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Ubiquitin Receptors and Protein Quality Control

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Abstract

Protein quality control (PQC) is essential to intracellular proteostasis and is carried out by sophisticated collaboration between molecular chaperones and targeted protein degradation. The latter is performed by proteasome-mediated degradation, chaperone-mediated autophagy (CMA), and selective macroautophagy, and collectively serve as the final line of defense of PQC. Ubiquitination and subsequently ubiquitin (Ub) receptor proteins (e.g., p62 and Ubiquilins) are important common factors for targeting misfolded proteins to multiple quality control destinies, including the proteasome, lysosomes, and perhaps aggresomes, as well as for triggering mitophagy to remove defective mitochondria. PQC inadequacy, particularly proteasome functional insufficiency, has been shown to participate in cardiac pathogenesis. Tremendous advances have been made in unveiling the changes of PQC in cardiac diseases. However, the investigation into the molecular pathways regulating PQC in cardiac (patho)physiology, including the function of most ubiquitin receptor proteins in the heart, has only recently been initiated. A better understanding of molecular mechanisms governing PQC in cardiac physiology and pathology will undoubtedly provide new insights into cardiac pathogenesis and promote the search for novel therapeutic strategies to more effectively battle heart disease.

Keywords

ubiquitin; proteasome; Ubiquilin; p62; autophagy

1. Introduction

A polypeptide must attain and maintain a proper conformation via folding and at times refolding, in order to function properly in the cell. However, proper folding of a polypeptide often is a complex task requiring not only a correct amino acid sequence but also delicate collaboration among multiple factors. Misfolding is inevitable. Approximately one third of newly synthesized polypeptides in the cell never make it to mature proteins [1]. The cell has therefore evolved a sophisticated set of co-translational and post-translational mechanisms to ensure that a newly synthesized polypeptide is properly folded and that unfolded/ misfolded proteins are either repaired or removed in a timely fashion. The co- and post-

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translational mechanisms to repair and remove misfolded proteins are what protein quality control (PQC) commonly refers to [2].

Targeted protein degradation in the cell is primarily carried out by the ubiquitin-proteasome system (UPS) and the autophagic-lysosomal pathway. The UPS is responsible for the degradation of most cellular proteins. By targeted and timely degradation of unneeded normal proteins, the UPS regulates virtually all cellular processes and functions. This type of degradation is known as regulatory degradation. I κ B, p53, and β -catenin are among the *bona fide* UPS substrates [3]. By degrading specific individual misfolded/damaged proteins, the UPS plays a pivotal role in PQC. UPS-mediated proteolysis includes two essential steps: (1) ubiquitination which tags the target protein molecule with a chain of ubiquitin (Ub) molecules via a cascade of enzymatic reactions, and (2) the degradation of the ubiquitinated protein by the proteasome (Figure 1). Both steps are highly regulated by a number of processes and factors. For instance, other post-translational modifications (e.g., phosphorylation, acetylation, and sumoylation) of a substrate protein molecule can either promote or block the ubiquitination of the substrate. De-ubiquitination, which removes Ub from ubiquitinated proteins via deubiquitinating enzymes (DUBs), counters ubiquitination and helps process ubiquitinated proteins at the proteasome [4]. Moreover, ubiquitinated proteins may need assistance from a family of Ub receptor proteins to reach the proteasome and/or other destinies [5]. Finally, the activity of the proteasome appears to be highly regulated, although the regulatory mechanisms are largely not yet delineated [6].

Autophagy is a mechanism by which cytoplasmic material is degraded in the lysosomal compartment. Based on the way cytoplasmic material is delivered into the lumen of lysosomes, autophagy is commonly classified into 3 types: microautophagy, chaperone-mediated autophagy (CMA), and macroautophagy [7]. In microautophagy, cytoplasmic materials are internalized by invagination of lysosomal membrane which then pinches off as a single-membrane bound vesicle into lysosomal lumen. In CMA, individual cytosolic proteins harboring a KFERQ or KFERQ-like motif are recognized and bound by cytosolic chaperone heat shock cognate 70 (Hsc70) and cochaperones. The chaperone-substrate complex binds to LAMP-2A (lysosomal membrane associated protein 2A) where the substrate is unfolded and translocated into the lysosome via polymerized LAMP-2A, and finally degraded in the lysosome lumen. Macroautophagy is a cellular process by which a portion of cytoplasm, sometimes including organelles, is engulfed and segregated by a double-membrane enclosed structure known as an autophagosome, for delivery to and degradation by the lysosome. During nutrient deprivation, macroautophagy and CMA are sequentially activated to digest a portion of cytoplasm for fuel and/or for provision of free amino acids to sustain synthesis of other proteins, thereby helping the cell survive temporarily the starvation. At baseline and under certain stress conditions, CMA can selectively degrade individual misfolded proteins while macroautophagy removes defective organelles and perhaps protein aggregates [7].

PQC, either endoplasmic reticulum (ER) associated or ER-independent, is carried out by an elaborate collaboration between molecular chaperones and targeted protein degradation (Figure 2) [2]. Chaperones play a critical role in folding nascent polypeptides, especially the larger proteins. Chaperones can bind to misfolded/unfolded proteins to help repair/refold them and, if repair fails, escort the terminally misfolded proteins for degradation by the UPS or perhaps by CMA. Misfolded/unfolded proteins tend to form aberrant aggregates if their production overwhelms the chaperones and/or the UPS. Assisted by microtubules, these aggregates can converge at the microtubule organizing center and form aggresomes [8]. Aggresomes are specialized structures and their formation is likely a protective mechanism to segregate misfolded proteins in a location that reduces toxicity. Too, the formation of aggresomes may promote bulk degradation. Aberrant protein aggregates or aggresomes are

inaccessible to the proteasome but may trigger the activation of macroautophagy [2]. Aberrant protein aggregation has been shown to impair UPS proteolytic function [9, 10]. As an indispensable part of proteostasis, PQC is essential in virtually all aspects of cellular function. Mounting evidence also suggests that PQC dysfunctions play an important role in a variety of common and life-threatening diseases such as neural degenerative disease, cancer [11], and more recently heart disease [12]. This review updates recent advancement in PQC research, with an emphasis on the role of major Ub receptor proteins in targeting misfolded proteins to various proteolytic pathways critical to PQC.

