Structural and Functional Diversities between Members of the Human HSPB, HSPH, HSPA, and DNAJ Chaperone Families[†]

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Received April 10, 2008; Revised Manuscript Received June 2, 2008

ABSTRACT: Heat shock proteins (HSPs) were originally identified as stress-responsive proteins required to deal with proteotoxic stresses. Besides being stress-protective and possible targets for delaying progression of protein folding diseases, mutations in chaperones also have been shown to cause disease (chaperonopathies). The mechanism of action of the "classical", stress-inducible HSPs in serving as molecular chaperones preventing the irreversible aggregation of stress-unfolded or disease-related misfolded proteins is beginning to emerge. However, the human genome encodes several members for each of the various HSP families that are not stress-related but contain conserved domains. Here, we have reviewed the existing literature on the various members of the human HSPB (HSP27), HSPH (HSP110), HSPA (HSP70), and DNAJ (HSP40) families. Apart from structural and functional homologies, several diversities between members and families can be found that not only point to differences in client specificity but also seem to serve differential client handling and processing. How substrate specificity and client processing is determined is far from being understood.

Heat shock proteins (HSPs)[1] were originally discovered as proteins that are upregulated upon and protective against proteotoxic stresses, i.e., situations that increase the fraction of proteins that are in a (partially) unfolded state, thereby enhancing their probability of forming intracellular protein aggregates that can lead to loss of cell function and eventually to cell death. We now appreciate that a variety of normal cellular processes (translation, transport over membranes) constantly challenge the cellular protein homeostasis and require protein quality control systems for assistance. In addition, diseases like Alzheimer's and Parkinson's disease, CAG-repeat diseases, and many heart diseases (e.g., atrial fibrillation) or physiological disturbances (e.g., hypoxia) are pathogenic because they disturb protein homeostasis. Finally, folding mutations may arise as a result of somatic mutations (aging) and genomic instability (cancer) requiring increased protein quality control.

The HSPs make up a group of structurally unrelated protein families (HSPA, HSPB, HSPC, HSPD, HSPH, and DNAJ) that play a prime role in protein homeostasis by binding to substrates at risk, thereby keeping them in a state competent for either refolding or degradation. As such, they belong to a much larger superfamily of molecular chaperones. The number of genes encoding the diverse HSP family members largely varies per organism. For HSPA, the number varies from three in *Escherichia coli*, 14 in *Arabidopsis thaliana*, and 12 in *Drosophila melanogaster* to 13 in *Homo sapiens*. For small HSP (sHSP), the number of genes is relatively high in plants and the same holds true for DNAJ (Table 1). Although originally identified as heat inducible proteins, many members are in fact not heat shock inducible. However, within each family, individual heat shock inducible proteins such as HSPB1, HSPA1, HSPH1, and DNAJB1 are found. Why the human genome contains so many members in most families (Table 2) sometimes with a high degree of sequence homology (HSPA and HSPH) but sometimes also with substantial sequence divergence in certain domains (HSPB and DNAJ) is largely unclear. Part of the redundancy may relate to the intracompartmental distribution of the diverse family members and the requirement of their activities in these different compartments. Also, some HSPs exhibit tissue or development specific expression (Table 3). On one hand, this may reflect the ability to specifically regulate expression of the same activity and function. On the other hand, this suggests that chaperones are not merely promiscuous in terms of clients and indicates a strong need for specialized chaperones under these conditions. The fact that promis-

Table 1: Occurrence of HSP Gene Numbers in Different Species

	HSPA/H	DNAJ	HSPB	genome size (bp)
H. sapiens	13/4	41	11	3.3×10^9
D. melanogaster	12/2	36	11	1.2×10^8
A. thaliana	14/4	89	19	1.1×10^8
Saccharomyces cerevisiae	14/2	22	2	1.2×10^7
E. coli	3	6	2	4.6×10^6

[†] Funding was provided by Innovatiegerichte Onderzoeksprogramma IOP-genomics Grant IGE03018.

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[1] Abbreviations: HSP, heat shock protein; sHSP, small heat shock protein; NCBI, National Center for Biotechnology Information. In this review, we will use the NCBI gene names to refer to the various human Hsp families and their members: HSPA = HSP70, HSPB = small HSP, HSPC = HSP90, HSPD = HSP60, HSPH = HSP110, and DnaJ = HSP40.

10.1021/bi800639z CCC: $40.75
Published on Web 06/17/2008

Table 2: Alternative Names and Gene Identifiers of the Human HSP Gene Families

	gene name	protein name	alternative name	human GeneID
HSPB	*HSPB1*	HSPB1	CMT2F, HMN2B, HSP27, HSP28, Hsp25, HS.76067, DKFZp586P1322	3315
	HSPB2	HSPB2	MKBP, HSP27, Hs.78846, LOH11CR1K, MGC133245	3316
	HSPB3	HSPB3	HSPL27	8988
	HSPB4	HSPB4	αA-crystallin, CRYAA, CRYA1	1409
	HSPB5	HSPB5	αB-crystallin, CRYAB, CRYA2	1410
	HSPB6	HSPB6	HSP20, FLJ32389	126393
	HSPB7	HSPB7	cvHSP, FLJ32733, DKFZp779D0968	27129
	HSPB8	HSPB8	H11, HMN2, CMT2L, DHMN2, E2IG1, HMN2A, HSP22	26353
	HSPB9	HSPB9	FLJ27437	94086
	HSPB10	HSPB10	ODF1, ODF, RT7, ODF2, ODFP, SODF	4956
	HSPB11	HSPB11	Hsp16.2	51668
HSPH	*HSPH1*	HSPH1	HSP105	10808
	HSPH2	HSPH2	HSPH4 APG-2, Hsp110	3308
	HSPH3	HSPH3	HSH4L APG-1	22824
	HSPH4	HSPH4	HYOU1	10525
HSPA	*HSPA1A*	HSPA1A	HSP70-1, HSP72, HSPA1	3303
	HSPA1B	HSPA1B	HSP70-2	3304
	HSPA1L	HSPA1L	hum70t, hum70t	3305
	HSPA2	HSPA2	heat shock 70 kDa protein-2	3306
	HSPA5	HSPA5	BIP, GRP78, MIF2	3309
	HSPA6	HSPA6	heat shock 70 kDa protein 6 (HSP70B′)	3310
	HSPA7	HSPA7		3311
	HSPA8	HSPA8	HSC70, HSC71, HSP71, HSP73	3312
	HSPA9	HSPA9	GRP75, HSPA9B, MOT, MOT2, PBP74, mot-2	3313
	HSPA12A	HSPA12A	FLJ13874, KIAA0417	259217
	HSPA12B	HSPA12B	RP23-32L15.1, 2700081N06Rik	116835
	HSPA13	HSPA13	Stch	6782
	HSPA14	HSPA14	HSP70-4, HSP70L1, MGC131990	51182
DNAJ	*DNAJA1*	DNAJA1	DJ-2, DjA1, HDJ2, HSDJ, HSJ2, HSPF4, hDJ-2	3301
	DNAJA2	DNAJA2	DNJ3, mDj3, Dnaj3, HIRIP4	10294
	DNAJA3	DNAJA3	Tid-1, Tid1I	9093
	DNAJA4	DNAJA4	Dj4, Hsj4	55466
	DNAJB1	DNAJB1	HSPF1, Hsp40	3337
	DNAJB2	DNAJB2	HSJ1, HSPF3	3300
	DNAJB4	DNAJB4	Hsc40	11080
	DNAJB5	DNAJB5	Hsc40, Hsp40-3	25822
	DNAJB6	DNAJB6	Mrj, mDj4	10049
	DNAJB7	DNAJB7	Dj5, mDj5	150353
	DNAJB8	DNAJB8	mDj6	165721
	DNAJB9	DNAJB9	Mdg1, mDj7, ERdj4	4189
	DNAJB11	DNAJB11	Dj9, ABBP-2	51726
	DNAJB12	DNAJB12	Dj10, mDj10	54788
	DNAJB13	DNAJB13	Tsarg	374407
	DNAJB14	DNAJB14	EGNR9427, FLJ14281	79982

