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LARGE POTENTIALS OF SMALL HEAT SHOCK PROTEINS

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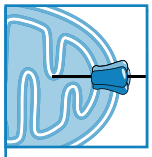

Mymrikov EV, Seit-Nebi AS, Gusev NB. Large Potentials of Small Heat Shock Proteins. *Physiol Rev* 91: 1123–1159, 2011; doi:10.1152/physrev.00023.2010.—Modern classification of the family of human small heat shock proteins (the so-called HSPB) is presented, and the structure and properties of three members of this family are analyzed in detail. Ubiquitously expressed HSPB1 (HSP27) is involved in the control of protein folding and, when mutated, plays a significant role in the development of certain neurodegenerative disorders. HSPB1 directly or indirectly participates in the regulation of apoptosis, protects the cell against oxidative stress, and is involved in the regulation of the cytoskeleton. HSPB6 (HSP20) also possesses chaperone-like activity, is involved in regulation of smooth muscle contraction, has pronounced cardioprotective activity, and seems to participate in insulin-dependent regulation of muscle metabolism. HSPB8 (HSP22) prevents accumulation of aggregated proteins in the cell and participates in the regulation of proteolysis of unfolded proteins. HSPB8 also seems to be directly or indirectly involved in regulation of apoptosis and carcinogenesis, contributes to cardiac cell hypertrophy and survival and, when mutated, might be involved in development of neurodegenerative diseases. All small heat shock proteins play important "housekeeping" roles and regulate many vital processes; therefore, they are considered as attractive therapeutic targets.

I. INTRODUCTION

A large group of the so-called heat shock proteins was initially discovered in different *Drosophila* tissues during recovery following a transiently mild and sublethal increase of normal core body temperature (318). Synthesis of these proteins was accompanied by the increased tolerance not only to high temperature (heat shock), but also to many other unfavorable conditions such as hypoxia, ischemia, and stress factors like endotoxins, heavy metals, organic solvents, and reactive oxygen species. Enhanced resistance to different kind of stresses was described in a great variety of organisms including bacteria, plants, and animals, and it was shown to be due to the ability of heat shock proteins to protect the cell against different kind of stresses. Some of these proteins are constitutively expressed in the cell and are responsible for quality control of protein folding, whereas expression of other proteins of this group is strongly increased in response to unfavorable conditions.

The modern classification of the human heat shock proteins contains the following main groups: HSPH (former name HSP110), HSPC (HSP90), HSPA (HSP70), HSPD/E (HSP60/HSP10) and CCT (TRiC), DNAJ (HSP40), and HSPB (small HSP or sHSP) (163, 334). Functioning of some of these proteins (HSPH, HSPA, HSPD/E, CCT) is ATP dependent, and they are involved in cotranslational or posttranslational protein folding. Other proteins (such as DNAJ) (334) play the role of cochaperones modulating activity of the other heat shock proteins (HSPA), whereas the small heat shock proteins (HSPB) are responsible for the binding of improperly folded protein-substrates and their further transfer to the ATP-dependent chaperones or to the protein degradation machines like proteasomes or autophagosomes (137, 334). Heat shock proteins form a complex network, as many of the members seem to interact and cross-react with each other. In this way the resulting network efficiently controls protein folding, preventing the accumulation of denatured or improperly folded proteins in the cell (94, 136, 334). Heat shock proteins play an important "housekeeping" role, and therefore, any damages in the heat shock proteins network are very dangerous for the cell. Recent investigations indicate that the small heat shock proteins participate in regulation of many intracellular processes, and mutations of certain members of this protein family lead to cell dysfunctions that correlate with development of different congenital diseases (22, 194, 311). These facts have attracted the interest of many scientists, and several comprehensive reviews (6, 7, 88, 101, 131, 132, 136, 137, 145, 221, 232, 277, 288, 334) highlighted different aspects of the structure and function of the small heat shock proteins. However, information in this field is accumulating very rapidly, and new roles of sHSP, important for physi-

ology and medicine, have emerged recently. This review deals with the description of three small heat shock proteins, namely, HSPB1, HSPB6, and HSPB8, with special emphasis on the functioning of these proteins under normal and stress-related conditions and their probable participation in different physiological processes.

II. STRUCTURE AND PROPERTIES OF SMALL HEAT SHOCK PROTEINS

The large family of small heat shock proteins is expressed throughout nearly all kingdoms (except for certain bacteria species) and contains a large number of proteins with monomer molecular mass in the range of 12–43 kDa (114, 136). All proteins of this family contain the so-called α-crystallin domain, a region of ~90 residues (185) that is homologous to the corresponding region in the primary structure of the main eye lens proteins αA- and αB-crystallin. This domain is considered as an important hallmark of small heat shock protein, independent of their origin and nature (165). The α-crystallin domain consists of six to eight β-strands organized in two β-sheets flanked by the NH_2- and COOH-terminal regions, which are variable in length and structure. Very often the NH_2-terminal region contains a number of sites phosphorylated by different protein kinases (137, 334) **(FIG. 1)**. As a rule, the small heat shock proteins tend to form highly mobile oligomers [starting from easily dissociating dimers (HSPB8) to multimers of more than 20 subunits (HSPB1, HSPB5)] of different size and composition, and this significantly complicates structural investigations. At present, the three-dimensional structure has been determined only for HSP16.5 of *Methanococcus jannaschii* (174), HSP16.9 of *Triticum aestivum* (326), and Tsp36 of parasitic flatworm *Taenia saginata* (300). In all of these cases, a short β6 strand of one monomer interacts with the β2 strand of another monomer (174, 300, 326). A dimer is the building block of large oligomers that are stabilized by the interaction of mobile NH_2- and COOH-terminal regions with an α-crystallin core of a neighboring dimer. Depending on the length and orientation of the

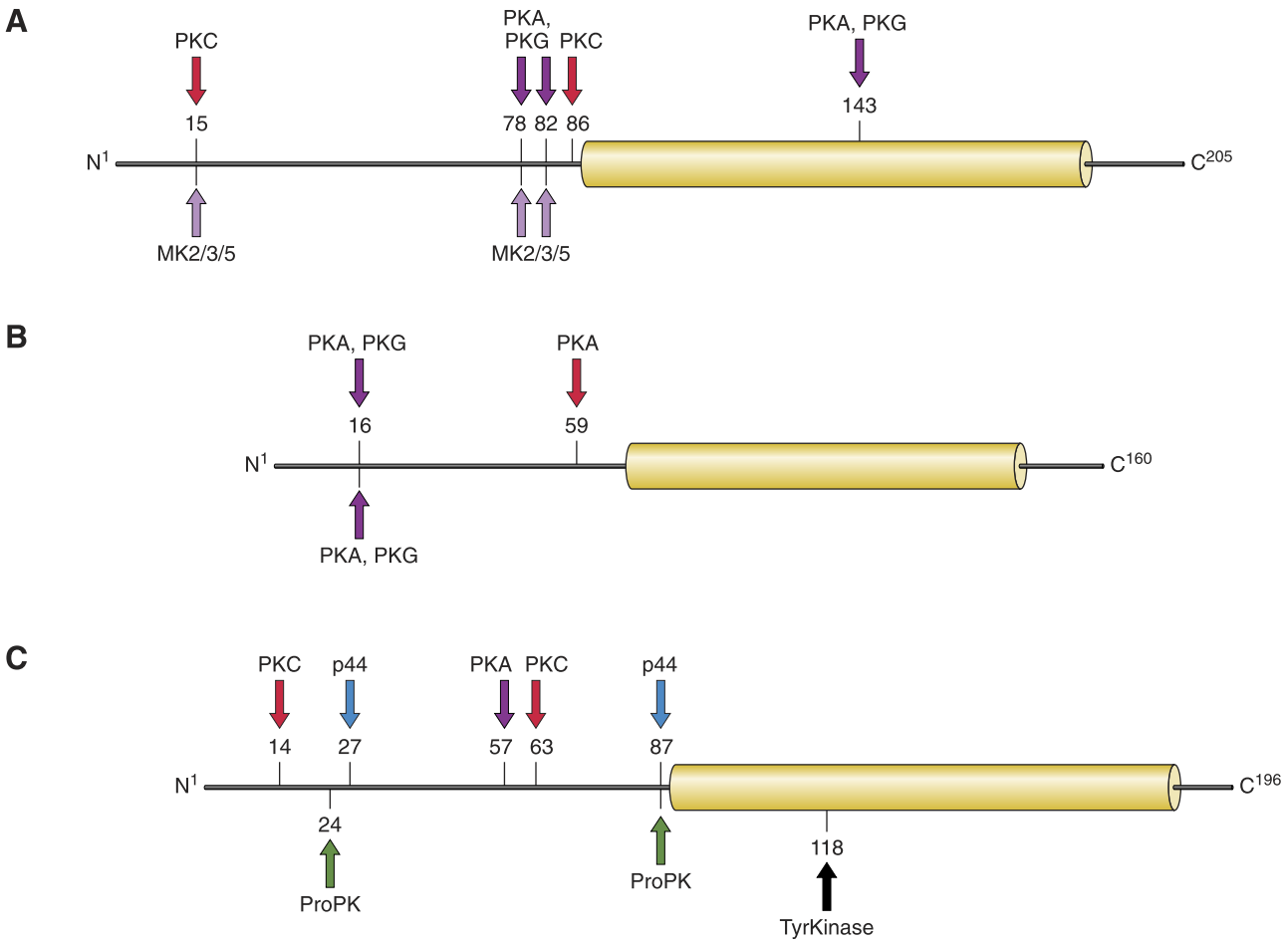

FIGURE 1. Linear representation of HSPB1 (*A*), HSPB6 (*B*), and HSPB8 (*C*). α-Crystallin domain is shown as yellow cylinder. Differently colored arrows above each scheme represent the sites phosphorylated in vitro; arrows beneath the scheme represent the sites determined in vivo. PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; p44, ERK1 protein kinase; MK2/3/5, mitogen-activated protein kinase activated protein kinases; ProPK, Pro-directed protein kinases; TyrKinase, protein kinases phosphorylating Tyr.

flexible NH_2- and COOH-terminal extensions, the small heat shock proteins can form different oligomers **(FIG. 2)**. In the case of HSP16.5 of *M. jannaschii* , 24 monomers form a hollow sphere (174), in the case of HSP16.9 of *T. aestivum* and HSP21 of *Arabidopsis thaliana* chloroplasts, 12 monomers form two hexameric rings (191, 326), and in the case of Tsp36 of *T. saginata,* two dimers shown in **FIGURE 2*C*** (each monomer of this dimer contains 2 α-crystallin domains) tend to form a tetramer (300).

The primary structure of all crystallized small heat shock proteins of *Archaea*, plants, and flatworm markedly differ from their mammalian counterparts. All attempts to crystallize full-size human proteins have been until now unsuccessful. However, three-dimensional structures were determined for the α-crystallin domain lacking the flexible NH_2- and COOH-terminal regions of bovine and human αA- and αB-crystallins and rat HSPB6 (13, 188). Very recently, the structure of full-size αB-crystallin (HSPB5) was determined by means of solid-state NMR and small-angle X-ray scattering (161). In all these cases, monomers have very short loops connecting β5 and β7 strands and therefore cannot exchange β-strands. The contacts between monomers inside of a dimer are provided by the interaction of antiparallel oriented elongated β7 strands belonging to two neighboring α-crystallin domains (13, 188). Exact orientation of the flexible NH_2- and COOH-terminal ends remains unknown, but it is thought that the polydispersity of large oligomers of human small heat shock proteins could be due to at least one of the following: the sliding of two antiparallel β-strands in the region of monomer-monomer contacts (188), different orientations of the flexible COOH-terminal regions (221), and/or different orientations and interaction of flexible NH_2-terminal regions with the α-crystallin core domain (13). Therefore, human small heat shock proteins have very flexible quaternary structure, and, depending on intracellular conditions and posttranslational modifications (phosphorylation, *S*-thiolation, glyoxylation, etc.), they can undergo structural changes leading to either reversible changes in oligomeric substructure (19) or reversible cycles of dissociation and association (221). Both of these processes modulate the interaction of HSPB with unfolded target proteins and their chaperone-like activity (136, 137, 221). Having similar primary structure and being presented in a rather high concentration in the cell, different small heat shock proteins can interact with each other to generate hetero-oligomers of various sizes and compositions (21, 30, 39, 305, 309). This ability to form oligomers highly heterogeneous in size and composition dramatically expands the spectrum of sHSP properties and functions in the cell.

The human genome encodes 10 members of the HSPB family **(TABLE 1)**. These proteins have molecular mass in the range of 17.0–28.4 kDa and contain a conservative α-crystallin domain. Some of these proteins (HSPB1, HSPB5, HSPB6, HSPB8) are ubiquitously expressed in practically all cell types, whereas others (HSPB3, HSPB4, HSPB7, HSPB9, and HSPB10) are less abundantly expressed and their level is high only in certain tissues (314, 330). Practically all members of this family have chaperone-like activity in vitro. This means that sHSP prevent aggregation of partially denatured protein in the test tube. Unfortunately, we know very little about whether the chaperone-like activity is really a physiologically important property of sHSP and whether this activity plays a crucial role in protecting the cell from accumulating aggregates of denatured or unfolded proteins. In addition, each member of this family has unique properties. Thus all members of HSPB family are important for cell functioning under normal and stress conditions. Since HSPB plays diverse and important roles, many mutations of these proteins described in the literature correlate with the development of a number of congenital diseases such as cataracts, different types of myopathy, and certain neurodegenerative disorders **(TABLE 2)**. Detailed mechanisms underlying development of these diseases remain elu-

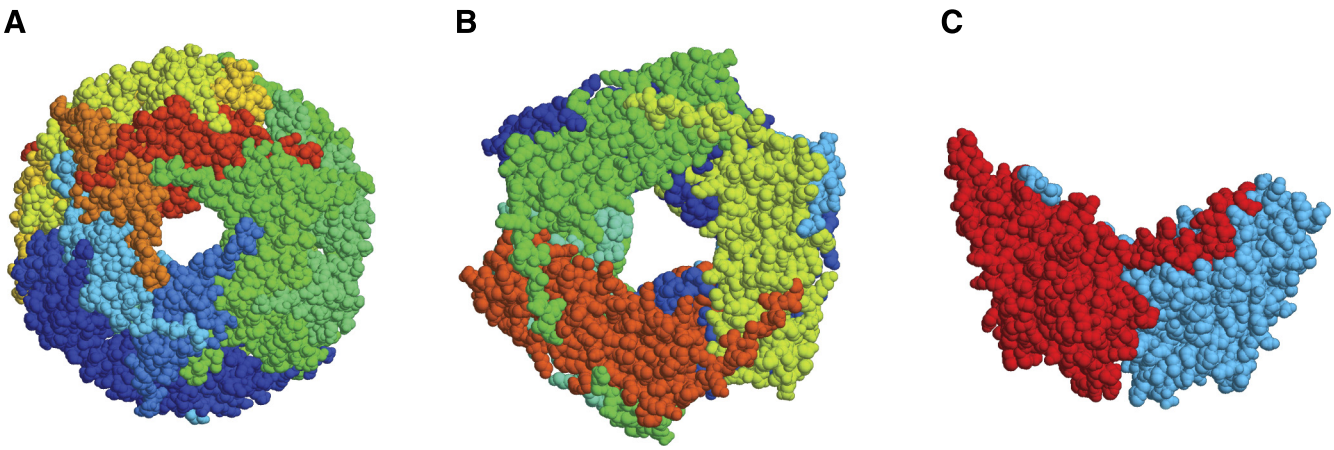

FIGURE 2. Oligomeric structure of HSP16.5 of *Methanococcus jannaschii* (*A*) (PDB accession no. 1SHS) (24 subunits differently colored), HSP16.9 of *Triticum aestivum* (*B*) (1GME) (12 subunits differently colored), and Tsp36 of *Taenia saginata* (*C*) (2BOL) (dimer composed of two monomers each containing two α-crystallin domains). Figures were prepared by RasMol program.