2. Ubiquitination of misfolded proteins

Ub is the earliest described and best studied small modifier protein that can be covalently attached to the side chain of a lysine residue of the target protein via a process known as ubiquitination. This is carried out by an ATP-dependent cascade of enzyme reactions catalyzed by the Ub-activating enzyme (E1), Ub-conjugating enzymes (E2), and Ub ligases (E3) (Figure 2). Ubiquitination is utilized in many cellular pathways, both proteolytic and non-proteolytic, depending on the length and topology of Ub moieties. Usually, a chain of 4 or more Ub's linked through preferentially lysine 48 (K48) serves as a signal for degradation of the target protein by the proteasome [13]. The substrate specificity of ubiquitination is conferred by the E3. While a Ub E3 can be a single protein, it more often consists of multiple proteins as exemplified by the SCF (Skp1-Cullin1-Fbox) type of E3 complexes. An E3 enzyme contains the HECT, the RING, or the U-box domain [14].

A mature degradation signal (also known as a degron) in a protein molecule is required to trigger Ub E3 binding and subsequent ubiquitination of the protein. Therefore, factors that have an effect on degron maturation, on the availability and binding of the E3 to the target protein, or on the activation of the E3 can all modulate the attachment of Ub to target proteins. For instance, phosphorylation of I κ B and β -catenin at certain residues is required for the maturation of their degrons and subsequent degradation by the UPS [15].

The ER-associated PQC is responsible for the quality control of proteins passing through the ER. ER-associated degradation (ERAD) represents the critical final step and is performed primarily by the UPS. The ubiquitination step of ERAD is relatively well understood. Terminally misfolded ER proteins are sensed by ER resident chaperones, unfolded as they are being retro-translocated to the cytosol by the translocation complex on the ER membrane, and the unfolded polypeptide is immediately ubiquitinated by E3 complexes anchored on the ER membrane. HRD1 (also known as Synoviolin), Parkin, and CHIP (C-terminus of Hsp70-interacting protein) are among the identified E3s of ERAD in mammals [16].

By contrast, the degrons and corresponding Ub ligases of terminally misfolded cytosolic proteins are much less understood. Nonetheless, exiting progress has been made to address these areas in the past several years. Damaged or misfolded cytosolic or nuclear proteins likely trigger ubiquitination in multiple ways, such as the exposure of a cryptic degradation signal and/or a patch of hydrophobic residues that are normally buried in the interior of the native conformation [15]. In yeast, Ub ligase San1 targets misfolded nuclear proteins for degradation by recognition of exposed hydrophobicity in its substrates [17]. Exposure of as few as five continuous hydrophobic residues can trigger recognition by San1. The degradation targeted by San1 is fundamentally protective, as the same study showed the exposed hydrophobicity recognized by San1 can also cause aggregation and cytotoxicity [17]. Further, the nuclear located San1 appears to be able to participate in the degradation of cytosolic misfolded proteins with the assistance of heat shock protein (HSP) 70 to translocate the substrates to the nucleus [18, 19]. CHIP, a U-box type of E3, is capable of

interacting with HSPs as well, making it an important player in targeting misfolded cytosolic proteins for degradation in metazoans [20]. Indeed, CHIP knockout mice display accelerated aging [21]. In *S. cerevisiae*, ribosome-associated Ub ligase Ltn1 was shown to specifically target nascent non-stop polypeptides for degradation, in which the poly-lysine sequence derived from translation of the poly(A) tail of mRNA lacking a stop codon signals Ltn1 for ubiquitination [22]. Using model substrates and chemically imposed proteotoxic stress in yeast, Ubr1, a RING family Ub ligase and best known for its role in the “N-end rule” pathway, was recently demonstrated to target misfolded cytosolic proteins for degradation by the UPS in a chaperone dependent manner that was independent of its function in the well-described N-end rule [18, 23]. However, more recently, Fang et al. reported that cytosolic Hul5 (HECT Ub ligase 5), not Ubr1, plays a major role in the ubiquitination and degradation of cytosolic misfolded proteins in yeast [24]. Therefore, these recent discoveries suggest that multiple Ub ligases are responsible for targeting cytosolic misfolded proteins for degradation by the proteasome in yeast. It will be important to test whether the mammalian counterparts of these yeast E3s play a similar role in PQC in mammals. Consistent with a critical role of CHIP in cardiac PQC, CHIP deficient mice show exacerbated cardiac injury in response to myocardial infarction and inhibition of CHIP ligase activity promotes apoptosis in diabetic hearts [25, 26]. The Casitas b-lineage lymphoma (c-Cbl), an adaptor protein with an intrinsic Ub ligase activity, has been found to participate in the degradation of focal adhesion and myofibrillar proteins in cardiomyocytes [27]. AMP-activated protein kinase (AMPK) plays a central role in regulating metabolism in the heart during nutrient deprivation by activating not only autophagy but also the UPS. AMPK increases the expression of muscle atrophy F-box protein (MAFbx) or Atrogin-1 and muscle RING finger protein 1 (MuRF1) in the heart [28]. Mice deficient of MuRF1 develop more cardiac hypertrophy in response to pressure overload [29]. More recently, loss-of-function mutations of MuRF1 are linked to human hypertrophic cardiomyopathy [30], underscoring the importance of Ub ligases in cardiac function.

3. Deubiquitination in PQC

Ubiquitination of a specific protein in the cell is countered by deubiquitination. The latter removes Ub from ubiquitinated proteins by DUBs. The mammalian genome encodes ~100 DUBs but only a few of them have been studied. Nevertheless, DUBs are implicated in the regulation of numerous cellular functions, including cell cycle regulation, DNA repair, proteasome- and lysosome- dependent proteolysis, transcription, kinase activation, microbial pathogenesis, and more [31]. In most cases, DUBs contain not only catalytic domains but Ub binding domains and various protein-protein interaction domains as well. These modular domains contribute to the binding and recognition of different Ub chain linkages and help assemble multi-protein complexes that localize DUBs and assist in substrate selection [4]. Another recurring theme is that DUBs associate with Ub E3 containing complexes. There appears to be specificity in DUB and E3 pairing [4]. DUB association usually negatively regulates Ub chain length and substrate turnover [32]; however, an exception has been noted in the CHIP-Ataxin-3 pairing. Ataxin-3 is a recently identified DUB and its poly-glutamine (poly-Q) expansion has been linked to the neurodegenerative disease spinocerebellar ataxia type 3 (SCA3, also known as Machado-Joseph disease) [33]. Similar to CHIP, Ataxin-3 suppresses poly-Q pathogenicity [34]. Ataxin-3 does trim long Ub chains but not to chains of 4 or fewer Ubs [33, 35]. Counter intuitively, Ataxin-3 facilitates substrate degradation [36]. Emerging evidence suggests that Ataxin-3 collaborates with CHIP, and perhaps other E3s [37], to promote rather than inhibit clearance of ubiquitinated misfolded proteins [38]. The model posits that (1) upon engaging with a misfolded protein via HSPs, CHIP recruits E2 enzyme Ube2w, which in turn monoubiquitinates both CHIP and the HSP-bound misfolded protein; (2) Ataxin-3 is recruited to the monoubiquitinated CHIP via its Ub interacting motif (UIM) and is monoubiquitinated by Ube2w, which in turn stimulates

