**HSP27 Multimerization Mediated by Phosphorylation-sensitive Intermolecular Interactions at the Amino Terminus**

(Received for publication, October 15, 1998, and in revised form, January 8, 1999)

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Distinct biochemical activities have been reported for small and large molecular complexes of heat shock protein 27 (HSP27), respectively. Using glycerol gradient ultracentrifugation and chemical cross-linking, we show here that Chinese hamster HSP27 is expressed in cells as homotypic multimers ranging from dimers up to 700-kDa oligomers. Treatments with arsenite, which induces phosphorylation on Ser15 and Ser90, provoked a major change in the size distribution of the complexes that shifted from oligomers to dimers. Ser90 phosphorylation was sufficient and necessary for causing this change in structure. Dimer formation was severely inhibited by replacing Ser90 with Ala90 but not by replacing Ser15 with Ala15. Using the yeast two-hybrid system, two domains were identified that were responsible for HSP27 intermolecular interactions. One domain was insensitive to phosphorylation and corresponded to the C-terminal α-crystallin domain. The other domain was sensitive to serine 90 phosphorylation and was located in the N-terminal region of the protein. Fusion of this N-terminal domain to firefly luciferase conferred luciferase with the capacity to form multimers that dissociated into monomers upon phosphorylation. A deletion within this domain of residues Arg2–Tyr23, which contains a WDPF motif found in most proteins of the small heat shock protein family, yielded a protein that forms only phosphorylation-insensitive dimers. We propose that HSP27 forms stable dimers through the α-crystallin domain. These dimers further multimerize through intermolecular interactions mediated by the phosphorylation-sensitive N-terminal domain.

Mammalian heat shock protein 27 (HSP27, also called HSP25) belongs to the phylogenically conserved small heat shock protein (smHSP) family that includes HSP25. Phosphorylation of HSP27 during stress or growth factor stimulation regulates actin polymerization and modulates filament stability or reorganization (3, 9, 13, 38). A second activity has been described for the oligomeric HSP27 complex. In *vitro*, high molecular weight recombinant HSP27 complexes can absorb heat-denatured proteins on their surface, preventing their aggregation and keeping them in a folding-competent state. The subsequent action of other chaperone proteins such as HSP70 leads to the renaturation of the unfolded proteins (39, 40). Although not yet directly demonstrated, the mechanism proposed for this activity as well as studies performed with other smHSP suggested that the chaperone activity is limited to the oligomeric complexes (39–41). Both the chaperone and actin modulation activities could explain the protective action of HSP27 in *vivo*. A first activity is restricted to monomeric HSP27. In solution, purified monomers behave as F-actin cap-binding proteins and inhibit actin polymerization (35–37). Only unphosphorylated HSP27 could block actin polymerization, hence providing a mechanism to explain the *in vivo* observations that phosphorylation of HSP27 during stress or growth factor stimulation regulates actin polymerization and modulates filament stability or reorganization (3, 9, 13, 38). A second activity has been described for the oligomeric HSP27 complex. *In vitro*, high molecular weight recombinant HSP27 complexes can absorb heat-denatured proteins on their surface, preventing their aggregation and keeping them in a folding-competent state. The subsequent action of other chaperone proteins such as HSP70 leads to the renaturation of the unfolded proteins (39, 40). Although not yet directly demonstrated, the mechanism proposed for this activity as well as studies performed with other smHSP suggested that the chaperone activity is limited to the oligomeric complexes (39–41). Both the chaperone and actin modulation activities could explain the protective action of HSP27 in *vivo*. However, specific chaperone functions regulating the activity or the stability of specific target proteins may also contribute to the homeostatic functions of HSP27. For example, oligomeric HSP27 binds to activated protein kinase B, a protein that lies in a survival pathway during stress (42–44). Monomers and dimers of HSP27 bind to granzyme A, a protease...
ase involved in granule-mediated cell lysis (45). Furthermore, a close relative of HSP27, MKBP, binds and modulates the activity of the myotonic dystrophy protein kinase (46). The role of HSP27 phosphorylation and multimeric state in modulating these interactions is unknown.

