Proteolysis within the cardiac sarcomere is a constantly evolving area of research. Three major pathways of proteolysis have been identified as being active within the cardiac sarcomere, namely the ubiquitin-proteasome system, autophagy, and the calpain system. The role of ubiquitin-proteasome system-mediated proteolysis in cardiovascular health and disease has been known for some time; however, it is now apparent that other proteolytic systems also aid in the stabilization of cardiac sarcomere structure and function. This minireview focuses on the individual as well as cooperative involvement of each of these three major pathways of proteolysis within the cardiac sarcomere.

Maintenance of the structure and function of the cardiac sarcomere is essential to health and longevity. This is illustrated by cardiovascular diseases (for example, the various cardiomyopathies) and conditions in which an element of sarcomeric dysfunction is central to the underlying pathology. Ensuring proper function of the cardiac sarcomere requires precise control of protein synthesis, processing, and degradation. Protein degradation (proteolysis) within the heart is achieved by three main systems, which are illustrated in Fig. 1: 1) the ubiquitin-proteasome system (UPS)²; 2) autophagy/lysosomal degradation; and 3) the calpain system. These three systems degrade proteins using complementary yet distinct mechanisms. The UPS functions by targeting specific proteins and labeling them with multiple ubiquitin molecules, which then allows recognition and subsequent degradation by the 26 S proteasome. The process of autophagy degrades larger structures that the UPS is often unable to handle, such as damaged organelles and misfolded sarcomere proteins. Calpain-mediated proteolysis is mediated by a family of calcium-dependent, non-lysosomal cysteine proteases that are expressed ubiquitously within all cells and whose function in muscle appears to involve both atrophic and hypertrophic pathways. Together, these three systems play an essential role in the maintenance of sarcomeric function in the face of physiological and pathological stimuli. Although the study of proteolysis in the cardiac sarcomere is not new (1–4), the identification of the specific mechanisms involved is still evolving, with much of what we know being inferred from studies carried out in skeletal muscle. In this minireview, we will take data from both skeletal and cardiac muscle studies and use them to discuss some of the recent literature describing the individual and sometimes coordinated roles of these proteolytic systems in the context of cardiomyocyte function and dysfunction.

The UPS in Cardiac Proteolysis

The UPS plays a critical role in protein turnover in the heart, mainly in association with cardiac atrophy, but also in the inhibition of cardiac hypertrophy and remodeling associated with cardiac stress (5–7). The UPS utilizes a series of enzymes to construct covalently linked polyubiquitin chains on lysine residues of targeted protein substrates that then “mark” these proteins for degradation (Fig. 1) (8). Proteasomal degradation of myofibrillar proteins was first demonstrated by Solomon and Goldberg (9), who showed that purified myosin, actin, troponin, and tropomyosin can be degraded in a proteasome-dependent process. Since then, it has been shown that up-regulation of the expression and activity of various components of the UPS is commonly associated with multiple models of skeletal muscle atrophy, including nutrient deprivation, unloading, diabetes, uremia, and cancer (10–12). It is now also clear that the UPS plays a significant role in atrophy occurring in the heart by either physiological or pathological means (13).

The substrate specificity of the ubiquitination process occurs at the level of the E3 ubiquitin ligases, which bind directly to the protein that is targeted for degradation (Fig. 1) (14, 15). To date, at least nine ubiquitin ligases have been described in the heart (16). Included in this group are the MuRF (muscle RING finger) family of proteins and atrogin-1/MAFbx (muscle atrophy F-box) (10, 17–19), muscle-specific ubiquitin ligases that serve both protective and possibly maladaptive roles in the development of common cardiac conditions, including cardiac atrophy.

MuRF Proteins—The three MuRF family members of ubiquitin ligases localize to the M-line and Z-disc of the sarcomere (17, 20, 21) and appear to play an important role in the regulation of structure and function of skeletal and cardiac muscle. Although the MuRF proteins share a high degree of structural similarity and are capable of heterodimerizing, the functions attributed to each of these ubiquitin ligases is quite different. Whereas MuRF2 and MuRF3 are critical for maintenance and normal contractile function (22, 23), MuRF1 is required to activate skeletal muscle atrophy (10) and inhibits pathological cardiac hypertrophy (24) but has no identified role in normal cardiac growth. MuRF3, the first MuRF family member to be identified, associates with microtubules and plays an important role in muscle development and integrity (17, 25). MuRF1 and MuRF2 (but not MuRF3) interact with titin, troponin T, myo-

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2. The abbreviations used are: UPS, ubiquitin-proteasome system; MLC, myosin light chain; TAC, transaortic cuff; MHC, myosin heavy chain; I/R, ischemia/reperfusion; LC3, light chain 3; PE, phosphatidylethanolamine.