cuity may not be an essential feature of Hsp/chaperone activity is further supported by the existence of different HSP families and family members within the same compartment (e.g., the cytosol) (Table 3). In this review, we focus on the structural, sequence, and functional divergence within the HSPA, HSPH, DNAJ, and HSPB families either in terms of client specificity or client processing.

The classical model of the HSP chaperone functions is primarily based on cell-free experiments with human HSPA1 (or HSPA8), HSPB1, HSPH1, and DNAJB1 and work on their orthologues in *E. coli*, yeast, and mouse. In this model, un- or misfolded proteins bind to these HSPs both directly or sequentially (Figure 1). In naïve cells that were not stressed before, the instant chaperone action toward increases in proteotoxic stresses is obtained by constitutively expressed members such as cellular stores of small heat shock proteins (HSPB). It is thought that HSPB members are stored in oligomeric complexes that dissociate into smaller-sized molecules upon stress (*1, 2*). This shift in oligomeric size allows for binding of unfolded proteins, which effectively neutralizes the chance for nonspecific interaction of the unfolded substrate with other proteins. HSPB members are ATP-independent chaperones and require other partners for further client processing (see below), which depending on the substrate and/or chaperone partners can be either refolding or degradation. How this distinction in client processing is regulated is not yet clear. Clearly, transfer to the HSPA/B machine has been suggested to promote folding in vitro as well as in living cells. This ATP-dependent chaperone constantly shuttles between an ATP-bound and an ADP-bound state in which it has different affinities for unfolded proteins (*3*). Substrates enter the HSPA complex in the ATP-bound configuration. In this configuration, HSPA has a high substrate on/off rate, meaning low substrate affinity. Upon binding, ATP is hydrolyzed which stabilizes the affinity of HSPA for its substrate, a reaction which is regulated by cofactors like DNAJs and CHIP. Subsequently, nucleotide exchange is stimulated (BAG-1, HSPBP1, and HSPH), resulting in an ATP-bound HSP70 complex followed by substrate release. Unfolded proteins may also directly enter the HSPA chaperone machine or with the assistance of HSPA cochaperones like DNAJ and HSPH. In fact, there are several modulators of the HSPA ATP cycle (HSPH, DNAJ, HIP, CHIP, BAG3, and HSPBP1),

Table 3: Properties of the Human HSP Families

	level of sequence identity (%)	molecular size (kDa)	chromosome location	tissue distribution	subcellular localization	clients/substrates	associated disease
HSPB1	(100)	22.8	7q11.23	ubiquitous	cytosol	cytoskeletal components, ubiquitin, cytochrome *c*	Charcot-Marie-Tooth disease, distal hereditary motor neuropathy
HSPB2	36	20.2	11q22-q23	heart and skeletal muscle	cytosolic granules/ mitochondria	myotonic dystrophy protein kinase	
HSPB3	23	17	5q11.2	muscle	unknown		
HSPB4	36	19.9	21q22.3	eye lens	cytoplasm		cataract
HSPB5	38	20.2	11q22.3-q23.1	ubiquitous	cytosol/nucleus	cytoskeletal components	cataract, desmin-related myopathy
HSPB6	34	17.1	19q13.12	heart, muscle, brain	cytosol	14-3-3 γ, Bax	
HSPB7	20	18.6	1p36.23-p34.3	heart and skeletal muscle	cytosol/nucleus	α-filamin	upregulated in muscular dystrophy
HSPB8	34	21.6	12q24.23	muscle, brain, keratinocytes, placenta	cytosol/plasma membrane	BAG-3	Charcot-Marie-Tooth disease, distal hereditary motor neuropathy
HSPB9	19	17.5	17q21.2	testis	cytosol/nucleus	DynLT1	upregulated in certain tumors
HSPB10	17	28.4	8q22.3	testis	sperm cell tails		
HSPB11	13	16.3	1p32.1-p33	unknown	cytosol/nucleus	Hsp90	upregulated in certain tumors
HSPH1	(100)	96.9	13q12.3	ubiquitous	cytosol/nucleus		
HSPH2	63	94.3	5q31.1-q31.2	ubiquitous	cytosol/nucleus		
HSPH3	58	94.5	4q28	testis, brain, kidney, liver, lung, spleen	cytosol/nucleus		
HSPH4	25	111.3	11q23.1	ubiquitous	endoplasmic reticulum (ER)		
HSPA1A	(100)	70.0	6p21.3	ubiquitous	cytosol	promiscuous	upregulated in certain tumors
HSPA1B	99	70.0	6p21.3	ubiquitous	cytosol	promiscuous	upregulated in certain tumors
HSPA1L	88	70.4	6p21.3	testis	cytosol		rs2075800 G allele associates with sarcoidosis
HSPA2	83	70.0	14q24.1	testis/ubiquitous	cytosol/nucleus		upregulated in certain tumors
HSPA5	60	71.0	9q33-q34.1	ubiquitous	ER	ATF6	
HSPA6	81	71.0	1q23	brain, liver, ovary, saliva	cytosol/nucleus		in the proximity of a susceptibility locus for schizophrenia
HSPA7	ND	ND	1q23.3	unknown	unknown		in the proximity of a susceptibility locus for schizophrenia
HSPA8	85	70.9	11q24.1	ubiquitous	cytosol/nucleus	many growth factors	upregulated in certain tumors
HSPA9	45	73.7	5q31.1	B cell, brain, liver, ovary, platelet, saliva	mitochondria	mitochondrial proteins, p53	
HSPA12A	14	141.0	10q26.12	endothelia, brain, heart, kidney, muscle, testis	unknown		associates with atherosclerosis
HSPA12B	18	75.7	20p13	endothelia, ubiquitous	unknown		associates with atherosclerosis
HSPA13	20	51.9	21q11	unknown	microsomes		
HSPA14	27	54.8	10p14	unknown	unknown		
DNAJA1	(100)	44.9	9p13-p12	ubiquitous	cytosol	promiscuous	
DNAJA2	54	45.7	16q11.1-q11.2	brain, heart, kidney, liver	cytosol	promiscuous	
DNAJA3	24	52.5	16p13.3	fetus, mammary gland, B cell	mitochondria		protects against dilated cardiomyopathy
DNAJA4	73	44.7	15q24.1	brain	membranes	promiscuous	
DNAJB1	(100)	38.2	19p13.2	ubiquitous	cytosol	promiscuous	protects against various neuronal misfolding diseases
DNAJB2	27	35.6/30.6	2q32-q34	heart, muscle, brain	cytosol/ER		
DNAJB3	24	26.7	1 D (Mm)	testis	unknown		
DNAJB4	65	37.8	1p31.1	ubiquitous	unknown	G protein β subunit	
DNAJB5	62	39.1/26.9	9p13.2	brain, heart, liver, pancreas, skeletal muscle, spleen	unknown		
DNAJB6	27	36.1	7q36.3	ubiquitous	cytosol/nucleus	keratin-18	
DNAJB7	24	35.4	22q13.2	ubiquitous	unknown		
DNAJB8	23	25.7	3q21.3	testis	unknown		
DNAJB9	19	25.5	7q31	ubiquitous	ER		
DNAJB10	23	30.6/28.6	1 (Mm)	unknown	unknown		
DNAJB11	30	40.5	3q28	ubiquitous	ER	APOBEC1	
DNAJB12	18	41.9	10q22.2	blood plasma	unknown		
DNAJB13	48	36.1	11q13.4	fetus, spermatozoa, testis	unknown		
DNAJB14	17	42.5/33.5	4q23	unknown	unknown		