In the present study, we studied the relationships between the phosphorylation and the oligomerization properties of HSP27. Chinese hamster HSP27 (HaHSP27) is phosphorylated on two serine residues, Ser15 and Ser90. We show here that phosphorylation on Ser90 is sufficient and necessary to cause HSP27 to shift from a 700-kDa multimeric structure to dimers. Two homotypic binding domains were identified in HSP27. A first one, located within residues 95–186 of HaHSP27 (87–178 in HuHSP27), mediates dimerization and is insensitive to phosphorylation. A second one includes a small conserved stretch in the extreme amino terminus and is destabilized by phosphorylation of Ser90. We show that the N-terminal domain of HSP27 is sufficient to confer firefly luciferase with a phosphorylation-sensitive multimerization capacity.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—pSVHa27WT codes for wild type HaHSP27. It contains the HaHSP27 sequences from pH8 (8) inserted at the HindIII site of the vector pSV7. Other HaHSP27 constructs were made from a derivative of pSVHa27WT, pSVHa27Mu, in which a MuI restriction site was created by introducing a silent mutation in the third codon of HaHSP27. pSVHa27AA, pSVHa27EE, pSVHa27SA, pSVHa27AS, pSVHa27AE, and pSVHa27EA express phosphorylation site mutants of HaHSP27, the last two letters in the names corresponding to the replacement of amino acid residues at position 15 and 90, respectively. Mutations were introduced in pSVHa27Mu by polymerase chain reaction using specific synthetic oligonucleotide primers replacing the serine codon AGC by the alanine codon GCC or the glutamate codon GAA. pSVHa27 was prepared by deleting the fragment MuI–RrnI in pSVHa27Mu, yielding a protein lacking residues 5–23. pCMVHa27–109-Luc expresses firefly luciferase (produced in NIH3T3 cells transfected with pH8 (49)) at 30 °C, the reaction was stopped by adding one volume of 1 M TRIS-HCl containing 10% SDS and 10 mM EDTA. Aliquots were analyzed by electrophoresis on a 3–10% SDS-polyacrylamide gel. Cross-linked HaHSP27 species were detected by immunoblotting with antibody to HSP27.

**Cell Culture, Transfection, and Lysis**—Transfections were done using 103 103 HuHSP27 cells transfected with pH8 (~106) at 30 °C. The positions of the 20 S (700 kDa) and 15 S (340 kDa) proteasomes were determined by measuring luciferase activity. The position of p38/ stress-activated protein kinase 2 (38 kDa) was determined using an antibody against the C-terminal sequence PPLQEEMES of murine p38.

**Glutaraldehyde Cross-linking**—The cell lysates were mixed with one volume of 0–6.8% glutaraldehyde in water. After incubation for 30 min at 30 °C, the reaction was stopped by adding one volume of 1 M TRIS-HCl containing 10% SDS and 10 mM EDTA. Aliquots were analyzed by electrophoresis on a 3–10% SDS-polyacrylamide gel. Cross-linked HaHSP27 species were detected by immunoblotting with antibody to HSP27.

**Two-hybrid Screening and Analyses**—Two-hybrid assays were performed in the CLONTECH Matchmaker Library user manual. Full-length Chinese hamster or human HSP27 cDNAs were cloned in the pBTM116 (TRP1) vector to produce the bait fusion protein LexA-HaHSP27 or LexA-HuHSP27 (50). The LexA-HaHSP27 construct was used to screen a HeLa cell cDNA library constructed at the EcoRI-XhoI site of the GAL4 activation domain plasmid pGADGH (LEU2) (CLONTECH). The LexA-HuHSP27 protein was used to screen a human kidney cDNA library constructed at the EcoRI site of the GAL4 activation domain plasmid pGAD10 (LEU2) (CLONTECH). Screening was performed by sequential transformation of bait and library vectors in the Saccharomyces cerevisiae reporter strain L40 (MATa trpl1 leu2 his3 lys2::lexA-HIS3 ura3::lexA-lacZ (50, 51). Colonies that arose on Trp-/Leu-/His- selective plates were replica-plated, and one set was transferred to filter disks (Whatman Inc., Clifton, N.J.) by freezing and thawing, and a β-galactosidase assay was performed by incubation at 30 °C with 0.1 M NaPO4, 10 mM KC1, 1 mM MgSO4, 0.27% β-mercaptoethanol, 0.33 mg/ml 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside, pH 7.0. LEU2 plasmids were isolated from the blue colonies and restested by co-transfection with Ras(V12) or lamin C fused to LexA to eliminate false positive clones (52). Inserts from the plasmids were pGAD10 plasmids of the remaining true positive clones were sequenced to identify sequences of proteins belonging to members of the smHSP family (HaHSP27 and aA- and aB-crystallin).

