ACTA MYOLOGICA 2022; XLI: p. 59-75
doi:10.36185/2532-1900-071

ORIGINAL ARTICLES

The role of BAG3 in dilated cardiomyopathy and its association with Charcot-Marie-Tooth disease type 2

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Bcl2-associated athanogene 3 (BAG3) is a multifunctional cochaperone responsible for protein quality control within cells. BAG3 interacts with chaperones HSPB8 and Hsp70 to transport misfolded proteins to the Microtubule Organizing Center (MTOC) and degrade them in autophagosomes in a process known as Chaperone Assisted Selective Autophagy (CASA). Mutations in the second conserved IPV motif of BAG3 are known to cause Dilated Cardiomyopathy (DCM) by inhibiting adequate removal of non-native proteins. The proline 209 to leucine (P209L) BAG3 mutant in particular causes the aggregation of BAG3 and misfolded proteins as well as the sequestration of essential chaperones. The exact mechanisms of protein aggregation in DCM are unknown. However, the similar presence of insoluble protein aggregates in Charcot-Marie-Tooth disease type 2 (CMT2) induced by the proline 182 to leucine (P182L) HSPB1 mutant points to a possible avenue for future research: IPV motif. In this review, we summarize the molecular mechanisms of CASA and the currently known pathological effects of mutated BAG3 in DCM. Additionally, we will provide insight on the importance of the IPV motif in protein aggregation by analyzing a potential association between DCM and CMT2.

Key words: Chaperone Assisted Selective Autophagy (CASA), Dilated Cardiomyopathy (DCM), Charcot-Marie-Tooth disease (CMT), Bcl2-associated athanogene 3 (BAG3), protein quality control (PQC), IPV motif, oligomerization, protein aggregation

Received: April 26, 2022
Accepted: June 13, 2022

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How to cite this article: Yerabandi N, Kouznetsova VL, Kesari S, et al. The role of BAG3 in dilated cardiomyopathy and its association with Charcot-Marie-Tooth disease type 2. Acta Myol 2022;41:59-75. https://doi.org/10.36185/2532-1900-071

Abbreviations

AAA+ family: ATPases associated with various cellular activities; ACD: alpha-crystallin domain; ADP: adenosine diphosphate; ALP: autophagy lysosomal pathway; ATP: adenosine triphosphate; BAG: Bcl2-associated athanogene; BAG1: Bcl2-associated athanogene 1; BAG3: Bcl2-associated athanogene 3; CASA: Chaperone Assisted Selective Autophagy; CHIP: C-terminus of Hsc70 Interacting Protein; CMT: Charcot-Marie-Tooth disease; CMT1: Charcot-Marie-Tooth disease type 1; CMT2: Charcot-Marie-Tooth disease type 2; CTR: C-terminal region; DCM: Dilated cardiomyopathy; ER: endoplasmic reticulum; HOPS: homotypic fusion and protein sorting; Hsc70: heat shock cognate protein 70; HSP: heat shock protein; Hsp70: heat shock protein 70; HSPB8: small heat shock protein B8; IPV motif: Ile-Pro-Val motif; JDP: J domain protein; LIR: LC3-interacting region; MTOC: microtubule-organizing center; NBD: nucleotide binding domain; NSF: N-ethylmaleimide-sensitive factor; NTR: N-terminal region; P209L: proline 209 to leucine muta-

tion; P182L: proline 182 to leucine mutation; PQC: protein quality control; PxxP: proline-rich center of BAG3; SBD: substrate-binding domain; sHSP: small heat shock protein; SNARE: soluble NSF (N-Ethylmaleimide-Sensitive Factor) attachment protein receptor; SYNPO2: synaptopodin-2; TPR: tetratricopeptide repeat; UBA: ubiquitin-associated (domain); UPP: ubiquitin-proteasome pathway; UPS: ubiquitin–proteasome system; VPS18: vacuolar protein sorting-associated protein 18.

Introduction

Protein quality control (PQC) is an essential cellular function that is responsible for maintaining protein homeostasis [1-3]. Preserving protein stability has special significance within myocytes as muscle contractions increase heat and tension within cells [1,4-6]. The contractions induce thermal and oxidative stresses within myocytes, which make proteins more prone to becoming unfolded or mutated [3]. Chaperones play a role in cellular maintenance by inhibiting aggregation of non-native proteins and refolding misfolded proteins to their native state [1,2,5]. However, if a non-native protein cannot be remodeled, the chaperone system directs the substrate to the ubiquitin proteasome system (UPS) or the autophagy lysosome system [2,3,5-7]. These pathways maintain the stability of proteins within the cell by eliminating waste that can damage cellular structure and functions [2,7]. Short-lived misfolded proteins can be degraded in UPS, but when non-native proteins aggregate under acute stress, they are relocated to the autophagy lysosome system [3,7].

Chaperone Assisted Selective Autophagy (CASA), or BAG3-mediated macroautophagy, is a selective type of macroautophagy within the autophagy lysosome system that serves to maintain protein stability within myocytes, neurons, and other mechanically strained tissue cells [3]. The CASA complex consists of the co-chaperone BAG3, heat shock protein 70 (Hsp70), small heat shock protein B8 (HSPB8), E3 ubiquitin ligase CHIP, cytoskeletal motor protein dynein, autophagy receptor p62, and autophagosome-forming synaptopodin-2 (SYNPO2) [2,3,5]. Upon failure of a chaperone's refolding ability and the impairment of the ubiquitin proteasome pathway, CASA will be activated in order to degrade non-native proteins within the cell [1,3,6,8]. Maintaining protein homeostasis is essential within myocytes in order to prevent the accumulation of misfolded proteins, which can interfere with the cell's function and ultimately result in apoptosis [1,4-6]. Muscle contractions increase heat and tension within the cell, and as a result, the thermal and oxidative stresses within myocytes can cause proteins to become unfolded or misfolded through mutations [3]. The chaperone system works to prevent the excessive buildup of misfolded proteins by either assisting with refolding or directing the proteins towards the ubiquitin proteasome pathway or the autophagy lysosomal pathway [1,3,5,6]. The role of protein quality control (PQC) has been essential in preventing myopathy and neuropathy [3-5].

The Bcl2-associated athanogene (BAG) family of proteins are a group of co-chaperones that are essential to chaperone activity [1,9]. The BAG family includes six BAG proteins with the conserved BAG domain in each one [1]. These proteins are involved in many cellular pathways including apoptosis, macroautophagy, cytoskeleton organization, and motility [10,11]. Bcl2-associated athanogene 3 (*BAG3*) is a 575 amino acid co-chaperone from the BAG family that participates in the autophagy lysosomal pathway by interacting with Hsc70/Hsp70 and HSPB8 and similar small chaperones [1,3,4,9]. This protein has a WW domain involved in binding to diverse proteins, the PxxP motif responsible for microtubule-based retrograde transport, two Ile-Pro-Val (IPV) motifs involved in macroautophagy through interaction with small heat shock proteins (sHSPs), and the conserved BAG domain involved in apoptosis and the inhibition of proteasomal degradation [2,3,12,13] (Fig. 1). Within the CASA complex, Hsp70 binds to the BAG domain of BAG3 to redirect the misfolded proteins to the autophagy lysosomal pathway [5,9,10]. Similarly, HSPB8, along with other small heat shock proteins like HSPB6, binds to the two IPV motifs for the purpose of maintaining protein stability [1,2,4,9,14,15]. Dynein, a cytoskeletal motor protein, binds to the PxxP motif of BAG3 and facilitates the transportation of the CASA complex to the microtubule organizing center [5]. SYNPO2 binds to the WW domain and plays a role in autophagosome formation [12].

The interaction of BAG3 with chaperones is essential for chaperone activity [9]. Under physiological stress within the cell, the co-chaperone interacts with the chaperone Hsp70 as a cellular response to maintain protein homeostasis [1,3,5,13]. Hsp70 is part of the family of heat shock proteins (HSPs), which are ATP-dependent chaperones that are involved in the refolding of misfolded proteins within the cell [16,17]. HSPs consist of three domains including the N-terminal nucleotide binding domain (NBD), substrate-binding domain (SBD), and C-terminal region (CTR) [3,16]. Hsp70 also has an interdomain linker, which facilitates communication between the NBD and SBD, allowing for conformational changes in each domain [16]. The N-terminal region contains a nucleotide binding cleft for ATP and ADP binding, which dictates the protein's ability to bind to substrates [3,9,16]. BAG3 functions as a nucleotide exchange factor by regulating the ATPase cycle of Hsp70 [1,5,9]. The co-chaperone binds to the nucleotide binding domain and facilitates ADP to ATP exchange in order to release the refolded client and allow a new client to

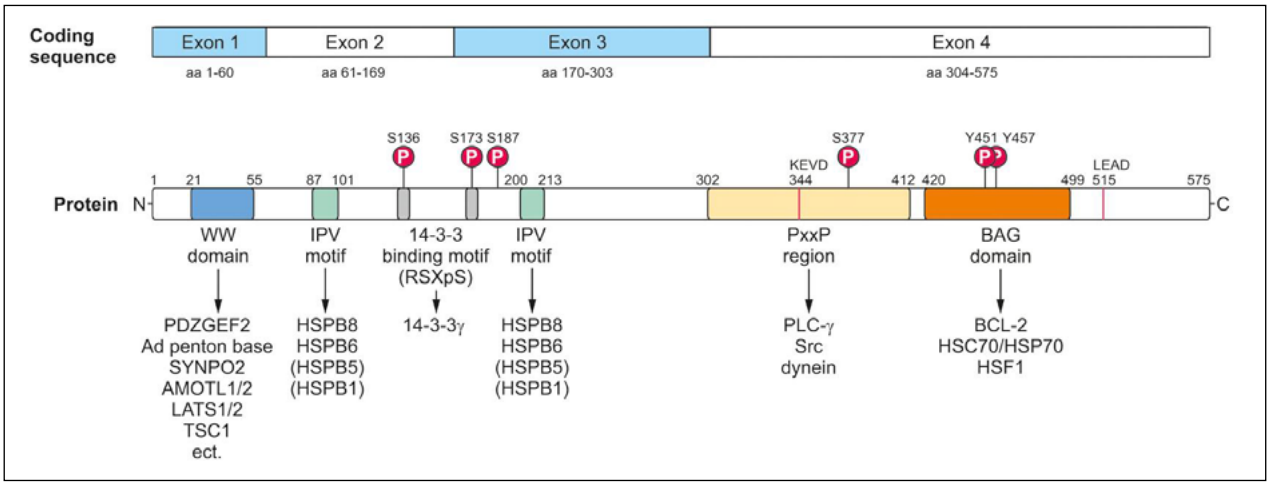

Figure 1. BAG3 consists of many domains, allowing the multifunctional protein to attach to various proteins and participate in several cellular functions. The different regions of BAG3 include the WW domain, two conserved IPV motifs, two 14-3-3 binding motifs, PxxP region, and the conserved BAG domain, which are encoded by four exons. The WW domain is located within the N-terminal region, while the conserved BAG domain is located within the C-terminal region. The phosphorylation sites of BAG3 have been marked in six locations at serine and tyrosine amino acids. The PxxP region contains a caspase cleavage site (KEVD) along with the C-terminal region (LEAD) (reproduced from the open source, from Stürner, Behl, 2017, mod.) [3].

