Essential role of the N-terminal WD/EPF motif in the phosphorylation-activated protective function of mammalian Hsp27 *

Jimmy R. Thériault ‡ §, Herman Lambert ‡, Aura T. Chávez-Zobel § ¶, Gabriel Charest ||, Pierre Lavigne || and Jacques Landry ‡ †

‡Centre de recherche en cancérologie de l'Université Laval, L'Hôtel-Dieu de Québec, Centre hospitalier universitaire de Québec, 9 rue McMahon, Québec (QC), Canada G1R 2J6,

¶Universidad Centroccidental Lisandro Alvarado, Decanato de Medicina, Departamento de Morfología, Sección de Anatomía Microscópica, Av. Libertador con Av. Andrés Bello, Barquisimeto, Estado Lara, Venezuela

and

|| Département de pharmacologie Faculté de médecine, Université de Sherbrooke, Sherbrooke (QC), Canada J1H 5N4

*This work was supported by the Canadian Institutes of Health Research, Grant MT-7088 and the Canada Research Chair in Stress Signal Transduction.

§ Supported by a studentship from the Société de recherche sur le cancer, Inc. (J.R.T.), the Fonds de la recherche en Santé du Québec/Fonds Québécois de la recherche sur la nature et les technologies (J.R.T.) and, the Fundación Gran Mariscal de Ayacucho and the Universidad Centroccidental Lisandro Alvarado, Venezuela (A.T.C.Z).

†To whom correspondence should be addressed. Tel.: 418-691-5281; Fax: 418-691-5439. Email: jacques.landry@med.ulaval.ca
Footnotes:

1The abbreviations used are: CS, citrate synthase; Hsp, heat shock protein; sHsp, small HSP;

2Research Collaboratory for Structural Bioinformatics Protein Databank = PDB # 1GME_A

3Research Collaboratory for Structural Bioinformatics Protein Databank = PDB # 1GME_B

Running title:  Hsp27 phosphorylation: effect on structure and thermoprotection.
ABSTRACT

Hsp27 is expressed at high level after mild heat shock and contribute to making cells extremely resistant to subsequent treatments. The activity of the protein is regulated at the transcriptional level, but also by phosphorylation, which occurs rapidly during stress and is responsible for causing the dissociation of large 700 kDa Hsp27 oligomers into dimers. We investigated the mechanism by which phosphorylation and oligomerization modulate the protective activity of Chinese hamster Hsp27. In contrast to oligomer dissociation, which only required Ser90 phosphorylation, activation of Hsp27 thermoprotective activity required the phosphorylation of both Ser90 and Ser15. Replacement of Ser90 by Ala90, which prevented the dissociation of the oligomer upon stress, did cause a severe defect in the protective activity. Dissociation was however not a sufficient condition to activate the protein since replacement of Ser15 by Ala15, which caused little effect in the oligomeric organization of the protein, also yielded an inactive protein. Analyzes of mutants with short deletions in the N-terminus identified the Hsp27 WD/EPF or PF-rich domain as essential for protection, maintenance of the oligomeric structure and in vitro chaperone activity of the protein. In the light of a three-dimensional model of Hsp27 based on the crystallographic structure of wheat Hsp16.9, we propose that the conserved WD/EPF motif of mammalian Hsp27 mediates important intramolecular interactions with hydrophic surfaces of the $\alpha$-crystallin domain of the protein. These interactions are destabilized by Ser90 phosphorylation, making the motif free to interact with heterologous molecular targets upon the additional phosphorylation of the nearby Ser15.
INTRODUCTION

Heat shock treatments lead to a rapid and specific induction of several genes whose products are collectively known as of heat shock proteins (Hsp)\textsuperscript{1} (1). A direct correlation has been demonstrated between the accumulation of Hsp after the treatment and the acquisition of cellular resistance to heat shock and other toxic stresses (1-4). HspB1/Hsp27 (called thereafter Hsp27) is one of the most efficient thermoprotective Hsp when overexpressed alone following gene transfection (5). Hsp27 is also very efficient in protecting cells against various cytotoxic conditions such as exposure to oxidative stress, cancer chemotherapeutic agents or agonists of the death receptors tumor necrosis factor \( \alpha \) or Fas (5-8). Hsp27 has generated much interest because in addition to accumulating to high levels within a few hours after stress, the protein already present at the time of treatment is phosphorylated within minutes in a stress-sensitive signaling pathway involving the MAP kinase p38 (9-12). It has been suggested that such a rapid phosphorylation is important to set up a prompt homeostatic mechanism helping to survive stress and accordingly many studies have shown an essential role of phosphorylation in the protective activity of Hsp27 (13).

Hsp27 belongs to the small HSP (sHsp) family also called the HspB family, which have representatives in virtually all organisms. In human, the family includes 10 members comprising HspB1/Hsp27, HspB2/MKBP, HspB3/HspL27, \( \alpha \)- and \( \beta \)-crystallin, Hsp20, HspB7/cvHsp27, HspB8/H11/Hsp22, HspB9 and HspB10/Odf1 (13-15). The major characteristic of these proteins is the presence at their carboxyl-terminus of a sequence of about 100 residues called the \( \alpha \)-crystallin domain. The N-terminus is less well conserved, but a small proline-phenylalanine-rich region containing one or two WD/EPF motifs, is found in many of the sHsp proteins (16-18). Hsp27, like most studied sHsp, is found under normal conditions in large oligomers with a molecular mass of
some 700 kDa (17). Structural studies have been difficult because the oligomeric structure is highly dynamics, the high molecular oligomer being in equilibrium with dimers or tetramers and showing a high rate of subunit exchange (19). Nonetheless, the crystal structure of two sHsp has been determined to date, revealing that oligomers of sHsp form as a result of multiple interactions in the \(\alpha\)-crystallin domain, stabilized in some but not all cases, by interaction with hydrophobic sequences of the N-terminal (20-22). In the case of Hsp27, the oligomer is formed of an estimated number of 24 subunits and deletion studies confirmed the role of molecular interactions at the N-terminus in the stability of the oligomers (17). Studies, both \textit{in vitro} with recombinant proteins and \textit{in vivo} with endogenous or ectopically expressed proteins, indicated that phosphorylation causes the dissociation of the oligomers into dimers that are suggested to be the basic building unit of sHsp (17,20,21,23).

