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REVIEW

Emerging roles of molecular chaperones and co-chaperones in selective autophagy: focus on BAG proteins

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Abstract Macroautophagy is a catabolic process by which the cell degrades cytoplasmic components through the lysosomal machinery. While initially acknowledged as a rather unspecific bulk degradation process, growing lines of evidence indicate the selectivity of macroautophagy pathways in the removal of misfolded or aggregated proteins. How such substrates are recognized and specifically targeted to the macroautophagy machinery has become a hotspot of investigation, and recent evidence suggests that here molecular chaperones and co-chaperones play a central role. One emerging pathway is mediated by the co-chaperone protein Bcl-2-associated athanogene 3 (BAG 3) which seems to utilize the specificity of molecular chaperones (heat-shock proteins) towards non-native proteins as basis for targeted macroautophagic degradation. In this short review, we focus on the molecular interplay between the macroautophagy system and molecular chaperones and highlight the relevance of the pathway mediated by BAG3 to aging and age-associated protein-misfolding diseases.

Keywords Chaperones · Proteasome · Autophagy · BAG3 · HSP

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Introduction

To maintain the integrity of the proteome in the cellular environment, the quality of proteins must be permanently controlled and dysfunctional and irreversibly damaged proteins must be readily and efficiently eliminated. Central players of the cellular protein quality control (PQC) system and protein homeostasis are molecular chaperones that sense misfolded proteins and, when native folding fails, direct them to intracellular protein turnover pathways [1]. The main pathway cells use to degrade misfolded proteins is the ubiquitin–proteasome system (UPS), where substrates are targeted to the proteasome, a barrel-shaped proteolytic complex [2]. In recent years, several studies indicate that in addition to the UPS, a lysosomal degradation pathway known as macroautophagy has essential functions in PQC [3, 4]. Although macroautophagy was initially thought to be an unspecific bulk degradation process, today we know that additional macroautophagy pathways exist that remove substrates in a highly selective manner [5]. However, it is still enigmatic how substrates are specifically recognized and targeted to the macroautophagy machinery in these pathways and this open question has become a hot topic of investigation in recent years. This short overview will outline recent advances in our understanding of selective macroautophagy pathways with special emphasis on the role of molecular chaperones and co-chaperones, which have been recently shown to play an important role in the selection of substrates and their targeting to the macroautophagy system. In particular, we will focus on BAG3 (Bcl-2-associated athanogene 3) mediated macroautophagy degradation of misfolded substrates. Finally, we will discuss the importance of this new emerging pathway with respect to aging and protein aggregation diseases characterized by a disturbed protein homeostasis.

Protein degradation

Misfolded proteins are directed to intracellular protein degradation systems when a native folding state cannot be reached. The main protein degradation system in a cell is the UPS, where substrates are marked for degradation by ubiquitination and thereupon degraded by the proteasome, a barrel-shaped protein complex that performs fast proteolysis reactions. For degradation, proteins must enter the central cavity of the proteasome through a 13-Å wide entrance channel [2]. Thus, proteins must be unfolded to get degraded by the proteasome since a globular protein would, due to the steric conditions, not fit through the narrow entrance channel. An insufficient catabolic potential arises when substrates to be degraded are prone to form non-dissociable aggregates that cannot be processed by proteasomes. Indeed, it has been shown that aggregate-prone proteins, such as the polyglutamine (polyQ)-expanded huntingtin, which is associated with the neurodegenerative Huntington disease (HD), are poor proteasome substrates and in the aggregated state they even impair proteasome function [6]. Hence, for such and other substrates an alternative catabolic mechanism is needed and recent studies underline here a critical role for the macroautophagy system.

