stressed under conditions that did not affect microtubules or microfilaments. Therefore, we followed mitochondria and several cytoskeletal structures under identical conditions and show here that mitochondria appear to be most strongly associated with the intermediate filament network in CEF.

The heat-shock-induced morphological changes in CEF occur in the *absence* of the nonphysiological drugs and agents that have been used previously to study the interactions of the cytoskeleton and intracellular organelles. The heat shock condition used here is a brief exposure to 45°C, a temperature normally experienced by birds in flight and one that is only 10% higher than the normal bird temperature. Thus we suggest that a mild heat shock may represent a physiologically relevant tool for studying the dynamic interactions of the cytoskeleton with intracellular organelles.

MATERIALS AND METHODS

Materials

Fibroblasts were prepared from 11 day chicken embryos and cultured in Eagle's minimal essential medium (MEM) containing 3% fetal bovine serum. Rhodamine 123 was from Molecular Probes, Inc., Eugene, OR and stock solutions were prepared at 5 mg/ml in ethanol. Rabbit anti-chicken vimentin antibody [Bennett et al., 1978] was a gift from Dr. Harold Holtzer, University of Pennsylvania School of Medicine (Philadelphia, PA). Monoclonal anti-chicken HSP 47 antibody [Nagata et al., 1986] was a gift from Dr. Kazuhiro Nagata, Kyoto University, Japan. Rabbit anti-yeast HSP 60 [McMullin and Hallberg, 1988] was a gift from Dr. Richard Hallberg, Syracuse University (Syracuse, NY). Rabbit anti-P1 [Gupta and Dudani, 1987] was a gift of R.S. Gupta, McMaster University, Hamilton, Ontario. Monoclonal anti-β-tubulin antibodies were from Amersham Corp. Arlington Heights, IL or from Sigma Chemical Co., St. Louis, MO. Fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgG and rhodamine isothiocyanate-labeled goat anti-rabbit antibodies were from Boehringer Mannheim, Indianapolis, IN. FITC-labeled goat anti-rat IgG antibodies were from Cappell Laboratories, Malvern, PA.

Heat Shock

Subconfluent secondary CEF were grown on glass coverslips at 37°C for 1–2 days. For DIC microscopy the culture medium was changed to MEM without phenol red indicator dye and prior to heat shock the medium was buffered with 10 mM Hepes, pH 7.2. The coverslips were sealed, mounted on a temperature controlled (within 0.1°C) stage, and the temperature raised to 45°C. For fluorescent staining, coverslips were placed in sealed petri dishes and cells, in medium supplemented with 10 mM Hepes, pH 7.2, heat shocked at 45°C in a thermostat-controlled water bath. For recovery after heat shock, cells were incubated at 37°C.

Differential Interference Contrast Microscopy

Real-time changes in cellular morphology were observed with a Zeiss Axiovert microscope equipped with differential interference contrast optics and video enhancement, as described by Schnapp [1986], and recorded on three-quarter-inch video tape. Intermittent recordings were made of single cells grown at 37°C (normal growth temperature), during an increase in temperature to 45°C (heat shock temperature), during continuous heating at 45°C for 40 min, and during a 2 h recovery period at 37°C.

Cytochemistry

Rhodamine 123 dye (final concentration of 10 μg/ml) was added to live cells on coverslips and incubated for 10 min at 37°C. The coverslips were washed three times with phosphate buffered saline (PBS) and immediately examined

with a Leitz Laborlux microscope using fluorescein optics. For immunocytochemical staining, CEF on coverslips were rinsed with PBS and fixed with 4% paraformaldehye in PBS for at least 30 min at room temperature and placed at 4°C. Cells were permeabilized with 0.5% Triton X-100 in PBS, rinsed with 1 mg/ml glycine in PBS, and pretreated with 5% normal goat serum in 25 mM Tris, pH 7.5, 150 mM NaCl containing 25 mg/ml BSA (buffer A). Microtubules, intermediate filaments, endoplasmic reticulum, and mitochondria were stained by indirect immunofluorescence using a mouse monoclonal antibody to β-tubulin (diluted 1:500), rabbit anti-vimentin antibodies (diluted 1:100), rat anti-HSP 47 antibody (diluted 1:100), and rabbit anti–HSP 60 antibody (diluted 1:100), respectively. Primary antibodies were added for 3 h at room temperature. Coverslips were washed free of excess primary antibody with PBS and incubated for 2 h with fluorescently labeled secondary antibodies diluted 1:100. All primary and secondary antibodies were diluted in buffer A. For double labeling, cells were fixed with cold methanol and treated with buffer A. Cells were stained with rabbit anti-P1 antibodies followed by rhodamine conjugated goat anti-rabbit IgG. Cells were then washed, treated with 1 mg/ml unconjugated goat anti-rabbit antibodies, and fixed with 2% paraformaldehyde in phosphate-buffered saline for 30 min. Following this treatment, cells were treated with buffer A and incubated either with rabbit anti-vimentin antibody or with monoclonal antibody to β-tubulin followed by FITC-conjugated goat–anti-rabbit antibody or anti-mouse antibody, respectively. Stained cells were mounted in 30% glycerol in PBS, pH 8.3, containing 10 mg/ml n-propyl gallate and examined with a Leitz Laborlux microscope equipped with epifluorescence illumination. Photographs of double-labeled cells were obtained using a Nikon microscope equipped with epifluorescence illumination. We thank Dr. A. Loewy (Department of Anatomy and Neurobiology, Washington University School of Medicine) for assistance with the latter microscopy.

