Journal of Cell Science 110, 1431-1440 (1997)
Printed in Great Britain © The Company of Biologists Limited 1997
JCS8125

COMMENTARY

Molecular chaperones and the cytoskeleton

P. Liang and T. H. MacRae*

Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada

*Author for correspondence (e-mail: tmacrae@is.dal.ca)

SUMMARY

Heat shock proteins, first observed because they are preferentially synthesized by organisms exposed to heat or other physiological stress, are also synthesized constitutively. These proteins are divided into several families, namely, HSP100, 90, 70, 60 (chaperonin), and the small heat shock/α-crystallin proteins. They enjoy a wide phylogenetic distribution and are important because they function as molecular chaperones, able to mediate many cellular processes through an influence on higher order protein structure. For example, molecular chaperones assist in the transport of proteins into mitochondria and chloroplasts, as well as influencing clathrin lattice dynamics, viral replication and transcriptional activation. Under conditions of stress, some molecular chaperones prevent denaturation of proteins while others may dissociate protein aggregates, refolding monomers derived therefrom or directing their proteolytic destruction. We present in this review an analysis of the emerging literature on the relationship between molecular chaperones and the cytoskeleton, a collection of polymeric structures consisting of microtubules, microfilaments and intermediate filaments. A recent development in this field is identification of the TCP-1 complex as the eukaryotic cytoplasmic chaperonin which directs folding of cytoskeletal proteins such as α/β/γ-tubulin, actin and centractin. Moreover, the TCP-1 complex is a centrosomal component, apparently involved in the nucleation of microtubules. Other molecular chaperones recognize one or more cytoskeletal elements and in most cases they modulate the assembly of and/or provide protection for their constituent proteins. For example, HSP70 protects the centrosome and perhaps intermediate filaments during heat shock, and like HSP90, it binds to microtubules. Small heat shock proteins interact with microfilaments and intermediate filaments, affect their polymerization and guard them from heat shock by a phosphorylation-dependent mechanism. We conclude that molecular chaperones have different but cooperative roles in the formation and function of the eukaryotic cell cytoskeleton.

Key words: Stress protein, Molecular chaperone, Cytoskeleton

INTRODUCTION

Tissières et al. (1974) demonstrated a rapid and selective increase in the synthesis of a specific group of proteins following exposure of *Drosophila* larvae to temperatures above those optimal for growth. These were referred to as heat shock proteins (HSPs) due to the method of induction, but subsequent studies revealed that metals, amino acid analogs and anoxia cause a similar response. HSPs, also called stress proteins (Morimoto et al., 1991; Parsell and Lindquist, 1994), are divided into five major families, HSP100, 90, 70, 60, and the small HSP (sHSP)/α-crystallins, according to their size, structure and function (Craig et al., 1994; Morimoto et al., 1991, 1994). HSPs exist in all organisms from bacteria to humans and they are among the most conserved proteins known (Schlesinger, 1990). As a result of HSP synthesis, organisms gain tolerance to insult, a phenomenon termed induced thermotolerance or stress tolerance (Parsell and Lindquist, 1994). In addition to their production in stressed cells, HSPs are present in and essential for the well being of unstressed cells (Hendrick and Hartl, 1993, 1995).

Most proteins, after either translation or denaturation, cannot spontaneously attain their functional conformation, in part because of extremely high intracellular protein concentrations which favor misfolding or aggregation. Instead, they are assisted in this process by molecular chaperones. A molecular chaperone is ‘a protein that binds to and stabilizes an otherwise unstable conformer of another protein and, by controlled binding and release of the substrate protein, facilitates its correct fate in vivo: be it folding, oligomeric assembly, transport to a particular subcellular compartment, or controlled switching between active/inactive conformations’ (Ellis and van der Vies, 1991). Molecular chaperone function is probably the major role of the HSPs (Craig et al., 1994; Ellis, 1987; Ellis and van der Vies, 1991; Georgopoulos and Welch, 1993; Hendrick and Hartl, 1995; Martin et al., 1992). As one example, HSP70 is a family of stress-inducible and constitutive proteins, the latter usually termed HSC70, approximately 70 kDa in molecular mass and found in diverse cellular compartments of many

organisms. HSP70 binds to nascent polypeptide chains on ribosomes, maintains the membrane translocation-competent state of precursor proteins, assists protein transport into mitochondria and the endoplasmic reticulum, and protects proteins under stress. A unifying theme behind these actions is ATP-dependent binding of HSP70 to short hydrophobic regions of 6-9 amino acid residues on target proteins in extended or unfolded conformation, but the peptide-peptide binding may have little influence on the specificity of HSP70 function (Craig et al., 1994; Georgopoulos and Welch, 1993; Hartl and Martin, 1992; Hendrick and Hartl, 1995; James et al., 1997).

The HSP60 or chaperonin family, including GroEL from bacteria, rubisco-binding protein from chloroplasts, HSP60 from mitochondria, and the t-complex polypeptide 1 (TCP-1 complex) from eukaryotic cytosol, is a group of proteins with distinct ring-shaped, or toroid (double donut) quaternary structure (Ellis and van der Vies, 1991). One important activity of chaperonin is apparently to mediate the native folding of proteins in an ATP-dependent manner, and a model to describe this process through cooperation of HSP70 and HSP60 is available (Frydman and Hartl, 1996; Hendrick and Hartl, 1995; Martin et al., 1992; Martin and Hartl, 1994; Morimoto et al., 1991). It is proposed, although agreement is incomplete (Bukau et al; 1996), that HSP70 and its cofactor HSP40 interact with nascent polypeptide chains and prevent (mis)folding until a domain able to form a stable structure is synthesized. Mature proteins are then produced in the central cavity of the chaperonin toroid complex by ATP-dependent cycles of release and rebinding. Of additional interest, the TCP-1 complex may function outside its influence on protein folding per se, as suggested by its interaction with actin in growth cones, a cell compartment devoid of ribosomes (Roobol et al., 1995).

HSP90 is an abundant, essential, cytosolic protein under normal conditions, and it increases markedly during stress (Criag et al., 1994; Georgopoulos and Welch, 1993; Morimoto et al., 1991, 1994). Studies in vitro suggest that HSP90 suppresses aggregation or denaturation of citrate synthase and casein kinase II, while in vivo it not only has general chaperone activity, but it targets specific proteins with maturational and regulatory capacities (Craig et al., 1994; Rutherford and Zuker, 1994; Bose et al., 1996; Redmond et al., 1989). In one example, HSP90 in a complex with several other proteins engages steroid receptors, perhaps maintaining them in an inert, folded state, able to bind with and react to the correct steroid signal (Redmond et al., 1989; Rutherford and Zuker, 1994; Freeman et al., 1996; Kimura et al., 1995; Bose et al., 1996). HSP100/Clp proteins protect organisms under extreme stress conditions, and in the absence of HSP104 for example, the survival of heat shocked yeast is reduced several orders of magnitude. The HSP100/Clp proteins function as chaperones, solubilizing protein aggregates in an ATP-dependent fashion and leading either to the renaturation of individual proteins or to their proteolytic degradation (Craig et al., 1994; Parsell and Lindquist, 1994; Horwich, 1995; Schirmer et al., 1996). Small heat shock/α-crystallins, with molecular masses of 15 to 30 kDa, are characterized by a conserved region of primary structure termed the α-crystallin domain (de Jong et al., 1993). The sHSP/α-crystallins exhibit chaperone activity in vitro and thermoprotection in vivo (Arrigo and Landry, 1994; Horwitz, 1992; Landry et al., 1989; Lavoie et al., 1993a; Parsell and Lindquist, 1994; Muchowski et al., 1997). Most sHSP/α-crystallins are heat inducible, but some are synthesized in unstressed conditions, usually in a tissue-specific manner as cells develop. By illustration, the four *Drosophila* sHSP/α-crystallin genes are differentially expressed during development, although they are coordinately activated by heat shock (Pauli et al., 1989). Additionally, a sHSP/α-crystallin from *Artemia* is produced only in embryos undergoing encystment and it disappears quickly once larvae develop (Liang et al., 1997).