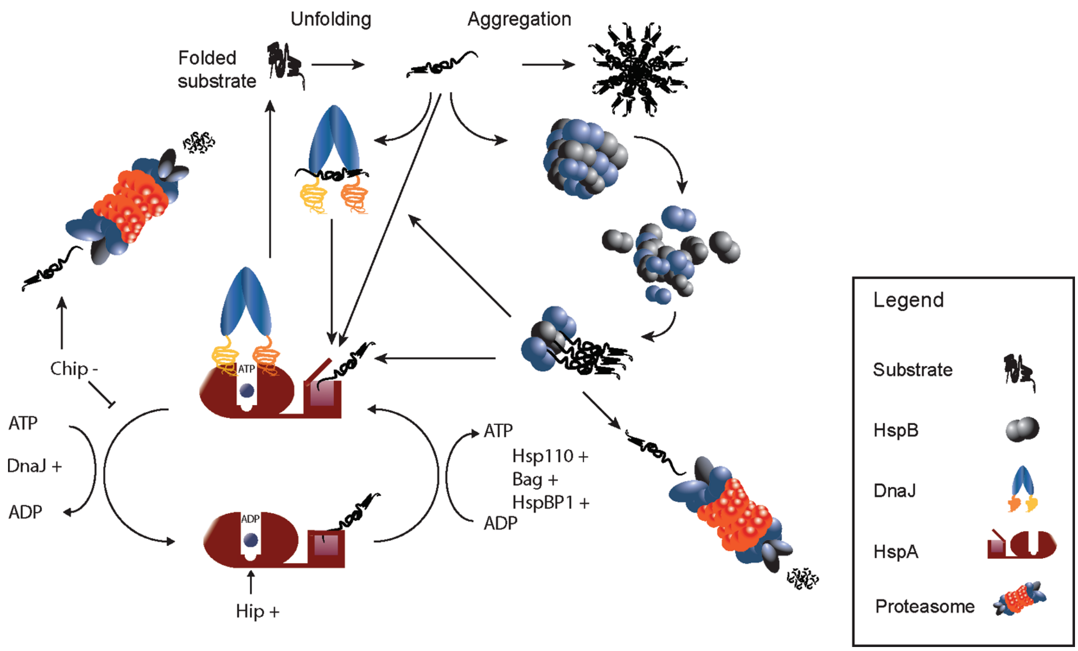

FIGURE 1: Schematic representation of HSP-mediated client processing. A folded substrate is unfolded upon a proteotoxic stress event. This unfolded substrate either aggregates or binds HSPs like HSPB, DNAJ, or HSPA. Both HSPB and DNAJ are thought to eventually hand over the substrate to the HSPA machine capable of binding and releasing the substrate. Released substrates that still expose hydrophobic patches to the exterior are bound again by HSPs, whereas substrates without such hydrophobic patches are not recognized. Substrates can also be targeted to the proteasome degradation system by HSPB1 and CHIP or other uncharacterized mechanisms.

which not only modulate the cycle but also may confer client specificity to the HSPA machine and/or affect the fate of its client.

HSPB FAMILY

Small heat shock proteins (sHSPs or HSPB in mammals) are low-molecular mass chaperones (Table 3) found in every kingdom. sHSPs are characterized by the presence of a conserved crystallin domain flanked by a variable N-terminus and C-terminus. The N- and C-termini, together with part of the crystallin domain, are involved in substrate binding (Figure 2). The majority of structural information about sHSPs comes from studies performed in archaea and plants which show that sHSPs form large symmetrical complexes composed of several dimers. Dimers are formed through strand exchange between a β-sheet extending loop present in the crystallin domain. These interactions are further strengthened by the C-terminal extensions. Together with the N-terminal extensions, this allows for buildup and stabilization of the higher oligomeric structure. The homo- and/or heterogeneous oligomeric complexes are believed to be reservoirs, which under stress can dissociate into smaller multimers that are generally assumed to be the active units. Upon dissociation into dimers (Figure 1), hydrophobic residues in the N-terminus, C-terminus, and crystallin domain become exposed, allowing interaction with substrate molecules (*4*) and preventing their irreversible aggregation. Further processing of the bound substrate is carried out by other HSP families, directing it for either refolding or degradation (Figure 1).

