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Review

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The BAG3-dependent and -independent roles of cardiac small heat shock proteins

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Small heat shock proteins (sHSPs) comprise an important protein family that is ubiquitously expressed, is highly conserved among species, and has emerged as a critical regulator of protein folding. While these proteins are functionally important for a variety of tissues, an emerging field of cardiovascular research reveals sHSPs are also extremely important for maintaining normal cardiac function and regulating the cardiac stress response. Notably, numerous mutations in genes encoding sHSPs have been associated with multiple cardiac diseases. sHSPs (HSPB5, HSPB6, and HSPB8) have been described as mediating chaperone functions within the heart by interacting with the cochaperone protein BCL-2-associated anthanogene 3 (BAG3); however, recent reports indicate that sHSPs (HSPB7) can perform other BAG3-independent functions. Here, we summarize the cardiac functions of sHSPs and present the notion that cardiac sHSPs function via BAG3-dependent or -independent pathways.

Introduction

The small heat shock proteins (sHSPs), also referred to as HSP family B (HSPB), belong to the superfamily of HSPs and play critical roles within the cell. sHSPs are characterized by their small molecular weight, ranging from 15 to 40 kDa, as well as by a highly conserved domain of approximately 80 amino acids, the α -crystallin domain (ACD) (1, 2). Although most sHSPs can be assembled into large, multimeric complexes that vary in size and contain up to 24 to 40 subunits, they can also exist in the form of monomers and dimers (1). The human genome encodes 10 sHSPs (HSPB1–HSPB10) (3), all of which exhibit distinct tissue-specific expression profiles. Four sHSP members (HSPB5–8) are highly expressed in the heart (4). Mutations in sHSP-encoding genes have been associated with human cardiac disease (5–18), highlighting the important role of sHSPs in maintaining cardiac function.

The sHSPs were initially recognized as a first line of defense against protein aggregation to maintain protein homeostasis (19, 20). sHSPs are ATP-independent chaperones, which function as “holdases” to bind to and stabilize denatured or non-native proteins against aggregation. Additionally, they facilitate subsequent protein renaturation in cooperation with “foldases,” the ATP-dependent, high-molecular-weight chaperones (19, 21). Thus far, BCL-2-associated anthanogene 3 (BAG3), a cochaperone protein, is the only protein known to bridge ATP-independent sHSPs and the ATP-dependent HSP70 family to form ternary complexes (22–26). Recent studies also suggest that BAG3 is not a passive scaffolding factor, in that the chaperone-like functions of cardiac sHSPs are highly dependent on BAG3. Among the 4 cardiac sHSPs, HSPB5, HSPB6, and HSPB8, but not HSPB7, interact with BAG3 (27). Moreover, the protein stability of HSPB5, HSPB6, and HSPB8, but not HSPB7, is dependent on BAG3 (28, 29). Recent evidence also reveals that HSPB7 carries a unique BAG3-independent function. In this Review, we summarize recent findings from studies focused on understanding the diverse functions of cardiac sHSPs as well as the importance of their interaction with BAG3.

BAG3 and its interacting sHSPs

BAG3 is a chaperone protein that is highly expressed in the heart and is associated with the development of human cardiomyopathy (30–36). The conserved BCL-2-associated anthanogene domain binds to the nucleotide-binding domain of members of the HSP70 family and helps release ADP from the chaperone to facilitate nucleotide cycling (26). BAG3 also contains multiple unique domains and motifs that facilitate interactions with different proteins, including a WW (Trp-Trp) domain at the amino terminus, a central proline-rich (PxxP) region, with 2 Ile-Pro-Val (IPV) motifs between the WW domain and the PxxP region (37, 38).

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These multifaceted interactions enable BAG3 to assemble large multichaperone complexes (22–24) that play essential roles in cardiac protein homeostasis, cardiac structure, and cardiac function (39, 40).