Ataxin-3's DUB activity [39, 40]; (3) likely a second E2 (e.g., UbcH5) is recruited to the complex and elongates the Ub chain on the misfolded protein; (4) once the Ub chain reaches 4 Ubs or longer, Ataxin-3 moves to bind the polyubiquitinated substrate and trims the polyubiquitin chain and/or limits further chain extension; (5) upon completion of ubiquitinating the misfolded protein by CHIP, Ataxin-3 deubiquitinates CHIP, thereby effectively stopping the ubiquitination cycle; and (6) after CHIP is deubiquitinated it becomes free for another round of substrate ubiquitination while Ataxin-3 might escort the ubiquitinated substrate to its destination [38]. In addition to promoting degradation of misfolded proteins, Ataxin-3 has also been found to promote aggresome formation of misfolded proteins [41]. These studies illustrate a significant role of DUBs in PQC.

DUBs in cardiovascular (patho)physiology have rarely been studied. Accompanied with increased total ubiquitinated proteins, mRNA and protein expression of ubiquitin carboxyl-terminal hydrolase (UCH) were significantly increased in end-stage failing human hearts with dilated cardiomyopathy [42]. More recently, Abro1 (also known as KIAA0157), a subunit of the BRISC (BRCC36-containing isopeptidase complex) DUB enzyme, was found predominantly expressed in the heart. Furthermore, Abro1 expression is significantly upregulated in mouse hearts with myocardial ischemia/reperfusion. Compared with tissue samples from healthy donor hearts, Abro1 protein expression is markedly increased in the infarct area but not the remote area of explanted human hearts with ischemic heart disease. This study further demonstrated that upregulation of Abro1 leads to K63-specific deubiquitination of specific protein targets [43], consistent with the known DUB property of BRISC [44]. The pathophysiological significance of altered expression of DUBs in the heart remains to be investigated.

4. Ub receptors and delivery of polyubiquitinated proteins to the proteasome

The 19S proteasome subunit Rpn10/S5a serves as an intra-proteasomal polyubiquitin receptor. It binds polyubiquitin via its Ub interacting motif (UIM). Hence, some proteins after being ubiquitinated, especially those located in the immediate vicinity of the 26S proteasome, may be recognized and degraded by the proteasome directly. However, perhaps more often, ubiquitinated proteins require the assistance of extra-proteasomal Ub receptors to be delivered to the proteasome. These Ub receptors must be able to bind polyubiquitinated proteins as well as directly interact with the proteasome. The UBA-UBL family proteins, such as Rad23 and the Ubiquilin (Ubqln) family (mammalian orthologs of yeast Dsk2), are ideally suited for this task. They are characterized by a Ub-like (UBL) domain at the N-terminus and a Ub associated (UBA) domain at the C-terminus [5]. The UBA domain binds polyubiquitinated proteins while the UBL domain can bind to the 26S proteasome through the Rpn10/S5a subunit UIM of the 19S proteasome [45]. Interestingly, p62/SQSTM1 has a C-terminal UBA domain and could serve as a Ub receptor as well, although it does not contain a *bona fide* UBL domain. Rather, the N-terminal of p62 has a Phox/Bem1p (PB1) domain that folds similarly to the UBL domain and can also interact with the proteasome via S5a [46, 47].

Very few reported studies have investigated into the delivery of ubiquitinated proteins to the proteasome or other proteolytic pathways in the heart. This area of research should be emphasized in the endeavor to better understand cardiac PQC and its role in cardiac pathogenesis. As implicated frequently by the co-existence of increased total ubiquitinated proteins with normal or even increased proteasome activities in diseased hearts, the delivery of ubiquitinated proteins to the proteasome appears to be deficient in diseased hearts [10, 48, 49].

4.1 The Ubiquilin family of Ub receptors

The human genome contains five Ubiquilin genes: *Ubqln1*, *2*, *3*, *4*, and *Ubqln1* (Ubiquilin-like), located on 4 different chromosomes. Ubiquilin genes are highly conserved among mammals. *Ubqln1* is ubiquitously expressed; *Ubqln2* and *4* are expressed in most tissues including the heart; however, *Ubqln3* is only expressed in testis [50]. Ubqln proteins share a high degree of sequence and domain structural homology. They all harbor a UBA domain in the C-terminus and a UBL domain in the N-terminus. Located between the UBL and UBA domains is a central region containing multiple STI1 (stress-inducible heat shock chaperonin-binding motif) motifs that may confer Ubqln with chaperon-like functions (Figure 3A). Hence, Ubqln is purported to accelerate the delivery of polyubiquitinated proteins, such as presenilin-1 (PS1) and mutant Huntingtin, to the 26S proteasome for degradation [51].

Ubqln1 intronic polymorphism has been associated with increased risk for late-onset Alzheimer's disease (AD), possibly through affecting *Ubqln1* alternative splicing in the brain [52]. Immunohistochemistry using anti-Ubqln1 antibody showed increased expression of Ubqln1 in neurofibrillary tangles and Lewy bodies of AD and Parkinson's disease affected brains, respectively [53]. Multiple missense mutations of *Ubqln2* were recently linked to dominant X-linked juvenile and adult onset amyotrophic lateral sclerosis (ALS) and ALS/dementia. The *Ubqln2* mutations were found to impair protein degradation [54]. A more recent report showed that primary lung adenocarcinomas have more *Ubqln1* mRNA than adjacent normal lung tissue and higher *Ubqln1* mRNA levels are associated with shorter survival of lung cancer patients [55]. These lines of clinical evidence strongly suggest an important role for Ubiquilin in human pathogenesis and underscore the significance to better understand the function of the Ubiquilin family in physiology and pathology.