Molecular chaperones influence several cell components one of which is the cytoskeleton, an assortment of filamentous and tubular polymers composed of microtubules, microfilaments and intermediate filaments. Microtubules are hollow cylinders constructed of tubulin, a heterodimeric protein comprised of α- and β-tubulin (Sackett, 1995; Amos, 1995; Ludueña et al., 1992; MacRae and Langdon, 1989). Microtubules often emanate from discrete regions of variable structure known as microtubule-organizing centers (MTOCs). The centrosome, consisting of centrioles and pericentriolar material, is the most common organizer of microtubules in mammalian cells (Archer and Solomon, 1994; Schatten, 1994), while yeast exhibit a functionally analogous but structurally different structure termed the spindle pole body (Marschall et al., 1996). A third tubulin isotype, γ-tubulin, is involved in the nucleation of microtubules at MTOCs and is also found as cytosolic complexes within eukaryotic cells (Moudjou et al., 1996; Joshi and Palevitz, 1996; Raff, 1996; Joshi, 1994). In concert with microtubule-associated proteins (MAPs) that bind to the microtubule surface (Hirokawa, 1994; MacRae 1992a,b; Chapin and Bulinski, 1992), the MTOC determines the intracellular distribution of microtubules, and thus their function. The microfilaments, sometimes called actin filaments or F-actin, are formed from a small family of closely related, monomeric actin isoforms (Mitchison, 1992; Carlier, 1991; Rubenstein, 1990). The spatial arrangement and function of microfilaments are regulated by many different accessory proteins (Pollard et al., 1994; Bretscher, 1991). Intermediate filaments assemble from fibrous proteins supercoiled into rope-like cables, but unlike tubulin and actin, there are at least five major classes of intermediate filament proteins, each characteristic of specific tissues or cell types (Fuchs and Weber, 1994).

Although varied in molecular composition, the activities of cytoskeletal elements overlap with and are influenced by one another. Functions of the cytoskeleton, modulated by polymerization dynamics and spatial organization, include determination of cell shape, partitioning of organelles and molecules by intracellular transport mechanisms mediated by mechanochemical accessory proteins, division and motility. Clearly, proper synthesis and assembly of cytoskeletal elements are vital to the cell, and disruption of these macromolecular complexes is often disastrous. It is our purpose in this review to analyze the emerging evidence in support of the proposal that molecular chaperones facilitate formation of the cytoskeletal elements, influence their function and protect the cytoskeleton during stress, thus providing an indispensable service to the cell.

THE FOLDING OF CYTOSKELETON PROTEINS BY THE TCP-1 COMPLEX

HSP60 chaperonins in bacteria, mitochondria and chloroplasts are well studied, but their equivalent in the eukaryotic cytosol,

a heteromeric assembly of several polypeptides uninducible by stress and termed the TCP-1 complex (also called TriC, CCT, C-cpn) was discovered more recently (Frydman et al., 1992; Gupta, 1990; Lewis et al., 1992; Trent et al., 1991; Willison and Kubota, 1994; Lewis et al., 1996; Melki and Cowan, 1994; Kim et al., 1994; Tian et al., 1995, 1996; Melki et al., 1996). The complex, composed of TCP-1 and related proteins in the range of 50 to 65 kDa (Kubota et al., 1994) has a double-ring quaternary structure resembling that of GroEL. TCP-1 has distant but significant sequence homology to the HSP60 chaperonins (Gupta, 1990, 1995), suggesting an evolutionary relationship between these proteins. This possibility was strengthened by the discovery of an archaebacteria heat shock protein, TF55, with quaternary structure the same as GroEL but a greater sequence similarity to TCP-1 (Gupta, 1990, 1995; Trent et al., 1991). An early indication that tubulin is a target of the TCP-1 complex was the observation of abnormal cytoskeleton structures in a cold-sensitive TCP-1 yeast mutant (Ursic and Culbertson, 1991). The number of genes now know to be in the TCP-1 family has risen to eight and at least four of these genes, *TCP1*, *BIN2*, *BIN3* and *ACN2*, or *CCT1-CCT4*, are necessary for the normal function of tubulin and actin (Stoldt et al., 1996; Chen et al., 1994; Vinh and Drubin, 1994; Ursic et al., 1994; Miklos et al., 1994). Both in vitro (Yaffe et al., 1992) and in vivo (Sternlicht et al., 1993), nascent tubulin and actin enter a 900 kDa TCP-1-containing complex from which assembly-competent forms of the proteins emerge. Folding of α- and β-tubulin, but not actin, requires protein cofactors in addition to TCP-1 (Gao et al., 1992, 1993). Two of these, termed D and E, are homologues of yeast proteins that, when mutated, affect microtubule function (Tian et al., 1996). A third protein, cofactor A, or p14, is highly conserved in vertebrates and has a structural/functional homologue in yeast termed Rb12p (Archer et al., 1995). Recent work indicates cofactor A is a chaperone that interacts in a nucleotide-independent manner with a β-tubulin folding intermediate liberated from TCP-1 and that it not only has a role in the folding process but may sequester β-tubulin when it is present in excess (Melki et al., 1996; Llosa et al., 1996). The isolated mouse TCP-1 complex also catalyzes the ATP-dendent conversion of urea-denatured actin into its native state in vitro (Gao et al., 1992). Besides α/β-tubulin and actin, the centrosome-related proteins γ- tubulin and centractin are folded by the TCP-1 complex (Melki et al., 1993).

Tubulin and actin are consistently found in the TCP-1 complex favoring the idea that they are its major substrates (Sternlicht et al., 1993; Yaffe et al., 1992); correspondingly, the quantity of TCP-1 in cells is estimated to be only slightly more than required for the folding of actin and tubulin. However, this does not mean that they are the only important TCP-1 substrates; rather it may reflect the intracellular abundance of these proteins. Assuming that the TCP-1 complex recognizes specific proteins, why does it favor actin and tubulin? As one possibility, a β-tubulin peptide enriched in hydrophobic residues and proline, may provide a region for binding of this protein to the TCP-1 complex (Dobrzynski et al., 1996). The domain, termed the 'interactive core', encompasses residues from about position 150 to 350. Other regions located toward the carboxy terminus may be required for removal of β-tubulin from the TCP-1 complex (Dobrzynski et al., 1996). Interestingly, of the cytoskeletal components that interact, with the TCP-1 complex, each contains a carboxy-terminal peptide RK(A,C,T)F/KRAF, a sequence with a TCP-1 homologue (Burns and Surridge, 1994), and these sequences may mediate separation of substrate and chaperone. This is apparently followed, at least for tubulin, by transfer to a second chaperonin, for which residues 137-152 are important, perhaps for binding and/or hydrolysis of GTP (Zabala et al., 1996).

In other reactions possibly not directly related to folding, the TCP-1 complex associates with tubulin during its transport along neurites (Carden and Roobol, 1995; Roobol et al., 1995), as shown upon differentiation of ND7/23 cells to a neuronal phenotype. Chaperonins containing TCP-1 (TCPα) enter neuritic processes and they are enriched at the leading edge of growth cone-like structures where they co-localize with G-actin. On the other hand, chaperonins with the components (TCPβ, ε and γ) remain largely in the perikaryal cytoplasm demonstrating specificity in the transport function of these proteins. Another interesting observation concerning chaperonin function is its cosynthesis with tubulin during cilia recovery in *Tetrahymena* (Soares et al., 1994). The results suggest that the chaperonin assists folding of newly synthesized tubulin and/or other components of the cilia. Finally, the TCP-1 complex, identified as chromobindin A, reacts with the membrane of chromaffin granules and it may have a role in vesicle transport and/or fusion (Creutz et al., 1994). Thus, in eukaryotic cells chaperonin function exemplified by the TCP-1 complex is undoubtedly wider than indicated by its association with cytoskeletal elements.

ARE MOLECULAR CHAPERONES REQUIRED FOR MICROTUBULE FUNCTION?

Several HSP70s were characterized as microtubule-associated proteins (MAPs) before they were identified as molecular chaperones. A 70 kDa MAP from NIL8 cells, co-localizing with microtubules during interphase and mitosis, belongs to the HSP70 family (Weller, 1988). Similarly, a 68 kDa protein from HeLa cells, while unable to stimulate tubulin assembly, co-purifies with this protein through two cycles of polymerization and depolymerization (Weatherbee et al., 1980). The 68 kDa protein is a homologue of the mammalian HSC70-related protein, thermin, found in the cytoskeletal fractions of rat spinal cord, porcine brain and various cell lines (Wang et al., 1980). β-Internexin, a rat brain MAP extracted from taxol-stabilized microtubules, is identical to HSC70 (Green and Liem, 1989). A 70 kDa HSP in the green alga *Chlamydomonas* binds to the microtubule-containing axoneme and concentrates at the distal ends of flagella where axoneme assembly occurs (Bloch and Johnson, 1995). Incubation of axonemes with ATP, but not AMP or AMP-PNP, a non-hydrolyzable ATP analog, releases the HSP70. Extraction under ionic conditions, known to free capping structures that link microtubule distal ends to the flagellar membrane, liberates axonemal-bound HSP70. The authors propose that HSP70 directs tubulin and other proteins to the flagellar tip where the chaperone participates in construction of the axoneme. Additionally, because tubulin and HSP70 are well conserved across species boundaries the studies support a widespread role for this chaperone in cytoskeleton formation.