There are numerous pieces of evidence that phosphorylation or changes in the supramolecular organization modulate the activity of Hsp27. The capacity to modulate actin microfilament dynamics was the first biochemical activity described for Hsp27. In a cell-free assay, small Hsp27 species behave as actin capping proteins that regulate actin polymerization in a phosphorylation-dependant manner (24,25). \textit{In vivo}, overexpression of wild type or pseudo-phosphorylated Hsp27 but not a non-phosphorylatable mutant induced an enhanced-accumulation of stress fibers in response to growth factors or mild cytotoxic stress (6,26-29), an effect which in the case of oxidative stress or cisplatin treatment can lead to actin filament dependent cell blebbing (30). In other contexts, this activity results into a phosphorylation-dependent protection of the actin filaments against severe stress such as cell treatment with the actin polymerization inhibitor cytochalasin-D. Hsp27 also possesses a chaperone-like activity preventing the thermal aggregation of protein \textit{in vitro} (31,32). Like other sHsp, Hsp27 has no protein folding activity, but it can adsorb
heat-denatured protein keeping them in a folding-competent state (33). The role of phosphorylation in this function is not totally clear. In yeast, both small and large oligomers participate in chaperone activity of the Hsp27 homologue, Hsp26 (34). It is proposed that small oligomers bind denatured proteins during hyperthermia. After heat shock, they re-associate into large oligomers with the misfolded proteins (34). A sHsp from Cyanobacteria seems to be able to bind denatured proteins both in its small and large oligomeric forms, however, dissociation of the oligomers is required for releasing the sHsp from its substrate and thus for refolding to occur (35). On the contrary, although evidence suggest that mammalian Hsp27 bind denatured proteins in a way similar to yeast Hsp26, it was reported that small phosphorylated oligomers of mammalian Hsp27 have reduced chaperone activities (23). Multimerization is however not required for the chaperoning activity of βB-crystallin (36). Phosphorylation has also been shown to regulate the inhibition by Hsp27 of a caspase-independent cell death process mediated by Daxx and Ask1 (37). Not surprisingly considering all the evidence for a regulation of Hsp27 activity by phosphorylation, phosphorylation/dissociation has also been shown to affect the protective activity of Hsp27 at the level of survival. Phosphorylation appears essential for protecting against heat shock-induced loss of clonogenic activity. It is also essential for protection against the toxic action of a number of anticancer drugs (6,27,38). In contrast, the non-phosphorylated and oligomeric species of high molecular weight are better at protecting against tumor necrosis factor or oxidative stress, suggesting different mode of protection of Hsp27 against different stresses (39,40).

Hsp27 is phosphorylated on 2 (rodent) or 3 (human) serine residues by MAPKAP kinase-2, a kinase activated by p38 in a cascade of activation/phosphorylation reactions involving, in the case of heat shock, MKK3/6 and Ask1 (9,11,41-44). Phosphorylation of Ser90 (Chinese hamster
sequence) is sufficient and necessary to induce the dissociation of the Hsp27 oligomers into the basic dimeric subunits (17). The role of Ser15 phosphorylation is however unknown. Hsp27 with Ser15 replaced by Glu15 to mimic phosphorylation or Hsp27 in which Ser90 is replaced by Ala90 so that only Ser15 is phosphorylatable, are expressed in cells as high molecular oligomers only slightly smaller that the wild type protein in the presence or absence of stress, suggesting that the major role of this phosphorylation site is not to regulate oligomerization (17). In the present study, we examined the role of phosphorylation at Ser15 and Ser90 in the protective function of Hsp27 against heat shock-induced cell killing. Our results suggest an essential role for both phosphorylation events, one (Ser90) in dissociating the oligomeric structure thereby exposing an active site at the N-terminus and the other (Ser15) in derepressing this active site. An important site for activity was mapped to the hydrophobic motif WD/EPF, which in wheat sHSP, Hsp16.9, corresponds to a sequence predicted to play an important role in the supramolecular organization of the protein.
EXPERIMENTAL PROCEDURES

**Plasmids** pSVHa27WT contains wild type Chinese hamster Hsp27 sequence from pH8 inserted in HindIII sites of pSVT7 vector (45). The phosphorylation mutant constructs pSVHa27AA, pSVHa27EE, pSVHa27AE, pSVHa27EA were as described before (17) whereas pSVHa27DD was constructed as described later. The two last letters of the phosphorylation mutant constructs correspond to the amino acid residue substituted at position 15 and 90, respectively. Alanine (A), glutamate (E) or aspartate (D) have been added to mimic non-phosphorylatable (A) or pseudo-phosphorylated (E or D) serine residues. pSVHa27Δ5-23 was described before (17). All other Hsp27 deletants/mutants were constructed especially for this study using a PCR-based Site-Directed Mutagenesis Kit (ExSite™, Stratagene, La Jolla, CA). Briefly, polymerase chain reactions were performed on pSVHa27WT as template using two oligonucleotides covering the boundaries of the deletion or the mutation. After amplification, the reaction products were digested with DpnI (Invitrogen, USA) and the polymerase chain reaction products were ligated with T4 DNA ligase (Invitrogen, USA). The plasmid for expression of the chimeric Hsp27-luciferase protein (pCMVHa27-luciferase) was constructed from the full-length Hsp27 insert of pSVHa27WT inserted at the HindIII/XhoI restriction sites of the pCMV-luciferase vector (17). pCMVHa27-luciferase was then used as template to produce the Hsp27 deletants in fusion with luciferase.

**Preparation of recombinant Hsp27 proteins** Vectors for the expression of the recombinant Hsp27 were prepared by insertion of the Hsp27 wild type, mutated, or deleted sequences at the BamH1 restriction site of pGEX4T3 vector (Amersham Biosciences). The resulting plasmids were subcloned in *Escherichia coli* strain BL21 Codon+ RIL (Stratagene) producing glutathione S-transferase -Hsp27 fusion proteins (GST-Hsp27) after addition of isopropyl-1-thio-β-D-
galactopyranoside 0.15 mM for 4 hours at 30°C. Bacteria were lysed in a buffer containing 50 mM HEPES pH 7.5, 0.45 M NaCl, 5 mM dithiothreitol, the Complete protease inhibitor (Roche), 1.25 mg/ml lysozyme and 11.2 μg/ml Dnase I (Sigma). Extraction of the GST-Hsp27 proteins from the inclusion bodies was done by 1 h incubation with vigorous shaking at 37 °C in a 25 mM phosphate buffer pH 7.4 containing 150 mM NaCl, 1 mM EDTA, 2 % (v/v) Triton X-100, 5 mM dithiothreitol and the Complete protease inhibitor. The solubilized GST-Hsp27 proteins were affinity purified using Glutathione-Sepharose 4B beads (Amersham Biosciences) and digested with thrombin (Sigma, St-Louis, MO) overnight at room temperature yielding recombinants Hsp27 with the two additional residues serine and glycine before the N-terminal methionine. The proteins were purified to homogeneity by anion exchange chromatography on a Mono Q column (Amersham Biosciences). The protein concentration was determined using a extinction coefficient of 1.87 for a 1 mg/ml solution at 280 nm (46). The size distribution of the multimeric complexes formed by the recombinant proteins was determined by chromatography on Superose 12 HR column (Amersham Bioscience).