Functional Diversity. HSPB1. HSPB1 (HSP27) is one of the most well-studied members of the family and can exist as a high-molecular mass (e.g., hexadecamers) or low-molecular mass (e.g., tetramers and dimers) structure. Under nonstressed situations, a high-molecular mass form is the most predominant species. During heat stress, its level decreases with a concurrent increase in the amount of two low-molecular mass phosphorylated forms. HSPB1 can be phosphorylated at three sites (figure 2) which regulates its activity. A pseudophosphorylated mutant of HSPB1 (HSPB1-3D) shows a decrease in in vitro chaperone activity, either implying the oligomeric sHSP structure as a key for in vitro chaperone action or pointing to a need for shuttling between dimers and oligomers. The latter is supported by data from cellular studies showing that overexpression of the pseudophosphorylated HSPB1 increases the cellular chaperone capacity (*5*). In this setting, HSPB1 may also form mixed oligomers with endogenously expressed HSPB members. Interestingly, a nonphosphorylatable mutant was ineffective in cells in increasing chaperone activity. Considering the in vitro and cellular data together, shuttling between dimers and (heterogeneous) oligomers seems to be required for substrate binding and protection against aggregation (Figure 1). However, the nonphosphorylatable mutant is still able to protect against oxidative stress, suggesting that either this stress directly causes (phosphorylation-independent) changes in oligomeric structure or nondynamic oligomeric structures can also act cytoprotectively. Once activated by phosphorylation and oligomeric changes, HSPB1 can bind non-native substrates. For substrate release, it requires the help of ATP-dependent chaperones (HSPA) or proteases (HSP104) (*6*) that further process the client. Consistent with this notion, the increase in the level of refolding mediated by transfected HSPB1 was prevented by inhibiting the HSPA chaperone machine (*5*). When refolding via the HSPA machine is not possible, HSPB1-bound substrates might be ubiquitynated and targeted to the 26S proteasome for degradation. Besides its role in assisting refolding and proteasomal targeting of soluble (denatured) proteins, one of the best-characterized functions of HSPB1 is its ability to interact with several

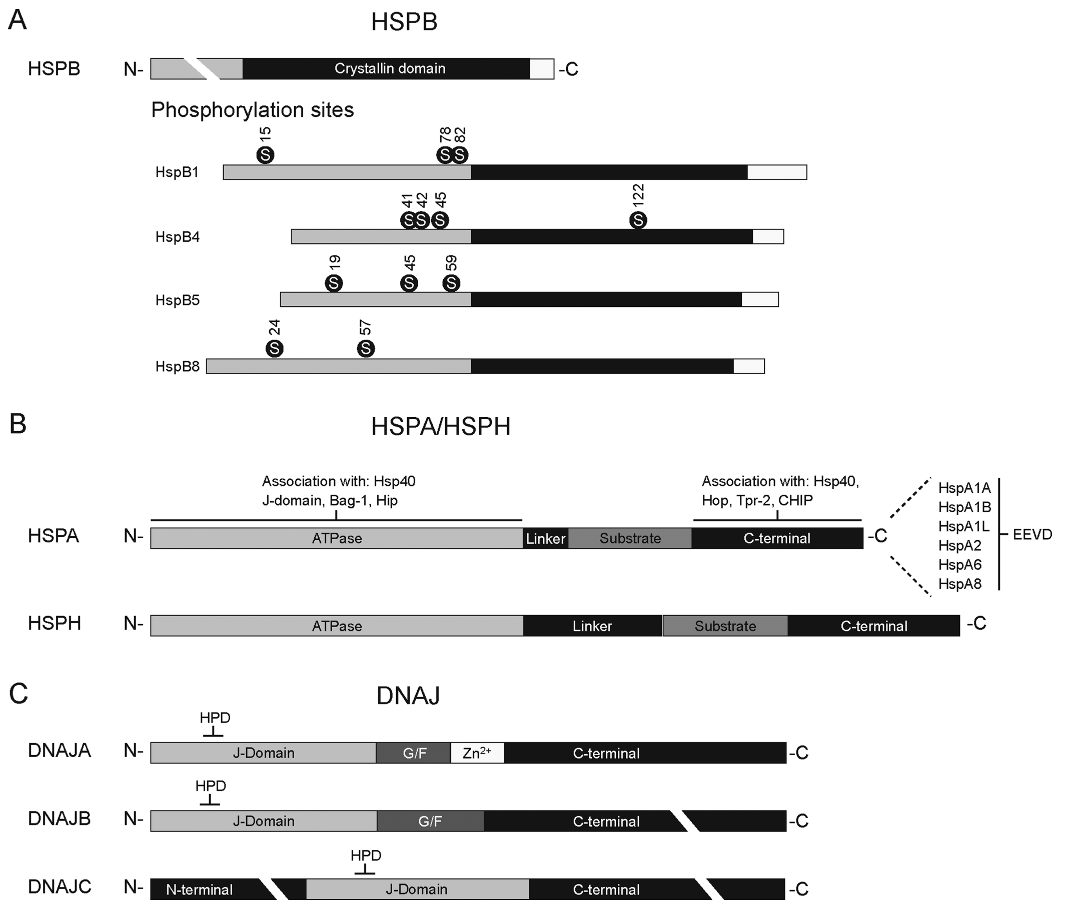

FIGURE 2: Linear representation of HSPB, HSPA/HSPH, and DNAJ proteins.

cytoskeletal components, including actin, intermediate filaments, and microtubules (*7*). In muscle tissue, HSPB1 is found in association with sarcomeres and suggested to be cardioprotective. In neuronal cells, overexpression of a mutated form of HSPB1 (S135F), which is associated with distal hereditary peripheral neuropathies (*8*), resulted in neurofilament network disruption, further confirming the critical role of this protein in cytoskeleton stabilization. Finally, after (heat) stress, HSPB1 shows a reversible nuclear accumulation into SC35 splicing speckles, structures implied in RNA processing (*5*). Also, other HSPB members like HSPB5 and HSPB7 (M. J. Vos, unpublished observations) have been found to localize to these structures. Localization of HSPB1 to these nuclear structures is not associated with refolding of heat-denatured nuclear proteins but overlaps with sites of nuclear protein degradation (*5*).

