Molecular Chaperones and the Centrosome

A ROLE FOR HSP 73 IN CENTROSOMAL REPAIR FOLLOWING HEAT SHOCK TREATMENT*

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In the accompanying paper (Brown, C. R., Doxsey, S. J., Hong-Brown, L. W., Martin, R. L., and Welch, W. J. (1996) J. Biol. Chem. 271, 824-832) two molecular chaperones, hsp 73 and TCP-1, were shown to be integral components of the centrosome. Here we show that heat shock treatment adversely affects both the structure and function of the centrosome, and that hsp 73 plays a role in the repair of the organelle. After heat shock treatment, the centrosome could not be identified via indirect immunofluorescence and cells were unable to support microtubule regrowth. During recovery from heat shock, a strong correlation between the return of staining of three centrosomal antigens (hsp 73, TCP-1, and pericentrin) and the recovery of microtubule regrowth properties was found. Incubation of cells with glycerol, a protein protective agent, prevented the heat induced alterations in the structure/function of the centrosome. Likewise, the recovery of the structure and function of the centrosome after heat shock treatment was significantly accelerated in cells first made thermotolerant. We provide evidence that this process is related to the levels of hsp 73 since: 1) microinjection of hsp 73 antibody blocked centrosomal reassembly and microtubule regrowth abilities following heat shock; and 2) microinjection of purified hsp 73 protein prior to heat shock treatment accelerated both the repair and function of the organelle, similar to that observed for thermotolerant cells.

Exposure of cells to thermal treatments is known to result in a compromise or inhibition of various cellular processes. Examples include the transient arrest of protein synthesis, inhibition of respiration and a corresponding increased demand on glycolysis for energy production, alterations in RNA splicing, alterations in the integrity of the nucleus and a shut down of new ribosomal biogenesis, delays in cell cycle progression and an inability of cells to enter into mitosis, a refractory response of the cells to other external stimuli including mitogens, and alterations in the organization of different components of the cytoskeleton (reviewed in Ref. 2). One suspects that many of these aforementioned cellular processes that are adversely affected after heat shock treatment are a result of thermal denaturation and/or inactivation of essential proteinaceous components. Presumably the increased synthesis and accumulation of heat shock proteins following thermal stress functions to either repair and/or replace these proteinaceous components. In line with this idea is the fact that many of the heat shock proteins now have been shown to function as molecular chaperones, intimately involved in facilitating the synthesis, folding, and overall maturation of most, if not all cellular proteins (reviewed in Refs. 3 and 4). Moreover, increased levels of the stress proteins correlate directly with an enhanced capacity of the cell to repair and/or restore the activities of those pathways adversely affected as a consequence of the heat shock insult (5, 6).

In the preceding report (1) we demonstrated yet another biological process which likely involves the participation of members of the heat shock protein/molecular chaperone family. Specifically, we found that both hsp 73 and the TCP-1 protein, a component of the cytosolic chaperonin complex, are present within the centrosome, the major microtubule-organizing center in animal cells. Using both in vitro and in vivo approaches we presented evidence implicating an essential role of the TCP-1 protein in the organelles' ability to initiate the growth of the microtubule network. In contrast, we found no evidence for a role of hsp 73 in mediating microtubule growth off the centrosome.

Here we have examined the consequences of heat shock treatment both on the organization and function of the centrosome. Similar to what others have observed (8–11), we found that heat shock treatment adversely affects the integrity of the centrosome. For example, after heat shock treatment there was no observed staining of the centrosome using antibodies to hsp 73, TCP-1, or the centrosomal antigen, pericentrin. Consistent with this apparent loss of centrosome integrity was an inability of the heat shock-treated cells to support the re-growth of their microtubule-based cytoskeleton. During recovery from the heat shock treatment, hsp 73, TCP-1, and pericentrin staining of the centrosome gradually returned, and the cells now again appeared competent to support microtubule regrowth. Like other cellular processes which are adversely affected by heat shock treatment, the recovery of the structure and function of the centrosome after heat shock was accelerated in cells first made thermotolerant. Via microinjection experiments, we show that this accelerated recovery of centrosomal integrity in the thermotolerant cells is correlated with the levels of hsp 73. Furthermore, we demonstrate that cells can be made functionally thermotolerant, at least in terms of centrosomal recovery, by injecting purified hsp 73 protein. Hence, we suspect that hsp 73 functions in facilitating the reorganization of the centrosome after heat shock treatment.