Further two-hybrid assays were performed between pairs of GAL4 activation domain and LexA binding domain plasmids. GAL4 plasmids containing full-length HuHSP27, aA- and aB-crystallin cDNA, and N-terminal deletion mutants of HuHSP27 and aB-crystallin were obtained from the screenings described above. LexA plasmids were prepared by recloning the insert of the GAL4 plasmids into pBTM116. Additional GAL4 and LexA C-terminal deletion mutants of HuHSP27 and aB-crystallin were obtained after digesting their corresponding full-length cDNA with HindIII (yielding HuHSP27 (1–101) and aB-crystallin (1–77)) and recloning in pBTM116 and pGADGH. All DNA constructs were confirmed by DNA sequencing. Interactions were determined by co-transfecting the pBTM116 and pGADGH or pGAD10 constructs in pairs L40. Positive interactions were determined by the ability of the transformed yeast to grow on Trp-/Leu-/His- media and for their capacity to express β-galactosidase (blue colonies) within 5 h.

**RESULTS**

**Modulation of HSP27 Size Distribution by Phosphorylation**—CCL39 cell extracts were fractionated by ultracentrifugation on glyceral gradient, and each fraction was analyzed for the presence of HSP27 by immunoblotting. HSP27 sedimented as complexes of homogeneous sizes distributed between the top of the gradient and the peak corresponding to a molecular mass of about 700 kDa (Fig. 1A). To better assess the nature of the HSP27 complex, increasing concentrations of glutaraldehyde was added to the cell extracts, and the cross-linked products were analyzed by SDS-polyacrylamide gel electrophoresis and HSP27 immunoblotting (Fig. 1B). With increasing concentrations of glutaraldehyde, HSP27 was cross-linked progres-
sively in species showing a uniform ladder distribution of sizes with apparent molecular masses in multiples of 28 kDa (determined by linear regression analysis of the position of the cross-linked products). These data indicate that no other proteins were associated stoichiometrically with HSP27 and, thus, that in situ the HSP27 complex is mainly a homopolymer. Some 700-kDa species, dimers, and monomers resisted cross-linking even at the highest concentration of glutaraldehyde. At this concentration, glutaraldehyde started to produce a general cross-linking of all proteins as revealed from the fainting of all protein bands on the Coomassie-stained gel (data not shown). These properties agreed with the sedimentation profile data and suggested that HSP27 was expressed in cells as large polymers of about 700 kDa in equilibrium with smaller species, mainly monomers and dimers.

HaHSP27 is phosphorylated on Ser<sup>15</sup> and Ser<sup>90</sup> by MAPKAP kinase 2, a serine kinase activated by the stress sensitive SAPK2/p38 kinase (20, 22). To analyze changes in the size distribution of HSP27 upon phosphorylation, CCL39 cells were exposed to arsenite for 2 h before extraction. Such treatment induced almost complete phosphorylation of HSP27 (data not shown) and a dramatic change in the size distribution profile of HSP27. After treatment, essentially all HSP27 was recovered in the first few fractions at the top of the glycerol gradient, between stress-activated protein kinase 2 (38 kDa) and firefly luciferase (62 kDa), and most of it could not be cross-linked in larger species than dimers (Fig. 1).

To confirm that phosphorylation was directly involved in modulating the supramolecular organization of HSP27, phosphorylation mutants of HaHSP27 were prepared by replacing Ser<sup>15</sup> and Ser<sup>90</sup> with alanine to mimic nonphosphorylatable serine residues (HaHSP27-AA) or with glutamate to mimic constitutively phosphorylated residues (HaHSP27-EE). These constructs were expressed in NIH 3T3 cells, and their size distribution was investigated as above. NIH 3T3 cells were chosen as recipient cells because they express negligible amounts of endogenous HSP27. As expected, HaHSP27-AA had a sedimentation profile and cross-linking pattern similar to those obtained with wild type HSP27 in untreated cells (Fig. 2) but did not shift to smaller sizes upon treatments that induce phosphorylation (not shown). In contrast, HaHSP27-EE was found almost exclusively as dimers and monomers in both control (Fig. 2) and treated cells (not shown). The relative importance of each site of phosphorylation was investigated by expressing single site mutants. HaHSP27, in which serine 15 was changed for alanine (HaHSP27-AS), behaved as the wild type protein (Fig. 3, B versus A) but did not shift to smaller sizes upon stress (Fig. 3, B versus A). The data indicated that phosphorylation of serine 90 was sufficient and that phosphorylation of serine 15 was not required to cause the ultrastruc-
tural changes in HaHSP27. In contrast, HaHSP27 in which serine 90 was converted to alanine (HaHSP27-SA) had a normal size distribution in control cells and did not produce dimers or monomers after phosphorylation (Fig. 3C). This indicated that phosphorylation of serine 15 was not sufficient and that phosphorylation of serine 90 was necessary for the production of small molecular weight species. The results were confirmed by analyzing HaHSP27-EA and HaHSP27-AE double mutants. As expected, HaHSP27-EA was found mostly in large oligomeric complexes, whereas HaHSP27-AE was mostly found at the top of the gradient (Fig. 3D). We reproducibly observed that phosphorylated HaHSP27-SA and HaHSP27-EA yielded a peak slightly smaller than control HSP27. There is therefore a possibility that phosphorylation of serine 15 might affect the stability of the very high molecular weight species, causing a size shift in the 500–800-kDa range.