Mutations of PS1 and PS2 are linked to both early-onset AD and dilated cardiomyopathy [56, 57]. Ubqln1, also known as PLIC-1 (protein linking integrin associated protein with cytoskeleton 1), is a human homolog of yeast Dsk2. Ubqln1 is a cytosolic protein and was identified as a PS1 and PS2 interacting protein that promotes PS protein accumulation in the cell [53]. It was subsequently shown that overexpression of Ubqln1 decreases the degradation of co-expressed PS2, likely through the binding of the Ubqln1 UBA domain to the Ub chains conjugated to PS2. Ubqln1 proteins colocalize with Ub positive structures in cells and Ubqln1 proteins are present within the inner core of aggresomes [58]. Additionally, Ubqln1 UBA domain shows similar binding affinity to K48 and K63 linked polyubiquitin chains [59]. More recent studies show that Ubqln1 is upregulated in the cell by increased protein misfolding and regulates aggresome formation via its UBL domain [60]. The role of Ubqln1 in AD pathogenesis is not limited to its influence on PS1 and PS2, where it participates in the processing of the amyloid precursor protein (APP), as additional findings reflect that Ubqln1 may function as a molecular chaperone to modulate APP trafficking and Abeta secretion. In this way it could protect against aggregation of APP and thereby reduce toxicity associated with APP [61, 62]. The association of *Ubqln1* polymorphisms with late-onset AD may be rare, but Ubqln1 protein levels are significantly decreased in late onset AD patient brains [62]. Taken together, these findings suggest that diminished Ubqln1 function may contribute to AD pathogenesis. Additionally, Ubqln1 is involved in ERAD as part of a trimeric complex with erasin and p97/VCP [63], consistent with its proposed role as a link between ubiquitination machinery and the proteasome [64]. In addition to delivery of ubiquitinated proteins to aggresomes or aggregates [60, 65], Ubqln1 also plays a role in delivery of proteins to lysosomes via both macroautophagy and CMA. Ubqln1 is degraded during macroautophagy and CMA [66]. Ubqln1 may also facilitate autophagosome maturation and promote cell survival during nutrient starvation [67]. In cellular and invertebrate models of Huntington disease, Ubqln1 protects against poly-Q-induced cell

death with unknown mechanisms [68, 69]. More recently, Ubqln1 was found to interact with an anti-apoptotic BCL2-like protein BCL2L10/BCLb, and promote monoubiquitination and stability of BCLb in human cancer cell lines [55].

Contradicting to findings from most studies using cell culture and *C. elegans* that suggest a protective role of Ubqln1 against neurodegeneration, a recent study using *Drosophila* indicates that Ubqln interacts and antagonizes PS [70]. Ubiquitously silencing the *Ubqln* gene in somatic cells is lethal during early development, indicating that Ubqln is essential to *Drosophila* early development. Silencing *Ubqln* in the developing wing results in viable flies with wing defects. The wing defects were attenuated by decreasing PS expression and exacerbated by overexpression of PS. Similarly, early pupal lethality resulting from the silencing of *presenilin* is partially rescued by simultaneous silencing of *Ubqln* [70].

Ubqln1 can yield at least 4 alternative splicing variants (TV1 through TV4) in human brain [71]. Compared with the full length (i.e., TV1), TV2 lacks exon 8 which harbors one of the four STI motifs. TV3 lacks exons 2, 3, and 4, and therefore is missing the majority of the UBL domain and one STI1 motif. TV4 contains the first 3 exons and a 32 amino acid insertion resulting from the frame-shift after the exon 3/5 junction (Figure 3A). Most of the experimental studies on Ubqln1 published so far used TV1; hence the functional significance of TV2, TV3, and TV4 is much less clear. Nevertheless, emerging evidence suggests both overlapping and distinctive functions among TV1, TV2, and TV3. Under tunicamycin-induced ER stress, TV1, TV2, and most prominently TV3, but not TV4, were shown to attenuate the induction of proapoptotic C/EBP homologous protein (CHOP) and improve cell survival. This suggests that like TV1, TV2 and especially TV3 can confer cytoprotection under certain stress conditions. The *Ubqln1* intronic polymorphism associated with late-onset AD increases the expression of TV2 in patient brains [52]. Full length Ubqln1 but not TV2 promotes the aggregation of and co-aggregates with TDP43 (43-kDa TAR DNA-binding domain protein) [65], a major protein present in the Ub-positive cytoplasmic aggregates in neurons of patients with frontal-temporal lobular dementia and ALS. A study using *Drosophila* has demonstrated that like overexpression of *Drosophila* ubiquilin, overexpression of either human full length *Ubqln1* or the AD-associated allele (i.e., TV2) in the *Drosophila* eye, leads to adult-onset age-dependent retinal degeneration. More importantly, the retinal degeneration is more severe when TV2 is overexpressed [70]. It has been reported that the UBL domain of Ubqln1 regulates aggresome formation in a cell model of poly-Q disease [60]. Given that TV3 lacks most of the UBL domain, TV3 may not be able to promote aggresome formation. However, Viswanathan and co-workers showed recently that co-expression of TV3, but not TV1, stabilize full-length PS1 and that co-expression of PS1 with TV1 or TV3 enhances aggresome formation [71]. It remains to be fully elucidated whether changes in the expression of Ubqln1 variants account for its multifaceted functions reported thus far in a variety of cellular processes. It should also be pointed out that studies on Ubqln functions reported to this point have used either cell cultures or invertebrate animals. Conclusions drawn from these studies sometimes contradict one another and remain to be confirmed and/or clarified in higher species.

We have recently examined protein expression of Ubqln1 in the heart of a mouse model of desmin-related cardiomyopathy (DRC) that was created by cardiomyocyte-restricted overexpression of a human desmin-related myopathy linked 7-amino acid deletion (R172-E178) mutant desmin (D7-des) [72]. As shown in Figure 3B, the non-transgenic mouse hearts express the full length Ubqln1 (i.e., TV1) and two additional higher molecular weight species with an apparent molecular weight of approximately 80 and 90 kDa, respectively. In the D7-des transgenic hearts, the TV1 and the 80 kDa species were markedly upregulated; and more interestingly, two other species that are smaller than TV1 and not expressed in the non-transgenic controls, were also prominently expressed with even a greater abundance

than TV1. It is unknown but will be important to determine whether the higher molecular weight species are post-translationally modified Ubqln1 and too, if the upregulated lower molecular weight species are shorter alternative splicing forms of Ubqln1 or simply proteolytic products of the higher molecular weight species. The functional significance of Ubqln in cardiomyocytes and the heart remains to be explored.

4.2 p62/Sequestosome-1

p62/SQSTM-1 contains a C-terminal UBA domain and an N-terminal PB1 domain. The latter exhibits self-aggregation, implicating a role for p62 in the aggregation of ubiquitinated proteins. The UBA domain of p62 preferentially binds K63-linked Ub chains. p62 also contains a LC3-interacting region (LIR), linking p62 to autophagosome formation [73–75]. Hence, p62-mediated aggregation may prepare ubiquitinated proteins for macroautophagy-mediated removal, thereby protecting the cell from the more toxic free form or oligomeric species of misfolded proteins [76–78]. However, this postulate remains to be tested in the heart [79, 80]. Whether p62 mediated accumulation and aggregation of ubiquitinated proteins are protective or detrimental appears to depend on a number of variables including tissue type and functional status of macroautophagy [81]. p62 is degraded by macroautophagy and thus accumulates in the cell upon autophagy inhibition. Upregulation of p62 during proteasome inhibition may contribute to compensatory autophagy activation but paradoxically, accumulation of p62 during chronic autophagy inhibition has been shown to hinder the delivery of ubiquitinated proteins to the proteasome and thereby impair their degradation by the proteasome [82]. This suggests that p62 may serve a unique context-dependent role in the cross-talk between the UPS and macroautophagy (Figure 4C), though whether this is the case in cardiomyocytes remains to be explored.