enter the cleft [1,9,16,17]. BAG3 also interacts with the class of small heat shock proteins (sHSPs), which differ from other heat shock proteins since they function as "holdases" rather than enzymes [2,4,9,10]. In other words, sHSPs bind to and stabilize misfolded proteins, preventing aggregation [2,4,10]. There are ten proteins in the sHSP family (HSPB1 to HSPB10), which all contain the conserved alpha-crystallin domain (ACD), a flexible N-terminal region (NTR), and a C-terminal region (CTR) containing the IXI (IPV) motif [2,4-6]. BAG3 binds to the β4/β8 hydrophobic groove within the ACD of HSPB8 during autophagy [2,9].

CASA is prone to errors when the BAG3 gene contains a mutation, resulting in the production of a mutant BAG3 protein [3]. Mutations of proline in codon 209 within the second IPV motif have been identified to cause forms of myopathies, including dilated, distal, and peripheral neuropathy [1-3,13-15,18]. The P209Q, P209S, and P209L missense mutations have been associated with the development of myofibrillar myopathy [13]. Biopsies of patients with myopathy and peripheral neuropathy reveal the aggregation of misfolded proteins within the cells [13]. The proline 209 to leucine (P209L) BAG3 mutant has been specifically associated with the development of the clinical conditions Dilated Cardiomyopathy (DCM) and Charcot-Marie-Tooth (CMT) disease [11,13,19]. Patients with this disease are observed to have an accumulation of misfolded proteins in cardiomyocytes, which clinically leads to weakening of the heart muscle [3,11]. This toxic gain-of-function mutant is disrupting CASA, resulting in the aggregation of insoluble misfolded proteins and P209L mutants at aggresomes [13,20]. The relocation of the CASA complex to these aggresomes is preventing the degradation of misfolded proteins and reducing availability of essential proteins within the CASA complex [13].

Chaperone system

HSPB8 is known to serve as the "first line of defense" in the chaperone system that is activated in response to heat stress [2,17]. Functioning as "holdases," these ATP-independent chaperones form sHSP-substrate complexes [2,4,17]. These complexes have a different morphology and a smaller size compared to the aggregates that form when sHSPs are not present [17]. When a protein becomes denatured, its hydrophobic segments are no longer hidden deep within the 3D structure of the protein but instead become vulnerable to its surroundings [17]. As a result, HSPB8 is able to use its hydrophobic ACD to interact with the hydrophobic segments of denatured proteins [21]. This interaction serves to stabilize misfolded proteins and prevent them from aggregating before Hsp70 processing or degradation [4,17].

Subsequently, ATP-dependent Hsp70 displaces sHSPs from the sHSP-substrate complexes by competitively binding to the substrates [17]. It has been discovered that Hsp70 plays a role in permanently disassociating the complexes [17]. By outcompeting sHSPs in binding to the substrates, Hsp70 initiates protein refolding and contrib-

utes to disaggregation efforts within the cell [16,17]. Hsp70 and Hsp100, belonging to the AAA+ family ("ATPases associated with various cellular activities"), commonly work together in order to refold substrates from the sHSP–substrate complexes [8,17]. The scheme of these interactions is presented on Figure 2.

Chaperone-assisted selective autophagy

Formation of HSPB8-Hsp70-BAG3-CHIP complex

When the chaperones are unable to refold the denatured proteins back into their native state, these proteins need to be degraded before they interfere with the cell's function [1,2,5,22]. Ubiquitination is an essential process by which misfolded proteins can be selectively identified for degradation [22]. This post-translational modification involves tagging proteins that need to be degraded with the molecule ubiquitin, signaling the protein's degradation [22]. E3 Ubiquitin Protein Ligase CHIP is an Hsp70-interacting protein that is significant for ubiquitination [2,5,19,22]. CHIP is a member of the U-Box-containing E3 ubiquitin ligases family in which the conserved U-Box domain is responsible for the formation of multi ubiquitin chains [1,5,22]. The protein also contains an N-terminus tetratricopeptide repeat (TPR) domain, enabling E3 ubiquitin ligase to interact with chaperones like Hsp70 and Hsp90 [22]. CHIP binds to the CTR of Hsp70 and selectively ubiquitylates the substrates of the chaperone, directing the misfolded proteins toward Ubiquitin-Proteasome System (UPS) or Autophagy Lysosomal pathway (ALP) [1,2,4,22]. As a result, CHIP functions as a link between the proteasome and autophagy systems [1,2,22].

CASA requires the association of CHIP with chaperone Hsp70 and the ubiquitination of the chaperone's substrate [1,5,22]. The ubiquitination of protein aggregates recruits co-chaperone BAG3 and chaperone HSPB8 to the complex [19]. BAG3 binds to Hsp70 at its nucleotide binding domain, allowing it to regulate the ATPase cycle of the chaperone which controls substrate interaction [1-3,9]. The attachment of BAG3 to Hsp70 triggers ADP to ATP exchange, which changes the conformation of the substrate-binding domain of Hsp70 to an open state, promoting substrate release [2,3,9,16,17]. N-terminal conserved domain (J domain) proteins (JDPs) are responsible for ATP hydrolysis, which favors the closed state of the substrate-binding domain, allowing misfolded proteins to be held in place [3,9,16,17] (Fig. 3).

The co-chaperone BAG3 simultaneously binds to the hydrophobic groove in the ACD of HSPB8 and the NBD of Hsp70, but BAG3 does not directly bind to CHIP or the protein aggregate within the CASA complex [2,3,9]. Instead, these components are bound to Hsp70. Ubiquitination is also essential for autophagy receptor p62 recruitment at the microtubule-organizing center (MTOC) for phagophore expansion [1,5,14,15,23]. Co-chaperone BAG3 is crucial for the direction of the tagged substrates to autophagic degradation, and it competes directly with BAG1 for binding to Hsp70 [1,2,5]. Co-chaperone BAG1, also a member of the conserved BAG domain family, binds to

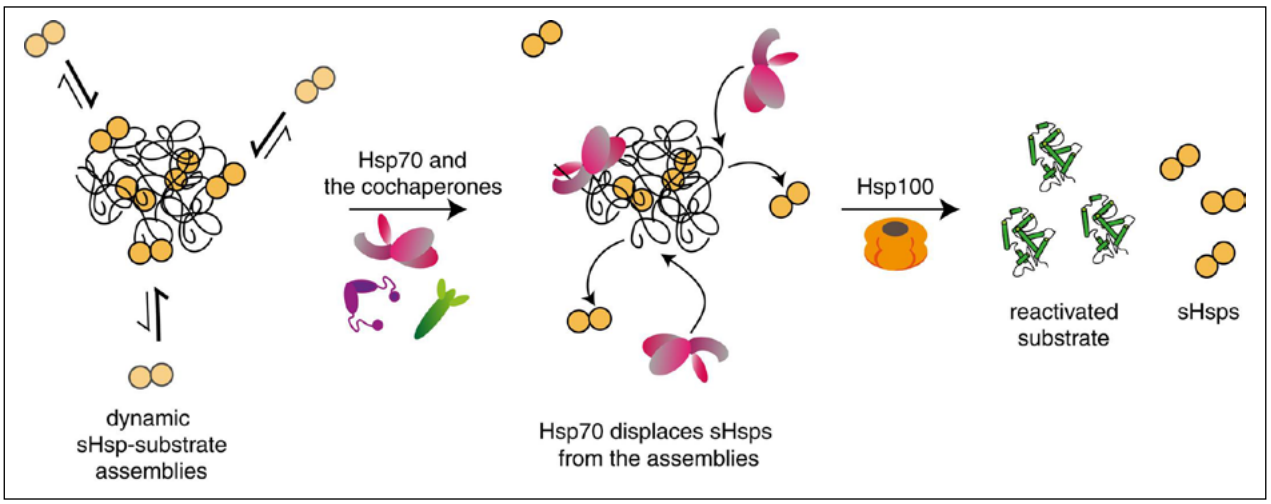

Figure 2. sHSP-substrate complexes are formed in which the outer surface consists of dynamic sHSPs in equilibrium shifted toward sHSPs being bound. These assemblies contain a stable sHSP-substrate center, which initially remains intact when Hsp70 and cochaperones displace the dynamic sHSPS in the outer surface. After successfully outcompeting the superficial sHSPs in binding to substrates, Hsp70 and Hsp100 identify the misfolded proteins and begin refolding, causing the sHSP–substrate assemblies to disassociate (reproduced from the open source, from Żwirowski et al., 2017, mod.) [17].