Domains Responsible for HSP27-HSP27 Interactions—Information on the nature of the intermolecular binding domains of HSP27 was obtained in a two-hybrid screen made using full-length HaHSP27 or HuHSP27 fused to the LexA DNA-binding domain as bait. The plasmid libraries of fusions between the activation domain of Gal4 and cDNA from either HeLa cell or human kidney were screened in a yeast reporter system. In the HeLa cell library, 111 out of 193 clones that were confirmed as real HSP27 interacting proteins were full-length or N-terminal deletants of HuHSP27. The others corresponded to unidentified gene products distinct from HSP27. In the kidney library, 55 out of 92 real interacting partners were identified as full or partial length clones of HuHSP27. All the others were derived from the homologous α-crystallin proteins (29 αB-crystallin and 8 αA-crystallin), confirming previous studies indicating that HSP27 can form heterologous complexes with α-crystallins (53). In the HeLa cell library, all HuHSP27 variants were deleted at the N terminus, and the shortest clone started at residue 87; in the kidney library, all N-terminal deletants started before residue 87, and the most severe C-terminal deletant ended at residue 178. All αA-crystallin clones were full-length; the shortest αB-crystallin clone was an N-terminal deletant starting at residue 74 (corresponding to residue 102 in HaHSP27 and 94 in HuHSP27). A C-terminal αB-crystallin deletant ended at 162 (192 in HaHSP27; 184 in HuHSP27).

This defined tentatively the HuHSP27 peptide containing residues 94–178 (corresponding to HaHSP27 residues 102–186) as a domain sufficient for HSP27-HSP27 interactions. Accordingly, we found that a C-terminal HuHSP27 peptide containing residues 87–205 gave a positive signal in the two-hybrid system when co-expressed with either full-length HuHSP27 or αA- or αB-crystallin. The HuHSP27 C-terminal peptide also interacted with itself and with a C-terminal peptide of αB-crystallin-(74–175) but not with the N-terminal residues 1–101 of HuHSP27 or 1–77 of αB-crystallin (Table I). This suggested the involvement of a C-terminal-C-terminal interaction in smHSP oligomerization.

To form multimeric structures, the C-terminal region of HSP27 must contain either a multivalent binding domain or two distinct monovalent binding domains. Another possibility is that a binding domain also exists in the N-terminal domain. This possibility was directly tested in the two-hybrid system. As shown in Table I, a peptide containing the first 101 residues of HuHSP27 did interact with full-length HuHSP27. However, no interaction was detected when the peptide was co-expressed with the C-terminal end. Similarly, an N-terminal peptide of αB-crystallin did interact with full-length HuHSP27 and with αA- and αB-crystallin but did not interact with the C-terminal peptide of either HuHSP27 or αB-crystallin. The results suggested that the N-terminal domain of smHSP also contains an intermolecular binding domain, probably mediating N-terminal-N-terminal interactions. It was not possible to test directly this interaction, since the fusion protein made of the N-terminal end of HuHSP27 or αB-crystallin and the transactivation domain yielded by itself a false positive signal.