**Cell culture and transfection** NIH 3T3 cells were grown at 37°C in a 5% CO2 humidified atmosphere, in high glucose DMEM medium supplemented with 10% calf serum. Cells were plated 24 h before transfection at a concentration of 16 000 cells/cm² in a 25-cm² culture flask (in vivo thermoprotective assay) or 4000 cells/cm² in 100 mm Petri dish (glycerol gradient and glutaraldehyde cross-linking analysis). Transfection by calcium phosphate was performed as previously reported with varying amount of plasmids (0-5 μg) in 25-cm² culture flask and 0-10 μg of plasmids in 100 mm Petri dish (5). Chloroquine (50 μM) was added for the first 5 h of transfection.
**Glycerol gradient ultracentrifugation** Cell lysates were prepared 72 h after transfection by brief sonication in 25 mM HEPES buffer, pH 7.4, containing 3.33% glycerol, 1 mM EDTA, 1 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride at 4°C. Supernatant was cleared by centrifugation at 17 000 g for 5 min. at 4°C. The lysates (0.5 ml) were loaded on the top of a 12.6 ml linear gradient of glycerol (10-40%) made in 25 mM HEPES buffer, pH 7.4, 1 mM EDTA and 1 mM dithiothreitol (17). The tubes were centrifuged at 30 000 rpm for 18 h in a SW40 rotor (Beckman) at 4°C. The gradients were fractionated in approximately 44 fractions. Aliquots were diluted (1:4) in Tris/Glycine/SDS buffer (25 mM Tris, 192 mM glycine and 0.01% SDS), dot-blotted on a nitrocellulose membrane and probed for Hsp27 using an antibody developed against the C-terminal tail of Chinese hamster Hsp27 (Upstate). Antigen-antibody complexes were detected with 125I-labeled goat anti rabbit IgG. Detection and quantification were done using a Storm imaging system from Molecular Dynamics, Inc. (Sunnyvale, CA) and recombinant Hsp27 (5ng/µl) as standard.

**Hsp27 thermoprotective activity** The cells were transfected with graded amount of plasmids in order to obtain varying expression levels of the protein. The cells were trypsinized 24 h after transfection and plated in at a concentration of 1, 5 or 25 x 10⁴ cells per 25-cm² culture flasks. Twenty hours later, the cells were heat shocked for 2.5 h in a water-bath maintained at 44°C and were returned to 37°C to allow for colony formation. Colonies were stained 13 days later with a solution of 0.25 % (w/v) trypan blue and 5 % (v/v) acetic acid. Cellular survival was evaluated by counting colonies that contained more than 50 cells. Hsp27 concentration was determined in control groups of transfected cells at the time of heat shock. Quantification was performed by western blot using the anti-C-terminal Hsp27 antibody as described above after electrophoresis of total cell extracts. A standard curve was generated using recombinant Hsp27 as standard (5 ng/µl).
Luciferase activity—Aliquots (10 mL) of each protein extract were diluted in 300 mL of 25 mM glycyl/glycine buffer, pH 7.8 containing 10 mM MgSO₄, 2 mM ATP, and 1 mM dithiothreitol. Luciferase activity was measured in a Berthold Lumat 9501 luminometer for 30 sec after the addition of 100 mL of D-luciferin at a final concentration 50 µM.

In vitro thermal aggregation assay—The thermal denaturation kinetics of citrate synthase (CS) were measured from the light scattering at 320 nm during incubation at 43°C using a Varian spectrophotometer (model Cary 1 Bio) equipped with a temperature controlled cell holder. CS was diluted at a concentration of 150 µM (monomer) in a solution of 40 mM Hepes pH 7.5 in absence or presence of various concentration of Hsp27.

Three-dimensional modeling of Hsp27—The secondary structure of Chinese hamster Hsp27 (HaHsp27) was calculated with the web-based software JNET (ref. 47, http://www.compbio.dundee.ac.uk), and PROFsec and PHDsec (ref. 48, http://www.predictprotein.org) and aligned to wheat Hsp16.9 structure using Superfamily 1.6 (ref. 49, http://supfam.org). With this alignment, residues of Hsp27 were transferred onto the coordinate of the corresponding residues of the structure of Hsp16.9 (PDB codes: 1GME_A², 1GME_B³) using the HOMOLOGY module of INSIGHT II software (Accelrys). Loops that are located between the conserved secondary structure elements were also generated using the HOMOLOGY module. The potential energy of the model of Hsp27 was then minimized using FDISCOVER. Calculations were done on an Octane2 computer (Silicon Graphics, Inc.). Molecular drawing were made with the software Ribbons (50).
RESULTS

Phosphorylation of both Ser15 and Ser90 is necessary for full Hsp27 thermoprotective activity. NIH 3T3 cells were transfected with plasmids encoding various phosphorylation mutants of Chinese hamster Hsp27 and subjected 48 h later to a severe heat shock treatment at 44°C for 150 min. In control sham-transfected NIH 3T3 cells or in cells transfected with empty vectors, such treatments yielded a survival in the order of 10^-5 to 10^-4. In the various experiments, between 1 and 10 cells out of 100000 plated cells could grow into colonies after heat shock. In agreement with the known thermoprotective activity of Hsp27, transient transfection with graded amounts of Hsp27-WT plasmids yielded a dose-dependant increase in survival (Fig. 1a). The pseudo-phosphorylated Hsp27-EE (Fig. 1a) and Hsp27-DD (data not shown) mutants, in which the Ser15 and Ser90 had been replaced by glutamate or aspartate, respectively, were also highly protective; the dose-response curve obtained for these mutants was identical to that obtained with Hsp27-WT. In contrast, the non-phosphorylatable Hsp27-AA protein (Ser15 and Ser90 replaced by Ala residues) yielded very little protection even at the highest level of expression obtained (Fig. 1a). The role of the individual phosphorylation sites was evaluated by determining the protective activity of Hsp27 double mutants having one phosphorylation changed to Ala and the other to Glu (Hsp27-AE and Hsp27-EA). Both mutants had a highly reduced activity, protecting similarly to the Hsp27-AA mutant (Fig. 1b). It was concluded that phosphorylation at both Ser15 and Ser90 was required for full Hsp27 thermoprotection.

The region between amino acids 15 to 27 is required for cellular protection of Hsp27 against heat shock. The importance of Ser15 phosphorylation in Hsp27 protective activity suggested that a region located at the N-terminal might be essential for thermoprotection. To determine whether the N-terminus of Hsp27 could protect when expressed alone, cells were
transfected with a construct containing the first 109 residues of Hsp27. Such construct yielded no protein expression and thus could not be evaluated for protection (data not shown). To resolve the protein expression problem, we fused different HSP27 N-terminus inserts to luciferase used as a carrier protein. As a control, we also made a construct coding for a fusion protein of the full length Hsp27 and luciferase (Hsp27-luc). Transfection of the Hsp27-luc construct yielded a level of luciferase activity that was comparable that obtained upon transfection of luciferase alone. At maximal expression obtained, over 200-fold increase in survival was found with the Hsp27-luciferase chimera as compared to luciferase alone (Fig. 2). A construct containing the first 49 residues of Hsp27 fused to luciferase (Hsp27(1-49)-luc) was expressed at very low level and yielded inconclusive results (data not shown). A longer construct containing the first 87 residues of Hsp27 (Hsp27(1-87)-luc), was expressed at higher levels and, at corresponding concentrations, yielded a level of protection similar to Hsp27-luc (Fig. 2).