Table 1. *Some properties of human small heat shock proteins*

Name	Synonyms	UnitProtKB Database Accession Number	Chromosome Location	Number of Amino Acid Residues	Tissue Distribution	Heat Induction	Associated Diseases
HspB1	Hsp25, Hsp27, Hsp28	P04792	7q11.23	205	Ubiquitous	Yes	Charcot-Marie-Tooth disease type 2, distal hereditary motor neuropathy
HspB2	MKBP, myotonic dystrophy protein kinase binding protein	Q16082	11q22-q23	182	Cardiac and skeletal muscle	No	Myotonic dystrophy, different forms of neuropathology
HspB3		Q12988	5q11.2	150	Cardiac and skeletal muscle	No	Motor neuropathy
HspB4	αA-Crystallin	P02489	21q22.3	173	Eye lens	No	Cataract
HspB5	αB-Crystallin	P02511	11q22.3-q23.1	175	Ubiquitous	Yes	Cataract, desmin-related and myofibrillar myopathy
HspB6	Hsp20, p20	O14558	19q13.12	157	Ubiquitous	No	Dilated cardiomyopathy(?)
HspB7	cvHsp, cardiovascular heat shock protein	Q9UBY9	1p36.23-p34.3	170	Cardiac and skeletal muscle	?	Upregulated in muscular dystrophy
HspB8	Hsp22, H11 protein kinase, product of E2IG1 gene	Q9UKS3	12q24.23	196	Ubiquitous	Cell type dependent	Charcot-Marie-Tooth disease type 2, distal hereditary motor neuropathy
HspB9		Q9BQS6	17q21.2	159	Testis	?	Upregulated in certain tumors
HspB10	ODF1, outer dense fiber proteins	Q14990	8q22.3	250	Testis	?	?

sive; however, it is thought that HSPB might play important roles in the development of these disorders.

The literature concerning the structure, properties, and physiological role of all HSPB is extensive and cannot be overviewed in detail. We chose three members of the HSPB family, namely, HSPB1, HSPB6, and HSPB8, and using these proteins as an example, we try to illustrate what a large potential the small heat shock proteins have in the cell.

III. SMALL HEAT SHOCK PROTEIN HSPB1 (HSP25, HSP27, OR HSP28)

Human HSPB1 was described in the beginning of the 1980s when it was found that incubation of HeLa cells at elevated temperature was accompanied by synthesis of a previously unknown protein with apparent molecular mass of 27 kDa (142). The primary structure of HSPB1 is highly homologous to that of α-crystallin, the main eye lens protein (141). The genes coding similar proteins were detected earlier in many other species, and their products were marked as HSP25, HSP27, or HSP28. The human *HSPB1* gene is located in chromosome 7q11.23, and two pseudogenes are detected in the ninth and X-chromosomes (302). The protein product of this gene is referred to as HSP27, and called HSPB1 (166) in the modern classification.

A. Tissue and Cellular Distribution of HSPB1 and Factors Affecting Its Expression

HSPB1 is ubiquitously expressed in all human tissues with the highest level of expression in skeletal, smooth, and cardiac muscles (305) where its content can reach up to 3 mg/g of total proteins (170). Expression of HSPB1 can be dramatically changed during ontogenesis (211) and under certain pathological conditions such as cardiac diseases (120, 211, 343), renal ischemia (293), and nonobese type 2 diabetes (233). An especially large increase of HSPB1 expression was detected in many types of tumors (10, 65, 118, 148). However, these correlations are not universal, and in certain tumors (such as esophageal carcinoma that ends in adenocarcinomas, adrenal adenoma), the level of HSPB1 is decreased (65, 204). Increased expression of HSPB1 in tumors is accompanied

Table 2. *Mutation of human small heat shock proteins associated with congenital diseases*

Protein	Mutations	Disease	Reference Nos.
HSPB1 (HSP27)	P39L	Distal hereditary motor neuropathy	98, 146, 153, 159, 173
	G84R		
	L99M		
	R127W		
	S135F		
	R140G		
	K141Q		
	T151I		
	T180I		
	P182S		
	P182L		
	R127W	Charcot-Marie-Tooth disease type 2	98, 146, 210, 313
	S135F		
	R136W		
	T180I		
HSPB3	R7S	Motor neuropathy	181
HSPB4 (αA-crystallin)	W9X	Different forms of cataracts	24, 80, 129, 130, 133, 172, 202, 212, 259, 280
	R12C		
	R21W		(Reviewed in 145, 187, 194)
	R21L		
	R49C		
	R54C		
	F71L		
	G98R		
	R116C		
	R116H		
HSPB5 (αB-crystallin)	R11H	Different forms of cataracts	23, 58, 80, 172, 203
	R56W		
	R120G		
	D140N		
	A171T		
	450delA		
	R120G	Dilated cardiomyopathy	154, 254
	G154S		
	R157H		
	S21AfsX24	Myofibrillar myopathy and desmin-related myopathy	284, 285, 331
	R120G		
	Q151X		
	G154S		
	464delCT		
HSPB6 (HSP20)	P20L	Abrogation of cardioprotective effect of HSPB6	237
HSPB8 (HSP22)	K141N	Distal hereditary motor neuropathy	157
	K141E		
	K141N	Charcot-Marie-Tooth disease type 2	313

by elevated cellular resistance to a number of chemotherapeutic drugs (doxorubicin, colchicine, and vincristine) (66, 118, 328). Certain hormones and biologically active compounds also affect HSPB1 expression leading either to a decreased (186, 354) or increased (59) level of HSPB1 in the cell. Addition of heavy metals [Hg(II)] (299), organic solvents, or elevated temperatures (18, 142, 144, 179) are also accompanied by increased ex-

pression of HSPB1. The temperature-dependent increase of HSPB1 expression seems to be dependent on the functioning of heat shock factors (HSF) which interact with two regulatory heat shock elements (HSE) in the structure of *HSPB1* gene (reviewed in Refs. 277, 301).

Cellular localization of HSPB1 seems to be dependent on the state of the cell. In weakly differentiated cardiomyocytes, HSPB1 is predominantly located in the cytosol, whereas in differentiated cells this protein was found in I-bands and Z-discs. Morphological methods indicate that in mature cardiomyocytes HSPB1 is colocalized with actin (144, 198, 211). Combined utilization of indirect immunofluorescence and cell fractionation lead to the conclusion that under normal conditions in keratinocytes, HSPB1 is predominantly located in the cytosol and smaller portions of this protein are located in the membrane and cytoskeletal fractions. Heat shock is accompanied by translocation of HSPB1 to the nucleus and increasing of HSPB1 level in the cytoskeleton (219). Different morphological methods (38, 204, 262, 323) as well as different methods of cell fractionation (38, 205, 262, 276, 323) demonstrated similar distribution of HSPB1 in many different cells (although certain differences were reported for HSPB1 distribution in different human breast cancer cell lines; Ref. 262). Stress-induced translocation of HSPB1 to the cytoskeleton and nucleus is thought to be important for protection of the cell from unfavorable conditions and improves cell survival.

B. Physicochemical Properties and Structure of HSPB1

Human HSPB1 (UniProt accession no. P04792) contains 205 residues **(TABLE 1)**. Its structure consists of an NH_2-terminal sequence that includes the so-called WDPF-motif, conservative α-crystallin domain, and a short COOH-terminal sequence. Both WDPF-motif and α-crystallin domain seem to be important for HSPB1 oligomerization (190). HSPB1 contains the single Cys residue (Cys137) located in the β7 strand of α-crystallin domain. This strand plays an important role in the monomer-monomer interaction, and Cys residues of two neighboring monomers can form intermolecular disulfide bonds leading to accumulation of cross-linked HSPB1 dimers (234, 357).

HSPB1 tends to form variable size oligomers having apparent molecular masses of up to 800 kDa (60, 139, 190, 273). The state of oligomerization, among others, depends on posttranslational modifications (e.g., phosphorylation and thiolation) of HSPB1. Phosphorylation leads to formation of small oligomers (139, 169, 273), or even monomers of HSPB1 (200). However, existence of stable monomers of HSPB1 remains ambiguous since experimental data using microcalorimetry (89) and sedimentation (96) indicate that the dimers are the minimal structural units of large HSPB1 oligomers. The size of oligomers formed by HSPB1 is also dependent on the pH and temperature. For instance, increase in the temperature or decrease in the pH leads to the formation of large oligomers of HSPB1 both in vitro (60, 199) and in vivo (37).

C. Posttranslational Modifications of HSPB1

HSPB1 undergoes different types of posttranslational modifications. For instance, HSPB1 is the main methylglyoxal (MGO) modified protein in mesanglial kidney cells (243), in endothelial cells under conditions of hyperglycemia (282), in brunescent cataract lenses (242), and in different cancer cells that are characterized by high level of glycolysis (275, 325). Modification of Arg188 by methylglyoxal leads to the formation of argininopyrimidine, which is accompanied by a change in the oligomeric state of HSPB1 (275) and increase of its chaperone-like activity, as measured in vitro (235). Modification by methylglyoxal reduces the in vitro interaction of HSPB1 with cytochrome *c* (243); however, at the same time, this modification increases the antiapoptotic activity of HSPB1 (242, 275, 325) by a mechanism that is not completely understood.

Under conditions of oxidative stress and during ischemia and reperfusion, HSPB1 undergoes *S*-thiolation (91, 92) and formation of mixed disulfides between Cys137 of HSPB1 and low molecular mass thiols such as glutathione, cysteine, homocysteine, etc. (92, 358). *S*-thiolation leads to dissociation of large HSPB1 oligomers (92). Moreover, formation of mixed disulfide with glutathione can precede cross-linking of two neighboring HSPB1 monomers via formation of disulfide bond between two Cys137 (92).

HSPB1 undergoes multisite phosphorylation catalyzed by a number of different protein kinases (183), and Ser15, Ser78, and Ser82 are the main sites that are phosphorylated in vivo **(FIG. 1*A*)**. In the past, there have been controversial data regarding the identity of protein kinase that is responsible for phosphorylation of these sites; however, there is a general agreement upon the fact that MK2 (MAPKAP2) kinase is definitely involved in this process (11, 193, 303). Phosphorylation by MK2 leads to dissociation of the large oligomers of HSPB1 and formation of smaller oligomers, probably dimers (139, 169, 190) or tetramers (273). Phosphomimicking mutant harboring three aspartate residues in place of Ser15, Ser78, and Ser82 (the so-called "3D mutant") has similar, but not identical properties to phosphorylated HSPB1 (139). This mutant was used for analyzing the effect of phosphorylation on different HSPB1 properties. Phosphorylation (or phosphomimicking mutations) affects not only the oligomeric state but also the chaperone-like activity of HSPB1. The data regarding the effect of phosphorylation on chaperone-like activity of HSPB1 are rather controversial and seem to depend on the nature of

model protein substrate and on the origin of the small heat shock protein (245). Chaperone-like activity is an integral parameter that depends on the number of sites binding denatured protein, on the affinity of these sites to the model protein substrate, as well as on the ability of small heat shock proteins to undergo conformational changes, which affects oligomer dynamics and are often accompanied by association/dissociation of large HSPB1 oligomers. Because phosphorylation might affect any of these processes and because these effects depend both on the nature of small heat shock proteins and their substrates, it is difficult to make any straightforward conclusions on the effect of phosphorylation on chaperone-like activity of HSPB1 or its orthologs.

D. Mutations of HSPB1 and Their Biomedical Significance

All mutations described for HSPB1 can be divided into two groups. The first group consists of mutations inside of the α-crystallin domain, whereas the second group combines mutations outside of this domain. Many mutations of the first group (R127W, S135F, R136W, R140G, K141Q) (1, 194) are located in the β7-strand or in the loop connecting the β5 and β7 strands. This part of the molecule is involved in the intersubunit contacts, and mutations in this part of the molecule might affect monomer-monomer interaction and formation of high-order oligomers (234). Indeed, mutations S135F and R136W lead to dissociation of HSPB1 dimers without a dramatic effect on the structure of large oligomers (1). These mutants possessed higher chaperone-like activity and were more effective than the wild-type HSPB1 in protecting the cells against heat shock (1). At the same time, expression of these mutants correlates with the development of certain neurodegenerative diseases (1). These results remain unexplained; however, it is possible that these mutations affecting the structure and hydrophobicity of HSPB1 modify interaction of HSPB1 with different target proteins and/or interfere with transfer of target proteins to ATP-dependent chaperone systems. As already mentioned, mutations S135F and R136W lead to increased hydrophobicity, and this can affect solubility of HSPB1. Indeed, these mutations increase the probability of formation of insoluble aggregates inside of the cell (159). Mutation S135F increases interaction of HSPB1 with HSPB6 and probably leads to accumulation of protein aggregates (112). It still remains unclear whether the toxic effect induced by these mutations in the α-crystallin domain is due to the decrease of putative chaperone-like activity (decreased protein quality control function) or to the accumulation of sHSP aggregates. Overexpression of fused protein consisting of enhanced green fluorescent protein (EGFP) and S135F mutant (EGFP-S135F mutant) leads to disruption of the neurofilament net and decreased survival compared with overexpression of EGFP-wild type HSPB1 (98, 194). Thus certain mutations inside of the α-crystallin domain impair intersubunit interaction and affect oligomerization of HSPB1. As a rule, mutations inside of the α-crystallin domain (L99M, R127W, S135F, R136W, R140G, K141Q) correlate with the development of distal hereditary motor neuropathy (dHMN) and/or Charcot-Marie-Tooth (CMT) type 2 disease (22, 146, 194).

Mutations outside of the α-crystallin domain of HSPB1 are less thoroughly investigated. However, it is well-known that the certain areas of the variable NH_2- and COOH-terminal parts of small heat shock proteins play an important role in formation of high-order oligomers (57, 221, 317). Mutation P182L promotes formation of insoluble aggregates and completely abolishes the thermoprotective effect of HSPB1 (159). Mutation G84R located in the NH_2-terminal part, in close vicinity to the sites of HSPB1 phosphorylation (Ser78 and Ser82), is also accompanied by formation of insoluble aggregates, the appearance of which seems to be dependent on improper interaction of HSPB1 subunits. Mutations in the variable NH_2- and COOH-terminal ends (P39L, G84R, P182L) usually correlate with the development of distal hereditary motor neuropathy.

Although the correlations between HSPB1 mutations and congenital diseases are well-established, detailed mechanisms underlying participation of HSPB1 in the development of these diseases remain enigmatic. Further investigations are required to understand the role of HSPB1 in these diseases.

E. Interaction of HSPB1 With Other Small Heat Shock Proteins

In earlier publications, it has been shown that HSPB1 is copurified with αB-crystallin (HSPB5) and HSPB6, and it was supposed that these three proteins form tight complexes with each other and do not practically interact with HSPB2 and HSPB3 (168, 170, 305, 356). The complexes formed by HSPB1 and αA- or αB-crystallin detected both in vitro and in vivo have high molecular mass (~600–800 kDa) (30, 356) and easily exchange their subunits (30). Under in vitro conditions, HSPB1 forms two types of complexes with HSPB6 (39, 41). Formation of these complexes is temperature dependent. Both complexes with apparent molecular masses of 100–150 and 250–300 kDa contain equimolar quantities of HSPB1 and HSPB6 (39). The interaction of HSPB1 and HSPB8 in vitro and especially in vivo remains controversial. Earlier investigations indicated that HSPB8 interacts only with phosphomimicking mutant of HSPB1, but not with the wild-type protein (21). Later, weak interaction of the wild-type HSPB1 and HSPB8 was also established (309, 310). It is possible that formation of hetero-oligomeric complexes (which is especially possible in the case of simultaneous high expression of different

small heat shock proteins in the same cell) will significantly change the structure and properties of each component and by this means affect their participation in different intracellular processes.

F. Control of Protein Folding and Interaction of HSPB1 With Different Protein Partners

HSPB1 (as well as other small heat shock proteins) possesses chaperone-like activity and prevents aggregation of improperly folded or partially denatured proteins in vitro. HSPB1 and its orthologs interact with a number of different model substrate proteins such as citrate synthase, rhodanese, α-glucosidase, insulin, etc., forming highly soluble and stable complexes (304). Similar highly soluble complexes were described for HSPB1 or its orthologs with many partially denatured enzymes (such as malate dehydrogenase, alcohol dehydrogenase, lactate dehydrogenase, luciferase, lysozyme) and α-lactalbumin. In addition to these model substrates, HSPB1 interacts with and prevents aggregation of thermally denatured F-actin and S1 fragment of myosin (213, 256, 257). HSPB1 also interacts with intrinsically disordered proteins such as sinuclein and tau proteins, as well as with amyloid peptides, and modulates their irreversible aggregation and development of certain neurodegenerative diseases (see next section). All of these data mean that HSPB1 interacts with a plethora of different partially denatured or misfolded proteins and prevents their aggregation. This type of interaction might be of great importance in conferring thermotolerance and resistance to any other types of stress (221). Mutations and modification of oligomeric states induced by phosphorylation affect interaction of HSPB1 with target proteins, thus modifying its chaperone-like activity (reviewed in Ref. 221). For instance, different kinds of stresses induce phosphorylation of HSPB1 by MAPKAP2 (MK2) protein kinase (38, 205, 276). This process often correlates with the intracellular translocation of HSPB1 from the cytosol to the nucleus, contractile apparatus, or cytoskeleton (38, 205, 219, 276, 323). This translocation can be important for the protection of the actin cytoskeleton from stress-induced disruption and damages (121, 151), for protection of certain nuclear proteins, or accelerated elimination of denatured proteins (38). It is thought that the client proteins, which are bound to HSPB1 in a partially unfolded state, can be transferred to ATP-dependent chaperones such as HSP70-HSP40 or HSP100 where they are refolded (94, 136, 137).