HSPB4 and HSPB5. HSPB4 (αA-crystallin) and HSPB5 (αB-crystallin) are both strongly expressed in the eye lens, where together they maintain lens transparency. Mutations in both HSPB4 and HSPB5, causing protein instability and aggregation, result in congenital cataract development (*9*) (Table 3). The amount of HSPB5 is also expressed in striated muscle where, together with HSPB1, it can be found in association with sarcomeric structures. In line with this, mutations in HSPB5 have been linked to muscle and cardiac myopathies (*9*). Like HSPB1, HSPB4 and HSPB5 have chaperone activity in vitro for which a dimer comprising only the crystallin domain was found to be sufficient (*10*). Also, HSPB4 and HSPB5 can be phosphorylated on different serines (Figure 2). Recombinant HSPB5-3D forms oligomers that are smaller than those formed by wild-type HSPB5. Whereas the three-dimensional (3D) mutant exhibited a reduced chaperone activity toward heat-denatured luciferase both in vitro and in cells (*2*), it exhibited an enhanced chaperone activity toward heat-induced aggregation of citrate synthase and amyloid fibril formation of α-synuclein (*11*). These data strongly suggest that HSPB5 exhibits substrate specificity. Interestingly, the formation of mixed oligomers between HSPB5 and HSPB5-3D reduced the total chaperone capacity of HSPB5-3D. Thus, subunit exchange and the ratio between both phosphorylated and unphosphorylated subunits may be a key modulator of chaperone activity. An R120G mutation of HSPB5 was found to be associated with desmin-related myopathy (*9*). This mutation results in hyperphosphorylation and being prone to aggregation which prevents nuclear entry and reduces chaperone activity both in vitro and in vivo (*12, 13*). HSPB5, like HSPB1, plays an important role in stabilization of the cytoskeleton, which is also dependent on its phosphorylation state.

HSPB8. HpsB8 (HSP22/H11/E2IG1) is strongly expressed in striated and smooth muscles, brain, and keratinocytes. Like HSPB1, HSPB5 and HSPB8 can be phosphorylated in vitro (Figure 2). In contrast to HSPB1 and HSPB5, phosphorylation only marginally affects the tertiary and quaternary structure of HSPB8. Both wild-type and phosphorylated HSPB8 exist as low-molecular mass oligomers. Phosphorylation of HSPB8 in vitro severely lowers its chaperone activity toward denatured insulin and rhodanase. In view of HSPB1 and HSPB5, where phosphorylation increases chaperone activity and reduces oligomeric size, phosphorylated HSPB8 is present in slightly larger oligomeric structures than wild-type HSPB8. This could explain the different effect phosphorylation has on HSPB8 chaperone activity (*14*). In vitro, HSPB8 can form high-molecular mass hetero-oligomers by interacting with other members of the HSPB family. In vivo, HSPB8 forms a stable and stoichiometric complex with the cochaperone Bag3. Interestingly, HspB8 stability depends on its association with Bag3 (*15*). In contrast to the case with HSPB1 and HSPB5, which interact and stabilize cytoskeletal elements, no direct association of HSPB8 with actin and microtubules has yet been reported. Rather, a specific role for HSPB8 in autophagy is emerging (*15*). Recently, mutations in HSPB8 (K141E and K141N) have been associated with hereditary peripheral neuropathies (*16*). The mutations decrease the level of HSPB8 oligomer dissociation, reduce chaperone activity in vitro (*17*), and weaken the ability of HSPB8 to clear polyglutamine proteins in cells.

Other Members and Their Diversity. Besides HSPB1, HSPB4, HSPB5, and HSPB8, seven more HSPB family members are found in humans. The 11th member, only recently identified, has been named HSP16.2 but will here be termed HSPB11 (Tables 2 and 3).

HSPB2, also known as myotonic dystrophy protein kinase binding protein (MKBP), interacts with the myotonic dystrophy protein kinase (DMPK), for which mutations have been linked to the development of myotonic dystrophy (*18*). In muscle cells, HSPB2 forms an oligomeric complex with HSPB3, expression of which is induced during muscle differentiation (*19*). Due to its interaction with DMPK and its ability to enhance its kinase activity, a role for HSPB2 in muscle maintenance has been suggested (*18*).

HSPB6 (HSP20) is strongly expressed in smooth muscles and seems to play a role in muscle relaxation (*20*) and cardioprotection. In vitro, HSPB6 exists mainly as dimers. In the presence of HSPB1, it can oligomerize to form higher-molecular mass complexes with molecular masses of 100−300 kDa. HSPB6 can be phosphorylated at serine 16, and mimicking its phosphorylation (S16D mutant) resulted in decreased chaperone activity in vitro. Recently, HSPB6 was reported to interact in a phosphorylation-dependent manner with 14-3-3 proteins that function as regulators of a wide variety of processes (*21*). HSPB7 (cvHSP) is expressed in heart and skeletal muscle. Analysis of aging muscle shows a large increased level of expression of both HSPB7 and HSPB5 (*22*). This could reflect a cellular adaptation to higher-proteotoxic stress conditions related to muscle degeneration. HSPB7 upregulation is also found in muscular dystrophy-affected diaphragm muscle, again linking high stress levels with HSPB7 induction.

Next to the muscle-associated HSPB members, two members (HSPB9 and HSPB10) are exclusively expressed in testis. Through its C-terminus, HSPB9 interacts with DynLT1, which is a light chain component of dynein (*23*), one of the energy-dependent tubulin motor proteins. However, the physiological significance of this interaction is not yet known. The other testis specific member, HSPB10 (sperm outer dense fiber protein 1, ODFP/ODF1), seems to fulfill a structural function in the flagellar axoneme cytoskeleton of sperm cells (*24*), which may indicate additional substrate specificity. However, further structural and functional information is still lacking.

The recently reported HSPB11 (HSP16.2) was shown to form oligomeric complexes and to prevent the aggregation of in vitro denatured aldolase and glyceraldehyde-3-phosphate dehydrogenase in accordance with the chaperone model of HSPB1 and HSPB5. HSPB11 overexpression protected against etoposide-induced cell death which correlated with a decreased level of release of mitochondrial cytochrome *c* into the cytosol. Inhibiting HSP90 function completely abrogated the protective effect of HSPB11 (*25*). This would suggest that at least in the case of HSPB11, interaction with other chaperone machines besides HSPA1A may contribute to functional specificity and cellular functioning.

Concluding Remarks. The major regulatory mechanism of sHSPs involves phosphorylation and oligomeric redistribution. This can result in a subcellular redistribution and influence chaperone activity and even substrate specificity. The outcomes of these modifications can, however, be diverse and are only known for a few members. Besides playing a key role in the response to (external) stresses, HSPB members are also crucial for normal cellular functioning, especially in muscle tissue and the eye lens. This is emphasized by several diseases caused by mutations in several HSPB members. Inversely, the functional activity of some HSPB members may be exploited for future therapeutic intervention in combating protein folding diseases like heart diseases (atrial fibrillation and ischemia) and neurodegenerative diseases (Alzheimer's, amyotrophic lateral sclerosis, and CAG-repeat diseases).