In general, autophagy is a ubiquitous and evolutionarily conserved process in eukaryotes that degrades cytosolic components by the lysosome. So far, three distinct types of autophagy have been described: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) [7, 8]. Autophagy also includes a process called mitophagy. Mitophagy selectively eliminates organelles (mitochondria) to regulate their number and maintain organelle quality and has been reviewed elsewhere [9]. Macroautophagy is a multi-step process by which cytosolic material is sequestered in a double-layered membrane structure, the autophagosome, and delivered to the lysosome for degradation. Macroautophagy is considered as an unspecific robust degradation process. Microautophagy is similar to macroautophagy, but involves direct sequestration of cytosolic components by invagination of the lysosomal membrane. CMA is a highly selective lysosomal pathway that removes a distinct subset of proteins containing a pentapeptide lysosome-targeting motif (KFERQ). These substrates are directly translocated into the lysosome after docking to the lysosomal receptor LAMP2A and being unfolded by a chaperone complex containing the constitutively expressed heat-shock cognate (HSC) 70 protein and the co-chaperones BAG1, Hip, Hop, and HSP40/DNAJB1 [10].

Among these autophagy types in particular, macroautophagy has recently attracted a lot of attention as it became evident that, in addition to the UPS, macroautophagy plays an essential role in sustaining protein homeostasis in various tissues, including the nervous system [3, 4, 11]. Moreover, in subsequent studies, it has been demonstrated that disease-related cytotoxic aggregate-prone proteins such as polyQ-expanded huntingtin, responsible for HD, and mutant SOD1, causally involved in amyotrophic lateral sclerosis (ALS), are specifically removed through the macroautophagy pathway and that stimulating general macroautophagy can exert beneficial effects in different cellular and in vivo disease models [12–14]. But how are unfolded/misfolded substrates specifically eliminated by macroautophagy, a process thought to be rather unspecific?

Targeted protein degradation: molecular chaperones get in touch

For targeted degradation, unfolded/misfolded substrates must be specifically detected in the cell and transferred to the protein degradation systems. Detection of such substrates can occur through molecular chaperones, a group of specialized proteins that bind with high affinity to solvent-exposed, unstructured and hydrophobic regions of non-native proteins [15]. Many molecular chaperones are up-regulated under protein denaturing conditions like heat stress and therefore are referred to as heat-shock proteins (HSP) [16, 17]. Binding of chaperones enables unstructured polypeptides to fold into the native three-dimensional structure. However, when the native state cannot be reached, like in the case of disease-related mutated proteins (e.g., polyQ-expanded huntingtin and mutant SOD1), molecular chaperones can also promote subsequent substrate degradation [18–21]. Our knowledge about how chaperones regulate triage decision between substrate folding and degradation increased significantly in recent years by studying the HSP70 chaperone system.

The HSP70 chaperone system comprises the main chaperone HSP70 and a large group of co-chaperones and co-regulators that modulate the HSP70 folding machinery in a positive or negative manner [16, 17] (Fig. 1). Substrate binding and release by HSP70 is regulated by an ATP-consuming cycle controlled by nucleotide-exchange factors (NEFs). In the ATP-bound state, HSP70 shows rapid substrate association and dissociation kinetics. Hydrolysis of bound ATP to ADP stabilizes the interaction of HSP70 with a substrate. Release of bound ADP from HSP70 and rebinding of ATP triggers the dissociation of the chaperone-substrate complex and the release of the substrate. Amongst the co-chaperones that control the ATP-consuming cycle of HSP70 are, for example, HSP40/DNAJs and HIP and the NEFs HSPBP1 and BAG1. By their J-domain all DNAJs enhance the ATPase activity of HSP70, but they can influence in different ways the fate of their bound substrate;