Lactate Measurements

CEF were grown to almost confluency on 60 mm plates in MEM supplemented with 3% fetal bovine serum. Immediately prior to the experiment, the cell medium was changed to MEM containing 5 mM Hepes, pH 7.2, without fetal bovine serum. Samples of the medium were taken 1 h after incubation at 37°C, after a 1 h heat shock at 45°C, and at 1 h intervals during recovery at 37°C. Lactate concentrations in the cell culture medium were measured fluorometrically based on the reduction of NAD to NADH by lactic dehydrogenase in the presence of glutamic-pyruvic transaminase. The absence of serum had no effect on the morphological changes noted for mitochondria during heat shock and recovery as detected by rhodamine 123 staining. We thank Joyce Carter (Department of Molecular Biology and Pharmacology, Washington University School of Medicine) for performing the lactate assays.

RESULTS

Real-Time Events in Heat Shocked Fibroblasts

Video-enhanced DIC microscopy of CEF growing at 37°C showed smooth-appearing cell nuclei and nucleoli (Fig. 1A) and the cytoplasm filled with long tubular organelles that extended from the nuclear membrane to the most peripheral regions of the cell (Fig. 1D). We subsequently identified these organelles as mitochondria based on their labeling by rhodamine 123, a mitochondria specific dye, and by monoclonal antibodies to HSP 60, a protein constitutively present only in mitochondria [Johnson et al., 1980; Gupta and Dudani, 1987].

As the temperature of the cells was raised to 45°C the nucleus became granular in texture, the nucleoli swelled, and the nuclear membrane thickened (compare nuclei in Fig. 1A to 1B). In the cell cytoplasm the tubular mitochondria moved away from the cell periphery toward the nuclear region (Fig. 1B) where they appeared shorter and rounder. After 30 min at 45°C the periphery of the cytoplasm was cleared of these organelles and several mitochondria fused to form larger rounded structures (Fig. 1E, arrow). During a 1 h recovery period at 37°C after a 1 h heat shock, mitochondria started to return to their normal cytoplasmic distribution, moving away from the perinuclear region (Fig. 1F). Almost complete recovery of the normal mitochondria morphology and cytoplasmic distribution was observed in cells that had been heat shocked for 1 h at 45°C and incubated at 37°C overnight (Fig. 1C).

Cytochemical Identification of Mitochondria in Heat Shocked Fibroblasts

We rigorously established the identity of the heat shock sensitive membranous organelles as