MATERIALS AND METHODS

Cell Culture and Immunofluorescence—HeLa cells were used to examine the distribution of centrosomal antigens before and after heat shock treatment. Owing to their larger size and well spread morphology, COS cells were used to examine microtubule regrowth. Cells used in all experiments were grown in Dulbecco's modified Eagle's medium supplemented with 10% bovine calf serum at 37 °C. The temperature and time of duration of heat shock treatments are presented in the

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relevant figure legends. For indirect immunofluorescence experiments, cells growing on glass cover slips were either directly placed into fixative (−20°C absolute methanol for 3–5 min), or in most cases the cells were first extracted with nonionic detergents and then directly fixed in methanol. Extraction was done by incubating the cells in microtubule stabilization buffer (MSB: 80 mM Pipes,1 pH 6.9, 5 mM EGTA, 1 mM MgCl2 supplemented with 0.05–0.1% Triton X-100 for 2 min at room temperature. A rabbit polyclonal antibody to hsp 73 (12), a rabbit polyclonal TCP-1 antibody (1, 13), a human anti-pericentrin antibody (14), and a mouse monoclonal antibody specific for α-tubulin (15) were used as primary antibodies. Visualization of primary antibodies was done by subsequent incubation with the appropriate fluorescein-conjugated secondary antibody (all from Cappel Laboratories). All antibodies were diluted into 5 mg/ml BSA in phosphate-buffered saline.

Microtubule Regrowth—Cells growing on glass cover slips were treated with nocodazole (20 μg/ml) for 90 min at 37°C. The culture medium was then removed and the cells were washed with and further incubated in fresh culture medium for the times indicated in the figure legends. The cells were then extracted with microtubule stabilization buffer supplemented with 0.05–0.1% Triton X-100 and the distribution of the microtubules determined by indirect immunofluorescence using the anti-α-tubulin antibody.

Effects of Heat Shock on the Centrosome and Microtubule Growth—HeLa cells were subjected to a 43°C/90-min heat shock treatment and then returned to 37°C for various times. The cells were extracted and fixed as described above and the distribution of hsp 73, TCP-1, and pericentrin determined by indirect immunofluorescence. To examine the effects of heat shock treatment on microtubule regrowth, COS cells were heat shocked at 43°C/90 min. The cells were returned to 37°C and then analyzed for their ability to regrow their microtubule network after nocodazole treatment exactly as described above. For some experiments, cells were first made thermotolerant by exposure to a 43°C/90-min shock treatment and subsequent recovery at 37°C for 12 h. Finally, to determine the effects of compounds like glycerol which help in protecting proteins from thermal denaturation, HeLa and COS cells were heated in the presence of 1 mM glycerol and both the staining for centrosomal antigens and microtubule regrowth capabilities were examined as described above.

Microinjection Experiments—For microinjection experiments, the hsp 73 antibody (12) was first purified by DEAE-Affi-Gel blue chromatography. A control, cdls were injected with a similar concentration of purified rabbit anti-goat antibody. The antibodies, at −10 mg/ml in 0.5 × phosphate-buffered saline, were injected into the cells while still in the presence of nocodazole. After a 15-min incubation period, the medium was removed, the cells were extensively washed with fresh Dulbecco’s modified Eagle’s medium, and then further incubated in Dulbecco’s modified Eagle’s medium for an additional period of time to allow for microtubule regrowth. For all experiments, the cells were lightly extracted with MSB supplemented with 0.05% Triton X-100 for 1–2 min and then fixed by immersion in cold absolute methanol. The distribution of tubulin was determined by indirect immunofluorescence using an anti-α-tubulin antibody, while injected cells were identified by staining with fluorescein-conjugated goat anti-rabbit secondary antibody. Similarly, the effects of microinjecting purified hsp 73 protein on the recovery of centrosome staining and microtubule regrowth was performed. Cells were injected with approximately 1–2 mg/ml purified hsp 73 protein and the resultant effects on the structure and function of the centrosome were examined. As a control, cdls were injected with a similar concentration of bovine serum albumin.