Mutations in the *SQSTM1* gene that cause loss of the UBA domain or change the amino acid sequence in the UBA domain of the p62 protein have been linked to human Paget disease of bone. Additionally, most recently, a number of novel missense and deletion mutations of the *SQSTM1* have been found in patients with familial or sporadic ALS [83]. The disease has also been linked to mutations in the UBA-containing proteins Ubqln2 (*see section 4.1 for details*) and Optineurin [84], and further suggests that mishandling of ubiquitinated proteins might be an important pathogenic mechanism of ALS.

Multiple lines of evidence support p62 as a sensor of proteotoxic stress. First, expression of aggregation-prone proteins impairs the proteasome but upregulates p62 transcripts and proteins [79, 85, 86]. Second, p62 is selectively degraded by autophagy but not the UPS [87], and accumulates upon autophagic or lysosomal inhibition [88, 89]. Third, pharmacologically induced proteasome inhibition also increased p62 expression [90, 91]. Finally, p62 is increased in mouse hearts with defects in UPS- and/or autophagy-mediated proteolysis [89, 92].

The transcription of p62 can be regulated by NRF2 (nuclear factor erythroid-derived 2-related factor 2), a transcription factor that regulates the expression of a number of anti-oxidant genes (Figure 4A). In non-stressed condition, NRF2 protein is constitutively degraded by the UPS under the control of the cullin3-Keap1 Ub ligase complex. The interaction between Keap1 and NRF2 is disrupted by oxidative stress, resulting in the stabilization and activation of NRF2 [93, 94]. In turn, NRF2 binds the antioxidant responsive element (ARE) in the p62 promoter and activates p62 expression [95]. Interestingly, it was shown recently that when upregulated, p62 interacts with Keap1 at the NRF2 binding site, consequently freeing up NRF2 and allowing its activation of downstream genes, including p62. Therefore, p62 can control its own expression in a feed-forward fashion by regulating NRF2 activation [95].

The (patho)physiological significance of p62 in PQC resides largely in its mediating the formation and selective degradation of aggresomes. According to a recent model (Figure 4B) [96], p62 recognizes the non-functional ubiquitinated proteins through its UBA domain and promotes protein aggregation via its ability to oligomerize through PB1 domain, thus reducing the toxicity of soluble misfolded proteins. Finally, p62 helps deliver the aggregates for autophagic degradation through both LC3-interacting region (LIR) and PB1 domains [97]. In many organs/systems, including the heart, this model has not been fully established, but it is well supported by recent findings that expression of misfolded proteins in cardiomyocytes upregulates p62 in the heart and too, that p62 knockdown reduces the formation of protein aggregates and ubiquitinated proteins and aggravates stress-induced cell injury in cultured cardiomyocytes [79].

The activity of p62 in selective autophagy appears to be controlled by its phosphorylation status. Phosphorylation at Ser403 of the p62 UBA domain, as mediated by casein kinase 2 (CK2), enhances the affinity of the UBA domain for polyubiquitin chains and thus promotes inclusion body formation and efficient autophagic degradation of polyubiquitinated proteins [98].

5. Proteasome-mediated degradation

The proteasome is a large multi-subunit protease found in the cytosol, both free and attached to the ER, and in the nucleus of eukaryotic cells [99]. Generally, a functional proteasome consists of two subcomplexes: a 20S proteolytic core particle and the regulatory particle that binds at one or both ends of the 20S. The regulatory subcomplex can be the 19S proteasome, the 11S proteasome, or both [100, 101]. It is generally believed that the 19S associated 20S proteasome (i.e., the 26S) mediates housekeeping protein degradation. Some studies, but not others, have suggested that the 20S is sufficient to degrade a subset of proteins (e.g., oxidized proteins) independent of the 19S proteasome and ubiquitination [102]; however, it is generally accepted that the degradation of a polyubiquitinated protein requires the 19S. The 19S recognizes and binds the polyubiquitinated substrate or its shuttle, deubiquitinates the substrate for Ub recycling, and unfolds the substrate and channels the unfolded polypeptide into the 20S proteasome where proteolysis takes place. As described in *Section 1.4*, some polyubiquitinated proteins are recognized and taken by the proteasome directly, while others require delivery by UBL-UBA bivalent shuttles to reach the 19S proteasome. The direct uptake is likely mediated by the direct interaction of the UIMs of Rpn10/S5a and Rpn13 with the polyubiquitin chain of the substrate, as Rpn10 shows marked preference for polyubiquitin over UBLs. The polyubiquitinated substrate-bearing shuttles (e.g., Rad23, Dsk2) appear to dock directly onto Rpn1. In the proteasome, Rpn1 is directly bound by Rpn10 and this binding is stabilized by the association of Rpn2. Through Rpn2, Rpn13 binds to this complex [103]. Rpn10 is a nonconventional proteasome subunit, existing in intra- and extra- proteasomal pools. Extra-proteasomal Rpn10 has been shown to restrict the access of shuttle protein Dsk2 to the proteasome, alleviating stress imposed by overexpressed Dsk2 [104].

RPN11, a stoichiometric DUB subunit of the 19S proteasome, is responsible for the removal of the Ub chain during proteasome-mediated degradation of polyubiquitinated proteins [105]. RPN11 removes the Ub chain *en bloc* by cutting at the base of the ubiquitin chain, recycles ubiquitin, frees the substrate, and promotes substrate translocation into the 20S proteasome. Mammalian 19S proteasomes are reversibly associated with two other DUBs, UCH37 and USP14. In contrast to RPN11, USP14 and UCH37 trim the Ub chain from its tip distal to the substrate, therefore shortening the chain rather than removing the chain *en bloc*. Recently, a small chemical compound (IU1) capable of inhibiting USP14 deubiquitination was shown to enhance proteasome mediated degradation of some

substrates, including several proteins associated with neural degenerative diseases [106]. This represents the invent of the first pharmacological proteasome activator, although proteasome inhibitors are used clinically to treat certain forms of cancer [107]. It remains to be determined whether IU1 or alike can enhance proteasome function in animals, and whether it is effective in treating disease with proteasome functional insufficiency (PFI).