All of these data are consistent with a model in which HSP27 would possess two homotypic interacting domains located in the N-terminal and C-terminal region, respectively. A prediction of this model is that deletion of either domain should produce a protein that forms smaller multimer. Although the N-terminal domain of smHSP is poorly conserved, a short conserved WDPF motif (see Fig. 6) is observed at the extreme N terminus of most smHSP (26). A plasmid containing coding sequences of HaHSP27 in which codons corresponding to residues Arg2-Tyr23 were deleted (Δ5–23HSP27) was expressed in NIH-3T3 cells and analyzed by chemical cross-linking and ul-
tracentrifugation. Δ5–23HSP27 sedimented as a discrete peak at the top of the gradient and could not be significantly cross-linked in species larger than dimers (Fig. 4). Phosphorylation had little influence on the sedimentation profile or the cross-linking properties of the protein, suggesting that the dimers formed though C-terminal interactions are insensitive to serine 90 phosphorylation.

A number of small deletions in the C-terminal domain were produced and tested for expression in NIH-3T3 cells. The constructs produced Triton-insoluble proteins that could not be analyzed further, suggesting that a major structural domain responsible for the stability or solubility of the protein is contained in these sequences. A fusion protein was therefore engineered containing the N-terminal sequences 5–109 of HaHSP27 (NH) fused to luciferase (LUC). Luciferase is a monomeric protein of about 62 kDa. NIH-LUC (theoretical molecular mass of 75 kDa) formed multimers of more than 350 kDa in NIH-3T3 cells. Upon treatment with arsenite, the luciferase activity was shifted to fractions corresponding to 75 kDa, consistent with a phosphorylation-induced dissociation of the multimers into monomers (Fig. 5). The results indicated that the N-terminal end contains the phosphorylation-sensitive binding domain of HSP27.

### DISCUSSION

On the basis of the primary structure of smHSP from different organisms including plant, yeast, bacteria and mammalian, the position of introns in their respective genes, and the localization of well conserved amino acid residues, it has been suggested that smHSP are composed of two distinct domains (see Refs. 25 and 26 and Fig. 6). The C-terminal domain, also called the \(\alpha\)-crystallin domain, is well conserved between the various species and constitutes the signature for proteins of this family. In contrast, the N-terminal domain is very weakly conserved except near the N terminus, where a similar hydrophobic motif is often observed. The rest of the N-terminal domain is highly variable both in length and in composition and probably forms at its end a connecting peptide to the C-terminal domain. Most smHSP end with a C-terminal tail of varying length. Alignments of mammalian HSP27 with other smHSP basally identify the two major domains. The C-terminal domain of HuHSP27 extends from residue 88 to 183 (from 96 to 191 in HaHSP27). In rodent HSP27, it has been determined by two-dimensional NMR that the last 18 residues of the C terminus form a highly flexible tail. The last 10 residues form a flexible peptide in \(\alpha\)-A- and \(\alpha\)-B-crystallins (54, 55). Human and rodent HSP27 are almost identical except for the presence of a shorter connecting peptide between the N- and C-terminal domains in the human protein. Both rodent and human HSP27 contain a site of phosphorylation located at serine 15 in the conserved hydrophobic motif. HuHSP27 has two sites of phosphorylation in the connecting peptide (serines 78 and 82). In rodents, the residue corresponding to serine 78 is replaced by an asparagine residue.
do not exclude the possibility that HSP27 can interact with other proteins in the cells, but they suggest that such an association is not stoichiometric. The most abundant form of HSP27 oligomers as revealed by ultracentrifugation has a molecular mass of about 700 kDa, which corresponds also to the size of the major cross-linked species obtained at high concentrations of glutaraldehyde. Some dimeric and monomeric species, however, appeared to resist cross-linking, and species with molecular mass lower than 700 kDa distributed along the glycerol gradient, suggesting that the structure might be highly dynamic, small species being in equilibrium with bigger ones. Such a high dynamic property has also been described for the structure of αA-crystallin, in which exchange of subunits between different oligomers occurs at high rates (56). Our results showed that upon phosphorylation of serine 90 but not of serine 15, most HSP27 complexes reduced also to dimers. Phosphorylation of serine 90 may shift the equilibrium for the formation of HSP27 complexes toward smaller species, perhaps by destabilizing the association of the dimers into higher molecular weight structures. Since mostly dimers were seen after phosphorylation, one could conclude that the dimer is the building block of the HSP27 complex. This conclusion is in line with that of Dudich et al. (57), who showed that the temperature dependence of the excessive heat capacity of the denaturation of HSP27 corresponds to a protein with a molecular mass of 50 kDa, suggesting that the dimer structure is a minimum cooperative subunit of the protein. The conclusion is also in agreement with the crystallographic data recently obtained for a distantly related bacterial smHSP, MJHS16.5 (58). The analysis revealed a 24-subunit hollow spherical complex made of three asymmetric units of eight subunits, in which the strongest contacts occur between dimers. Although many of the residues involved in subunit contact of MJHS16.5 are not conserved in HSP27, many of the structural features such as the basic symmetry may be conserved. A 24-mer of HSP27 would yield a size of 648 kDa, in close agreement with our estimated size of about 700 kDa.

