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Chaperone-assisted proteostasis is essential for mechanotransduction in mammalian cells

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Maintaining the dynamic proteome of a living cell in the face of an ever-changing environment depends on a fine-tuned balance of protein synthesis and protein degradation. Molecular chaperones exert key functions during protein homeostasis (proteostasis). They associate with nonnative client proteins following synthesis or damage and facilitate client sorting and folding. When client proteins are terminally misfolded, chaperones cooperate with protein degradation systems to dispose of such clients. This dual proteostasis activity of chaperones is essential for maintaining cell function under normal growth conditions and becomes even more important under stress conditions such as heat and oxidative stress. The recent identification of chaperone-assisted selective autophagy (CASA) as a tension-induced autophagy pathway highlights the critical role of molecular chaperones in mechanically strained cells and tissues. The CASA complex, assembled by the cochaperone BAG3, coordinates protein degradation and protein synthesis in response to mechanical force. Here we describe the composition and function of this chaperone complex in mammals and discuss its relevance for tissue homeostasis and the regulation of cell adhesion, migration and proliferation. We provide a unifying concept for the function of BAG3, which integrates its involvement in muscle maintenance, tumor formation and virus infection.

Living cells and tissues are constantly subjected to mechanical tension.[1] This is apparent for force-generating tissues such as muscles, and for cells that have to resist force such as blood-filtering kidney cells. However, intracellular tension is also generated in adherent and migrating cells through cell-cell and cell-matrix contacts.[2,3] Cells respond to mechanical tension by reinforcing cell attachment sites and by strengthening the cytoskeleton.[4] Moreover, tension can represent a physiological stimulus, governing for example stem cell differentiation, immune cell recruitment and tumor cell development.[3,5,6] Considerable progress has been made in recent years regarding our understanding of how mechanical signals are transduced into biochemical and genetic responses within cells. Diverse cytoskeleton components were shown to undergo tension-induced conformational changes.[2,3,7-9] This often affects the functional interplay with signaling proteins, pointing to a role of the involved cytoskeleton components as mechanosensors. In addition, transcription regulators, such as YAP1 and WWTR1/TAZ, were found to be activated under tension to induce the expression of proteins involved in cell adhesion.[4] Yet, how mechanosensing is ultimately linked to transcription regulation remains poorly understood. Notably, we recently identified a chaperone complex, which coordinates tension sensing, transcription regulation and the degradation of mechanically damaged proteins in mammalian cells.[10]

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Role of Molecular Chaperones in Protein Folding and Degradation

Molecular chaperones are defined by the ability to associate with nonnative proteins and prevent protein aggregation (**Fig. 1**).[11] They assist in the sorting and folding of newly synthesized and damaged proteins, and facilitate the assembly of protein complexes. Accordingly, biology textbooks describe molecular chaperones usually as cellular protein folding factors. However, in recent years it has become increasingly clear that some key chaperones, i.e., Hsp70 and Hsp90 family members, very actively participate in protein degradation.[12,13] When a client protein is unable to attain its native conformation, the chaperones can initiate client disposal by different pathways (**Fig. 1**).[12] This includes sorting to the proteasome, a large proteolytic complex, following the attachment of a ubiquitin chain to the client protein.[12] Alternatively, nonnative clients can be targeted for lysosomal degradation by two distinct autophagy pathways, i.e., chaperone-assisted selective autophagy (CASA) and chaperone-mediated autophagy (CMA). During CASA, nonnative clients are initially ubiquitylated and then enclosed in autophagosomes, which eventually fuse with lysosomes (**Fig. 1**).[12] In contrast, CMA involves the ubiquitin-independent translocation of chaperone clients, which display a KFERQ consensus-degradation motif, directly across the lysosome membrane.[12,14]

Considering the degradation-promoting activity of molecular chaperones, it becomes apparent that the common textbook definition of these proteins needs to be revised. Instead of being protein folding factors, many chaperones rather act as surveillance proteins. They constantly scan the cellular interior for the presence of nonnative proteins. The chaperones will bind to these

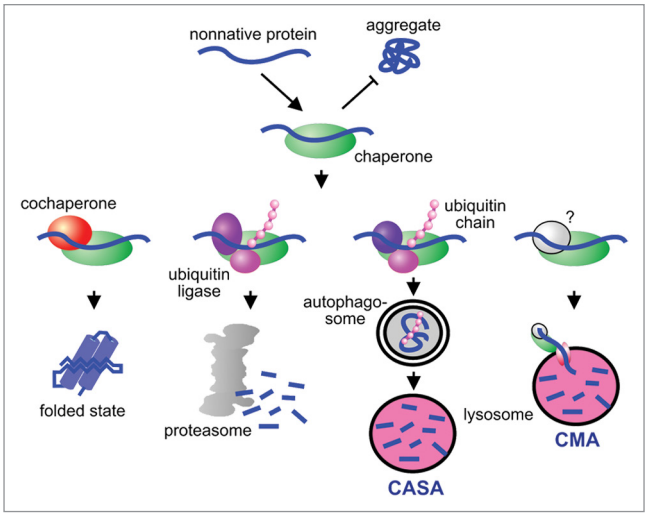

Figure 1. Molecular chaperones bind nonnative proteins and prevent their aggregation. Folding or degradation of the client protein is initiated in conjunction with regulatory cochaperones. Association with an ubiquitin ligase leads to ubiquitin chain formation on the chaperone-bound client. This induces client sorting to the proteasome or triggers the autophagic engulfment of the client during chaperone-assisted selective autophagy (CASA). On the latter pathway, the client is eventually degraded in lysosomes. During chaperone-mediated autophagy (CMA) the client is directly translocated across the lysosome membrane. The cochaperone requirement during CMA remains to be determined (?).