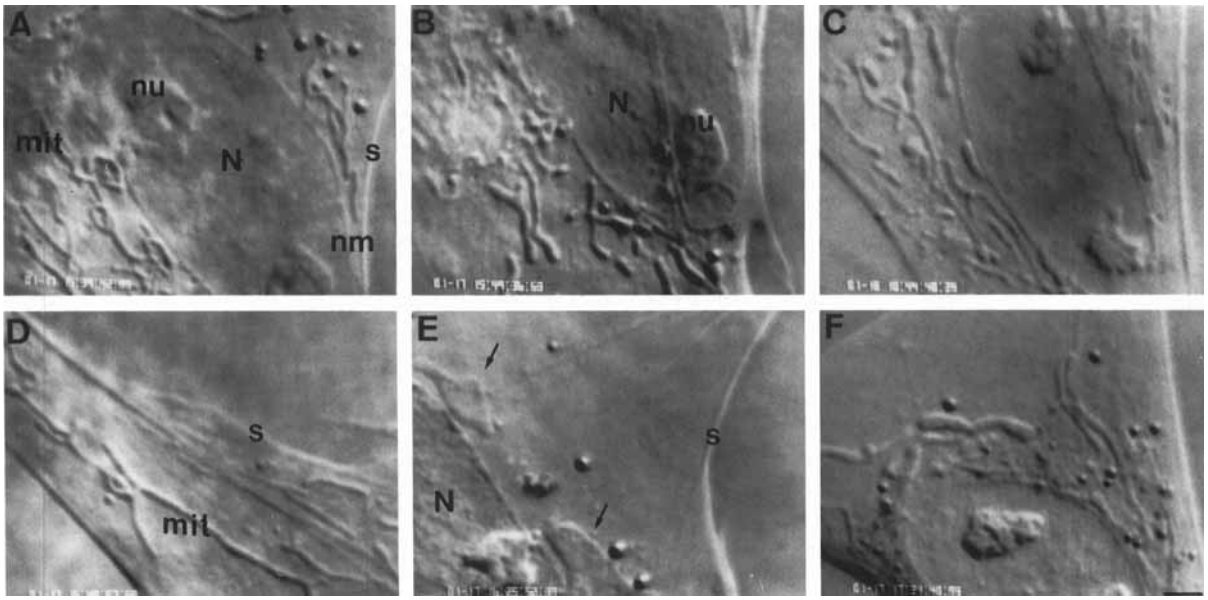

Fig. 1. Video-enhanced differential interference contrast microscopy of normal, heat shocked, and recovering CEF Panels **A, B, D,** and **E** are of the same cell Panels A (nuclear region) and D (cytoplasmic periphery) were taken at 37°C before heat shock Panels B (nuclear region) and E (cytoplasmic periphery) were taken about 5 min and 40 min after increasing the temperature to 45°C, respectively Panel **F** is a cell heat shocked for about 40 min and incubated 1 h at 37°C Panel **C** is a cell from a separate coverslip that was heat shocked at 45°C for 1 h and allowed to recover overnight at 37°C before observation mit, mitochondria, N, nucleus, nm, nuclear membrane, nu, nucleolus, s, cell surface membrane Arrows in panel E show fused mitochondria Bar = 1 μm

mitochondria by staining live cells with rhodamine 123, a vital stain specific for intact mitochondria [Chen, 1988; Bereiter-Hahn, 1990]. The dye revealed long tubular organelles distributed throughout the cytoplasm to the periphery of CEF cultured at 37°C (Fig. 2A). As early as 20 min after the temperature stress mitochondria aggregated in the perinuclear region and by 60 min the fluorescence pattern of bright overlapping punctate images made identification of individual mitochondria within the perinuclear region difficult (Fig. 2B). In cultures allowed to recover overnight from a 1 h heat shock, many mitochondria redistributed throughout the cytoplasm in a pattern similar to untreated cells although some remained as shorter and thicker organelles still clustered around the nucleus (Fig. 2C vs. 2A). These effects on mitochondrial distribution and morphology were also observed in cells stressed by treatment with sodium arsenite (100 μM for 3 h), a chemical that we have shown previously leads to changes virtually identical to those in heat shocked cells [Collier and Schlesinger, 1986; data not shown].

We also used antibodies specific for the mitochondrial protein, HSP 60, to confirm the distribution of mitochondria in normal, heat shocked, and recovered cells. HSP 60 is localized to the inner compartment of the mitochondria where it participates in the refolding of oligomeric proteins transported across the mitochondria membrane [Cheng et al., 1989; Pfanner et al., 1988]. Indirect immunofluorescent staining of fixed CEF by anti–HSP 60 antibodies showed a pattern of long, thick, wavy cytoplasmic organelles in normal cells, a clustering at the perinuclear region in heat shocked cells, and a relatively normal cytoplasmic distribution in heat shocked and recovered cells (data not shown, but see Fig. 3 for heat shocked cells).

Direct Comparison of Mitochondria and Intermediate Filaments in Heat Shocked Fibroblasts

We reported previously that a 30 min heat shock of CEF rapidly collapses the extended intermediate filament network in the cytoplasm to the perinuclear region of the cell that is followed by a slower recovery to a normal state in cells heat shocked and then incubated at normal temperatures [Collier and Schlesinger, 1986]. This response was strikingly similar to the translocation and aggregation of mitochondria described above; therefore, we carried out a