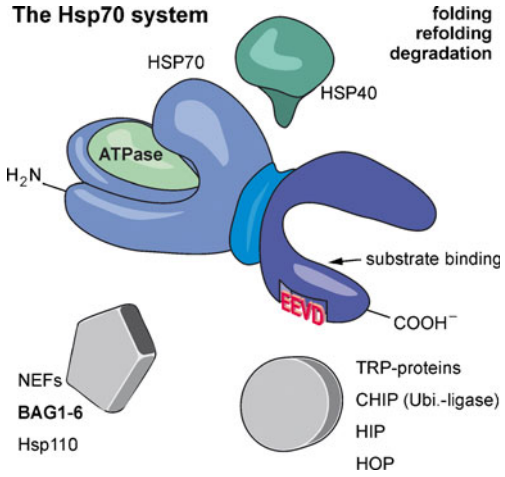

Fig. 1 The HSP70 chaperone system. The HSP70 chaperone system consists of the main chaperone HSP70 and a large group of co-chaperones and co-regulators and is involved in protein folding, refolding and protein degradation. The HSP70 system can be considered as surveillance system and first line of defense upon protein-misfolding [15]. Substrate binding and release by HSP70 is regulated by an ATP-consuming cycle [24]. HSP70 folding activity and its mediation of protein degradation are regulated by co-chaperones that control the ATP-consuming cycle (for example HSP40 and HIP) and by other HSP70-binding co-factors, among which is the ubiquitin ligase CHIP. Also BAG1 and BAG3 bind to HSP70 and modulate its function, linking HSP70 to the proteasome (BAG1) and to macroautophagy (BAG3) [18, 20, 21, 36]

indeed, whereas DNAJB1 generally favors substrate folding, DNAJB2-bound clients are specifically degraded by the proteasome [22]. Hip stabilizes the ADP-bound state of HSP70 and cooperates with HSP70 in protein folding, whereas BAG1 inhibits the HSP70 chaperone activity, competing with the stimulatory action of Hip [23]. Other HSP70-binding co-factors are the ubiquitin ligases CHIP and parkin, which provide a link between HSP70, molecular co-chaperones (e.g., BAG1) and the proteasome system, often resulting in client substrate ubiquitylation and degradation by the proteasome [15, 24, 25] (Fig. 1). An extensive description of the HSP70 machinery, the exact structural/functional relationship between HSP70 and its various co-factors has been excellently presented elsewhere [24]. With respect to the numerous NEFs, the BAG proteins are described as the most complex NEFs [24]. The human BAG family of proteins comprises six members that are localized in the cytosol and/or in the nucleus. All members of the BAG family of proteins specifically interact with HSP70 via their evolutionary conserved BAG domain located within their C-terminus [26]. The BAG proteins act as important mediators of HSP70-assisted protein degradation pathways [26]. They mediate nucleotide exchange on HSP70 thereby inducing the release of a chaperone-bound substrate [27]. BAG proteins are believed to be able to couple the release of chaperoned substrates to distinct downstream cellular processes. For example, depending on the type of substrate BAG1 can either participate in proteasome-mediated degradation [28], or lysosomal-mediated degradation (for KFERQ-containing proteins via chaperone-mediated autophagy [10]). Another member of the BAG family, BAG3, now emerges to specifically control protein degradation by macroautophagy [18, 21]. BAG3 binds to two molecular chaperones: HSC70/HSP70 and HSPB8. The exact relation between BAG3, HSC70/HSP70, and HSPB8 in substrate recognition, binding and targeting to autophagic vacuoles for degradation has however not yet been fully elucidated (see below).

BAG3: a mediator of selective macroautophagy

BAG3 has a modular structure comprising a WW domain at the N-terminus, a proline-rich region (PxxP) in the central region and the BAG domain at the C-terminus. The WW domain is a protein interaction module known to bind proline-rich ligands, including the adenovirus penton base protein [29] and the PDZ domain containing guanine nucleotide-exchange factor 2 (PDZGEF2) [30]. The PxxP motif has been described as a docking site for interaction with SH3 (Src homology 3) and, indeed, it has been shown that BAG3 binds to PLC-γ (phospholipase C-γ), a SH3 domain containing protein involved in growth signal transduction [26, 31]. Recently, two IPV motifs (Ile-Pro-Val) were identified between the WW domain and the PxxP region that mediate the stoichiometric interaction of BAG3 with the small heat-shock protein (sHSP/HSPB) HSPB8 and, with lower affinity, with other members of the sHSP/HSPB family, namely HSPB5 and HSPB6 [18, 32, 33]. These IPV motifs, together with the HSP70-binding BAG domain, allow BAG3 to assemble large multichaperone complexes (for domain structure overview see [34]).