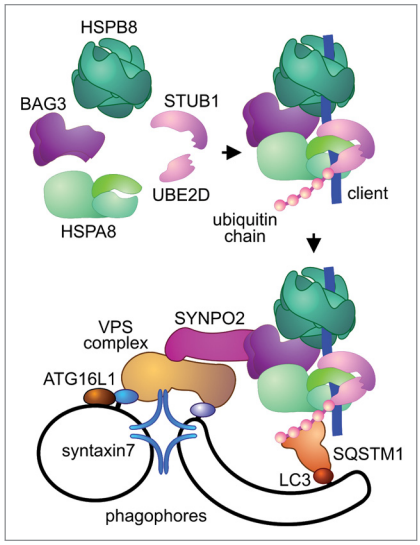

Figure 2. The CASA chaperone complex comprises the chaperones HSPA8 and HSPB8 and the cochaperones BAG3 and STUB1. STUB1acts as a chaperone-associated ubiquitin ligase and cooperates with the ubiquitin-conjugating enzyme UBE2D during the ubiquitylation of the chaperone-bound client. Autophagosome formation during CASA depends on an interaction of BAG3 with SYNPO2, which interacts with a VPS protein-containing membrane fusion complex. This complex mediates the tethering of ATG16L1 and LC3 positive autophagosome precursor membranes (phagophores) in preparation for syntaxin7-mediated membrane fusion. Binding partners of the VPS complex on the target membranes (blue) remain to be identified. Through simultaneous interactions with BAG3 and the VPS complex, SYNPO2 triggers the formation of an autophagosome membrane around the client-loaded CASA complex.

proteins in order to prevent aggregation and then facilitate either folding or degradation.[11] The mode of action of the chaperone is determined in this situation by cochaperones, which regulate client binding and provide a link to other folding factors or to degradation systems (**Fig. 1**).[15] Defined chaperone-cochaperone complexes are thus specifically engaged in distinct cellular processes. In mechanically strained cells and tissues the CASA chaperone-cochaperone complex mediates autophagosome formation.[10,16]

Chaperone-Assisted Selective Autophagy (CASA)

The CASA complex comprises the molecular chaperones HSPA8/HSC70 and HSPB8/HSP22 and the cochaperones BAG3 and STUB1/CHIP (**Fig. 2**).[16] HSPA8 represents a constitutively expressed member of the Hsp70 chaperone family localized in the cytoplasm and nucleus of mammalian cells. HSPB8 belongs to the family of small heat shock proteins, which, although small in size (~20-kDa), form large oligomeric assemblies that stabilize nonnative proteins. Further processing of the stabilized protein occurs in cooperation with Hsp70 chaperones.[17] The cochaperone BAG3 facilitates the functional interplay between HSPA8 and HSPB8, because it possesses non-overlapping binding sites for both chaperones.[16] Client loading onto HSPA8 might be facilitated by the J-domain cochaperone DNAJB6, found to be associated with the CASA complex in muscle cells.[18] J-domain cochaperones are often the first components of the chaperone machinery, which recognize client proteins and regulate the ATP-driven chaperone cycle of Hsp70 proteins in a manner allowing high-affinity client binding.[11] Once bound to the chaperone complex the client is modified by attachment of an ubiquitin chain. This appears to be mediated preferentially by the chaperone-associated ubiquitin ligase STUB1 in conjunction with ubiquitin-conjugating enzymes of the UBE2D family (**Fig. 2**).[16] However, STUB1 might not be the only ubiquitin ligase involved in CASA, as protein degradation is not significantly affected in STUB1 deficient mice and functional redundancy has been reported for chaperone-associated ubiquitin ligases.[19,20] Following ubiquitylation, BAG3 facilitates the autophagic degradation of CASA-bound clients by multiple means. Based on an association with the microtubule-motor dynein it directs clients into aggregate-like structures suited for efficient autophagic digestion.[21] Moreover, the cochaperone stimulates the recognition of ubiquitylated clients by the autophagic ubiquitin adaptor SQSTM1/p62.[16,22,23] The adaptor possesses a ubiquitin binding site and also a domain that interacts with LC3 on autophagosome precursor membranes (phagophores). Simultaneous binding to ubiquitylated clients and to phagophores allows the adaptor to induce autophagic engulfment (**Fig. 2**).[23] Autophagosome formation during CASA also depends on SYNPO2 (synaptopodin-2/myopodin).[10] SYNPO2 has been described as a cytoskeleton adaptor protein in skeletal muscle and heart,[24,25] and as a tumor suppressor in bladder and prostate.[26-28] It interacts through a

PPPY motif with the WW domain of BAG3 and at the same time contacts the vacuolar protein sorting homolog VPS18 through an N-terminal PDZ domain (**Fig. 2**).[10] VPS18 is a core component of diverse protein complexes, which tether intracellular membranes in preparation for fusion.[29-31] The SYNPO2-associated VPS protein complex seems to be specifically involved in the tethering of phagophore membranes, because corresponding marker proteins, i.e., ATG16L1 and LC3, were detectable in SYNPO2 immunocomplexes.[10] In addition, these complexes contain the SNARE protein syntaxin7, which was recently shown to facilitate phagophore fusion.[32] Taken together, SYNPO2 apparently acts as a coupling factor between the client-processing CASA chaperone complex and a membrane tethering and fusion machinery that provides autophagosome membranes (**Fig. 2**). The data establish a functional paradigm how ubiquitylation can be linked to autophagosome formation during selective autophagy.