The 11S proteasome is formed by either homopolymerization of PA28 γ or heteropolymerization of PA28 α and PA28 β . The association of the 11S with the 20S was studied extensively as a player in antigen processing that modulates peptide cleavage in the 20S [108, 109]. However, it is now recognized that the 11S may play a greater role than promoting antigen processing [110]. Our laboratory has recently demonstrated that PA28 α is essential to the degradation of a missense (R120G) mutant α B-crystallin (CryAB^{R120G}), a *bona fide* misfolded protein linked to human disease [111]. We have further shown that forced PA28 α overexpression (PA28 α OE) suffices to benignly enhance proteasome-mediated removal of a surrogate as well as a *bona fide* misfolded protein in cultured cardiomyocytes and transgenic mice, via up-regulating 11S proteasomes and likely increasing hybrid proteasomes [111, 112].

Three peptidase activities, chymotrypsin-like, trypsin-like, and caspase-like, are identified in the 20S proteasome and harbored in the β 5, β 2, and 1 subunits, respectively. Upon viral infection or treatment of interferon γ , these subunits can be replaced by their respective inducible counter parts: β 5i, β 2i, and β 1i, forming immunoproteasomes. Immunoproteasomes display higher peptidase activities and presumably enhance antigen processing during viral infection. Interestingly, interferon stimulation also increases oxidative stress to the cell, resulting in an increase in the production of polyubiquitinated proteins and protein aggregates when the immunoproteasome is impaired [113]. The immunoproteasome seemingly facilitates the clearance of damaged proteins and protects against oxidative stress [113–115]. The induction of synthesis of immunoproteasomes, PA28 α and PA28 β , as well as 20S proteasomes by oxidative stress has been observed [115]. These new findings suggest a novel role of 11S proteasomes and immunoproteasomes in PQC under stress conditions.

In yeast, pharmacological and genetic inhibition of the proteasome triggers Rpn4-mediated transcriptional upregulation of proteasome genes, increasing new proteasome synthesis to enhance recovery of proteasome activities [116]. A concerted increase in the synthesis of proteasomal subunits in response to proteasome inhibition was also observed in cultured mammalian cells and implicated in the heart with PFI [117, 118]. Nuclear factor erythroid-derived 2-related factor 1 (NRF1) was recently demonstrated to mediate this feedback loop in mouse embryonic fibroblasts and mouse brain [119, 120]. NRF1 is constitutively degraded in both the cytoplasm, likely via the Hrd1-p97/VCP mediated ERAD pathway, and in the nucleus by β TrCP mediated ubiquitination and degradation [121]. Another F-box protein, Fbw7, was also shown to mediate NRF1 degradation [122]. Upon proteasome inhibition, NRF1 protein accumulates and translocates to the nucleus where NRF1 binds to the antioxidant response elements (ARE) in the promoter of proteasome genes [123]. The role of NRF1 in cardiac PQC remains to be determined.

Proteasome activities are not only determined by proteasome abundance and associated partners, but are also regulated by posttranslational modifications (PTMs) of proteasome subunits. A myriad of PTMs that occur to the proteasome was comprehensively reviewed recently by Scruggs *et al.* [6]. It will be extremely important to delineate the signaling pathways that regulate these PTMs as well as the functional consequence and (patho)physiological significance of the PTMs in cardiac proteasomes.

Proteasome dysfunction is associated with common and devastating diseases; hence, normalizing proteasome function is potentially an important therapeutic strategy to treat these diseases. A better understanding of proteasome function regulation is essential to developing such a strategy.

6. Lysosomal degradation in PQC

Among the three types of autophagy, CMA and macroautophagy are capable of targeted removal of misfolded proteins and thereby play an important role in PQC. Approximately 30% of cytosolic proteins carry a KFERQ motif. In most cases, this pentapeptide motif recognized in CMA is likely buried by the folding of native proteins. However, misfolding, partial unfolding, or in some cases conformational changes resulting from PTMs can all potentially expose the CMA targeting motif and trigger CMA [124]. Hence, CMA can selectively target specific misfolded protein molecules for degradation by the lysosome. CMA activities are reduced during aging due mainly to decreases of LAMP-2A protein abundance, the substrate receptor and translocator for CMA. Restoration of CMA by preventing the decrease of LAMP-2A in aging livers has been shown to improve the ability of hepatocytes to handle protein damage and preserve liver function in aged mice [125]. Blockade of CMA accumulates oxidized proteins and protein aggregates in a cell and renders the cell more vulnerable to a variety of stressors [126]. Cells with impaired macroautophagy show constitutive activation of CMA [127]. Several neurodegenerative disease associated proteins, in their wild type form, are degraded by CMA but their mutant forms often impair CMA [128, 129]. The role of CMA in cardiac physiology and pathophysiology remains to be explored.

Extensive attention has been drawn to macroautophagy in the past several years. Macroautophagy delivers substrates to the lysosome via autophagosomes. The formation of autophagosomes is regulated by a cascade of events, including Atg7 (autophagy-related protein 7)-dependent conjugation of Atg5 to Atg12, and subsequent lipidation of LC3I (light chain 3 I) to form LC3II. The conversion of LC3I to LC3II is coupled with the translocation of LC3 from the cytosol to autophagic membranes. LC3II remains with autophagosomes as they fuse with lysosomes to form autolysosomes. Thus, LC3II protein levels and distribution, including the use of a variety of fluorescence protein fused LC3 [130–132], are widely utilized to assess autophagosomes and/or autolysosomes. Their capabilities have contributed to the explosion of this area of research.

Notably, many earlier reports claimed activation or increase of macroautophagy based solely on a snapshot of increased abundance of autophagosomes, without examining the dynamics of the whole process. The conclusion of these studies requires re-examination as the presence of more autophagosomes may result from either increased production or decreased clearance. The latter can be caused by impaired fusion of autophagosomes with lysosomes or lysosomal deficiency. Hence, autophagic flux must be carefully assessed to decipher autophagic activities.