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tilin, myosin light chain (MLC) 2, and T-cap (26). However, so far, MuRF1 is the only MuRF family member closely implicated in the regulation of myofibrillar proteolysis, having been linked to both cardiac atrophy and hypertrophy.

The role of MuRF1 in muscle atrophy was first discovered when its expression level was increased in several models of skeletal muscle atrophy (10). Gene transcripts from the gastrocnemius muscle of mice subjected to denervation, immobilization, unweighting, or exposure to the cachectic agents IL-1 and dexamethasone (all inducers of atrophy) were analyzed for changes in the pattern of transcription. MuRF1 (and atrogin-1/MAFbx, discussed below) was up-regulated in all models of atrophy, identifying this gene as an important regulator of skeletal muscle atrophy. Further proof of the involvement of MuRF1 in muscle atrophy came with the finding that MuRF1/H11002 mice fail to undergo muscle atrophy to the same extent as wild-type mice following muscle denervation (10). Because MuRF1 is also expressed in cardiac tissue, interest in its ability to regulate cardiac muscle atrophy burgeoned following the results obtained in atrophied skeletal muscle. This interest was intensified with the discovery that MuRF1 protein levels are increased in human cardiac tissue isolated from patients undergoing therapeutic atrophy following placement of a left ventricular assist device as treatment to decrease pressure over-

![Diagram of the integrated proteolytic system in the heart](image-url)
load associated with heart failure (13). Subsequently, using two different models of cardiac atrophy, a definitive role for MuRF1 in the regulation of cardiac atrophy was determined. First, the role of MuRF1 in therapeutic atrophy (i.e. reversal of hypertrophy) was verified using a model of transaortic cuff (TAC) reversal (13). Following the release of TAC in wild-type mice, hearts return to baseline size as early as 4 days. However, in MuRF1−/− mice, cardiac hypertrophy is only fractionally reversed even after 4 weeks of TAC reversal, indicating that MuRF1 is essential for this form of cardiac atrophy. The involvement of MuRF1 in cardiac atrophy associated with a decrease in muscle mass from the base line was also examined in mice treated with a continuous infusion of dexamethasone. Wild-type mice continuously infused with dexamethasone undergo significant cardiac atrophy, losing a large degree of cardiac mass (13). In comparison, MuRF1−/− mice are largely impervious to dexamethasone treatment, having little to no loss of cardiac mass. Interestingly, in the case of therapeutic atrophy in humans and mouse models, as well as in dexamethasone-induced cardiac atrophy, the levels of MuRF1 are significantly elevated in cardiac tissue, consistent with the findings from skeletal muscle studies.

The precise role that MuRF1 plays in muscle atrophy is not known; however, recent studies suggest that MuRF1 may be involved in the initial proteolytic destabilization of the myofibril, thereby allowing subsequent degradation of the individual myofibrillar component proteins in skeletal muscle. The seminal study of Solomon and Goldberg (9) demonstrating proteasomal degradation of myofibrillar proteins suggested that when actin and myosin are in a complex, they are somewhat impervious to proteasomal degradation. This study further suggested that before the UPS can degrade the individual components of the myofibril, the myofibril itself has to be disassembled or destabilized. As will be discussed below, numerous studies have been performed to identify the proteolytic agent or system responsible for the initial degradation of the myofibril, with both calpain and caspases being nominated as the cooperative degradation system (Fig. 1) (27, 28). However, there is now also some evidence to suggest that MuRF1 is capable of degrading the constituent proteins of the myofibril. Extracts prepared from denervation-induced atrophic gastrocnemius muscle reveal that the first myofibrillar proteins to undergo MuRF1-associated degradation are cardiac myosin-binding protein C, MLC1, and MLC2, whereas the myosin heavy chain (MHC) remains initially undegraded (29). The MLCs bind to the globular head of myosin, and evidence suggests that thick filament stabilization requires MLC1 and MLC2 (30, 31). Although MHC is initially protected from ubiquitination in myofibrils, it eventually undergoes MuRF1-dependent degradation. These results suggest a very different mechanism of myofibril proteolytic degradation than what has previously been suggested, and it remains to be determined whether this stepwise degradation of support proteins (for example, MLC1 and MLC2) is essential for MHC degradation and muscle atrophy. It also remains to be determined how the degradation of the thin filament proteins occurs, as in this study, the loss of MuRF1 ubiquitin ligase activity did not impact their degradation. This in itself is an interesting finding given the fact that cardiac troponin I (a thin filament protein) has been identified previously as a MuRF1 substrate (32). It is possible that this difference reveals a subtlety between skeletal versus cardiac muscle atrophy.