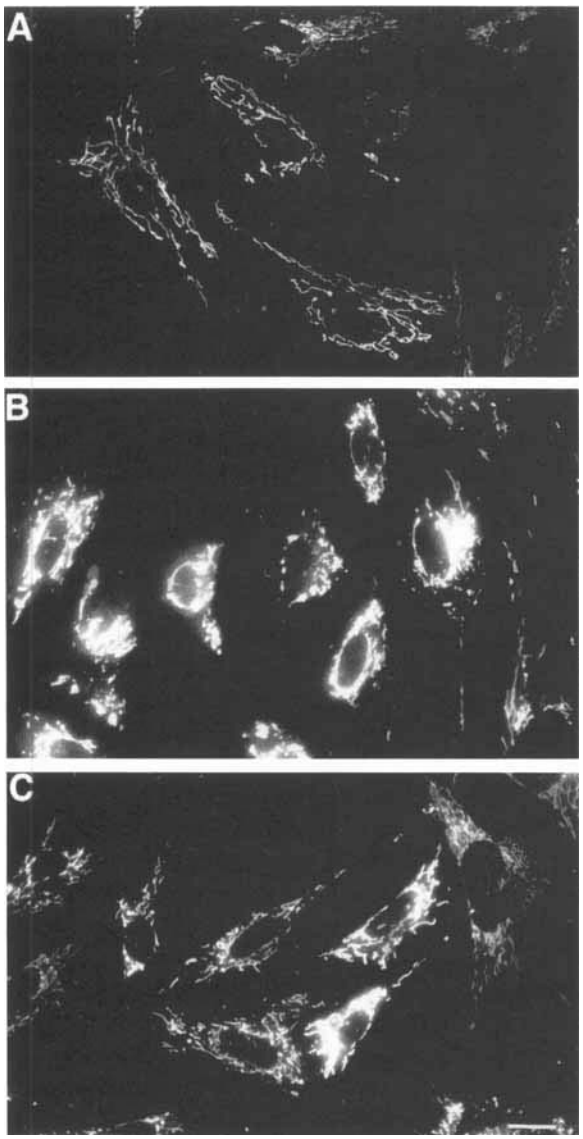

Fig. 2. Mitochondria in normal, heat shocked, and recovered CEF Live CEF were stained with rhodamine 123 **A:** Normal cells **B:** Cells heat shocked for 1 h at 45°C **C:** Cells heat shocked for 1 h at 45°C and incubated at 37°C overnight Bar = 25 μm

direct comparison of mitochondrial movement and changes in the intermediate filament network in cells subjected to the same treatment of heat shock and recovery from stress. Cells were double labeled with anti-vimentin antibodies to follow the intermediate filament network and antibodies to P1, the HSP 60 protein, to follow the mitochondria. In heat shocked CEF, the distribution of mitochondria closely aligned with the intermediate filaments (Fig. 3A,B). We also examined heat shocked cells double labeled with anti-tubulin and anti-P1 antibodies. In sharp contrast to the results with the intermediate filaments, the microtubule network remained virtually intact (Fig. 3C) under heat shock conditions that led to accumulation of the mitochondria in the perinuclear region (Fig. 3D). It is clear from these results that, under identical conditions of heat shock, the distribution of the mitochondria closely align with the intermediate filament network.

Cytochemical Characterization of the Endoplasmic Reticulum

The pattern of the endoplasmic reticulum in the cell cytoplasm after heat shock and recovery was examined by indirect immunofluorescence using antibodies to HSP 47, a collagen-binding protein recently shown to be localized to the endoplasmic reticulum [Nagata et al., 1986]. Normal cells showed a granular bright staining in the perinuclear region and a reticulated pattern that extended to the periphery of the cytoplasm (Fig. 4A). This pattern was retained in heat shocked cells (Fig. 4B); thus, no gross morphological changes occurred in the endoplasmic reticulum of CEF heat shocked for 1 h at 45°C. In other studies designed to test the function of the endoplasmic reticulum, we followed the glycosylation pattern of the vesicular stomatitis virus G glycoprotein in normal and heat shocked cells. No differences were observed in the SDS-PAGE gel analysis of G protein that had been radiolabeled in infected cells under normal or heat shocked conditions (data not shown).

Effect of Taxol on Mitochondrial Distribution in Heat Shocked Fibroblasts

To further examine the role of microtubules and their involvement in mitochondrial movement in heat shocked cells, we pretreated CEF for 5 h at 37°C with 2 μM taxol, a drug that stabilizes microtubules. In normal CEF, this treatment led to the characteristic pattern of microtubules described for this drug—namely a "stiffening" of the microtubule network (data not shown). Taxol-treated cells heat shocked for 1 h in the presence of the drug still showed the typical translocation of mitochondria from the cell periphery to the perinuclear region (Fig. 5A). Importantly, the mitochondria retained the capacity to return to their normal cytoplasmic location in taxol-treated cells that were recovering from a heat shock (Fig. 5B).