HSP70 attaches to polymerized tubulin at the carboxy-

terminal residues, 431- 444, which are included in the site that is recognized by MAPs (Sánchez et al., 1994). Moreover, HSP70 and MAP1B contain tubulin binding motifs of similar sequence and a 65 kDa HSP70 from beet yellow virus interacts with purified microtubules, exhibiting an association constant similar to that determined for MAP1and MAP2. Removal of a short C-terminal domain from α- and β-tubulin by subtilisin digestion abolishes its reaction with the 65 kDa HSP (Karasev et al., 1992). It seems that members of the HSP70 family combine with microtubules through a MAP-like motif. In line with this idea, the identification of mutants resistant to tubulin-reactive drugs and possessing a modified form of hsc70 (Ahmad et al., 1990), implies that this heat shock protein regulates tubulin polymerization. HSP70 may prevent microtubule formation by acting as an antagonist of MAPs which are able to promote tubulin assembly and stabilize microtubules (Vallee, 1990). Inhibition of tubulin polymerization could be necessary for cell division and differentiation, times when a highly dynamic cytoskeleton is favoured. These effects of HSP70 on microtubules can be examined further by assembling purified tubulin in vitro in the presence and absence of the protein, and by either expressing exogenous or disrupting intrinsic HSP70 genes within cells.

Besides a direct interaction with tubulin, HSP70 apparently affects microtubules through association with tau, a microtubule-associated protein (Kirby et al., 1994; Wallace et al., 1993). Heat shocked neuronal PC12 cells produce hyperphosphorylated tau, leading to structures similar to the neurofibrillary tangles found in the brains of individuals with Alzheimer's disease. Tangles form because the hyperphosphorylated tau does not bind to HSP70 (hsp72 in PC12 cells) and is available for interaction with microtubules, whereas normal tau is sequestered in a stable complex with HSP70 (Wallace et al., 1993). It was proposed that newly synthesized tau associates with HSP70 and is protected from phosphorylation, whereas mature tau does not complex with this chaperone and is available for modification. This conclusion is strengthened by comparing normal cells to those with acquired thermotolerance. Production of HSP70 in the former occurs much later than in the latter upon heat shock, and in each case the period of maximal HSP70 concentration correlates with the time at which tau is protected from phosphorylation. Moreover, when HSP70 action is inhibited by L-azetidyl 2-carboxylic acid, tau is phosphorylated suggesting that HSP70 shields tau from enhanced phosphorylation during heat shock. Because abnormal phosphorylation of tau disrupts its ability to promote tubulin assembly, HSP70 may, through its effect on tau, promote tubulin assembly, an outcome opposite to that proposed earlier in this review for binding of HSP70 to microtubules.

Antibodies against tubulin and HSP90 immunoadsorb HSP90 and tubulin, respectively, from L cell cytosol preparations. Use of these antibodies also demonstrated the colocalization of HSP90 and microtubules in cultured cells (Sanchez et al., 1988). Immunofluorescence experiments with Ishikawa (Fostinis et al., 1992) and rat pulmonary endothelial cells (Czar et al., 1996) confirmed the association of HSP90 with microtubules, and possibly with cytokeratin intermediate filaments, but not with microfilaments (Redmond et al., 1989). HSP90 may be involved in microtubule-based movement because the protein binds to cytosolic steroid receptors which undergo intracellular translocation as a prerequisite for carrying out their function (Craig et al., 1994). Moreover, because it is dispersed amongst the cytoskeletal elements, the possibility exists that different HSP90 isoforms interact with each type of polymer (Czar et al., 1996).

Exposure in vitro to a temperature of 50°C disassembles microtubles and inactivates tubulin (Coss et al., 1982). Incubation of dividing CHO cells at 45.5°C destroys the spindle and undermines the contractile ring and the midbody-cytoplasmic bridge complex to varying degrees, depending on the length of treatment. Division is prevented and tetraploid cells arise. In mouse T lymphocytes, hyperthermia damages microtubule organization and impairs cytolytic activity (Knox et al., 1991). However, protection of microtubules by HSPs has not been reported, although HSP70 and HSP90 associate with these structures. If future study excludes thermoprotection, then it is likely that HSP70 regulates tubulin assembly/disassembly rather than serving as a chaperone for this protein.

Although evidence links the TCP-1 complex, HSP70 and HSP90 with tubulin/microtubules, much less is known about their relationship with the small heat shock/α-crystallins. Atomi and Arai (1996) reported, in abstract form, that αB-crystallin may influence tubulin polymerization. In other work it was shown that exposure of C6 glioma cells to drugs that cause depolymerization of microtubules, increases their content of αB-crystallin, but not HSP27 and HSP70 (Kato et al., 1996). The amplification of αB-crystallin is preceded by enhanced production of mRNA for this protein. However, induced transcription and translation are inhibited if cells are incubated with taxol, a drug that stabilizes microtubules, suggesting that disassembly is required for promotion of αB-crystallin synthesis. Moreover, the induction of αB-crystallin is suppressed by staurosporine, an inhibitor of protein kinases. Immunostaining reveals that soluble tubulin and αB-crystallin colocalize to the periphery of cells subjected to microtubule depolymerization, but interaction of the two proteins has not been demonstrated. No role is proposed for αB-crystallin in treated cells, but it is tempting to speculate that it associates with tubulin preserving the latter for assembly upon removal of the drug. Whether an equivalent physiological situation occurs in cells not exposed to drugs is unknown.

MOLECULAR CHAPERONES ARE CENTROSOMAL COMPONENTS

The centrosome, an important MTOC, nucleates microtubules and determines their polarity (Joshi, 1994; Joshi and Palevitz, 1996; Raff, 1996). Heat shock can alter the pericentriolar material of the centrosome leading to mitotic dysfunction (Vidair et al., 1993). Centrosomal labelling by anti-pericentrin antibody, for example, decreases immediately after heating tolerant and nontolerant CHO cells at 45°C for 4-18 minutes. When returned to 37°C, pericentrin staining reappears gradually in both types of cells, but distribution in nonthermotolerant cells is abnormal. Heat reduces the extent of microtubule nucleation, but centriole number is not perturbed, indicating that the pericentrolar material is affected. Such findings imply that thermotolerant cells have an enhanced capacity to repair damage to centrosomes, signifying a contribution by molecular chaperones to this process.

Molecular chaperones have been identified at the centrosome. A 72 kDa HSP associates with centrosomes in various species, including dinoflagellates and humans (Perret et al., 1995), while Brown et al. (1996a,b) have shown that a 73 kDa HSP and TCP-1 exist in centrosomes throughout the cell cycle. There is a strong correlation between increased centrosome staining by anti-HSP73 antibody and the appearance of microtubules during recovery from heat shock. Moreover, microinjection of anti-HSP73 antibody blocks centrosome assembly following heat treatment. In contrast, microinjection of HSP73 prior to heating accelerates recovery of centrosome function in a manner similar to that observed in themotolerant cells. Although details are lacking, HSP73 appears to have a role in centrosome repair, which in turn affects the intracellular organization of microtubules. Incubation in vitro with an antibody to TCP-1, but not to HSP73, blocks microtubule growth from centrosomes. Similarly, injection of antibody to TCP-1 inhibits establishment of microtubule networks in cells after removal of nocodazole, while antibody to HSP73 has no effect. Coupled with the observation that yeast TCP-1 mutants have several microtubule abnormalities (Ursic and Culbertson, 1991), the results indicate that TCP-1 assists in microtubule nucleation, a fundamental activity of centrosomes.

MOLECULAR CHAPERONES MODULATE ACTIN ASSEMBLY

There is evidence that HSP90 and HSP100 cross-link microfilaments (Koyasu et al., 1986). HSP70 may stabilize actin filaments in cultured cells (Macejak and Luftig, 1991), it has been proposed to coaggregate with actin in ATP-deprived cells (Kabakov and Gabai, 1994), and an HSP70-related actin capping protein, aginactin, has been found in *Dictyostelium* amoebae (Weeds and Maciver, 1993). Aginactin could link cell movement to cytoskeletal reorganization via its response to external stimuli. Also, HSP70, through its association with the TCP-1 complex (Kubota et al., 1994; Lewis et al., 1992), has the potential to modulate actin formation and dynamics in an indirect way. However, members of the sHSP/α-crystallin family probably exert a greater influence on actin filaments than do other molecular chaperones. Miron et al. (1988, 1991) showed that a protein termed 25 kDa IAP from turkey smooth muscle inhibits actin polymerization in vitro, perhaps because it attaches to the barbed-end of microfilaments and is devoid of nucleating activity. IAP has 80% similarity to human small heat shock-like protein (HSPL27) from heart, speculated to stabilize actin filaments in myocytes (Lam et al., 1996), and in cultured chicken fibroblasts it increases up to 15-fold during heat shock at 45°C. A transient but prominent reduction in microfilament number occurs in turkey smooth muscle cells, but a direct correlation between increased IAP synthesis and actin disassembly could not be made because deterioration of actin filaments is apparent before an appreciable amount of IAP accumulates. Moreover, the change in microfilaments is transient while IAP persists upon continued exposure of cells to heat. Indeed, it could be argued, based on more recent studies of HSP27 (discussed later), that IAP assists in the recovery of actin filament networks in these cells.