To identify more precisely the N-terminal region responsible for thermoprotection, cells were transfected with Hsp27 constructs containing various deletions at the N-terminus. Deletion of the region between amino acids 5 to 23 (Hsp27Δ5-23) totally eliminated the activity of Hsp27, whereas deletion of residues 50 to 79 (Hsp27Δ50-79) yielded a protein with a protection curve similar to that of wild type Hsp27 (Fig. 3a). The result suggested that the region in or around the residues 5 to 23 was involved in protection against heat shock. Removal of the region between the first methionine and residue 14 (Hsp27 Δ2-14) or between residues 27 to 80 (Hsp27Δ28-79) had no notable effect on Hsp27 protective function (Fig 3b). A severe N-terminal deletant was constructed after eliminating both sequences 2-14 and 28-79. Hsp27Δ2-14Δ28-79 was as thermoprotective as Hsp27WT (Fig 3c). We concluded that the region confined between residues
15 to 27 contains a motif that is necessary and sufficient (together with the α-crystallin C-terminal domain) for protection.

**Role of phosphorylation and residues 15-27 on the chaperone activity of Hsp27** We next evaluated the effects of the same mutations/deletions on the *in vitro* chaperone activity of Hsp27. Recombinant Hsp27 mutants and deletants produced in bacteria were used in an *in vitro* thermal aggregation assay using CS as substrate. For each conditions, the kinetics of denaturation of CS at 43°C was measured for up to 40 minutes by monitoring at intervals the turbidity (light scattering at 320 nm) of the protein solution containing CS alone, compared to CS in solution with increasing concentrations of the various Hsp27 proteins. All concentrations were calculated considering monomeric proteins. We found very little difference in the activities of Hsp27WT, Hsp27AA or Hsp27EE (Fig. 4a). In all 3 cases, the fifty-percent protection level was attained at a Hsp27/CS molar ratio of about 1 to 1.5, suggesting that whatever the molecular organization of the Hsp27 mutants, 2 subunits of Hsp27, phosphorylated or not, were required to keep the CS dimers in solution. We also examined the activity of the N-terminal deletants. Hsp27D2-14 and Hsp27D28-79 were very little affected in their chaperone activity compared to Hsp27WT, whereas HSP27D5-23 had no chaperone-like activity for a Hsp27/Cs molar ratio of up to 8. (Fig. 4b). Hence the region between the residues 15 to 27 appears essential for Hsp27 to prevent the thermal aggregation of CS *in vitro*.

**The region between amino acids 15 to 27 is required for Hsp27 oligomerization** We next evaluated the oligomerization capacity of the various N-terminal deletants. Soluble extracts from cells transfected with the various constructs were analyzed by ultracentrifugation on glycerol gradients and the distribution of the Hsp27 mutants evaluated by Western blot. As described previously (17), Hsp27WT sedimented as a large complex with a major peak at a molecular mass
corresponding well to the predicted size of about 650 kDa for a 24-mer complex (Fig. 5a), whereas Hsp27Δ5-23 sedimeted almost exclusively as dimers of some 50 kDa (data not shown and ref. 17), suggesting that sequences located in this region were also essential for maintaining the Hsp27 molecule in its polymerized state. Like the Hsp27Δ5-23 deletant, Hsp27Δ18-30 could not form a significant amount of large molecular species, most of the proteins distributing in fractions corresponding to tetramers (about 100 kDa) and dimers (about 50 kDa) (Fig. 5b). In contrast, Hsp27Δ28-79 formed like Hsp27WT large oligomers corresponding probably also to 24-mers (about 500 kDa). Hsp27Δ2-14 had also the capacity to form large oligomers, some of which being as large as those formed by Hsp27WT. However, the distribution was broader extending to the lower molecular weight region, perhaps suggesting a more dynamic or destabilized oligomeric structure. Interestingly, this pattern was similar to that obtained with the Hsp27EA mutant (17) suggesting that deletion of residues 2-14 might have the same effect on the structure than the phosphorylation of S15. Interestingly, the double Hsp27Δ2-14Δ28-79 deletant sedimented almost normally, suggesting that the sequences 15 to 27 provided the properties responsible for the stabilization of the oligomers by the N-terminal (Fig. 5c).

To further confirm that residues between position 15 and 27 were essential role for oligomerization, we analyzed the size of the complexes formed by purified recombinant HSP27 using HPLC size-exclusion chromatography. Purified Hsp27WT eluted predominantly at a position expected for a dodecamer of some 325-kDa (Fig. 6a). As expected since Hsp27WT was not phosphorylated, a very similar profile was obtained with the non-phosphorylatable mutant HSP27-AA (Fig. 6c). As reported before (46), the size of the multimer of Hsp27 appeared to be affected by protein concentration. Indeed, a significant proportion of HSP27 oligomers fractionated as 24-mers (about 700 kDa) when Hsp27 was analyzed at higher concentrations.
(Fig. 6b). It thus appeared that the 24-mer complexes are labile \textit{in vitro}, and that the dodecamers represents a more stable complex. In the case of Hsp27WT, oligomers of smaller size never became predominant even at lower sample concentrations (data not shown). In contrast, the pseudo-phosphorylated Hsp27EE mutant eluted mainly as dimers and tetramers, reproducing well the \textit{in vivo} situation, although a shoulder of higher molecular weight species was also apparent (Fig. 6d). HSP27 \[\text{28-79}\] (Fig. 6e) was able to form 24- and 12-subunits complexes as we observed with Hsp27WT. Hsp27\[\text{2-14}\] (Fig. 6f) formed dodecamers, however, Hsp27\[\text{5-23}\] (Fig. 6g) and \[\text{18-30}\] (Fig. 6h) formed dimers and tetramers, respectively. Hence, like \textit{in vivo}, the residues between position 15 and 27 were essential for the supramolecular organization of recombinant Hsp27 \textit{in vitro}.