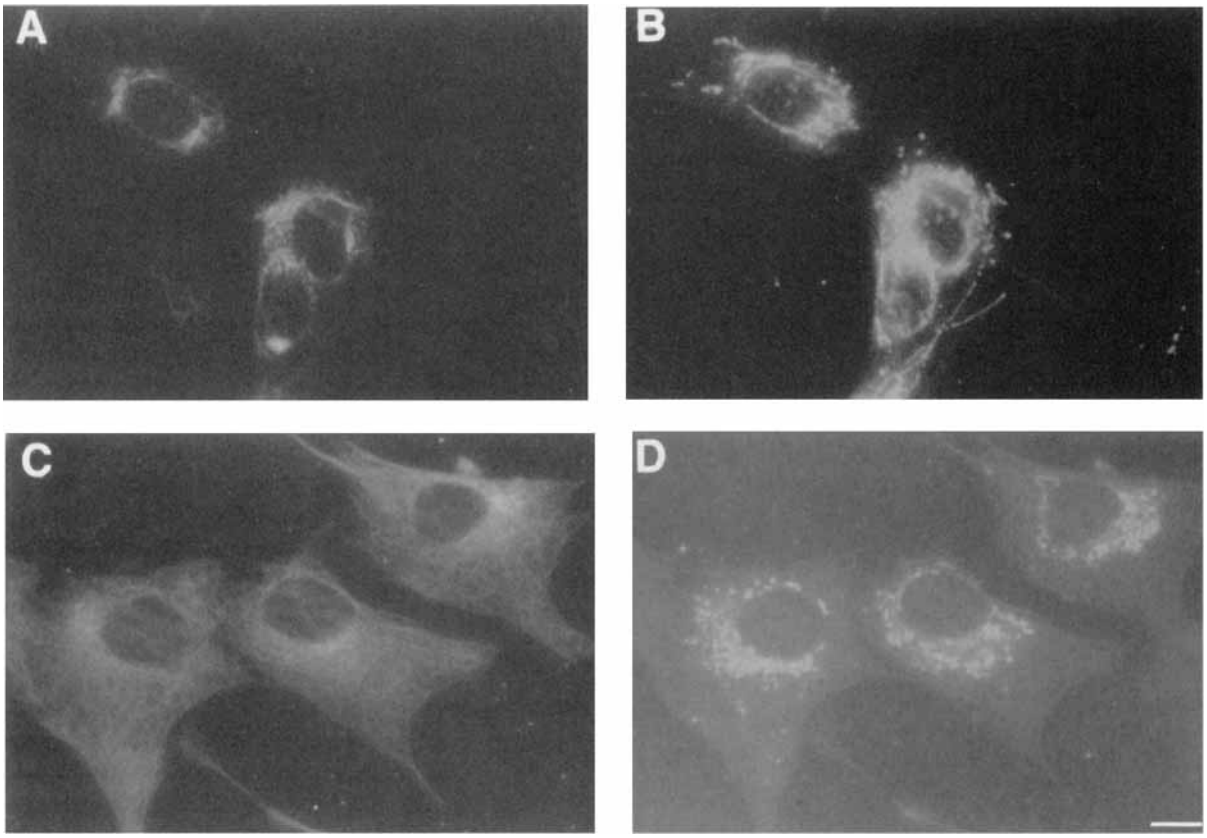

Fig. 3. Double immunofluorescent staining with antibodies to vimentin or tubulin and the mitochondrial matrix protein, P1, of CEF heat shocked for 1 h. **A:** Cells stained for intermediate filaments. **B:** Same cells as in A stained for mitochondria. **C:** Cells stained for microtubules. **D:** Same cells as in C stained for mitochondria. Bar = 10 μm.

Effect of Actinomycin D on Mitochondrial Distribution in Heat Shocked Fibroblasts

Cells heat shocked in the presence of actinomycin D are unable to induce heat shock proteins although many of the other effects of heat shock, including the intermediate filament collapse, still occur [Collier and Schlesinger, 1986]. Similarly, cells treated with this drug showed the same kind of mitochondria translocation to the perinuclear region of the cell after heat shock (Fig. 5C). However, the presence of actinomycin D during heat shock inhibited recovery of the mitochondria to their normal cytoplasmic location (Fig. 5D). This response was identical to that seen for intermediate filaments in cells heat shocked in the presence of actinomycin D where the recovery of normal intermediate filament network morphology was inhibited if transcription was blocked during the stress [Collier and Schlesinger, 1986].

Changes in Mitochondrial Function in Stressed Cells

The major role of mitochondria is to convert energy from oxidative breakdown of a variety of carbon sources to the ATP required for cell growth and maintenance. The results described here suggest that this mitochondrial function might be altered in the heat shocked cell. In fact, Maresca and Carratu [1990], using an oxygen electrode, detected a rapid loss of mitochondrial oxidative phosphorylation after heat shock of *Histoplasma capsulatum* in suspension culture. It was not possible to utilize this technique to directly measure changes in the mitochondrial oxidative function for our tissue culture system. Altered mitochondria function might also be revealed by changes in ATP levels, but we reported earlier that ATP and phosphocreatine concentrations do not change significantly in CEF heat shocked under the same conditions as

