Autophagy 7:7, 1-4; July 2011; © 2011 Landes Bioscience

This manuscript has been published online, prior to printing. Once the issue is complete and page numbers have been assigned, the citation will change accordingly.

BAG3 and friends

Co-chaperones in selective autophagy during aging and disease

Christian Behl

Institute for Pathobiochemistry; University Medical Center; Johannes Gutenberg University; Mainz, Germany

©2011 Landes Bioscience. Do not distribute

There is a reciprocal change in the expression of two members of the BAG (Bcl-2-associated athanogen) family, BAG1 and BAG3, during cellular aging and under acute stress ("BAG1-BAG3-switch"). BAG3 was recently described as a mediator of a novel macroautophagy pathway that uses the specificity of heat shock protein 70 (HSP70) to misfolded proteins and also involves other protein partners, such as HSPB8. Also crucial for induction and execution of autophagy are sequestosome-1/p62 (SQSTM1/p62) and LC3, an autophagosome-associated protein. In this novel pathway, BAG3 mediates the targeting and transport of degradation-prone substrates into aggresomes via the microtubule-motor dynein. Interestingly, aggresome-targeting by BAG3 does not depend on substrate ubiquitination and is, therefore, involved in the clearance of misfolded proteins that are not ubiquitinated.

Introduction

Protein homeostasis is crucial for function and survival of cells. Through their lifetime, cells face constantly changing and sometimes unfriendly conditions that challenge the two basic protein degradation systems, the proteasome and autophagy (hereafter referred to as macroautophagy). In the last decade, the latter especially has received an overwhelming attention. Macroautophagy, involving the sequestration of organelles and macromolecules into autophagosomes, is an evolutionarily conserved process of vital importance. Cellular protein and organelle homeostasis depend on autophagic clearance. A failure of this basic quality control system leads to disease.[1,2] Based on mounting evidence excellently reviewed elsewhere,[3] it is now common sense that the maintenance of cellular proteostasis through macroautophagy is not just a crude, nonselective bulk degradation process, but rather displays a sophisticated portfolio of selectivity provided by different molecular strategies. A great advance in the autophagy field was (1) the discovery of the autophagy adaptors SQSTM1/p62 and NBR1 (neighbor of Brc1), and their role as cargo receptors and functional interfaces for the degradation of ubiquitinated protein substrates and (2) the description of the direct interaction between SQSTM1/p62 and NBR1 with LC3, a protein of the autophagosome.[3] A distinct type of macroautophagy that also involves chaperone proteins is the so-called chaperone-mediated autophagy (CMA).[4,5] CMA is different from macroautophagy because in this process (1) no autophagosome structures are formed and (2) targets are soluble cytosolic proteins carrying the pentapeptide KFERQ as a protein sequence motif. CMA represents, therefore, a highly specific and selective degradation mechanism and, interestingly, the KFERQ motif is present in approximately 30% of cytosolic proteins. The chaperone proteins HSC70, HSP90 and the co-chaperones BAG1, HIP, HOP and HSP40/DNAJB1 [6,7] are crucial cooperation partners in CMA. In a concerted action of this protein complex, the CMA substrates are directly transported via the lysosomal receptor LAMP-2A into

Key words: aging, BAG3, HSPB8, p62, LC3, aggresome

Submitted: 04/14/2011

Accepted: 04/15/2011

DOI:

Correspondence to: Christian Behl;
Email: cbehl@uni-mainz.de

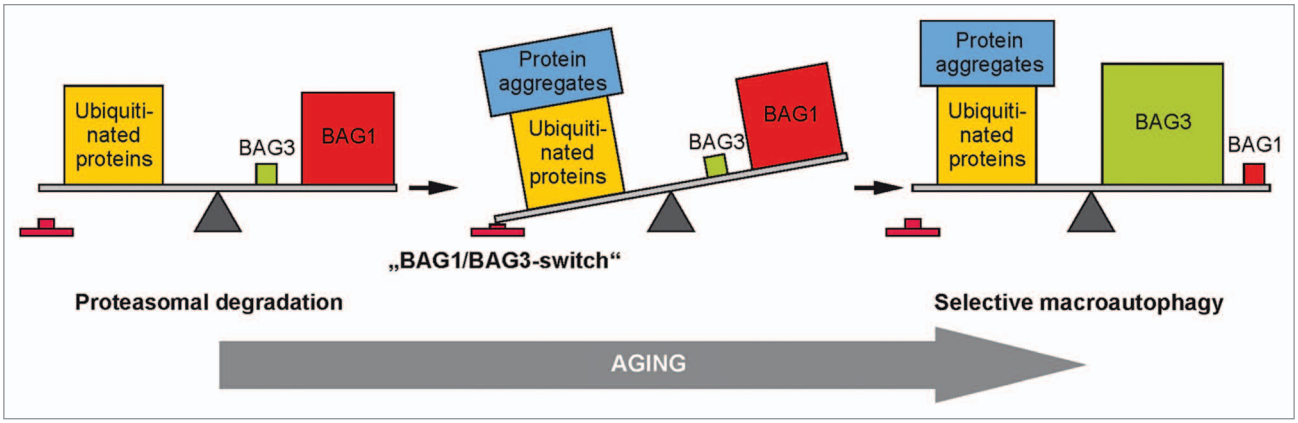

Figure 1. The shift from BAG1 to BAG3 to maintain intracellular proteostasis under enhanced protein stress. To maintain the cellular proteostasis, the regular turnover of degradation-prone proteins is usually carried out by the proteasomal pathway. In young cells, the co-chaperone BAG1 interacts with the HSP70 system and is essential for the transfer of polyubiquitinated proteins to the proteasome. During cellular aging there is an increasing demand for protein degradation due to the accumulation of misfolded proteins and protein aggregates that cannot enter the proteasome system. This age-associated protein stress that also occurs under acute oxidative stress or upon pharmacological proteasome inhibition, leads to an enhanced expression of the co-chaperone BAG3. The increased BAG3/BAG1 ratio induces the recruitment of the macroautophagy pathway for PQC mainly mediated by a multichaperone complex consisting of BAG3, HSPB8 and HSP70. The BAG1/BAG3-switch may function as a physiological adaptation to the changing protein degradation demand during aging and also in protein aggregation disorders.[8,9,11-13]

the lysosomal lumen where degradation occurs.[4,5]

The BAG1-BAG3-Switch: Shifting from Proteasome to Autophagy

In the search for basic biochemical differences of young and old cells, the expression of two members of the BAG family, BAG1 and BAG3, was found to be reciprocally regulated during cellular aging and under acute oxidative or proteasomal stress ("BAG1/BAG3-switch").[8] Under physiological conditions the protein quality control (PQC) mediated by the activity of HSP70 towards misfolded proteins is mainly achieved by BAG1-dependent proteasomal degradation of polyubiquitinated (polyUb)-proteins. Under pathophysiological conditions (aging, acute stress) with an accumulation of misfolded and aggregated proteins and a concomitant decrease in proteasomal degradation efficacy, BAG3-mediated selective macroautophagy is turned on, representing an on-demand autophagy pathway (**Fig. 1**).

BAG3-Mediated Selective Autophagy: A Concerted Action of Chaperones and Co-Chaperones as an Adaptive Physiological Response

Driven by an increased BAG3 expression, protein homeostasis in aged (or challenged) cells is stabilized by the recruitment of the macroautophagy pathway involving polyUb-substrates, HSP70, HSPB8, SQSTM1/p62 and LC3.[8,9] A multichaperone protein complex consisting of BAG3-HSPB8-HSP70 was found as controlling the selective degradation of protein substrates such as polyUb-proteins and disease proteins including polyQ-huntingtin and superoxide dismutase-1 (SOD1) mutants linked to amyotrophic lateral sclerosis (ALS).[8-13] More recently, it was found that degradation-prone proteins that are not ubiquitinated are also clients of this BAG3-mediated autophagic clearance pathway.[13]

Based on its modular domain structure, BAG3 is a highly promiscuous molecule. It has two WW domains at the N terminus, a proline-rich region (PxxP) in the center and the BAG domain at the C terminus. The complete domain structure has been recently summarized.[14] Interaction of BAG3 with the small heat-shock protein HSPB8 occurs through Ile-Pro-Val motifs.[9,15] Therefore, BAG3 can interact with many partners and, in addition to selective macroautophagy, is involved in several key cellular processes, including apoptosis, cell proliferation and adhesion.[14] An essential prerequisite of BAG3-HSBP8-HSP70-mediated macroautophagy is the specificity of HSP70 chaperones for misfolded proteins. The HSP70 chaperone system is very complex and involved in protein folding, protein degradation and protein function.[16-18] It comprises HSP70 as the key chaperone and many co-chaperones and co-regulators.[19,20] Binding of substrates to, and substrate release from, HSP70 is controlled by an ATP-consuming cycle and the folding as well as the degradation activity of HSP70 are regulated by co-chaperones that directly affect the turnover of ATP at HSP70.[16]