\textbf{Three-dimensional modeling of Hsp27 structure}-- Hsp27 is composed of two domains: a well conserved C-terminal domain (called the \[\alpha\]-crystallin or Hsp20 domain, PFAM accession number: PF00011) and a less conserved N-terminal domain having homologous region only in subfamily members. A proline/alanine-rich segment of low complexity and varying length connects the two regions (11,16,51,52). Secondary structure calculations for Hsp27 were done using 3 different algorithms that take into account the alignments of multiple family members (see \textit{Experimental Procedures}). Essentially identical results were obtained, all software predicting that the \[\alpha\]-crystallin domain of Hsp27 was mostly composed of \[\beta\]-strands that aligned very well with the known secondary structure of Hsp16.9 (Fig. 7a), a sHsp from wheat whose structure has recently been resolved experimentally (20). The position of the \[\beta\]-strands in Hsp27 also matched quite well the equivalent secondary structures in the sHSP Hsp16.5 from \textit{Methanococcus jannaschii} (data not shown). However, the primary sequence of Hsp27 show low homology with
the bacterial proteins and, like Hsp16.9, appears to lack the $\beta$1-strand present in Hsp16.5 (20,21). We therefore used the crystal structure of Hsp16.9 as a template to get insights into the three-dimensional structure of Hsp27. The alignment used for modeling was based on the predicted secondary structures and the homology of the primary sequences using the software Superfamily (49). This resulted in an alignment very similar to that proposed before (20), except for an intriguing difference at the N-terminal end. The N-terminal SXXFDPF motif of Hsp16.9, which, as shown by crystallographic analyses, mediates essential inter- and intramolecular interactions in the oligomeric organization of this sHSP, somehow replacing the $\beta$1-strand of Hsp16.5, aligned with a high degree of homology (9/14 homologous residues) with the sequences around the W$_{16}$EPF motif of Hsp27.

The three-dimensional structure of the monomer and the relative orientation of the monomers in the dimer of Hsp27 were based on the corresponding monomeric and dimeric structures of Hsp16.9 (PDB codes: 1GME_A, 1GME_B). The results indicated that Hsp27 could accurately be modeled on this basis for most of the sequence. There are two major differences between the model of Hsp27 and the experimentally determined structure of Hsp16.9. First, the difference in size of the loop between the $\beta$5 and $\beta$7 strands, which is longer by 7 residues in Hsp16.9. Secondly, Hsp27 has a much longer connecting sequence between the N- and C-terminal terminal region (42 more residues) (Fig. 7b). The model predicts that like in Hsp16.9, the strands ($\beta$2-$\beta$8) of the crystallin domain of Hsp27 are organized as a sandwich of two antiparallel $\beta$-sheets formed by the strands $\beta$2, $\beta$3, $\beta$9 and $\beta$8 on one side of the sandwich and $\beta$7, $\beta$5 and $\beta$4 on the other. The model also predicts that the sequence W$_{16}$EPF plays an important role in this structure making intramolecular interactions and patching a hydrophobic pocket formed by residues Ile96, Gln98, Tyr150, Thr 151 and Pro153 at the interface of the strands $\beta$2 and $\beta$7 (Fig. 7c).
phosphorylatable Ser90 provides an explanation for the phosphorylation-induced shift in the structure of the molecule. In the monomer, Ser 90 is found near the middle of the two $\beta$-sheets and, in the dimer, at the interface between the 2 molecules, the two Ser90 facing each other. It can be predicted that phosphorylation of Ser90, by introducing negative charges, could generate highly destabilizing repulsion forces at the heart of the dimerization interface likely destabilizing the multimer and forcing the establishment of a new equilibrium in the phosphorylated dimer. Even if it is shorter than the one found in the structure of Hsp16.9, the loop between $\beta$5 and $\beta$7, is close to the $\beta$2 strand of the opposing monomer and may, like in Hsp16.9, be implicated in the formation of the dimeric form of Hsp27. In sum the model provides explanation supporting the important role that is played by the motif WD/EPF and the influence of phosphorylation in the structure and function of Hsp27.
DISCUSSION

Hsp27 is expressed in cells as homopolymers of sizes ranging from 2- to 24-mers depending on the phosphorylation state of the protein. In unstressed cells, Hsp27 is mostly found unphosphorylated and assembled in 24-mers (17). Upon stress that activates the p38 kinase pathway and leads to Hsp27 phosphorylation, Hsp27 dissociates into dimers (9,12,17). Our results show that the pseudo-phosphorylated Hsp27EE mutant, an almost exclusive dimeric molecule, provides full thermoprotection whereas the non-phosphorylatable Hsp27AA, found mostly as large 24-mers, provides very little protection, suggesting that phosphorylation-induced dissociation is an important step in the activation of the thermoprotective activity of Hsp27. In theory, by inducing the dissociation of the oligomers into dimers, the physiological effect of phosphorylation could be simply to increase by 12-fold the molar concentration of the Hsp27 protective units. This was an attracting possibility because phosphorylation is maximally induced within 20 min of heat shock, and vanished by 5-6 h at the time when a real 10-fold increase in the Hsp27 monomeric concentration had occurred as a result of transcriptional activation (53). Phosphorylation would thereby provide in a few minutes an efficient increase in Hsp27 concentration, which otherwise takes hours with transcription. In our transfection experiments, we never attained a sufficiently high molar concentration of Hsp27AA to really test whether the 24-mers of Hsp27AA have an activity per mole equal to the dimeric Hsp27EE. However, this seems totally unlikely. In fact, the existence of a phosphorylation-activated event other than the increase in molar concentration was clearly demonstrated by comparing the protective activity of Hsp27EE and Hsp27AE mutant. Both mutants are expressed as dimers because phosphorylation of Ser90 is sufficient to cause the dissociation of the Hsp27 oligomer (17). However, the Hsp27AE mutant was much less efficient
than the Hsp27EE mutant to protect the cells, suggesting that both Ser15 and Ser90 phosphorylation were important for the protective activity of Hsp27.