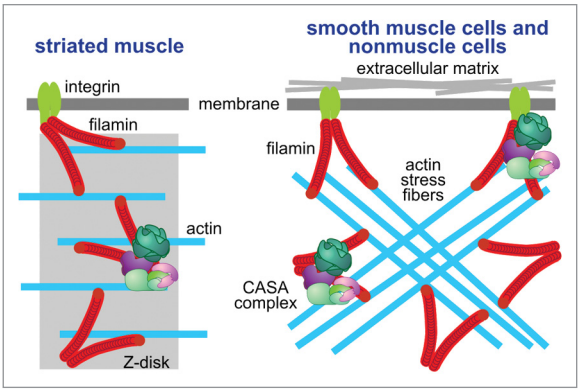

Figure 3. The CASA complex is associated with Z-disks in striated muscles and with actin stress fibers in smooth muscle and nonmuscle cells. It senses the mechanical unfolding of the actin-crosslinking and anchoring protein filamin (red) and initiates the autophagic disposal of mechanically damaged filamin.

CASA is Essential for Cytoskeleton Maintenance

The CASA complex is closely associated with the actin cytoskeleton (**Fig. 3**). In striated skeletal muscles, the complex localizes at Z-disks, force-bearing structures, where actin filaments are anchored and crosslinked.[16] In smooth muscle and nonmuscle cells the complex is detectable along actin stress fibers that form when tension is generated inside cells during adhesion and migration.[9,10] In all cases, association reflects a critical role of the CASA complex in maintaining the actin cytoskeleton under mechanical tension. The CASA-inducing cochaperone BAG3 is indeed essential for the adhesion and migration of diverse cell types ranging from embryonic fibroblasts to kidney epithelial cells and breast cancer cells.[33,34] Moreover, BAG3-deficient mice suffer from a rapid disintegration of Z-disks in contracting muscles after birth, leading to a progressively developing muscle weakness.[35] The mice die within four weeks because of heart and lung failure, in agreement with an essential function of the CASA machinery in muscle maintenance.

In 2009, Selcen and Engel were the first to identify a missense mutation in human BAG3, which leads to dominant childhood muscle weakness.[36] Similar to the findings obtained for BAG3-deficient mice, a progressive deterioration of Z-disk architecture was observed in muscles of affected patients, causing limb and axial muscle weakness, cardiomyopathy and respiratory insufficiency at about ten years of age. Other pathogenic mutations within BAG3 were identified in subsequent years and the CASA-inducing cochaperone is now recognized as a causative agent in muscular dystrophy and cardiomyopathy.[37-39] Furthermore, limb-girdle muscular dystrophy was recently shown to be caused by mutations in the CASA-associated cochaperone DNAJB6.[18] CASA-mediated protein degradation is obviously necessary for muscle homeostasis in humans.

What is the critical client of CASA in mechanically strained cells, which needs to be degraded in order to maintain the cytoskeleton? To address this question, we incubated the isolated cytoskeleton of differentiated muscle cells with purified CASA components.[16] The assumption was that the CASA machinery should be able to mediate the release of clients from the cytoskeleton as a prerequisite for their autophagic sorting to lysosomes. In this assay, BAG3 specifically triggered the dissociation of the actin-crosslinking protein filamin from the isolated cytoskeleton.[16] Filamin is a homodimer comprised of two ~250-kDa rod-like structures, each formed by an N-terminal actin-binding domain and 24 immunoglobulin (Ig)-like domains.[40] Filamin crosslinks actin filaments at Z-disks and along actin stress fibers, and exerts actin-anchoring functions through interactions with cell surface attachment proteins such as integrins (**Fig. 3**).[40,41] Notably, mechanical tension of a magnitude prevalent in adherent and migrating cells results in a loss of interdomain interactions and in an unfolding of individual Ig domains of the filamin rod, leading to a significant extension of the molecule.[7,41-43] This enables filamin to act as a flexible linker between actin filaments under mechanical strain. Tension-induced conformational changes and unfolding also affect the interaction of filamin with diverse signaling proteins, in line with a role of the actin-crosslinking protein as a mechanosensor.[40,41,44] Mutations in filamin indeed cause skeletal and heart muscle weakness.[45,46] We could show that BAG3 binds to a mechanosensitive region of filamin, comprising Ig domain 19–21, and facilitates the recruitment of HSPA8 and HSPB8 to this site.[10] The chaperones then monitor tension-induced unfolding and damage of filamin, and initiate its autophagic disposal in cooperation with BAG3 and STUB1. In adherent smooth muscle cells, filamin is indeed degraded by autophagy in a BAG3 and STUB1 dependent manner.[10,16] Moreover, filamin forms large proteinaceous aggregates in muscles of transgenic mice deficient for the lysosome membrane protein LAMP2, in which autophagosome-lysosome fusion is blocked.[16] Apparently, filamin needs to be continuously degraded by CASA in mechanically strained cells and tissues in order to maintain the actin cytoskeleton.

BAG3 is a Dual Function Proteostasis Factor

Cell and tissue maintenance under tension depends not only on the disposal of mechanically damaged cytoskeleton

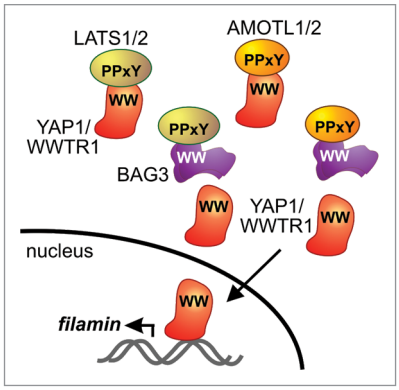

Figure 4. BAG3 utilizes its WW domain to interact with PPxY motif-containing components of the Hippo signaling network, i.e., LATS1/2 and AMOTL1/2. These proteins are usually involved in the cytoplasmic retention of the transcriptional regulators YAP1 and WWTR1, which control filamin expression. BAG3 binding to LATS1/2 and AMOTL1/2 abrogates the cytoplasmic retention and induces YAP1/WWTR1-mediated *filamin* transcription under tension.