HSP70 CHAPERONING MACHINE

General Introduction. Whereas the HSPB chaperones (HSPB1, HSPB4, and HSPB5) may form the primary line of defense under stress, the HSPA chaperone machine, especially HSPA1A/B, and its cochaperone, DNAJB1, are the strongest stress inducible proteins. As an ATP-dependent chaperone machine, it can act on substrates bound to HSPB oligomers after being induced (see Figure 1). However, several members, including some stress-induced members, are also expressed under nonstress conditions (HSPA8, DNAJA1, HSPA1A/B, and DNAJB1). The human genome encodes 13 different HSPA family members, four HSPH family members, and 41 different DNAJ family members (*26, 27*) (Table 2). HSPA proteins are highly homologous to the four members of the HSPH (HSP110) family. In fact, HSPA4 and HSPA4L are currently annotated as HSPA members in the NCBI gene database but are more homologous to HSPH1 and therefore here are termed HSPH2 and HSPH3. In addition, a fourth HSPH member (Grp170) is present in the endoplasmic reticulum (ER) and here termed HSPH4.

Typically, HSPA proteins consist of an N-terminal ATPase domain of 45 kDa and a C-terminal substrate binding domain of 25 kDa. The ATPase and the C-terminal domain are separated by a small linker domain for HSPA members and a longer linker domain for HSPH members (Figure 2). This linker domain couples the nucleotide hydrolysis to the opening and closing of the substrate binding cavity (*28*). Why HSPH proteins have an extension of the linker domain is currently unknown. Clearly, like HSPA members, HSPH proteins can bind substrates. However, alone they cannot release substrates, which is typical for HSPA members.

Several important sites within the domains described above of the HSPA protein have been mapped with a high degree of precision. Different cofactors bind to different domains of the HSPA protein: DNAJ binds to both the ATPase and the extreme C-terminal EEVD domain. Deletion or mutation of the EEVD motif causes a disruption of the interdomain communication, resulting in an enhanced intrinsic ATPase activity, leading to a reduced level of binding of substrates. Also, this deletion resulted in complete abrogation of DNAJ binding to and regulation of HSPA (*29*). Interestingly, the HSPH proteins show deviations from this motif and hence may not functionally interact with DNAJ proteins. Cofactors BAG-1 and Hip bind only to the ATPase domain, whereas Hop, Tpr-2, CHIP, and DNAJ bind to the C-terminal domain (Figure 2) (*30*). HSPA proteins constantly shuttle between an ATP-bound and an ADP-bound state (Figure 1). DNAJ members stimulate the hydrolysis of ATP. Hip stabilizes the ADP-bound HSPA complex, whereas HSPH, BAG-1, and HSPBP1 stimulate ADP−ATP nucleotide exchange. The E3 ubiquitin ligase cofactor CHIP can inhibit the ATP hydrolyzing capacity of HSPA. Finally, Hop does not act on the HSPA ATPase cycle as such but links the HSPA chaperone to the HSP90−HSPC chaperone complex. Details about these cofactors and how these cofactors affect the fate of HSPA bound substrates have been described elsewhere (*30*).

HSPA FAMILY

Paradigm According to HSPA1A/B and HSPA8. Crystallographic evidence shows that both HSPA and DNAJ resemble molecular clamps (*31*). However, the way by which they bind substrates is significantly different. The substrate binding domain of HSPA proteins consists of a short β-sandwich motif which can be locked by the α-helical lid structure. The β-sandwich contains a hydrophobic core of four or five amino acids with two flanking basic residues (*32*). Only a short linear polypeptide fits within the substrate binding domain of the monomeric HSPA protein, and therefore, it binds only a short stretch of peptides. Because HSPA recognizes very short hydrophobic peptides, it is thought that it can bind a wide variety of substrates. It must be mentioned, however, that these findings are all based on a small number of HSPA proteins like *E. coli* DnaK and DNAJ, bovine HSP70/HSPA1A, and yeast DNAJ family members Ydj1 and Sis1. As both the HSPA and DNAJ families are quite diverse, it is likely that the proposed models do not account for all possible cellular HSPA−DNAJ complexes and that some of the different cytosolic HSPA complexes are adapted for a limited range of cellular substrates.

Functional Diversities and Copartner Specificity. A couple of the HSPA members were recently reviewed elsewhere (*33*) and are therefore described in less detail here. Features of different HSPA members are summurized in Table 3.

HSPA1A and HSPA1B differ by only two amino acids and are believed to be fully interchangeable proteins. Both proteins have been termed HSP70i (or HSP72) (Table 2) and are the strongest stress inducible HSPA members. HSPA8, the cognate HSPA and previously termed Hsc70 (or HSP73), is expressed in all cell types. It is considered to be the essential “housekeeping” HSPA member. HSPA1L and HSPA2 are two cytosolic family members with a high level of expression in the testis, and HSPA2 has been shown to be required for spermatogenesis (*34, 35*). Their biochemical mode of action is currently unknown. HSPA6 is a poorly studied, stress inducible protein that is lacking in rodents. It is not expressed under normal conditions and only induced upon severe heat stress, where it is thought to act as a final proteotoxic resistance buffer (*36*). HSPA7 is considered a pseudogene as its transcription product is terminated after 367 amino acids. As a complete conserved HSPA protein can originate by bypassing a frame shift at codon position 340, it might also be a true gene which is highly homologous to HSPA6. HSPA9, the mitochondrial HSPA member (HSP75), and HSPA5, the ER-localized HSPA chaperone (BiP), are thought to act in a similar manner in their respective compartments like HSPA8 in the cytosol. In line with this, cofactors such as ER (DNAJC1, DNAJB11, DNAJB9, and DNAJC10) and mitochondrion specific DNAJs (DNAJA3) as well as ER (HSPH4/Grp170/HYOU1) and mitochondrion (HMGE) specific nucleotide exchange factors have been found (*37, 38*). A small HSPA-like protein, STCH, which we propose to call HSPA13 (Table 2), has been found attached to microsomes (*39*) and may fulfill HSPA8-like roles here. HSPA12A and HSPA12B are two distantly related proteins found in atherosclerotic lesions (*40*).

HSPA14 (HSP70L1) is the smallest HSPA protein and interacts with MPP11, the human ortholog of Zuo1, a cytosolic ribosome-associated chaperone that acts together with Ssz1p and the Ssb proteins in yeast as a chaperone for nascent polypeptide chains during translation (*41*).