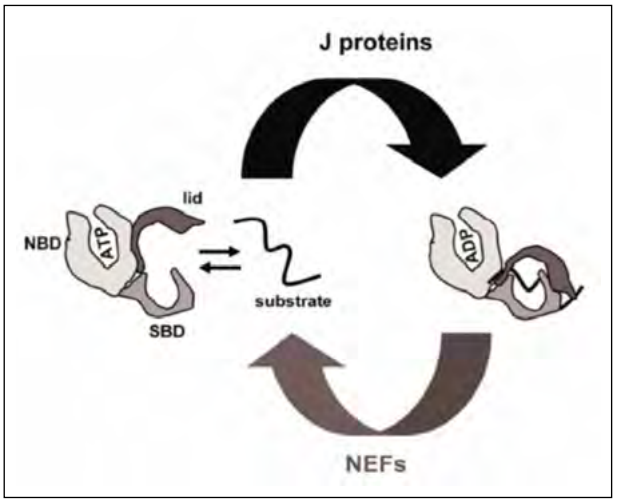

Figure 3. ATPase cycle of Hsp70. When ADP binds to Hsp70, SBD is configured to its closed conformation and has higher affinity for substrates. The lid domain closes onto the substrate that is bound to the SBD. NEFs catalyze the release of ADP and the binding of ATP to the NBD, which induces an open configuration of the lid domain. Hsp70 now has lower affinity for the substrate, leading to substrate release from the SBD. J-domain proteins are important for facilitating ATP hydrolysis to produce ADP. The ADP-bound-state once again configures for the lid domain to close upon a new substrate within the cleft (reproduced from the open source, from Kabani, Martineau, 2008, mod.) [24].

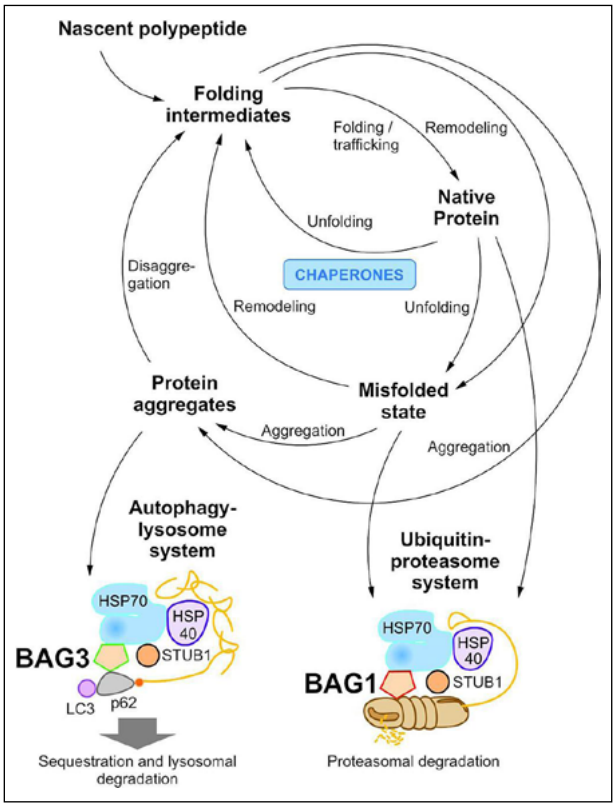

Figure 4. Under proteotoxic stress conditions, native proteins can become unfolded into a misfolded protein state. Molecular chaperones, such as the HSP family, bind to the misfolded proteins inhibiting aggregation and initiating refolding. If successfully remodeled, the misfolded protein will become a native protein. If refolding fails, non-native proteins may also be degraded by UPS, involving the binding of BAG1 to the Hsp70-substrate complex. Upon aggregation of misfolded proteins, protein aggregates are directed to the autophagy system with the recruitment of BAG3 (reproduced from the open source, from Stürner, Behl, 2017, mod.) [3].

Hsp70-CHIP complexes in a similar fashion as BAG3 and transports ubiquitinated substrates for proteasomal degradation [1,2,5,22]. Removal of proteins by proteasome is most common under physiological conditions in which UPS maintains PQC within the cell [1,25]. However, upon acute stress, the proteasomal pathway is inhibited as it is ineffective at degrading insoluble and larger protein aggregates [1,7]. As a result, during proteasome inhibition, BAG3 is upregulated and facilitates removal of protein aggregates by autophagy, preventing the accumulation of more aggregates [1,2,7,8,14,15]. This inverse relationship between the expression of BAG3 and BAG1 is known as the "BAG1–BAG3 switch" [1,2,7]. Overall, when cells are under oxidative stress or heat, the expression of BAG3 increases, causing misfolded proteins to be directed towards CASA more frequently [1,5,7,8,14,15] (Fig. 4).

Complete assembly of CASA complex

After HSPB8, BAG3, and Hsp70 have assembled, the misfolded proteins need to be transported to the Microtubule-Organizing Center (MTOC) located near the nucleus of the cell [3,5,8]. Substrates for autophagy are transported and sequestered at the MTOC, where autophagosome nucleation occurs [3,5]. Therefore, the relocation of substrates to MTOC allows for the efficient removal of the insoluble proteins in a space concentrated with autophagosomes [13]. The HSPB8 BAG3 Hsp70 complex interacts with the dynein motor complex in order to be transported along microtubules to the MTOC [3,5,8,25]. First, the 14-3-3 adaptor protein binds to BAG3 in regions RSQS136 and RSQS173 (between two IPV motifs) and it functions as a bridge between BAG3 and motor protein dynein [3,8,25]. 14-3-3 adaptor protein is from a family of multifunctional, cytosolic, ubiquitous proteins that bind to over 200 different proteins [25]. It is important for PQC due to its role in transporting the CASA complex via microtubules [25]. 14-3-3 proteins form homo- and hetero-dimers with monomers arranged in an antiparallel direction [25]. As a

result, in a 14-3-3 dimer, the ligand binding grooves of both monomers are on opposite ends, allowing more than one protein to bind to the dimer [25]. Therefore, the adaptor protein binds to BAG3 while simultaneously binding to dynein [3,25].

Dynein, a member of a family of cytoskeletal motor proteins, hydrolyzes ATP, generating a force that will move cargo protein towards the minus-end of a microtubule [26]. As a result, dynein facilitates the retrograde transport of BAG3 and its cargo protein along microtubules to the MTOC [3,5,8,25]. At the MTOC, autophagy receptor p62 binds to the ubiquitin chain on the misfolded protein [3,5,23]. p62/SQSTM1 (sequestosome 1) is a multifunctional protein and an autophagy substrate that delivers misfolded proteins to both UPS and lysosomal autophagy [23]. p62 is involved in many other cellular pathways including cell signaling, cell division, and redox processes [27]. Upon inhibition of UPS, the expression and phosphorylation of p62 significantly increases, which allows the receptor to bind to ubiquitinated proteins and deliver them for degradation via autophagy [23] (Fig. 5). p62 is degraded along with other misfolded proteins in autophagy, and as a result, impaired autophagy restricts p62 and ubiquitinated protein degradation [5,23]. The autophagy receptor uses its C-terminal ubiquitin-associated (UBA) domain to interact with ubiquitin chains on misfolded proteins, and then delivers these proteins to either proteasomes using its PB1 domain or autophagy using its LC3-interacting region (LIR) [2,5,23,28] (Fig. 5).

Additionally, autophagosome-forming SYNPO2 attaches to the WW domain of BAG3, completing the assembly of the CASA complex [12]. BAG3 and SYNPO2 interacting is important for the maturation of phagophores and the fusion of autophagosomes and lysosomes [12]. SYNPO2 uses its PDZ domain to physically connect BAG3 client complexes with membrane fusion complexes in autophagosomes [12]. Overall, p62 and SYNPO2 both allow for CASA complexes to interact with autophagosomes for protein degradation [12].

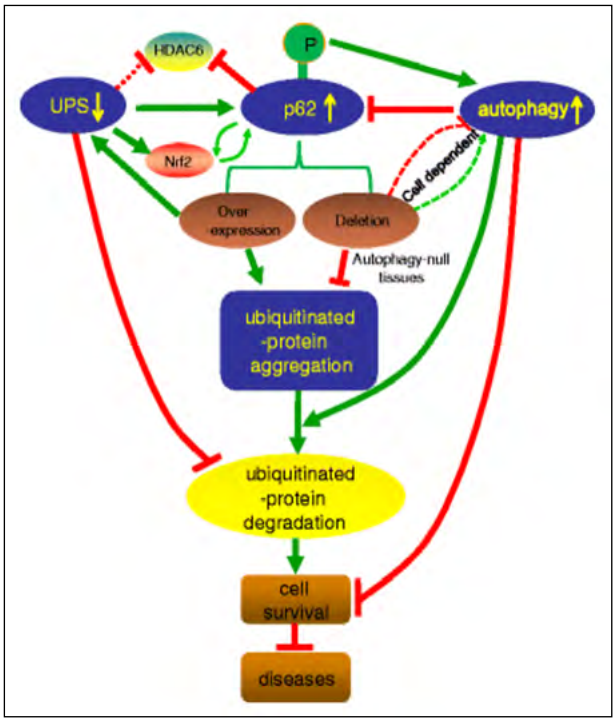

Figure 5. p62 overexpression protects the proteasome stability of a cell by increasing the number of ubiquitinated protein aggregates that are sent to the autophagy lysosomal pathway. A deficiency in UPS decreases the number of ubiquitinated proteins degraded by UPS, leading to increased p62 expression and phosphorylation on S405 and S409. Upon UPS inhibition, the levels of Nrf2 increases, inducing p62 overexpression. The greater concentration of p62 better enables it to compete with Nrf2 for Kea1, forming p62-Keap1 complexes, which assist with the formation of ubiquitinated protein aggregates for degradation by autophagy. HDAC6 is critical in the autophagy lysosomal pathway, but its synthesis is directly inhibited by UPS inhibition and p62 overexpression. However, p62 overexpression can be inhibited by an increase in autophagy. Therefore, the ratio of p62 to HDAC6 is essential for cellular stability (reproduced from the open source, from Liu et al., 2016, mod.) [23].