In several recent studies, the multichaperone complex BAG3-HSPB8-HSP70 was found to control the selective degradation of misfolded proteins by macroautophagy, including polyQ-expanded huntingtin and mutant SOD1 [18, 20, 21, 35, 36]. This seems to be mediated by BAG3-HSPB8-HSP70 in cooperation with the macroautophagy receptor protein p62/SQSTM1. The protein p62/SQSTM1 is a stress-regulated multi-adaptor protein that can bind simultaneously to ubiquitin and the autophagosome membrane-associated protein LC3 [37]. The identification of autophagy receptors, such as p62/SQSTM1 and NBR1 has provided a molecular link between ubiquitination and autophagy [38]. Through self-oligomerization, which is stimulated by ubiquitin binding, p62/SQSTM1 sequestrates ubiquitinated substrates in form of inclusion bodies. These inclusions are then specifically engulfed by the autophagosome membrane by recruiting LC3 [37, 39]. BAG3 has

been shown to recruit HSP70 to p62/SQSTM1 [21, 35] and, by inducing nucleotide exchange on HSP70, it may be suggested that BAG3 stimulates transfer of HSP70-bound substrates to the autophagy receptor. Consistent with this mechanistic model, BAG3 was found to stimulate the degradation of ubiquitinated substrates by macroautophagy in a p62/SQSTM1-dependent manner and this was associated with the formation of BAG3-positive sequestration structures containing p62/SQSTM1 and ubiquitinated substrates [21]. The HSP70-interacting ubiquitin ligase CHIP was also found in the complex together with HSPB8, HSP70 and BAG3 [35] suggesting that this complex could ubiquitinate chaperoned substrates and target them to the macroautophagy pathway via p62/SQSTM1. This is further suggested by the finding that BAG3, in complex with HSPB8-HSC70 and CHIP, binds to mutated SOD1 and targets it to macroautophagy for degradation [20]. Thus, the simultaneous interaction of BAG3 with molecular chaperones, like HSPB8 and HSC70/HSP70, would allow this multichaperone complex to recognize and bind to misfolded substrates. An interaction with p62/SQSTM1 would enable BAG3 to specifically target the bound substrates to the autophagosomes for degradation. However, from recent findings, it emerges that an additional mechanistic step may participate to increase the selectivity of the BAG3-HSPB8-HSP70 mediated macroautophagy degradation.

Indeed, it has been shown that mammalian cells direct and concentrate both aggregated proteins and autophagic vacuoles at the microtubule organizing center (MTOC) to increase the specificity and efficiency of clearance of aggregated proteins by macroautophagy. Aggregate-prone proteins like, e.g., polyQ-expanded huntingtin and mutated SOD1, are retrogradely transported along microtubules in a dynein-dependent manner to the MTOC, where they are assembled in protein structures called aggresomes [40]. Aggresomes are thought to act as staging grounds for the disposal of protein aggregates by macroautophagy [41, 42]. Also autophagic vacuoles are retrogradely transported to the perinuclear region, where they can easily engulf the aggregated proteins [43]. As a consequence, impairment of dynein-mediated transport results in defective degradation by macroautophagy of aggregate-prone proteins and participates in the progression of several diseases, including ALS [44]. Interestingly, we found that BAG3 directly associates with the minus-end directed microtubule motor dynein [36] and mediates the selective transport of misfolded proteins to the aggresome. As a nucleotide-exchange factor, hereby BAG3 may stimulate substrate transfer from HSP70 to the dynein motor complex, thus promoting transport of misfolded proteins to the aggresome (Fig. 2). This may further participate to ensure the selective targeting and degradation of misfolded substrates by macroautophagy. While awaiting additional original data, this hypothesis may be further supported by our observations that: (1) binding of BAG3 to dynein is mediated by its PxxP domain [36] and (2) deletion of BAG3 PxxP domain abrogates its ability to facilitate the clearance of polyQ-expanded huntingtin [45].