HSP26 from yeast disrupts microfilaments, and mixing equimolar amounts with G-actin decreases F-actin fivefold under standard polymerization conditions (Rahman et al., 1995). Inhibition of actin assembly, at least for murine HSP25, is greatest when the protein occurs as a nonphosphorylated monomer, whereas multimeric nonphosphorylated complexes of HSP25, and phosphorylated monomers, fail to inhibit actin assembly (Benndorf et al., 1994). Interestingly, the motif G-[V/I]-L-T-$[X]_3$-P, shared by sHSP/α-crystallins and thought to have a role in the formation of chaperone multimers (Kaukinen et al., 1996), appears in a conserved domain of actin important for polymerization. Similar secondly structures may exist in these regions of actin and HSP26. Thus, it is possible that some sHSP/α-crystallins inhibit assembly by competing with G-actin for incorporation into the F-actin polymer, thereby capping microfilaments at their elongating ends. However, other studies show a slight stimulation of actin assembly by α-crystallin, suggesting it does not cap microfilaments (Wang and Spector, 1996).

There is a rapid, reversible loss of stress fibers in CHO cells exposed to hyperthermia (Glass et al., 1985). The appearance of intranuclear actin-containing inclusion bodies is part of the morphological change in heat-shocked rat fibroblast cells and similar rod-like structures are found in the nucleus of other cells experiencing different types of stress (Welch and Suhan, 1985; Iida et al., 1986). In allied studies, immunofluorescence staining reveals that HSP27 is enriched in a cytoplasmic region of highly motile fibroblasts characterized by actin polymerization, and that overexpression of HSP27 in CHO fibroblasts partially suppresses cytochalasin D-induced microfilament disruption (Lavoie et al., 1993a,b). Modulation of microfilament stability by HSP27 is phosphorylation-dependent and HU27 Chinese hamster cells that overexpress wild-type HSP27 exhibit increased F-actin at the cortex, elevated pinocytotic activity and enhanced survival after heating (Lavoie et al., 1993a, 1995). In contrast, mutant HU27pm3 cells induced to synthesize high levels of nonphosphorylatable HSP27 have less cortical F-actin, decreased pinocytotic activity and low survival after thermal shock. Greater amounts of HSP27, but not its nonphosphorylatable form, accelerate the reappearance of microfilaments after treatment with the drug, cytochalasin D. Also, phosphorylatable HSP27 protects against oxidative stress, preventing actin fragmentation and cell death, properties not shared by the nonphosphorylatable pm3 HSP27 (Huot et al., 1996). The authors propose that early during stress, phosphorylation regulates the effect of HSP27 on actin dynamics, resulting in greater microfilament stability, accelerated recovery of filament number, and higher overall cell survival.

That phosphorylation of sHSP/α-crystallins influences their interaction with actin is indirectly supported by two reports. Loktionova et al. (1996) demonstrated the redistribution of HSP27 in human endothelial cells to stress fibers within 15-30 minutes of heat shock, concurrent with increased HSP27 phosphorylation and before the stress-induced disruption of the microfilaments. Additionally, when the human promyelocytic leukemic cell line, HL-66, is induced by exposure to phorbol ester myristate to differentiate into a macrophage-like cell, the amount of phosphorylated HSP28 increases, and there is colocalization of HSP28 and actin, probably at the leading edge of the cell (Minowada and Welch, 1995). However, the specific association of the phosphorylated chaperones with microfilaments and their enhanced stability as a result of interaction were not demonstrated in either case. On the other hand, recent

data on α-crystallin contradict the findings obtained through study of Chinese hamster cells, and suggest more than one mechanism whereby phosphorylation affects the sHSP/α-crystallins (Wang and Spector, 1996). In this case, α-crystallin and either of its αA or αB-isoforms, prevent depolymerization of microfilaments by cytochalasin D, but this protective effect is decreased by phosphorylation. In the absence of drug, phosphorylation has little effect on the ability of α-crystallin to either stabilize microfilaments or prevent heat-induced aggregation of microfilaments. Thus, not only does α-crystallin appear to function differently from HSP27, it may exhibit alternate activites in the presence and absence of cytochalasin. Because phosphorylation of sHSP/α-crystallins is dependent on MAPKAP kinase 2, it is possible that HSP27 is part of a pathway for signal transduction that ultimately regulates the organization of microfilaments (Kaukinen et al., 1996; Knauf et al., 1994).

MOLECULAR CHAPERONES AND INTERMEDIATE FILAMENTS

Members of the HSP70 (Liao et al., 1995), HSP90 (Fostinis et al., 1992; Czar et al., 1996) and sHSP/α-crystallin (Leicht et al., 1986; Nicholl and Quinlan, 1994; Quinlan et al., 1996) families interact with intermediate filaments. HSP70 immunoprecipitates with keratins 8 and 18 (K8/18), a pair of intermediate filament proteins preferentially synthesized in glandular epithelia. The K8/18-HSP70 complex is disrupted in a Mg-ATP-dependent manner and reconstituted in vitro using purified HSP70 and K8/18. The association of HSP70 is stronger with soluble than with polymerized K8/18 and this property increases with heat stress, but the protein has little effect on intermediate filament protein assembly in vitro. The nature of the relationship between HSP70 and K8/18 remains uncertain (Liao et al., 1995).

The in vitro polymerization of glial fibrillary acidic protein (GFAP) and vimentin is repressed in an ATP-independent fashion by α-crystallin (Nicholl and Quinlan, 1994), a major lens structural protein in vertebrate eye. α-Crystallin is expressed in non-lenticular tissues as well where it functions as a sHSP (Arrigo and Landry, 1994; Horwitz, 1992). Inhibition of intermediate filament protein assembly is unrelated to α-crystallin phosphorylation and the α-crystallin polypeptides αA1, αA2, αB1 and αB2 are equally effective in this activity. Nonfilamentous vimentin in extracts of lens precipitates with α-crystallin upon reaction with anti-vimentin antibodies, and the nonassembled pool of GFAP is increased by adding α-crystallin to preformed filaments, supporting the idea that α-crystallin binds preferentially to soluble forms of both intermediate filament proteins. A small portion of α-crystallin localizes to intermediate filaments, and examination of negatively stained samples with the electron microscope shows a regular pattern, about one particle per GFAP molecule. In one unusual case, α-crystallin coats intermediate filaments composed of CP49 and filensin forming the beaded filament, a structure unique to the vertebrate lens (Quinlan et al., 1996). There are diverse reasons for the interaction of α-crystallins with intermediate filament proteins (Nicholl and Quinlan, 1994). One attractive hypothesis is that α-crystallin selectively removes intermediate filaments at transition stages during cell differentiation through binding preferentially to their soluble precursor proteins. Because there are several major types of intermediate filament proteins it may be necessary to eliminate a pre-

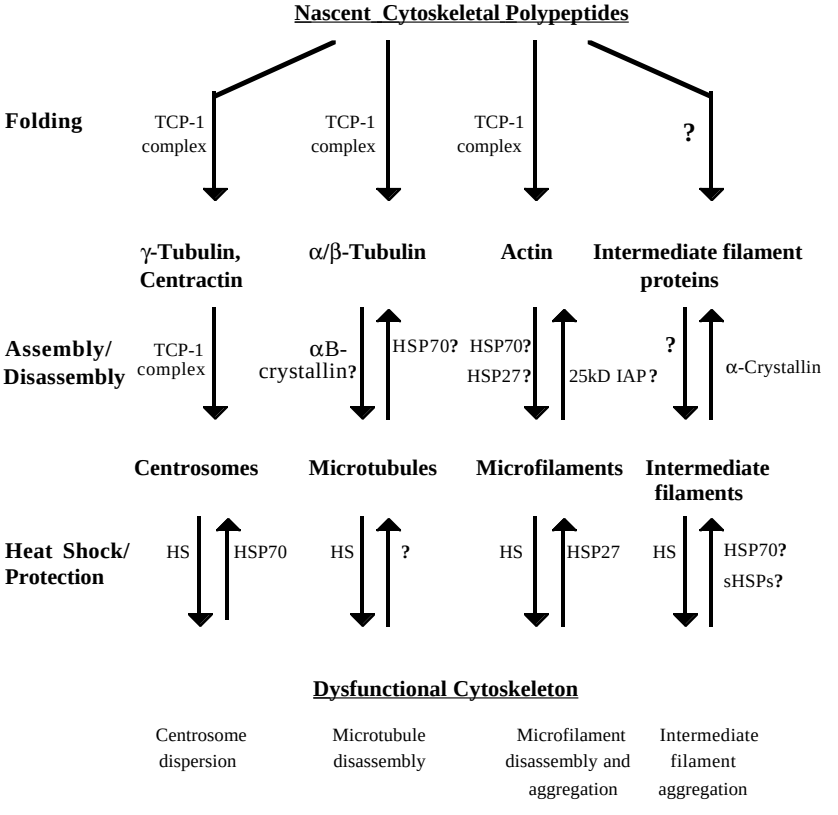

Fig. 1. Schematic representation of the relationship between molecular chaperones and the cytoskeleton during protein folding, assembly/disassembly and heat shock. When a molecular chaperone is known to affect a process, it is noted beside the arrow(s) representing the process. A question mark beside the name of a protein means that the proposed function is tentative. A question mark in the absence of a protein designation means that no chaperone has been shown to affect the process indicated by the arrow(s). Heat shock, HS.