Autophagosome and autolysosome formation

Once the entire CASA complex has been assembled, autophagosome nucleation is initiated at MTOC, enabling the misfolded proteins to be degraded in autolysosomes [23,28]. An autophagosome is a spherical double-layered structure that is responsible for engulfing damaged cellular components and fusing with lysosomes (autolysosome) to break down the components [3,28]. Prior to autophagosome formation, phagophores, crescent-shaped double membrane that fuses to form a double-membrane vesicle, must form [3,28]. A phagophore is formed by vesicle precursors that fuse together into a double-membraned sac coated with LC3-II proteins, which are common biomarkers for autophagosomes due to their role in substrate selection and autophagosome formation [1,5,12,23,29]. LC3 proteins are accessible in two forms in the cell: LC3-I is in the cytoplasm and assists with the elongation of the autophagosome during formation and LC3-II is membrane-bound and helps with docking cargo proteins to the autophagosome for degradation [23,28-30].

Autophagosome formation involves triggering the expansion of a phagophore (premature autophagosome) via interaction between SYNPO2 and VPS18 and SNARE containing protein complex, which are membrane fusion machinery attached to phagophores [12,31] (Fig. 6). SNARE

proteins are known to mediate vesicle fusion into a phagophore by connecting the two membrane sites [31]. VPS18 is a subunit from the homotypic fusion and protein sorting (HOPS) complex that functions as a membrane tether by facilitating the docking and fusion process of autophagosomes [12,31]. Attached to BAG3, SYNPO2 initiates autophagosome nucleation by interacting with this membrane fusion machinery on the phagophore [12]. Autophagy receptor p62 is responsible for delivering the cargo protein to the autophagosome for degradation [1,5,23,28]. p62, bound to the ubiquitin chain of the misfolded protein, loads the cargo protein into the autophagosome by attaching itself to membrane-bound LC3-II on the phagophore [1,2,5,23,28]. After this interaction, p62 and the ubiquitinated misfolded protein detach from the CASA complex and bind to the maturing phagophore using LC3-II [1,2,5,23]. Once the phagophore has fully transformed into an autophagosome, p62 and the misfolded protein are sequestered in the autophagosome, ready for degradation [1,3,5,14,15]. Eventually, the autophagosome will fuse with a lysosome, forming an autolysosome [1,3,14,15]. Inside this structure, the hydrolytic enzymes of the lysosome will degrade the misfolded protein and p62 [1,3,5]. The CASA complex will disintegrate into its individual proteins until it's signaled to form again by the presence of protein aggregates. The proper functioning of this pathway is needed to maintain the structure of the Z disc by regulating the removal and production of filamin within cardiomyocytes [1,3,14,15,28] (Fig. 6). Without sufficient degradation, protein aggregates would accumulate within the cell and interfere with its functions, eventually leading to cell death [3,5]. The complete scheme of interactions in the functional CASA pathway is depicted in Figure 7.

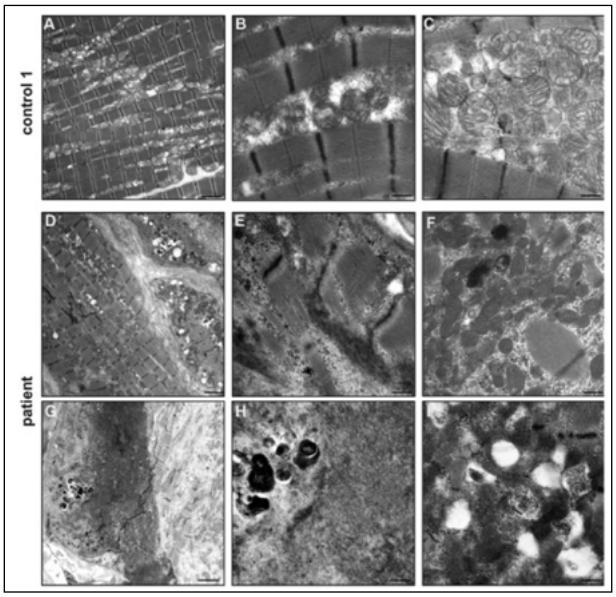

Figure 6. Ultrastructural analysis of cardiac tissue from control 1 (A-C) and BAG3 P209L-mutant patient (D-I). (D) BAG3-mutant cardiac tissue has altered Z-disk structure and extreme myofibrillar disintegration with cells of varying diameters and vacuoles. (E) Recurring Z-disk alterations and substantial electron dense material originating from Z-disks. (F) Central accumulation of mitochondria and visible lipofuscin granules. (G,H) Bundles of electron-dense material are abundant and partly surrounded by electron dense vacuoles. (I) In spaces between myofibrillar bundles, empty vacuoles and glycogen or electron dense vacuoles are present. Scale: 2.5 µm (A,D,G) and 0.5 µm (B,C,E,F,H,I) (reproduced from the open source, from Schänzer et al., 2018, mod.) [33].

The effect of BAG3 P209L mutant on CASA

The BAG3 P209L mutant is inhibiting the cell's innate response to protein instability, resulting in cells with an abundance of protein aggregates [11,20]. Specifically, the mutant is preventing the delivery of misfolded proteins to the autophagosomes at MTOC, causing the accumulation of these proteins as well as the unavailability of CASA components [11,13,19,20]. It has been discovered that the point mutation in codon 209 of the second IPV motif is not impairing the ability of BAG3 to interact with other proteins in the CASA complex [13,32]. In vitro, human studies found that mutant BAG3 retains its ability to bind to HSPB8 and Hsp70 but has reduced binding affinity for HSPB8 [13,32]. One such study found an increase in the interaction between autophagy receptor p62 and the CASA complex with mutant BAG3 although there is no direct interaction between the mutant and p62 [13]. These results suggest that the entire CASA complex is able to fully assemble with or without the P209L mutant [13]. It is unclear whether BAG3 P209L can impair autophagosome formation. A mice model of BAG3 variants observed higher levels of SOD1_G923A in the insoluble fraction of cells with Pro209 mutation compared to other BAG3 variants, indicating that the CASA complex recognizes misfolded proteins but is unable to degrade them [13]. Despite the proper assembly of the CASA complex, cells with mutant P209L are associated with the clustering of misfolded proteins into insoluble aggregates [11,20,32]. It appears that the complexes with mutant BAG3 are unable to deliver the cargo protein to the autophagosomes, significantly decreasing the effectiveness of CASA [19,32]. The accumulation of CASA complexes at MTOC results from the inefficient degradation of misfolded proteins [13]. As a gain-of-function mutation, the proline to leucine alteration in codon 209 is known to decrease the solubility of BAG3, as observed in human, in-vitro studies, and therefore may be responsible for the aggregation of BAG3 and other misfolded proteins [13,20]. BAG3 co-aggregates with other components in