components. Degraded components also need to be replaced by newly synthesized molecules in order to maintain the cellular architecture. Intriguingly, BAG3 is not only involved in the autophagic degradation of filamin under tension, but also participates in filamin transcription.[10] The cochaperone is able to interact with components of the Hippo signaling network, which controls the development and growth of tissues and was recently linked to mechanotransduction[4,47,48] (The term "Hippo" refers to the key kinase that is involved in this network in *Drosophila melanogaster*). Downstream targets of the signaling network include the transcription regulators YAP1 and WWTR1/TAZ, which are activated in response to mechanical tension and induce the expression of proteins involved in cell adhesion, including filamin.[4] The activity of the transcription regulators is restricted by the network proteins LATS1/2 and AMOTL1/2. These proteins possess PPxY motifs that contact WW domains present in YAP1 and WWTR1. In this way LATS1/2 and AMOTL1/2 mediate the cytoplasmic retention of the transcription regulators and attenuate target gene expression (**Fig. 4**).[47,48] Intriguingly, BAG3 can engage in interactions with the PPxY-containing YAP1/WWTR1 inhibitors through its own WW domain.[10] As a consequence, the transcription regulators are released, migrate into the nucleus and activate gene expression (**Fig. 4**).[10] The data reveal BAG3 as a positive regulator of YAP1/WWTR1-mediated transcription. This enables BAG3 to stimulate filamin synthesis under tension. The cochaperone thus acts as a dual function proteostasis factor in mechanically strained cells and tissues. On one hand, BAG3 mediates the autophagic degradation of mechanically damaged filamin in conjunction with SYNPO2, and on the other hand, it binds YAP1/WWTR1 inhibitors to induce a transcriptional response for compensating autophagic disposal. It remains to be seen whether BAG3 cooperates with its chaperone partners during transcription regulation. In any case, the dual function of the cochaperone is essential for maintaining the actin cytoskeleton under tension.[10,16]

Tension-Dependent Regulation of CASA Activity

BAG3 is a stress-inducible cochaperone under control of the heat shock transcription factor HSF1.[49,50] BAG3 expression is upregulated in mammalian cells under oxidative and heat stress or upon exposure to heavy metals.[22,49,51] We observed that mechanical tension also leads to HSF1 activation and increased BAG3 expression.[10] Similar to the situation in heat-stressed cells, the tension-induced unfolding of cytoskeleton proteins and mechanosensors apparently causes a depletion of the available pool of chaperones in mechanically strained cells. This in turn induces the HSF1-mediated compensatory expression of chaperones, cochaperones and proteostasis factors, including BAG3 and other CASA components. In this way, CASA activity is constantly adjusted to the level of tension within the actin cytoskeleton.

Physiological Role of CASA

It was previously observed that mechanically strained tissues such as skeletal muscle, heart, lung, bone and kidney highly rely on autophagy for their development and maintenance.[52,53] Transgenic mice unable to express key autophagy factors often develop severe pathologies affecting these tissues. For example, LAMP2-deficient mice, in which autophagosome-lysosome fusion is blocked, suffer from skeletal muscle weakness and severe cardiomyopathy.[54] A similar pathology is observed in patients with Danon disease, which is caused by functional impairment of human LAMP2.[55] Mutations in p62, which acts as an autophagic ubiquitin adaptor during CASA, are frequently found in Paget disease of bone.[52] Furthermore, a cell-specific knockdown of the autophagy initiation factor ATG5 in podocytes, which form the kidney filtration barrier, causes glomerulosclerosis accompanied by an accumulation of ubiquitinated proteins in transgenic mice.[56] These are only few examples that illustrate the physiological importance of autophagic degradation in mechanically strained tissues. It remains to be seen whether in all cases CASA-mediated degradation of filamin lies at the heart of the observed pathologies, as was shown for LAMP2-deficiencies.[16] Still, the finding that autophagy is necessary for maintaining the architecture of the actin cytoskeleton under mechanical tension provides a novel conceptual framework for our understanding of these pathologies.

Beth Levine and coworkers recently identified an exercise-induced autophagy pathway in muscles, which is regulated by the anti-apoptotic protein BCL2.[57] Acute exercise leads to a rapid induction of autophagy in skeletal and cardiac muscle of mice. Induction is accompanied by the disruption of an inhibitory complex between BCL2 and the autophagy initiation factor beclin1. Expression of a mutant form of BCL2 that stably associates with beclin1 abrogates exercise-induced autophagy.[57] Because mutant mice also show altered glucose metabolism, the authors conclude that exercise-induced and BCL2-regulated autophagy is required for muscle glucose homeostasis. Still, it will be interesting to investigate the relationship between this pathway and CASA, which is upregulated in contracting mouse muscles and may, therefore, also represent an exercise-induced autophagy pathway.

The ability to resist mechanical force and to respond to mechanical signals is a vital feature of immune cells. These cells circulate in the blood and are recruited to lymphoid organs and peripheral sites of injury, infection and inflammation.[3] Notably, tension-regulated adhesion and migration of immune cells involves induction of BAG3 expression and filamin turnover.[10] Moreover, pharmacologic inhibition of autohagy attenuates immune cell adhesion, migration and resistance to shear stress.[10] The data point to an essential function of CASA in immune cell activation and recruitment.

Although we focus here on the role of CASA in mechanotransduction, it should be mentioned that CASA is also essential for protein homeostasis in neuronal cells. In fact, the CASA-inducing cochaperone BAG3 was initially linked to the autophagic degradation of pathologic forms of the huntingtin protein, which cause Huntington's disease.[58] Moreover, it was observed that BAG3 expression is induced in aged neuronal cells in correlation with an increased autophagic clearance of oxidatively damaged proteins.[22] CASA seems to protect the brain against a pathologic accumulation of misfolded proteins.