viously synthesized protein which in the new phenotype is detrimental. Support for this proposal arises from the observation that desmin, an intermediate filament protein not usually found in lenses, upsets normal function of this tissue and leads to cataract formation (Bloemendal, 1991). In cardiac muscle, on the other hand, α-crystallin may organize desmin at the Z-band as part of its normal function (Ganote and Armstrong, 1993).

Heat shock causes the spatial arrangement of intermediate filaments to change from a fine thread-like network to a large, perinuclear aggregate (Weatherbee et al., 1980; Falkner et al., 1981; Parsell and Lindquist, 1994). In thermotolerant cells, integrity of the intermediate filament network is enhanced, and maintenance of this organization is required for establishment of increased heat resistance (Lee and Lai, 1995). For example, when 9L brain tumor cells are treated before heat-shock with withangulatin A (WA), a drug that disrupts vimentin intermediate filaments, they do not acquire thermotolerance, while those treated after receiving a heat shock gain protection. The vimentin intermediate filaments in cells exposed to WA prior to heating are arranged in tight perinuclear aggregates unable to return to their normal distribution upon recovery. In contrast, intermediate filaments in cells treated with WA subsequent to thermal stress resume a dispersed organization. HSC70 co-localizes with vimentin during these spatial changes but whether it has a role in stabilization of intermediate filaments remains unsettled (Lee and Lai, 1995). In heat shocked *Drosophila*, on the other hand, sHSPs (HSP28, 26, 23, and 22) have the same distribution as vimentin-like intermediate filament proteins in Kc cells and salivary glands (Leicht et al., 1986). Although no purpose has been proposed, the sHSPs may, as for microfilaments, protect intermediate filaments upon exposure to elevated temperatures.

CONCLUSIONS

Interpretation of data related to the role of molecular chaperones in the formation and protection of cytoskeletal elements can be difficult (Bukau et al., 1996; Rutherford and Zucker, 1994). Within cells, the protein synthesis and folding take place concurrently at the ribosome surface. Both processes are rapid, they occur in a protein-rich mileau and they are subject to many different influences. As a case in point, it is not always possible to differentiate in vivo the individual effects of molecular chaperones that act cooperatively and/or sequentially on nascent proteins. The problem is partially overcome by analysis of cells with mutated chaperones, but this approach is limited to those organisms for which genetic manipulation is possible. Studies in vitro, while allowing defined conditions, suffer from limitations imposed by an artificial environment. Protein concentration and diversity in vitro are often much lower than in the cytoplasm and only one of many cooperative reactions influencing protein folding may be under study. Thus, a molecular chaperone might seem unrelated to a particular cytoskeletal component only because a cochaperone is missing. Conversely, molecular chaperones tend to recognize hydrophobic regions exposed to the aqueous environment, a generalized mechanism providing ample opportunity for false interactions. This concern is especially pertinent in an in vitro situation, where relatively high levels of a limited number of proteins dissolved in low salt nonphysiological buffers may drive inappropriate reactions. Artifactual protein-protein associations are also encountered when searching cells for MAPs (MacRae, 1992b), an example which provides a strong cautionary message to the study of chaperone-substrate interactions.

In spite of the problems associated with their study available evidence proves that molecular chaperones assist in the formation of cytoskeletal proteins, while influencing the organization and function of microtubules, microfilaments and intermediate filaments (Fig. 1) For example, the TCP-1 complex folds α-, β-, and γ-tubulin, actin, and centractin; additionally, it affects microtubule nucleation by the centrosome. Members of the HSP70 family facilitate repair of the centrosome and perhaps intermediate filaments during heat shock, and they bind to microtubules, but the consequence of this latter relationship is unresolved. The sHSP/α-crystallins interact with microfilaments and intermediate filaments, regulate their dynamics and protect microfilaments from heat, while HSP90 associates with microtubules and possibly intermediate filaments. Clearly, molecular chaperones perform different but cooperating roles in the regulation of cytoskeleton function, but many questions concerning the interplay of molecular chaperones and the cytoskeleton remain. Clarification of these issues requires a better understanding of both molecular chaperones and the cytoskeletal elements, as well as continued study of the interaction between these two groups of proteins. Such work is ongoing in several laboratories and these efforts are certain to yield interesting results.

Financial support for this work was provided by a Natural Sciences and Engineering Research Council of Canada Grant to T.H.M. and an Izaak Walton Killam Memorial Scholarship to P.L.

REFERENCES

Ahmad, S., Ahuja, R., Venner, T. J. and Gupta, R. S. (1990). Identification of a protein altered in mutants resistant to microtubule inhibitors as a member of the major heat shock protein (hsp70) family. *Mol. Cell. Biol*. **10**, 5160-5165.

Amos, L. A. (1995). The microtubule lattice – 20 years on. *Trends Cell Biol*. **5**, 48-51.

Archer, J. E., Vega, L. R. and Solomon, F. (1995). Rb12p, a yeast protein that binds to β-tubulin and participates in microtubule function in vivo. *Cell* **82**, 425-434.

Archer, J. and Solomon, F. (1994). Deconstructing the microtubule-organizing center. *Cell* **76**, 589-591.

Arrigo, A. P. and Landry, J. (1994). Expression and function of the low-molecular-weight heat shock proteins. In *The Biology of Heat Shock Proteins and Molecular Chaperones* (ed. R. I. Morimoto, A. Tissières, and C. Georgopoulos), pp. 335-374. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Atomi, Y. and Arai, H. (1996). α-B Crystallin associates with MT/tubulin in L6 cells and has chaperone activity for MT/tubulin. *Mol. Biol. Cell* **7**, 46a.

Benndorf, R., Hayeß, K., Ryazantsev, S., Wieske, M., Behlke, J. and Lutsch, G. (1994). Phosphorylation and supramolecular organization of murine small heat shock protein HSP25 abolish its actin polymerization-inhibiting activity. *J. Biol. Chem.* **269**, 20780-20784.

Bloch, M. A. and Johnson, K. A. (1995). Identification of a molecular chaperone in the eukaryotic flagellum and its localization to the site of microtubule assembly. *J. Cell Sci.* **108**, 3541-3545.

Bloemendal, H. (1991). Disorganization of membranes and abnormal intermediate filament assembly lead to cataract. *Invest. Ophthalmol. Vis. Sci.* **32**, 445-455.

Bose, S., Weikl, T., Bügl, H. and Buchner, J. (1996). Chaperone function of Hsp90-associated proteins. *Science* **274**, 1715-1717.

Bretscher, A. (1991). Microfilament structure and function in the cortical cytoskeleton. *Annu. Rev. Cell Biol*. **7**, 337-374.

Brown, C. R., Doxsey, S. J., Hong-Brown, L. Q., Martin, R. L. and Welch, W. J. (1996a). Molecular chaperones and the centrosome. A role for TCP-1 in microtubule nucleation. *J. Biol. Chem.* **271**, 824-832.

Brown, C. R., Hong-Brown, L. Q., Doxsey, S. J. and Welch, W. J. (1996b). Molecular chaperones and the centrosome. A role for HSP 73 in centrosomal repair following heat shock treatment. *J. Biol. Chem.* **271**, 833-840.

Bukau, B., Hesterkamp, T. and Luirink, J. (1996) Growing up in a dangerous environment: a network of multiple targeting and folding pathways for nascent polypeptides in the cytosol. *Trends Cell Biol.* **6**, 480-486.

Burns, R. G. and Surridge, C. D. (1994). Functional role of a consensus peptide which is common to α-, β-, and γ-tubulin, to actin and centractin, to phytochrome A, and to the TCP1 chaperonin protein. *FEBS Lett.* **347**, 105-111.

Carden, M. J. and Roobol, A. (1995). Neuronal aspects of cytosolic chaperonin complexes: structures implicated in the production of functional cytoskeletal proteins. *Biochem. Soc. Trans.* **23**, 70-76.