BAG3 in Aggresome Targeting also Works Independently of Protein Substrate Ubiquitination and Interacts with Dynein

BAG3 is not only a powerful trigger of macroautophagy, but rather is also involved in the active transport of protein substrates inside the cell. Selective autophagy of damaged, misfolded and aggregated proteins strongly requires the concentration and isolation of degradation substrates away from other cytosolic components. The retrograde transport of substrates along microtubules via the cytoplasmic dynein motor complex constitutes such a sequestration of degradation substrates in a special perinuclear compartment, called the aggresome.[21] A link of aggresome formation and the

©2011 Landes Bioscience. Do not distribute.

macroautophagy pathway is well established.[22-24] Interestingly, we were now able to show that BAG3 directly associates with the microtubule motor dynein and mediates the selective transport of misfolded proteins to the aggresome.[13] Binding of BAG3 to dynein is mediated by its PxxP domain.[13] Based on these findings, we propose the following scenario: BAG3 acts as a nucleotide-exchange factor and directly stimulates substrate transfer from HSP70 to the dynein motor complex. Therefore, BAG3 directly promotes transport of misfolded proteins to the aggresome. When the native state of an aggregation-prone protein, such as polyQ-huntingtin or SOD1, cannot be reached via the protein quality control activity of the HSP70 system, the BAG3-HSP8-HSP70 complex promotes targeting of these disease proteins to the aggresome and their subsequent degradation.[8,9,12,13] Interestingly, deletion of the PxxP domain inhibits the ability to clear polyQ-expanded huntingtin,[9] which further supports the BAG3 specificity of this process. Enhanced protein aggregation is a pathological hallmark of aging and various neurodegenerative diseases, such as Huntington disease, Parkinson disease and ALS. The disturbance of dynein-mediated transport and, therefore, aggresome formation, results in inefficient degradation of aggregation-prone proteins by macroautophagy and is associated with the progression of various disorders, including ALS.[25]

Usually, specific aggresome targeting is achieved by the ubiquitination of degradation-prone proteins, which are then recognized by ubiquitin adaptor proteins that bind to dynein, such as the deacetylase HDAC6.[26,27] But, because many misfolded proteins in aggresomes are not ubiquitinated,[22] selective loading of cargo onto dynein should also occur independently of ubiquitin signaling. Here, BAG3 might play a crucial role.[13]

BAG3 in Macroautophagy: is there a Need for a New Name and What's Next?

The identified novel pathway of aggresome targeting and selective macroautophagy mediated by BAG3 in concert with other co-chaperones is an additional example of the involvement of chaperone molecules in autophagy. In recent years, multiple chaperone-associated and chaperone-assisted degradation pathways have been described, including CAP (chaperone-assisted proteasomal degradation), CASA (chaperone-assisted selective autophagy) and CMA.[28] As mentioned before, CMA depends on specific pentapeptide motifs in the substrates and thus is highly selective and specific. Consequently, CMA also belongs to the pathways that can be subsumed as chaperone-mediated selective autophagy. The term CASA was originally coined for the chaperone-assisted selective autophagy mediated by the Drosophila BAG3 ortholog Starvin.[10] More specifically, as pointed out here, we and others have now shown the involvement of BAG3 and the BAG3-HSPB8-HSP70-chaperone complex in selective macroautophagy in mammalian cells,[8,9,11] satisfying the introduction of a new name for this process. For instance: BAG3- or BAG3-HSPB8-HSP70-specific selective autophagy. Does this make sense? One can easily imagine that there might be additional interesting chaperones and proteins that may enter the stage as mediators and regulators of selective macroautophagy pathways. But to my understanding much more important than the terminology are the following issues: (1) Molecular and functional integration of key players of various autophagy pathways at the proteome level and extension of recent studies[29] for a better understanding of the overall fine-tuning of cellular autophagy. (2) A better and detailed knowledge of the role of various autophagy pathways during aging and disease. (3) An analysis of tissue-specific regulation of the various macroautophagy mechanisms, since it is clear that proteostasis regulation depends on aging and is different in different tissues.[30] (4) The quest for possibilities to specifically and selectively induce a single autophagy pathway when thinking of preventive and therapeutic approaches for disorders, where a disturbed protein clearance by autophagy might be fundamental.