A Unifying View on the Multifaceted Cochaperone BAG3

Ever since its identification as a BCL2-associated protein[59] and HSPA8 cochaperone[60] in 1999, the CASA-inducing cochaperone BAG3 has been analyzed in seemingly very different functional settings. The finding that BAG3 exerts anti-apoptotic activity in conjunction with BCL2[59] led to a series of studies that focused on the role of the cochaperone in tumor development.[61] Indeed, BAG3 is overexpressed in several tumors, including glioblastoma, acute lymphoblastic leukemia and prostate carcinoma, where it sustains cell survival and therapy resistance.[62,63] Moreover, the cochaperone regulates the adhesion and motility of tumor cells.[33,34,64] Other studies revealed the physiological relevance of BAG3 in muscle cells and identified mutations in the cochaperone as the cause for muscle dystrophy and cardiomyopathy.[36-39] Finally, the identification of BAG3 as a cochaperone of HSPA8 and HSPB8 stimulated research on its role in proteostasis, unravelling its function in chaperone-assisted selective autophagy.[10,16,21,22,58,60]

A unifying concept for the function of this cochaperone is now emerging based on the recent identification of BAG3 as a transducer of mechanical signals, which coordinates autophagosome formation and YAP1/WWTR1-mediated transcription under tension.[10] According to this concept, association of BAG3 with BCL2 might reflect an involvement of the cochaperone in regulating early steps of autophagosome formation, because BCL2 engages in inhibitory interactions with the autophagy initiation factor beclin1.[65,66] The oncogenic activity of BAG3 might be attributed to its role as an activator of YAP1 and WWTR1. The two transcription regulators drive oncogenic transformation and epithelial to mesenchymal transition, which is a critical step during tumor progression.[47,48] It is also noteworthy in this regard that extracellular matrix stiffness promotes a malignant phenotype of epithelial cells.[67] Changes in the expression of BAG3, which senses extracellular matrix stiffness in cooperation with its chaperone partners,[10] might therefore directly contribute to oncogenic transformation. This is certainly in agreement with the essential regulatory function of BAG3 in tumor cell adhesion and migration.[33,34] Hence, BAG3-mediated mechanotransduction may not only be essential for muscle maintenance but may also explain the eminent role of the cochaperone in tumor formation.

Several studies revealed BAG3 as a critical regulator of viral replication and growth. Affected viruses include varicella-zoster virus (VZV),[68] polyomavirus JC,[69] Epstein-Barr virus (EBV),[70] herpes simplex virus (HSV)[71] and HIV.[72] In many cases, depletion of BAG3 by small interfering RNA inhibits virus replication. It is important to note in this regard that many viruses exploit autophagy pathways to facilitate their entry and replication.[73,74] Therefore, the role of BAG3 during virus infection might also be related to the function of the cochaperone as an autophagy inducer.

Outlook

The identification and functional characterization of the CASA machinery revealed the importance of chaperone-assisted proteostasis in mechanically strained cells and tissues. Still, many important questions remain to be answered. We do not know yet the full repertoire of client proteins that are degraded by CASA. Mechanisms that underlie the physiological regulation of CASA components and the formation of autophagosomes during CASA only begin to emerge. The future analysis of the CASA machinery will certainly continue to provide important insights into very diverse areas of biology and biomedicine, including virology, immunology, mechanobiology and cancer research.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Eyckmans J, Boudou T, Yu X, Chen CS. A hitchhiker's guide to mechanobiology. Dev Cell 2011; 21:35-47; PMID:21763607; http://dx.doi.org/10.1016/j.devcel.2011.06.015
2. Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science 2005; 310:1139-43; PMID:16293750; http://dx.doi.org/10.1126/science.1116995
3. Alon R, Dustin ML. Force as a facilitator of integrin conformational changes during leukocyte arrest on blood vessels and antigen-presenting cells. Immunity 2007; 26:17-27; PMID:17241958; http://dx.doi.org/10.1016/j.immuni.2007.01.002
4. Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, et al. Role of YAP/TAZ in mechanotransduction. Nature 2011; 474:179-83; PMID:21654799; http://dx.doi.org/10.1038/nature10137
5. Yim EK, Sheetz MP. Force-dependent cell signaling in stem cell differentiation. Stem Cell Res Ther 2012; 3:41; PMID:23114057; http://dx.doi.org/10.1186/scrt132
6. Jaalouk DE, Lammerding J. Mechanotransduction gone awry. Nat Rev Mol Cell Biol 2009; 10:63-73; PMID:19197333; http://dx.doi.org/10.1038/nrm2597