RESULTS

In the accompanying article (1) we demonstrated, via indirect immunofluorescence analysis, that a portion of both hsp 73 and TCP-1 localized to the centrosome in cells maintained at 37°C. Because heat shock treatment results in rather dramatic changes in the intracellular distribution of hsp 73, we were curious to know whether the protein would remain associated with the centrosome after heat shock. HeLa cells subjected to a 43°C/90-min heat shock treatment were returned to 37°C for 30 min and then analyzed for the distribution of the centrosomal protein, pericentrin, as well as for both hsp 73 and TCP-1 (Fig. 1). The cells were first lightly extracted with low concentrations of nonionic detergent to remove that portion of hsp 73 and TCP-1 not present within the centrosome. In contrast to the situation with cells grown at 37°C, after heat shock treatment we no longer were able to identify the centrosome using the autoimmune sera 5051 which recognizes pericentrin (Fig. 1, C and F). Similarly, analysis of the heat shock-treated cells with either the hsp 73 or TCP-1 antibodies (Fig. 1, panels B and E, respectively) failed to stain a perinuclear structure corresponding to the centrosome (compare the staining pattern here with that of cells shown in Figs. 2 and 8, or Fig. 4 of the preceding paper).

We next examined whether staining of the centrosome would return when the cells were allowed to recover from the heat shock treatment. Cells were heat shock treated as described above, returned to 37°C, and at various times thereafter examined for the reappearance of centrosomal staining using the three different antibodies used in Fig. 1. At the very earliest times after return of the heat shock-treated cells back to 37°C (e.g. 30–60 min), we still were unable to identify a centrosome via indirect immunofluorescence. Only after 3–4 h of recovery from the heat shock treatment could we now begin to detect what appeared to be a return of centrosomal staining by all three antibodies (Fig. 2).

Previous work has established that the centrosome serves as an anchor for microtubules and is a site of nucleation for microtubule growth (16, 17). For example, others have shown that microtubules regrow from the centrosome following the removal of various drugs (e.g. nocodazole) which cause depolymerization of the microtubule network (18, 19). Because heat shock treatment resulted in an apparent disappearance of the centrosome, we were interested to know whether the cells after heat shock could still support microtubule regrowth. COS cells growing at 37°C were treated with nocodazole for 90 min to depolymerize the microtubule network. Verification that the drug did in fact result in a loss of filamentous tubulin was confirmed by first extracting the nocodazole-treated cells with

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1 The abbreviations used are: Pipes, 1,4-piperazinediethanesulfonic acid; BSA, bovine serum albumin.
nonionic detergent (to remove that portion of tubulin not present within the microtubules) and then performing indirect immunofluorescence staining using an anti-tubulin antibody. As is shown in Fig. 3B, the nocodazole treated and detergent-extracted cells showed absolutely no tubulin staining. However, if the drug was removed and the cells then provided a 30-min recovery period, regrowth of the microtubule network was observed (Fig. 3C). When the cells were subjected to a 43°C/90-min heat shock treatment, no obvious changes in the distribution of the microtubules was observed (Fig. 3D). Subsequent exposure of the heat shock-treated cells to nocodazole for 90 min again resulted in the depolymerization of the microtubules (Fig. 3E). However, in contrast to the situation at 37°C, cells first subjected to the heat shock treatment and then treated with nocodazole were unable to regrow their microtubule cytoskeleton within 30 min following removal of the drug (data not shown). Only when the heat shock-treated cells were allowed a 4-h recovery period in the absence of the drug could we observe any indications of microtubule regrowth (Fig. 3F). Even then, the relative extent of the microtubule regrowth was significantly less than that observed for the cells maintained at 37°C.