Carlier, M. -F. (1991). Actin: protein structure and filament dynamics. *J. Biol. Chem.* **266**, 1-4.

Chapin, S. J. and Bulinski, J. C. (1992). Microtubule stabilization by assembly-promoting microtubule-associated proteins: a repeat performance. *Cell Motil. Cytoskel.* **23**, 236-243.

Chen, X., Sullivan, D. S. and Huffaker, T. C. (1994). Two yeast genes with similarity to TCP-1 are required for microtubule and actin function in vivo. *Proc. Nat. Acad. Sci. USA* **91**, 9111-9115.

Coss, R. A., Dewey, W. C. and Bamburg, J. R. (1982). Effects of hyperthermia on dividing Chinese hamster ovary cells and on microtubules in vitro. *Cancer Res.* **42**, 1059-1071.

Craig, E. A., Weissman, J. S. and Horwich, A. L. (1994). Heat shock proteins and molecular chaperones: mediators of protein conformation and turnover in the cell. *Cell* **78**, 365-372.

Creutz, C. E., Liou, A., Snyder, S. L., Brownawell, A. and Willison, K. (1994). Identification of the major chromaffin granule-binding protein, chromobindin A, as the cytosolic chaperonin CCT (chaperonin containing TCP-1). *J. Biol. Chem.* **269**, 32035-32038.

Czar, M. J., Welsh, M. J. and Pratt, W. B. (1996). Immunofluorescence localization of the 90-kDa heat-shock protein to cytoskeleton. *Eur. J. Cell Biol.* **70**, 322-330.

de Jong, W. W., Leunissen, J. A. M. and Voorter, C. E. M. (1993). Evolution of the α-crystallin/small-heat shock protein family. *Mol. Biol. Evol.* **10**, 103-126.

Dobrzynski, J. K., Sternlicht, M. L., Farr, G. W. and Sternlicht, H. (1996). Newly-synthesized β-tubulin demonstrates domain-specific interactions with the cytosolic chaperonin. *Biochemistry* **35**, 15870-15882.

Ellis, R. J. (1987). Proteins as molecular chaperones. *Nature* **328**, 378-379.

Ellis, R. J. and van der Vies, S. M. (1991). Molecular chaperones. *Annu. Rev. Biochem.* **60**, 321-347.

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

Fostinis, Y., Theodoropoulos, P. A., Gravanis, A. and Stournaras, C. (1992). Heat shock protein HSP90 and its association with the cytoskeleton: a morphological study. *Biochem. Cell Biol.* **70**, 779-786.

Freeman, B. C., Toft, D. O. and Morimoto, R. I. (1996). Molecular chaperone machines: chaperone activities of the cyclophilin Cyp-40 and the steroid aporeceptor-associated protein p23. *Science* **274**, 1718-1720.

Frydman, J., Nimmesgern, E., Erdjument-Bromage, H., Wall, J. S., Tempst, P. and Hartl, F. -U. (1992). Function in protein folding of TRiC, a cytosolic ring complex containing TCP-1 and structurally related subunits. *EMBO J.* **11**, 4767-4778.

Frydman, J. and Hartl, F. U. (1996). Principles of chaperone-assisted protein folding: differences between in vitro and in vivo mechanisms. *Science* **272**, 1497-1502.

Fuchs, E. and Weber, K. (1994). Intermediate filaments: structure, dynamics, function, and disease. *Annu. Rev. Biochem.* **63**, 345-382.

Ganote, C. and Armstrong, S. (1993). Ischaemia and the myocyte cytoskeleton: review and speculation. *Cardiovasc. Res.* **8**, 1387-1403.

Gao, Y., Thomas, J. O., Chow, R. L., Lee, G. -H. and Cowan, N. J. (1992). A cytoplasmic chaperonin that catalyzes β-actin folding. *Cell* **69**, 1043-1050.

Gao, Y., Vainberg, I. E., Chow, R. L. and Cowan, N. J. (1993). Two cofactors and cytoplasmic chaperonin are required for the folding of α- and β-tubulin. *Mol. Cell. Biol.* **13**, 2478-2485.

Georgopoulos, C. and Welch, W. J. (1993). Role of the major heat shock proteins as molecular chaperones. *Annu. Rev. Cell Biol.* **9**, 601-634.

Glass, J. R., DeWitt, R. G. and Cress, A. E. (1985). Rapid loss of stress fibers in Chinese hamster ovary cell after hyperthermia. *Cancer Res.* **45**, 258-262.

Green, L. A. D. and Liem, R. K. H. (1989). β-Internexin is a microtubule-associated protein identical to the 70-kDa heat-shock cognate protein and the clathrin uncoating ATPase. *J. Biol. Chem.* **264**, 15210-15215.

Gupta, R. S. (1990). Sequence and structural homology between a mouse t-complex protein TCP-1 and the 'chaperonin' family of bacterial (GroEL, 60-65 kDa heat shock antigen) and eukaryotic proteins. *Biochem. Int.* **20**, 833-841.

Gupta, R. S. (1995). Evolution of the chaperonin families (Hsp60, Hsp10 and Tcp-1) of proteins and the origin of eukaryotic cells. *Mol. Microbiol.* **15**, 1-11.

Hartl, F. U. and Martin, J. (1992). Protein folding in the cell: The role of molecular chaperones hsp70 and hsp60. *Annu. Rev. Biophys. Biomol. Struct.* **21**, 293-322.

Hendrick, J. P. and Hartl, F. -U. (1993). Molecular chaperone functions of heat-shock proteins. *Annu. Rev. Biochem.* **62**, 349-384.

Hendrick, J. P. and Hartl, F. -U. (1995). The role of molecular chaperones in protein folding. *FASEB J.* **9**, 1559-1569.

Hirokawa, N. (1994). Microtubule organization and dynamics dependent on microtubule-associated proteins. *Curr. Opin. Cell Biol.* **6**, 74-81.

Horwich, A. L. (1995). Resurrection or destruction? *Curr. Biol.* **5**, 455-458.

Horwitz, J. (1992). α-Crystallin can function as a molecular chaperone. *Proc. Nat. Acad. Sci. USA* **89**, 10449-10453.

Huot, J., Houle, F., Spitz, D. R. and Landry, J. (1996). HSP27 phosphorylation-mediated resistance against actin fragmentation and cell death induced by oxidative stress. *Cancer Res.* **56**, 273-279.

Iida, K., Iida, H. and Yahara, I. (1986). Heat shock induction of intranuclear actin rods in cultured mammalian cells. *Exp. Cell Res.* **165**, 207-215.

James, P., Pfund, C. and Craig, E. A. (1997). Functional specificity among Hsp70 molecular chaperones. *Science* **275**, 387-389.

Joshi, H. C. (1994). Microtubule organizing centers and γ-tubulin. *Curr. Opin. Cell Biol.* **6**, 55-62.

Joshi, H. C. and Palevitz, B. A. (1996). γ-Tubulin and microtubule organization in plants. *Trends Cell Biol.* **6**, 41-44.

Kabakov, A. E. and Gabai, V. L. (1994). Heat-shock proteins maintain the viability of ATP-deprived cells: what is the mechanism? *Trends Cell Biol.* **4**, 193-196.

Karasev, A. V., Kashina, A. S., Gelfand, V. I. and Dolja, V. V. (1992). HSP70-related 65 kDa protein of beet yellows closterovirus is a microtubule-binding protein. *FEBS Lett.* **304**, 12-14.

Kato, K., Ito, H., Inaguma, Y., Okamoto, K. and Saga, S. (1996). Synthesis and accumulation of αB crystallin in C6 glioma cells is induced by agents that promote the disassembly of microtubules. *J. Biol. Chem.* **271**, 26989-26994.

Kaukinen, K. H., Tranbarger, T. J. and Misra, S. (1996). Post-germination-induced and hormonally dependent expression of low-molecular-weight heat shock protein genes in Douglas fir. *Plant Mol. Biol.* **30**, 1115-1128.

Kim, S., Willison, K. R. and Horwich, A. L. (1994). Cytosolic chaperonin subunits have a conserved ATPase domain but diverged polypeptide-binding domains. *Trends Biochem. Sci.* **19**, 543-548.

Kimura, Y., Yahara, I. and Lindquist, S. (1995). Role of the protein chaperone YDJ1 in establishing Hsp90-mediated signal transduction pathways. *Science* **268**, 1362-1365.

Kirby, B. A., Merril, C. R., Ghanbari, H. and Wallace, W. C. (1994). Heat shock proteins protect against stress-related phosphorylation of tau in neuronal PC12 cells that have acquired thermotolerance. *J. Neurosci.* **14**, 5687-5693.