7. Rognoni L, Stigler J, Pelz B, Ylänne J, Rief M. Dynamic force sensing of filamin revealed in single-molecule experiments. Proc Natl Acad Sci USA 2012; 109:19679-84; PMID:23150587; http://dx.doi.org/10.1073/pnas.1211274109
8. Carisey A, Tsang R, Greiner AM, Nijenhuis N, Heath N, Nazgiewicz A, et al. Vinculin regulates the recruitment and release of core focal adhesion proteins in a force-dependent manner. Curr Biol 2013; 23:271-81; PMID:23375895; http://dx.doi.org/10.1016/j.cub.2013.01.009
9. Galkin VE, Orlova A, Egelman EH. Actin filaments as tension sensors. Curr Biol 2012; 22:R96-101; PMID:22321312; http://dx.doi.org/10.1016/j.cub.2011.12.010
10. Ulbricht A, Eppler FJ, Tapia VE, van der Ven PF, Hampe N, Hersch N, et al. Cellular mechanotransduction relies on tension-induced and chaperone-assisted autophagy. Curr Biol 2013; 23:430-5; PMID:23434281; http://dx.doi.org/10.1016/j.cub.2013.01.064
11. Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. Nature 2011; 475:324-32; PMID:21776078; http://dx.doi.org/10.1038/nature10317
12. Kettern N, Dreiseidler M, Tawo R, Höhfeld J. Chaperone-assisted degradation: multiple paths to destruction. Biol Chem 2010; 391:481-9; PMID:20302520; http://dx.doi.org/10.1515/bc.2010.058
13. Buchberger A, Bukau B, Sommer T. Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. Mol Cell 2010; 40:238-52; PMID:20965419; http://dx.doi.org/10.1016/j.molcel.2010.10.001
14. Arias E, Cuervo AM. Chaperone-mediated autophagy in protein quality control. Curr Opin Cell Biol 2011; 23:184-9; PMID:21094035; http://dx.doi.org/10.1016/j.ceb.2010.10.009
15. Arndt V, Rogon C, Höhfeld J. To be, or not to be-molecular chaperones in protein degradation. Cell Mol Life Sci 2007; 64:2525-41; PMID:17565442; http://dx.doi.org/10.1007/s00018-007-7188-6
16. Arndt V, Dick N, Tawo R, Dreiseidler M, Wenzel D, Hesse M, et al. Chaperone-assisted selective autophagy is essential for muscle maintenance. Curr Biol 2010; 20:143-8; PMID:20060297; http://dx.doi.org/10.1016/j.cub.2009.11.022
17. Haslbeck M, Franzmann T, Weinfurtner D, Buchner J. Some like it hot: the structure and function of small heat-shock proteins. Nat Struct Mol Biol 2005; 12:842-6; PMID:16205709; http://dx.doi.org/10.1038/nsmb993
18. Sarparanta J, Jonson PH, Golzio C, Sandell S, Luque H, Screen M, et al. Mutations affecting the cytoplasmic functions of the co-chaperone DNAJB6 cause limb-girdle muscular dystrophy. Nat Genet 2012; 44(S1-2):450-5; PMID:22366786; http://dx.doi.org/10.1038/ng.1103
19. Dai Q, Zhang C, Wu Y, McDonough H, Whaley RA, Godfrey V, et al. CHIP activates HSF1 and confers protection against apoptosis and cellular stress. EMBO J 2003; 22:5446-58; PMID:14532117; http://dx.doi.org/10.1093/emboj/cdg529
20. Morishima Y, Wang AM, Yu Z, Pratt WB, Osawa Y, Lieberman AP. CHIP deletion reveals functional redundancy of E3 ligases in promoting degradation of both signaling proteins and expanded glutamine proteins. Hum Mol Genet 2008; 17:3942-52; PMID:18784277; http://dx.doi.org/10.1093/hmg/ddn296
21. Gamerdinger M, Kaya AM, Wolfrum U, Clement AM, Behl C. BAG3 mediates chaperone-based aggresome-targeting and selective autophagy of misfolded proteins. EMBO Rep 2011; 12:149-56; PMID:21252941; http://dx.doi.org/10.1038/embor.2010.203
22. Gamerdinger M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU, Behl C. Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. EMBO J 2009; 28:889-901; PMID:19229298; http://dx.doi.org/10.1038/emboj.2009.29
23. Shaid S, Brandts CH, Serve H, Dikic I. Ubiquitination and selective autophagy. Cell Death Differ 2013; 20:21-30; PMID:22722335; http://dx.doi.org/10.1038/cdd.2012.72
24. Linnemann A, van der Ven PF, Vakeel P, Albinus B, Simonis D, Bendas G, et al. The sarcomeric Z-disc component myopodin is a multiadapter protein that interacts with filamin and alpha-actinin. Eur J Cell Biol 2010; 89:681-92; PMID:20554076; http://dx.doi.org/10.1016/j.ejcb.2010.04.004
25. Weins A, Schwarz K, Faul C, Barisoni L, Linke WA, Mundel P. Differentiation- and stress-dependent nuclear cytoplasmic redistribution of myopodin, a novel actin-bundling protein. J Cell Biol 2001; 155:393-404; PMID:11673475; http://dx.doi.org/10.1083/jcb.200012039
26. Yu YP, Luo JH. Phosphorylation and interaction of myopodin by integrin-link kinase lead to suppression of cell growth and motility in prostate cancer cells. Oncogene 2011; 30:4855-63; PMID:21643011; http://dx.doi.org/10.1038/onc.2011.200
27. Jing L, Liu L, Yu YP, Dhir R, Acquafondada M, Landsittel D, et al. Expression of myopodin induces suppression of tumor growth and metastasis. Am J Pathol 2004; 164:1799-806; PMID:15111326; http://dx.doi.org/10.1016/S0002-9440(10)63738-8
28. Sanchez-Carbayo M, Schwarz K, Charytonowicz E, Cordon-Cardo C, Mundel P. Tumor suppressor role for myopodin in bladder cancer: loss of nuclear expression of myopodin is cell-cycle dependent and predicts clinical outcome. Oncogene 2003; 22:5298-305; PMID:12917631; http://dx.doi.org/10.1038/sj.onc.1206616
29. Wickner W. Membrane fusion: five lipids, four SNAREs, three chaperones, two nucleotides, and a Rab, all dancing in a ring on yeast vacuoles. Annu Rev Cell Dev Biol 2010; 26:115-36; PMID:20521906; http://dx.doi.org/10.1146/annurev-cellbio-100109-104131