BAG3 physically and functionally links HSP70 and sHSPs. BAG3 has been shown to directly interact with sHSPs, including HSPB5, HSPB6, and HSPB8, in cells overexpressing BAG3 and via glutathione-*S*-transferase (GST) pull-down assays (22), isothermal titration calorimetry assays (23), and coimmunoprecipitation experiments (24, 41). The 2 IPV motifs in the intermediate region of BAG3 have been shown to directly bind 2 sHSP molecules (22–24), whereas the BAG, WW, and PxxP domains are not essential for this interaction. However, the sHSP-binding site for BAG3 has not been unequivocally identified. All mammalian sHSPs consist of a highly conserved central β -sheet containing an α -crystallin domain and highly variable C- and N-terminal extensions (Figure 1). One study mapped the BAG3-binding region of HSPB8 to residues 149–169, encompassing the last 2 β -strands of the α -crystallin domain by generating HSPB8-HSPB1 chimeras (41). This experiment was based on the premise that HSPB1 does not bind BAG3 in pull-down assays when overexpressed in mammalian cells (42). However, more quantitative isothermal calorimetry titration experiments reported an HSPB1-BAG3 interaction, but this affinity was weaker than what was previously measured between BAG3 and other sHSPs (23). Therefore, future studies are required to identify the sHSP-binding sites for BAG3 and to understand the physiologically relevant variation in binding affinities between BAG3 and cardiac sHSPs. Binding with BAG3 promotes the deoligomerization of sHSPs, likely because BAG3 competes with the self-interactions among sHSPs, which normally stabilize large oligomers (23). Forming a complex with HSP70 through BAG3 is essential to the chaperone function of sHSPs because sHSPs lack the enzymatic activity that appears to be required for active remodeling or refolding by the other categories of chaperones (23, 39, 43, 44). Thus, the sHSPs-BAG3-HSP70 complex is essential for denatured proteins to refold to protect against protein aggregation. Our lab recently discovered that without BAG3, or when the BAG domain carries a glutamic acid to lysine (E455K) mutation, which decreases the interaction between BAG3 and HSP70, HSPB5, HSPB6, and HSPB8 become unstable, consequently leading to accumulation of insoluble proteins and dilated cardiomyopathy (DCM) (28). However, the levels of HSPB7, which does not interact with BAG3 (27), were not changed in BAG3-deficient or E455K-mutant mice (28). Interestingly, the binding affinity between specific sHSPs and BAG3 (23, 27) correlates with the magnitude of protein reduction we observed in mice with cardiomyocyte-specific knockout (cKO) of BAG3 or mice expressing the E455K mutation (28, 29) (Figure 2), suggesting that the cardiac functions of HSPB5, HSPB6, and HSPB8, but not HSPB7, are largely dependent on BAG3. In the following sections (as well as summarized in Table 1), we will discuss the cardiac-specific roles of sHSPs, which we have grouped based upon their dependence on BAG3.

HSPB8. HSPB8 (also known as protein kinase H11, α C-crystallin, and HSP22) is a ubiquitous 22-kDa sHSP that is predominantly expressed in the heart and skeletal muscles (45, 46). Elevated expression of HSPB8 in the heart has been observed in animal models and patients with various forms of cardiac disease, including both acute and chronic myocardial ischemic injuries, as well as pressure overload–induced cardiac hypertrophy (45, 47, 48). Most cardiac HSPB8 has been found in complexes of high molecular masses, ranging from 25 kDa up to 670 kDa and greater (49). Structure and biochemistry studies determined that, similar to other sHSPs, HSPB8 interacts with itself and/or with other sHSPs to form homo- and hetero-oligomeric complexes (49, 50). Although HSPB8 was initially reported to have protein kinase activity, a series of thorough experiments demonstrated that it lacks kinase activity. In fact, HSPB8 functions as a molecular chaperone to regulate protein quality control (reviewed in ref. 51). Given the predominant expression of this sHSP in the heart under basal conditions and its elevated expression in various forms of cardiac disease (45–48), researchers have long believed that HSPB8 is important for maintaining cardiac function at baseline and in response to cardiac stress. Initial reports sought to overexpress HSPB8 in cardiomyocytes and analyze the cardiac phenotype at baseline and in response to myocardial infarction. A 6- to 7-fold increase in HSPB8 expression in cardiomyocytes causes a compensated cardiac hypertrophy phenotype, with preserved contractile function at 2 to 3 months of age under basal conditions (45, 52). These results are consistent with observations of a dose-dependent prohypertrophic effect of HSPB8 overexpression in cultured cardiomyocytes (53, 54). In response to myocardial infarction stress, HSPB8 overexpression is as powerful as ischemic preconditioning at preventing myocardial infarction and thereby offers preemptive cytoprotection by defending the myocardium against cell death and by promoting the metabolic switch through the IP3K/AKT, PKC ϵ , AMPK, and hypoxia-inducible factor 1- α pathways (52). Loss-of-function studies reveal that HSPB8 is not essential for normal cardiac development or baseline cardiac function; however, the loss of HSPB8 accelerates cardiac dysfunction and remodeling and leads