Although biochemical details of many HSPA members have yet to be identified, recent systems biology approaches in yeast indicated that two distinct chaperone networks with specialized function exist. One molecular chaperone network may protect the proteome against environmental stress (HSP), and the second deals with protein translation (CLIPS) and is associated with ribosomes (*42*). In line with these findings, one may speculate that HSPA1A/B, HSPA1L, and HSPA6/HSPA7 belong to the HSP network whereas HSPA5, HSPA8, HSPA9, and HSPA14 belong to the CLIPS network.

HSPH FAMILY

Paradigm According to HSPH1. In vitro, HSPH1 has been shown to suppress the aggregation of denatured luciferase, resulting in enhanced refolding of luciferase. However, rabbit reticulocyte lysate was always required as a source of cofactors after the denaturation to stimulate refolding in these assays, indicating that HSPH members are good suppressors of irreversible aggregation but lack the release activity typical of HSPA proteins and necessary for the stimulation of protein refolding. Consistently, biochemical evidence from yeast Sse1 and mammalian HSPH2 showed that HSPH proteins are poor ATPases (*43*).

Recently, it was found that HSPH members act as nucleotide exchange factors for both mammalian and yeast HSP70 proteins (*43*). This is surprising as different nucleotide exchange factors (BAG-1 and HSPBP1) had already been identified in the mammalian cytosol (*44, 45*). Interestingly, none of the nucleotide exchange factors shows significant primary sequence homology to *E. coli* nucleotide exchange factor GrpE, suggesting that they have evolved independently. However, as HSPH proteins are known to have the capacity to hold (unfolded) proteins in a folding competent state (unlike BAG-1 and HSPBP1), they might act as coupling factors between substrate loading and nucleotide exchange for the refolding of specific substrates similar to the coupling of HSPA substrate loading and ATP hydrolysis by DNAJ proteins. Like the other nucleotide exchange factors, HSPH members interact with HSPA members in the ADP configuration and stimulate the dissociation of ADP. The subsequent rebinding of ATP induces the dissociation of HSPH−HSPA complexes (*43*). Via stimulation of the nucleotide exchange of the HSPA complex, it was shown that HSPH accelerates the HSPA-mediated folding of firefly luciferase (*43*). As both a heat inducible substrate holder and heat inducible nucleotide exchange factor, HSPH may be particularly relevant under (heat) stress conditions during which it may hold substrates (like HSPB) which can be passed on to HSPA for further handling after the stress.

Functional Diversities. Besides HSPH1 (HSP110), there are three other HSPH family members in humans: HSPH2 (HSPA4/APG-2), HSPH3 (HSPA4L/APG-1), and HSPH4 (HYOU1/Grp170). Features of the different HSPH members are summarized in Table 3. Currently, the extent to which the different HSPH members functionally overlap is unknown. While one of them, HSPH4, the grp170 orthologue, is found in the ER (*46*) where it is likely to fill the role of a nucleotide exchange factor for HSPA5, the other three family members are found in the cytosolic/nuclear compartment. HSPH1 and HSPH2 are expressed ubiquitously; HSPH3 is mainly expressed in testis, and HSPH3 knockout mice show defects in spermatogenesis (*47*), suggesting a unique role for this protein within the testis, maybe in conjunction with HSPA1L or HSPA2.

DNAJ SUPERFAMILY

All eukaryotic cells contain DNAJ proteins which are known to stimulate the ATPase domain of HSPA chaperones. The common domain that defines this family is the J domain that stimulates the HSPA ATPase domain. In the human genome, at least 41 different DNAJ-encoding genes have been identified (*27*). The exact protein partners of the different DNAJ proteins as well as the exact cellular functions are currently unknown for most of its members. DNAJ proteins are divided in three subfamilies (Figure 2); type A proteins are the closest human orthologues of the *E. coli* DNAJ and contain, besides an extreme N-terminal J domain, a glycine/phenylalanine-rich region, a cysteine-rich region, and a variable C-terminal domain. Type B proteins contain all the domains listed above with the exception of the cysteine-rich region, and type C DNAJ proteins contain only the J domain that is not necessarily restricted at the N-terminus but can be positioned at any point within the protein.

Paradigm According to DNAJB1. The J domain is highly conserved and folded in an α-helical secondary structure. A conserved sequence motif (HPD) in the J domain has been shown to be critical for accelerating the ATPase activity of HSP70. Adjacent to the J domain, a glycine/phenylalanine-rich region is believed to function as a flexible spacer that separates the N-terminal J domain from the rest of the molecule. In the center of the molecule, the cysteine-rich domain contains four cysteine-rich repeats that fold around two zinc atoms. The C-terminal domain folds in a β-plated sheet structure and is involved in dimerization as well as in substrate binding and presentation (*48*).

DNAJA proteins dimerize in a V-like structure (*31*), and the binding motif of *E. coli* DNAJ consists of a hydrophobic core of eight residues enriched for arginine, aromatic amino acids, and large aliphatic hydrophobic residues positioned in the middle of each monomer. Although each monomer contains only a short binding motif, dimer formation gives rise to a relatively large β-sheet projection. Each of the monomers binds part of the unfolded substrate and holds it in an extended conformation between the middle of the two monomers. HSP70 binds the DNAJ dimer at the tips of the V-like structure and takes over the substrate for binding and release cycles. Although DNAJB members also can form dimers, they differ structurally from DNAJA dimers; e.g., whereas DNAJA1 forms compact dimers in which the N- and C-termini face each other, DNAJB4 forms a dimer in which only the C-termini of the two monomers are in contact (*49*). These structural differences may very well relate to differences in substrate binding or selection.

Functional Diversities. Comparative studies on 13 cytosolic J proteins in yeast (*50*) revealed that the J domains from a variety of different classes of J proteins could complement the severe growth defects in yeast lacking the DNAJ protein Ydj1. This demonstrates that the stimulation of the ATPase activity of yeast SSA1 is sufficient for many cellular processes. On the other hand, the phenotypes of four other DNAJ deletions (*cwc23*, *sis1*, *jjj1*, and *jjj3*) could be rescued by expression of only the deleted genes, indicating that these proteins carry out highly specialized and unique tasks. For mammals, domain swapping experiments have confirmed that the J domain can be exchanged between various DNAJ proteins with preservation of the biological function (*51*). Even more, the J domain from DNAJB1 could complement the J domain of yeast Ydj1 (*52*). This all indicates that the J domain is highly conserved and not likely responsible for any functional diversity. Rather, it suggests that the J domain is only required to recruit HSPA members to specific microenvironments. Here, the C-termini of the DNAJ family members would provide substrate and/or functional specificity. Clearly, in vitro, type A DNAJ molecules have substrate binding activity (*53*) and thus may function in the delivery of substrate to HSPA partners. It is still under debate if type B members also exhibit substrate binding activity, although most studies now support this idea. Whether all DNAJ members can stimulate the nucleotide cycle of HSPA machines and/or have any a preference for specific HSPA members is yet unclear.