In addition to the essential role of non-selective macroautophagy in dealing with energy crisis, selective macroautophagy facilitates cytoplasmic quality control by targeted removal of defective or surplus organelles such as mitochondria (mitophagy) or protein aggregates (aggrephagy). The best characterized pathway for mitophagy is that mediated by PTEN-induced putative protein kinase 1 (PINK1) and Ub ligase Parkin. PINK1 accumulated on damaged mitochondria recruits cytosolic Parkin to the depolarized mitochondria via an unknown mechanism [133]. The translocated Parkin then promotes ubiquitination of mitochondrial proteins. Parkin is capable of catalyzing the formation of both K48 linked and K63 linked Ub chains [134]. There is significant evidence to support a role of both lysine

linkages in triggering mitophagy. One model posits that Parkin mediates K48-linked ubiquitination and proteasomal degradation of mitochondrial membrane proteins, thereby triggering mitophagy. Supporting this model, proteasome inhibition blocks mitophagy of damaged mitochondria [135, 136]. A second model emphasizes the importance of Parkin in promoting the K63-linked polyubiquitination of mitochondrial substrate(s) to recruit the ubiquitin receptor p62/SQSTM1, to the mitochondria. The UBA domain of p62 preferentially binds K63 Ub chains and through polymerization via its PB1 domain, p62 mediates the clustering of damaged mitochondria, in a manner analogous to the aggregation of polyubiquitinated proteins. It has been proposed that p62 helps recruit phagophore to damaged mitochondria through interacting with LC3 in the phagophore; however, a recent study shows that p62 mediates Parkin-triggered mitochondrial clustering but is dispensable for mitophagy [137]. Emerging evidence has also raised the possibility that PINK1 and Parkin may be able to directly recruit autophagic machinery to the mitochondria [134]. Consistent with its quality control role, mitophagy protects the heart under stress conditions. Huang *et al.* reported a critical role of Parkin and p62 mediated mitophagy in cardiac protection by ischemic preconditioning [138]. A mechanism for the p53-TIGAR (TP53-induced glycolysis and apoptosis regulator) pathway to exacerbate ischemic cardiac damage appears to inhibit mitophagy [139].

Aggrephagy is a term coined for selective disposal of protein aggregates by macroautophagy [140]. When misfolded proteins escape from the surveillance of the proteasome and presumably CMA, they form aberrant aggregates. The smaller aggregates can further converge into larger aggregates which can be transported via microtubules to the microtubule organizing center to form aggresomes. The latter are considered a protective structure to segregate harmful misfolded proteins to a place more suitable for lysosomal degradation via aggrephagy. Polyubiquitin conjugates are enriched in aberrant aggregates and aggresomes. Proteins such as p62, ALFY (autophagy-linked FYVE protein), and likely NBR1 (neighbor of BRCA1 gene) are frequently found in protein aggregates and are believed to be there because they mediate both the construction and degradation of the aggregates. As a Ub receptor, p62 can bind polyubiquitinated proteins via its UBA domain. Self-polymerization of p62 via its PB1 domain may contribute to the clustering of smaller aggregates to larger ones such as aggresomes. As mentioned earlier, p62 can recruit autophagosome membrane to the aggregates by interacting LC3 via its LIR motif. In DRC mouse hearts, both p62 and macroautophagy are concurrently upregulated [79, 141]. The upregulation appears to be adaptive, as reducing autophagy exacerbated CryAB^{R120G}-based DRC in mice [141]. In cultured cardiomyocytes, p62 knockdown decreased aggresome formation and LC3-II levels, and aggravated cytotoxicity caused by overexpression of DRC-linked mutant proteins [79]. Moreover, increasing macroautophagy by Atg7 overexpression reduced aggregates and cytotoxicity caused by CryAB^{R120G} overexpression in cultured cardiomyocytes [142].

Notably, electron microscopic examination of DRC mouse hearts which contain abundant large protein aggregates, could detect autophagosomes containing amorphous electron dense materials or mitochondria but could not identify typical autophagosomes that engulf a large protein aggregate, even after lysosomes were inhibited [79, 143]. This implicates that in at least cardiomyocytes, large protein aggregates are not directly removed by autophagy, but rather through two alternative scenarios: either macroautophagy catches small protein aggregates on their way to the aggresomes or misfolded proteins are broken off aggresomes before they can be engulfed by autophagosomes.

7. PQC inadequacy in cardiac pathogenesis

The existence of PQC inadequacy in cardiac disease is best supported by seminal findings that pre-amyloid oligomers are prevalent in a large subset of failing human hearts with idiopathic cardiomyopathy, but not in normal control hearts [57, 144]. The significance of PQC inadequacy in pathogenesis is well illustrated by proteinopathy, a family of disease caused by increased production and/or decreased removal of misfolded proteins and featured by the presence of aberrant protein aggregates in the affected cells. A *bona fide* cardiac proteinopathy is DRC [145]. DRC is the cardiac aspect of desmin-related myopathy and is pathologically characterized by the presence of desmin-positive protein aggregates in cardiomyocytes [12]. Assisted by UPS function reporter mice [146], severe cardiac PFI has been detected in mouse models of DRC [10, 147]. The defect resides primarily in delivery of ubiquitinated proteins into the proteasome as increased ubiquitin conjugates are concurrent with increased peptidase activities of the 20S proteasome in these mouse hearts. Additional studies have suggested that aberrant protein aggregation plays an important role in causing PFI in DRC hearts [10, 148]. Cardiac PFI resulting from acute regional myocardial ischemia/reperfusion injury was recently unveiled [149]. PFI and an impaired autophagic-lysosomal pathway were suggested in a mutant myosin-binding protein C knock-in mouse model of hypertrophic cardiomyopathy [150]. Impaired autophagosome clearance in cardiomyocytes was observed in experimental myocardial ischemia/reperfusion injury [151]. These reports illustrate the commonality of PQC inadequacy in animal models of heart diseases.

The establishment of a mouse model of a benign enhancement of cardiac proteasome function by overexpression of PA28 α in cardiomyocytes has made it possible for the first time to demonstrate the pathophysiological significance of PFI [111]. Enhancing proteasome function by overexpression of PA28 α significantly diminished aberrant protein aggregation and delayed the premature death of a mouse model of DRC; moreover, overexpression of PA28 α markedly reduced myocardial I/R injury in intact mice [111]. Consistent with these findings, prevention of proteasome impairment was shown to be essential to the protective role of ischemic preconditioning [152]. These are very important advancements although DRC *per se* is not a common disease. This is because the DRC mice represent valuable models for pathogenic elucidation and therapeutic exploration of cardiac proteinopathies. Increasing reports suggest that a large subset of common heart diseases likely share pathogenic mechanisms with cardiac proteinopathy. Aberrant protein aggregates are also observed and considered the trigger of autophagy activation in the pressure overloaded mouse heart [153]. Significant increases in myocardial ubiquitinated proteins are invariably observed in failing human hearts and virtually all animal models of heart diseases, including pressure overloaded hearts [12]. Both elevated levels of ubiquitinated proteins and the presence of aberrant protein aggregates are consistent with PFI, but the sufficiency of proteasome function in a pressure overloaded heart remains to be directly tested.