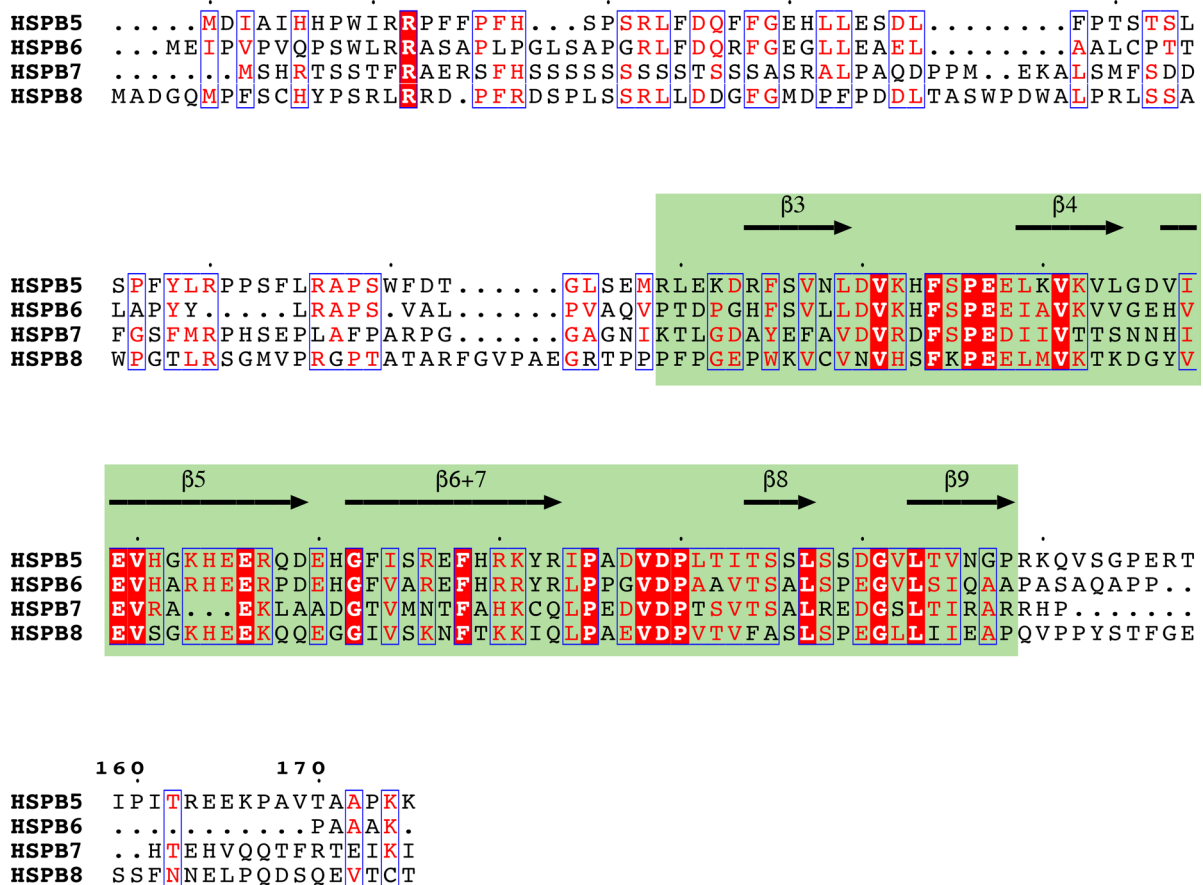


Figure 1. Comparison of cardiac sHSP sequences. Reference sequences of human sHSPs were retrieved from Uniprot database and aligned using Kalign (129). Secondary structure of the α -crystallin domain (green box) was annotated based on NMR structure of α B-crystallin (PDB ID: 2KLR) (130).

to heart failure in the pressure overload-induced model (55). Mechanistic studies point out that HSPB8 deletion impairs the translocation and phosphorylation of STAT3, as well as gene expression downstream of STAT3 signaling (55).

K141N and K141E, 2 dominant missense mutations that target the same lysine 141 residue in the highly conserved α -crystallin domain of HSPB8, are associated with autosomal dominant distal hereditary motor neuropathy and axonal Charcot-Marie-Tooth disease (56–58). Interestingly, the K141N and K141E mutations in HSPB8 reduce its ability to bind to BAG3 (59) but promote binding with its homo- and hetero-oligomeric partners (56). HSPB8^{K141N}-knockin mice display late-onset muscle atrophy and severe axonal degeneration. This phenotype is likely because of a toxic gain of function of the mutant protein, because *Hspb8*-knockout mice do not have a discernable phenotype at baseline (57). Notably, the levels of HSPB8 and BAG3 are significantly reduced in the sciatic nerve of 2-month-old HSPB8^{K141N}-expressing mice (57). Although there are no reports regarding the cardiac function of HSPB8^{K141N} mutation in patients or HSPB8^{K141N}-knockin mice, cardiac-specific expression of HSPB8^{K141N} in Tg mice results in mild hypertrophy and a slight reduction of cardiac function (60).