30. Epp N, Rethmeier R, Krämer L, Ungermann C. Membrane dynamics and fusion at late endosomes and vacuoles--Rab regulation, multisubunit tethering complexes and SNAREs. Eur J Cell Biol 2011; 90:779-85; PMID:21683469; http://dx.doi.org/10.1016/j.ejcb.2011.04.007
31. Bröcker C, Kuhlee A, Gatsogiannis C, Balderhaar HJ, Hönscher C, Engelbrecht-Vandré S, et al. Molecular architecture of the multisubunit homotypic fusion and vacuole protein sorting (HOPS) tethering complex. Proc Natl Acad Sci USA 2012; 109:1991-6; PMID:22308417; http://dx.doi.org/10.1073/pnas.1117797109
32. Moreau K, Ravikumar B, Renna M, Puri C, Rubinsztein DC. Autophagosome precursor maturation requires homotypic fusion. Cell 2011; 146:303-17; PMID:21784250; http://dx.doi.org/10.1016/j.cell.2011.06.023
33. Iwasaki M, Homma S, Hishiya A, Dolezal SJ, Reed JC, Takayama S. BAG3 regulates motility and adhesion of epithelial cancer cells. Cancer Res 2007; 67:10252-9; PMID:17974966; http://dx.doi.org/10.1158/0008-5472.CAN-07-0618
34. Iwasaki M, Tanaka R, Hishiya A, Homma S, Reed JC, Takayama S. BAG3 directly associates with guanine nucleotide exchange factor of Rap1, PDZGEF2, and regulates cell adhesion. Biochem Biophys Res Commun 2010; 400:413-8; PMID:20800573; http://dx.doi.org/10.1016/j.bbrc.2010.08.092
35. Homma S, Iwasaki M, Shelton GD, Engvall E, Reed JC, Takayama S. BAG3 deficiency results in fulminant myopathy and early lethality. Am J Pathol 2006; 169:761-73; PMID:16936253; http://dx.doi.org/10.2353/ajpath.2006.060250
36. Selcen D, Muntoni F, Burton BK, Pegoraro E, Sewry C, Bite AV, et al. Mutation in BAG3 causes severe dominant childhood muscular dystrophy. Ann Neurol 2009; 65:83-9; PMID:19085932; http://dx.doi.org/10.1002/ana.21553
37. Norton N, Li D, Rieder MJ, Siegfried JD, Rampersaud E, Züchner S, et al. Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy. Am J Hum Genet 2011; 88:273-82; PMID:21353195; http://dx.doi.org/10.1016/j.ajhg.2011.01.016
38. Arimura T, Ishikawa T, Nunoda S, Kawai S, Kimura A. Dilated cardiomyopathy-associated BAG3 mutations impair Z-disc assembly and enhance sensitivity to apoptosis in cardiomyocytes. Hum Mutat 2011; 32:1481-91; PMID:21898660; http://dx.doi.org/10.1002/humu.21603
39. Lee HC, Cherk SW, Chan SK, Wong S, Tong TW, Ho WS, et al. BAG3-related myofibrillar myopathy in a Chinese family. Clin Genet 2012; 81:394-8; PMID:21361913; http://dx.doi.org/10.1111/j.1399-0004.2011.01659.x
40. Nakamura F, Stossel TP, Hartwig JH. The filamins: organizers of cell structure and function. Cell Adh Migr 2011; 5:160-9; PMID:21169733; http://dx.doi.org/10.4161/cam.5.2.14401
41. Ehrlicher AJ, Nakamura F, Hartwig JH, Weitz DA, Stossel TP. Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. Nature 2011; 478:260-3; PMID:21926999; http://dx.doi.org/10.1038/nature10430
42. Lad Y, Kiema T, Jiang P, Pentikäinen OT, Coles CH, Campbell ID, et al. Structure of three tandem filamin domains reveals auto-inhibition of ligand binding. EMBO J 2007; 26:3993-4004; PMID:17690686; http://dx.doi.org/10.1038/sj.emboj.7601827
43. Chen H, Zhu X, Cong P, Sheetz MP, Nakamura F, Yan J. Differential mechanical stability of filamin A rod segments. Biophys J 2011; 101:1231-7; PMID:21889461; http://dx.doi.org/10.1016/j.bpj.2011.07.028
44. Zhou AX, Hartwig JH, Akyürek LM. Filamins in cell signaling, transcription and organ development. Trends Cell Biol 2010; 20:113-23; PMID:20061151; http://dx.doi.org/10.1016/j.tcb.2009.12.001
45. Kley RA, Serdaroglu-Oflazer P, Leber Y, Odgerel Z, van der Ven PF, Olivé M, et al. Pathophysiology of protein aggregation and extended phenotyping in filaminopathy. Brain 2012; 135:2642-60; PMID:22961544; http://dx.doi.org/10.1093/brain/aws200
46. Ferrer I, Olivé M. Molecular pathology of myofibrillar myopathies. Expert Rev Mol Med 2008; 10:e25; PMID:18764962; http://dx.doi.org/10.1017/S1462399408000793
47. Sudol M, Harvey KF. Modularity in the Hippo signaling pathway. Trends Biochem Sci 2010; 35:627-33; PMID:20598891; http://dx.doi.org/10.1016/j.tibs.2010.05.010
48. Salah Z, Aqeilan RI. WW domain interactions regulate the Hippo tumor suppressor pathway. Cell Death Dis 2011; 2:e172; PMID:21677687; http://dx.doi.org/10.1038/cddis.2011.53
49. Franceschelli S, Rosati A, Lerose R, De Nicola S, Turco MC, Pascale M. Bag3 gene expression is regulated by heat shock factor 1. J Cell Physiol 2008; 215:575-7; PMID:18286539; http://dx.doi.org/10.1002/jcp.21397
50. Song S, Kole S, Precht P, Pazin MJ, Bernier M. Activation of heat shock factor 1 plays a role in pyrrolidine dithiocarbamate-mediated expression of the co-chaperone BAG3. Int J Biochem Cell Biol 2010; 42:1856-63; PMID:20692357; http://dx.doi.org/10.1016/j.biocel.2010.07.021
51. Pagliuca MG, Lerose R, Cigliano S, Leone A. Regulation by heavy metals and temperature of the human BAG-3 gene, a modulator of Hsp70 activity. FEBS Lett 2003; 541:11-5; PMID:12706811; http://dx.doi.org/10.1016/S0014-5793(03)00274-6
52. Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. Cell 2011; 147:728-41; PMID:22078875; http://dx.doi.org/10.1016/j.cell.2011.10.026