The role of the BAG3-selective autophagy pathway in aging and protein aggregation diseases

Cellular aging is associated with an enhanced accumulation of misfolded proteins and protein aggregates permanently challenging the PQC and the protein degradation capacity of the cell. Different extent of an age-associated decrease in the activity of the UPS has been shown in a variety of tissues [46–49]. As proteasome activity is decreasing during aging [50], the degradation demand is increasing. And, indeed, enhanced macroautophay has been observed in aged primary human fibroblasts and in aged mouse brain tissue, which has been suggested to compensate for the reduced proteasomal-mediated degradation of misfolded and/or damaged proteins [21]. Moreover, aggregated proteins can impair the proteasome system directly [6] and misfolded and polyubiquitinated proteins that form aggregates and cannot be handled by the UPS need, finally, to be taken care of by macroautophagy [51]. We showed previously that the co-chaperones BAG1 and BAG3 represent key players of cellular PQC by stimulating the turnover of polyubiquitinated proteins by proteasomal and autophagic degradation pathways, respectively [21]. Interestingly, the decreased proteasomal activation and the increased macroautophagy activation in aged cells/tissue have been correlated with decreased levels of BAG1 and increased levels of BAG3 [21]. The age-related increased BAG3/BAG1 ratio triggers the recruitment of the macroautophagy pathway as supplementary avenue for PQC in addition to the proteasome [21]. Therefore, the expression shift from BAG1 to BAG3 (increasing levels of expression of BAG3 and decreasing levels of expression of BAG1, an event referred to as “BAG1/BAG3-switch”) during aging but also upon acute stress (e.g. proteasome inhibition, oxidative stress) can be considered as a physiologically important adaptive response (Fig. 3).

Enhanced protein aggregation is also a pathological hallmark of neurodegenerative diseases, protein aggregate myopathies and peripheral neuropathies. Neurodegenerative disorders include Huntington disease, characterized by the aggregation of polyQ-expanded huntingtin, Parkinson Disease, associated with α-synuclein aggregates, and ALS developing aggregates of mutated forms of proteins SOD or TDP-43. Relevant myopathies are the desmin-related myopathy showing aggregation of mutated R120G HSPB5 [52, 53] and several forms of muscular dystrophy, charac-