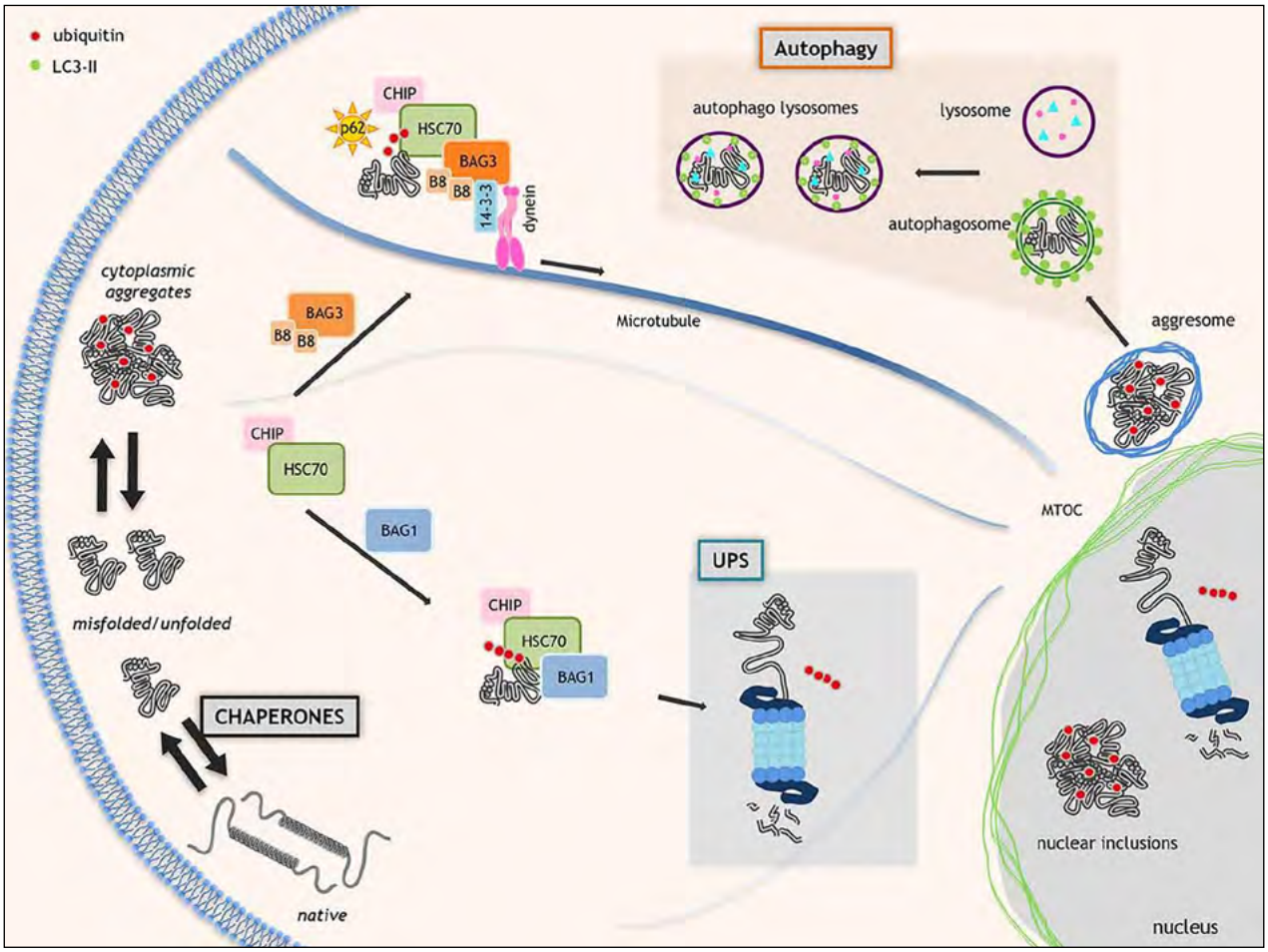

Figure 7. Protein quality control in neurons and other mechanically strained tissue cells. Upon the misfolding or unfolding of a protein, molecular chaperones bind to these non-native proteins and facilitate the process of refolding. However, if refolding is unsuccessful, the HSC70 chaperone is responsible for routing the misfolded protein to one of two pathways for degradation. The binding of BAG1, a nucleotide exchange factor from the Bcl2-associated athanogene family to the HSC70 CHIP complex allows for the inhibition of HSC70's chaperone activity and the polyubiquitination of the client by CHIP. This interaction directs the misfolding protein to UPS, in which a proteasome breaks down the client. Conversely, the binding of BAG3 and HSPB8 to the complex reroutes the substrate to CASA. In this pathway, 14-3-3 protein and dynein are responsible for facilitating the transportation of the CASA complex along microtubules to MTOC, site of autophagosome formation. Here, autophagy receptor p62/SQSTM1 attaches to the polyubiquitin chain of the misfolded protein and directs the client to the autophagosome through its interaction with the LC3 receptor. The misfolded protein is degraded once a lysosome fuses with the autophagosome, forming an autolysosome (reproduced from the open source, from Cristofani et al., 2019, mod.) [5].

the CASA complex, contributing to the large aggregates found within affected cells [11,13.32]. It has been determined that the collective aggregation of PQC proteins can be attributed to the interaction between BAG3 and Hsp70 as eliminating the BAG domain ceases the accumulation of misfolded proteins [13,32] (Fig. 7).

In addition to the excessive formation of aggregates, the P209L mutant condition is also classified with trapping PQC proteins, including Hsp70 and HSPB8, into aggresomes [13,32]. Aggresomes are a cellular response to the overload of misfolded proteins that involves the formation of circular bodies that hold protein aggregates in an attempt to concentrate them in one location [13]. These inclusion bodies can be toxic to the cell when they accumulate, which can ultimately lead to cell death. Besides storing misfolded proteins, aggresomes in human cell lines with mutant P209L BAG3 have also been shown to relocate BAG3 and other essential chaperones, reducing the number of available chaperones in the cell [13,32]. As a result, misfolded proteins have less access to functional chaperones, promoting unregulated aggrega-

tion [13,32]. One possible explanation for the formation of insoluble aggregates and sequestration of PQC components is that BAG3 is unable to properly function as a nucleotide exchange factor [13,34]. As previously mentioned, BAG3 is responsible for the ADP to ATP exchange within the ATPase cycle of Hsp70, which signals client release [9,13]. When ADP is bound to the nucleotide binding cleft in the NTR, P209L mutant BAG3 may not be able to exchange the nucleotide for ATP, resulting in no conformational change to the open state of the substrate-binding cleft [13,16]. As a result, the continuous closed state of this cleft will not allow the misfolded protein within the SBD to be released into the autophagosome, disrupting the process of CASA [13]. This may delay the dissociation of PQC components in the CASA complex, and ultimately increase the likelihood of the complex becoming engulfed by an aggresome [13] (Fig. 8).

HSPB1 IPV motif, Charcot-Marie-Tooth disease, and their association with dilated cardiomyopathy

Charcot-Marie-Tooth (CMT) disease is the most common form of inherited peripheral neuropathy, a group of neurodegenerative diseases characterized by damaged motor and sensory neurons in the peripheral nervous system [35-38]. More than 80 genes with mutations linked to CMT disease have been identified, contributing to the diversity of the genetic disorders classified as CMT disease [39]. Individuals with CMT disease experience sensory and motor deficits that progress over time [35,36,40,41]. CMT can be divided into two types, CMT1 and CMT2: CMT1 (demyelinating CMT) is caused by impaired myelin sheath while CMT2 (axonal CMT) is caused by damaged neuronal axons [35-37,40,42,43]. Mutations within the genes of small heat shock proteins, including HSPB1, HSPB8, and HSPB3, are commonly associated with causing CMT2 or distal hereditary motor neuropathy (dHNM) [37,39-45]. CMT2 disease-causing mutations within the sHSP family affect self-interaction among sHSPs, impairing oligomerization and the chaperone capacity of these chaperones [36,42,46].

Like BAG3, HSPB1 has a conserved IPV (also called IXI) motif located within its C-terminal region that also interacts with the β4/β8 groove of another HSPB1 dimer [6,44,46-48]. Mutations in HSPB1 are known to cause Charcot-Marie-Tooth disease type 2 (CMT2), but the mutation proline 182 to leucine (P182L) within the IXI motif is similarly associated with protein insolubility and protein aggregates [38,41,42,45,49,50]. The HSPB1 P182L variant is

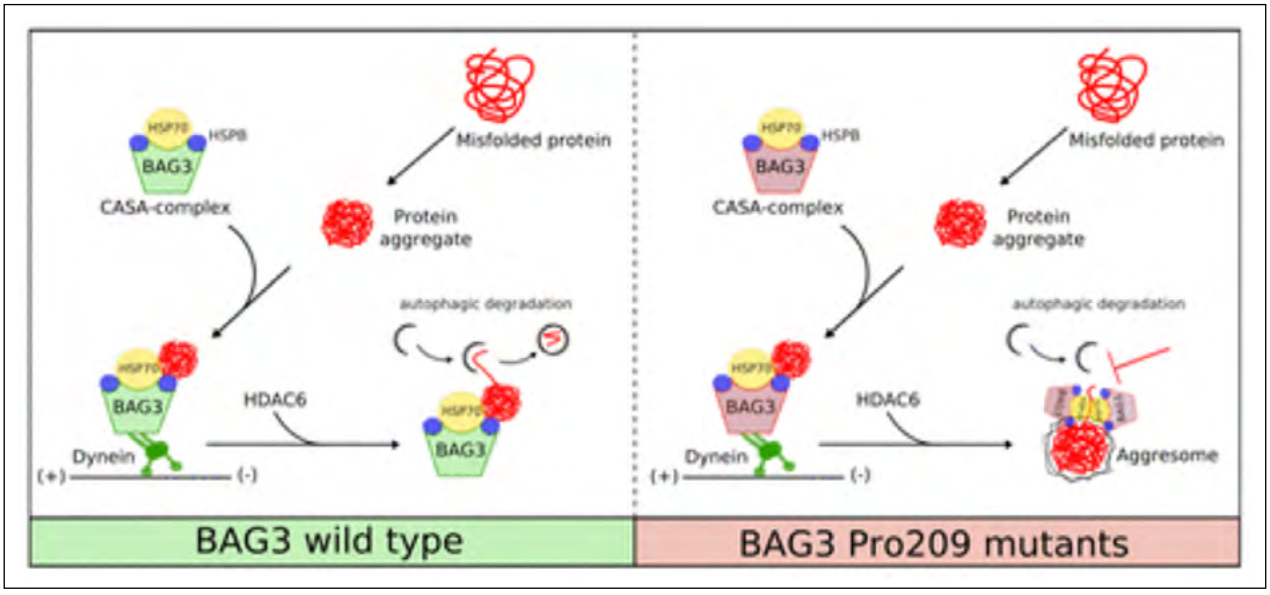

Figure 8. In the presence of BAG3 wild type, the CASA complex assembles, attaches to a protein aggregate, to an autophagosome in MTOC, where the aggregate becomes degraded. The P209L mutation increases the aggregation propensity of BAG3 and may impair the ATPase cycle of Hsp70, causing Hsp70 to be unable to release the protein aggregate. As a result, the protein aggregate may remain bound to the CASA complex and not be delivered to an autophagosome, leading to the accumulation of CASA complexes in MTOC. These complexes become relocated to aggresomes, decreasing the accessibility of essential and functional proteins, including Hsp70, HSPB8, and p62 (reproduced from the open source, from Adriaenssens et al., 2020, mod.) [13].

classified as rare but severe as onset occurs within the first five years of life compared to adult onset of HSPB1 variants with mutations in the ACD [42,49]. It was discovered that the P182L variant promotes aggregation of client proteins and overexpression can lead to the formation of aggresomes with HSPB1 [38,45,49,50]. This may lead to speculation of whether there is a possible similarity between DCM, caused by mutant P209L BAG3, and CMT2, caused by mutant P182L HSPB1.

Self-interaction among small heat shock proteins

Point mutations associated with CMT2 within sHSPs may have toxic effects on self-interaction and self-assembly, which are considered unique and essential properties among the sHSP family [41]. All sHSPs form dimers and/or oligomers through intradimer or interdimer interactions [46-48]. sHSP oligomerization is dynamic with each subunit in equilibrium between dimeric and oligomeric states, enhancing sHSP chaperone activity [21] (Fig. 9).

HSPB1 and HSPB5 are known to form larger homooligomers (oligomers formed from dimers of the same protein) compared to the rest of the sHSP family which form smaller protein structures [6,46,47]. Heterooligomeric complexes (oligomers formed from dimers of different proteins) can be formed as well, especially between HSPB2 and HSPB3, HSPB5 and HSPB6, and HSPB1 and HSPB5 [6,40,46,47]. sHSPs common to CASA, HSPB6 and HSPB8, mostly exist as homodimers due to their lack of an IXI motif but can selectively bind to dimers of other sHSPs [6,46,47].