Our data suggest that HSP27 oligomerizes through the interaction of two distinct binding domains. Both domains appear to act as independent binding modules that are required for oligomerization up to high molecular weight complexes. Analyses in the two-hybrid interaction assay system localized one of the binding domains between residues 94 and 178 of HuHSP27 (corresponding to HaHSP27 residues 102–186). This region corresponds almost exactly to the a-crystallin domain. It starts 6 residues after the position corresponding to intron I in a-crystallins and ends 7 residues before the beginning of the C-terminal flexible tail found in HSP27 (55). Although there is little known about the structure of HSP27, in several studies a role for this conserved domain as the building block of the quaternary structure of other members of this family has been suggested. In particular, the sequence extending between Tyr109 and Leu120 in αA-crystallin (Tyr135–Leu144 in HaHSP27; Tyr141–Leu152 in HaHSP27) was suggested to be the cooperative subunit of the protein. The conclusion is also in line with that of Dudich et al. (57), who showed that the temperature dependence of the excessive heat capacity of the denaturation of HSP27 corresponds to a protein with a molecular mass of 50 kDa, suggesting that the dimer structure is a minimum cooperative subunit of the protein. The conclusion is also in agreement with the crystallographic data recently obtained for a distantly related bacterial smHSP, MJHS16.5 (58). The analysis revealed a 24-subunit hollow spherical complex made of three asymmetric units of eight subunits, in which the strongest contacts occur between dimers. Although many of the residues involved in subunit contact of MJHS16.5 are not conserved in HSP27, many of the structural features such as the basic symmetry may be conserved. A 24-mer of HSP27 would yield a size of 648 kDa, in close agreement with our estimated size of about 700 kDa.
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REFERENCES

1. Arrigo, A. P., and Landry, J. (1994) in The Biology of Heat Shock Proteins and Molecular Chaperones (Morimoto, R. I., Tissières, A., and Georgopoulos, C., eds) pp. 335–373, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

2. Landry, J., Chretien, P., Lambert, H., Hickey, E., and Weber, L. A. (1989) J. Cell. Biol. 106, 7–15

3. Lavoie, J. N., Lambert, H., Hickey, E., Weber, L. A., and Landry, J. (1995) Mol. Cell. Biol. 15, 505–516

4. Knauf, U., Jakob, U., Engel, K., Buchner, J., and Gaestel, M. (1994) EMBO J. 13, 54–60

5. Mehlen, P., Preville, X., Charreyron, P., Brislay, J., Klemenz, R., and Arrigo, A. P. (1995) J. Immunol. 154, 363–374

6. Garrido, C., Oltav, P., Fromentin, A., Hamann, A., Arrigo, A. P., Chauvet, M., and Mehlen, P. (1995) J. Biol. Chem. 270, 2661–2667

7. Mehlen, P., Schulze-Osthoff, K., and Arrigo, A. P. (1996) J. Biol. Chem. 271, 16510–16514

8. Lavoie, J. N., Gingras-Breton, G., Tanguay, R. M., and Landry, J. (1993) J. Biol. Chem. 268, 3430–3429

9. Lavoie, J. N., Hickey, E., Weber, L. A., and Landry, J. (1995) J. Biol. Chem. 268, 24210–24214

10. Huot, J., Houle, F., Spitz, D. R., and Landry, J. (1996) Cancer Res. 56, 273–279

11. Huot, J., Houle, F., Marieau, F., and Landry, J. (1997) Circ. Res. 80, 383–392

12. Schaffer, C., Ross, E. B., Bragado, R. M., Grohewski, G. E., Ernst, S. A., and Williams, J. A. (1996) J. Biol. Chem. 271, 47210–47214