If unfolded client proteins cannot be refolded by ATP-dependent chaperones, then they can be presented to proteasomal-dependent degradation, a process in which HSPB1 seems to play an important role. Recently published data (247, 248) indicate that under stress conditions HSPB1 promotes ubiquitylation of certain proteins, which increases the rate of their proteasomal degradation (359). In addition, there is substantial indirect evidence indicating tight interaction between the proteasomal system and HSPB1. For instance, it is known that the cell death induced by administration of proteasome inhibitor can be at least partly prevented by overexpression of HSPB1 (55). Moreover, HSPB1 protected astrocytes against stress, induced by proteasomal inhibitor MG-132, and was able to retard the accumulation of aggresomes in the cell (27, 125). These data indicate that HSPB1 is somehow involved in ubiquitin-dependent degradation of client proteins in 26S proteasomes. It is also thought that HSPB1 can participate in ubiquitin-independent proteasomal degradation of client proteins in 20S proteasomes, and this seems to be especially important for misfolded proteins accumulated after oxidative stress (215).

Although HSPB1 predominantly interacts with misfolded proteins, this does not mean that HSPB1 is unable to interact and modulate activity and/or intracellular location of native proteins. Of special interest are the data on HSPB1 modulation of protein kinase activity. It is postulated that HSPB1 binds and activates Akt/protein kinase B possessing antiapoptotic activity and at the same time inhibits the prodeath JNK pathway (reviewed in Ref. 301). The murine ortholog of HSPB1 directly interacts with protein kinase C-δ, inhibiting its activity and translocation to the membrane and thus blocking apoptosis (196). In rabbit smooth muscle, HSP27 (ortholog of HSPB1) seems to modulate association and translocation of RhoA and protein kinase C-α (26). Serving as an adapter, HSP27 can provide an interface for interaction between two signaling proteins and by this means might participate in the transduction of different signals. This hypothesis agrees with recently published data indicating that HSPB1 is involved in regulation of cell growth by affecting signaling through extracellular signal-regulated protein kinase (218). HSPB1 can also participate in signal transduction interacting with the androgen receptor and facilitating transcriptional activity of this receptor (360). Interaction of HSPB1 (or its orthologs) with protein kinases and steroid receptor is modulated by HSPB1 phosphorylation, thus providing additional mechanisms of regulation (183, 301). The majority of the above-cited data were predominantly obtained from the cells overexperessing HSPB1 (or its phosphomimicking mutants) and interaction of HSPB1 with different protein kinases, as well as modulation of their activity upon interaction, was demonstrated either by coimmunoprecipitation or by using more or less specific protein kinase inhibitors. These approaches are useful for establishing certain correlations; however, further detailed investigations are needed to confirm evidence of the direct participation of HSPB1 in regulation of signal transduction.

G. HSPB1 and Neurodegenerative Disorders

Many neurodegenerative disorders are accompanied by accumulation of insoluble protein aggregates or amyloid fi-

brils in neurons or glia cells. Trying to avoid accumulation of protein aggregates, the cell increases expression of HSPB1 and αB-crystallin. In neurons of patients with Alzheimer's and Parkinson's disease, HSPB1 is colocalized with Lewy's bodies (222, 241), neurofibrillar tangles (236), and senile plaques formed by β-amyloid (290, 346). HSPB1 is able to prevent α-sinuclein aggregation in the culture of neurons overexpressing α-sinuclein and thus increases neuron survival (241, 361). Increased expression of HSPB1 in the case of dementia with Lewy's bodies is probably one of the ways for decreasing cytotoxicity induced by misfolded and aggregated α-synuclein (241). Similar effects were detected in the case of transgenic mice with amyotrophic lateral sclerosis. Increased levels of HSPB1 and αB-crystallin were detected in neurons of the spinal cord of these mice (333), and overexpression of HSPB1 in these animals increased survival of motoneurons (286). HSPB1 (as well as HSPB6 and αB-crystallin) retards formation of amyloid fibrils by D-$A\beta_{1-40}$ (but not by less toxic $A\beta_{1-42}$) in experiments in vitro (346).

HSPB1 interacts with hyperphosphorylated tau proteins that form neurofibrillar tangles in neurons of patients with Alzheimer's disease. Interaction with HSPB1 promotes tau dephosphorylation and induces proteasomal degradation of phosphorylated tau, probably by inducing changes in tau conformation (289). Moreover, HSPB1 prevents apoptosis of neuronal cells induced by accumulation of hyperphosphorylated tau (289). However, the data on colocalization of HSPB1 with neurofibrillar tangles formed by tau proteins in patients with Alzheimer's disease remain controversial (236, 346).

There are experimental data indicating neuroprotective action of HSPB1 in Huntington's disease (253, 301, 351); however, in this case its effect seems not to be due to prevention of protein aggregation, but rather to its ability to decrease the level of reactive oxygen components (ROS) in the cell (253, 301, 351).

The probable role of HSPB1 in the development of different neurodegenerative diseases is described in a number of recently published papers (93, 194, 264, 301, 311). However, detailed molecular mechanisms underlying participation of HSPB1 in Alzheimer's disease, amyotrophic lateral sclerosis, and Parkinson's and Huntington's diseases remain elusive. The main effect of HSPB1 in these disorders is probably due to its ability to prevent formation of amyloid fibrils and aggregates of misfolded proteins. However, other mechanisms (for instance, antioxidant activity) cannot be excluded.

H. HSPB1 in Apoptosis and Carcinogenesis

The data of literature indicate that the small heat shock proteins might be important in the signal transduction leading to apoptosis (6, 67). Apoptosis signaling comprises two pathways, intrinsic and extrinsic. The intrinsic pathway is based on the cytochrome *c*-dependent activation of caspases, special proteases which are involved in cleavage and processing of more than 300 different cellular proteins (106). Different signals (such as γ-irradiation, oxidative stress, or antineoplastic agents) lead to liberation of cytochrome *c* from mitochondria. In the cytosol, cytochrome *c* interacts with apoptotic protease activating factor-1 (Apaf-1), procaspase-9, and dATP forming the so-called apoptosome. Formation of the apoptosome leads to activation of procaspase-9, which in turn cleaves procaspase-3, the main caspase effector during apoptosis **(FIG. 3)**. It is supposed that HSPB1 can affect different stages of this process. First, HSPB1 seems to be able to prevent the release of cytochrome *c* from mitochondria (251). As mentioned earlier, indirect data indicate that HSPB1 interacts with and modulates activity of many protein kinases, and among them phosphatidylinositol 3-kinase (PI3-K). This protein kinase activates protein kinase Akt, phosphorylating Bax which prevents pore formation in the outer mitochondria membrane and thus prevents liberation of cytochrome *c* from mitochondria (138). In addition, HSPB1 seems to inhibit the apoptosis-signal regulated kinase 1 (Ask1)-JNK pathway which through Bax leads to cytochrome *c* leakage (301) **(FIG. 3)**. Second, HSPB1 directly interacts with cytochrome *c* leaving the mitochondria and by this means prevents apoptosome formation (35). It is still controversial whether HSPB1 affects both routes of caspase-3 activation or interacting with procaspase-3 inhibits its activation without affecting apoptosome formation and activation of caspase-9 (246). Activity of caspases is regulated by inhibitor of apoptosis proteins (IAPs), which in turn are inhibited by the so-called second mitochondria-derived activator of caspases (Smac/Diablo) **(FIG. 3)**. HSPB1 seems to be able to inhibit the release of Smac/Diablo from mitochondria and by this means can also induce inhibition of caspases, preventing apoptosis (54).

The extrinsic pathway of apoptosis is dependent on special membrane receptors. For instance, Fas receptor plays an important role in induction of apoptosis activating two different signal pathways. Binding of special ligand (FasL) promotes interaction of Fas receptor with Fas-associated-death-domain (FADD) protein which activates procaspase-8 and downstream caspases (caspases 3, 6, and 7) (51, 52) and cleaves Bid leading to accumulation of truncated Bid (tBid). tBid then modulates interaction of Bax with the mitochondrial membrane (9) **(FIG. 3)**. It is postulated that HSPB1 prevents translocation of activated Bid (tBid) to the mitochondrial membrane and thus inhibits apoptosis (9). On the other hand, binding of Fas-ligand promotes interaction of Fas receptor with another protein, called Daxx. Daxx-dependent induction of

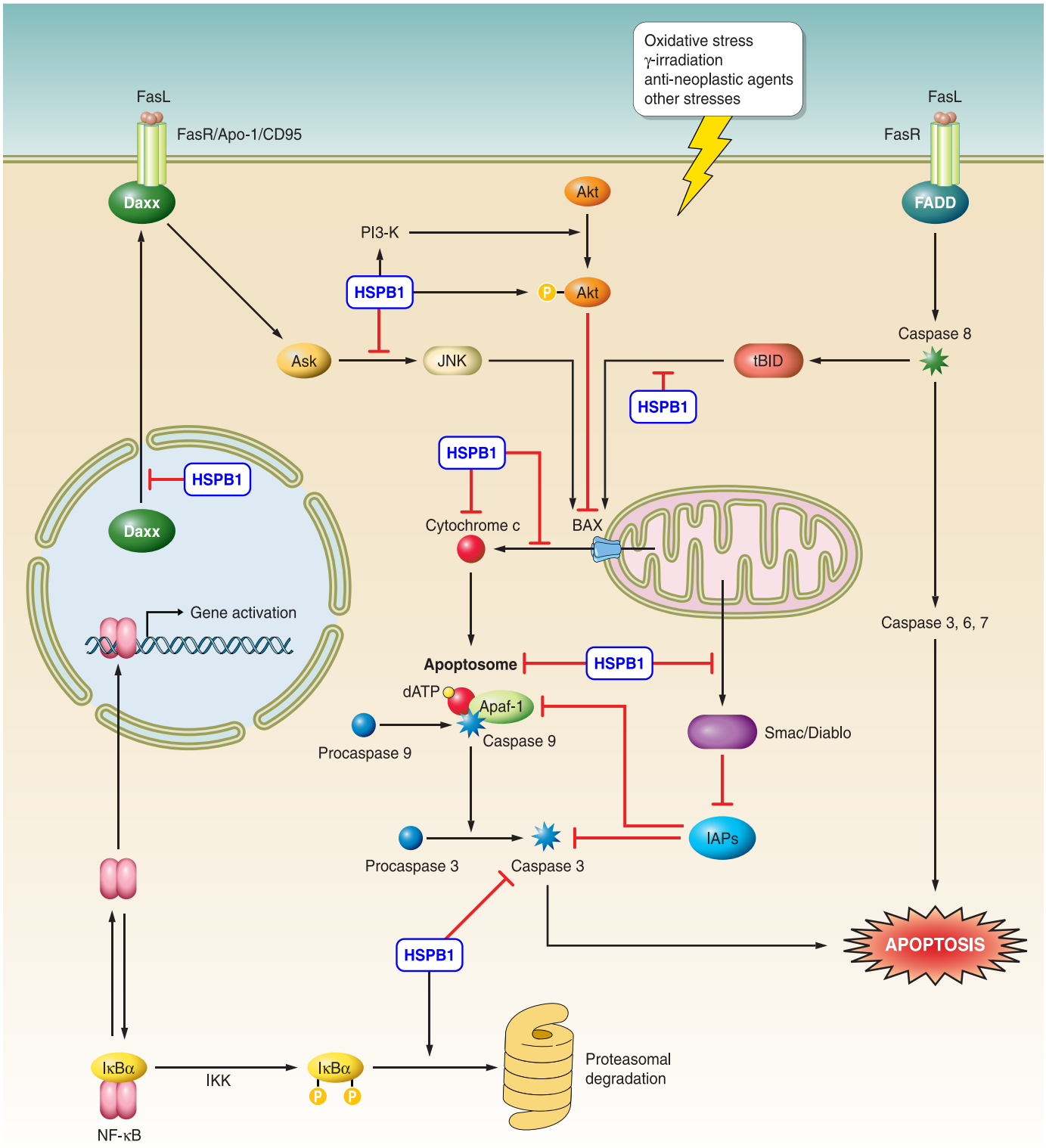

FIGURE 3. Involvement of HSPB1 in regulation of apoptosis. See text for details and explanation of abbreviations.

apoptosis relies on translocation of Daxx from the nucleus to the cytosol, and its interaction with Fas receptor is probably followed by activation of apoptosis signal-related kinase 1 (Ask1). Upon signal-induced phosphorylation, HSPB1 migrates from cytosol to nucleus, i.e., in the direction that is opposite to the direction of Daxx movement. Phosphorylated HSPB1 is supposed to interact with Daxx, preventing its translocation to the cytosol, and thus inhibits binding of Daxx to Fas receptor and Daxx-dependent apoptosis (51, 52). This suggestion was

not confirmed in a recently published paper (70), and one cannot exclude that HSPB1 somehow affects not only the Daxx-dependent pathway but can also affect the FADD-dependent activation of caspase-8.

Recently published papers indicate that HSPB1 is effective in preventing of apoptosis induced by different agents. Treatment of HeLa cells with Fas agonist antibodies and etoposide was accompanied by shifting HSPB1 towards formation of large differently phosphorylated oligomers that somehow affect mitochondria and cytochrome *c* release (252). Staurosporine and cytochalasin D induce rapid and transient formation of differently phosphorylated small HSPB1 oligomers, which later on associate, forming large oligomers. It is thought that transiently formed small HSPB1 oligomers somehow prevent F-actin disruption and interact with special protein targets, whereas large oligomers formed later affect cytochrome *c* liberation and caspase cascade activation (252). Thus the mechanisms of apoptosis are very complicated and involve many different proteins. The hypothesis of HSPB1 direct participation in regulation of apoptosis is very attractive; however, it is predominantly supported by indirect often controversial experimental data. Therefore, further investigations are needed to establish a putative role of HSPB1 in regulation of apoptosis.

Nuclear factor κB (NF-κB) is an important transcription factor regulating cellular survival, proliferation, and differentiation. Under normal conditions in the absence of cellular stress, it interacts with endogenous inhibitors that prevent its translocation to the nucleus and inhibits its transcriptional activity. Phosphorylation of NF-κB inhibitor (I-κBα) by protein kinase complex I-κB kinase (IKK) promotes I-κBα ubiquitylation and proteasomal degradation. This process is enhanced by HSPB1, which forms tight complexes with ubiquitylated I-κBα and 26S proteasome (248) **(FIG. 3)**. Thus activation of transcriptional activity of NF-κB can be another pathway providing for antiapoptotic activity of HSPB1.

The data presented correlate with the hypothesis that HSPB1 is an important regulator of proliferation and apoptosis and therefore may be of great importance in carcinogenesis. Expression of HSPB1 is significantly elevated in a number of tumors (10, 65, 118, 148), and increased expression of HSPB1 usually correlates with increased resistance to cytotoxic (antineoplastic) compounds (10, 65, 328). Increased resistance of tumors to cancer therapy induced by HSPB1 overexpression seems to depend on many factors. As already mentioned, HSPB1 plays an important "housekeeping" role and protects the cell against different kinds of stresses (including chemotherapy) by preventing accumulation of aggregated proteins, modulating the redox state of the cell, protecting the cytoskeleton, modulating transcription, regulating apoptosis, and probably enhancing the DNA repair in cancer cells (65).

I. HSPB1 and Oxidative Stress

Oxidative stress is accompanied by increased levels of toxic ROS, such as peroxides and free radicals. Oxidative stress is characteristic for many human diseases such as atherosclerosis, viral infection, and Alzheimer's disease and seems to be one of the most important processes in aging.

The small heat shock proteins increase cell viability under different unfavorable conditions including oxidative stress (151, 228, 229, 249, 273). Under conditions of oxidative stress, overexpression of HSPB1 typically leads to a significant decrease of basal levels of ROS and ROS production (6, 119, 227, 228, 260). It is assumed that HSPB1 increases the level of reduced glutathione, an event that depends on the presence of high molecular mass unphosphorylated oligomers of HSPB1 (227, 228). This elevated level of reduced glutathione could be the result of HSPB1-induced increase in the activity of glucose-6-phosphate dehydrogenase, glutathione reductase, glutathione transferase, and glutathione peroxidase, which are vital for the maintenance of intracellular redox potential (8, 97, 260). Inhibition of glutathione synthesis abrogates the protection induced by HSPB1 overexpression (97). It is also thought that HSPB1 and αB-crystallin decrease the intracellular level of iron, and by this means they interfere with formation of hydroxyl radicals via the Fenton reaction (6, 8). Detailed mechanisms of the antioxidant activity of HSPB1 still remain elusive. When specifically targeted, the antioxidant activity seems to be based on the modulation of activity of the above-mentioned enzymes, while when this activity is nonspecific, it is predominantly based on the control of protein folding and prevention of protein aggregation. In addition to these functions, HPSB1 may also serve as a buffer during oxidation reactions. The single Cys residue of HSPB1 can form mixed disulfide with glutathione (92, 358). Replacement of this Cys residue with Ala slightly decreased the HSPB1 antioxidant properties (81); however, under certain conditions this mutation decreased the antiapoptotic activity of HSPB1 (35, 250).