The structure of a monomer in a small heat shock protein consists of three domains: NTR, ACD, and

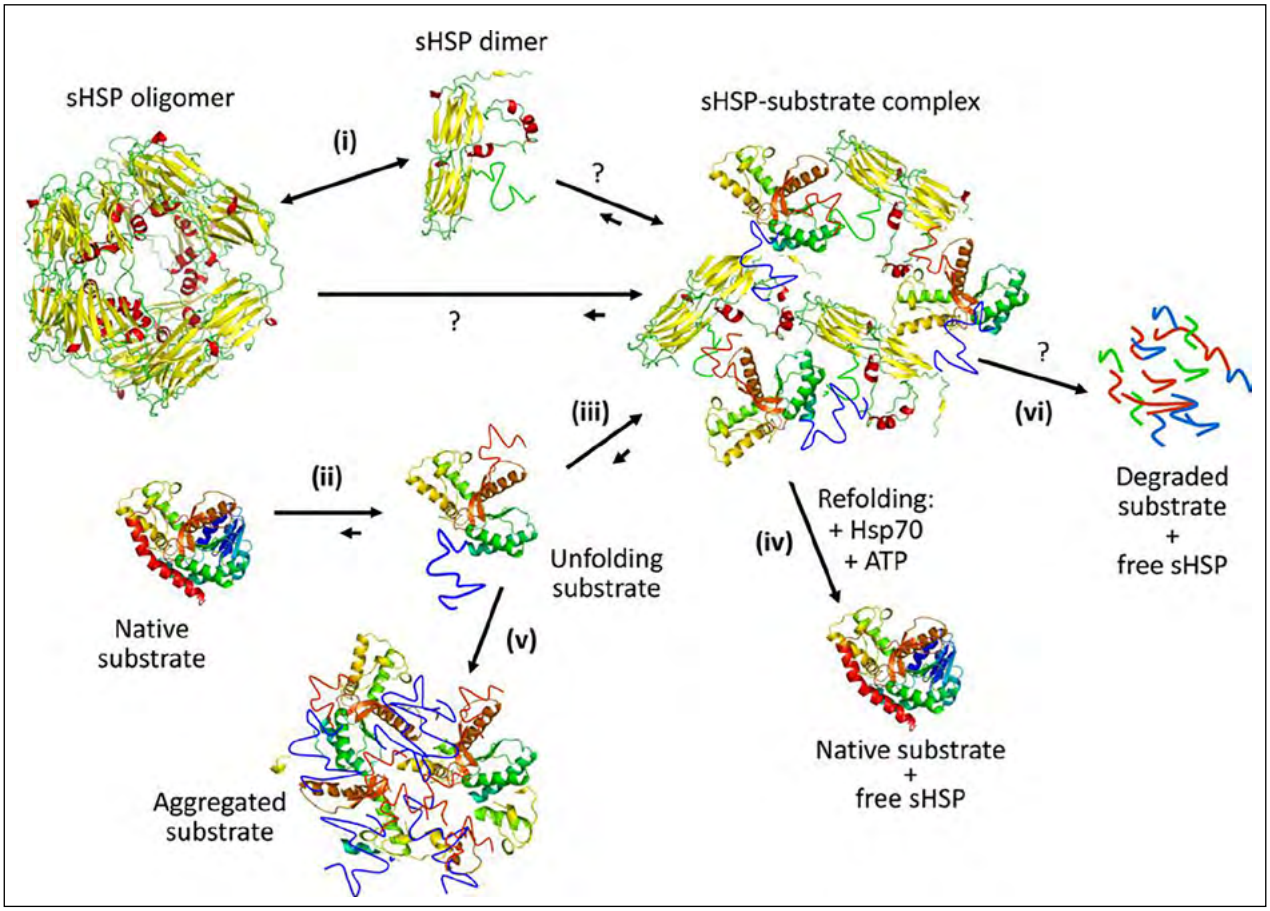

Figure 9. Model for sHSP chaperone activity. (i) sHSP dimers and monomers continuously alternate between being bound in an oligomer or released into the environment. (ii) Misfolded and unfolded proteins expose hydrophobic regions to which sHSPs bind using a hydrophobic groove in the ACD, forming large substrate-sHSP complexes (iii). (iv) The denatured substrate can be refolded by Hsp70, ATP, and a co-chaperone which displace the sHSP-substrate complex and attach to the substrate. (v) Lack of adequate sHSPs can result in the formation of protein aggregates, which are harder to eliminate. (vi) Cellular proteases can also degrade the substrate from an sHSP-substrate complex, but this pathway is less well-known. This model does not elucidate all possible sHSP proteins actions (reproduced with the permission of the publisher, from Basha et al., 2012, mod.) [21].

CTR [6,21,44,46-48]. The disordered NTR and CTR have binding sites that adhere to the grooves of the structured and conserved ACD [6,21,44,47]. Competition for binding to the grooves within ACD is high but each interaction is weak, creating "tethered" and "untethered" states among the disordered regions [6,46]. ACD is known to serve as a binding region for many different proteins, including BAG3, as well as segments of other sHSPs [6]. There are three main types of self-interactions that the ACD facilitates: ACD ACD, CTR ACD, and NTR ACD [6,21,44,46-48]. These interactions are essential for oligomer assembly and subunit exchange, which contribute to chaperone activity [21,44,46]. ACD to ACD interaction forms the basis of dimerization among all sHSPs [6,21,44,46-48]. The ACD β6/β7 strands of two monomers weakly interact in an antiparallel alignment [6,21,46,48]. The formation of a sHSP dimer creates two β4/β8 groove binding sites for the NTR and CTR segments of sHSPs or for aggregation-prone proteins, with each groove located within a monomer [6,21,46,48]. The hydrophobic chains within these grooves interact with the hydrophobic regions of the denatured proteins, reducing their propensity for aggregation until the proteins can be refolded or degraded [21]. The structure of the identical edge grooves are distinctive because the compacted side chains within the β4 and β8 strands form a binding site that looks like two holes separated by a wall [6]. In addition, the monomer-to-monomer interaction among sHSPs forms one dimer interface groove near the β6 and β7 strands [6,24,46] (Fig. 10).

The CTR is a less conserved region of small heat shock proteins that can serve as a "solubility tag" [38,46]. Its length varies from protein to protein, but it shares the common features of being flexible and disordered [44,46,49]. Some sHSPs contain a short IXI motif within the CTR that is most commonly associated with the formation of higher order oligomers [6,44,46,47]. HSPB1, HSPB2, HSPB4, and HSPB5 are all known to have this 3-letter sequence, allowing them to assemble into larger homoligomers due to the motif's crucial role in oligomerization [6,46,49]. CTR to ACD interaction takes place at the inter-dimer level, meaning from the IXI motif of one sHSP dimer to the ACD of another dimer [6,44,47,49]. Due to the knob-like structure of the β4/β8 groove, the IXI motif binds to the edge groove of the ACD in a knob-into-hole fashion [6,46]. Besides forming larger order oligomers, the CTR ACD interaction is also known to recruit and exchange monomers within an oligomer [44,46,48,51] (Fig. 10). One study divided this process into two steps [51]. First, the unbound CTRs within a sHSP oligomer bind to the β4/β8 groove of a subunit within a free dimer [44,51]. Then, the free dimer dissociates into two monomers [51]. One of the dimers within the oligomer dissociates and forms a heterodimer with a subunit from the new dimer [51]. Therefore, the CTR's IXI

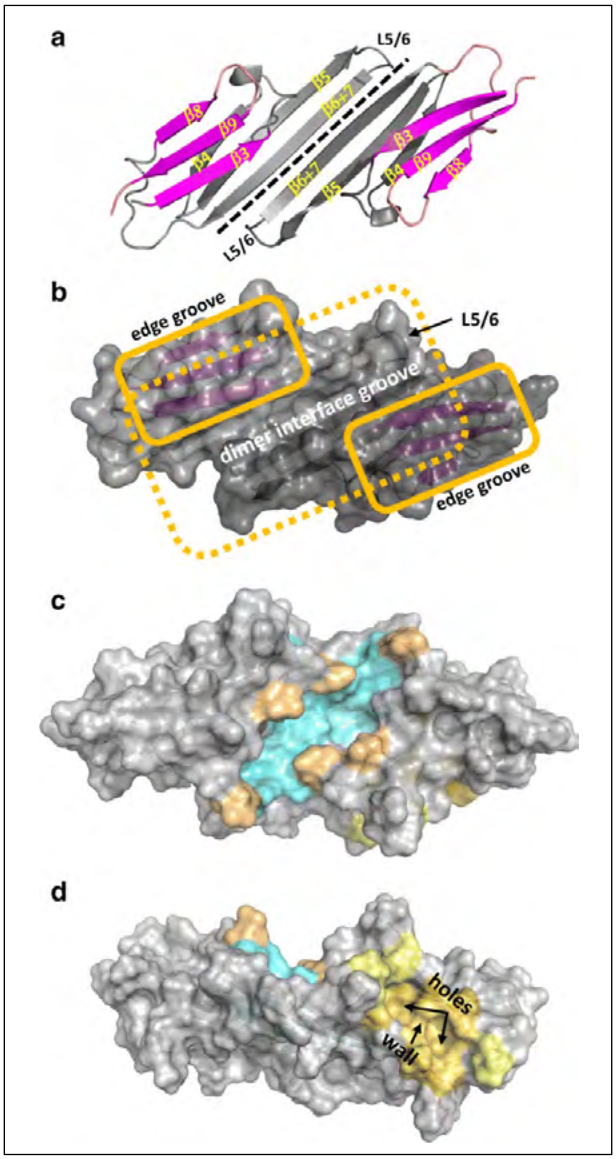

Figure 10. Structure and grooves of alpha-crystallin domain (ACD) within sHSPs. (A) ACD dimer with β-strand and L5/6 loops connecting β5 strands to β6+7 strands. The dashed line represents the dimer interface. The β-strand is divided into a "bottom" sheet with 6β strands (in gray) consisting of β4, β5, and β6+7 from both sHSP monomers [6]. The "top" sheet is composed of 3β strands (in magenta), including β3, β8, and β9 [6]. (B) Surface front view of the dimer interface groove formed by the β6+7 strands. The L5/6 loops on either side of the groove are in "loop-up" position [6]. The 6-stranded β sheet is indicated by the dashed yellow line and the 3-stranded β sheets are indicated by the solid yellow line. (C) Color coded portions of the dimer interface groove from the same front view. Cyan residues represent the floor of the groove and buff residues represent the sides of the groove. (D) Top view of ACD structure shows hydrophobic β4/β8 groove (edge groove) with the two holes and wall identified (reproduced from the open source, from Klevit, 2020, mod.) [6].

motif interacts with the hydrophobic groove of the ACD, which is essential for oligomerization and monomer exchange [51] (Fig. 11). This study's conclusions and models were made based on observations from the expression of sHSP constructs in *Escherichia coli* and evidence from previous studies [51]. As a result, it is important to note that there are limitations in applying the process described above to humans. Small heat shock proteins that lack the IXI motif in the CTR, like HSPB6 and HSPB8, mostly exist as small homodimers, but they can also form heterooligomers with other sHSPs that have the IXI motif [6,9,51].