13. Piotrowicz, R. S., and Levin, E. G. (1997) J. Biol. Chem. 272, 25920–25927

14. Piotrowicz, R. S., Weber, L. A., Hickey, E., and Levin, E. G. (1995) FASEB J. 9, 1079–1084

15. Mehlen, P., Mehlen, A., Gotet, J., and Arrigo, A. P. (1997) J. Biol. Chem. 272, 31657–31665

16. Rousseau, S., Houle, F., Landry, J., and Huot, J. (1997) Oncogene 15, 1619–2177

17. Landry, J., Lambert, H., Zhou, M., Lavoie, J. N., Hickey, E., Weber, L. A., and Anderson, C. W. (1992) J. Biol. Chem. 267, 794–803

18. Gaestel, M., Schroder, W., Benndorf, B., Lippmann, C., Buchner, K., Hacho, F., Erdmann, V. A., and Bieilka, H. (1991) J. Biol. Chem. 266, 14721–14724

19. Guay, J., Lambert, H., Gingras-Breton, G., Lavoie, J. N., Huot, J., and Landry, J. (1997) J. Cell. Sci. 110, 357–368

20. Rouse, J., Cohen, P., Triguon, S., Morange, M., Alonso-Llamazares, A., Zamanillo, D., Huot, T., and Nechada, A. R. (1994) Cell 78, 1027–1037

21. Stokoe, D., Engel, K., Campbell, D. G., Cohen, P., and Gaestel, M. (1992) FEBS Lett. 313, 307–313

22. Huot, J., Lambert, H., Lavoie, J. N., Guimond, A., Houle, F., and Landry, J. (1995) Eur. J. Biochem. 237, 416–421

23. Landry, J., and Huot, J. (1995) Biochem. Cell. Biol. 73, 703–707

24. de Jong, W. W., Hendriks, W., Mulders, J. W., and Bloemendal, H. (1989) Trends Biochem. Sci. 14, 365–368

25. de Jong, W. W., Leunissen, J. A., Leenen, P. J., Zweers, A., and Versteeg, M. (1988) J. Biol. Chem. 263, 25924–25931

26. de Jong, W. W., Leunissen, J. A., and Voorter, C. E. (1993) Mol. Cell. Biol. 13, 103–126

27. Hickey, E., Brandon, S., Ede, S., Sadis, S., Smale, G., and Weber, L. A. (1986) Gene (Amst.) 43, 147–154

28. van den Ijssel, P. R., Smulders, R. H., de Jong, W. W., and Bloemendal, H. (1996) Ophthalmic Res. 28, Suppl. 1, 39–43

29. Arrigo, A. P., Suhani, J. P., and Welch, W. J. (1988) Mol. Cell. Biol. 8, 5059–5071

30. Behlke, J., Lutsch, G., Gaestel, M., and Bieilka, H. (1991) FEBS Lett. 288, 199–202

31. Kato, K., Hasegawa, K., Goto, S., and Inaguma, Y. (1994) J. Biol. Chem. 269, 11274–11278

32. Mehlen, P., Mehlen, A., Guillet, D., Peville, X., and Arrigo, A. P. (1995) Cell. Biochem. Biophys. 28, 225–235

33. Mehlen, P., and Arrigo, A. P. (1994) Eur. J. Biochem. 221, 327–334

34. Chauffour, S., Mehlen, P., and Arrigo, A. P. (1996) Cell Stress Chaperones 1, 225–235

35. Miron, T., Wilchek, M., and Geiger, B. (1988) Eur. J. Biochem. 178, 543–553

36. Miron, T., Vancompernole, K., Vandekerckhove, J., Wilchek, M., and Geiger, B. (1991) J. Cell Biol. 114, 2659–2666

37. Benndorf, R., Hayess, K., Rayanzetsev, S., Wieske, M., Behlke, J., and Lutsch, G. (1994) J. Biol. Chem. 269, 20783–20784

38. Zhu, Y., O'Neill, S., Saklatvala, J., Tassi, L., and Mendelsohn, M. E. (1994) J. Biol. Chem. 269, 3715–3723

39. Jakob, U., Gaestel, M., Engel, K., and Buchner, J. (1993) J. Biol. Chem. 268, 1517–1520

40. Ehrnsperger, M., Graber, S., Gaestel, M., and Buchner, J. (1997) EMBO J. 16, 212–229

41. Leroux, M. R., Melki, R., Gordon, B., Batelier, G., and Candido, E. P. (1997) J. Biol. Chem. 272, 24646–24656
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9385