The literature about the antioxidant effect of HSPB1 is enormous and cannot be completely summarized. Therefore, we will present only a few examples. It has been shown that accumulation of huntingtin fragment carrying expanded polyGln tail is accompanied by increased ROS production. Accumulation of protein aggregates and increased levels of ROS might be important factors in Huntington's disease development (351). Large oligomers of HSPB1 decrease the level of ROS in cells, producing huntingtin fragments, without significantly affecting protein aggregation. Therefore, it was supposed that HSPB1 prevented the cell death predominantly by modulating ROS levels (351). It is

interesting that phosphorylation-induced dissociation of large oligomers decreases the protective effect of HSPB1.

Viral infection is often accompanied by oxidative stress leading to cell death. Replication of certain Herpes viruses is significantly enhanced in the presence of HSPB1 (215). This seems to be due to an antioxidant effect of HSPB1 leading to a decreased level of carbonylated proteins (214). In addition, HSPB1 increases proteasome-dependent degradation of oxidized proteins (8) and stimulates accumulation of oxidized proteins in special foci inside of the infected cell nucleus (214). Thus, in the case of viral infection, antioxidant properties of HSPB1 are dangerous for the cell and promote viral replication.

The cytotoxic compound doxorubicin induces cancer cell death by increasing ROS level. However, doxorubicin-induced ROS level is dangerous for normal cardiac cells (329). Heat-induced expression of HSPB1 protected cardiac cells from doxorubicin toxicity by decreasing the level of ROS and by stabilizing actin cytoskeleton (329). The authors of this publication do not propose a clear molecular mechanisms of the antioxidant potential of small heat shock proteins, but theorize that sHSP can somehow affect the activity of aconitase, which might be responsible for ROS production (329).

HSPB1 somehow protects actin cytoskeleton against damages induced by oxidative stress (150, 151) or heat shock (206, 207). Unfortunately, it is still unknown how the HSPB1 antioxidant activity and the protection of actin cytoskeleton are connected. However, it is thought that oxidative stress can lead to oxidation of Met residues, thiolation and oxidation of Cys residues of certain actin-binding proteins (for example, β-thymosin), and of actin itself and thus modulate reorganization of the cytoskeleton (71).

Summing up, protection against oxidative stress induced by HSPB1 may rely on a number of different mechanisms, including *1*) decrease of ROS level which is achieved by modulation of the glutathione system, *2*) the nonspecific chaperone-like activity that prevents aggregation of partially denatured proteins by their renaturation or proteasome-dependent degradation, and *3*) protection of the cytoskeleton.

It is worthwhile to mention that the cellular context in which HSPB1 is expressed strongly affects its antioxidant properties and therefore, under special conditions, HSPB1 can sensitize certain cell lines to oxidative stress (reviewed in Refs. 6, 8). Hence, the mechanism of antioxidant effect of HSBP1 is very complicated: it is not only cell- and stress type-dependent, but also based on unrelated biological processes such as nonspecific chaperone-like activity of HSPB1, regulation of redox state of the cell, buffering of oxidation reactions, and/or a combination of all above-mentioned processes.

J. Effect of HSPB1 on Cytoskeleton

In the early 1990s, avian HSP25 (ortholog of human HSPB1) was recognized as an inhibitor of actin polymerization (230, 231). Later on inhibition of actin polymerization was ascribed for nonphosphorylated monomers of murine HSP25, whereas phosphorylated monomers and nonphosphorylated multimers did not affect actin polymerization (20). It is worthwhile to mention that these effects were observed only for the HSP25 isolated from the Ehrlich ascites tumor cells, but not for the corresponding recombinant protein. Two short peptides of murine HSP25, containing residues 43–57 and 92–106, were shown to be effective inhibitors of actin polymerization, however, the increase of the length of these peptides diminished their inhibitory activity (344). These papers became classic and now it is generally believed that HSPB1 directly interacts with actin, and, depending on the phosphorylation and oligomerization status, differently affects organization of actin filaments (105, 232).

However, many important details underlying the effect of HSPB1 on actin cytoskeleton remain controversial (reviewed in Ref. 131). It is supposed that the effect of HSPB1 on actin polymerization can be due either to direct interaction of HSPB1 with G-actin (336, 344), or to the capping of barbed ends of actin filaments (230, 231). Indeed, at very high concentrations, recombinant unphosphorylated HSP27 decreased the rate of polymerization of pyrenyl maleimide modified actin (336), and it is thought that unphosphorylated HSPB1 interacts with G-actin inhibiting its polymerization (90). Formation of the HSPB1-G-actin complex made possible crystallization of G-actin; however, HSPB1 was not evident in the resulting crystal structure (336) and complex formation was observed only at nonphysiological high concentrations of HSPB1. In contrast, HSPB1 did not affect F-actin denaturation recorded by scanning microcalorimetry and was unable to form tight complexes with intact F-actin (256, 257). Moreover, many attempts to reproduce phosphorylation-dependent effects of HSPB1 on actin polymerization were unsuccessful. Human HSPB1 had no effect on actin polymerization, whereas its phosphomimicking mutants only slightly increased the rate of actin polymerization (43). Recombinant avian HSP25 and its phosphomimicking mutants were mostly ineffective in modulation of intact actin polymerization (244). These data seem to be inconsistent with the oversimplified suggestion that under in vivo physiological conditions HSPB1 (or its orthologs) directly interacts with G-actin or induces capping (or uncapping) of barbed ends of actin filaments (105, 232) and/or associates with the lateral side of actin filaments (85, 86).

At the same time, there is little doubt that HSPB1 effectively protects the actin cytoskeleton from many different kinds of stresses and that heat shock, ischemia, ATP depletion, oxidative stress, and action of certain hormones and biologically active compounds are accompanied by increased expression of HSPB1 and its translocation to contractile apparatus and different elements of the actin cytoskeleton (38, 105, 180, 271, 301, 327). The mechanism of HSPB1 translocation and its protective effect on actin cytoskeleton remain enigmatic. However, one can put forward several hypotheses. First, the literature indicates that HSPB1 (and its orthologs) interacts with many actin-binding proteins such as tropomyosin (295), caldesmon (152), and troponin T (95), and functional association of HSPB1 with integrin-mediated actin assembly was also reported (3, 277). Interaction with many of these proteins is dependent on HSPB1 phosphorylation (295). Therefore, depending on the state of phosphorylation, HSPB1 can interact with certain actin-binding proteins that mediate its binding to actin filament in the cell. Indirect binding of HSPB1 can affect interaction of actin filaments with other proteins, some of which can be involved in fragmentation of actin filaments. Thus migration of HSPB1 to actin filaments may protect them from fragmentation. Second, denatured actin forms tight complexes with HSPB1 (244, 256, 257), and therefore, any damages will attract HSPB1 from the cytosol to actin filaments and interaction with actin (and probably with other target proteins) is also dependent on HSPB1 phosphorylation. Third, as mentioned earlier, HSPB1 plays an important role in regulation of redox states in the cell and thus can protect actin cytoskeleton from oxidative damage (71). Antioxidant activity of HSPB1 also depends on its oligomeric state (6) and can be regulated by means of HSPB1 phosphorylation. It is worthwhile to mention that the literature on the interaction of HSPB1 with actin cytoskeleton is very abundant and controversial and cannot be discussed in detail. The reader is directed to the excellent, recently published reviews, addressing this problem more thoroughly (105, 232, 301).

In addition to interaction with the actin filaments, HSPB1 can be involved in interaction with other cytoskeleton proteins. For instance, it was shown that HSPB1 interacts with vimentin and nestin, intermediate filament proteins (162, 195), and by this means affects cell adhesion and protects the cell from cadmium-induced poisoning. Mutations of glial fibrillary acidic protein lead to formation of aggregates called Rosenthal fibers, which attract and sequester HSPB1 (263). Depletion of HSPB1 might attenuate resistance of astrocytes to different types of stress and increase the probability of apoptosis (263).

It is thought that HSPB1 is able to interact with tubulin and microtubules (143) and by this means modulate the cytoskeleton. In addition, HSPB1 seems to interact with hyperphosphorylated tau protein and induce either its dephosphorylation or proteasomal degradation (182). Thus HSPB1 may be able to modulate practically all elements of the cytoskeleton and would thus play an important role in its regulation and remodeling.

K. HSPB1 as a Therapeutic Target

The data presented indicate that HSPB1 may be involved in regulation of multiple processes in cells. Therefore, it may be a potentially important therapeutic target. For instance, geranylgeranylacetone increasing HSPB1 expression can be useful for the treatment of atrial fibrillation, whereas atovastin modulating HSPB1 phosphorylation can be potentially useful in the case of ischemic heart disease (36). Since HSPB1 affects many different processes, uncontrolled increase of HSPB1 expression can be both useful and dangerous for the cell. As mentioned earlier, increased expression of HSPB1 often correlates with increased resistance to anticancer drugs. Therefore, experiments were aimed toward inhibiting HSPB1 expression by using anti-sense or nucleotide-based therapies to sensitize cancer cells to apoptotic inducers (reviewed in Refs. 7, 160). On the other hand, controlled increase of HSPB1 expression is associated with protection against ischemia, and electroinjection (or other methods of delivery) of HSPB1 in retinal ganglion cells protects them from apoptosis (240) and might be useful for treatment of certain neurodegenerative diseases. As well, HSPB1 probably can protect hepatocytes against liver ischemia/reperfusion injury (353). Since many properties of HSPB1 depend on its oligomeric state, it seems reasonable to search for compounds that will affect the equilibrium between the small and large oligomers of HSPB1 or to search for inhibitors or activators of protein kinases and protein phosphatases involved in the phosphorylation of this protein.

HSPB1 together with αA- and αB-crystallin belongs to a group of the most thoroughly investigated small heat shock protein. At present, there are more than 2,000 references in the PubMed Database dealing with HSPB1 (HSP27), and therefore, it is impossible to analyze all peculiarities of its structure and properties. Readers are directed to many useful reviews dealing with the detailed description of structure, properties, and the role that HSPB1 might play in different cell processes (6, 8, 9, 36, 131, 232, 277, 301).

IV. SMALL HEAT SHOCK PROTEIN HSPB6 (HSP20 OR HEAT SHOCK 20 kDa-LIKE PROTEIN, P20)

HSPB6 was described in 1994 as a protein highly homologous to αB-crystallin (168). Further investigations revealed that this protein belongs to the family of small heat shock proteins, and its primary structure is closely related to the primary structure of αA- and αB-crystallin (HSPB4, HSPB5) and HSP25/27 (HSPB1) (110, 166).

A. Expression and Location of HSPB6

HSPB6 belongs to the group of ubiquitously expressed small heat shock proteins (314) (TABLE 1) with the highest level of expression in smooth muscles (myometrium) (69, 320), red and white skeletal muscles (149, 178), and cardiomyocytes (84). The level of HSPB6 expression in rat hindlimb muscles (soleus) is increased in the course of ontogenesis (155). Similar results were obtained in the case of pig heart and skeletal muscle (330). Expression of HSPB6 does not seem to be heat-inducible (179, 305, 314) (TABLE 1) and therefore probably does not depend on the action of heat shock factor (HSF-1); however, many other experimental and physiological factors seem to affect the level of HSPB6 in the cell. For instance, spinal cord isolation and denervation lead to decreased levels of HSPB6 in rat soleus, plantaris, and adductor longus muscle (149, 155). At the same time, congestive heart failure (84) and exercise training (28) were accompanied by increased levels of HSPB6 in dog and rat hearts. Increased levels of HSPB6 were also detected in obstructed rat bladder (15). This means that the level of HSPB6 in the cell is strictly regulated and the mechanism of regulation can be different in different tissues. Moreover, HSPB6, as with many other members of sHSP family, undergoes posttranslational modifications (e.g., phosphorylation), and there are these modifications rather than transcriptional activation that might provide fine tuning of HSPB6 activity in the cell.

Intracellular location of HSPB6 was analyzed in a number of investigations; however, the results still remain controversial. Confocal microscopy revealed that in swine carotid artery HSPB6 is present throughout the cytoplasm, with some focal regions containing more HSPB6 than the remaining cytoplasm (269). In rat heart HSPB6 was also predominantly localized in cytosolic fraction; however, part of HSPB6 was colocalized with actin in transverse bands (255). Stimulation of smooth muscle was not accompanied by significant changes of subcellular distribution of HSPB6 in vascular smooth muscles (32, 34); however, other publications indicate that hormone-induced phosphorylation of HSPB6 was accompanied by its translocation from cytosol to actin filaments in cardiomyocytes (99, 100) and carotid artery (266). HSPB6 is a typical intracellular protein; therefore, endothelial injury is marked by reduced levels of HSPB6 in vessel walls with concomitant increase of HSPB6 level in plasma of cardiomyopathic hamster (184). This means that at least part of HSPB6 remains free in cytosol and is liberated upon injury; however, detailed intracellular distribution of HSPB6 remains poorly analyzed and requires further investigation.

B. Physicochemical Properties of HSPB6

As already mentioned, HSPB6 is a typical representative of the large family of small heat shock proteins (163, 314). This means that human HSPB6 contains the so-called α-crystallin domain and has a low subunit molecular mass (TABLE 1). Physicochemical properties of HSPB6 isolated from other species (rat, mouse, bovine) are similar to those of the human counterpart. The oligomeric state of HSPB6 still remains a controversial issue. Early publications (168, 324) indicate that isolated HSPB6 (previously called HSP20) forms two types of interconvertible oligomers with apparent molecular masses in the range of 43–67 and 300–470 kDa being in equilibrium with each other. Fractionation of crude muscle homogenate by sucrose density gradient centrifugation (168) or by size-exclusion chromatography (32) indicate that HSPB6 migrates in the fractions with apparent molecular masses close to 68 and 540 kDa and that after treatment of carotid strips by forskolin and 3-isobutyl-1-methylxanthine (IBMX) (leading to an increased level of cAMP) the apparent molecular mass of HSPB6 oligomers was significantly decreased (32). These data lead to the widespread conclusion that like other small heat shock proteins, HSPB6 tends to form high-molecular-mass oligomers that undergo dissociation upon phosphorylation (88, 277). We assume that this conclusion is not completely correct. Later investigations performed on isolated untagged human HSPB6 indicate that this protein forms only small-molecular-mass oligomers (55–60 kDa) and that mutation mimicking phosphorylation did not affect its oligomeric state (39, 41). Moreover, HSPB6 interacts with many protein partners such as other small heat shock proteins (39, 111) and 14–3-3 (61) forming complexes with high molecular mass. Phosphorylation can affect the size of these complexes and, by this means it leads to changes of molecular mass of fractions containing HSPB6. However, this does not mean that phosphorylation directly affects the quaternary structure of HSPB6 (283).

C. HSPB6 Phosphorylation

On two-dimensional electrophoresis, HSPB6 forms multiple spots with similar molecular masses and different pI (17, 42, 342). This fact might indicate that HSPB6 undergoes multisite phosphorylation. Rat and swine HSPB6 are phosphorylated at Ser157 by a PI3-K-dependent pathway, and this phosphorylation might be dependent on insulin stimulation (341). In swine carotid artery, phosphorylation of Ser157 was constitutively high and was not dependent on forskolin application (226). It is worthwhile to mention that the primary structure at the corresponding residue is modified in bovine HSPB6, and Ser/Thr residue is lacking in this position in the case of human HSPB6. Stimulation of bovine carotid artery by phorbol ester or application of forskolin and IBMX was accompanied by phosphorylation of HSPB6 at a site(s) located in the peptide LPPGVDPAAVTSALSPEG containing residues 123–140 (16). The third site of phosphorylation, namely, Ser59, was detected after in vitro phosphorylation of isolated rat HSPB6 by cAMP-dependent protein kinase (16). To our knowledge, it is still unknown

whether this site is phosphorylated in vivo. Finally, the fourth site, namely Ser16, is phosphorylated both in vivo and in vitro by cyclic nucleotide-dependent protein kinases (16, 99, 266) **(FIG. 1*B*)**. The level of phosphorylation at this site is increased after different kinds of stimulation, and phosphorylation of this site significantly affects interaction of HSPB6 with target proteins and influences many physiologically important events (see below).

D. Chaperone-Like Activity of HSPB6

The ability to prevent aggregation of partially denatured protein seems to be one of the most important features of all small heat shock proteins (221, 334). Originally it was believed that HSPB6 is a poor chaperone and is less effective than αB-crystallin in preventing reduction-induced aggregation of insulin in vitro (324). At the same time, the thermoprotective effect induced by overexpression of HSPB6 was comparable with that induced by overexpression of αB-crystallin (322). Later published data indicated that under certain conditions the chaperone-like effect of HSPB6 measured with different model protein substrates in vitro was comparable to that of αB-crystallin or HSPB8 (41, 234). Thus, at present, the data on the chaperone-like activity of HSPB6 remain contradictory, since it is difficult to make a quantitative comparison of chaperone-like activity of different small heat shock proteins. However, we might conclude that under certain conditions HSPB6 (as other small heat shock proteins) may effectively prevent aggregation of partially denatured proteins.