Atrogin-1/MAFbx—Unlike MuRF1, no specific sarcomeric protein substrates have been identified for atrogin-1/MAFbx. However, mice deficient in atrogin-1/MAFbx fail to undergo skeletal muscle atrophy to the same extent as wild-type mice, suggesting that atrogin-1/MAFbx does play a role in proteolysis of sarcomeric proteins, although whether this is a direct or indirect effect has not been established (10). Atrogin-1/MAFbx is also involved in the inhibition of both calcineurin-dependent and calcineurin-independent cardiac hypertrophy (33, 34). Identifying the substrate(s) of atrogin-1/MAFbx and the specific role that this muscle-specific ubiquitin ligase plays in muscle atrophy is an ongoing pursuit of investigators.

**Autophagy in Cardiac Sarcomere Proteolysis**

Autophagy is the process through which proteins, organelles, and invading pathogens are removed via lysosome-mediated degradation. Although there is a constant low level of autophagic activity under normal conditions (35), autophagy in the heart is up-regulated in response to stressors such as ischemia/reperfusion (I/R) injury, cardiac hypertrophy, heart failure, and nutrient deprivation (36). Autophagy requires a cascade of evolutionarily conserved proteins (Atg proteins) that comprise two conjugation pathways: 1) the Atg12-Atg5 pathway and 2) the light chain 3 (LC3)-phosphatidylethanolamine (PE; or Atg8-PE) pathway (Fig. 1) (37). Autophagy begins with the formation of an isolation membrane, which elongates in a stepwise manner to eventually surround the molecule or organelle that is slated for degradation in a double-membrane organelle called a phagosome (Fig. 1). In order for this to happen, Atg12/Atg5 or Atg8 (LC3) is conjugated to Atg7, Atg10, Atg5, or Atg3 via lysine residues, forming complexes essential for the recruitment of LC3 and the formation of the membranes needed to form the phagosomes. Once the targeted protein or organelle is fully contained, the inner membrane of the phagosome fuses with the lysosome, thereby allowing degradation to occur.

Autophagy is an important mechanistic role in normal cardiac function especially in aging, as well as an adaptive mechanism used to withstand cardiac stress. The importance of autophagy in normal cardiac function is illustrated by the phenotypes of the numerous Atg-deficient mice that have been engineered. Cardiac-specific deletion of Atg5 in adult animals results in disorganization of the sarcomere and misalignment and agglutination of mitochondria, as well as cardiac hypertrophy, left ventricular dilatation, and contractile dysfunction (38). In contrast, cardiac-specific deletion of Atg5 during cardiogenesis results in no functional deficits (38). However, over time, these mice develop signs of cardiac failure, with significant increases in left ventricular dimension and a decrease in fractional shortening (39). Ultrastructural examination of hearts from Atg5−/− mice reveals disorganized sarcomeric structure as well as collapsed mitochondria as early as 3 months of age, notably at a time when cardiac dysfunction is not yet apparent. Interestingly, severe cardiac dysfunction can be induced much earlier in Atg5−/− mice by induction of pressure overload (38).
Autophagy is associated with both positive and negative influences on cardiac function. It has long been known that autophagy increases in the heart after I/R injury. Over 30 years ago, an increase in autophagic vesicles was seen following I/R injury to fetal mouse heart cells in culture (40). Numerous studies since then have alluded to the possibility that autophagy represents a protective mechanism in the heart following I/R, possibly by reducing cell apoptosis (41–43). HL-1 myocytes subjected to I/R injury in culture exhibit increased cell death when autophagy is inhibited and increased cell survival when autophagy is enhanced (44). Likewise, cardiomyocytes subjected to either I/R or glucose deprivation (a component of ischemia) fare better when autophagic pathways are enhanced rather than inhibited (45, 46). However, there are also reports of autophagy promoting cell death after I/R injury. Isolated cardiomyocytes in which the Atg protein Beclin-1 is blocked survive better after I/R insult than do cells in which the autophagic pathway is left intact (47). Beclin-1 contains a pro-apoptotic BH3 domain and becn1−/− mouse hearts exhibit reduced numbers of apoptotic cells following I/R injury, supporting the theory that autophagy is in fact detrimental to cell survival following I/R injury (46, 48, 49). These various reports suggest that autophagy can have dual roles in the heart following I/R injury. Indeed, it has been shown that autophagic pathways can be protective in cultured cells following ischemia, yet continued activation of autophagy can become detrimental at the onset of reperfusion (46).