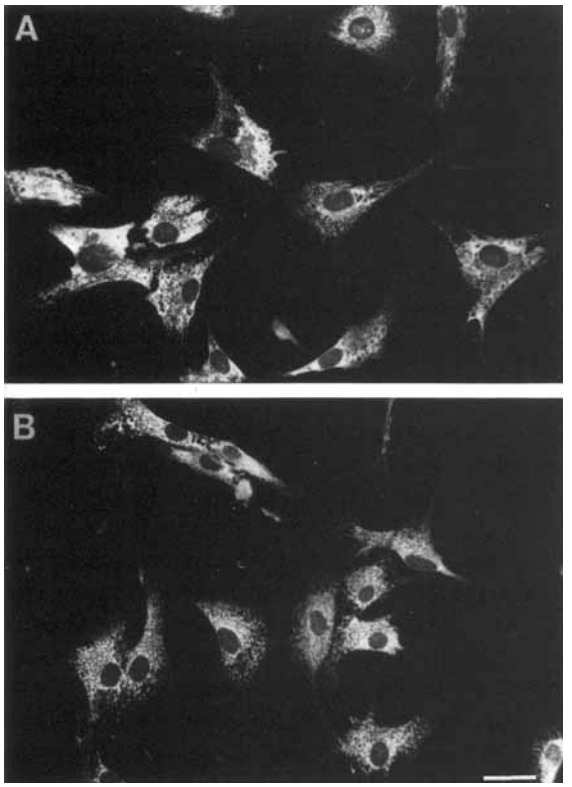

Fig. 4. Endoplasmic reticulum in normal and heat shocked CEF Fixed CEF were stained with antibodies to HSP47 **A:** Normal cells **B:** Heat shocked cells Conditions for heat shock are described in the legend to Figure 2 Bar = 30 μm

those used here [Schlesinger et al., 1989]. Cells can also generate ATP by glycolysis, a biochemical pathway that leads to accumulation of lactic acid; thus, changes in the latter after heat shock would be an indirect measure of the capacity of mitochondria to provide ATP. Determination of lactic acid secreted into the medium of heat shocked cells showed a sharp increase over that normally present (Fig. 6). Intracellular lactate levels were similar in normal and heat shocked cells (data not shown). These data indicate that the stress-induced morphological changes in mitochondria were accompanied by altered organelle function.

DISCUSSION

Video-enhanced DIC microscopy has permitted—for the first time—a view of the dynamic events occurring in a cell as it experiences a heat shock stress. The most heat shock sensitive structures visualized by this methodology were the chromatin, the nucleolus, the nuclear membrane, and the mitochondria. The alterations in nuclear structures were predicted based on earlier results of both morphological and biochemical studies [reviewed in Nover, 1991]. However, the extent of the changes in mitochondria was unexpected even though these organelles have been shown by electron microscopy to become enlarged after a heat shock [Welch and Suhan, 1985]. Shortly after the cell was exposed to a heat shock, these normally plastic, snake-like, and highly motile organelles rapidly translocated to the perinuclear region of the cell. Upon recovery at 37°C the mitochondria slowly regained a normal distribution.

A major factor in determining motility and distribution of mitochondria is considered to be the cytoskeleton but it is unclear which network plays the primary role [Bereiter-Hahn, 1990; Chen, 1988; Traub, 1985]. A correlation between the distribution of mitochondria and microtubules suggests that mitochondrial localization and motility are strongly influenced by the microtubule network [Couchman and Rees, 1982; Heggeness et al., 1978; Smith et al., 1977; Wang and Goldman, 1978] but the close spatial arrangement of microtubules and intermediate filaments [Ball and Singer, 1981; Chen et al., 1981; Summerhayes et al., 1983; Welch and Suhan, 1985] also implicates the latter as an important determinant of mitochondrial distribution. In cultured cells treated with antimitotic drugs a simultaneous depolymerization of microtubules and disruption of mitochondria [Heggeness et al., 1978; Mose-Larsen et al., 1982; Summerhayes et al., 1983; Toh et al., 1980] have been observed, but these drugs also collapse the intermediate filament network leading to a perinuclear aggregation. In some cases [Chen et al., 1981; Summerhayes et al., 1983; Toh et al., 1980], but not all [Ball and Singer, 1982; Chen et al., 1981; Couchman and Rees, 1982; Mose-Larsen et al., 1982], the mitochondrial localization correlated with the perinuclear ring of intermediate filaments. In other examples, the mitochondrial distribution was examined under experimental conditions that disrupted only a single cytoskeletal network. Treatment of cultured cells with the protein synthesis inhibitor, cycloheximide, aggregated the intermediate filament network without affecting microtubules [Sharpe et al., 1980]. Using similar conditions with CV-1 cells, Chen et al., [1981] showed that both the intermediate filament and mitochondrial localizations were disrupted; however, Ball and Singer [1982] observed a correla-