The NTR of sHSPs is longer than the CTR, and as a result, it has more well-defined regions that bind to the ACD's hydrophobic grooves [6,44]. One study divided the NTR into six regions with the distal region, aromatic region, conserved motif, tryptophan-rich region, insertion, and boundary region [46]. These regions were defined by applying hydrogen deuterium exchange mass spectrometry (HDXMS) to human HSPB1 dimer and nuclear magnetic resonance (NMR) to short segments of NTR, which revealed specific interactions between the ACD and NTR [46]. NTR interactions with the ACD include the distal region bound to the edge groove, the aromatic region bound to L3/4 and L5/6, and the conserved and boundary regions bound to the dimer interface groove [6,46]. Due to the highly disordered state of the NTR, this domain is able to bind to the ACD at the intramonomer, intradimer, and interdimer levels [46,47] (Fig. 12). The NTR has been identified as crucial for oligomerization due to its flexible interactions [21,44,46-48]. Additionally, this domain exists in a bound and unbound state, which may regulate client binding among sHSPs [6,46]. The tethered state is proposed to restrict the binding of misfolded proteins and limit chaperone function, while the untethered state may enhance chaperone function by allowing client proteins to bind [6]. A schematic of the role of sHSP self-interactions in oligomer assembly is depicted in Figure 13.

Mutations in HSPB1 and their effect on protein stability

Seven to eight percent of mutations causing CMT2 are located in HSPB1, which equates to about 30 HSPB1 mutants [50]. In-vitro and in-vivo studies of HSPB1 mutants have noted the formation of larger HSPB1 oligomers, whether the mutation is located in the NTR, CTR, or ACD [36-38,41,49,50]. All mutants are more likely to aggregate at lower temperatures [50]. Larger HSPB1 oligomers are only formed at higher concentrations when the mutation is located in the ACD [37]. At lower concentrations, HSPB1 ACD mutants are known to disassociate from the oligomeric structure [37]. Additionally, HSPB1 has three sites of phosphorylation in its NTR that are associated with the dissociation of an HSPB1 oligomer [50,52] (Fig. 14). In-vitro and mass-spectrometry studies have discovered that phosphorylation-induced disassembly is resisted by HSPB1 NTR mutants, whereas HSPB1 ACD mutants more readily dissociate upon phosphorylation compared to wild type HSPB1 [50,52]. This can be explained by the fact that the NTR of HSPB1 is phosphorylated in response to stress and serves as an instigator of oligomer disassembly [50,52]. The larger oligomeric structure of wild type HSPB1 dissociates into smaller complexes with increased availability to bind to misfolded proteins and therefore increased chaperone function [41,50,52]. However, when HSPB1 has a mutation in the NTR, the stress-induced phosphorylation is disrupted, which prevents the reduction in oligomer size [50]. As a result, the β4/β8 groove will likely remain occupied and not be able to bind to as many misfolded proteins [52]. Lastly, mutations in any domain alter the protein structure of HSPB1, leading to a change in chaperone capacity [49].

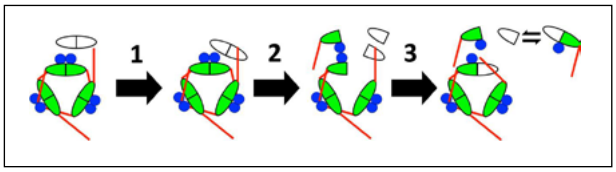

Figure 11. Model for subunit exchange within sHSPs. Unbound ACD dimer is white, and within the oligomer, NTR is blue, CTR is red, and ACD is green. (1) Free CTR within the oligomer recruits an unbound ACD dimer to the oligomer. (2) A subunit within the oligomer and the recruited subunit dissociates. (3) A heterodimer is formed with one new subunit and one original subunit (reproduced from the open source, from Delbecq et al., 2015, mod.) [51].

P182L-induced protein aggregation

Like other HSPB1 mutants, in-vitro and in-vivo studies have established that the P182L mutant forms larger oligomeric complexes than wild type HSPB1 and other HSPB1 mutants do [38,41,42,49]. These massive structures may contain only P182L mutants or possibly a combination of mutant and wild type HSPB1 [42]. Despite the formation of larger structures, a lower affinity between the IXI motif and ACD has been observed in P182L mutants [38,42]. One study noted that the amino acid Pro182 in the wild type contributes to a rigid binding-friendly conformation more often than the mutation with codon Leu 182 [42]. As a result, lack of this rigidity has made it easier for the P182L mutant to escape from the binding-friendly conformation, which lowers the interaction between IXI motif and ACD [42]. This conclusion was reached using 200 ns of all-atom molecular dynamics (MD) simulations performed on fragments of wild type and P182L HSPB1 [42]. Another paper explained the contradiction of a lower binding affinity yet a larger oligomeric structure through discoveries of oligomerization at the molecular level [38,42].

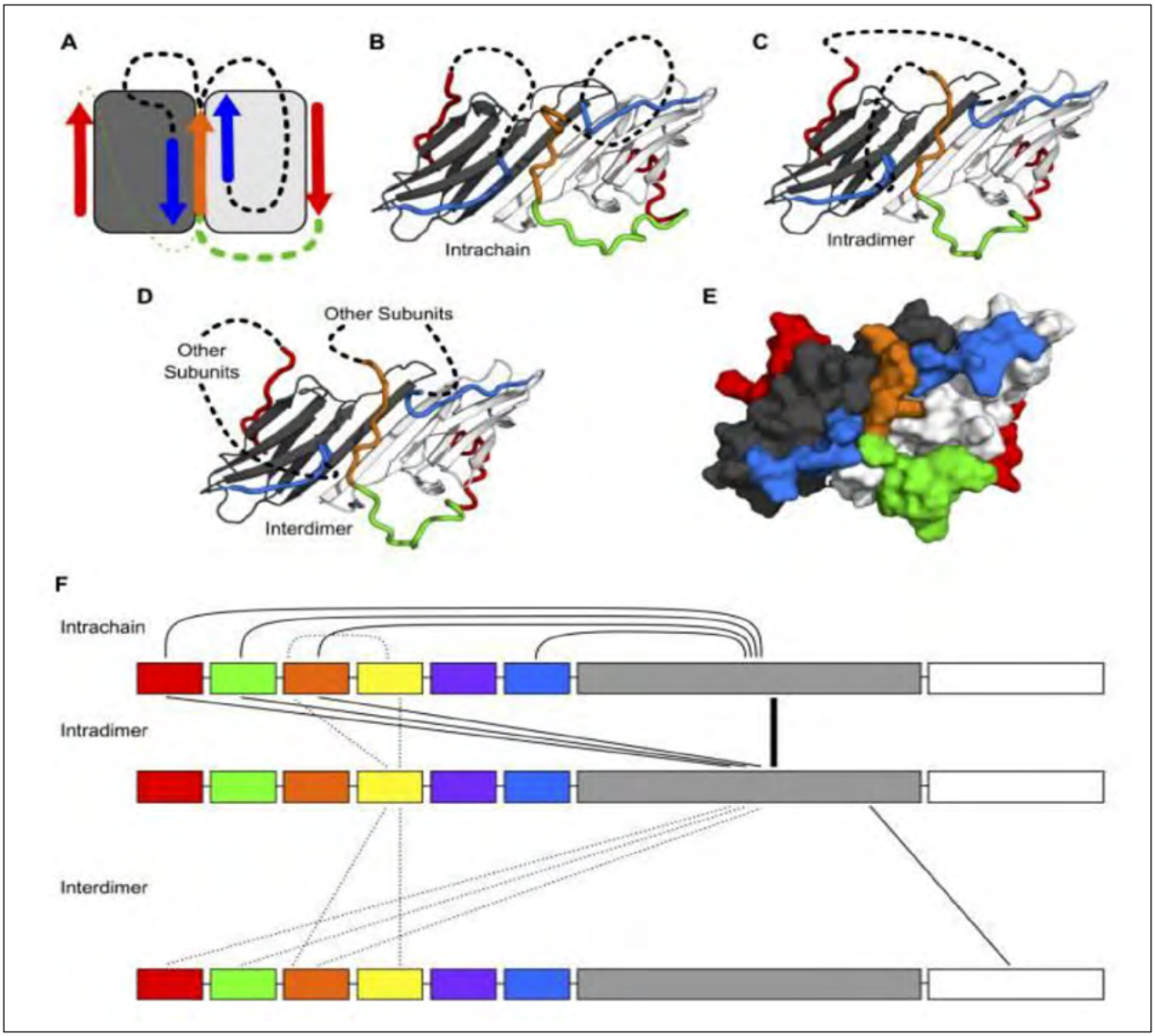

Figure 12. Representation of the NTR-ACD interactions. (A,B) intrachain, (C) intradimer, and (D) interdimer interactions between sHSPs. (E,F) Structure of NTR with distal region in red, aromatic region in green, conserved region in orange, Trp-rich region in yellow, insertion region in purple, and boundary region in blue. ACD is in gray and CTR is in white. Interactions indicated by solid lines are supported by evidence from the study and the dotted lines are interactions that the researchers "believe are likely to occur" [46]. This figure demonstrates the degree of flexibility in NTR's interactions within an sHSP or with other sHSPs (reproduced from the open source, from Clouser et al., 2019, mod.) [46].