HSPB6. Kato et al. first identified HSPB6 (also known as HSP20 and P20) in the skeletal muscles of rats and humans (61). Similar to HSPB8, HSPB6 is ubiquitously expressed but highly enriched in cardiac, skeletal, and smooth muscle tissues (61–63). It is well accepted that HSPB6 forms homo- or hetero-oligomeric complexes that may undergo stress-induced dissociation accompanied by increased chaperone activity (61). In addition to a conserved α -crystallin domain, HSPB6 contains a homology sequence of troponin I, a domain that inhibits platelet aggregation, and a consensus motif (RRAS) for PKA/PKG-dependent phosphorylation at Ser16 (reviewed in ref. 64). The expression levels of HSPB6 and its phosphorylation at Ser16 are increased in β -adrenergic agonist-stimulated cardiomyocytes (65), postinfarcted hearts (66, 67), and failing hearts (67, 68). Reports from in vivo and in vitro studies indicate that HSPB6 protects cardiomyocytes against apoptosis,

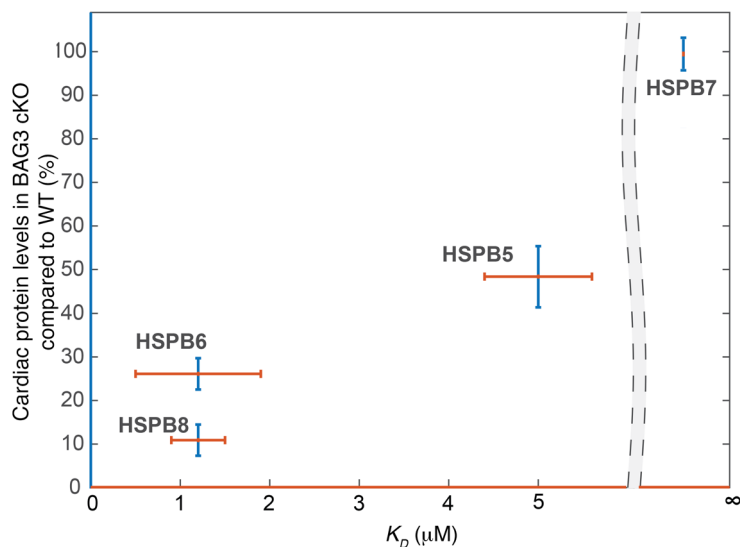


Figure 2. Reduction of cardiac sHSP levels in BAG3-cKO mice correlates with their affinity for BAG3. The x axis (red lines) shows the dissociation constant (K_D) between sHSP and BAG3. A lower K_D value indicates a stronger interaction between the respective sHSP and BAG3. K_D values between sHSP and BAG3 were obtained from Raush et al. (23), and Vos et al. (27) reported the absence of affinity (infinite K_D) between HSPB7 and BAG3. The y axis (blue lines) shows cardiac levels of sHSP protein in BAG3-cKO mice relative to WT mice. Cardiac levels of sHSPs in BAG3-cKO mice were obtained from Fang et al. (28). Infinite K_D indicates no binding.

and phosphorylation at the Ser16 site enhances HSPB6-mediated cardioprotection (63, 64). Two comprehensive reviews have summarized these studies of the cardiac-specific roles of HSPB6 (please refer to reviews in refs. 63 and 64). Here, we discuss HSPB6 mutation(s) associated with human cardiomyopathy, as well as several genetic mouse models involved in studying the cardiac function of HSPB6.

Multiple studies have shown that S10F and P20L substitutions in HSPB6 are associated with human DCM (6, 7). Computational structure analysis indicates that the S10F mutation may change the content of the helix, strand, and loop in the secondary structure of HSPB6, which may consequently affect its function (7). Liu et al. (7) generated HSPB6^{S10F}-Tg mice, with 10-fold overexpression of mutant HSPB6 in the heart, to investigate cardiac-specific effects. Compared with non-Tg controls, male HSPB6^{S10F}-Tg mice developed DCM at 6 months of age, and their survival rate declined rapidly by 11 months (7). Interestingly, cardiac overexpression of HSPB6^{S10F} induces lethal peripartum cardiomyopathy in female Tg mice (8). The P20L mutation is located 4 amino acids downstream of the PKA/PKG-dependent Ser16 phosphorylation site (6). In vitro studies reveal diminished phosphorylation and the loss of cytoprotective properties in HSPB6^{P20L}-expressing cells (6). Qian et al. studied a cardiac-specific Tg model with 7-fold overexpression of a phospho-null, mutant HSPB6 (HSPB6^{S16A}, referred to as S16A-Tg), and their findings supported a cardioprotective role for the phosphorylated form of HSPB6. In comparison with non-Tg controls, S16A-Tg mice are more sensitive to I/R injury, as evidenced by a lower contractile function recovery rate, increased necrosis and apoptosis, and decreased autophagy (67). Moreover, Edwards et al. reported that overexpression of the phosphomimetic form of HSPB6 (HSPB6^{S16D}) in vivo confers protection from β -agonist-induced apoptosis in the heart (69). In addition, overexpression of WT HSPB6 in the heart protects against I/R injury as well as cardiac stress from LPS or ISO treatment (70–72). Together, these studies suggest that HSPB6 may constitute a new therapeutic target for ischemic heart diseases; however, analysis of knockout and knockin mouse models is required to unequivocally study the cardiac role of HSPB6.