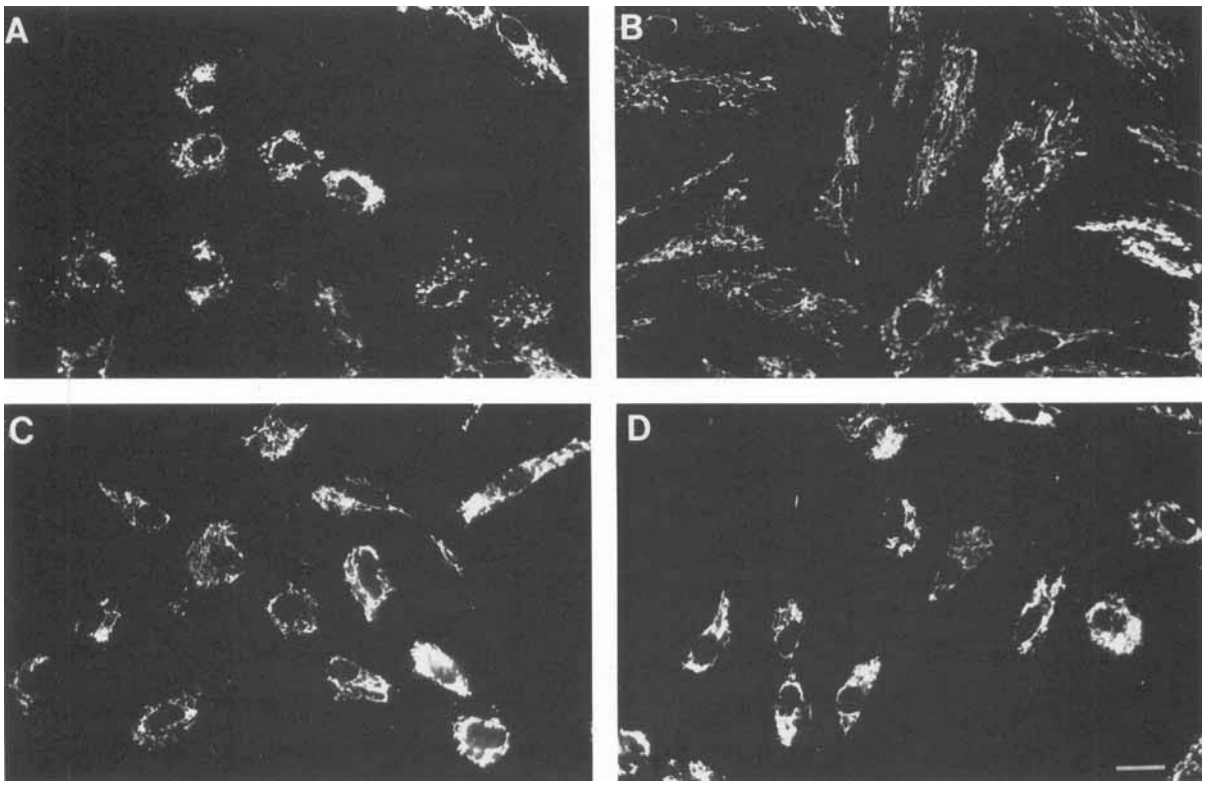

Fig. 5. Mitochondria in taxol-treated and actinomycin D–treated CEF. **A:** Taxol-treated cells heat shocked for 1 h. **B:** Taxol-treated cells heat shocked and incubated at 37°C overnight. **C:** Actinomycin D–treated cells heat shocked for 1 h. **D:** Actinomycin D–treated cells heat shocked for 1 h and incubated at 37°C for 6 h. Cells were stained with rhodamine 123. Bar = 25 μm.

tion between mitochondria and microtubule distribution in cycloheximide-treated human lung fibroblasts. A selective collapse of intermediate filaments was detected in reovirus-infected CV-1 cells and this was accompanied by a redistribution of the mitochondria [Sharpe et al., 1982]. Microinjection of antibodies specific to an intermediate filament associated protein aggregated this cytoskeletal network and disrupted mitochondrial distribution in CV-1 cells [Chen et al., 1981; Summerhayes et al., 1983] but had no effect on mitochondria in gerbil fibroma cells [Summerhayes et al., 1983]. Intermediate filaments collapsed in rat kidney cells transformed by Rous sarcoma virus but mitochondrial distribution correlated with microtubules [Ball and Singer, 1982]. The opposite result was detected in rat fibroblasts stressed by a 3 h treatment at 43°C as the mitochondria collected around the cell's nucleus along with the collapsed intermediate filaments and no severe effect on microtubules was detected. A severe heat shock of mouse mammary epithelial cells led to aggregation of both the keratin-containing intermediate fila-

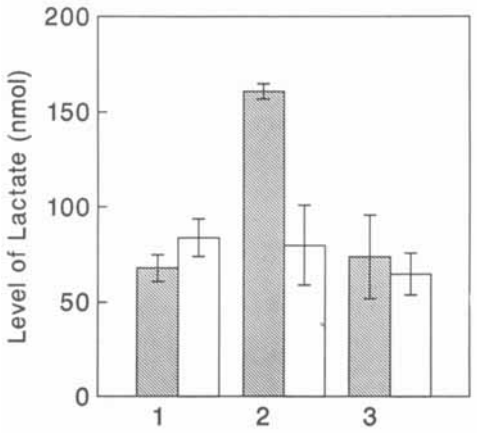

Fig. 6. Levels of lactic acid released from normal, heat shocked, and recovered CEF. The levels of lactic acid released into medium of CEF cultures were measured after a 1 h incubation at 37°C (**column 1**), after transfer to 45°C for 1 h (**column 2**), and after a 1 h recovery period at 37°C (**column 3**). For comparison, a second set of cultures was held at 37°C for 3 h and the amount of lactic acid released into the medium was measured at 1 h intervals (**clear columns**). Data are expressed as the net amount of lactic acid released during the 1 h interval. Samples from triplicate sets of monolayers were assayed. The experiment was repeated three times with similar results.