42. Konishi, H., Matsuzaki, H., Tanaka, M., Takeura, Y., Kuroda, S., Ono, Y., and Kikkawa, U. (1997) FEBS Lett. 410, 493–498

43. Downward, J. (1998) Curr. Opin. Cell Biol. 10, 262–267

44. Franke, T. F., Kaplan, D. R., and Cantley, L. C. (1997) Cell 88, 435–437

45. Beresford, P. J., Jaju, M., Friedman, R. S., Yoon, M. J., and Lieberman, J. (1998) J. Immunol. 161, 161–167

46. Suzuki, A., Sugiyama, Y., Hayashi, Y., Nyu-i, N., Yoshida, M., Nonaka, I., Ishiura, S., Arahata, K., and Ohno, S. (1998) J. Cell Biol. 140, 1113–1124

47. Michels, A. A., Nguyen, V. T., Konings, A. W., Kampinga, H. H., and Bensaude, O. (1995) Eur. J. Biochem. 234, 382–389

48. Yang, Y., Fruh, K., Ahn, K., and Peterson, P. A. (1995) J. Biol. Chem. 270, 27687–27694

49. de Wet, J. R., Wood, K. V., DeLuca, M., Helinski, D. R., and Subramani, S. (1987) Mol. Cell. Biol. 7, 725–737

50. Bartel, P. L., Chien, C., Sternaglanz, R., and Fields, S. (1993) in Cellular Interactions in Development: A Practical Approach (Hartley, D. A., ed) pp. 153–179, IRL Press, Oxford

51. Holenberg, S. M., Sternaglanz, R., Cheng, P. F., and Weintraub, H. (1995) Mol. Cell. Biol. 15, 3815–3822

52. Vojtek, A. B., Hollenberg, S. M., and Cooper, J. A. (1993) Cell 74, 205–214

53. Zantema, A., Verlaan-De Vries, M., Maasdam, D., Bol, S., and van der Eb, A. (1992) J. Biol. Chem. 267, 12936–12941

54. Carver, J. A., Aquilina, J. A., Truscott, R. J., and Ralston, G. B. (1992) FEBS Lett. 311, 113–149

55. Carver, J. A., Esposito, G., Schwedersky, G., and Gaestel, M. (1995) FEBS Lett. 369, 305–310

56. Bova, M. P., Ding, L. L., Horwitz, J., and Fung, B. K. (1997) J. Biol. Chem. 272, 29511–29517

57. Dudich, I. V., Zar’yalov, V. P., Pfeil, W., Gaestel, M., Zar’yalova, G. A., Denesyuk, A. I., and Korpela, T. (1995) Biochim. Biophys. Acta 1253, 163–168

58. Kim, K. K., Kim, R., and Kim, S. H. (1998) Nature 394, 595–599

59. Berengian, A. R., Bova, M. P., and McHaourab, H. S. (1997) Biochemistry 36, 9951–9957

60. Merck, K. B., Horwitz, J., Kersten, M., Overkamp, P., Gaestel, M., Bloemendal, H., and de Jong, W. W. (1995) Mol. Biol. Rep. 18, 209–215

61. Farnsworth, P. N., Frauwirth, H., Groth-Vasselli, B., and Singh, K. (1998) Int. J. Biol. Macromol. 22, 175–185

62. Carver, J. A., Aquilina, J. A., and Truscott, R. J. (1994) Exp. Eye Res. 59, 231–234

63. Merck, K. B., De Haard-Hoekman, W. A., Oude Esink, B. B., Bloemendal, H., and de Jong, W. W. (1992) Biochim. Biophys. Acta 1130, 267–268

64. Haley, D. A., Horwitz, J., and Stewart, P. L. (1998) J. Mol. Biol. 277, 27–35

65. Ehrnsperger, M., and Gaestel, M. (1998) in Molecular Chaperones in the Life Cycle of Proteins: Structure, Function and Mode of Action (Fink, A. L., and Goto, Y., eds) pp. 533–575, Marcel Dekker, New York

66. Plater, M. L., Goode, D., and Crabbe, M. J. (1996) J. Biol. Chem. 271, 28558–28566

67. Boyle, D., Gopalakrishnan, S., and Takenoto, L. (1993) Biochem. Biophys. Res. Commun. 192, 1147–1154

68. Harrison, C. J. (1997) Structure 5, 1261–1264

69. Horovitz, A. (1998) Curr. Opin. Struct. Biol. 8, 93–100