HSPB5. HSPB5 (also referred to as α B-crystallin) was initially identified as an abundant component of ocular lenses and is thus referred to as α B-crystallin (73, 74). However, further studies have demonstrated that α B-crystallin expression is not restricted to the eye. Various tissues, including the cardiac muscle, express α B-crystallin at a level that is equal to 3% to 5% of the total soluble protein (73, 75, 76). During cardiac ischemia stress, α B-crystallin rapidly translocates from the cytosol to the myofibrils (77–79). An important binding partner of α B-crystallin in cardiac myofibrils is titin (77, 80, 81). α B-crystallin interacts with the extensible N2B region of titin (77, 80, 81), which is exclusively found in cardiac isoforms and

Table 1. Summary of BAG3 and sHSPs

	Affinity (K_p) for BAG3	Percentage reduction in BAG3-cKO heart	Lethality of gKO mice	Cardiac phenotype in Tg models	Cardiac phenotype in knockout models	Associated mutants in human cardiac disease
BAG3	N/A	~90% (28)	Yes. Die within postnatal 3–4 weeks (28, 30, 128)	Tg WT human BAG3 overexpression leads to cardiac dysfunction (131).	DCM cKO mice (28)	Multiple mutations in BAG3 have been associated with cardiomyopathy (32, 33, 36, 132): E455K, V468M, R395fxX48, S385QfsX56, R309X, Q251RfsX56, P209L
BAG3-dependent sHSPs						
HSPB8	^A 1.2 ± 0.3 μM (23)	~90% (28)	No (55)	WT protein Tg: at baseline, compensated cardiac hypertrophy phenotype with preserved contractile function (45, 52); in I/R, decrease in infarct size, apoptosis (52); K141N-mutant Tg: mild hypertrophy and slightly reduced cardiac function at baseline (60)	gKO baseline: no phenotype; gKO in pressure overload model: decreased cardiac function; increased HF and mortality (55)	N/A
HSPB6	^A 1.2 ± 0.7 μM (23)	~70% (28)	N/A	WT protein Tg: at baseline, ex vivo contractility increased (70); in I/R, ISO, or LPS: decrease in infarct size, hypertrophy, fibrosis, apoptosis, & necrosis (70–72); S16D-mutant (phosphomimetic form) Tg: protection from β-agonist-induced apoptosis (69); S16A-mutant (phosphonull) Tg: more sensitive to I/R injury (67); S10F-mutant Tg: lethal DCM in male; lethal peripartum cardiomyopathy in female (7, 8).	No studies on single HSPB6-KO animals have been published. Knockdown of HSPB6 in isolated myocytes via antisense methods: decreased fractional shortening and contraction rates (71)	P20L (6) or S10F (7) mutations have been associated with DCM.
HSPB5	^B 5.0 ± 0.6 μM (23)	~50% (28)	No (98)	WT protein Tg: in I/R, decreased apoptosis and necrosis (96); R120G-mutant Tg: desmin-related cardiomyopathy (109)	HSPB5/HSPB2–double-KO mice: at baseline, no cardiac phenotype; in I/R ex vivo, increased apoptosis, necrosis, and stiffening (82); decreased contractile function (100); In vivo: infarct size (102)	In R120G (5), D109H (11), Q151X (18), 464delCT (18): DRMs; in R157H (12) and G154S (13): late-onset DCM (incomplete penetrance).
BAG3-independent sHSP						
HSPB7	Absent (27)	Not changed (28)	Yes. Die between E11.5 and E12.5 (125)	N/A	gKO and cKO embryos display small hearts, thinner ventricular wall. Abnormal actin bundles exist in gKO and cKO embryonic hearts (125). In icKO, sudden arrhythmic death within 10 days after ablation of HSPB7 (126)	SNPs in 3'UTR associated with DCM; no coding variant has been identified (36).