We wondered whether these heat induced alterations in both the structure and function of the centrosome was a general manifestation of cells undergoing a stress response. Specifically, increased expression of heat shock proteins has been observed in cells exposed to other agents, many of which like heat shock treatment lead to the intracellular accumulation of abnormally folded proteins (2). Relevant examples include the exposure of cells to different amino acid analogs (20, 21) or puromycin (22), an antibiotic which results in the premature release of nascent polypeptides. Cells either were exposed to an amino acid analogue of proline (azc), or to puromycin, and then examined for their distribution of pericentrin, hsp 73, or TCP-1 via indirect immunofluorescence. In contrast to the effects of heat shock treatment, cells treated with either azc or puromycin still exhibited strong centrosome staining of all three of the centrosome antigens. In addition, the azc- or puromycin-treated cells exhibited microtubule regrowth capabilities similar to that observed for the control, unstressed cells (data not shown). Hence we suspect that the alterations in the structure and function of the centrosome following heat shock treatment are not simply a consequence of the cells having activated a stress response. Rather, we suspect that heat shock treatment adversely affects one or more components, likely proteinaceous in nature, that are important for maintaining the integrity of the centrosome.

To further examine the idea that heat shock adversely affects one or more centrosomal proteins, we took advantage of earlier work demonstrating that the activation of a heat shock response could be significantly dampened if the cells were exposed to high concentrations of glycerol before being subjected to thermal treatments (23). The idea being that at high concentration, glycerol acts as a type of “chemical chaperone,” reducing the overall extent of heat-induced protein denaturation which eventually leads to the activation of the stress response. Cells therefore, were incubated in media containing 1 M glycerol for 1 h and then subjected to a 43°C/90-min heat shock treatment. The cells were returned to 37°C and the relative extent of heat shock protein expression analyzed via Western blotting using an antibody specific for the most highly inducible heat shock protein, hsp 72. In contrast to the cells heated in the absence of glycerol, those cells incubated in the

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**Fig. 2.** Hsp 73 and TCP-1 antibody staining of the centrosome occurs during recovery of the cells after heat shock treatment. HeLa cells growing on glass coverslips were subjected to a 43°C/90-min heat shock treatment and then returned to 37°C for 4 h. Following nonionic detergent extraction and methanol fixation, the simultaneous distribution of hsp 73 and the centrosome, or TCP-1, and the centrosome was analyzed exactly as described in the legend to Fig. 1. Panels A-C show the phase-contrast (A), anti-hsp 73 staining (B), and anti-centrosome staining (C). Panels D-F show phase-contrast (D), anti-TCP-1 staining (E), and anti-centrosome staining (F). The position of centrosomes are indicated by arrowheads.

**Fig. 3.** Effects of heat shock on the ability of cells to regrow their microtubule network after nocodazole treatment. COS cells maintained at 37°C, or subjected to a 43°C/90-min heat shock treatment followed by a 90-min incubation at 37°C, were extracted with nonionic detergent and then immediately fixed and stained with an anti-tubulin antibody. Primary antibodies were visualized by subsequent incubation with a rhodamine-conjugated goat anti-mouse antibody. Panels A and D, microtubule staining in the 37°C and heat shock-treated cells, respectively. In parallel, cells growing at 37°C, or cells subjected to a 43°C/90-min heat shock treatment and returned to 37°C, were incubated with nocodazole (20 μg/ml) for 90 min at 37°C. Immediately following the drug treatment, the cells were extracted with nonionic detergent, fixed, and analyzed via incubation with the anti-tubulin antibody. Panels B and E, tubulin staining in the 37°C cells and heat shock-treated cells, respectively, treated with nocodazole. Finally, both the nonheated and heat shock-treated cells were incubated with nocodazole for 90 min, the culture medium containing the drug was removed and the cells extensively washed with and further incubated in complete medium for varying times to allow for microtubule regrowth. Microtubule regrowth was analyzed via indirect immunofluorescence using the anti-tubulin antibody as described above. Panel C, 37°C cells 30 min after removal of nocodazole. Panel F, heat shock-treated cells 4 h after removal of nocodazole.
presence of 1 M glycerol and then heat shock-treated exhibited little or no induction of hsp 72 (data not shown). Moreover, in the glycerol-treated cells subjected to heat shock treatment, the cells were returned to 37°C for 30 min and then fixed and analyzed by staining with the 5051 antibody specific for the centrosome. For microtubule regrowth experiments, COS cells growing on coverslips were incubated in the presence of 1 M glycerol and subjected to a 43°C/90-min heat shock treatment. At the end of the heat shock treatment, the cells were returned to 37°C and then incubated with nocodazole. Following a 90-min nocodazole treatment, the culture medium was removed, and the cells extensively washed with and further incubated in complete medium for 30 min to allow for microtubule regrowth. Microtubule regrowth was analyzed by staining with the α-tubulin antibody as described earlier. Panels A and B, phase and fluorescent micrographs of HeLa cells subjected to heat shock in the presence of 1 M glycerol and stained for the centrosomal 5051 antibody. Panels C and D, phase and fluorescent micrographs of tubulin staining of the COS cells heat-shock treated in the presence of 1 M glycerol and then analyzed for microtubule regrowth using the nocodazole assay.