E. Interaction of HSPB6 With Other Small Heat Shock Proteins

As already mentioned, the primary structure of different sHSP is rather conservative, and all members of this family contain the α-crystallin domain (221, 277, 334) and therefore potentially can interact with each other. Interaction of HSPB6 with αB-crystallin (HSPB5) is well documented (29, 168, 305), and it seems probable that these proteins form tight hetero-oligomeric complexes. HSPB6 also interacts with HSPB1 (HSP27) forming equimolar hetero-oligomeric complexes with apparent molecular masses of ~100–150 and 250–300 kDa (39, 41). Formation of these complexes is temperature dependent, and inside of these complexes, HSPB1 and HSPB6 mutually affect the structure of each other (39). Recently published data indicate that HSPB6 is able to interact with HSPB8 (111), another small heat shock protein that is also closely related to αB-crystallin (HSPB5) and HSPB1 (110). In addition, HSPB6 seems to interact with HSPB2 (29), i.e., with the protein only remotely related with the two above mentioned small heat shock proteins. This makes HSPB6 universal in its interaction with other sHSP.

A number of other potential protein targets of HSPB6 were described in the literature and among them are actin (33), α-actinin (316), 14-3-3 (61, 87), protein phosphatase 1 (72), Bag3 protein (117), Bax protein (102), and certain protein kinases (103, 104). We will discuss the validity of these reports and their potential importance analyzing physiological functions of HSPB6.

F. Participation of HSPB6 in Regulation of Smooth Muscle Contraction

Early investigations have shown that the activation of cyclic nucleotide-dependent protein kinases (cAMP- and/or cGMP-dependent protein kinases) and/or inhibition of protein phosphatases is accompanied by phosphorylation of HSPB6 at Ser16, and this event somehow correlates with relaxation of trachealis and carotid artery smooth muscle (16, 17, 266). Relaxation induced by HSPB6 phosphorylation was not accompanied by dephosphorylation of myosin light chains (266, 350) and therefore was denoted as "force suppression" (265). The experimental data indirectly indicate that the force suppression somehow relies on the interaction of phosphorylated HSPB6 with actin filaments. In the late 1990s, two diametrically opposite hypotheses were put forward. The first hypothesis advocated by Rembold et al. (266) suggested that the primary structure of HSPB6 contains the sequence that is similar to the inhibitory peptide of troponin I. Unphosphorylated HSPB6 weakly interacts with actin filaments and does not significantly affect their structure. Phosphorylation induces conformational changes of HSPB6, which binds to and inactivates thin filaments **(FIG. 4*A*)**. Therefore, phosphorylated myosin heads are unable to form productive complexes with actin, and this leads to force suppression without significant decreases of the myosin light chain phosphorylation (115, 226, 265, 268). According to this hypothesis, HSPB6 phosphorylation induced by heat pretreatment (239, 267), hypoxia (115), or activation of cyclic nucleotide-dependent protein kinases (266) leads to regional inactivation of thin filaments or inhibits myosin binding at either the thin- or thick-filament level (226) **(FIG. 4*A*)**.

An alternative hypothesis intending to explain the mechanism of HSPB6-induced relaxation of smooth muscle was proposed by Brophy et al. (33). These authors postulated that HSPB6 is an actin-binding protein (33). In an unphosphorylated state, HSPB6 predominantly interacts with F-actin, whereas after phosphorylation, HSPB6 predominantly binds G-actin (33) **(FIG. 4*B*)**. Histochemical and immunochemical methods indicate that under certain conditions HSPB6 interacts with α-actinin, and HSPB6 phosphorylation decreases its interaction with this cytoskeletal protein (316). It was thought that unphosphorylated HSPB6 interacts with F-actin and stabilizes its binding to other cytoskeleton elements, thus providing conditions for effective contraction **(FIG. 4*B*)**. After phosphorylation,

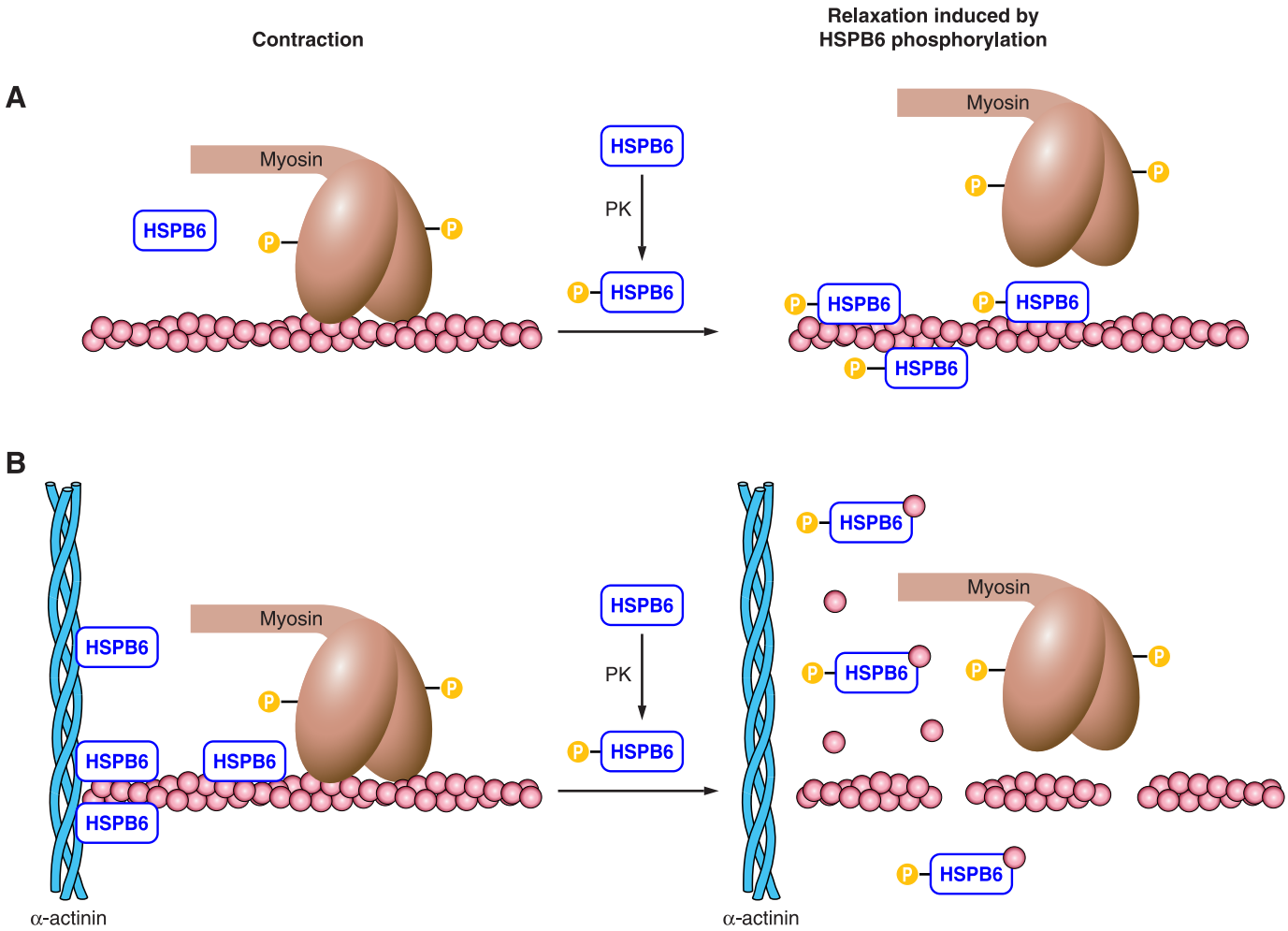

FIGURE 4. Oversimplified schemes illustrating hypothetical mechanisms of smooth muscle relaxation induced by phosphorylated HSPB6. *A*: unphosphorylated HSPB6 weakly interacts with actin and therefore does not prevent interaction of phosphorylated myosin with actin and muscle contraction. Phosphorylated HSPB6 interacts with actin and, like troponin, prevents productive interaction of phosphorylated myosin with actin. *B*: unphosphorylated HSPB6 binds to F-actin and cytoskeleton proteins (i.e., α-actinin), thus stabilizing contractile apparatus and providing for force-generating interaction of phosphorylated myosin and actin. Phosphorylation induces dissociation of HSPB6 from F-actin and cytoskeleton proteins leading to partial disassembling of contractile apparatus and smooth muscle relaxation. PK, protein kinases; P-HSPB6, phosphorylated HSPB6; P-myosin, myosin with phosphorylated regulatory light chains.

HSPB6 dissociates from actin and α-actinin, thus leading to destabilization (partial actin depolymerization) and reorganization of cytoskeleton and smooth muscle relaxation (31, 316, 349, 350) **(FIG. 4*B*)**.

Although these hypotheses contradict one another (see, for example, Refs. 132, 226, 265), both of them are based on the assumption that HSPB6 interacts with actin filaments and somehow affects the structure or stability of thin filaments. By using cosedimentation, we analyzed the interaction of HSPB6 with isolated F-actin, actin filaments containing different regulatory proteins or myofibrils (40). Under all conditions used, the stoichiometry monomer HSPB6/monomer actin was less than 0.04, and this stoichiometry was independent of mutation (S16D) mimicking phosphorylation of HSPB6. These results agree with the fact that the total concentration of actin in smooth muscle is close to 1,100 μM (266), whereas the total monomer concentration of HSPB6 is in the range of 100–200 μM (168). Moreover, HSPB6 is predominantly located in the cytosol of smooth muscle (32, 269, 320), with only a small portion of HSPB6 bound to the cytoskeleton (269). All these facts make very improbable any hypothesis postulating the direct effect of HSPB6 on the structure and properties of thin filaments. Since there is little doubt that in the smooth muscle phosphorylation of HSPB6 correlates with relaxation and force suppression (17, 225, 266, 349, 350), one should theorize that HSPB6 indirectly affects the properties of thin (or thick) filaments.

Since the phosphorylated form of HSPB6 induced relaxation, it was reasonable to analyze the effect of a short peptide, containing the site of HSPB6 phosphorylation, on the contractile activity of smooth muscle. A short peptide

consisting of 11 residues forming the so-called protein transduction domain (PTD) and 13 residues of the NH_2-terminal part of HSPB6 (including Ser16 which is phosphorylated) was synthesized, and its effect on smooth muscle strips and fibroblasts was analyzed (108). This peptide entered both types of the cell and induced relaxation of norepinephrine precontracted rabbit aortic smooth muscle. Similar results were obtained with phosphorylated full-sized HSPB6 containing a special TAT protein transduction domain (the so-called TAT-pHSP20) on the NH_2-terminal end. This full-sized construct (as well as a short penetrating peptide containing phosphorylated Ser16 of HSPB6) effectively prevented vasospasm of human umbilical artery (107). Furthermore, TAT-pHSP20 inhibited contraction of rabbit aortic and human saphenous vein segments and inhibited platelet aggregation (223). Saphenous vein is often used for peripheral vascular reconstruction; however, vasospasm and intimal hyperplasia can lead to early and delayed graft failure. Transducible phosphorylated peptide of HSPB6 was shown to prevent vasospasm and to inhibit cellular migration without significant effect on cell proliferation (315). This makes phosphorylated peptide of HSPB6 a potentially useful tool for some surgical manipulations.

Expression of connective tissue growth factor (CTGF) is dependent on the proper organization of actin cytoskeleton (208). Therefore, modulation of keloid fibroblast cytoskeleton might affect expression of CTGF and collagen which are induced by transforming growth factor-β1 (TGF-β1). Recently published data indicate that the phosphorylated transducible peptide of HSPB6 (the so-called AZX100) decreased TGF-β1-induced expression of CTGF and collagen in human keloid fibroblasts and improved collagen organization in dermal wounds in a Siberian hamster scarring model (208). These facts make cell penetrating phosphorylated peptide and phosphorylated transducible HSPB6 a potentially useful tool for many different physiological and medical applications. A recently published paper describes coexpression of TAT-HSP20 and catalytic domain of cGMP-dependent protein kinase in *Escherichia coli* and provides a simple method for obtaining phosphorylated transducible HSPB6 in preparative quantities (109).

A special investigation was undertaken to understand the mechanism of action of phosphorylated peptide of HSPB6. Treatment of Swiss 3T3 cells with penetrating phosphorylated peptide of HSPB6 was followed by the loss of actin stress fibers and focal adhesion complexes (87, 109). It was shown that in Swiss 3T3 cells phosphorylated peptide of HSPB6 (and probably intact phosphorylated HSPB6) interacts with universal adapter protein 14-3-3 and therefore can compete and displace phosphorylated cofilin from its

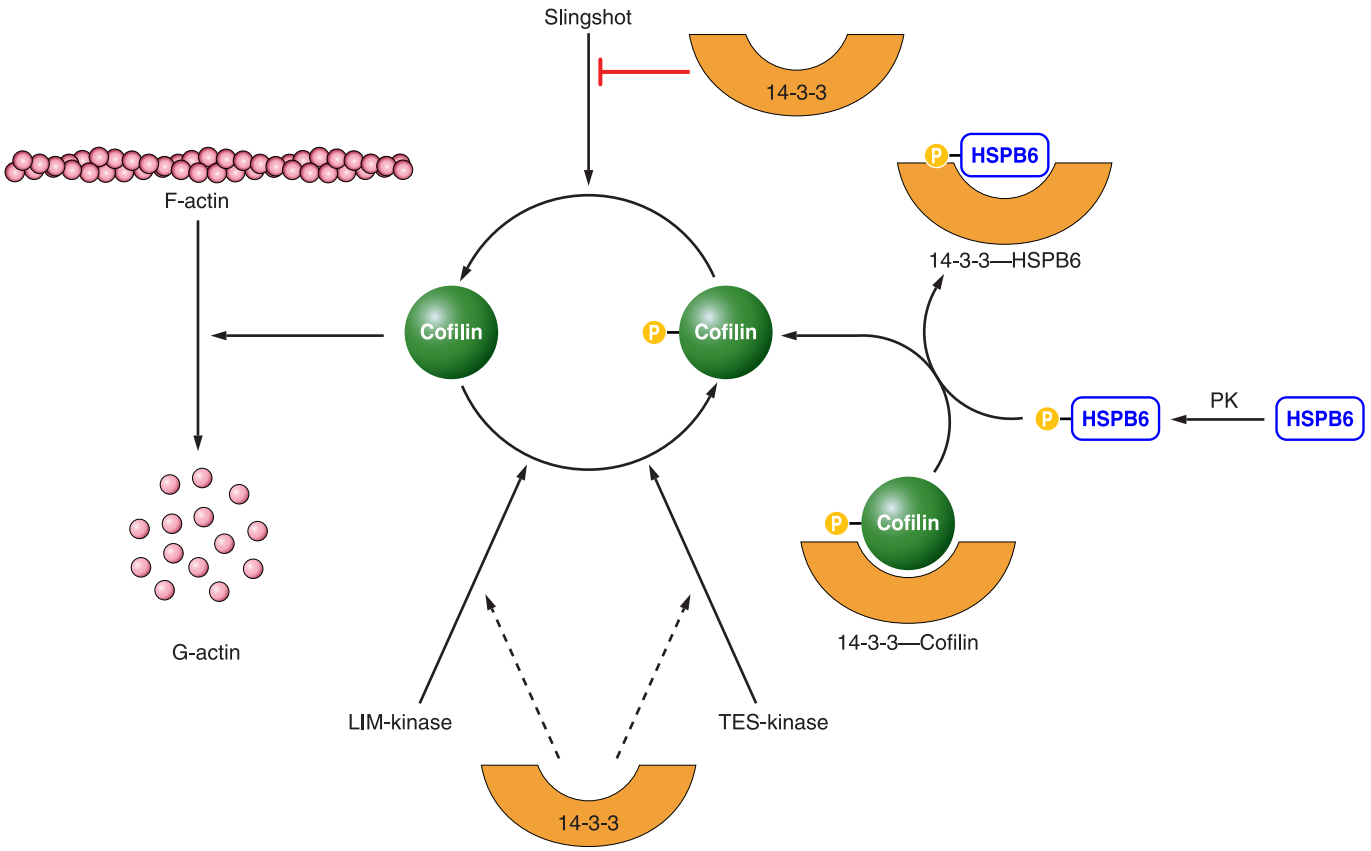

FIGURE 5. Interaction of phosphorylated HSPB6 with 14-3-3 and probable role of this interaction in regulation of smooth muscle relaxation and cell motility. Phosphorylated proteins are marked by P.

complex with 14-3-3 (FIG. 5). Displaced phosphorylated cofilin undergoes dephosphorylation by slingshot protein phosphatase and induces fragmentation and depolymerization of actin filaments leading to muscle relaxation (87, 88) (FIG. 5). Our data indeed indicate that the full-sized phosphorylated HSPB6 [not only phosphorylated peptide used by Dreiza et al. (87)] tightly interacts with 14-3-3 (61). Taking into account importance of direct interaction of 14-3-3 with phosphorylated HSPB6, a special high-throughput fluorescence polarization assay was recently developed for screening pharmacological compounds able to compete with HSPB6 for its interaction with 14-3-3 (2).