^AHigh level of binding affinity for BAG3. ^BMedium level of binding affinity for BAG3. gKO, global knockout; I/R, ischemia/reperfusion; HF, heart failure; LPS, lipopolysaccharide; ISO, isoproterenol; DRMs, desmin-related myopathies; icKO, inducible cardiomyocyte-specific knockout; SNPs, single-nucleotide polymorphisms.

regulates cardiac elasticity and diastolic function (82–85). α B-crystallin can also translocate to the mitochondria, where it inhibits mitochondrial permeability transition pore opening, thus stabilizing mitochondrial membrane potential and inhibiting apoptosis (86–89). In the heart, α B-crystallin undergoes at least 2 types of posttranslational modification, phosphorylation and *O*-GlcNAcylation (an *O*-linked attachment of the monosaccharide β -*N*-acetyl-glucosamine), which potentially regulate α B-crystallin translocation and cellular function (88, 90–95). In vitro studies of adenovirus-mediated overexpression of α B-crystallin in neonatal cardiomyocytes, as well as in vivo Tg models of α B-crystallin overexpression, have elucidated a cardioprotective role of α B-crystallin under myocardial ischemia (96, 97).

The α B-crystallin-encoding and HSPB2-encoding genes are in close proximity to one another; therefore, generation of α B-crystallin-knockout mice also disrupts HSPB2 (98, 99). At baseline, mice lacking both α B-crystallin and HSPB2 (double-knockout [DKO] mice) do not have an obvious phenotype in the early stages of their lives; however, these animals begin to die at 30 weeks of age, and less than 20% of the DKO mice survive to 40 weeks due to skeletal muscle defects (82, 98–100). In-depth analysis revealed that α B-crystallin and HSPB2 deficiency activates the nuclear factor of activated T cells/calcineurin (NFAT/calcineurin) pathway, leading to the development of cardiac hypertrophy during stress-free or minimal-stress conditions, and exacerbates cardiac dysfunction in response to pressure overload. Additionally, α B-crystallin overexpression attenuates the hypertrophic response to pressure overload via suppression of the NFAT/calcineurin pathway (101). In ex vivo Langendorff-perfused experiments, DKO hearts exhibit lower contractile recovery along with an increase in necrosis and apoptosis after I/R (99, 100). In contrast, Benjamin et al. reported smaller infarct sizes in DKO hearts compared with those from WT controls in both in vivo and ex vivo I/R injury models (102). Thus far, no single α B-crystallin cKO model has been generated.

Numerous mutations in the α B-crystallin-encoding gene have been associated with cardiomyopathy (5, 9–18). The first α B-crystallin mutation to be identified was the R120G mutation, which was discovered in a large multigenerational French family. This mutation causes cataracts, hypertrophic cardiomyopathy, and DRMs, in which the formation of intracellular aggregates containing both desmin and α B-crystallin are observed in muscle fibers (5). A D109H mutation in α B-crystallin was identified in a 2-generation family, with 5 affected individuals who displayed a very similar clinical phenotype to those described in the R120G family, suggesting a similar pathogenic mechanism (11). Additionally, Q151X and 464delCT mutations are linked to DRM (18), and R157H and G154S mutations have been reported in patients with late-onset DCM (12, 13). However, these 2 mutations have incomplete penetrance of the cardiac phenotype, indicating that unknown genetic modifier(s) may be required to manifest a cardiomyopathy phenotype in patients carrying R157H or G154S mutations (13, 14). Although the characterization of the effects of most α B-crystallin mutations is limited, the functional consequences of the R120G mutation have been extensively investigated. The R120G mutation resides in the ACD of α B-crystallin, and protein structure studies reveal that this mutation results in an irregular structure that stabilizes a closed-groove dimer, forms large oligomers, and is prone to aggregation (11, 103–105). In vitro assays have shown that the chaperone function of R120G-mutant α B-crystallin is significantly reduced or abolished (104, 106). The R120G mutation also markedly enhances the binding affinity between α B-crystallin and desmin filaments (107). The combined loss of chaperone function and the strong binding with desmin leads to aggregation of intermediate filament proteins with the mutant α B-crystallin, as well as the formation of inclusion bodies in cells (104, 107, 108).