One of the triggers of autophagy within the heart is the accumulation of protein aggregates (50). Protein aggregates can form via a number of mechanisms, including the failure of the UPS to clear ubiquitinated proteins (Fig. 1) (50). A link between the proteasome and autophagy systems is now well recognized, with studies demonstrating that ubiquitinated protein aggregates can be substrates for autophagic degradation (51–53). There are numerous reports demonstrating that inhibition of the UPS leads to increased autophagy and that suppression of autophagy leads to an increase in ubiquitinated protein aggregates (54–56). It is worth noting, however, that many of these studies were carried out in neuronal systems, so whether or not these same mechanisms exist in muscle cells still remains to be determined.

These studies demonstrate that there is cross-talk between the UPS and autophagy systems; however, there is also evidence of coordinated activity of these two systems that is regulated via the same transcription factors yet is still functionally independent of one another (57, 58). Myotubes transfected with a constitutively active version of the forkhead transcription factor FoxO3 exhibit heightened proteolysis that includes proteasomal and autophagic degradation (58). Forkhead transcription factor proteins activate atrogin-1/MAFbx, increasing proteasomal degradation associated with this ubiquitin ligase (59). However, FoxO3a can also induce the expression of many autophagy-related genes, including LC3b, Atg12l, Atg4b, and becn1, in both myotubes in culture and mouse muscles atrophied from either denervation or fasting (58). Hence, it is conceivable that the processes of proteasomal and autophagic proteolysis occur simultaneously in some tissues.

Calpain Involvement in Proteolysis within the Cardiac Sarcomere

The calpain family of Ca2+-dependent, non-lysosomal cysteine proteases consists of at least 15 proteins, all of which are coded for by an independent gene (60, 61). The two most well studied forms of calpain are calpain-1 (μ-calpain) and calpain-2 (m-calpain), named for their responsiveness to either micromolar or millimolar quantities of Ca2+, respectively (62). Both of these calpains are found in modest amounts within the myocardium, where they have been linked to the degradation of myofibrillar proteins such as the troponins, tropomyosin, and titin (63–65). Another of the calpains, calpain-3 is specific to skeletal muscle, where it has been proposed to aid in the disassembly of the multiprotein complexes that make up the sarcomere, prior to the UPS-mediated degradation of the individual sarcomeric proteins (66, 67).

The earlier studies by Solomon and Goldberg (9) demonstrating that actin and myosin, when in a complex, are resistant to proteasomal degradation raised the possibility that the intact myofibril must first be disassembled before degradation of the component proteins can take place. Since that report, other investigators have published studies that seem to support this theory and suggest that calpains might be the degradative system responsible for myofibril destabilization (Fig. 1). For instance, HL-1 cardiomyocytes induced to tachy pace (mimicking the effects of atrial fibrillation) exhibit a gradual yet significant decrease in cardiac troponins that is prevented by the calpain-specific inhibitor PD150606 (68). Likewise, atrial tissue isolated from patients suffering from atrial fibrillation demonstrates a striking correlation between the degree of cardiac troponin degradation and the level of calpain activity. Interestingly, in both the tachy-paced myocytes and the isolated atrial tissue, actin is spared from calpain-mediated degradation, suggesting that the targeting of troponin by calpain is a preliminary step in myofibrillar degradation, with proteolysis of actin and myosin being carried out by another proteolytic system. Evidence for this theory of combined action of the proteasomal and calpain systems has previously been reported in the process of muscle remodeling in response to altered contractile activity, injury, and eccentric exercise (69–72). In a mouse model of muscle remodeling, in which non-pathological atrophy is induced followed by stimulation of muscle regrowth, mice deficient in the muscle-specific calpain-3 exhibit deficits in both the rates of atrophy and regrowth compared with wild-type mice (67). Whereas muscle reloading induces an elevation in both ubiquitinated proteins and calpain-3 in the muscles of wild-type mice, muscles isolated from calpain-3-deficient mice exhibit no increase in ubiquitination at the reloading stage, suggesting that calpain is necessary for ubiquitination of myofibrillar proteins and that it acts upstream of the UPS (67).

Further support for the theory of cooperation between the calpain system and proteasomal degradation of sarcomeric proteins comes from studies using cultured cardiomyocytes in which calpain-1 is expressed via adenoviral transfection (73). These cells exhibit heightened proteolysis of specific substrates (including desmin and troponin I), increased overall protein ubiquitination, and accelerated protein turnover, all of which
can be inhibited by coexpression of the endogenous calpain inhibitor calpastatin (73). Likewise, transgenic mice overexpressing cardiac calpain-1 exhibit substrate-specific proteolytic activity, including hyperubiquitination of cardiac proteins and increased 26 S proteasome activity. Conversely, loss of endogenous cardiac calpain expression in transgenic mice overexpressing the inhibitor calpastatin decreases ubiquitination of myocardial proteins without actually affecting the overall proteasome activity, suggesting that the calpain contribution to proteolysis in this instance is upstream of UPS involvement (73).