53. King JS. Mechanical stress meets autophagy: potential implications for physiology and pathology. Trends Mol Med 2012; 18:583-8; PMID:22981844; http://dx.doi.org/10.1016/j.molmed.2012.08.002
54. Tanaka Y, Guhde G, Suter A, Eskelinen EL, Hartmann D, Lüllmann-Rauch R, et al. Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. Nature 2000; 406:902-6; PMID:10972293; http://dx.doi.org/10.1038/35022595
55. Nishino I, Fu J, Tanji K, Yamada T, Shimojo S, Koori T, et al. Primary LAMP-2 deficiency causes X-linked vacuolar cardiomyopathy and myopathy (Danon disease). Nature 2000; 406:906-10; PMID:10972294; http://dx.doi.org/10.1038/35022604
56. Hartleben B, Gödel M, Meyer-Schwesinger C, Liu S, Ulrich T, Köbler S, et al. Autophagy influences glomerular disease susceptibility and maintains podocyte homeostasis in aging mice. J Clin Invest 2010; 120:1084-96; PMID:20200449; http://dx.doi.org/10.1172/JCI39492
57. He C, Bassik MC, Moresi V, Sun K, Wei Y, Zou Z, et al. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. Nature 2012; 481:511-5; PMID:22258505; http://dx.doi.org/10.1038/nature10758
58. Carra S, Seguin SJ, Lambert H, Landry J. HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag3, a stimulator of macroautophagy. J Biol Chem 2008; 283:1437-44; PMID:18006506; http://dx.doi.org/10.1074/jbc.M706304200
59. Lee JH, Takahashi T, Yasuhara N, Inazawa J, Kamada S, Tsujimoto Y. Bis, a Bcl-2-binding protein that synergizes with Bcl-2 in preventing cell death. Oncogene 1999; 18:6183-90; PMID:10597216; http://dx.doi.org/10.1038/sj.onc.1203043
60. Takayama S, Xie Z, Reed JC. An evolutionarily conserved family of Hsp70/Hsc70 molecular chaperone regulators. J Biol Chem 1999; 274:781-6; PMID:9873016; http://dx.doi.org/10.1074/jbc.274.2.781
61. Rosati A, Graziano V, De Laurenzi V, Pascale M, Turco MC. BAG3: a multifaceted protein that regulates major cell pathways. Cell Death Dis 2011; 2:e141; PMID:21472004; http://dx.doi.org/10.1038/cddis.2011.24
62. Staibano S, Mascolo M, Di Benedetto M, Vecchione ML, Ilardi G, Di Lorenzo G, et al. BAG3 protein delocalisation in prostate carcinoma. Tumour Biol 2010; 31:461-9; PMID:20535599; http://dx.doi.org/10.1007/s13277-010-0055-3
63. Romano MF, Festa M, Petrella A, Rosati A, Pascale M, Bisogni R, et al. BAG3 protein regulates cell survival in childhood acute lymphoblastic leukemia cells. Cancer Biol Ther 2003; 2:508-10; PMID:14614315; http://dx.doi.org/10.4161/cbt.2.5.524
64. Kassis JN, Guancial EA, Doong H, Virador V, Kohn EC. CAIR-1/BAG-3 modulates cell adhesion and migration by downregulating activity of focal adhesion proteins. Exp Cell Res 2006; 312:2962-71; PMID:16859681; http://dx.doi.org/10.1016/j.yexcr.2006.05.023
65. Zhou F, Yang Y, Xing D. Bcl-2 and Bcl-xL play important roles in the crosstalk between autophagy and apoptosis. FEBS J 2011; 278:403-13; PMID:21182587; http://dx.doi.org/10.1111/j.1742-4658.2010.07965.x
66. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 2005; 122:927-39; PMID:16179260; http://dx.doi.org/10.1016/j.cell.2005.07.002
67. Huang S, Ingber DE. Cell tension, matrix mechanics, and cancer development. Cancer Cell 2005; 8:175-6; PMID:16169461; http://dx.doi.org/10.1016/j.ccr.2005.08.009
68. Kyratsous CA, Silverstein SJ. BAG3, a host cochaperone, facilitates varicella-zoster virus replication. J Virol 2007; 81:7491-503; PMID:17475647; http://dx.doi.org/10.1128/JVI.00442-07
69. Basile A, Darbinian N, Kaminski R, White MK, Gentilella A, Turco MC, et al. Evidence for modulation of BAG3 by polyomavirus JC early protein. J Gen Virol 2009; 90:1629-40; PMID:19282432; http://dx.doi.org/10.1099/vir.0.008722-0
70. Young P, Anderton E, Paschos K, White R, Allday MJ. Epstein-Barr virus nuclear antigen (EBNA) 3A induces the expression of and interacts with a subset of chaperones and co-chaperones. J Gen Virol 2008; 89:866-77; PMID:18343826; http://dx.doi.org/10.1099/vir.0.83414-0
71. Kyratsous CA, Silverstein SJ. The co-chaperone BAG3 regulates Herpes Simplex Virus replication. Proc Natl Acad Sci USA 2008; 105:20912-7; PMID:19088197; http://dx.doi.org/10.1073/pnas.0810656105
72. Rosati A, Khalili K, Deshmane SL, Radhakrishnan S, Pascale M, Turco MC, et al. BAG3 protein regulates caspase-3 activation in HIV-1-infected human primary microglial cells. J Cell Physiol 2009; 218:264-7; PMID:18821563; http://dx.doi.org/10.1002/jcp.21604
73. Kirkegaard K. Subversion of the cellular autophagy pathway by viruses. Curr Top Microbiol Immunol 2009; 335:323-33; PMID:19802573; http://dx.doi.org/10.1007/978-3-642-00302-8_16
74. Kudchodkar SB, Levine B. Viruses and autophagy. Rev Med Virol 2009; 19:359-78; PMID:19750559; http://dx.doi.org/10.1002/rmv.630