A significant amount of work has established that cells provided a relatively mild heat shock treatment subsequently acquire a “thermotolerant” phenotype (5, 6). Such heat tolerant cells now are capable of withstanding subsequent heat shock treatments which would otherwise lead to cell death. It is generally accepted that there is a direct correlation between the increased expression and accumulation of the various stress proteins produced during the initial or “priming” heat shock exposure and the enhanced protection provided to the cell. Consequently, we examined whether cells first rendered thermotolerant would exhibit an increased protection provided to the centrosomal proteins maintaining the integrity and function of this organelle.

Fig. 4. Glycerol protects the centrosome from the adverse effects of heat shock. HeLa cells growing on glass coverslips were incubated for 1 h with Dulbecco’s modified Eagle’s medium containing 1 M glycerol and then subjected to a 43°C/90-min heat shock treatment while still in the presence of glycerol. The cells were returned to 37°C for 30 min and then fixed and analyzed by staining with the 5051 antibody specific for the centrosome. For microtubule regrowth experiments, COS cells growing on coverslips were incubated in the presence of glycerol and subjected to a 43°C/90-min heat shock treatment. At the end of the heat shock treatment, the cells were returned to 37°C and then incubated with nocodazole. Following a 90-min nocodazole treatment, the culture medium was removed, and the cells extensively washed with and further incubated in complete medium for 30 min to allow for microtubule regrowth. Microtubule regrowth was analyzed by staining with the α-tubulin antibody as described earlier. Panels A and B, phase and fluorescent micrographs of HeLa cells subjected to heat shock in the presence of 1 M glycerol and stained for the centrosomal 5051 antibody. Panels C and D, phase and fluorescent micrographs of tubulin staining of the COS cells heat-shock treated in the presence of 1 M glycerol and then analyzed for microtubule regrowth using the nocodazole assay.

Fig. 5. Thermotolerant cells subjected to heat shock treatment exhibit an increased ability to support microtubule regrowth after nocodazole treatment and a faster return of centrosomal staining. COS cells growing on glass coverslips at 37°C were made thermotolerant by a 43°C/90-min heat shock treatment and a 12-h recovery at 37°C. Both the thermotolerant cells as well as non-tolerant cells were subjected to a 43°C/90-min heat shock treatment, returned to 37°C, and then incubated with nocodazole for 90 min. The medium containing the drug was removed and the cells extensively washed with and further incubated in complete medium for only 1 h before analyzing the distribution of the microtubules. Panels A and B, phase and fluorescent micrographs analyzing the regrowth of the microtubules in nontolerant cells subjected to heat shock. Panels C and D, phase and fluorescent micrographs analyzing microtubule regrowth in the thermotolerant cells subjected to heat shock. In Panels E and F are the phase and fluorescent micrographs showing the staining of the centrosome with the 5051 centrosome-specific antibody in the thermotolerant cells subjected to the 43°C/90-min heat shock treatment and then returned to 37°C for 30 min. The position of centrosomes are indicated by arrowheads. Note that the re-appearance of centrosomal staining is faster in the thermotolerant cells (within 30 min) as compared to the nontolerant cells (4 h as shown in Fig. 2) after heat shock.