DNAJA Family. The human genome contains four different members of the DNAJA family, and general features are summarized in Table 3. Interestingly, it was found that whereas both DNAJA2 and DNAJA4 could stimulate the hydrolysis of ATP on HSPA1A, the DNAJA2−HSPA1A combination but not the DNAJA4−HSPA1A combination was able to support refolding of denatured luciferase. This indicates that at least DNAJA2 and DNAJA4 may have differential substrate specificity in that DNAJA4 is unable to bind and load denatured luciferase onto the HSPA1 chaperone (*54*).

DNAJB Family. The DNAJB subfamily, DNAJB1 in particular, has been most extensively studied in mammalian cells. It has been found to cooperate with both HSPA1A and

HSPA8 in luciferase refolding in vitro and in living cells (*55*). DNAJB4/HLJ1 and DNAJB5 are two DNAJB proteins with unknown function that are close paralogs of DNAJB1. DNAJB4 shows full-length homology to DNAJB1 and is also known as Hsc40, a non-heat inducible constitutively expressed member proposed to act as a housekeeping HSP40/DNAJ protein just as Hsc70/HSPA8 is proposed as the housekeeping equivalent of the stress inducible HSPA1.

DNAJB2/HSJ-1 is also relatively well-studied and is expressed as two isoforms. The long isoform is targeted to the cytosolic face of the ER by C-terminal geranylgeranylation, while the short isoform is found in the cytosolic and nuclear compartment (*56*). Both proteins contain a ubiquitin interaction motif which is suggested to sort misfolded clients for HSPA8-dependent proteasomal degradation (*57*).

The DNAJB family members DNAJB9/ErdJ4 and DNAJB11/ErdJ3 are ER specific DNAJ members that collaborate with HSPA5 (*58, 59*). Both proteins are induced upon ER stress (*58, 59*). This implies that DNAJB9 and DNAJB11 likely fulfill roles equivalent to that of DNAJB1 in the cytosol.

DNAJB6/MRJ-1, DNAJB7, and DNAJB8 are three homologous proteins which share, besides a J domain, a high degree of sequence homology in the C-terminus. This C-terminal domain does not show any homology with known domains in the Pfam database (data not shown). DNAJB6 binds keratin 18 and was found to be important in preventing toxic keratin aggregation which interferes with placental development (*60*). DNAJB6 has also been shown to suppress the toxic aggregation of mutant Huntington, supporting the idea that (some) DNAJB family members can bind substrates. Whether this holds true for DNAJB7 and DNAJB8 as well and whether this requires collaboration with HSP70 machines remain to be elucidated.

Finally, there are three more diverse DNAJB members, DNAJB12, DNAJB14, and a testis specific DNAJB13/Tsarg3, that have hardly been studied. DNAJB12 and DNAJB14 exhibit a high level of sequence similarity in the C-terminus and contain C-terminal DUF1977 (domain of unknown function 1977). DNAJB13 contains a C-terminal domain homologous to DNAJB1, DNAJB4, and DNAJB5 (Pfam, data not shown). General features of the DnaJB family are summarized in Table 3.

DNAJC Family. With more than 23 members, the DNAJC family represents the largest of the three subfamilies. The family is very diverse in both amino acid composition and protein length (Table 3) with the DNAJ domain being the only common feature (Figure 2). Only a dozen proteins have been studied, including DNAJC1/ErDj1, SEC63/ErDj2, and DNAJC10/ErDj5 proteins, all ER specific DNAJ proteins that associate with HSPA5. The extent to which these HSPA5 factors show functional overlap or specificity is currently unknown. Another member, DNAJC19/TIMM14, is part of the mitochondrial TIM23 preprotein translocase that stimulates the ATPase activity of the mitochondrial HSPA protein (HSPA9), hereby supporting mitochondrial import of nuclear-encoded proteins. DNAJC21/ZRF1 is the ortholog of the yeast DNAJ protein called Zuo1, a ribosome-associated DNAJ protein important for translation in yeast. Furthermore, several other DNAJC family members such as DNAJC5/CSP, DNAJC6/auxilin, and DNAJC13/RME-8 seem to function in endocytosis and exocytosis. Both DNAJC6 and DNAJC13 have been shown to collaborate with HSPA8 in the process of endocytosis.

SUMMARY AND PERSPECTIVES

The human genome enot only ncodes a wide variety of HSP families but also a large number of individual proteins within each of these families. While the diversity is starting to be appreciated by a number of investigators, we yet have only faint clues about why such diversity exists. Housekeeping members may be primarily involved in cotranslational folding of proteins and/or transport of proteins across membranes, whereas some (inducible) members may perform more stress-related functions. Substrate specificity for the HSPA machine(s) may be, in part, evoked by specific cofactors (DNAJs and HSPH members). The different structures of the DNAJA and DNAJB C-termini may provide such possibilities for client specificities, but whether mammalian cells also make use of specific combinations between HSPA and DNAJ members remains to be elucidated. Determinations of the fate on client processing (toward folding or degradation) may not depend on this HSPA machine as such, although the presence of HSPA cofactors such as CHIP and BAG provides an easy link to the proteasome. Intriguingly, although binding of a client to some HSPB members can result in HSPA-dependent improved folding of (stress-denatured) clients, several HSPB members are linked to client degradation (proteasomal or autophagy) in both HSPA-dependent and -independent manners. The ability of nearly all HSPB members to associate with cytoskeletal elements, increasing their stability, suggests that HSPB members act as cytoskeleton specific chaperones. In addition, association of HSPBs with cytoskeletal elements may allow them to chaperone and transport un- or misfolded cytosolic proteins toward protein storage and/or degradation routes. However, much of this is still only speculative.

The ability of chaperones to handle unfolded proteins has challenged many researchers to test whether they could be used for prevention of the progression of protein folding diseases. Although this concept has had some support from in vitro work, so far studies with animal models have had limited success. More insight into the function of other individual HSP family members may help to elucidate better suppressors and/or strategies for these diseases. On the other hand, the finding that several neuropathies are related to mutations in HSP encoding genes (chaperonopathies) further supports their importance in neurodegeneration. Investigations into the molecular biochemical mechanisms by which these mutations lead to chaperonopathies will lead to an improved understanding of these neuropathologies and also improve our understanding of the normal function of the corresponding chaperones. Furthermore, searches for mutations in other family members or for alternative transcripts of individual HSP genes may lead to identification of causes for nonidiopathic cases of neuropathies or age-related decline in protein quality control and cellular aging.

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BI800639Z