There is also evidence refuting a role for calpain in myofibrillar destabilization prior to proteasomal degradation. Dystrophin-deficient mice overexpressing the calpain-specific inhibitor calpastatin exhibit the same degree of muscle wasting as dystrophin-deficient mice in which calpain is active, suggesting that the muscle atrophy associated with this mutation occurs independently of calpain-induced proteolysis. Similarly, in lysates of L6 muscle cells treated with staurosporine to induce caspase-3 activation, actin fragments can be isolated but only when the proteasome inhibitor MG132 is added to the incubation medium (presumably when the proteasome is left activated, it degrades the actin fragments) (27). The addition of the caspase inhibitor acetyl-DEVD-aldehyde to the medium prevents the accumulation of actin fragments in L6 muscle cell lysates, whereas calpain inhibitor 1 has no effect (27). Interestingly, this effect of activated caspase is thus far seen only in muscle proteolysis associated with catabolism. When calcium-activated proteases such as calpains are inhibited in isolated muscle preparations from rats subjected to a number of catabolic conditions (starvation, acidosis, muscle denervation, cancer), the rate of proteolysis is not suppressed, suggesting that alternative proteolytic pathways are in effect (74–78). Skeletal muscle isolated from rats that have been made acutely insulin-repressed, it degrades the actin fragments) (27). The addition of the caspase inhibitor acetyl-DEVD-aldehyde to the medium prevents the accumulation of actin fragments in L6 muscle cell lysates, whereas calpain inhibitor 1 has no effect (27). Interestingly, this effect of activated caspase is thus far seen only in muscle proteolysis associated with catabolism. When calcium-activated proteases such as calpains are inhibited in isolated muscle preparations from rats subjected to a number of catabolic conditions (starvation, acidosis, muscle denervation, cancer), the rate of proteolysis is not suppressed, suggesting that alternative proteolytic pathways are in effect (74–78). Skeletal muscle isolated from rats that have been made acutely insulin-deficient exhibits a decrease in PI3K activity that leads to an increase in caspase-3 activity (79). The decrease in PI3K in insulin-deficient muscle also leads to an increase in atrogen-1/MAFbx expression and activity via the stimulation of FoxO proteins as described above. In addition, as mentioned previously, MuRF1 is capable of degrading sarcomeric proteins from isolated mouse myofibrils including elements of the myosin thick filament, thereby contradicting the earlier premise that actomyosin complexes cannot be degraded by the UPS alone (29).

Summary

Proteolysis of the sarcomere and its component proteins occurs through the coordinated efforts of the UPS, the process of autophagy, and the activity of proteases such as calpain and caspases. Numerous studies have described the integral role of muscle-specific ubiquitin ligases and their targeted degradation of sarcomeric proteins in the regulation of many common cardiovascular diseases. Similarly, autophagy is considered a necessary process for the normal physiological workings of the heart, although its role (either beneficial or destructive) in cardiovascular diseases is still under scrutiny, with data linking the autophagic pathway of proteolysis to a number of cardiac pathologies, including hypoxia, I/R injury, myocardial infarction, and end-stage heart failure. Finally, sarcomeric proteolysis via calpain and caspase activation may be involved in cooperatively degrade proteins to allow their access to the UPS. Several lines of evidence have demonstrated that calpain and caspase activity cleaves the actomyosin sarcomere complex to release actin, allowing the UPS access to degrade specific sarcomere components. There is also accumulating evidence demonstrating that the individual processes of proteasomal and autophagic degradation work in concert to maintain protein quality control in various tissues, including the sarcomere, although the exact nature of the relationship between these proteolytic systems remains to be elucidated. Likewise, many of the details involving the interplay between the UPS and the calpain and caspase enzymes need to be better understood in terms of both normal maintenance of the sarcomere and cardiovascular diseases. Identifying and understanding the individual and cooperative roles that each of these proteolytic pathways plays in cardiovascular maintenance and health allow for the development of specifically targeted therapeutics that may be useful in the treatment of cardiovascular disease processes with a proteolytic element. This idea has already been tested with the use of proteasome inhibitors for certain cardiovascular diseases. However, the variable success of this treatment is perhaps reflective of the complex nature of proteolysis in the cardiac sarcomere.

REFERENCES