These now thermotolerant cells were subjected to a second 43°C/90-min heat shock treatment, returned to 37°C, and then treated immediately with nocodazole for 90 min. As was observed earlier, the control cells (i.e. no prior heat shock treatment) subjected to the 43°C/90-min heat shock treatment were significantly impaired with respect to their ability to regrow their microtubule cytoskeleton (Fig. 5B). In contrast, the thermotolerant cells when subjected to the same heat shock treatment exhibited little or no impairment in their ability to regrow their microtubule network following nocodazole treatment (Fig. 5D). Perhaps not too surprisingly, this accelerated recovery of microtubule regrowth capabilities in the thermotolerant cells was accompanied by an accelerated recovery of normal centrosome staining as determined by incubation of the cells with the anti-centrosome antibody 5051 (Fig. 5F). Although not shown, the thermotolerant cells also exhibited a faster recovery of centrosomal staining of both hsp 73 and shown).
TCP-1 following the heat shock treatment.

In our final set of experiments we examined whether hsp 73 might play a role in the normal function of centrosomes or alternately in the recovery of centrosome structure/function after heat shock. For these experiments microinjection protocols were used to modulate the levels of hsp 73 in the cells. COS cells growing on glass coverslips were treated with nocodazole to elicit the depolymerization of the microtubules, and the cells then injected with either a control antibody (rabbit anti-goat) or an antibody specific for hsp 73. Fifteen min later, the medium containing the nocodazole was removed, and the cells extensively washed with and further incubated in fresh culture medium for 30 min. Following this 30-min regrowth period, the cells were examined for their distribution of microtubules. As is shown in Fig. 6 injection of either the control antibody or the anti-hsp 73 antibody had no apparent adverse affect on the ability of the cells to regrow their network of microtubules.

Having found no effects of injected antibodies to hsp 73 on the ability of cells to regrow their microtubule network at 37 °C, we next turned our attention to the situation in cells after heat shock treatment. Here, we addressed the possibility that the enhanced capacity of thermotolerant cells to both reassemble their centrosome, and to support microtubule regrowth after heat shock treatment was associated with the higher levels of hsp 73 in the thermotolerant cells. COS cells were made thermotolerant via their exposure to a 43 °C heat shock treatment for 90 min, and the cells then returned to 37 °C for 12 h to allow for the increased expression and accumulation of hsp 73, along with the other heat shock proteins. These now thermotolerant cells maintained at 37 °C were treated with nocodazole for 70 min and then injected with the anti-hsp 73 antibody. Fifteen min later the medium containing the nocodazole was removed and the cells further incubated for 1 h to allow for the regrowth of their microtubules. Similar to the results obtained earlier, the hsp 73 antibody had no obvious affects on the ability of the thermotolerant cells, when maintained at 37 °C, to regrow their microtubule network (Fig. 7, A and B). Markedly different results were obtained, however, when anti-hsp 73 antibodies were microinjected into thermotolerant cells and the cells then subjected to a second heat shock treatment. While those thermotolerant cells not injected with the antibody were capable of regrowing their microtubule network immediately following the heat shock treatment (as...
was already shown in Fig. 5), microinjection of anti-hsp 73 completely abolished the ability of the thermotolerant cells to support microtubule regrowth after the heat shock treatment (Fig. 7, C and D). Moreover, the injection of the hsp 73 antibody similarly blocked the recovery of centrosome staining, as assayed using the centrosome-specific antisera 5051, in the tolerant cells after heat shock (Fig. 7, E and F). Taken together, these observations indicate that the enhanced ability of thermotolerant cells to both recover the organization and function of the centrosome after heat shock treatment is dependent on the levels of hsp 73.