The molecular pathways by which PQC inadequacy causes cardiac remodeling and failure have yet to be determined. Nevertheless, PQC inadequacy caused by either proteasome inhibition or deficiency of CryAB, the most abundant small HSP in the heart, can activate the calcineurin-NFAT (the nuclear factors of activated T-cells) pathway [154, 155], a well-established signaling pathway for cardiac pathological remodeling [156].

8. Concluding remarks

Targeted proteolysis including proteasome-mediated degradation, CMA, and selective macroautophagy plays a pivotal role and serves the final line of defense in PQC in the cell. Ubiquitination and subsequently Ub receptor proteins, such as p62 and Ubqln, appear to be

common factors for the triage of misfolded proteins to specific quality control destinies, including the proteasome, lysosomes, and perhaps aggresomes, as well as for triggering mitophagy to remove defective mitochondria. PQC inadequacy, particularly cardiac PFI, has been shown to participate in cardiac pathogenesis. However, molecular pathways regulating PQC in cardiac (patho)physiology, including the function of most Ub receptor proteins in the heart, has just begun to be investigated. Notably, systemic administration of proteasome inhibitors was shown by some studies to suppress cardiac hypertrophy without affecting heart function [157, 158]. This seems to contradict the central role of the UPS in cardiac PQC and raises a controversy that warrants further investigation. A better understanding of molecular mechanisms governing PQC in cardiac physiology and pathology will undoubtedly yield new insights into cardiac pathogenesis and shine light on the search for novel therapeutic strategies to more effectively combat heart disease, the leading threat to human health and life.

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Highlights

- Protein quality control minimizes the level and toxicity of misfolded proteins.
- Failing human hearts often display protein quality control inadequacy.
- Substrate delivery to the proteasome appears to be deficient in diseased hearts.
- Ubiquitin receptors help deliver ubiquitinated proteins to the proteasome.
- The role of ubiquitin receptors in cardiac (patho)physiology is yet to be defined.

Figure 1. An illustration of the ubiquitin-proteasome system-mediated proteolysis

A substrate protein molecule is first covalently tagged with a chain of ubiquitin (Ub) protein molecules, a process known as ubiquitination which is performed by a cascade of enzymatic reactions catalyzed sequentially by E1 (Ub activating enzyme), E2 (Ub conjugating enzyme), and E3 (Ub ligase). The conjugated Ub can be removed from the substrate via a process known as deubiquitination which counters ubiquitination and is performed by deubiquitinating enzymes (DUBs). Ubiquitinated substrates may be directly recognized and bound by Rpn10/S5a of the 19S proteasome, but often require extraproteasomal Ub receptor proteins (i.e., UBA-UBL proteins) to be delivered to the 26S proteasome (26S) and degraded by the latter.

Figure 2. An illustration of intracellular protein quality control (PQC)

Chaperones assist in protein folding and help to maintain protein integrity. The ubiquitin-proteasome system (UPS) is responsible for the degradation of most proteins, normal and abnormal, in the cell. The highly regulated process involves attachment of a ubiquitin (Ub) chain by E1 (Ub activating enzymes), E2 (Ub conjugating enzymes), and E3 (Ub ligase) to the targeted protein, a process known as ubiquitination. The Ub-tagged protein is then transferred to the proteasome for degradation. Recognition and target degradation of misfolded proteins can be carried out by the UPS as well, and additionally by chaperone-mediated autophagy (CMA). CMA unfolds and translocates individual misfolded proteins to the lysosome for degradation through the formation of a chaperone-substrate complex involving chaperone heat shock cognate 70 (Hsc70). Misfolded proteins that have escaped the surveillance of the UPS and CMA form aggregates and aggresomes. Proteins in the aggregated forms can only be degraded by macroautophagy, in which aggregates or aggresomes are segregated by formation of a double-membrane autophagosome. The formed vesicle then fuses with a lysosome and the degradation of autophagosome contents ensues. In addition to targeting proteins for degradation, ubiquitination can direct proteins along other pathways through site specific and varying chain length ubiquitination.

Figure 3. A schematic illustration of the exon composition and domain structure of Ubiquilin-1 transcript variants (TV) and Ubiquilin1 protein expression in the heart of a mouse model of desmin-related cardiomyopathy

A, The full-length form of ubiquilin-1 (TV1) contains 11 exons. A ubiquitin-like (UBL) domain at the N-terminus of ubiquilin-1 protein is encoded primarily by exon 2 (shaded in red) and binds to the proteasome. The C-terminal ubiquitin-associated (UBA) domain is coded by exon 11 (shaded in blue) and binds poly-ubiquitinated proteins. In variable central region, there are 4 STI1 motifs (indicated with purple bars) located respectively in exons 4, 5, 7, and 8. TV2 lacks exon 8 (shaded in green), causing deletion of one of the 4 STI motifs. TV3 lacks exons 2, 3 and 4, and thus the majority of the UBL domain. TV4 consists of the first 3 exons. A frame shift leading to a 32-amino acid insertion after the exon 3/5 junction creates a unique short C-terminus (dark blue) and therefore TV4 lacks the UBA domain. **B**, Western blot analyses of myocardial Ubqln1 in mice. Total myocardial proteins extracted from three pairs of D7-des transgenic (TG+) and non-TG (-) mice were fractionated using 12% SDS-PAGE under the standard denatured and reduced conditions, transferred onto PVDF membrane, and immuno-probed for Ubqln1 (using the upper part of the membrane, >40 kDa) and for GAPDH (using the lower part of the membrane, <40 kDa). The band marked as "Ubqln1 TV1" shows the same mobility as a full length murine Ubqln1 transgenic protein (data not shown). Bands a and b show a molecular weight substantially higher than the TV1, whereas bands c and d display a molecular weight clearly lower than TV1.

Figure 4. Models of p62 in cardiac protein quality control

(A) Upregulation of p62 in response to proteotoxic stress sequesters NRF2 from its interaction with Keap1, leading to the stabilization and activation of NRF2, which in turn induces p62 expression. (B) Proteasome functional insufficiency leads to the accumulation of protein aggregates and compensatory activation of selective autophagy in a p62-dependent manner. Casein kinase 2 (CK2)-mediated p62 phosphorylation regulates selective degradation of protein aggregates by autophagy. Autophagy activation protects cardiomyocytes from defective UPS-induced proteotoxic stress. (C) Defective autophagy accumulates p62, which binds ubiquitinated proteins and promotes their aggregation, hindering proteasomal proteolysis. Both defective autophagy and impaired UPS function are detrimental to cardiomyocyte function and survival. (Adopted from Figure 1 of Su *et al.* [96], with permission)