Cardiac-specific expression of α B-crystallin R120G recapitulates desmin-related cardiomyopathy in Tg mice (109). These animals display severe cardiomyopathy, early death, aberrant desmin expression, and α B-crystallin aggregation in a gene dosage-dependent manner (109). Tg mice with high expression of the R120G mutation exhibit 100% mortality by early adulthood, and those with modest expression of this mutation exhibit phenotypes that appear at a later stage but are strikingly similar to clinical features of desmin-related cardiomyopathies, including cardiac hypertrophy, overtly affected desmin filaments, and impaired myofibril alignment (109). These observations suggest a dosage- and time-dependent process in α B-crystallin R120G mutation-associated cardiomyopathy. However, the α B-crystallin R120G-knockin mouse model does not exhibit premature lethality like the R120G-Tg mice (110). Although R120G-knockin mice have been reported to develop skeletal muscle defects and cataracts similar to humans with the homologous mutation, no careful characterization of the heart in this mouse model has been carried out (110). Therefore, it is unclear whether the aberrant desmin and α B-crystallin aggregation observed in the skeletal muscle of R120G-knockin mice also occurs in the cardiac muscle or whether a late-onset cardiomyopathy would develop in the aged R120G-knockin mice (110).

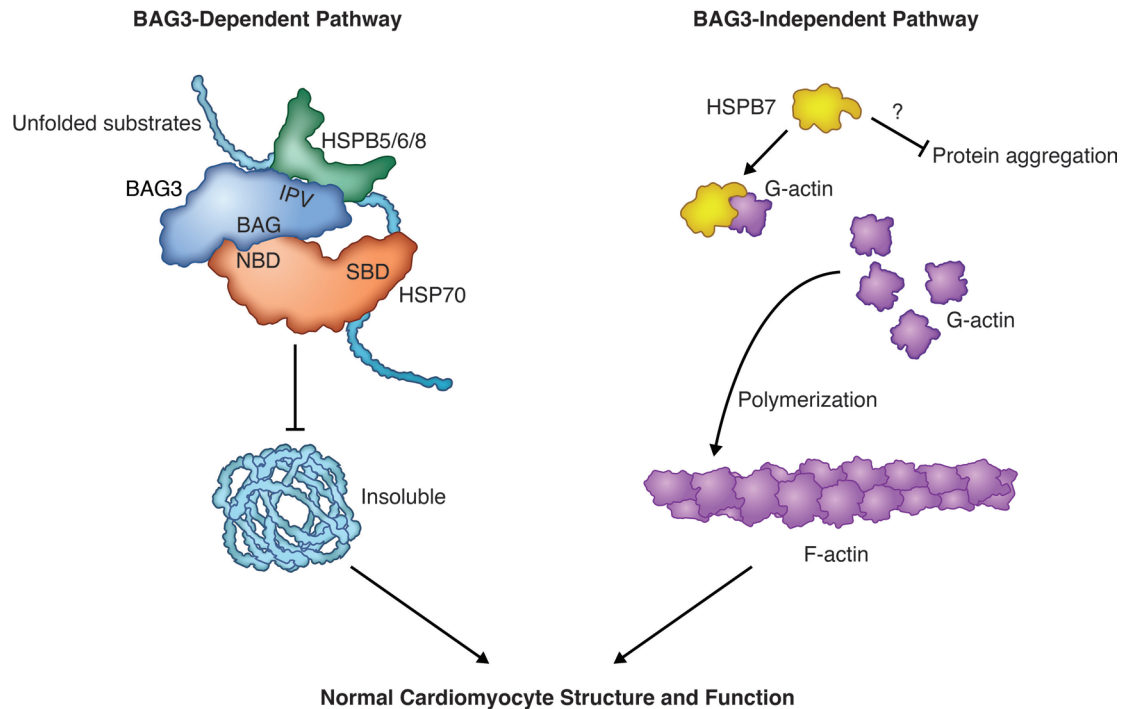


Figure 3. The BAG3-dependent and -independent pathways of cardiac sHSPs. HSPB5/6/8 represent the BAG3-dependent sHSPs (left), in which HSPB5/6/8 physically and functionally interact with BAG3 to form a multichaperone complex that prevents unfolded substrates from becoming insoluble in cardiomyocytes. HSPB7 represents the BAG3-independent cardiac sHSP (right) that binds to monomeric actin and limits its availability for polymerization. However, it is still unknown whether HSPB7 carries out chaperone activity in vivo. SBD, substrate-binding domain; NBD, nucleotide-binding domain. Illustrated by Rachel Davidowitz.

HSPB7- and BAG3-independent sHSP function

Besides collaborating with BAG3 and HSP70, some sHSPs have been shown to have diverse functions beyond their classically defined chaperone activity. These functions are independent of the HSP70-BAG3 complex (4, 27, 111–115) and include cytoskeleton assembly (111, 112), RNA splicing (113, 114), and HSP70 machinery-independent aggregation suppression (4, 27, 115). The most typical BAG3-independent sHSP is HSPB7, which is highly expressed in the heart (27, 116). Unlike HSPB5, HSPB6, and HSPB8, HSPB7 does not interact with BAG3 (27), and its stability is not impaired in BAG3-deficient cardiomyocytes (28). Upon overexpression in cells, HSPB7 does not form large oligomers (27). The ability of HSPB7 to prevent aggregation of polyglutamine (polyQ) proteins and the parkin (PARK2) C289G mutant is not dependent on the expression of heat shock factor 1 or HSP70. (27, 115). These phenomena all point out that the HSP70-BAG3 complex functions independently of HSPB7.

Many intronic SNPs have been identified in the HSPB7-encoding gene and have been found to be highly associated with HF, DCM, and idiopathic DCM in human patients (117–122), highlighting an important role for HSPB7 in maintaining cardiac function. Global depletion of Hspb7 in zebrafish disrupts normal cardiac morphogenesis (123, 124), and the essential role of HSPB7 in cardiomyocytes is further supported by the discovery that the global and cKO of HSPB7 in mice results in embryonic lethality between embryonic days 11.5 and 12.5 (E11.5–12.5) (29). Using biochemical assays, our laboratory recently revealed that HSPB7 plays a critical role in directly modulating actin filament length by binding to monomeric actin and limiting its availability for polymerization (125). Data from inducible, cardiomyocyte-specific-knockout HSPB7 (HSPB7-icKO) mice demonstrated that HSPB7 is also essential for adult cardiac function (126). HSPB7-icKO mice develop cardiac arrhythmia, and several cardiac intercalated disc proteins, such as connexin 43, desmoplakin, and N-cadherin, are downregulated, while filamin C protein aggregates are enriched in HSPB7-icKO murine hearts (126). It has also recently been reported that loss of HSPB7 in zebrafish or human cardiomyocytes leads to enhanced autophagic pathways (127). Inhibition of autophagy results in filamin C aggregation in HSPB7-null human cardiomyocytes and developmental cardiomyopathy in *hspb7*-null zebrafish embryos, suggesting that HSPB7 may be involved in regulating the autophagic processing of specific proteins within cardiomyocytes, but this remains to be fully explored (127).

Conclusion and future prospects

In this Review, we have summarized the diverse functions of sHSPs in the heart (Table 1) and have divided cardiac sHSPs into 2 distinct groups: BAG3-dependent (HSPB5, HSPB6, and HSPB8) and -independent (HSPB7) pathways (Figure 3). In BAG3-dependent pathways, HSPB5, HSPB6, and HSPB8 physically and functionally interact with BAG3 and form a multichaperone complex (22–26). These sHSPs are destabilized in cardiomyocytes lacking BAG3 function, with the magnitude of protein loss correlating with the binding affinity between the respective sHSP and BAG3 (refs. 23, 27–29, and Figure 2). Although almost all HSPB8 protein is lost upon BAG3 deletion, HSPB6 and HSPB5 levels are preserved at about 20% and 50%, respectively, in BAG3-deficient cardiomyocytes, implying that HSPB6 and HSPB5 may also carry BAG3-independent functions. To further elucidate potential BAG3-dependent roles of cardiac sHSPs, it will be interesting to investigate mice harboring genetically engineered mutations that disrupt interactions between BAG3 and sHSPs. Meanwhile, lack of basal phenotypes in mice harboring global deletions of HSPB8 (55), and HSPB5 and HSPB2 (82, 98–100), suggests potential genetic redundancy among HSPB5, HSPB6, and HSPB8. The generation of mice lacking all 3 BAG3-dependent sHSPs (HSPB5, HSPB6, and HSPB8) will be important to avoid potential compensation issues when addressing essential functions of these proteins in the heart.

Although these BAG3-dependent sHSPs mediate critical roles within the heart, we also summarized the impact HSPB7 has in the heart independent of BAG3. Three key pieces of evidence support the premise that HSPB7 exhibits BAG3-independent roles in cardiomyocytes. First, HSPB7 does not interact with BAG3 (27). Second, protein stability of HSPB7 is not dependent on BAG3 (27–29). Third, HSPB7-knockout mice die between E11.5 and E12.5 because of heart defects (29), a phenotype that is much severer than that of BAG3-knockout mice, which die immediately after weaning at 21 days after birth (27, 30, 128). HSPB7 directly modulates actin thin filament length in developing cardiomyocytes by binding monomeric actin and limiting its availability for polymerization (29). However, it remains unclear why or how loss of HSPB7 in adult cardiomyocytes results in accumulation of proteins, such as filamin C. Although Mymrikov et al. showed that HSPB7 does not exhibit chaperone activity in vitro (19), it is still unknown whether HSPB7 carries out chaperone activity in vivo or whether any other cochaperone proteins, analogous to BAG3, interact with HSPB7 to facilitate its potential protein quality control functions. Future studies of HSPB7-interacting partners may reveal novel functions of HSPB7 in adult cardiomyocytes.

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