Thus there are no doubts that both in vitro (61) and in vivo (87) phosphorylated HSPB6 or its phosphorylated peptide interacts with 14-3-3. However, this does not mean that the molecular mechanism of HSPB6 action is exclusively based on its ability to displace phosphorylated cofilin from its complex with 14-3-3 as was postulated in the literature (87, 88). First, 14-3-3 is reported to interact with a huge number (>200) of different protein targets (258) including phosphorylated LIM- (25) and TES-kinase (281) involved in cofilin phosphorylation as well as with phosphorylated slingshot protein phosphatase (297) participating in cofilin dephosphorylation. Interaction with 14-3-3 affects intracellular localization of LIM- and TES-kinases (25) (dashed lines on FIG. 5) and induces inhibition of slingshot phosphatase (297) (FIG. 5). Since the effect of HSPB6 on the interaction of 14-3-3 with protein kinases and protein phosphatases involved in cofilin regulation remains unknown, it is difficult to predict what would happen to cofilin after the appearance of phosphorylated HSPB6. Second, only ~30% of cofilin is phosphorylated (14), and the portion of phosphorylated cofilin bound to 14-3-3 in nonmuscle and muscle cells remains unknown. Therefore, it is difficult to believe that in both non-muscle (3T3 cells) and in smooth muscles (that significantly differ in actin content) liberation of cofilin from its complex with 14-3-3 will lead to similarly dramatic effects on cytoskeleton structure. Indeed, overexpression of phosphomimicking mutant of HSPB6 (S20D) had no effect on cytoskeleton reorganization and actin microfilament integrity in colonic smooth muscle (296). Third, 14-3-3 interacts with phosphorylated α- and β-chains of integrins (74, 197), as well as with phosphorylated β-catenin (201). Both integrins and catenins play an important role in cell-cell and cell-matrix interaction and in attachment of actin filaments to the cell membrane. Although the effect of phosphorylated HSPB6 on the interaction of 14-3-3 with integrins and catenin remains unknown, we cannot exclude that binding of HSPB6 may modify the interaction of actin filaments with focal contacts and by this means affect cell motility and smooth muscle contraction. Fourth, binding to 14-3-3, phosphorylated HSPB6 can indirectly affect activity of many protein kinases and protein phosphatases, which are also interacting with 14-3-3 (258). Liberated protein kinases and protein phosphatases could then modulate migration and contractile activity of different cells.

In summary, it is possible to conclude that the exact mechanism of HSPB6 action on smooth muscle relaxation and cell motility remains elusive. Suggestion that the relaxing effect of HSPB6 is explained exclusively by modulation of cofilin phosphorylation (87, 88) seems to be oversimplified (283). Although HSPB6 is not a genuine actin-binding protein [as it was suggested earlier (33, 266) and is believed presently (135, 355)], it can indirectly affect the structure and properties of contractile apparatus by binding to a universal adapter protein 14-3-3 (61) causing modulation of activity of different, yet not fully identified, cytoskeletal proteins and/or protein kinases and phosphatases (61, 283). As mentioned earlier, HSPB6 interacts with other small heat shock proteins. Formation of hetero-oligomeric complexes affects the oligomeric state (41, 168, 170) and properties of HSPB6 (39) and might also influence regulatory properties of HSPB6.

G. Cardioprotective Effect of HSPB6

Stimulation of cardiomyocites with isoproterenol was accompanied by phosphorylation of a number of proteins including troponin I (at short periods of stimulation) and HSPB6 (after sustained stimulation) (64). While prolonged treatment with isoproterenol led to apoptosis, overexpression of HSPB6 effectively prevented isoproterenol-induced apoptosis (100). It is worthwhile mentioning that the S16D mutant mimicking phosphorylation was even more effective than the wild-type HSPB6 in preventing cardiomyocyte apoptosis (100). HSPB6 was also effective in protection of cardiomyocytes against ischemia/reperfusion injury (102). It is thought that the injury leads to translocation of HSPB6 to actin filaments, and HSPB6 overexpression leads to actin filament stabilization (100, 126, 255). In addition, under unfavorable conditions, overexpression of HSPB6 leads to a strong antiapoptotic effect (99), which seems to be due to an increased protein ratio of Bcl-2/Bax and reduced caspase-3 activity (102). The antiapoptotic effect seems to be dependent on phosphorylation, since the recently described human HSPB6 mutant carrying mutation (P20L) close to the site of phosphorylation (Ser16) is poorly phosphorylated and has a decreased antiapoptotic activity (237). The detailed molecular mechanism of HSPB6 antiapoptotic action still remains unsolved; however, there are several lines of investigation designed to elucidate mechanisms of HSPB6 cardioprotection. First, it is thought that HSPB6 might interact with and regulate activity of several protein kinases. For example, HSPB6 seems to activate Akt and thus reduces doxorubicin-triggered oxidative stress and cardiotoxicity (104). On the other hand, HSPB6 seems to be able to inhibit the

apoptosis signal-regulating kinase/c-Jun-NH_2-terminal kinase/p38 pathway (103) and therefore attenuates β-agonist-mediated cardiac remodeling. Recently published data also indicate that genetically engineered rat mesenchymal stem cells containing the gene of HSPB6 are resistant to oxidative stress probably due to enhanced activation of Akt (338). These genetically engineered cells had increased secretion of growth factors, thus making modified mesenchymal stem cells very useful for treatment of myocardial infarction (338). The direct effect of HSPB6 on different protein kinases still remains debatable and may be a consequence of certain nonspecific upstream activities such as modulation of protein folding or autophagy. Second, phosphorylated HSPB6 might be involved in regulation of autophagy (261), and HSPB6 seems to participate in Bag3-dependent clearance of aggregated proteins (117). Therefore, HSPB6 might be involved in the removing of denatured and aggregated proteins, thus protecting the cell from apoptosis. Third, HSPB6 seems to interact with and to inhibit NF-κB, which is responsible for proinflammatory cytokine syntheses during sepsis (339). This effect of HSPB6 could protect against lipopolysaccharide (LPS)-induced cardiac apoptosis and dysfunction (323). Fourth, it is thought that the cardioprotective effect of HSPB6 can be at least partly due to its chaperone-like activity. For instance, deletion of the COOH-terminal extension of HSPB6, which is believed to be important for chaperone-like activity, decreased the cardioprotective effect of HSPB6 (158). It is also thought that HSPB6 contains sites important for binding of growth factors and may be important for their folding, processing, and prolonged half-life (338).

Many activities of HSPB6 are similar to the corresponding activities of HSPB1. For instance, both proteins possess antiapoptotic and antioxidant activities, might interact with and probably modulate activities of certain protein kinases, and demonstrate chaperone-like activity in vitro. However, mechanisms of action and sometimes even the final results of HSPB1 and HSPB6 activities can be completely different. HSPB1 prevents aggregation of misfolded proteins either by passing them to ATP-dependent chaperones (HSP70) (335) or by activating proteasomal degradation (247, 248), whereas HSPB6 prevents aggregation of misfolded proteins predominantly by stimulating autophagy (117, 335). Both HSPB1 and HSPB6 seem to be somehow involved in regulation of muscle contraction. However, phosphorylation of HSPB1 correlates with smooth muscle contraction (116, 224, 294, 296), whereas phosphorylation of HSPB6 promotes relaxation of smooth muscle (88, 266, 320, 349). HSPB1 and HSPB6 are highly expressed in muscles and form hetero-oligomeric complexes mutually affecting the structure and properties of each other (39, 41). This makes description of molecular mechanisms of HSPB6 action very complicated. However, the data of literature clearly indicate that HSPB6 plays an important role in cardioprotection.

H. HSPB6 as a Potential Regulator of Platelet Functions

At low concentration (0.01–0.1 mg/ml) HSPB6 inhibits the thrombin-induced aggregation of platelets (217). Further investigations revealed that HSPB6 somehow blocks thrombin-induced calcium entrance from outside of platelets (238), and this effect is at least partially explained by the fact that HSPB6 inhibits thrombin-induced activation of phospholipase C and accumulation of diacylglycerols which are important for activation of protein kinase C (184). Endothelial injury is accompanied by increased plasma level of HSPB6, which might participate in inhibition of platelet aggregation (184). Inhibition of platelet aggregation is specific for HSPB6 and αB-crystallin (HSPB5), whereas HSP27 (HSPB1) is ineffective in prevention of platelet aggregation (164, 216). Short peptides of αB-crystallin (HSPB5) (^{9}WIRRPFFPF^{17}) and of HSPB6 (^{11}WLRRASAPL^{19}) effectively prevent platelet aggregation induced by thrombin, botrocetin, ristocetin, but not by collagen and ADP (164, 216). The detailed mechanism of antiplatelet action of HSPB6 remains enigmatic; however, it is theorized that HSPB6 might prevent thrombin-induced activation of platelets, might affect platelet-fibrinogen interaction and platelet aggregation and/or inhibit platelet adhesion. Thus short peptides of αB-crystallin and HSPB6 are considered as potential pharmaceutical compounds for therapy of thrombosis and cardiovascular diseases (101, 164, 216).

I. Potential Participation of HSPB6 in Insulin-Dependent Regulation of Metabolism in Smooth and Skeletal Muscles

Addition of insulin to the strips of rat soleus or extensor digitorum longus was accompanied by phosphorylation of Ser157 of HSPB6 (340, 342). At the same time, addition of forskolin (or amylin) to the rat soleus or extenser digitorum longus inhibited insulin-induced phosphorylation of Ser157 of HSPB6 and at the same time activated phosphorylation of Ser16 (and of another unidentified site) in the structure of HSPB6 (340, 341). It was speculated that in rat skeletal muscles, insulin-induced phosphorylation of HSPB6 Ser157 somehow promotes glucose transport and by this means stimulates muscle contraction (340). At the same time, in the smooth muscle, insulin does not affect phosphorylation of Ser157, but unexpectedly increases phosphorylation of Ser16 (340) thus inducing relaxation of smooth vessel muscle and promoting glucose transportation to the peripheral organs. Molecular mechanisms underlying participation of

HSPB6 in insulin action remain poorly understood; however, overexpression of HSPB6 is accompanied by significant changes in insulin-dependent glucose transportation in cultured L6 myotube cells (342). Human HSPB6 does not contain residues homologous to Ser157 of the rat HSPB6, and therefore, insulin would not be expected to induce phosphorylation of human HSPB6. To our knowledge, effect of HSPB6 on insulin action in human remains uninvestigated.

In summary, it might be concluded that HSPB6 plays an important role in regulation of muscle contraction, in cardioprotection, in regulation of apoptosis and metabolism, and in protection against oxidative stress and many other processes which are described in a number of recently published reviews (88, 99, 101, 132, 277).

V. SMALL HEAT SHOCK PROTEIN HSPB8 (HSP22, H11 PROTEIN KINASE, OR GENE *E2IG1* PRODUCT)

HSPB8 was first described in 2000 in human melanoma cells, where it was designated as H11 protein kinase, similar to the protein kinase domain of the large subunit of herpes simplex virus type 2 ribunucleotide reductase (292). The same year, this protein was also detected among a small numbers of proteins that were expressed in response to estrogen in estrogen-responsive breast cancer cells (53). In this case, it was designated as a product of *E2IG1* gene, coding a novel member of the family of small heat shock proteins (53). A year later, HSPB8 was rediscovered by Benndorf et al. (21). This time it was recognized as a typical small heat shock protein containing α-crystallin domain and closely related to other members of the small heat shock protein family and especially to HSP27 (HSPB1) (309). Since the molecular mass of HSPB8 was close to 22 kDa, it was marked as HSP22 (21) in the early publications and renamed HSPB8 in modern publications (110, 166, 334).

A. Expression and Location of HSPB8

HSPB8 belongs to the small heat shock proteins ubiquitously expressed in practically all tissues (21, 123, 314, 330) **(TABLE 1)**. In the case of perinatal developing pig, the level of HSPB8 expression was found to be dependent on the stage of development (330). Two putative heat shock factor binding sites were detected 1,000 bases upstream of the HSPB8 translation start site; however, expression of HSPB8 was heat-inducible in the case of MCF-7 cells (63) and in cultured neurons (179) and was not dependent on heat shock in HeLa cells (63). This can be due to the fact that induction of the heat shock response is dependent on the severity of the stress and might be cell type specific (63, 123). As already mentioned, expression of HSPB8 is increased by estrogen in estrogen-receptor positive breast cancer cells (307), and the mRNA level of HSPB8 was higher in invasive than in preinvasive tumors (352). Different types of lesions, such as amyotrophic lateral sclerosis (4), experimental hybernating myocardium (77), and transient ischemia (78), were also accompanied by upregulation of the *HSPB8* gene in spinal cord and cardiac muscle. However, hypoxia at birth induced significant increase of the αB-crystallin (HSPB5) expression in piglet hearts without any significant increase of HSPB8 expression (209). All of these facts indicate that expression of HSPB8 is strictly regulated and is dependent on many poorly characterized factors.

The intracellular location of HSPB8 is not well characterized. However, the data of size-exclusion chromatography of extracts of estrogen receptor-positive breast cancer cells indicate that HSPB8 is detected predominantly in cytoplasm and is eluted in the fractions containing proteins with apparent molecular masses in the range of 30–670 kDa, and addition of estrogen affects distribution of HSPB8 on the size-exclusion chromatography (307). Immunochemical studies indicate that HSPB8 interacts with plasma membrane in human neuroblastoma cell line SK-N-SH and forms tight complexes with phospholipids located in the intracellular leaflet of biological membranes (62). Very often HSPB8 is colocalized with aggregates formed by partially denatured or improperly folded proteins. For instance, HSPB8 was detected in aggregates formed by αB-crystallin mutants whose expression correlates with certain types of inherited myofibrillar myopathy (291). HSPB8 can be trapped within inclusions formed by proteins with polyglutamine tails [fragment of huntingtin (Httg43Q)] and is able to prevent formation of aggregates formed by this protein (49). HSPB8 was detected in senile plaques and cerebral amyloid angiopathy which are pathological lesions of Alzheimer's disease and hereditary cerebral hemorrhage with amyloidosis of the Dutch type (347, 348). All of these observations might indicate that HSPB8 is involved in protecting the cell from accumulation of insoluble aggregates either by preventing aggregation or by promoting degradation of improperly folded proteins.

B. Physicochemical Properties of HSPB8

Human HSPB8 contains a conservative α-crystallin domain located in the COOH-terminal part of the protein molecule and has a calculated molecular mass 21.5 kDa (**FIG. 1*C***, **TABLE 1**). The primary structure of HSPB8 ortholog from other species is very similar to that of its human counterpart (21, 63). There are certain properties that distinguish HSPB8 from the other members of the family of small heat shock proteins. For instance, theoretical predictions indicate that the structure of HSPB8 is enriched in β-strands and unordered structures (63, 167) and that HSPB8 lacks the so-called β2 strand detected in many other small heat shock proteins (167, 171). The data of circular dichroism

spectroscopy also indicate that a rather large portion of HSPB8 is unordered (63, 175, 177). The primary structure of HSPB8 contains many Pro residues, and therefore, certain parts of HSPB8 seem to be able to form short stretches of the so-called polyproline type II (PPII) structures that contain two or three PXXP/PXP repeats (189). HSPB8 is rather resistant to thermal denaturation (171) and is very susceptible to proteolysis (167, 175). All these properties corroborate with theoretical predictions of disorder probability (167) and indicate that HSPB8 belongs to the group of intrinsically disordered proteins, a rapidly growing group of proteins playing important role in recognition, regulation, and cell signaling (321).

The data on oligomeric structure of HSPB8 remain controversial. On the basis of the data of ultracentrifugation in a glycerol gradient, it was postulated that HSPB8 is presented in the form of an extended monomer (63, 147), a viewpoint that is embraced in the UniProtKB/Swiss-Prot database. If this conclusion is correct, then HSPB8 is unique among other small heat shock proteins that tend to form either dimers or high-order oligomers (137, 221, 334). However, the data from chemical cross-linking experiments (175, 177) and the yeast two-hybrid system (309) indicate that HSPB8 forms dimers and high-order oligomers. If the cell extract is subjected to glycerol gradient ultracentrifugation, HSPB8 sediments as a dimer (or even as a tetramer) (56); in addition, the two-dimensional electrophoresis of cell extract indicates that HSPB8 is presented in the form of both monomer and dimer (21), and finally the data of size-exclusion chromatography of isolated HSPB8 indicate that it is presented in the form of an equilibrium mixture of monomers and dimers (167, 175).