ments and mitochondria [Shyy et al., 1989], and the kangaroo epithelial cell line, PtK2, treated with colchicine showed no change in either the mitochondria or distribution of keratins or vimentin-containing intermediate filaments [Summerhayes et al., 1983]. Human carcinoma cells show a perinuclear localization of mitochondria and intermediate filaments with normal cytoplasmic microtubules [Chen et al., 1984].

On the basis of this array of data, Chen [1988] concluded that "neither intermediate filaments nor microtubules have an absolute role in determining mitochondrial motility." Biochemical evidence for linkage between mitochondria and cytoskeleton has also been reported—in one case an intermediate filament associated protein bound to mitochondria [Mose-Larsen et al., 1982] and in another, a microtubule-associated protein bound to mitochondria [Gupta and Dudani, 1987].

Our data, summarized below, argue strongly that in primary cultures of chicken embryo fibroblasts, the intermediate filaments are more influential than microtubules in determining mitochondrial localization and motility; (1) the change in both the mitochondrial and intermediate filament network distribution occurred at similar times during heat shock; (2) both the mitochondria and intermediate filament network localized to the same region of the cell—the perinuclear space; (3) recovery after heat shock was accompanied by a slow return of both mitochondria and intermediate filaments to their normal cytoplasmic distribution. This latter observation is particularly significant because it implies that formation of an intermediate filament network is necessary for the normal cytoplasmic distribution of mitochondria. Double staining of cells with antibodies to vimentin and mitochondria showed a colocalization of these structures during all of the translocation events; (4) treatment of cells prior to heat shock with actinomycin D prevented the recovery process for both mitochondria and intermediate filaments even though the initial aggregation occurred. In addition, no changes appeared in the microfilament network at the time the mitochondria were profoundly perturbed and the microtubule network was only moderately altered under our conditions of heat shock. Most importantly, stabilization of microtubules by taxol neither prevented the mitochondrial movement to the perinuclear space nor affected their ability to recover their normal cytoplasmic location. Clearly, the ability of mitochondria to translocate in a cell with taxol-stabilized microtubules argues strongly that the mitochondria are not strongly associated with the microtubule network. There remains the possibility, however, that subtle changes in microtubules which may have occurred after our heat shock treatment but were not detected morphologically might also contribute to the alterations in the structure and localization of mitochondria.

The real-time changes in mitochondria were confirmed by staining live cells with rhodamine 123, a vital stain whose fluorescence depends on the mitochondrial membrane potential. The pattern of this dye's fluorescence in heat shocked cells showed a clustering in the perinuclear region. In addition, there was an apparent enhancement of the signal from the fluorescent probe. The mechanisms that might lead to the latter are unclear but it is probably not caused by an increase in the membrane potential [L.B. Chen, personal communication]. However, the pattern of rhodamine staining in heat shocked chicken fibroblasts was similar to that noted for cardiac muscle cells and some cancer cells [Summerhayes et al., 1982]. These latter use glycolysis rather than mitochondrial oxidative phosphorylation to generate ATP. Heat shocked chicken fibroblasts may also have shifted their source of ATP from mitochondria metabolism to glycolysis as indicated by the sharp increase in lactic acid formation. The latter result is similar to that reported previously for murine L929 cells in culture [Lanks et al., 1986]. A shift to glycolytic ATP formation after heat stress has been implicated in several other organisms based on changes in activities and levels of the glycolytic enzymes enolase, phosphoglycerate kinase, α-glycerophosphate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase [Iida and Yahara, 1985; Nickells and Browder, 1988; Piper et al., 1988; Vossen et al., 1983]. In cultures of *H. capsulatum,* heat shock induced a rapid uncoupling of oxidative phosphorylation [Maresca and Carratu, 1990].

The physiological significance of the intermediate filament network collapse and redistribution of the mitochondria to the perinuclear region during a stress is unclear. Other events, particularly in the nucleus, suggest that stress agents trigger a cell to initiate activities similar to those just prior to mitosis as a cell ceases many of its biosynthetic activities (i.e., DNA and RNA synthesis) and reorganizes for cell divi-

sion. Perhaps the cell is much less susceptible to damage by heat shock and other stress agents in this relatively quiescent state.

Studies of the heat shock response have led to the discovery of a number of basic biochemical events that occur normally in cell growth and development. Here we have shown that the relative selective effect of heat shock on the intermediate filament network has provided evidence that this cytoskeletal system is intimately associated with the mitochondria and may regulate their cellular distribution, morphology, and function.

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