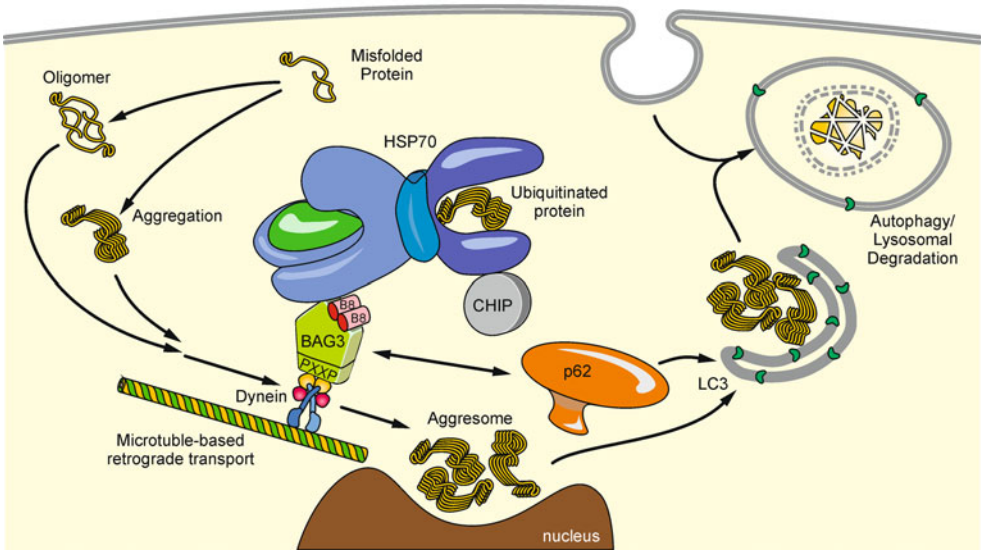

Fig. 2 Putative mechanism of action of the BAG3-HSPB8-HSP70 chaperone complex. Protein misfolding, either due to genetic mutation or to external stress (e.g. heat-shock, oxidative stress), leads to protein instability and aggregation. Misfolded aggregation-prone proteins are ubiquitinated and targeted by molecular chaperone and co-chaperone complexes to the proteasome (not shown) or macroautophagy for degradation. Amongst these complexes is the HSPB8-HSP70-CHIP-BAG3 chaperone complex. We hypothesize that in this complex the molecular chaperones HSPB8 and HSP70 might recognize and bind to the misfolded proteins, which could be ubiquitinated with the help of the E3 ubiquitin ligase CHIP. Bound substrates would be targeted to macroautophagy for degradation. This step would be accomplished with the help of two other key players that interact with BAG3: the dynein motor protein and the LC3-binding protein p62. The association of BAG3 with the dynein motor protein is mediated by the BAG3 PxxP region and would allow the dynein-mediated retrograde transport of the misfolded ubiquitinated proteins to the microtubule organizing center (MTOC). Here, aggregated proteins are assembled together to form the aggresome, a structure that act as staging grounds for the disposal of protein aggregates by autophagy. BAG3 interaction with p62, which binds both ubiquitinated substrates and the autophagy marker protein LC3 may ensure the specific targeting of the misfolded proteins to the autophagic vacuoles for degradation

terized by the aggregation of mutated dysferlin [54] or mutated BAG3 [55]. Protein aggregation has also been described in several forms of peripheral neuropathies, associated with mutated peripheral myelin protein 22, PMP2 [56, 57], mutated HSPB1 [58] or mutated HSPB8 [59], respectively. Interestingly, macroautophagy has been shown to participate in the clearance of the aggregates containing the mutated proteins dysferlin and PMP22 and