Using NMR spectroscopy, it was found that although the IXI motif and β4/β8 groove are located very close to each other, the IXI motif is generally unbound and unstructured in sHSP oligomers [38,42]. As a result, in a wild-type oligomer, the binding of two IXI motifs causes the exchange of a subunit within the oligomer [35,40]. When considering this model for mutant HSPB1, the P182L mutation likely causes slower subunit ejection [38,42]. Therefore, the faster recruitment of subunits compared to removal causes an increased oligomeric size [38,42]. Additionally, the same study identified the amino acid side chains of Ile181 and Val183 in the IXI motif as important for proper binding to the β4/β8 hydrophobic groove, and a lack of one of these side chains can disrupt binding [42]. The reduced binding affinity between the IXI motif and β4/β8 hydrophobic groove results in the CTR binding less frequently to the ACD of HSPB1 [38,42]. As previously stated, there are many sequences within the NTR that are competing with the CTR to bind to the β4/β8 groove [38,42]. As a result, it is likely that the CTR will become outcompeted by the

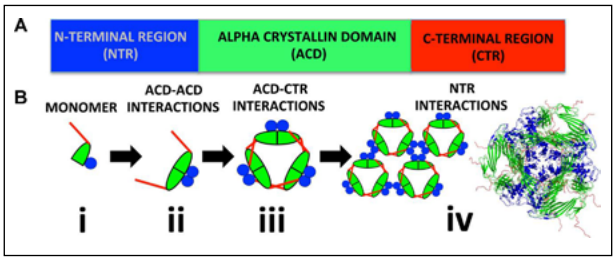

Figure 13. sHSP domains. (A) Schematic representation of sHSP with three domains: NTR in blue, ACD in green, and CTR in red. (B) Model of sHSP oligomerization. sHSP monomer (i) binds to another sHSP monomer via ACD–ACD interaction, forming a dimer (ii). A higher-order structure (iii) is formed by inter-dimer interactions between the CTR and ACD. NTR interactions between subunits contribute to the formation of an oligomer (iv). The structure of a final 24mer HSPB5 oligomer (reproduced from the open source, from Delbecq et al., 2015, mod.) [51].

NTR in binding to the ACD, significantly changing the structure of the HSPB1 oligomer [38,42].

Similar to DCM, cells infected with CMT2 have large insoluble aggregates that result from the failure to refold or degrade misfolded proteins, as observed in transgenic mice models [41,45,49,50]. Within these cells, wild type HSPB1 can form a heterooligomeric structure with P182L HSPB1 [38,42,45]. As a result, when mutant HSPB1 is relocated to cytoplasmic aggregates, wild type HSPB1 may become recruited as well [38,42,45]. This results in reduced availability of wild type HSPB1 proteins, causing more damage to the cell [38,42,45]. As a catalyst for a neurodegenerative disease, the P182L mutation in HSPB1 is also known to impair axonal transport in neurons [40,41,45,49,50]. The subunit of dynactin, p150, is essential for retrograde transport within cells as it mediates the binding between dynein and cargo protein [41,45]. p150 is observed to be less soluble in cells with mutant P182L, resulting in p150 becoming sequestered into aggresomes along with HSPB1 [45]. With an increasing amount of p150 becoming isolated, transport of cargo within the cell becomes infeasible, contributing to the buildup of aggregates and the inhibition of essential processes [45]. Therefore, the sequestration of p150 induced by mutant HSPB1 aggregation can impact the transport of certain molecules along the axon [45].

Conclusions and prospects

The similarities in the pathological mechanisms of BAG3-induced DCM and CMT2 can imply a possible association between the two diseases. P209L BAG3 mutant and P182L HSPB1 mutant are known to cause large, insoluble protein aggregates that impair the proteasome

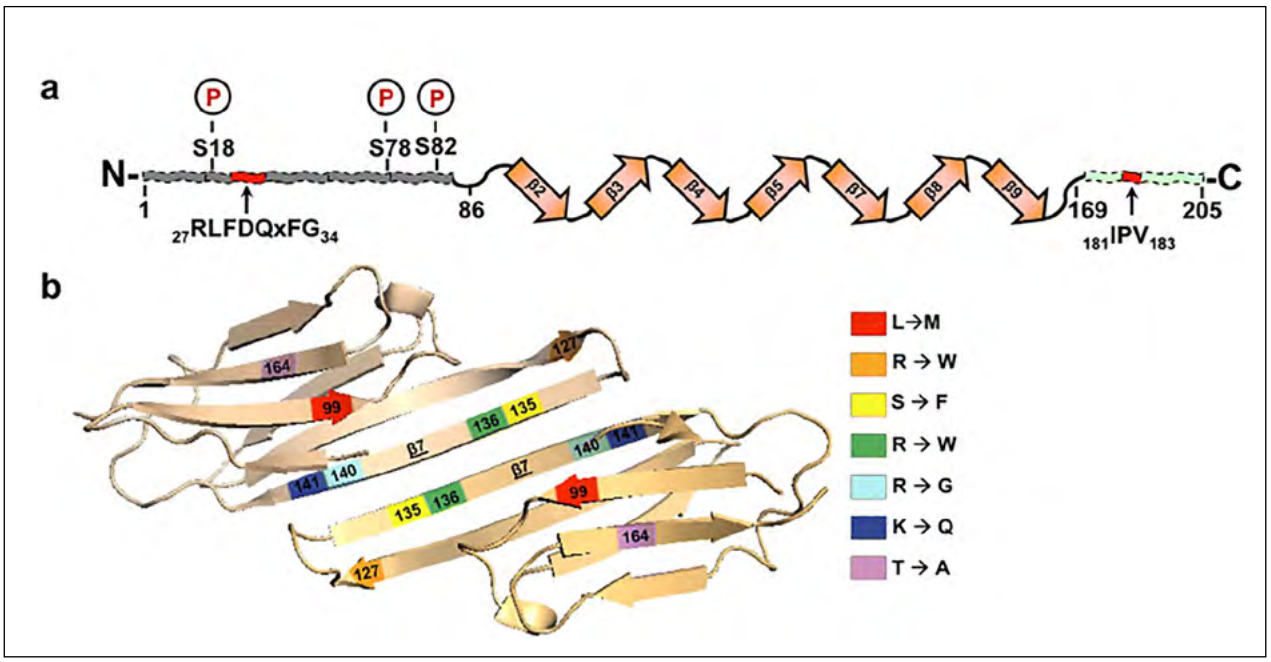

Figure 14. Representation of HSPB1 structure. (A) Primary structure: NTR (gray) has three phosphorylation sites and a conserved sequence of eight amino acids; ACD (orange) has seven β strands marked by arrows; CTR (green) has a conserved tripeptide (IPV) marked red. (B) Tertiary structure of HSPB1-ACD dimer. Marked with underlined β7 strands indicate the dimer interface with antiparallel alignment. Colored residues represent position of point mutations in HSPB1 associated with CMT (reproduced from the open source, from Muranova et al., 2020, mod.) [50].

stability of the cell [28,45,49,50]. These massive aggregates lead to the relocation of wild type BAG3 and HSPB1 proteins as well as other essential proteins, disrupting vital functions [38,45]. Additionally, the gain-of-function mutations reduce the solubility of each mutant, which may contribute to slower subunit exchange [38,49]. The importance of BAG3 and HSPB1 in regulating waste within cells makes them crucial for the chaperone network. The change in amino acid from proline to leucine in the IXI motif of both proteins causes diseases with severe clinical symptoms and aggregation-prone conditions [38,53]. Therefore, the significance of the IXI motif for BAG3 and HSPB1 and the similar pathological effects induced by the mutated motif hints at a potential association between DCM and CMT2. This calls for a closer understanding of the IXI motif and its role within the cell. Currently, specific details related to the role of the IXI motif in protein homeostasis within human cells are largely unknown.

The importance of the IPV motif in oligomerization has manifested itself in the self-interactions among sHSPs [34,49,54,55]. The IPV motif in the CTR binds to the ACD at an interdimeric level, facilitating the formation of larger oligomers and subunit exchange [6,49,55]. It outcompetes other protein partners of the ACD, promoting structurally stable oligomers [6,55]. It is known that sHSPs possessing this motif have the capability of forming larger oligomeric structures compared to sHSPs without this motif [9,55]. HSPB8 does indeed lack the IPV motif, but studies suggest that it can hetero-oligomerize with proteins containing the IPV motif, including other sHSPs and BAG3 [2,8,55-57]. Binding to BAG3 has been identified as essential for the chaperone capacity of HSPB8, which is reflected in its nature of predominantly forming a complex with BAG3 and Hsp70 as a dimer [2,55-57]. The dependency of HSPB8 on BAG3 may indicate the possibility of HSPB8 instability in the presence of a point mutation in the IPV motif of BAG3 [34,57]. The importance of the IPV motif in oligomerization further suggests the potential for a disruption in the oligomeric nature of HSPB8, other protein partners, and BAG3 itself [6,34]. Therefore, the role of the IPV motif in the sHSP family leaves implications for possible structural changes within HSPB8 when bound to P209L BAG3, which can harm the functioning of cardiomyocytes [34].

Due to its function and involvement in proteostasis, the IPV motif should be an area of interest for future studies that seek to discover the enigma behind protein aggregation in DCM and CMT2. A substantial portion of the existing evidence in this topic comes from in vitro, human models, and case studies, suggesting that, molecularly and clinically, DCM and CMT2 may be related to each other in humans. However, the abundance of transgenic mice models and in-vitro studies also point to some limitations in fully applying this association to the human field. We are hopeful that future research on the IPV motif may lead to uncovering the specific mechanism of DCM and CMT2 in humans and provide a complete picture of their relationship.

Acknowledgements

None.

Conflict of interest statement

The Authors declare no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

NY wrote the article; VLK and IFT suggested a topic and outline of the article; VLK made corrections in the article; SK supervised the medical part of the article.

Ethical consideration

The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki.

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