Proteinopathies are characterized by the accumulation of misfolded proteins, which ultimately interfere with normal cell function. While neurological diseases, such as Huntington disease and Alzheimer disease, are well-characterized proteinopathies, cardiac diseases have recently been associated with alterations in proteostasis. In this issue of the JCI, Fang and colleagues demonstrate that mice with cardiac-specific deficiency of the co-chaperone protein BCL2-associated athanogene 3 (BAG3) develop dilated cardiomyopathy that is associated with a destabilization of small HSPs as the result of a disrupted interaction between BAG3 and HSP70. Together, the results of this study suggest that strategies to upregulate BAG3 during cardiac dysfunction may be beneficial.
BAG3 plays a central role in proteostasis in the heart

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Protein aggregates and disease

Protein homeostasis (proteostasis) is essential for maintaining normal cell function (1, 2). Proper protein activity is achieved through rigorous coordination of synthesis, folding, and posttranslational modification, all of which are prone to malfunction in response to stress. For example, protein folding in the endoplasmic reticulum (ER) is easily disrupted in the presence of ER stress and oxidative stress. Disruption of proteostasis induces the accumulation of misfolded proteins, which then interfere with the normal function of cells through improper degradation, gain and/or loss of function, and aggregate formation. The adverse effects caused by altered proteostasis are collectively termed proteotoxicity, and pathological conditions resulting from proteotoxicity are collectively termed proteinopathies (3, 4). Proteostasis is particularly important for postmitotic cells, such as neurons and adult cardiomyocytes, that have negligible regenerative potential, because in these mature cells, proteotoxicity ultimately interferes with normal cell function. While neurological diseases, such as Huntington disease and Alzheimer disease, are well-characterized proteinopathies, cardiac diseases have recently been associated with alterations in proteostasis. In this issue of the JCI, Fang and colleagues demonstrate that mice with cardiac-specific deficiency of the co-chaperone protein BCL2-associated athanogene 3 (BAG3) develop dilated cardiomyopathy that is associated with a destabilization of small HSPs as the result of a disrupted interaction between BAG3 and HSP70. Together, the results of this study suggest that strategies to upregulate BAG3 during cardiac dysfunction may be beneficial.

These mechanisms include aggresome formation through retrograde transport of misfolded proteins, which depends on the interaction of BAG3 with dynein motors and the microtubule organization center (9), and stimulation of autophagy, which depends on BAG3 interaction with HSPB8. Both retrograde transport and autophagy facilitate the sequestration and removal of irreversibly misfolded proteins. BAG3 is also involved in chaperone-assisted selective autophagy (CASA). CASA is a cargo-selective form of autophagy that is mediated through the HSC70-BAG3-HSPB8 complex and allows ubiquitination of selected proteins recognized by HSC70 via the CHIP ubiquitin ligase and sequestration by autophagosomes through the LC3 adapter p62/SQSTM in the presence of synaptopodin 2 or DNAJB6 (10). In muscles, BAG3 is particularly important for maintaining the structure and integrity of sarcomeres through its interaction with HSC70 and an actin-capping sarcomeric protein (actin-capping protein β1) (11). In mice, a homozygous null mutation of Bag3 causes a fulminant form of dilated cardiomyopathy (DCM) (12). In addition, a genome-wide association study (GWAS) identified several mutations in Bag3, including an E455K mutation in the evolutionarily conserved BAG domain, that underlie a familial form of DCM (13). However, the molecular mechanism through which the loss of Bag3 function in cardiomyocytes leads to the development of DCM remains to be elucidated.

Loss of BAG3 function alters PQC in murine DCM

In this issue, Fang and colleagues used mice with cardiac-specific deletion of Bag3 (herein referred to as cKO mice) and mice with cardiac-specific expression of Bag3E455K (herein referred to as cMUT mice) and demonstrated that loss of function of Bag3 induces the development of DCM by attenuating PQC over a specific subset of proteins involved in metabolic and contractile functions in cardiomyocytes (14). Downregulation of Bag3 or expression of Bag3E455K disrupt-
ed the interaction between BAG3 and HSP70 and consequently downregulated several small HSPs (sHSPs), including HSPB5, HSPB6, and HSPB8, through protein destabilization. As a result, both protein refolding and degradation of selected HSP70-BAG3-HSPB8 complex-interacting proteins, many of which are involved in metabolism and cardiac contraction, were suppressed, resulting in accumulation of these proteins in the detergent-insoluble fraction (Figure 1).

Interestingly, large protein aggregates were not observed in cKO or cMUT mice, despite a drastic increase in the amount of proteins in the detergent-insoluble fraction. As BAG3 and HSPB8 are critical regulators of dynein-mediated retrograde transport (15), aggresome formation may be actively suppressed in these loss-of-function mouse models. As mentioned by Fang et al., the lack of aggresomes in BAG3-deficient mice strongly suggests that small and invisible protein aggregates, rather than large protein aggregates, are sufficient for the development of cardiac dysfunction. This proposed contribution of small aggregates to cardiac dysfunction in cKO and cMUT mice is similar to a study that demonstrated that the presence of soluble preamyloid oligomers (PAOs) is sufficient to induce cardiomyocyte dysfunction in desmin cardiomyopathy mouse models (16). It is possible that aggresome formation through BAG3-mediated retrograde transport may even be an adaptive process to protect cardiomyocytes from the toxicity caused by small protein aggregates.

**Remaining questions and future directions**

Although the study by Fang et al. clearly suggests that proper coordination of the actions of HSP70, BAG3, and HSPB8 is essential for the maintenance of PQC in the adult heart at baseline, several important issues remain to be addressed. For example, it remains unclear whether the selected proteins that accumulate in the detergent-insoluble fraction act as toxic molecules on their own. In a zebrafish model of myofibrillar myopathy, overexpression of BAG3P209L induces the formation of myofibrillar aggregates that sequester functional WT BAG3, thereby acting in a toxic gain-of-function manner (17). More extensive ultrastructural and biochemical analyses of the proteotoxicity associated with the different BAG3 mutants and of the presence of preamyloid oligomers would be helpful to address this issue. Alternatively, the depletion of some proteins in the soluble fraction may be more important and directly cause the functional deficit in the BAG3 loss-of-function mouse model, possibly because of the defect in retrograde transport. Despite the absence of large protein aggregates, BAG3 loss-of-function mice develop DCM.

![Figure 1. Putative functions of BAG3 in the heart and the underlying mechanisms of cardiomyopathy caused by loss of BAG3 function.](image-url)
or isoleucine, have been shown to alter BAG3 function. Specifically, BAG3\textsuperscript{V318I} has been shown to have improved binding to HSPB8 and to increase autophagy in limb muscles in response to ischemia compared with BAG3\textsuperscript{V318I} (23). These results are consistent with the notion that the PQC mechanisms controlled by endogenous BAG3 are involved in protection against myocardial stress. In fact, upregulation of BAG3 improves cardiac function in a mouse model of chronic myocardial infarction and reduces cardiomyocyte death in response to ischemia and reperfusion (8). Thus, interventions to upregulate the level of endogenous BAG3 should be further considered for treating diseases associated with cardiac stress. 

Transcription of BAG3 is regulated by NF-\kappaB and miR-371a-5p and in response to physical exercise (8). Interestingly, although both exercise and overexpression of ATG7 attenuate intracellular protein aggregates by inducing autophagy in desmin-related cardiomyopathy, exercise and ATG7 overexpression have the additive effects of improving cardiac function in another model of proteinopathy (24). Thus, combining multiple methods to enhance autophagy may allow for better control of cardiomyopathy caused by loss of function of BAG3. 

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