If in fact the overall amount of hsp 73 is a critical component responsible for the enhanced capacity of thermotolerant cells to recover centrosome structure/function after heat shock, we should be able to simply inject purified hsp 73 protein into normal (nontolerant) cells and thereby mimic a thermotolerant-like phenotype. To test this idea, COS cells growing at 37°C were injected with purified hsp 73. The cells then were subjected to a 43°C/90-min heat shock treatment and returned to 37°C. At various times thereafter the cells were examined, both for the presence of a centrosome, as well as their ability to regrow microtubules using the nocodazole regrowth assay. In those cells injected with purified hsp 73, a return of 5051 centrosomal staining was observed within 1 h after the heat shock treatment (Fig. 8, A and B). However, one should note the relatively large size of the recovering centrosome (e.g. compare the staining pattern in Fig. 8B with that shown for the 37°C cells in some of the earlier figures), an observation perhaps indicative of ongoing centrosome reassembly. By 2 h of recovery from the heat shock treatment, those cells injected with the hsp 73 protein now exhibited a pattern of centrosomal staining identical to that of the cells maintained at 37°C (Fig. 8, C and D). These results are in striking contrast to the results presented earlier showing that in nontolerant cells, the recovery of centrosomal staining required at least 4 h of recovery following the heat shock treatment (Fig. 2). To insure that this accelerated recovery of centrosomal staining in the cells injected with hsp 73 was not simply a consequence of increasing the overall levels of protein in the cell, similar microinjection experiments were performed using BSA. Here the injected BSA did not result in a faster return of 5051 centrosome staining after the heat shock treatment (Fig. 8, E and F). Rather, like the situation for the uninjected cells, the cells injected with BSA required an approximate 4-h recovery period before we could again detect the centrosome via the use of the 5051 antibody (data not shown).

Having shown that cells injected with the purified hsp 73 protein exhibited an accelerated recovery of centrosomal staining after heat shock, we next examined whether these cells might also display an accelerated recovery with respect to their ability to regrow their microtubule cytoskeleton. Cells were injected with either BSA or purified hsp 73, subjected to a 43°C/90-min heat shock treatment, returned to 37°C, and then immediately treated with nocodazole. Ninety min later the medium was removed and the cells washed with and further incubated in fresh culture medium. Those cells injected with BSA and then heat shock-treated were unable to support microtubule regrowth (Fig. 9, A and B). In contrast, cells injected with hsp 73 appeared fully competent for microtubule regrowth immediately following the heat shock treatment (Fig. 9, C and D). Thus by simply raising the relative intracellular levels of hsp 73, the cells now appear to have acquired a thermotolerant-like phenotype, at least as assayed by their enhanced capabilities to recover centrosome structure/function after heat shock.

**DISCUSSION**

Most of the recent studies examining the function of molecular chaperones have focused on their intimate role in protein synthesis and maturation (3, 4). However, it is becoming apparent that these proteins also participate in a number of other biological processes involving already synthesized or "mature" polypeptides. Indeed, studies presented here and in the accompanying manuscript (1) suggest novel roles for molecular chaperones as mediators of cytoskeletal and/or centrosomal assembly. While these results may appear to fall outside the currently defined roles for chaperones, they are, nevertheless, in line with results obtained by early workers in this field. For example, our first insights into the possible function of those heat shock proteins which function as molecular chaperones came from studies examining their role in facilitating the early events of phage replication in Escherichia coli (for a review, see Refs. 24 and 25). Here different members of the heat shock protein family were shown to be involved in mediating specific protein-protein interactions associated with the very earliest events of bacteriophage DNA replication, as well as the assembly of the bacteriophage head and tail structures. Similar to their proposed role in monomeric protein folding, the bacterial
heat shock proteins likely participated in these processes by facilitating changes in overall protein conformation needed for either the disassembly or assembly of higher ordered protein complexes.

In the results presented here and in the accompanying paper (1), we have shown that at least two molecular chaperones are integral components of the microtubule organizing center of animal cells, the centrosome. Likewise, evidence was presented that both proteins participate in different aspects regarding the structure and function of this organelle. In the case of TCP-1, antibodies to the protein were capable of blocking the initiation of microtubule growth off the centrosome. In the case of hsp 73, protein or BSA. Following the injection, cells were subjected to a 43°C/90-min heat shock treatment, returned to 37°C, and incubated with nocodazole. Ninety min later the culture medium containing the drug was removed, and the cells washed with and further incubated in complete medium for 1 h. The cells were then extracted, fixed, and processed for indirect immunofluorescence using the anti-tubulin antibody. Panels A and B, cells injected with BSA prior to the heat shock treatment. In panel A is shown the phase-contrast micrograph and in panel B the fluorescent micrograph of tubulin staining. Panels C and D, cells injected with hsp 73 protein prior to the heat shock treatment. In panel C is shown the phase-contrast micrograph and in panel D the fluorescent micrograph of tubulin staining. The arrowheads in panels C and D indicate a cell which was not injected with the hsp 73 protein.