Whereas an increase in the molar concentration of Hsp27 cannot explain the role of Ser90 phosphorylation, it is nonetheless likely that the essential role of Ser90 phosphorylation is to trigger the de-oligomerization of the protein. There is accumulating evidence that the N-terminus of sHsp is normally hidden within the native structure of the oligomer (21,54). Crystallographic analysis of the structure of the wheat sHSP, Hsp16.9, also suggested that the dissociation of the oligomer in dimers yields an increased exposure of the N-terminus (20). One very likely possibility is that phosphorylation of Ser90 causes a dissociation of the Hsp27 oligomer, exposing internal sequences in the N-terminal. Additional phosphorylation of Ser15 would then be required to fully activate the function. Both our mutational studies and our model of the structure of Hsp27 support this hypothesis and point out to the sequence S_{15}WEPF as being this important structural and functional element of the N-terminal. S_{15}WEPF of Hsp27 corresponds to the SXXF_{10}DPF motif of Hsp16.9, which have been shown to mediate important interactions with hydrophobic surfaces of the C-terminal α-crystallin domain. We confirmed the importance of this motif in the case of Hsp27 by showing that Hsp27 can be deleted of almost all its N-terminal sequences except for some 13 residues including S_{15}WEPF with little affect on the protective and oligomeric properties (Hsp27_{2-14}{28-79}), whereas a short deletion including S_{15}WEPF (Hsp27_{5-23}) affected both properties. Hence, phosphorylation of Ser90 which by its position is likely to destabilize both the internal structure of the monomer and the association of the monomers into dimers within the oligomer, may very well yield a dissociated dimer with organization that does not require the internal interaction of S_{15}WEPF with the α-crystallin domain. S_{15}WEPF would then be free to interact with an external substrate. Since nothing is known concerning the structure of the free
dimers, one can only speculate about the role of Ser15 phosphorylation in the activation of the dimeric molecule. Hsp27 deleted of 14 amino acid residues N-terminal of Ser15 (Hsp27\[2-14]) retained its full oligomeric and protective activity. It is interesting that the deletion \[2-14, which also removed the phosphorylation consensus site necessary for Ser15 by MAPKAP kinase-2 had no effect on thermoprotection whereas replacement of Ser15 to Ala totally abolished the protective function of Hsp27. One possibility is that the sequence on the N-terminal side of Ser15 has an autoinhibitory function in the Ser90 phosphorylated dimers. The sequences contained in residues 2-14 may compete with or inhibit directly or indirectly the protective action of S15WEPF and phosphorylation on Ser15 may relieve this inhibition. A number of different activities have been described for Hsp27, but the activity responsible for thermoprotection is not clear. Hsp27 has been reported to bind to cytochrome c and thereby prevent activation of the APAF-1 complex and cell death by apoptosis (55,56). Our analyses make it unlikely that cytochrome c might be an important target of the thermoprotective action of Hsp27. Indeed, a Δ15-51 deletant of human Hsp27, lacking the part of the WD/EPF domain that is essential for thermoprotection, could still associate with cytochrome c and block apoptosis induced by etoposide. Furthermore, a Δ51-88 deletant (Chinese hamster Δ49-80) which should be fully protective according to our result, was unable to bind cytochrome c and did not protect against cisplatin (56). Finally, we observed that the cytochrome c was not released and apoptosis (nuclear fragmentation) was not induced by heat shock in NIH 3T3 cells (data not shown). It is interesting that the human Hsp27Δ51-88 mutant, in spite of its incapacity to interact with cytochrome c, protected against oxidative stress (39). Furthermore, in contrast to their thermoprotective activity observed here, constitutively phosphorylated and dissociated oligomers could not protect against oxidative stress or Tumor Necrosis Factor \[, whereas unphosphorylatable and large Hsp27 oligomers were fully active (23). Together with our
results, this would indicate that the thermoprotective activity of Hsp27 (at the level of survival) is mechanistically different from its protective activity against cytochrome c-mediated apoptosis or oxidative stress.

Hsp27 protects microfilament during heat shock and phosphorylation of Hsp27 is required for this activity (27,29). An *in vitro* actin polymerization inhibiting activity of Hsp27 has been associated with mouse Hsp27 peptides containing residues 43-57 or 92-106 (57). Whereas a role for the region defined by the peptide 43-57 is not supported by our results, the identification of the second peptide is of potential interest. In fact, the region 92-106 corresponds to strands $\beta_2$ and $\beta_3$ and thus covers the region with which the WD/EPF motif interacts in the Hsp16.9-based Hsp27 structure model. The results clearly warrant further analyses with the complete protein and raise the possibility that this function of Hsp27 at the level of actin contributes to thermoprotection at the survival level.

Our study also provides correlative evidence that the same domain is required for Hsp27 to prevent protein aggregation *in vitro* and to protect cells *in vivo*, suggesting as already assumed in many studies that the chaperone function is the important pro-survival activity of Hsp27. There was, however, an important difference between the *in vitro* and *in vivo* activity of the various mutants: Hsp27AA protected against denaturation *in vitro* but did not protect the cells *in vivo*. A number of factors can explain this difference. One reason may be that the oligomeric structures of the recombinant Hsp27AA is less stable or has a higher exchange rate *in vitro* than *in vivo*. sHsp oligomers are known to be highly dynamic continuously exchanging dimer subunits with the environment (19). A higher rate of exchange *in vitro*, as supported by the finding that Hsp27AA was found *in vivo* as 24-mers compared to 12-mers *in vitro*, may result in a significant increase in the exposure to the environment of the active N-terminal domain. Another possibility is that
Hsp27AA can bind as oligomer to denatured substrates *in vivo* as well as *in vitro* and be able to prevent their aggregation, but that its more stable structure prevents the release of the denatured substrates, an essential and rate-limiting step in the process of renaturation (35). The capacity of Hsp27 to bind to denatured proteins and to keep them in solution constitutes only part of the requirement for chaperone activity. Our and previous results supports the idea that both the oligomeric sHsp and dimeric sHsp can bind denatured proteins. In the case of the dimers, binding to denatured proteins would be followed by the re-association into a higher oligomeric form together with the bound denatured substrates (23). Noncooperative binding of the denatured substrates by independent dimeric proteins also has been proposed (35). At any rates, renaturation appears to require the dissociation of the oligomers and this step might not be efficient *in vivo* in the case of the stable oligomers of Hsp27AA. It was recently reported that one major role of Hsp27 might be to target denatured protein to the proteasome degradation machinery. In that model, it is possible that Hsp27AA can bind denatured peptides but cannot interact with the proteasomes to initiate degradation (58).
Figure 1. Thermoprotective activities of phosphorylation mutants of Hsp27. NIH 3T3 cells were transfected with plasmids at varying concentrations in order to obtain varying levels of expression of Hsp27WT (□), Hsp27EE (△), Hsp27AA (□), Hsp27AE (○) and Hsp27EA (□). The cells were then plated at appropriate concentrations, heat shocked at 44°C for 150 min and returned at 37°C for colony formation. Results are presented as the number of colony forming cells that survived the treatment normalized for a total number of 10^6 heat shocked cells as a function of the level of expression of the exogenous Hsp27 in extracts of the transfected cells.

Figure 2. Thermoprotective activities of Hsp27-luciferase chimera. NIH 3T3 cells were transfected with plasmids at varying concentrations in order to obtain varying levels of expression of the proteins Hsp27-luc (□), Hsp27(1-87)-luc (△) or wild type luciferase (□). The cells were then plated at appropriate concentrations, heat shocked at 44°C for 150 min and returned at 37°C for colony formation. Results are presented as the number of colony forming cells that survived the treatment normalized for a total number of 10^6 heat shocked cells as a function of the level of luciferase activity in extracts of the transfected cells.
**Figure 3. Thermoprotective activities of Hsp27 deletants.** NIH 3T3 cells were transfected with plasmids at varying concentrations in order to obtain varying levels of expression of Hsp27WT ( ), Hsp27D5-23 ( ), Hsp27D50-79 ( ), Hsp27D2-14 ( ), Hsp27D28-79 ( ) and Hsp27D2-14D28-79 ( ). The cells were then plated at appropriate concentrations, heat shocked at 44°C for 150 min and returned at 37°C for colony formation. Results are presented as the number of colony forming cells that survived the treatment normalized for a total number of 10⁶ heat shocked cells as a function of the level of expression of the exogenous Hsp27 in extracts of the transfected cells.