Knauf, U., Jakob, U., Engel, K., Buchner, J. and Gaestel, M. (1994). Stress- and mitogen-induced phosphorylation of the small heat shock protein Hsp25 by MAPKAP kinase 2 is not essential for chaperone properties and cellular thermoresistance. *EMBO J.* **13**, 54-60.

Knox, J. D., Mitchel, R. E. J. and Brown, D. L. (1991). Effects of hyperthermia on microtubule organization and cytolytic activity of murine cytotoxic T lymphocytes. *Exp. Cell Res.* **194**, 275-283.

Koyasu, S., Nishida, E., Kadowaki, T., Matsuzaki, F., Iida, K., Harada, F., Kasuga, M., Saiki, H. and Yahara, I. (1986). Two mammalian heat shock proteins, HSP90 and HSP100, are actin-binding proteins. *Proc. Nat. Acad. Sci. USA* **83**, 8054-8058.

Kubota, H., Hynes, G., Carne, A., Ashworth, A. and Willison, K. (1994). Identification of six Tcp-1-related genes encoding divergent subunits of the TCP-1-containing chaperonin. *Curr. Biol.* **4**, 89-99.

Lam, W. Y., Tsui, S. K. W., Law, P. T. W., Luk, S. C. W., Fung, K. P., Lee, C. Y. and Waye, M. M. Y. (1996). Isolation and characterization of a human

heart cDNA encoding a new member of the small heat shock protein family-HSPL27. *Biochim. Biophys. Acta* **1314**, 120-124.

Landry, J., Chrétien, P., Lambert, H., Hickey, E. and Weber, L. A. (1989). Heat shock resistance conferred by expression of the human HSP27 gene in rodent cells. *J. Cell Biol.* **109**, 7-15.

Lavoie, J. N., Hickey, E., Weber, L. A. and Landry, J. (1993a). Modulation of actin microfilament dynamics and fluid phase pinocytosis by phosphorylation of heat shock protein 27. *J. Biol. Chem.* **268**, 24210-24214.

Lavoie, J. N., Gingras-Breton, G., Tanguay, R. M. and Landry, J. (1993b). Induction of Chinese hamster HSP27 gene expression in mouse cells confers resistance to heat shock. Hsp27 stabilization of the microfilament organization. *J. Biol. Chem.* **268**, 3420-3429.

Lavoie, J. N., Lambert, H., Hickey, E., Weber, L. A. and Landry, J. (1995). Modulation of cellular thermoresistance and actin filament stability accompanies phosphorylation-induced changes in the oligomeric structure of heat shock protein 27. *Mol. Cell. Biol.* **15**, 505-516.

Lee, Y. -C. and Lai, Y. -K. (1995). Integrity of intermediate filaments is associated with the development of acquired thermotolerance in 9L rat brain tumor cells. *J. Cell. Biochem.* **57**, 150-162.

Leicht, B. G., Biessmann, H., Palter, K. B. and Bonner, J. J. (1986). Small heat shock proteins of *Drosophila* associate with the cytoskeleton. *Proc. Nat. Acad. Sci. USA* **83**, 90-94.

Lewis, S. A., Tian, G., Vainberg, I. E. and Cowan, N. J. (1996). Chaperone-mediated folding of actin and tubulin. *J. Cell Biol.* **132**, 1-4.

Lewis, V. A., Hynes, G. M., Zheng, D., Saibil, H. and Willison, K. (1992). T-complex polypeptide-1 is a subunit of a heteromeric particle in the eukaryotic cytosol. *Nature* **358**, 249-252.

Liang, P., Amons, R., Clegg, J. S. and MacRae, T. H. (1997). Purification, structure and molecular chaperone activity in vitro of *Artemia* p26, a small heat shock/α-crystallin protein. *Eur. J. Biochem.* **243**, 225-232.

Liao, J., Lowthert, L. A., Ghori, N. and Omary, M. B. (1995). The 70-kDa heat shock proteins associate with glandular intermediate filaments in an ATP-dependent manner. *J. Biol. Chem.* **270**, 915-922.

Llosa, M., Aloria, K., Campo, R., Padilla, R., Avila, J., Sánchez-Pulido, L. and Zabala, J. C. (1996). The β-tubulin monomer release factor (p14) has homology with a region of the DnaJ protein. *FEBS Lett.* **397**, 283-289.

Loktionova, S. A., Ilyinskaya, O. P., Gabai, V. L. and Kabakov, A. E. (1996). Distinct effects of heat shock and ATP depletion on distribution and isoform patterns of human Hsp27 in endothelial cells. *FEBS Lett.* **392**, 100-104.

Ludueña, R. F., Banerjee, A. and Khan, I. A. (1992). Tubulin structure and biochemistry. *Curr. Opin. Cell Biol.* **4**, 53-57.

Macejak, D. G. and Luftig, R. B. (1991). Stabilization of actin filaments at early times after adenovirus infection and in heat-shocked cells. *Virus Res.* **19**, 31-46.

MacRae, T. H. and Langdon, C. M. (1989). Tubulin synthesis, structure, and function: what are the relationships? *Biochem. Cell Biol.* **67**, 770-790.

MacRae, T. H. (1992a). Towards an understanding of microtubule function and cell organization: an overview. *Biochem. Cell Biol.* **70**, 835-841.

MacRae, T. H. (1992b). Microtubule organization by cross-linking and bundling proteins. *Biochim. Biophys. Acta* **1160**, 145-155.

Marschall, L. G., Jeng, R. L., Mulholland, J. and Stearns, T. (1996). Analysis of Tub4p, a yeast γ-tubulin-like protein: implications for microtubule-organizing center function. *J. Cell Biol.* **134**, 443-454.

Martin, J., Horwich, A. L. and Hartl, F. -U. (1992). Prevention of protein denaturation under heat stress by the chaperonin Hsp60. *Science* **258**, 995-998.

Martin, J. and Hartl, F. -U. (1994). Molecular chaperones in cellular protein folding. *BioEssays* **16**, 689-692.

Melki, R., Vainberg, I. E., Chow, R. L. and Cowan, N. J. (1993). Chaperonin-mediated folding of vertebrate actin-related protein and γ-tubulin. *J. Cell Biol.* **122**, 1301-1310.

Melki, R. and Cowan, N. J. (1994). Facilitated folding of actins and tubulins occurs via a nucleotide-dependent interaction between cytoplasmic chaperonin and distinctive folding intermediates. *Mol. Cell. Biol.* **14**, 2895-2904.

Melki, R., Rommelaere, H., Leguy, R., Vandekerckhove, J. and Ampe, C. (1996). Cofactor A is a molecular chaperone required for β-tubulin folding: functional and structural characterization. *Biochemistry* **35**, 10422-10435.

Miklos, D., Caplan, S., Mertens, D., Hynes, G., Pitluk, Z., Kashi, Y., Harrison-Lavoie, K., Stevenson, S., Brown, C., Barrell, B., Horwich, A. L. and Willison, K. (1994). Primary structure and function of a second essential member of the heterooligomeric TCP1 chaperonin complex of yeast, TCP1β. *Proc. Nat. Acad. Sci. USA* **91**, 2743-2747.

Minowada, G. and Welch, W. (1995). Variation in the expression and/or phosphorylation of the human low molecular weight stress protein during in vitro cell differentiation. *J. Biol. Chem.* **270**, 7047-7054.

Miron, T., Wilchek, M. and Geiger, B. (1988). Characterization of an inhibitor of actin polymerization in vinculin-rich fraction of turkey gizzard smooth muscle. *Eur. J. Biochem.* **178**, 543-553.

Miron, T., Vancompernolle, K., Vandekerckhove, J., Wilchek, M. and Geiger, B. (1991). A 25-kD inhibitor of actin polymerization is a low molecular mass heat shock protein. *J. Cell Biol.* **114**, 255-261.

Mitchison, T. J. (1992). Compare and contrast actin filaments and microtubules. *Mol. Biol. Cell* **3**, 1309-1315.

Morimoto, R. I., Tissières, A. and Georgopoulos, C., editors (1991). *Stress Proteins in Biology and Medicine.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Morimoto, R. I., Tissières, A. and Georgopoulos, C., editors (1994). *The Biology of Heat Shock Proteins and Molecular Chaperones.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Moudjou, M., Bordes, N., Paintrand, M. and Bornens, M. (1996). γ-Tubulin in mammalian cells: the centrosomal and the cytosolic forms. *J. Cell Sci.* **109**, 875-887.

Muchowski, P. J., Bassuk, J. A., Lubsen, N. H. and Clark, J. I. (1997). Human αB-crystallin. Small heat shock protein and molecular chaperone. *J. Biol. Chem.* **272**, 2578-2582.

Nicholl, I. D. and Quinlan, R. A. (1994). Chaperone activity of α-crystallins modulates intermediate filament assembly. *EMBO J.* **13**, 945-953.