C. Putative Protein Kinase Activity and HSPB8 Phosphorylation

As already mentioned at the beginning, HSPB8 was described as an unusual protein kinase (H11 kinase) similar to the protein kinase domain of the large subunit (R1) of herpes simplex virus type 2 (HSV-2) ribonucleotide reductase (292). It was postulated that H11 kinase is able to undergo autophosphorylation and to phosphorylate exogenous protein substrates (myelin basic protein) (76, 292). These experiments were performed by using immunocomplexes of recombinant fusion HSPB8 carrying different tags rather than isolated unmodified HSPB8. Further investigations performed on isolated protein confirmed that HSPB8 undergoes autophosphorylation (63); however, the rate and the extent of phosphorylation were negligibly low (177). This might indicate that the protein kinase activity ascribed to HSPB8 was due to the presence of trace amounts of exogenous protein kinases contaminating immunocomplexes used in the initial experiments (177). This viewpoint was criticized, and it was postulated that H11 kinase possesses intrinsic protein kinase activity and is indeed similar to the protein kinase domain of the large subunit of herpes simplex virus ribonucleotide reductase (122). However, the literature indicates that the large subunit (R1) of herpes simplex virus type 2 ribonucleotide reductase does not possess intrinsic protein kinase activity and that the protein kinase activity earlier ascribed to this protein is due to the presence of contaminating exogenous protein kinases (192). Moreover, recently published data indicate that the R1 subunit of virus ribonucleotide reductase contains a fragment (located close to the putative protein kinase domain) that is similar to the α-crystallin domain that is common for all small heat shock proteins, and it is therefore possible that R1 possesses chaperone-like activity (50). These data mean that the structure of HSPB8 is indeed similar to the structure of certain parts of the R1 subunit of the virus ribonucleotide reductase due to the fact that both proteins contain α-crystallin domain; however, neither HSPB8 nor R1 subunit of ribonucleotide reductase possesses intrinsic protein kinase activity (176, 288). Despite all arguments and the absence of a firmly established relationship between protein kinases and any small heat shock proteins (114), HSPB8 is still considered by a few in the field to be a protein possessing intrinsic protein kinase activity (73, 127).

Belonging to the group of intrinsically disordered proteins, HSPB8 (167, 171) seems to be a good substrate for different protein kinases. Indeed, in vitro protein kinase C phosphorylates Ser14 and Thr63, ERK1 (p44) phosphorylates Ser27 and Thr87 **(FIG. 1*C*)**, whereas casein kinase 2 phosphorylates a number of unidentified sites (21). Under in vitro conditions cAMP-dependent protein kinase phosphorylates Ser57, and phosphorylation or site-directed mutation mimicking phosphorylation of Ser57 (S57D) or Ser24 (S24D) affect the quaternary structure and chaperone-like activity of HSPB8 (287). Modern proteomic methods provide important information on HSPB8 phosphorylation in vivo. Recently published data indicate that HSPB8 is phosphorylated at Ser24 (44, 75) and Ser(Thr)87 (332) **(FIG. 1*C*)**. In addition, Tyr118 of HSPB8 was found to be phosphorylated in non-small-cell lung cancer cells (270). The effect of phosphorylation on the structure and properties of HSPB8 remains poorly investigated.

D. Interaction of HSPB8 With Other Small Heat Shock Proteins

Initially HSPB8 was described as a novel small heat shock protein tightly interacting with a 3D mutant of HSPB1 (HSP27) mimicking the phosphorylation by MAPKAP2 kinase (21). Further investigations using different techniques revealed interaction of HSPB8 with itself, HSPB2, HSPB7, and HSPB1 (309). The strength of interaction depends on the protein partners. For instance, all methods [including modern proteomic approaches (274)] revealed tight interaction of HSPB8 with HSPB7. At the

same time, cross-linking and immunoprecipitation failed to reveal a tight interaction of HSPB8 with HSPB1, whereas the yeast two-hybrid system and fluorescence resonance energy transfer (FRET) indicate interaction between these proteins (309). Later, interaction of HSPB8 with HSPB6, αB-crystallin, and HSPB3 was also established (111).

HSPB8 prevents formation of large oligomers and aggregates of mutated (R120G) αB-crystallin, whose expression correlates with the development of desmin-related cardiomyopathy (279). HSPB8 interacts with three mutants of αB-crystallin (R120G, Q151X, and 464delCT), causing inherited myofibrillar myopathy and tending to form cytoplasmic aggregates (291). The rescue oligomeric organization of the R120G mutant thus retards or prevents formation of insoluble aggregates (56). All these facts indicate that due to one of its properties, chaperone-like activity or regulating of autophagy activity, HSPB8 plays an important role in protecting the cell from accumulation of aggregated proteins. Interaction of HSPB8 with protein partners seems to be regulated. For instance, mutation mimicking phosphorylation of Ser15 of HSPB1 or mutation mimicking phosphorylation of Ser16 of HSPB6 affects their interaction with HSPB8 (310). In summary, it may be concluded that HSPB8 interacts with different strengths with practically all (except of HSPB9 and HSPB10) small heat shock proteins. This interaction is strictly regulated and can play an important role in preventing aggregation of misfolded small heat shock proteins and their protein targets. Unfortunately, the stability and stoichiometry of the complexes formed by HSPB8 with different small heat shock proteins have not been analyzed in detail.

E. Chaperone-Like Activity of HSPB8

Similar to the other small heat shock proteins, HSPB8 effectively prevents aggregation of partially unfolded or denatured proteins in vitro. Chaperone-like activity of HSPB8 was demonstrated in vitro with a number of different model substrates (63, 175, 234). However, what is the most important is that HSPB8 is able to diminish or even to prevent the accumulation of protein aggregates in the cell.

HSPB8 prevents formation of the aggresomes formed by the R120G mutant of αB-crystallin, suggesting that HSPB8 has an authentic chaperone-like activity (56). Expression of the R120G mutant of αB-crystallin (HSPB5) correlates with the development of desmin-related cardiomyopathy **(TABLE 2)**, which is accompanied by accumulation of aggresomes containing desmin and mutated αB-crystallin in the form of preamyloid oligomer intermediates (279). Overexpression of HSPB8 was accompanied by reduction in amyloid aggregates leading to improved cardiac function and survival (278). Expression of two other αB-crystallin mutants, namely, Q151X (deletion of the COOH-terminal residues 151–175) and 464delCT (deletion of two nucleotides-induced frame shifting, leading to changes in the structure and the length of the COOH-terminal end), correlates with the development of myofibrillar myopathy **(TABLE 2)** and accumulation of aggregates of mutated αB-crystallin (291). HSPB8 interacted with all αB-crystallin mutants; however, its interaction with Q151X was not as strong as in the case with 464delCT or R120G mutants (291). This indicates that the efficiency of HSPB8 rescuing effect depends on the type of αB-crystallin mutant expressed in the cell.

HSPB8 was more effective than HSPB1 in preventing in vivo aggregation of polyglutamine containing proteins (fragment of huntingtin containing 43 Gln and androgen receptor containing 65 Gln residues) (49). HSPB8 interacts with amyloid β-peptides ($A\beta_{1-42}$ and $A\beta_{1-40}$) and inhibits the death of cardiovascular cells induced by Dutch-type $A\beta_{1-40}$ by reducing accumulation of amyloid peptides on the cell surface (347). The mechanism of protective action of HSPB8 in vivo remains elusive. It can be based on the following: *1*) the basal chaperone-like effect ascribed to all members of sHSP family, *2*) the regulation of autophagy or proteasomal degradation of improperly folded proteins, or *3*) the combination of both these effects. Regardless of the mechanism, we might conclude that in vivo HSPB8 interacts with partially unfolded proteins preventing their aggregation and protecting the cell from aggresome accumulation, thus improving cell survival.

Two natural mutations of human HSPB8, namely, K141E and K141N, have been described in the literature (157, 313) **(TABLE 2)**. Both missense mutations correlate with the development of distal hereditary motor neuropathy type II (dHMN) (157), whereas mutation K141N of HSPB8 seems to be responsible for autosomal dominant Charcot-Marie-Tooth disease type 2L (CMT2L) in a large Chinese CMT family (313). Expression of K141N or K141E mutants of HSPB8 in motor neurons resulted in neurite degeneration and in reduction in average length of neurites, but did not alter the phenotype of sensory and cortical neurons (156). Analysis of 112 familial and isolated patients revealed that the gene of HSPB8 is among four genes in which mutations correlate with the development of autosomal dominant distal hereditary motor neuropathy (82); however, families with mutations in the HSPB8 gene are rather rare (146). Lys141 of HSPB8 is homologous to Arg140 of HSPB1, Arg116 of HSPB4 (αA-crystallin), and Arg120 of HSPB5 (αB-crystallin) (167). Mutation R140G of HSPB1 correlates with the development of autosomal dominant or sporadic hereditary motor neuropathy (146), mutation R116C of HSPB4 is accompanied by the development of congenital cataract, whereas mutation R120G of HSPB5 correlates with the development of congenital cataract and/or desmin-related myopathy (22) **(TABLE 2)**. This means that the residues homologous to K141 from the primary struc-

ture of HSPB8 might be of special importance for stabilization of proper structure and normal functioning of several of the small heat shock proteins.

Indeed, mutation K141E is accompanied by significant changes in the secondary structure of HSPB8, increased susceptibility to proteolysis, and decreased chaperone-like activity measured in vitro (175). Mutations K141E and K141N also lead to decreased chaperone-like activity of HSPB8 measured on polyglutamine proteins as substrates in vivo (49). Transfection of COS cells with chimeric HSPB8 fused to enhanced fluorescent protein was accompanied by the accumulation of aggregates, and this process was especially pronounced in the case of K141E and K141N mutants (112, 157). Other small heat shock proteins (HSPB1, HSPB5, and HSPB6) are able to interact with mutated HSPB8 and to partially prevent HSPB8 aggregation (112). In summary, it may be concluded that mutations K141E and K141N affect the structure of HSPB8 leading to an increased tendency for self-aggregation and decreased chaperone-like activity. Physiologically, HSPB8 mutations might be responsible for alterations in the neurofilament networks and neuronal architecture, a cause for a large spectrum of neurological diseases (128, 311).

F. HSPB8 and Regulated Proteolysis of Partially Unfolded Proteins

Proteolytic degradation of unfolded proteins can be achieved either with participation of proteasomes or by macroautophagy. HSPB8 seems to be involved in both routes of regulated proteolysis of unfolded proteins.

In the case of cardiac hypertrophy induced by HSPB8 overexpression, the catalytic activity of 20S subunit of proteasome was significantly increased, and both 19S and 20S subunits of proteasome were redistributed from the cytosol to the nuclear periphery. Inhibition of proteasome activity reduced cardiac hypertrophy induced upon HSPB8 overexpression (140). It is thought that HSPB8 somehow affects stability and intracellular location of proteasomes and by this means activates proteolytic degradation of certain proteins which under normal conditions prevent cardiac hypertrophy (for example, Foxo3a or inducible cAMP early repressor) (140) **(FIG. 6)**.

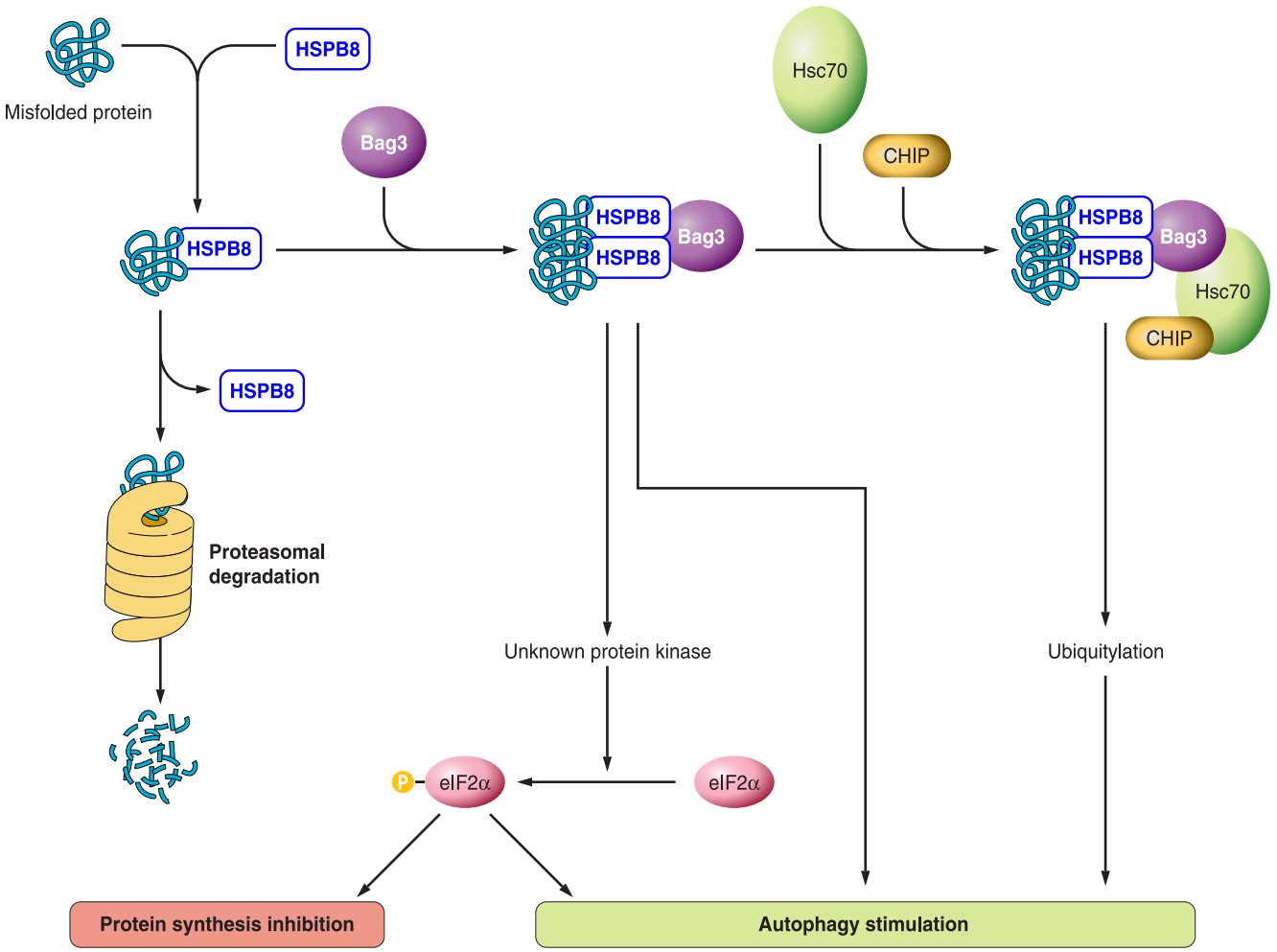

FIGURE 6. Participation of HSPB8 in regulated proteolysis of misfolded proteins. See text for details.

Recently published data indicate that HSPB8 interacts with Bag3, a stimulator of macroautophagy (47, 48). Bag3 is a member of a large family of the so-called cochaperones (312). The members of this family contain a conserved Bag-domain, interact with HSPA (HSP70), and modulate targeting of HSPA (HSP70) client proteins (117). Bag3 contains several different domains (220). For instance, the NH_2-terminal part of Bag3 contains a WW-domain responsible for interaction with PXXP regions of different target proteins and two pseudo repeats containing conserved IPV triads (117, 220). These IPV triads of Bag3 interact with the hydrophobic pocket formed by β4- and β8-strands of HSPB8 (and probably with the corresponding parts of HSPB6) and provides for formation of a complex with a stoichiometry of Bag3 to HSPB8 equal to 1:2 (117) **(FIG. 6)**. Located in the COOH-terminal part, PXXP and Bag domains are involved in the interaction of Bag3 with the γ-isoform of phospholipase C (PXXP domain) and with HSPA (HSP70) and Bcl2 (Bag-domain) (220). Recently published data indicate that Bag3 interacts with cytosolic chaperone CCT and is somehow involved in proper actin folding (113). Interacting with Bag3, HSPB8 is part of multiheteromeric complex formed by HSPB8, Bag3, Hsc70, and CHIP (chaperone-associated ubiquitin ligase) and promotes autophagic removal of misfolded proteins (one of which is superoxide dismutase) involved in the development of amyotrophic lateral sclerosis (68) **(FIG. 6)**. Thus Bag3 seems to be a very important adapter interacting with a number of proteins involved in the regulation of many different processes inside of the cell.