**Figure 4. Effect of Hsp27 deletants and mutants on the thermal aggregation of citrate synthase (CS).** CS was heated at 43°C for up to 30 min in the presence of various concentrations of recombinant Hsp27 (Hsp27WT, ); Hsp27EE, ); Hsp27AA, ); Hsp27D5-23, ); Hsp27D28-79 ( ), Hsp27D2-14 ( ) or immonoglobulin G (IgG, ). The relative light scattering was calculated as the ratio of the light scattering at 320 nm of the CS solution heated for 30 min in the presence of the test protein over the light scattering of the CS solution heated for the same period of time in the absence of other proteins. Heated alone, more than 90% of CS was aggregated after 30 min at 43°C. Concentrations are calculated for monomeric proteins (Hsp27, 27 kDa; CS, 44 kDa).
Figure 5. Size distribution of Hsp27 deletants expressed in NIH3T3 cells. NIH 3T3 cells were transfected with different plasmids to yield expression of Hsp27WT (□), Hsp27D2-14 (□), Hsp27D18-30 (□), Hsp27D28-79 (□) or Hsp27D2-14D28-79 (□). The cell extracts were fractionated by centrifugation on glycerol gradient, and HSP27 in each fraction was detected by immunoblot. Molecular mass markers are indicated at the top: 26 S proteasome (2000 kDa), 20 S proteasome (700 kDa), β-galactosidase (540 kDa), 15S proteasome (350 kDa), firefly luciferase (62 kDa), p38 (38 kDa).

Figure 6. Size distribution of recombinant Hsp27 mutants and deletants. Recombinant Hsp27 proteins (Hsp27WT, a-b; Hsp27AA, c; Hsp27EE, d; Hsp27D28-79, e; Hsp27D2-14, f; Hsp27D5-23, g; Hsp27D18-30, h) were prepared as described in Experimental Procedures and analyzed by size exclusion chromatography on Superose 12 HR column. Molecular weight standards are shown on the top axes: dimers of thyroglobulin (340 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa).
Figure 7. Three-dimensional model of Hsp27 based on wheat Hsp16.9. (A) Secondary structure characteristics of Chinese hamster Hsp27 (HaHsp27) were calculated and primary sequences were aligned to wheat Hsp16.9 (We 16.9) structure as described in the text. $b$-strands ($b_2^{\pm}b_9$, blue bars) and $\alpha$-helices (yellow bars) are indicated at the top for HaHsp27 sequence and bottom for We 16.9 sequence. Both proteins lack the $b_1$ strand present in *Methanococcus jannaschii* Hsp16.5 (21). The human Hsp27 sequence (HuHsp27) was aligned by eye to HaHsp27. Residues in human and Chinese hamster Hsp27 that are homologous (green background) or identical (red background) to Hsp16.9 are indicated. The red arrowheads marked the position of the phosphorylatable serines in HaHsp27. (B) Stereoview of the model of Hsp27 (grey and yellow) superimposed onto Hsp16.9 (blue). Grey ribbons represent the residues 2-14 and 28-79 in Hsp27, which are not essential in the structure and function and have no equivalence in Hsp16.9. Note that the two antiparallel $\alpha$-sheets align very well. Heavy atoms of residues Ser15 and W16EPF motif of Hsp27 are displayed in ball-and-stick representation using standard atom colors. N-ter27 and N-ter16.9 indicate the position of the N-terminus of Hsp27 and Hsp16.9, respectively. The C-termini are also marked (C-ter). (C) Stereoview of the monomeric Hsp27 illustrating the masking of the hydrophobic cluster formed by residues Ile96 and Gln98 on strand $b_2$, and Tyr150, Thr151 and Pro153 on strand $b_7$ (green ball-and-stick), by the W16EPF motif (yellow ball-and-stick). For clarity, residues 2-14 and 28-79 are not shown and the C-terminus has been truncated. (D) Stereoview of the dimeric $\alpha$-crystallin region of the model of Hsp27 showing the localization of Ser 90 near the middle of the two $\alpha$-sheets in the monomers and, moreover, near the interface of dimerization of the $\alpha$-crystallin domain, the Ser90 on each monomer facing each other. Phosphorylation of Ser90 (purple ball) would introduce negative charges that could generate highly destabilizing repulsions forces at the hearth of the dimerization interface likely destabilizing
the multimer and forcing a new equilibrium in the phosphorylated dimers. The view angle illustrates that even if it is shorter than the one found in the structure of Hsp16.9, the $\beta_6$ extension is close to $\beta_2$ strand of the opposing monomer and is likely implicated in the formation of the dimeric form of Hsp27. Heavy atoms of S90 and SWEPF motif are displayed in ball-and-stick representation using standard atom color.
REFERENCES