Parsell, D. A. and Lindquist, S. (1994). Heat shock proteins and stress tolerance. In *The Biology of Heat Shock Proteins and Molecular Chaperones.* (ed. R. I. Morimoto, A. Tissières, and C. Georgopoulos), pp. 457-494. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Pauli, D., Arrigo, A. -P., Vazquez, J., Tonka, C. -H. and Tissières, A. (1989). Expression of the small heat shock genes during *Drosophila* development: comparison of the accumulation of hsp23 and hsp27 mRNAs and polypeptides. *Genome* **31**, 671-676.

Perret, E., Moudjou, M., Geraud, M. L., Derancourt, J., Soyer-Gobillard, M. -O. and Bornens, M. (1995). Identification of an HSP70-related protein associated with the centrosome from dinoflagellates to human cells. *J. Cell Sci.* **108**, 711-725.

Pollard, T. D., Almo, S., Quirk, S., Vinson, V. and Lattman, E. E. (1994). Structure of actin binding proteins: insights about function at atomic resolution. *Annu. Rev. Cell Biol.* **10**, 207-249.

Quinlan, R. A., Carter, J. M., Sandilands, A. and Prescott, A. R. (1996). The beaded filament of the eye lens: an unexpected key to intermediate filament structure and function. *Trends Cell Biol.* **6**, 123-126.

Raff, J. W. (1996). Centrosomes and microtubules: wedded with a ring. *Trends Cell Biol.* **6**, 248-251.

Rahman, D. R. J., Bentley, N. J. and Tuite, M. F. (1995). The *Saccharomyces cerevisiae* small heat shock protein Hsp26 inhibits actin polymerization. *Biochem. Soc. Trans.* **23**, 77s.

Redmond, T., Sanchez, E. R., Bresnick, E. H., Schlesinger, M. J., Toft, D. O., Pratt, W. B. and Welsh, M. J. (1989). Immunofluorescence colocalization of the 90-kDa heat-shock protein and microtubules in interphase and mitotic mammalian cells. *Eur. J. Cell Biol.* **50**, 66-75.

Roobol, A., Holmes, F. E., Hayes, N. V. L., Baines, A. J. and Carden, M. J. (1995). Cytoplasmic chaperonin complexes enter neurites developing in vitro and differ in subunit composition within single cells. *J. Cell Sci.* **108**, 1477-1488.

Rubenstein, P. A. (1990). The functional importance of multiple actin isoforms. *BioEssays* **12**, 309-315.

Rutherford, S. L. and Zuker, C. S. (1994). Protein folding and the regulation of signaling pathways. *Cell* **79**, 1129-1132.

Sackett, D. L. (1995). Structure and function in the tubulin dimer and the role of the acidic carboxyl terminus. In *Proteins: Structure, Function and Engineering*, vol. 24 (ed. B. B. Biswas and S. Roy), pp. 255-302. Plenum Press, New York.

Sánchez, C., Padilla, R., Paciucci, R., Zabala, J. C. and Avila, J. (1994). Binding of heat-shock protein 70 (hsp70) to tubulin. *Arch. Biochem. Biophys.* **310**, 428-432.

Sanchez, E. R., Redmond, T., Scherrer, L. C., Bresnick, E. H., Welsh, M. J. and Pratt, W. B. (1988). Evidence that the 90-kilodalton heat shock protein is associated with tubulin-containing complexes in L cell cytosol and in intact PtK cells. *Mol. Endocrinol.* **2**, 756-760.

Schatten, G. (1994). The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. *Dev. Biol.* **165**, 299-335.

Schirmer, E. C., Glover, J. R., Singer, M. A. and Lindquist, S. (1996).

HSP100/Clp proteins: a common mechanism explains diverse functions. *Trends Biochem Sci*. **21**, 289-296.

Schlesinger, M. J. (1990). Heat shock proteins. *J. Biol. Chem.* **265**, 12111-12114.

Soares, H., Penque, D., Mouta, C. and Rodrigues-Pousada, C. (1994). A *Tetrahymena* orthologue of the mouse chaperonin subunit CCTγ and its coexpression with tubulin during cilia recovery. *J. Biol. Chem.* **269**, 29299-29307.

Sternlicht, H., Farr, G. W., Sternlicht, M. L., Driscoll, J. K., Willison, K. and Yaffe, M. B. (1993). The t-complex polypeptide 1 complex is a chaperonin for tubulin and actin in vivo. *Proc. Nat. Acad. Sci. USA* **90**, 9422-9426.

Stoldt, V., Rademacher, F., Kehren, V., Ernst, J. F., Pearce, D. A. and Sherman, F. (1996). The Cct eukaryotic chaperonin subunits of *Saccharomyces cerevisiae* and other yeasts. *Yeast* **12**, 523-529.

Tian, G., Vainberg, I. E., Tap, W. D., Lewis, S. A. and Cowan, N. J. (1995). Specificity in chaperonin-mediated protein folding. *Nature* **375**, 250-253.

Tian, G., Huang, Y., Rommelaere, H., Vandekerckhove, J., Ampe, C. and Cowan, N. J. (1996). Pathway leading to correctly folded β-tubulin. *Cell* **86**, 287-296.

Tissières, A., Mitchell, H. K. and Tracy, U. M. (1974). Protein synthesis in salivary glands of *Drosophila melanogaster*: relation to chromosome puffs. *J. Mol. Biol*. **84**, 389-398.

Trent, J. D., Nimmesgern, E., Wall, J. S., Hartl, F. -U. and Horwich, A. L. (1991). A molecular chaperone from a thermophilic archaebacterium is related to the eukaryotic protein t-complex polypeptide-1. *Nature* **354**, 490-493.

Ursic, D. and Culbertson, M. R. (1991). The yeast homolog to mouse *Tcp-1* affects microtubule-mediated processes. *Mol. Cell. Biol.* **11**, 2629-2640.

Ursic, D., Sedbrook, J. C., Himmel, K. L. and Culbertson, M. R. (1994). The essential yeast Tcp1 protein affects actin and microtubules. *Mol. Biol. Cell* **5**, 1065-1080.

Vallee, R. B. (1990). Molecular characterization of high molecular weight microtubule-associated proteins: some answers, many questions. *Cell Motil. Cytoskel.* **15**, 204-209.

Vidair, C. A., Doxsey, S. J. and Dewey, W. C. (1993). Heat shock alters centrosome organization leading to mitotic dysfunction and cell death. *J. Cell. Physiol.* **154**, 443-455.

Vinh, D. B. -N. and Drubin, D. G. (1994). A yeast TCP-1-like protein is required for actin function *in vivo*. *Proc. Nat. Acad. Sci. USA* **91**, 9116-9120.

Wallace, W., Johnson, G., Sugar, J., Merril, C. R. and Refolo, L. M. (1993). Reversible phosphorylation of tau to form A68 in heat-shocked neuronal PC12 cells. *Mol. Brain Res.* **19**, 149-155.

Wang, C., Asai, D. J. and Lazarides, E. (1980). The 68, 000-dalton neurofilament-associated polypeptide is a component of noneuronal cells and of skeletal myofibrils. *Proc. Nat. Acad. Sci. USA* **77**, 1541-1545.

Wang, K. and Spector, A. (1996). α-crystallin stabilizes actin filaments and prevents cytochalasin-induced depolymerization in a phosphorylation-dependent manner. *Eur. J. Biochem.* **242**, 56-66.

Weatherbee, J. A., Luftig, R. B. and Weihing, R. R. (1980). Purification and reconstitution of HeLa cell microtubules. *Biochemistry* **19**, 4116-4123.

Weeds, A. and Maciver, S. (1993). F-actin capping proteins. *Curr. Opin. Cell Biol.* **5**, 63-69.

Welch, W. J. and Suhan, J. P. (1985). Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. *J. Cell Biol.* **101**, 1198-1211.

Weller, N. K. (1988). A 70 kDa microtubule-associated protein in NIL8 cells comigrates with the 70 kDa heat shock protein. *Biol. Cell* **63**, 307-317.

Willison K. R. and Kubota, H. (1994). The structure, function, and genetics of the chaperonin containing TCP-1 (CCT) in eukaryotic cytosol. In *The Biology of Heat Shock Proteins and Molecular Chaperones* (ed. R. I. Morimoto, A. Tissières, and C. Georgopoulos), pp. 299-312. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Yaffe, M. B., Farr, G. W., Miklos, D., Horwich, A. L., Sternlicht, M. L. and Sternlicht, H. (1992). TCP1 complex is a molecular chaperone in tubulin biogenesis. *Nature* **358**, 245-248.

Zabala, J. C., Fontalba, A. and Avila, J. (1996). Tubulin folding is altered by mutations in a putative GTP binding motif. *J. Cell Sci.* **109**, 1471-1478.