The complex formed by HSPB8 and Bag3 activates phosphorylation of the α-subunit of translation initiator factor eIF2 (45, 46). Although the exact mechanism and the kind of protein kinases involved in eIF2α phosphorylation remain unknown, it is clear that phosphorylation of eIF2α inhibits general protein synthesis, while allowing essential protein synthesis and stimulating macroautophagy (45, 46) **(FIG. 6)**. This pathway seems to be independent of HSP70 and provides an alternative mechanism for HSPB8-dependent regulation of protein synthesis and degradation.

In summary, it may be concluded that HSPB8 might stimulate the activity and affect intracellular location of proteasomes (140) **(FIG. 6)**. HSPB8 with a cargo of misfolded protein can interact with Bag3. The complex consisting of Bag3, HSPB8, and misfolded proteins stimulates macroautophagy and provides for cell clearing of aggregated proteins (5, 47, 48) **(FIG. 6)**. In addition, by an unknown mechanism, this complex equally leads to phosphorylation of eIF2α, inhibition of protein synthesis, and stimulation of autophagy (45, 46) **(FIG. 6)**. Finally, HSPB8 in the complex with Bag3 can be also involved in the transportation of misfolded proteins to HSPA8 (Hsc70). In this complex, the target proteins (and probably Bag3) undergo ubiquitylation catalyzed by chaperone-associated ubiquitin ligase CHIP. Subsequent recruitment of autophagic ubiquitin adapter p62, which interacts with phagophore membrane, leads to degradation of misfolded proteins in autophagosomes (5) **(FIG. 6)**. Thus HSPB8 is believed to be in the center of many different processes involved in the regulation of proteolytic degradation of unfolded proteins (5).

G. HSPB8 in Carcinogenesis and Apoptosis

Treatment of human estrogen-sensitive breast cancer MCF-7 cells with 17β-estradiol (E_2) leads to the increased expression of certain genes and among them the *E2IG1* gene encoding for HSPB8 (53). Moreover, it was shown that E_2 and the metalloestrogen cadmium induced expression of HSPB8 in estrogen receptor-positive (MCF-7), but not in the estrogen receptor-negative (MDA-MB-231) cell lines (307). Estrogen-induced increase of HSPB8 expression was blocked by true antiestrogen (faslodex, fulvestrant), whereas tamoxifen, being a partial agonist and partial antagonist of estrogen receptors, only slightly affected HSPB8 expression (307). Analysis of preinvasive and invasive tumors from mice and of tissues from patients-matched normal, ductal carcinoma in situ and invasive ductal carcinoma revealed certain genes displaying higher expression in invasive lesions. These genes (including *HSPB8*) induced anchorage independence and increased cell proliferation (352). These were the same genes that were activated by 17β-estradiol and cyclin D1, and upregulation of these genes could be prevented by the addition of irreversible dual kinase inhibitor of erB1/erB2 signaling (352). Overexpression of cyclin D1 was detected in many cancers (and among them, breast cancer), and cyclin D1 level correlated with tumor invasiveness. These effects can be explained by cyclin D1 involvement in the regulation of cyclin-dependent protein kinases and by its less well-characterized effects on gene expression (319). Unexpectedly, increased expression of cyclin D1 was accompanied by enhanced radiation sensitivity, although the mechanism remains obscure. Trent et al. (319) found that radiation sensitivity, induced by cyclin D1, is dependent on the level of HSPB8 expression. Overexpression of HSPB8 leads to an increased radiation sensitivity, whereas underexpression of HSPB8 induced by small interfering RNA was accompanied by decreased radiation sensitivity. Thus, in certain types of human tumors, HSPB8 seems to be involved in anchorage independence, radiation sensitivity, and cell proliferation.

HSPB8 is expressed in melanoma cell lines and primary melanoma tissues at higher levels than in normal melanocytes (292). HSPB8 is overexpressed in stomach tumors, proliferating human keratinocytes, and rat pheochromocytoma (PC12) cells (124). In all these cases HSPB8 demonstrates antiapoptotic properties. However, this effect seems to be dependent on the cell type, conditions (presence or absence of agents inducing differentiation), and level of HSPB8 expression. In SK-MEL-2 (melanoma), PC-3 (pros-

tate cancer), and TC32 (Ewing's sarcoma family tumor) cells, DNA demethylation leading to increased expression of HSPB8 or transient transfection with HSPB8 expression vector was accompanied by caspase and $p38^{MAPK}$-dependent apoptosis (123). Thus, depending on the cell type and conditions, HSPB8 may display both anti- and proapoptotic activity.

RNA-binding proteins can also be involved in the regulation of proliferation and tumorogenesis, and recently published data indicate that HSPB8 interacts with at least two different RNA-binding proteins. Sam68 is an RNA-binding protein involved in transportation and processing of RNA. Overexpression of HSPB8 significantly inhibits rev response element-mediated and constitutive transport element-dependent reporter gene expression, and this effect seems to be due to direct interaction of HSPB8 with Sam68 (12). In addition, it is known that Sam68 interacts with cellular Src-kinase during mitosis (12). It is thought that the sites of interaction of Sam68 with Src-kinase and HSPB8 overlap. If this assumption is correct, then HSPB8 may be able to indirectly regulate localization and/or activity of Src-kinase in the cell. HSPB8 also interacts with Ddx20 (gemin3, Dp120), a protein possessing ATP-dependent RNA unwinding (helicase) activity (308). Moreover, Ddx20 binds to survival-of-motor-neurons protein (SMN), and both Ddx20 and SMN are involved in splicesome assembly and RNA processing. Since neuropathy-associated mutations (K141E and K141N) affect interaction of HSPB8 with Ddx20, this might lead to changes in the complex formation between Ddx20 and SMN and induce different forms of inherited motor neuron diseases (308).

H. HSPB8 and Cardiac Cell Hypertrophy and Survival

Transient ischemia is accompanied by increased expression of HSPB8, thus indicating possible involvement of this protein in cell survival (78). Coronary artery disease leads to chronic dysfunction of myocardium, which is referred to as hibernating myocardium. Under these conditions, special mechanisms should provide an effective survival program, and among other proteins, HSPB8 was upregulated in hibernating human myocardium (77). Upregulation of HSPB8 was observed only in the case of viable hibernating myocardium, but not in the case of infarction. These data suggest that HSPB8 plays an important protective role in the cause of reversible changes occurring in hibernating myocardium, but is ineffective in the case of irreversible injury (77). Transgenic mice with a sevenfold increase of HSPB8 expression were characterized by significant myocardial hypertrophy that was accompanied by activation of Akt and $p70^{S6}$ kinase (76). Thus overexpression of HSPB8 induces myocardial hypertrophy that might be due to modulation of certain protein kinases (76) and/or to modulation of proteasome activity (140). The transgenic mice with increased expression of HSPB8 were also characterized by increased expression of glucose transporter GLUT1 in plasma membrane of myocytes, increased glycogen content, and phosphoglucomutase activity in the heart (337). Therefore, HSPB8 was considered as "an integrative sensor in the cardiac adaptation to stress by coordinating cell growth, survival, and metabolism" (337).

The molecular mechanisms of HSPB8 action in cardiomyocytes seem to be very complicated. It is thought that HSPB8 produces its antiapoptotic and survival effects by activation of protein kinase Akt, and this effect may be due to direct interaction of HSPB8 with Akt (73, 134). In addition, HSPB8 possesses metabolic and survival properties that seem to be due to activation of AMP-dependent protein kinase, which is responsible for increased translocation of GLUT to the plasma membrane and increased myocardial glycogen content (73, 79). These features are efficient in protecting myocardium against irreversible damage and may be explained by the direct interaction of HSPB8 with AMP-dependent protein kinase and its translocation to perinuclear space (73). Finally, it is postulated that HSPB8 is able to produce preconditioning and survival effects by activating the ϵ-isoform of protein kinase C and the inducible nitric oxide synthase (73, 79). Recently published data indicate that in transgenic mice with cardiac-specific HSPB8 overexpression this protein seems to be involved in bone morphogenetic protein (BMP) signaling (306). It is suggested that HSPB8 activates the so-called "canonical" BMP pathway, where interaction of BMP with its receptors (BMPR-II) and Alk3 results in Smad1/5/8 phosphorylation. In addition, HSPB8 seems to activate the "noncanonical" pathway, promoting interaction of TGF-β-activated kinase (TAK1) with Alk3 and BMP receptor II (306). TAK1 activates PI3-K, which in turns activates protein kinase Akt. If all of these premises are correct, then HSPB8 is a central player, starting with direct interaction with bone morphogenetic protein receptor (and/or Alk3) and leading to regulation of activity and intracellular location of different protein kinases (such as Akt, AMP-dependent protein kinase, TGF-β-activated protein kinase, and ϵ-isoform of protein kinase C).

The situation becomes even more complicated since it has been shown that HSPB8 possesses a dual effect on cardiac myocytes. Transduction of low doses of adenovirus harboring HSPB8 was accompanied by an increase of cell size, whereas at high doses this construct induced apoptosis of cardiac myocytes (134). The hypertrophic effect was explained by the activation of PI3-K and Akt protein kinase, whereas the proapoptotic effect was explained by the HSPB8-induced inhibition of casein kinase 2 activity (134). Moreover, it was postulated that the hypertrophic effect is independent of putative protein kinase activity, whereas the proapoptotic effect depends on intrinsic protein kinase activity of HSPB8 (134). As mentioned earlier, the intrinsic

protein kinase activity of HSPB8 remains very questionable (176, 288) and, therefore, explanation of proapoptotic effect of HSPB8 by its protein kinase activity is problematic. We assume that the proapoptotic effect of HSPB8 can be at least partially explained by its interaction with Bag3. It is possible that at high concentration, HSPB8 forms tight complexes with Bag3, and by this means displaces Bcl2 from its complex with Bag3. Releasing of Bcl2 induces apoptosis.

I. HSPB8 and Immune Response

Literature indicates that the heat shock proteins, such as HSPA (HSP70), HSPC (HSP90), and HSPD (HSP60), might be involved in activation of antigen-presenting cells or mature dendtritic cells, indirect presentation, and chaperoning of antigen peptides. Binding of the complexes formed by heat shock proteins and corresponding peptides to receptors on the surface of antigen-presenting cells induces synthesis of certain interleukins, tumor necrosis factor α, and nitrogen oxide (NO) (298). Small heat shock proteins may also be involved in the stimulation of antigen-presenting cells. For instance, HSPB8 and αA-crystallin (HSPB4) were able to activate dendritic cells, activation that was TLR4 (toll-like receptor 4) dependent (272). Moreover, HSPB8 was abundantly expressed in synovial tissues from patients with rheumatoid arthritis, indicating possible involvement of HSPB8 in inflammatory processes in autoimmune diseases (272). In good agreement with this suggestion, it was found that HSPB8 (as well as HSPB6 and HSPB2/HSPB3) induces interleukin-6 production in cultured pericytes and astrocytes (345). Therefore, it is supposed that HSPB8 (and, probably, certain other sHSP) might be among key mediators of local inflammation associated with hereditary cerebral hemorrhage with amyloidosis of the Dutch type (345).

Thus HSPB8 plays important roles in many different physiological processes. Certain aspects of HSPB8 functioning that were not analyzed in this paper were reviewed in earlier published reviews (83, 147, 288).

VI. CONCLUSIONS AND FUTURE PERSPECTIVES

The data presented indicate that the small heat shock proteins are involved in many vital processes. These proteins play important "housekeeping" roles and protect the cell under different unfavorable conditions. The sHSP (HSPB) are located in the nodes of complex protein networks and by interacting with many different partners affect a plethora of different cellular processes. Activities of sHSP are finely regulated by posttranslational modifications and by their ability to form homo- and hetero-oligomeric complexes.

A summary of general aspects of small heat shock protein functioning includes the following. First, sHSP interact with partially unfolded or misfolded proteins and prevent their aggregation. This interaction depends on the binding of hydrophobic regions of sHSP and client proteins and is mainly unspecific; however, certain members of the sHSP family predominantly interact with certain protein substrates. Second, unfolded client proteins bound to sHSP can be transported to the ATP-dependent chaperones where they undergo refolding and renaturation. Third, sHSP may be involved in ubiquitylation followed by proteasomal degradation of irreversible unfolded proteins. Fourth, under certain conditions, sHSP promote autophagy and by this means also provide for elimination of unfolded proteins. Fifth, sHSP interact, stabilize, and increase (or decrease) activity of many regulatory proteins such as protein kinases and protein phosphatases. In addition, certain members of the sHSP family interact with universal adapter proteins such as 14-3-3 and Bag3. Interacting with regulatory and adapter proteins, sHSP can indirectly affect many intracellular processes.

Although all sHSP protect the cell under different unfavorable conditions and therefore seem to induce similar effects, the mechanism of action of different members of this protein family can be completely different. For instance, HSPB1 and HSPB5 predominantly address unfolded proteins to ATP-dependent chaperones and thereby provide for their refolding. If refolding is impossible, then HSPB1 promotes proteasomal degradation of unfolded client proteins. HSPB6 and HSPB8 seem to be less effective (or noneffective) in refolding of unfolded proteins. At the same time, these members of sHSP family might prevent aggregation of unfolded proteins by activating autophagy (HSPB6 and HSPB8) and/or by affecting phosphorylation of translation initiation factor eIF2α leading to selective inhibition of protein synthesis (HSPB8). In addition, different members of sHSP family might have different substrate specificity, and expression of distinct sHSP is regulated by a number of different factors. All these facts complicate detailed analysis of molecular mechanisms of sHSP action. Moreover, as mentioned earlier, many members of sHSP family might participate in formation of hetero-oligomeric complexes. Unfortunately, the composition, properties, and mechanism of functioning of these complexes remain practically uninvestigated.

Multiple protein-protein interactions form the basis for involvement of sHSP in regulation of many complex processes. For instance, regulating activity of certain enzymes, sHSP protect the cell from oxidative stress, which is of great importance in development of neurodegenerative diseases and viral invasion. Regulating redox state of the cell and interacting with certain proteins sHSP participate in regulation of apoptosis and proliferation. Interacting with proteins of cytoskeleton and controlling their oxidation, sHSP play important roles in regulation of muscle contraction and cell motility. Affecting activity of protein kinases, mod-

ulating apoptosis and proteolysis, sHSP demonstrate pronounced cardioprotective activity.

Although involvement of sHSP in regulation of multiple cellular processes is unquestionable, molecular mechanisms of their action remain largely enigmatic. Future investigations should be addressed to detailed analysis of the structure of human sHSP and mechanisms underlying sHSP functioning. Special efforts should be undertaken to investigate interaction of sHSP with ATP-dependent chaperones and their cochaperones, with different components of proteasomal and autophagosomal systems, protein kinases, and protein phosphatases, as well as with different adapter proteins such as Bag3 and 14-3-3. Mutations of sHSP often correlate with development of certain congenital diseases. Thus it is important to investigate the structure and properties of sHSP mutants and to analyze the role of sHSP mutations in different human disorders. Because sHSP are involved in different cellular processes, these proteins seem to be attractive therapeutic targets. Tissue-specific regulation of sHSP expression is of great importance in development of cancer and cardiovascular therapy. Development of tissue-specific drugs regulating sHSP phosphorylation and oligomerization might be important for cardioprotection, whereas development of penetrating peptides derived from sHSP can be valuable for cardiovascular surgery and regulation of platelet aggregation. Future investigations of the structure, properties, and mechanism of action of sHSP will provide information which will be important for understanding many physiological processes and will likely result in advances in medicine.

NOTE ADDED IN PROOF

During the preparation for publication of this review, many important experimental data and reviews appeared in the literature.

New data on the structure of oligomeric bacterial small heat shock protein (142a) and the three-dimensional structure of α-crystallin domain of R120G mutant of αB-crystallin (66a) and α-crystallin domains of HSPB1 and HSPB6 (14a) were recently published. Significant progress was achieved in constructing an atomic-level model of a large (24-subunit) multimer of αB-crystallin and in determination of molecular mechanisms regulating multimerization of small heat shock proteins (161a).

A potential role of HSPB1 in cardiovascular disease was critically analyzed in the review of Ghayour-Mobarhan et al. (121a).

Protein phosphatase type I was recognized as a potential target of HSPB6, and it was shown that HSPB6 regulates activity of protein phosphatase and the level of phospholamban phosphorylation, thus affecting sarcoplasmic reticulum calcium cycling and cardiac contractility (261a). A multifunctional protective effect of HSPB6 in different cellular processes was analyzed in the recently published review of Edwards et al. (93a).

Investigation of interaction of HSP8 with different protein targets was continued, and it was shown that HSPB8 might regulate expression of Sam68 and by this means regulate proliferative potential of glioblastoma cells (231a). Interaction of HSPB8 [as well as the R120G mutant of αB-crystallin (143a)] with Bag3 was discussed in the literature, and it was concluded that HSPB8 plays an important role in autophagic removal of misfolded proteins (67a, 273a, 335) and in protection of astrocytes against different protein aggregation diseases (282a).

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DISCLOSURES

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