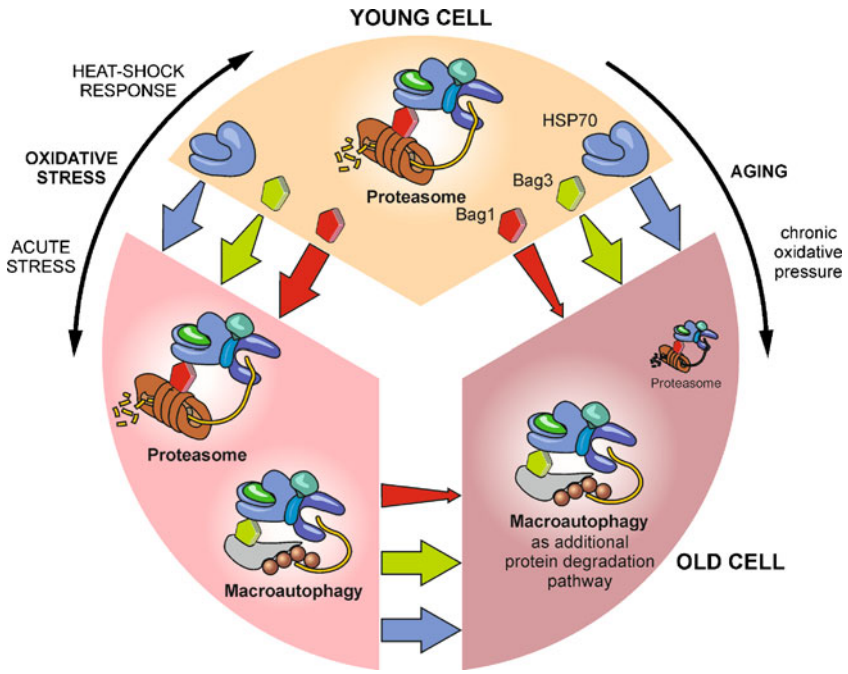

Fig. 3 "BAG1–BAG3-switch". BAG1 and BAG3 are key molecular players of the cellular PQC via the stimulation of the turnover of polyubiquitinated proteins (with respect to BAG3 including also non-ubiquitinated substrates) by proteasomal and autophagic degradation pathways, respectively. An increased BAG3/BAG1 ratio triggers the recruitment of the macroautophagy pathway as additional avenue for PQC. During aging and also upon acute stress (oxidative or proteasomal stress) there is an expression shift from BAG1 to BAG3. This BAG1/BAG3-switch can be considered as a physiologically important adaptive response to assist the clearance of misfolded aggregate-prone proteins and maintain protein homeostasis

has been demonstrated to protect against cellular toxicity and/or to decrease symptom severity/disease progression [54, 56, 57]. The finding that mutations in HSPB8 and BAG3, which aggregate themselves, cause dominant hereditary peripheral neuropathy and/or muscular dystrophy underscores a clear link between protein aggregation, PQC, specific chaperones and autophagy in neuronal and muscular disorders. This is further suggested by the observation that autophagic vacuoles typically accumulate in muscular disorders. Indeed, deregulated autophagy has been reported as cause for hereditary peripheral neuropathies and several genes associated with peripheral neuropathies play a role in lysosome biogenesis, maturation and/or vesicular trafficking (e.g., Rab7, LITAF/SIMPLE, MTMR2 and MTMR13) [60]. Taken together, these data reveal two important paradigms. First, deregulated autophagy may itself have dramatic consequences for neuronal and muscular cell function and viability and may cause or promote degeneration. Second, autophagy stimulation may be beneficial in many neuronal and muscular degenerative diseases. It is thus likely that the mutations in HSPB8 and BAG3 may affect their function in PQC and autophagy stimulation. Indeed, we recently showed that both mutated forms of HSPB8 associated with hereditary peripheral neuropathy (K141E and K141N) are less efficient than wild-type HSPB8 in decreasing the aggregation of several misfolded substrates (mutated huntingtin, mutated ataxin 3, associated with spinocerebellar ataxia 3 and mutated P182L-HSPB1, associated with hereditary peripheral neuropathy) [61]. This suggests that K141E and K141N are characterized by a loss of function in PQC and autophagy stimulation, which may participate in disease progression. Besides, both mutated HSPB8 and BAG3 are found in aggregates in cells. Their accumulation, together with their loss of function (and/or toxic gain of function), may further challenge the cells, contributing to an imbalance in protein homeostasis. Finally, the importance of the BAG3 function in the maintenance of protein homeostasis and cellular viability is also supported by the evidence that BAG3-mediated macroautophagy is essential for Z-disk maintenance in muscle and, indeed, knock-out of BAG3 in mice results in fulminant myopathy [35, 55, 62]. Why HSPB8 and BAG3 mutation affect selectively motor neurons and skeletal muscular cells, respectively, is still unknown. However, both these cell types are characterized by high energy demand (e.g. for protein/cargo transport and contraction, respectively) and might be more vulnerable to an imbalance of protein homeostasis and, ultimately, degeneration. Dissecting the precise role of the BAG3-HSPB8-HSC70 complex in autophagy modulation and how its function is affected by the disease-related mutations will allow a better understanding of protein aggregation diseases and myopathies. Moreover, the specific activation of selective macroautophagy in contrast to the general induction of various autophagy pathways at once might lead to future concepts of prevention and therapy of protein aggregation diseases.

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