Acknowledgements

I thank Drs. Andreas Kern, Tobias Morawe and Christine Ziegler for constructive input on the manuscript. Work in my lab is supported by grants from the Deutsche Forschungsgemeinschaft (DFG-FOR926, DFG-FOR1332) to C. Behl.

References

1. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell 2008; 132:27-42.
2. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature 2008; 451:1069-75.
3. Johansen T, Lamark T. Selective autophagy mediated by autophagic adapter proteins. Autophagy 2011; 7:279-96.
4. Dice JF. Chaperone-mediated autophagy. Autophagy 2007; 3:295-9.
5. Wong E, Cuervo AM. Integration of clearance mechanisms: the proteasome and autophagy. Cold Spring Harb Perspect Biol 2010; 2:6734.
6. Agarraberes FA, Dice JF. A molecular chaperone complex at the lysosomal membrane is required for protein translocation. J Cell Sci 2001; 114:2491-9.
7. Arias E, Cuervo AM. Chaperone-mediated autophagy in protein quality control. Curr Opin Cell Biol 2010; [Epub ahead of print].
8. 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.
9. 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.
10. 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.
11. Carra S, Seguin SJ, Landry J. HspB8 and Bag3: a new chaperone complex targeting misfolded proteins to macroautophagy. Autophagy 2008; 4:237-9.
12. Crippa V, Sau D, Rusmini P, Boncoraglio A, Onesto E, Bolzoni E, et al. The small heat shock protein B8 (HspB8) promotes autophagic removal of misfolded proteins involved in amyotrophic lateral sclerosis (ALS). Hum Mol Genet 2010; 19:3440-56.
13. 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.
14. McCollum AK, Casagrande G, Kohn EC. Caught in the middle: the role of Bag3 in disease. Biochem J 2010; 425:1-3.
15. Fuchs M, Poirier DJ, Seguin SJ, Lambert H, Carra S, Charette SJ, et al. Identification of the key structural motifs involved in HspB8/HspB6-Bag3 interaction. Biochem J 2010; 425:245-55.
16. Hartl FU, Hayer-Hartl M. Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 2002; 295:1852-8.
17. Lanneau D, Wettstein G, Bonniaud P, Garrido C. Heat shock proteins: cell protection through protein triage. ScientificWorldJournal 2010; 10:1543-52.
18. Young JC. Mechanisms of the Hsp70 chaperone system. Biochem Cell Biol 2010; 88:291-300.
19. Chang HC, Tang YC, Hayer-Hartl M, Hartl FU. SnapShot: molecular chaperones, Part I. Cell 2007; 128:212.
20. Tang YC, Chang HC, Hayer-Hartl M, Hartl FU. SnapShot: molecular chaperones, Part II. Cell 2007; 128:412.
21. Kopito RR. Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 2000; 10:524-30.
22. Garcia-Mata R, Bebok Z, Sorscher EJ, Sztul ES. Characterization and dynamics of aggresome formation by a cytosolic GFP-chimera. J Cell Biol 1999; 146:1239-54.

©2011 Landes Bioscience.
Do not distribute

23. Johnston JA, Ward CL, Kopito RR. Aggresomes: a cellular response to misfolded proteins. J Cell Biol 1998; 143:1883-98.
24. Webb JL, Ravikumar B, Rubinsztein DC. Microtubule disruption inhibits autophagosome-lysosome fusion: implications for studying the roles of aggresomes in polyglutamine diseases. Int J Biochem Cell Biol 2004; 36:2541-50.
25. Ravikumar B, Acevedo-Arozena A, Imarisio S, Berger Z, Vacher C, O'Kane CJ, et al. Dynein mutations impair autophagic clearance of aggregate-prone proteins. Nat Genet 2005; 37:771-6.
26. Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, Yao TP. The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. Cell 2003; 115:727-38.
27. Li G, Jiang H, Chang M, Xie H, Hu L. HDAC6 alpha-tubulin deacetylase: A potential therapeutic target in neurodegenerative diseases. J Neurol Sci 2011; 304:1-8.
28. Kettern N, Dreiseidler M, Tawo R, Hohfeld J. Chaperone-assisted degradation: multiple paths to destruction. Biol Chem 2010; 391:481-9.

©2011 Landes Bioscience.
Do not distribute.