1. Lindquist, S. (1986) Annu.Rev.Biochem. 55, 1151-1191
2. Li, G. C., and Werb, Z. (1982) Proc.Natl.Acad.Sci.USA 79, 3218-3222
3. Subjeck, J. R., and Sciandra, J. J. (1982) in Heat shock from bacteria to man (Schlesinger, M. J., Ashburner, M. J., and Tissières, A., eds), pp. 405-411, Cold Spring Harbor Laboratory, Cold Spring Harbor
4. Landry, J., Bernier, D., Chrétien, P., Nicole, L. M., Tanguay, R. M., and Marceau, N. (1982) Cancer Res. 42, 2457-2461
5. Landry, J., Chrétien, P., Lambert, H., Hickey, E., and Weber, L. A. (1989) J.Cell Biol. 109, 7-15
6. Huot, J., Houle, F., Spitz, D. R., and Landry, J. (1996) Cancer Res. 56, 273-279
7. Garrido, C., Ottavi, P., Fromentin, A., Hammann, A., Arrigo, A. P., Chauffert, B., and Mehlen, P. (1997) Cancer Res. 57, 2661-2667
8. Mehlen, P., Schulze-Osthoff, K., and Arrigo, A. P. (1996) J.Biol.Chem. 271, 16510-16514
9. Rouse, J., Cohen, P., Trigon, S., Morange, M., Alonso-Llamazares, A., Zamanillo, D., Hunt, T., and Nebreda, A. R. (1994) Cell 78, 1027-1037
10. Landry, J., Chrétien, P., Laszlo, A., and Lambert, H. (1991) J.Cell Physiol. 147, 93-101
11. Landry, J., Lambert, H., Zhou, M., Lavoie, J. N., Hickey, E., Weber, L. A., and Anderson, C. W. (1992) The Journal of Biological Chemistry 267, 794-803
12. Huot, J., Lambert, H., Lavoie, J. N., Guimond, A., Houle, F., and Landry, J. (1995) Eur.J.Biochem. 227, 416-427
13. 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
14. Fontaine, J.-M., Welsh, M. J., and Benndorf, R. (2003) Cell Stress Chaperones 8, 62-69
15. Kappé, G., Frank, E., Verschuure, P., Boelens, W. C., Leunissen, J. A. M., and de Jong, W. W. (2003) Cell Stress Chaperones 8, 53-61
16. Ehrnsperger, M., Buchner, J., 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
17. Lambert, H., Charette, S. J., Bernier, A. F., Guimond, A., and Landry, J. (1999) J Biol Chem 274, 9378-9385
18. de Jong, W. W., Leunissen, J. A. M., and Voorter, C. E. (1993) Mol.Biol.Evol. 10, 103-126
19. Bova, M. P., McHaourab, H. S., Han, Y., and Fung, B. K.-K. (2000) J Biol Chem 275, 1035-1042
20. van Montfort, R. L., Basha, E., Friedrich, K. L., Slingsby, C., and Vierling, E. (2001) Nat Struct Biol 8, 1025-1030
21. Kim, K. K., Kim, R., and Kim, S. H. (1998) Nature 394, 595-599
22. Koteiche, H. A., and McHaourab, H. S. (2002) FEBS Lett 519, 16-22
23. Rogalla, T., Ehrnsperger, M., Previle, X., Kotlyarov, A., Lutsch, G., Ducasce, C., Paul, C., Wieske, M., Arrigo, A. P., Buchner, J., and Gaestel, M. (1999) J Biol Chem 274, 18947-18956
24. Miron, T., Vancompernolle, K., Vandekerckhove, J., Wilchek, M., and Geiger, B. (1991) J.Cell Biol. 114, 255-261
29
25. Benndorf, R., Hayess, K., Ryazantsev, S., Wieske, M., Behlke, J., and Lutsch, G. (1994) *J.Biol.Chem.* **269**, 20780-20784
26. Lavoie, J. N., Hickey, E., Weber, L. A., and Landry, J. (1993) *J.Biol.Chem.* **268**, 24210-24214
27. Lavoie, J. N., Lambert, H., Hickey, E., Weber, L. A., and Landry, J. (1995) *Mol.Cell Biol.* **15**, 505-516
28. Guay, J., Lambert, H., Gingras-Breton, G., Lavoie, J. N., Huot, J., and Landry, J. (1997) *J.Cell Sci.* **110**, 357-368
29. Schafer, C., Clapp, P., Welsh, M. J., Benndorf, R., and Williams, J. A. (1999) *Am J Physiol* **277**, C1032-1043
30. Huot, J., Houle, F., Rousseau, S., Deschesnes, R. G., Shah, G. M., and Landry, J. (1998) *J Cell Biol* **143**, 1361-1373
31. Jakob, U., Gaestel, M., Engel, K., and Buchner, J. (1993) *The Journal of Biological Chemistry* **268**, 1517-1520
32. Horwitz, J. (1992) *Proc.Natl.Acad.Sci.U.S.A* **89**, 10449-10453
33. Ehrnsperger, M., Graber, S., Gaestel, M., and Buchner, J. (1997) *EMBO J.* **16**, 221-229
34. Haslbeck, M., Walke, S., Stromer, T., Ehrnsperger, M., White, H. E., Chen, S., Saibil, H. R., and Buchner, J. (1999) *Embo J* **18**, 6744-6751
35. Giese, K. C., and Vierling, E. (2002) *J Biol Chem* **277**, 46310-46318
36. Feil, I. K., Malfois, M., Hendle, J., van Der Zandt, H., and Svergun, D. I. (2001) *J Biol Chem* **276**, 12024-12029
37. Charette, S. J., Lavoie, J. N., Lambert, H., and Landry, J. (2000) *Mol Cell Biol* **20**, 7602-7612
38. Geum, D., Son, G. H., and Kim, K. (2002) *J Biol Chem* **277**, 19913-19921
39. Wytenbach, A., Sauvageot, O., Carmichael, J., Diaz-Latoud, C., Arrigo, A. P., and Rubinsztein, D. C. (2002) *Hum Mol Genet* **11**, 1137-1151
40. Mehlen, P., Hickey, E., Weber, L. A., and Arrigo, A. P. (1997) *Biochem Biophys Res Commun* **241**, 187-192
41. Dorion, S., and Landry, J. (2002) *Cell stress Chaperones* **7**, 200-206
42. Gaestel, M., Schroder, W., Benndorf, R., Lippmann, C., Buchner, K., Hucho, F., Erdmann, V. A., and Bielka, H. (1991) *The Journal of Biological Chemistry* **266**, 14721-14724
43. Stokoe, D., Engel, K., Campbell, D. G., Cohen, P., and Gaestel, M. (1992) *FEBS Lett.* **313**, 307-313
44. Dorion, S., Lambert, H., and Landry, J. (2002) *J Biol Chem* **277**, 30792-30797
45. Lavoie, J. N., Gingras-Breton, G., Tanguay, R. M., and Landry, J. (1993) *The Journal of Biological Chemistry* **268**, 3420-3429
46. Ehrnsperger, M., Lilie, H., Gaestel, M., and Buchner, J. (1999) *J Biol Chem* **274**, 14867-14874
47. Cuff, J. A., and Barton, G. J. (2000) *Proteins* **40**, 502-511
48. Rost, B., and Sander, C. (1993) *J Mol Biol* **232**, 584-599
49. Gough, J., Karplus, K., Hughey, R., and Chothia, C. (2001) *J Mol Biol* **313**, 903-919
50. Carson, M. (1997) *Methods in Enzymology* **277**, 493-505
51. Wistow, G. (1985) *FEBS Lett.* **181**, 1-6
52. de Jong, W. W., Leunissen, J. A., Leenen, P. J., Zweers, A., and Versteeg, M. (1988) *The Journal of Biological Chemistry* **263**, 5141-5149
53. Chrétien, P., and Landry, J. (1988) *J.Cell Physiol.* **137**, 157-166
54. Leroux, M. R., Melki, R., Gordon, B., Batelier, G., and Candido, E. P. (1997) *J Biol Chem* **272**, 24646-24656
55. Paul, C., Manero, F., Gonin, S., Kretz-Remy, C., Virot, S., and Arrigo, A.-P. (2002) *Mol. Cell. Biol.* **22**, 816-834
56. Bruey, J. M., Ducasse, C., Bonniaud, P., Ravagnan, L., Susin, S. A., Diaz-Latoud, C., Gurbuxani, S., Arrigo, A. P., Kroemer, G., Solary, E., and Garrido, C. (2000) *Nat Cell Biol* **2**, 645-652
57. Wieske, M., Benndorf, R., Behlke, J., Dolling, R., Grelle, G., Bielka, H., and Lutsch, G. (2001) *Eur J Biochem* **268**, 2083-2090
58. Parcellier, A., Schmitt, E., Gurbuxani, S., Seigneurin-Berny, D., Pance, A., Chantome, A., Plenchette, S., Khochbin, S., Solary, E., and Garrido, C. (2003) *Mol Cell Biol* **23**, 5790-5802
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7
Essential role of the N-terminal WD/EPF motif in the phosphorylation-activated protective function of mammalian Hsp27
Jimmy R. Thériault, Herman Lambert, Aura T. Chávez-Zobel, Gabriel Charest, Pierre Lavigne and Jacques Landry

J. Biol. Chem. published online March 21, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M402325200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts