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REVIEW

Universal Adapter Protein Bag3 and Small Heat Shock Proteins

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Abstract—Bag3 (Bcl-2-associated athanogene 3) protein contains a number of functional domains and interacts with a wide range of different partner proteins, including small heat shock proteins (sHsps) and heat shock protein Hsp70. The ternary Bag3–sHsp–and Hsp70 complex binds denatured proteins and transports them to phagosomes, thus playing a key role in the chaperone-assisted selective autophagy (CASA). This complex also participates in the control of formation and disassembly of stress granules (granulostasis) and cytoskeleton regulation. As Bag3 and sHsps participate in multiple cellular processes, mutations in these proteins are often associated with neurodegenerative diseases and cardiomyopathy. The review discusses the role of sHsps in different processes regulated by Bag3.

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INTRODUCTION

At present, the interests of many scientists are focused on adapter proteins that play a role of “hubs” coordinating interaction and functioning of many proteins. After binding with the adapter protein, the partner proteins can interact with their targets, thus forming protein ensembles, multiple components of which regulate each other’s activity and form a pipeline producing certain metabolites or providing efficient transmission of various signals. Bag3 is one of these adapter proteins. Bag family proteins are involved in the regulation of various intracellular pathways, including apoptosis, regulation of protein synthesis, quality control of protein folding, hormonal signal transduction, and many other vital processes.

Abbreviations: CASA, chaperone-assisted selective autophagy; Hsps, heat shock proteins; sHsp, small heat shock protein; TSC, tuberous sclerosis complex.

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DOMAIN STRUCTURE AND SOME PROPERTIES OF Bag3

Human genome contains six genes coding for Bag family proteins [1]. The name Bag is an abbreviation of *Bcl-2-associated athanogene*, because Bag proteins had been originally considered as partners of the important antiapoptotic protein Bcl-2 [1, 2]. All Bag family members contain one or several conserved Bag domain(s) consisting of 80-90 residues that provide interaction of these molecules with the Hsp70/Hsc70 heat shock proteins (Hsps). Bag proteins differ in their size and can include various functional domains that determine their activity mechanisms and processes they regulate [2]. In this review, we limited ourselves to discussing only one member of the Bag family, Bag3, and only briefly mentioned another member of this family, Bag1.

Human Bag3 contains 575 residues and is located predominantly in the cytosol [1], although a fraction of Bag3 molecules co-localizes with the contractile

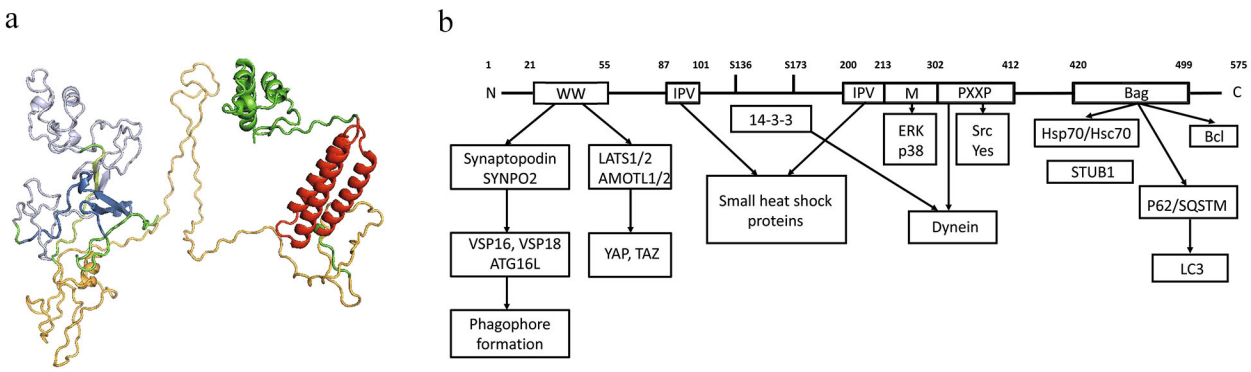

Fig. 1. *In silico* model of the 3D structure (PMDB ID: PM0083680) (a) and functional domains (b) of Bag3. a) Dark blue, WW domain; light blue, IPV domain; lemon yellow, short M domain; brown, long PXXP domain; red, Bag domain; green, parts of molecule not included in the functional domains. The main functional domains of Bag3 together with amino acids numbers are presented on the panel (b). b) The WW domain interacts with proteins containing poly-Pro sites. This domain interacts with synaptopodin (SYNPO2) and LATS1/2 protein kinases that regulate intracellular location of the transcription co-activators YAP and TAZ. The IPV domain contains two sites of small heat shock protein (sHsp) interaction and two sites of phosphorylation. After phosphorylation, Bag3 acquires the ability to interact with 14-3-3 protein and dynein (via 14-3-3). The M domain provides interaction with protein kinases p38 and JNK. The PXXP domain interacts with SH2 domain-containing proteins (Src and Yes). The Bag domain interacts with the Bcl family antiapoptotic proteins and Hsps of the Hsp70/Hsc70 family; it activates the ADP/ATP exchange in the nucleotide-binding site of Hsc70/Hsp70. Ubiquitin ligase STUB1 binds to Hsp70 and ubiquitinates protein substrates bound to this proteins complex. Bag3 also interacts with the autophagy receptor p62/SQSTM, which binds both ubiquitinated target proteins and LC3 protein located on the phagosome membrane.

apparatus in the region of Z-discs in skeletal and cardiac muscles [3]. The data on the 3D structure of the full-size Bag3 are lacking, although, the structure of conserved Bag domain (homologous for all Bag family members) of Bag1 in a complex with Hsc70 has been resolved by X-ray crystallography [4]. The 3D structure of the full-size Bag3 (PMDB ID: PM0083680) was modeled *in silico* [5] (Fig. 1a). According to this model, the most part of the Bag3 molecule is predominantly unfolded with a limited number of β-strands. Only the Bag domain has an ordered structure and includes three α-helices; there is also an additional α-helices at the C-terminus of Bag3 [5]. The theoretical model [5] and the data obtained by X-ray crystallography for the isolated Bag domain in a complex with Hsc70 [4] correlate well. Both theoretical and experimental data indicate that Bag3 has a predominantly unfolded structure and, therefore, belongs to the so-called intrinsically disordered proteins, which makes difficult its crystallization and analysis by X-ray crystallography, as well as by other methods.

Bag3 contains several functional domains, each responsible for the interaction with specific protein targets and capable of affecting the functioning of neighboring domains (Fig. 1b). The WW domain is located at the N-terminus of Bag3 (the name comes from Trp residues separated by 20-22 amino acids). This domain interacts with the poly-Pro motifs (PPPY, PPSY) found in certain proteins, such as synaptopodin (SYNPO2), protein kinases LATS1/2, and AMOTL1/2 adaptor proteins [6, 7]. Synaptopodin interacts with the autophagy proteins VPS16, VPS18, and ATG16 [7, 8]. Through synaptopodin, Bag3 can interact with proteins involved in the phagophore formation, which might be the reason why downregulation of synaptopodin synthesis inhibits chaperone-assisted selective autophagy (CASA) and suppresses elimination of phosphorylated tau protein in mature neurons [9]. Protein kinases LATS1/2, which also contain poly-Pro motifs in their structure, phosphorylate YAP and TAZ transcription co-activators [10, 11]. Under normal conditions the WW domain of Bag3 binds the AMOTL1/2 adapter proteins, LATS1/2 proteins kinases, and YAP/TAZ transcription co-activators forming a tight complex that makes possible phosphorylation of YAP/TAZ by LATS1/2. Phosphorylated YAP/TAZ proteins interact with the adapter protein 14-3-3 and either undergo proteolytic degradation or remain in cytosol [12]. In any case, they fail to reach the nucleus and to activate TEAD1-4 transcription factors. Proteotoxic stress leading to the accumulation of ubiquitinated proteins results in the LATS–YAP/TAZ–AMOTL complex dissociation, which prevents YAP/TAZ phosphorylation. YAP/TAZ activators move to the nucleus, activate TEAD1-4, and initiate transcription of certain genes [12, 13]. This regulatory mechanism consisting of activation/inactivation of LATS protein kinases followed by phosphorylation of transcription activators is similar to that occurring during activation of the HYPPO regulatory complex involved in cell differentiation, hormonal signal transduction, and cell–cell interactions [11, 12, 14]. The signaling through the HYPPO pathway can be regulated by mechanical stress induced by the contractile activity or cell translocation in viscous media. Hence, it was suggested that Bag3

acts as a mechanosensor that transduces mechanical signal using the above-described mechanism based on phosphorylation and translocation of YAP/TAZ in the cell [15].

The WW domain also interacts with tuberous sclerosis complex (TSC) through the PY motifs in TSC1 protein. In muscles, Bag3 localizes to the contractile apparatus, where it binds TSC1 which activates GTPase of the small G protein RHEB. RHEB with GDP in its active site inhibits mTORC1 and, therefore, suppresses protein synthesis in the vicinity of contractile apparatus. Simultaneously, this leads to the activation of CASA and promotes selective proteolysis of certain proteins, for instance, filamin. At the same time, TSC1 binding to the contractile apparatus reduces its concentration in the cytosol, thus leading to the activation of mTORC1 in the cytosol and activation of protein synthesis [16].

The WW domain is followed by the IPV domain (Fig. 1b). According to computer predictions, this domain has an unordered structure and contains only short β-strands [5]. This domain was named so because it includes two short sequences (residues 87-101 and 200-213) containing the IPIPV and IPVI motifs, respectively. These sequences are similar to the corresponding sequences in the C-terminal domains of some sHsps (HspB1, HspB4, and HspB5). The (I/V)P(I/V) motifs of sHsps interact with the hydrophobic groove formed by the β4 and β8 strands in the α-crystallin domain of neighboring subunits and, hence, play an important role in the formation of large oligomers of sHsps [17-19]. It was suggested that the IPV motifs of Bag3 can interact with the same hydrophobic β4-β8 groove in sHsps and form a tight complex with these proteins. We will discuss details of this interaction below.

The sequence between the two IPV motifs contains two Ser residues (Ser136 and Ser173) which, when phosphorylated, ensure Bag3 binding with 14-3-3 [20, 21]. This protein provides interaction of Bag3 with dynein. Therefore, Bag3 can bind dynein through the 14-3-3 protein and its own PXXP domain [20]. Such interaction with dynein at two different sites provides for the microtubule-associated transport of Bag3 and all bound proteins to aggresomes, which are intracellular centers of accumulation of aggregation-prone denatured proteins. Therefore, the IPV domain contains important elements of Bag3 structure and plays important role in the functioning of this protein.

The M and PXXP domains are located in the center of Bag3 molecule [6] (Fig. 1b). These domains contain the binding sites for different protein kinases. It is believed that the M domain binds p38 and ERK protein kinases, whereas the PXXP domain binds Yes and Src tyrosine kinases. Bag3 controls interaction of ERK with Dusp6 protein phosphatase; deletion of Bag3 results in the dissociation of Dusp6 leading to the constitutive ERK activation [22]. Non-receptor tyrosine kinases interact with Bag3 through their SH3 domains [23]. The binding of tyrosine kinases with the PXXP domain is dependent on Hsp70, which binds to the conserved neighboring Bag domain [23]. In the case of massive proteotoxic stress, i.e., upon accumulation of large quantities of denatured proteins, Hsp70 interacts with these denatured proteins and dissociates from Bag3, which affects interaction of non-receptor protein kinases with Bag3 and influences their intracellular location and activity.

By interacting with various protein kinases, Bag3 can regulate translocation of transcription activators from the cytosol and to the nucleus. As mention above, proteotoxic stress and, presumably, mechanical stress can lead to the dissociation of protein kinases LATS1/2 from the YAP/TAZ transcription activators bound to the WW domain of Bag3. As a result, the kinases cannot phosphorylate YAP at Ser127, which prevents the interaction between YAP/TAZ and 14-3-3, so that YAP and TAZ are no more retained in the cytosol and move to the nucleus [6]. Simultaneously, tyrosine kinases bound to the PXXP domain phosphorylate YAP at Tyr357, thus inhibiting its nuclear export and promoting its transcriptional activity [24].

To summarize the above, the M and PXXP domains of Bag3 interact with different protein kinases and affect their activity, making Bag3 an important participant in the transmission of hormonal signals. It should also be mentioned that the above-described HYPPO pathway is very complex and involves multiple protein kinases. For instance, the activity of LATS1/2 is regulated by proteins kinase MST1 and Salvado 1 complex (SAV1). The activity of MST1 depends on its phosphorylation by MAP kinases (MAP4K) which are under the hormonal control [10]. Therefore, Bag3 can indirectly participate in the transduction of hormonal signals by regulating the activity of protein kinases.

The conserved Bag domain contains three extended α-helices. It is located at the C-terminus of Bag3 (Fig. 1a) and is found in all Bag family members. It provides for the interaction with a number of protein partners. One of the most important partners is the heat shock proteins belonging to Hsp70/Hsc70 family. The Bag domain of Bag1 was co-crystallized with Hsc70, which allowed to demonstrate how this domain catalyzes exchange of adenine nucleotides in the active site of Hsc70 [4]. Bag1 and Bag3 can compete for Hsp70 binding [20, 25, 26], causing the switch between the pathways involved in the elimination of denatured proteins. As a rule, the complex formed by Bag1 and Hsp70 directs denatured proteins to the degradation in proteasomes, whereas the complex of Bag3 and Hsp70 usually provides degradation of denatured proteins in autophagosomes. Typically, individual proteins or small protein complexes are degraded in proteasomes, whereas large protein aggregates

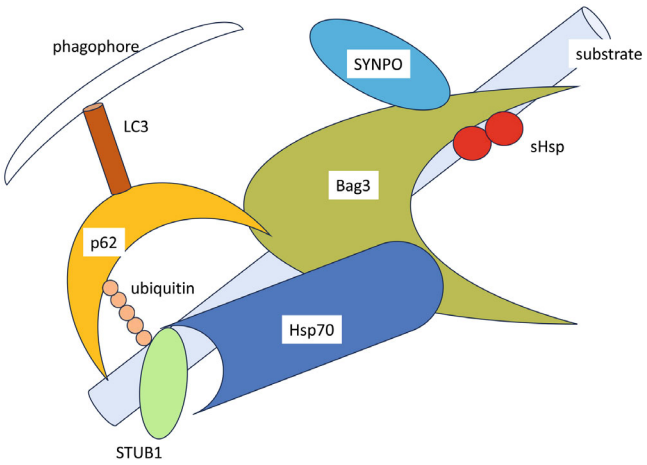

Fig. 2. Chaperone assisted selective autophagy (CASA) complex. Protein substrate (light blue cylinder) binds to HspB8 (two red circles) and the substrate-binding site of Hsp70 (dark blue). Hsp70 binds ubiquitin ligase STUB1 (light green oval) that transfer ubiquitin (small orange circles) onto the protein substrate. Bag3 (green crescent) interacts with the phagosome receptor p62 (yellow crescent) and tethers the protein complex to LC3 (brown cylinder) located on the phagophore membrane. Bag3 through its WW domain interacts with SYNPO2 (light blue oval), thus providing additional interaction with the ATG16 and VPS18/16 proteins of the autophagosomal complex and the Z-disk of myofibrils.

and organelles are degraded in autophagosomes [27]. The proteasomal and autophagosomal systems interact with each other in a quite complicated manner, as each of these systems can degrade the key components of the other one [27]. However, as a rule, inhibition of any of these systems results in the compensatory increase in the other system's activity. Aging or proteasome overload lead to the upregulation of Bag3 synthesis and promote selective degradation of denatured proteins in autophagosomes [20, 25]. The exchange of adenine nucleotides in the active site is necessary for proper Hsp70 functioning. Therefore, by acting as a nucleotide exchange factor, Bag3 plays a critical role in the regulation of Hsp70 activity. Because Bag3 forms tight complexes with Hsp70, a significant increase in its content can result in the inhibition (instead of activation) of the chaperone activity of Hsp70. This can be explained by the fact that chronic stress upregulates the synthesis of Bag3, which forms tight complexes with Hsp70, making its normal functioning impossible and decreasing its chaperone activity [28, 29]. Since the Bag domain plays a crucial role in the activity of Bag3, mutations in this domain are often associated with various inherited diseases, including different types of cardiomyopathies [13, 30].

Bag3 acts as a scaffold in the formation of complexes including sHsps bound to the IPV motifs and Hsp70 bound to the Bag domain, in which sHsps interact with denatured protein substrates. Hsp70 bound to Bag3 interacts with the E3 ubiquitin ligase STUB1 through tetratricopeptides in STUB1 [13], resulting in the formation of a complex consisting of Bag3, sHsps, Hsp70, STUB1, and denatured proteins that are ubiquitinated by STUB1. This multicomponent complex interacts with dynein and is transported to the microtubule organizing center (MTOC) close to nuclear envelope. The phagophore receptor protein p62/SQSTM1 (sequestosome 1) is added to this complex. This protein interacts with LC3 and participates in the phagophore formation [7, 31-33]. The fusion with the lysosome finishes the process of CASA (Fig. 2).

The Bag domain is not involved only in the binding of Hsp70, but also forms the contacts with the antiapoptotic proteins of the Bcl family (Bcl1, Bcl-x, Mcl-1) [34]. It is believed that Bag3 stabilizes Bcl family proteins and ensures their high antiapoptotic effect [35].

To summarize, Bag3 is the multidomain protein interacting with many different protein partners and participating in various cellular processes. Below, we will analyze the structure and properties of the IPV domain and discuss the role of this domain in the interaction with sHsps and other processes controlled by Bag3.

SMALL HEAT SHOCK PROTEINS AND Bag3

Human genome contains ten genes coding for sHsps [36-38]. sHsp monomers have a conserved structure and contain the α-crystallin domain (80-100 amino acids) flanked by the N- and C-terminal domains varying in size and structure. Some sHsps (HspB6, HspB7, and HspB8) exist as monomers or form small oligomers (dimers or tetramers) [39-41], whereas other sHsps form large oligomers of more than 20 subunits [42, 43]. sHsps prone to the formation of large oligomers contain in their C-terminal domains the so-called IPV motifs with the (I/V)P(I/V) sequence [18]. Residues of the IPV motif interact with the β4-β8 hydrophobic groove of the neighboring monomers, which stabilizes large sHsp oligomers [17, 19]. As already mentioned, Bag3 contains two IPV motifs (IPIPV and IPVI) in the fragments 87-101 and 200-213, respectively. It was reasonable to suggest that these motifs would also interact with the corresponding groove in the α-crystallin domain of sHsps. This hypothesis was checked experimentally. Using mutant forms of HspB6 and HspB8, Fuchs et al. [44] found that deletion of either IPV motifs had no effect on the Bag3 interaction with sHsps, whereas deletion of both motifs completely prevented the binding of HspB6 and HspB8 to Bag3. Moreover, mutations in the hydrophobic β4-β8 affected the interaction of sHsps with Bag3. In this first publication, the binding between Bag3 and sHsps was demonstrated only for HspB6 and HspB8. Later studies have thoroughly investigated the binding

of other sHsps with Bag3. Thus, the interaction of Bag3 with HspB1, HspB5, HspB6, and HspB8 was studied by the isothermal titration calorimetry method [45]. The highest affinity (K_d ~ 1.2 μM) was found for the Bag3 binding with HspB6 and HspB8, whereas the affinity of HspB1 was lower (K_d ~ 5-9 μM). Surprisingly, the binding stoichiometry (2 moles of sHsp per mole of Bag3) was the same for sHsps forming small (HspB6, HspB8) and large (HspB1, HspB5) oligomers [45]. This can be possible only if Bag3 possesses a very high affinity for sHsp monomers and, therefore, is capable to induce dissociation of large oligomers resulting in the extraction of monomers (or dimers) from oligomeric complexes. Later, this effect was described by Rauch et al. [45] who analyzed the interactions between Bag3 and HspB1. Similar effect was observed in the experiments on the Bag3 binding with HspB8 [46]. At high concentrations, HspB8 tended to form large complexes, in which monomers interacted weakly with each other. Affinity of Bag3 toward HspB8 monomers was higher than the affinity between HspB8 monomers. Therefore, Bag3 was able to extract HspB8 monomers from their large complexes. It should be mentioned that the K_d of the HspB8–Bag3 complex determined by the surface plasmon resonance varied between 2.4 and 4.6 nM and was significantly lower than that determined by isothermal titration calorimetry.

Several mutations of Pro209 located in the second IPV motif of Bag3 have been described. Thus, the P209L mutation is associated with myofibrillar myopathy, sensorimotor axonal neuropathy, axonal neuropathy, Charcot–Marie–Tooth disease of the second type, and cardiomyopathy. The P209Q mutation correlates with myofibrillar myopathy and sensorimotor axonal neuropathy. Finally, the P209S mutation was found in patients with the Charcot–Marie–Tooth disease of the second type [30, 47]. The P209L mutation results in approximately three-fold increase (from 0.45 up to 1.8 μM) in the K_d value for the Bag3 binding with the α-crystallin domain of HspB1 and decrease in the stoichiometry from ~2 to 1 moles of HspB1 α-crystallin domain per mole of Bag3 [48]. Pro209 mutations (P209/L,S,Q) reduce the binding of Bag3 with sHsps, but do not affect the first stages of CASA complex formation. Such defective complex is transported by dynein to the MTOC. At the final stage of CASA, the phagophore fuses with the lysosome, thus finishing the process of autophagy. In the case of Pro209 mutants, the formed protein complexes interact with histone deacetylase (HDAC6) and vimentin and form unusually highly stable aggresomes that contain immobilized Hsp70 and other proteins involved in CASA. This blocks selective autophagy and induces various pathological processes [3, 49]. Thus, mutations in the second IPV motif affect Bag3 interactions with sHsps, but have no effect on the initial stages of CASA complex formation and dynein-dependent transportation of CASA complex to aggresomes. At the same time, mutations in the second IPV motif block the final stages of autophagy and immobilize CASA components in aggresomes which are highly resistant to dissociation. Therefore, the second IPV motif not only provides the interaction between Bag3 and sHsps, but plays an important role in the normal functioning of the entire CASA complex.

Mutations I94F and R90XD in the first IPV motif have also been reported [30]. However, the data on the effect of these mutation on Bag3 functioning are lacking so far. In mouse Bag3, Ile81 is located close to the first IPV motif. In experiments conducted in mice, ischemia of limb arteries led to the ischemic myopathy, whose manifestations (e.g., limb necrosis) were more severe in the animals injected with an adenovirus coding for Bag3 with the Ile81Met mutations than in the mice injected with the adenovirus coding for the normal protein. The wild-type Bag3 with Ile at position 81 demonstrated better HspB8 binding than the Ile81Met mutant. The autophagic flux was more pronounced in the muscle cells of animals expressing the wild-type Bag3 vs. the mutant protein [50]. These data suggest that the IPV motives play an important role in the functioning Bag3. This agrees with the fact that point mutations in the IPV domain of human Bag3 (residues 55-213) are associated with dilated cardiomyopathy [13, 30].

Which sHsps interact with the IPV domain of Bag3? According to the initial point of view, the IPV motives in Bag3 interact only with the hydrophobic β4-β8 groove of the α-crystallin domain in Hsps [44]. If this hypothesis is correct, then taking into account a high homology of α-crystallin domains, all sHsps should interact with Bag3 with a similar affinity. However, the initial investigations [44] have shown that HspB8 and HspB6 interact with Bag3 with high affinity, whereas HspB1 had only very low affinity. At the same time later investigations have shown that the isolated α-crystallin domain of HspB1 has the highest affinity [45]. It was suggested that the specificity of interaction is determined by the hydrophobicity of the β4-β8 groove in the α-crystallin domain of sHsp [44], but this hypothesis has not received an experimental verification.

The K141E mutation in HspB8 is associated with distal motor neuropathy. The mutant protein was found in autophagosomes; however, these autophagosomes did not co-localize with the lysosomes. At the same time, the wild type HspB8 was observed in autophagosomes that co-localized with lysosomes [51]. This can indicate that mutations in the β6 strand of HspB8, i.e., relatively far from the β4 and β8 strands, can affect the affinity of HspB8 and its interaction with Bag3 [52, 53]. Thus, not only the β4 and β8 strands, but other sites of HspB8 as well can affect its interaction with Bag3.

Recently, four new HspB8 mutants were described with the shift in the reading frame starting from the residues 170, 173, 176, and 194 and leading to a significant increase in the length of the C-terminal peptide [54]. These mutants efficiently interacted with Bag3, but had a lower solubility in the isolated state or in a complex with Bag3 compared to the wild-type protein. This led to the suppression of CASA and development of distal myopathy with rimmed vacuoles (DMRV) [54].

There were attempts to elucidate the role of HspB8 in autophagy at the cellular level. For this, the cells were treated with a proteasome inhibitor and analyzed for the formation and accumulation of aggresomes. It was found that HspB8 affected interaction of Bag3 with p62/SQSTM1 presumably via regulation of p62 phosphorylation or oligomerization [55]. In any case, HspB8 affected the early stages of micro aggregate formation that later enter aggresomes and are subjected to autophagy. Hence, Bag3 and HspB8 cooperate in the processes of autophagy.

To understand the mechanisms of processes involving Bag3, it is necessary to identify its protein partners simultaneously interacting with this adapter protein. *In vitro* experiments have demonstrated that Bag3 can simultaneously bind both Hsp70 and sHsps, thus forming the ternary complex Hsp70–Bag3–sHsp. Moreover, HspB8 was found to affect the ability of Bag3 to regulate the ATPase of activity Hsp70 and to increase its chaperone activity, which was demonstrated using luciferase as a model substrate [45]. Thus, the representatives of two Hsp families (Hsp70 and sHsps) can simultaneously interact with Bag3 and influence each other's activity. This agrees with the fact that the knockout of Bag3 or expression of its E455K mutant (which does not interact with Hsp70) decreased the level of sHsps, especially, HspB6 and HspB8, which interact with Bag3 most efficiently [56, 57]. At the same time, the content of sHsps (HspB1 and HspB5) in the heart of transgenic mice overexpressing Bag3 was decreased [58]. The authors explained this fact by an increased turnover of sHsps due to the autophagy upregulation resulting in the sHsp degradation. It is probably impossible to combine and to compare the data obtained on different small heat shock proteins. For instance, overexpression of HspB5 in the primary culture of astrocytes or glioblastoma cells was accompanied by the increased synuclein aggregation [59]. The authors explain this unexpected effect by the competition between overexpressed HspB5 and HspB8 for Bag3, leading to the inhibition of autophagy and accumulation of synuclein aggregates. Therefore, it is impossible to exclude the probability that different sHsps can compete with each other and affect differently the functioning of Bag3. This conclusion agrees with the experimental results on the aggregation of huntingtin fragment coded by DNA containing 43 CAG repeats [60]. Aggregation of this fragment was effectively prevented by the Bag3–HspB8 complex, whereas HspB1 was unable to replace HspB8. Interestingly, the prevention of aggregation did not depend on the presence of Hsp70. It was concluded that for some substrates, the process of autophagy can be provided by Bag3 and HspB8 without participation of Hsp70 [60, 61].

Amyotrophic lateral sclerosis (ALS) is associated with the accumulation of protein aggregates. Among these proteins, there is a product of the *C9ORF72* gene. The ATG-independent translation of this gene produces a protein containing at the N-terminus short amino acid repeats coded by the G_4C_2 hexanucleotide sequence. These repeats (poly-PA, poly-GP, poly-GR, or poly-PR sequences) cause aggregation of the mutant proteins [62]. Cristofani et al. [62] showed that in immortalized NSC32 motoneurons, the modified proteins were accumulated in aggregates containing p62/SQSTM1 that slowly underwent autophagy, while overexpression of HspB8 significantly decreased formation of all possible types of aggregates. ALS can be also associated with the aggregation of certain RNA/DNA-binding proteins. TAR-DNA binding protein (TDP43) is located in the nucleus and can undergo proteolysis with the formation of TDP25 and TDP35 fragments. Because of the loss of nuclear localization signals (NLSs), these fragments are translocated to the cytosol where they aggregate and accumulate, leading to the ALS development. Overexpression of HspB8 prevented TDP25 and TDP35 aggregation in immortalized motoneurons [63]. The data of Italian scientists indicate that upregulation of HspB8 expression slowed down (or completely prevented) aggregation of TDP25, TDP43, mutant superoxide dismutase, and proteins containing short repeated dipeptides [64, 65]. Hence, HspB8 in a complex with other CASA system components can efficiently protect motoneurons from accumulation of detrimental protein aggregates. Therefore, development of small-molecular-weight compounds capable of upregulating HspB8 synthesis can be a promising approach in the treatment of many neurodegenerative diseases [64].

Stress can induce formation of the so-called stress granules. These granules are formed by RNA and RNA-binding proteins (FUS, TIA01, and hnRNPA1) and accumulate in the cytosol. They can undergo phase separation and form specific drops lacking a membrane. In the norm, stress granules disassemble after stress and release RNA. Under pathological conditions, stress granules can bind misfolded proteins or products of incomplete protein synthesis, which affects their structure and makes them more rigid. As a result, the granules do not dissociate and form aggregates detrimental for the cell functioning. Accumulation of such aggregates can lead to the development of neurodegenerative diseases, such ALS or frontotemporal dementia [66]. Immediately after the stress,

HspB8 dissociates from the complex with Bag3-Hsp70 and migrates to stress granules containing incompletely folded or denatures proteins [66]. When bound to the granules, HspB8 recruits the Bag3–Hsp70 complex, thus initiating and activating CASA. This mechanism is specific for HspB8 only and cannot be realized by other sHsps, e.g., HspB1 [66].

Mitosis is accompanied by the rearrangement of the entire cytoskeleton and actin filaments in particular. Bag3 is phosphorylated by the cyclin-dependent protein kinase 1 [67] and in a complex with HspB8 and p62/SQSTM1 binds the Arp2/3 protein complex, which controls polymerization and branching of actin filaments, as well as histone deacetylase 6 (HDAC6), that regulates another actin-binding protein, cortactin [68]. A decrease in the levels of HspB8 or/and Bag3 is accompanied by mitosis impairment and appearance of cells with two or more nuclei [69]. It was suggested that multifunctional Bag3 interacts with many protein partners and thus, regulates many intracellular processes. Interaction with HspB8 can switch the activity of Bag3 from the control of autophagy to the regulation of cytoskeleton [69]. Therefore, the protein quality control system not only prevents accumulation of protein aggregates and ensures their selective proteolysis, but can participate in the regulation of actin polymerization/depolymerization and even orientation of the mitotic spindle [70]. Interestingly, this activity depends on HspB8 and p62/SQSTM1, but not on Hsp70 [70].

CONCLUSION

Bag3 is a universal adapter protein capable of interacting with multiple protein partners. For instance, it can simultaneously bind Hsp70/Hsc70 and different sHsps. sHsps recognize and bind misfolded and denatured proteins that are then transferred to Hsp70 and undergo ubiquitination by ubiquitin ligase STUB1 bound to Hsp70. The complex composed of ubiquitinated protein substrate, Bag3, Hsp70, and sHsps is transferred by dynein to the MTOC, where it interacts with the autophagy receptors p72/SQSTM1 and LC3 on the phagophores. Later, the phagophores fuse with lysosomes in the process of CASA. The composition of such complex can vary and include different sets of components depending on the nature of the recognized protein substrate. Rather contradictory data indicate that all sHsps interact with Bag3. However, there is a consensus that HsB8 is a predominant Bag3 partner. In the future, it would be important to analyze the interactions of different sHsps with Bag3 and their involvement in the processes controlled by Bag3. Mutations in HspB8 and Bag3 fragments responsible for sHsp binding are often associated with neurodegenerative diseases and cardiomyopathies and mostly cause disturbance in the functioning of the CASA system. The CASA system is not only involved in the selective degradation of denatured proteins, but also plays an important role in granulostasis and cytoskeleton regulation.

Contributions. M.A.Z. collected the published data; L.K.M. and A.I.Z. analyzed the collected data and edited the manuscript, P.A.T.-K. and K.Yu.K. provided additional information on the interaction of Bag3 with protein kinases; N.B.G. wrote and edited the final version of manuscript.

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