Some support for this idea was our observations showing that incubation of the cells with high concentrations of glycerol, a compound known to protect proteins from thermal denaturation, resulted in the protection of centrosome integrity in cells subjected to heat shock.

To the long list of cellular processes which are adversely affected by heat shock treatment, we now can add alterations in the integrity of the centrosome after heat shock treatment of cultured Drosophila cells. The primary effects of heat shock treatment on the centrosome, these investigators concluded, was associated with changes in the organization of the so-called pericentriolar material which surrounds the centriolar portion of the centrosome. While further work is required, we suspect that hsp 73 and TCP-1, like the pericentrin protein, likely are components of this pericentriolar material.

Fig. 9. Microinjection of hsp 73 protein promotes rapid microtubule regrowth following heat shock. COS cells growing on etched coverslips as described above were microinjected with either hsp 73 protein or BSA. Following the injection, cells were subjected to a 43°C/90-min heat shock treatment, returned to 37°C, and incubated with nocodazole. Ninety min later the culture medium containing the drug was removed, and the cells washed with and further incubated in complete medium for 1 h. The cells were then extracted, fixed, and processed for indirect immunofluorescence using the anti-tubulin antibody. Panels A and B, cells injected with BSA prior to the heat shock treatment. In panel A is shown the phase-contrast micrograph and in panel B the fluorescent micrograph of tubulin staining. Panels C and D, cells injected with hsp 73 protein prior to the heat shock treatment. In panel C is shown the phase-contrast micrograph and in panel D the fluorescent micrograph of tubulin staining. The arrowheads in panels C and D indicate a cell which was not injected with the hsp 73 protein.

In the results presented here and in the accompanying paper (1), we have shown that at least two molecular chaperones are integral components of the microtubule organizing center of animal cells, the centrosome. Likewise, evidence was presented that both proteins participate in different aspects regarding the structure and function of this organelle. In the case of TCP-1, antibodies to the protein were capable of blocking the initiation of microtubule growth off the centrosome. In the case of hsp 73, protein or BSA. Following the injection, cells were subjected to a 43°C/90-min heat shock treatment, returned to 37°C, and incubated with nocodazole. Ninety min later the culture medium containing the drug was removed, and the cells washed with and further incubated in complete medium for 1 h. The cells were then extracted, fixed, and processed for indirect immunofluorescence using the anti-tubulin antibody. Panels A and B, cells injected with BSA prior to the heat shock treatment. In panel A is shown the phase-contrast micrograph and in panel B the fluorescent micrograph of tubulin staining. Panels C and D, cells injected with hsp 73 protein prior to the heat shock treatment. In panel C is shown the phase-contrast micrograph and in panel D the fluorescent micrograph of tubulin staining. The arrowheads in panels C and D indicate a cell which was not injected with the hsp 73 protein.

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identify a role for hsp 73 in microtubule nucleation off the centrosome. Nevertheless, hsp 73 is intimately associated with the centrosome during all stages of the cell cycle, suggestive that the protein is involved in some aspect of the organelle. One possibility is that hsp 73 participates in the process of centrosomal assembly. Perhaps, following the duplication of the centriole during S phase, hsp 73 mediates the assembly of the proteins which comprise the pericentriolar material. This idea, although admittedly speculative, would be consistent with our results showing a role for hsp 73 in the return of centrosomal structure and function following heat shock. Furthermore, the presence of hsp 73 (as well as TCP-1) in the centrosome during other stages of the cell cycle might facilitate the movement of proteins into and out of the organelle. Indeed, a number of proteins are known to transiently associate with the centrosome in a cell cycle related manner (26). To address these questions, we are currently utilizing defined invitro systems to examine the details by which chaperones might participate in the assembly and/or disassembly of the centrosome. Via such experiments, we hope to elucidate more fully the roles that molecular chaperones play in the biology of this rather intriguing organelle.

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